EXECUTIVE SUMMARY

1. INTRODUCTION AND GENERAL REMARKS

The Italian Institute of Technology (IIT) is a Foundation created to promote high tech research in Italy.

IIT was established jointly by the Ministry of Education, University and Research and the Ministry of Economy and Finance. The institute is open to the active participation of private and public organizations in order to encourage technological development and training in high technology.

IIT aims at becoming an institute of international excellence for scientific research in advanced technology, attracting researchers and experts from all over the world.

The purpose pursued by the Foundation is to promote the country's technological development and advanced training in technology, consistently with the national policies for science and technology, thus fostering and enhancing the national production system.

In order to pursue this result, the Foundation

– creates a world class research infrastructure based on a central research laboratory in Genova and on a multidisciplinary research network of internationally acknowledged institutions spread over the country.

– carries out advanced training programs as part of a broader multidisciplinary program;

– produces technological knowledge, related to new devices, methods, processes and techniques to create new services and new sectors of production, strategic for the competitiveness of the nation;

– pools researchers operating in different research institutes and establishes links with international centers of excellence;

– promotes the interaction between basic research and applied research;

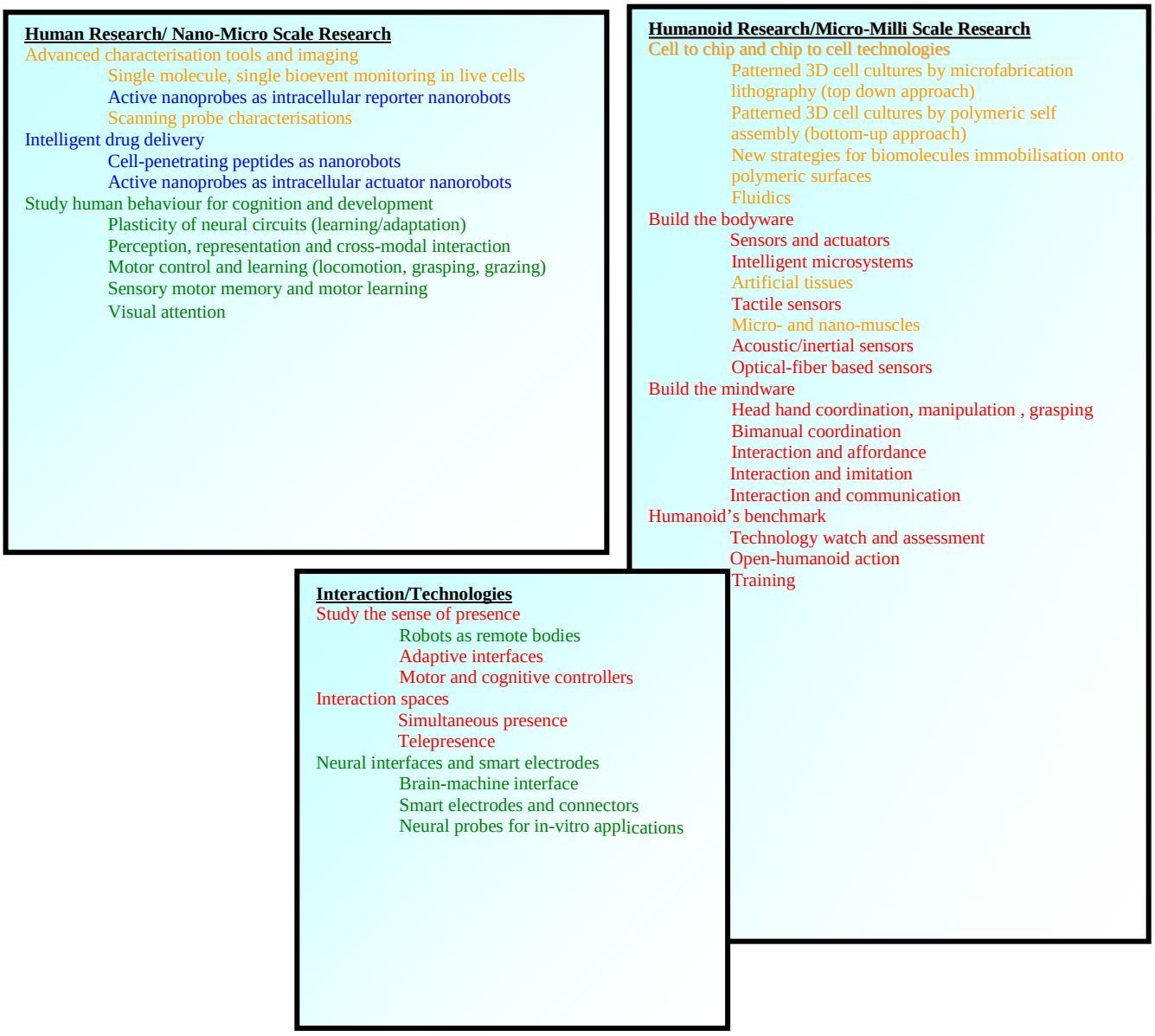
– disseminates transparent merit-based mechanisms of selection of researchers and projects in accordance with worldwide tested criteria.

IIT will simultaneously foster basic research and R&D joint actions with private entities, through two distinct branches, the IIT- basic research branch and the IIT applied research branch, having different focus and action schemes. The former is primarily based on the

central research lab of Genova (see section 3 and Annex 3) and on the multidisciplinary research network spread over the country (see section 4 and Annex 4). The latter develops high risk R&D programs jointly with companies, according either to bottom-up approach (demand-driven programs originating from technology transfer scouting in the districts, industrial sectors etc..) or to a top-down approach (call for proposal agreed with Confindustria, Ministero Attività Produttive, etc.). A description of such activities is given in section 7 of this document. Finally, the route for the establishment of a IIT PhD course in advanced technologies will be shortly outlined in section 8.

2. IIT SCIENTIFIC PROGRAM

Fig.1 Mainstream lines of the IIT research program



Robotics brain and cognitive sciences department
Neuroscience and brain technologies department
Drug discovery and development department
Nanobiotechnologies facilities

THE VISION

The aim of IIT is to develop research projects, capable of producing technological innovation, starting from fundamental research up to industrial research and development. Special attention will be given to projects where technology is considered a science and not just an engineering development.

In 2004 the Steering and Regulatory Committee and the Scientific Director broadly defined the fields in which IIT could focus its research activity. The Scientific Plan deals with a set of technologies referred to as "Humanoid Robotics" which include four highly interconnected technological platforms: Robotics Brain and Cognitive Sciences (RBCS), Neuroscience and Brain Technologies (NBT), Drug discovery and development (D3) and Nanobiotechnologies. Such research streams have to be considered as broad and non exclusive scientific areas, leaving room and freedom to the future IIT scientists to propose and develop their own scientific program, in the humanoid technology vision's frame.

Strong emphasis will be laid on design and fabrication of new devices for probing and studying living systems (photonics, electronics, nanoelectrodes interconnection, nanoparticle markers, tissue engineering in platform 4), which will be used to generate large sets of molecular and electrophysiological data from selected neural systems (platform 2). This will provide the background knowledge for computer simulation and, in the long term, for automation and robotics inspired to humanoid systems (platform 1). Finally the third platform will closely collaborate with other Departments within the IIT in the areas of neuropsychiatric disorders, intelligent nanomaterial-based drug delivery and drug discovery technology. The interplay of these platforms will be a distinguishing feature of the IIT.

Research will be carried out along the four streams autonomously, having dedicated staff and departments. A set of interconnected core facilities will be established and specific management policies will be accomplished to encourage and allow an interdisciplinary activity. According to its mission, the IIT research program will be technology oriented, though exploratory and visionary in the long term.

The program opens a broad area of research and applications in different fields, such as:

Learning and adaptation in robotics, visual, haptic and auditory perceptions, real time and remote control, human-robot interaction, sensors and actuators, biomimetic technologies, bionanotechnologies, artificial skin/tissues, drug delivery and discovery, nanoscale

designed materials, new characterization methods at molecular and atomic scale, brain-machine interfaces, neural plasticity etc.

More detailed information can be found in the Annex 1 and 2.

In what follows we briefly summarize some of the basic aspects of the four platforms.

– Platform 1: **Robotics Brain and Cognitive Sciences**

The advent of electronics and the development of Control Theory during the first half of the last century gave a tremendous acceleration to robotics, especially to the branch called Industrial Robotics, which is nowadays regarded as a mature and consolidated scientific field. Factory automation, for example, is one of the direct results of the advances in control theory, electronic and computer science in the last sixty years. Yet it was long ago acknowledged that robotic applications could go much further than factory automation. Modern robotic technologies include:

- Harsh environments' exploration, including space, marine and underwater, volcanoes, artic or desert regions;
- medical applications, including surgery, diagnostic and rehabilitation;
- service robotics, including cleaning and housekeeping, education and entertainment, demining, agricultural and harvesting, lawn mowing, surveillance, inspection, mining, construction, fire fighting, search and rescue, tour guides and office applications.

In perspective, robotics research will face issues such as the biologically compatible materials use, and of methods and operation principles approaching human capabilities. The coexistence of strong interdisciplinary research programs in nanotechnologies and in neurosciences will provide the ideal foundations for the third IIT program, which will be devoted to the study, design and fabrication of robotic systems based on biological mechanisms (such as learning, vision, hearing), materials and interconnections.

In order to exemplify the platform activity, the following research topics could be strategic areas of investigation:

- autonomous robotics;
- humanoid robotics;
- teleoperation.

– Platform 2: **Neuroscience and Brain Technologies (NBT)**

The main focus of research in neural sciences will be the development of new technologies for the study of brain function.

In order to exemplify the platform activity, the following research topics could be strategic areas of investigation:

- functional genomics;
- molecular structure and function synapses;
- from intercellular communication to cognitive functions;
- medical implications;
- networks sciences and biological computation.

- Platform 3: Drug Discovery and Development (D3)

The Department was launched on January 2007. Its mission is to discover and develop innovative medicines in the areas of brain disorders and inflammatory diseases. The D3 will accomplish this mission by creating an internal research engine, constituted by a multidisciplinary group of talented and creative scientists, and fostering the development of public-private partnerships aimed at accelerating the drug discovery process. It will closely collaborate with other Departments within the IIT in the areas of neuropsychiatric disorders, intelligent nanomaterial-based drug delivery and drug discovery technology. The research laboratories of the D3 are currently under construction.

– Platform 4: **Nanobiotechnologies**

The application of nanotechnologies to life sciences is a challenge for the next twenty years. This includes the development of new technologies. These could involve new concepts of biological matter to physical probes interconnection, and novel approaches to study cellular functions at the micro and nanoscale, aiming at a better understanding of the physiological behavior and of the mechanisms causing misfunction and disease. New tools for diagnostics and imaging, and new methods for intelligent drug delivery are in prospect as well. The nanobiotechnology platform will develop the enabling technologies needed for the accomplishment of such challenging results.

In order to exemplify the platform activity, the following research topics could be strategic areas of investigation:

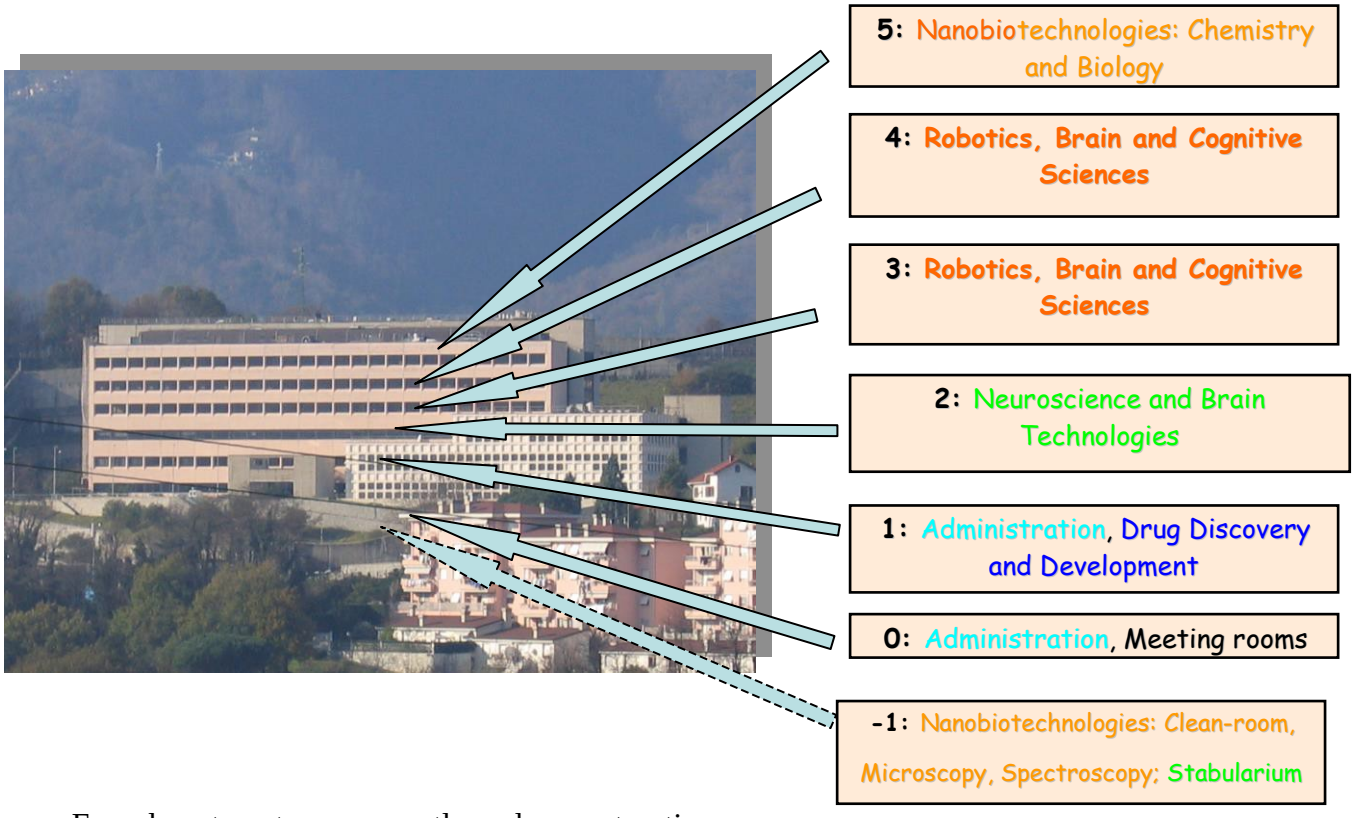
- cells-to-chip and chip-to-cells technologies;
- advanced characterization tools and imaging;
- intelligent drug delivery;
- artificial tissues;
- smart materials.

The four platforms represent the starting points of an ambitious research strategy which aims at developing cross-fertilization among scientific fields of great fundamental and applied potential. The implementation of such a strategy will need a coordinated effort between the IIT research infrastructure, a network of advanced research institutions over the country, the business community and the educational system. The main actions to be pursued are the following:

- constitution of the IIT thematic departments, at the central research laboratory in Genova-Morego,
- constitution of the IIT common facility labs, at the central research laboratory in Genova-Morego,
- constitution of the multidisciplinary research network, involving the major research institutions over the country
- preparation of the IIT applied research program with specific R&D actions in collaboration with industrial partners and business community.

3. CENTRAL RESEARCH LABORATORY AT GENOVA-MOREGO

The IIT Central Research Lab in Morego is designed to be one of the largest multidisciplinary research laboratories in the world. The location of the institute is close to Genova airport, about 15 minutes by car from the city centre. The building is a facility of about 30000 sqm (including external space) distributed over 5 floors and the underground level, fully equipped with power stations, air conditioning, continuity groups, services, etc. The building host a few research departments directed by internationally acknowledged scientists. The central research lab provide a common infrastructure consisting of dozen of advanced research laboratories, mechanical and electronic facilities, meeting and seminar rooms and restaurant. Up to about 400 people are expected to operate in the central research lab in the next three years.



Four departments are currently under construction:
ROBOTICS, BRAIN AND COGNITIVE SCIENCES (RBCS),
NEUROSCIENCE AND BRAIN TECHNOLOGIES (NBT),
DRUG DISCOVERY AND DEVELOPMENT (D3), and
NANOBIOTECHNOLOGIES.

The main technological goal of the **ROBOTICS, BRAIN AND COGNITIVE SCIENCES (RBCS)** department is to move ahead from traditional humanoid robots with mechanical hands and legs (hard-bodied systems) toward next generation hybrid systems realized with soft materials (soft-bodied systems) and with cognitive abilities allowing the interaction with humans in a natural way. This goal will be pursued through multidisciplinary research spanning from mechatronics, to advanced material, to the study of how humans learn, perceive, and act in a natural environment. The RBCS is structured into five main labs:
Humanoid platform, directed by Prof. Giulio Sandini (formerly at the University of Genova, Italy) and Prof. Darwin Caldwell (formerly at the University of Salford, UK), designing new generation, epigenetic and interacting platforms.
Human behaviour laboratory, directed by Prof. Giulio Sandini, exploring the brain mechanisms at the basis of motor behavior, learning and sensorimotor integration. New generation imaging systems will be developed for this purpose.
Interaction Laboratory, directed by Prof. Darwin Caldwell focusing on the study, development and testing of technologies required to create real, virtual or augmented human-robot interaction environments such as whole body haptic interfaces, exoskeletons and wearable systems.
Biotechnology laboratory, directed by Prof. G. Sandini, studying new technologies to produce brain probes and integrate artificial materials with the human body.
Targeted Robotics directed by Prof. Jean-Guy Fontaine (formerly at the University of Paris, France) and Prof. Darwin Caldwell fostering the development of industrially and socially relevant technologies through joint projects and labs with industries in all research areas of the department and addressing specifically the area of micro and macro teleoperation, telepresence and telexistence (tele 3x).
The five branches functionally integrate to form a unique interdisciplinary research infrastructure putting together a complete hardware-software strategy for humanoid robotics with the study of the brain mechanisms underlying motor control, learning and cognition. Brain-machine interfaces and advanced neuro-prosthetics will be investigated by interactively merging engineering and neurophysiology competencies, in synergy with the NBT.

The **NEUROSCIENCE AND BRAIN TECHNOLOGIES (NBT)** department, led by Prof. Fabio Benfenati (formerly at the University of Genova, Italy), has the objective to apply new

technologies to the study of the central nervous system. The mission of the NBT is to elucidate the genetic and epigenetic factors at the basis of synaptic transmission and plasticity, the learning and adaptation strategies of the nervous tissue and the relationships between neural molecules and information coding and processing in the brain. These research themes will be investigated:

at various levels of brain complexity from individual synapses to neural networks to the live animal by using advanced techniques coupling mouse genetics, patch-clamp/multi-electrode recordings and functional imaging of live neurons;

in experimental models of brain diseases (including epilepsy, addiction and neurodegenerative diseases) in synergy with the D3 department;

in bio-hybrid systems by generating neuro-electronic and bidirectional neuro-robotic interfaces. These neuron-to-chip systems will allow the study of the basic properties of simplified neuronal networks and the implementation of new neuron-based biosensors and neuroprosthetic interfaces. These topics will be investigated by interactively merging nanotechnology, engineering and neurophysiology competencies, in synergy with the NANOBIO and RBCS departments.

The laboratories and research infrastructures of the NBT are currently under construction.

The Department of **DRUG DISCOVERY AND DEVELOPMENT (D3)**, directed by Prof. Daniele Piomelli (from the University of California, Irvine, USA), was launched on January 2007. Its mission is to discover and develop innovative medicines in the areas of brain disorders and inflammatory diseases. The D3 will accomplish this mission by creating an internal research engine, constituted by a multidisciplinary group of talented and creative scientists, and fostering the development of public-private partnerships aimed at accelerating the drug discovery process. It will closely collaborate with other Departments within the IIT in the areas of neuropsychiatric disorders, intelligent nanomaterial-based drug delivery and drug discovery technology. The research laboratories of the D3 are currently under construction.

State of the art facilities will provide hardware and characterization support to the Lab's scientific activities.

The hardware facilities include:

- Mechanical workshop with advanced design and manufacturing capacities

- Electronic workshop with advanced design and fabrication capacities
- Animal facility with pharma test area, surgical area, in-vivo experiment area, etc.

The **NANOBIOTECHNOLOGIES FACILITIES** department will provide:

- Clean room with micro- and nano-fabrication and material processing
- Transmission electron microscopy facility
- Scanning electron microscopy facility
- Scanning probe (AFM/STM) microscopy facility
- Optical spectroscopy facility with fs, ps, ns and cw spectroscopy
- Polymer laboratory for composite systems
- Basic chemistry and characterization laboratory
- Colloidal Chemistry laboratory for synthesis of nanoparticles
- Super computer facility

FLOOR MAPS

- **Undergroud floor**

Nanobio Labs (Clean room, Microscopy Lab, Optical Labs) and Animal Facility

- **Ground floor**

Seminar room, Restaurant, Administration, Offices

- **First floor:**

Administration, D3 Labs, Offices

- **Second floor:**

NBT Labs, Offices

- **Third floor:**

RBCS Labs, Offices

- **Fourth floor:**

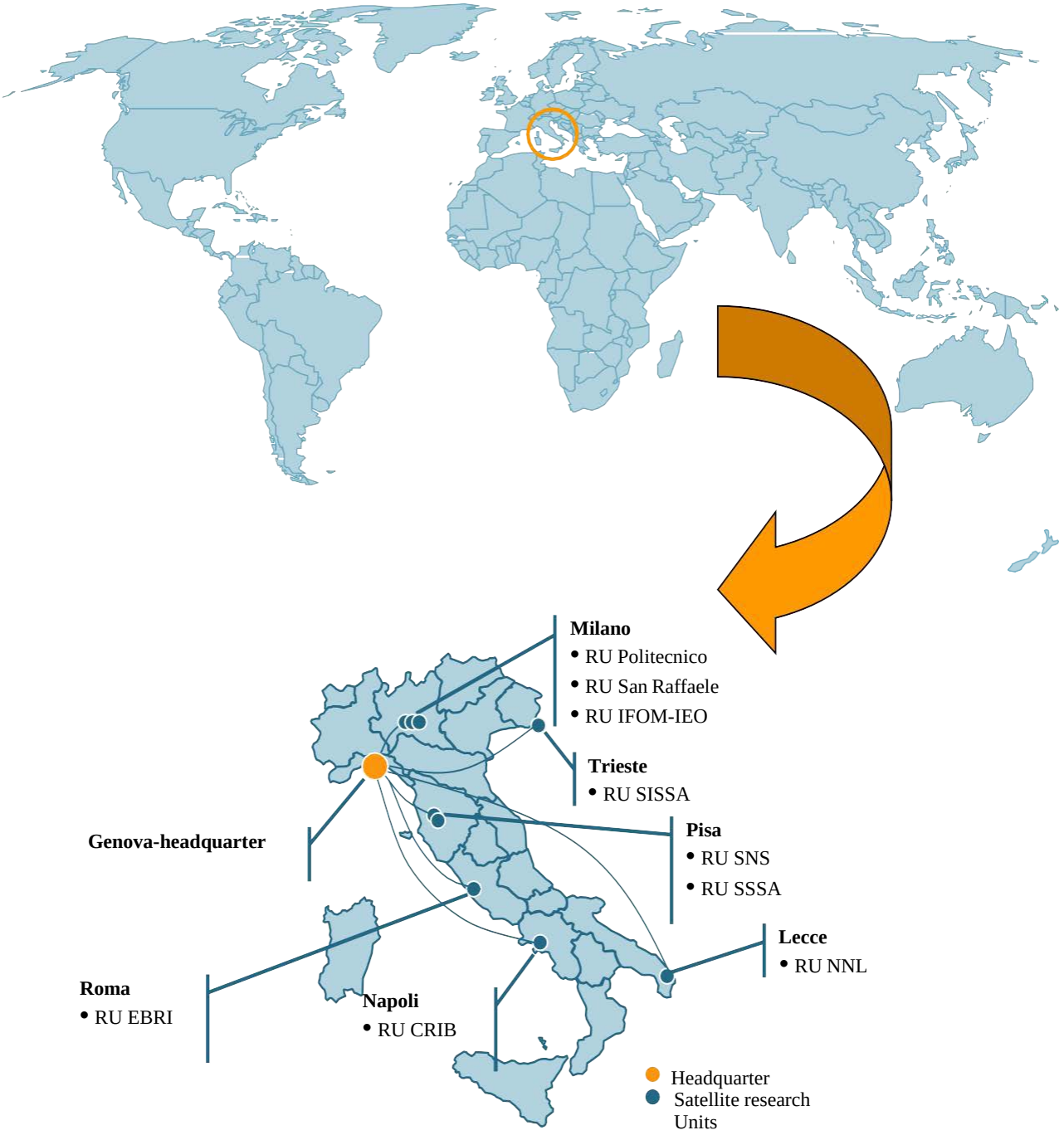
RBCS labs, Offices

- **Fifth floor:**

Nanobio Labs (Chemistry, Biology)

4. THE MULTIDISCIPLINARY RESEARCH NETWORK OF IIT

Fig.2



The Multidisciplinary Research Network consists of nine IIT Research Units hosted by internationally acknowledged research institutions (see Fig.2). Each Research Unit develops parts of the IIT research program according to specific agreements. These include training and

education of PhD students as well as experimental and theoretical research activities. The Research Units offer dedicated research infrastructures (laboratories, space, personnel) and know how relevant to the IIT program. On the other hand IIT supports the research with a dedicated budget, with the collaboration of the central research facility of Genova and of its research staff. The Research Units of the network are established for a period of five years, and their results are yearly evaluated by the IIT Scientific Committee.

The basic criteria for the choice of the Research Units are:

- Relevance of the research infrastructure made available by the host institution (staff, space, state of the art equipment);
- Internationally acknowledged reputation in the IIT field of interest (evaluated through impact factors, citation index, patents and publications over the last five years) ;
- Capability of raising public and private (national and international) funds;
- Capability of technology transfer and industrial R&D activities.

In general, each Research Unit makes available laboratories and infrastructures between 500 and 1000 sqm with state of the art equipment, and staff between 10 and 30 people (including researchers, PhD and technicians)

The Research Units list (from North to South) presently forming the MRN and the main research workpackages of each RU is listed in the following (see Annex 4 for detailed info about the research programs of each Research Unit):

1) Research Unit IIT – Politecnico di Milano

NANOBIOTECHNOLOGY

WP1 - Bioelectronics and biophotonic interfaces between cells and artificial systems

WP2 - Functional surfaces

WP3 - Organic materials for artificial bio-systems

WP4 - Molecular imaging

WP5 - Models and methods for local drug delivery from nano/micro structured materials

REHABILITATION

WP1 - Multisource neurophysiological information processing for innovative and personalized rehabilitation protocols

WP2 - Human machine interface for recovery of lost functions

WP3 - Robotic companion exploiting affective feedback for modeling emotional state of the patient and adapting the rehabilitation treatment

2) Research Unit IIT – Institute of Molecular Oncology Foundation-European Institute of Oncology (IFOM-IEO)

WP1 – Protein interaction dynamics and systems biology of macromolecular networks

WP2 – Light microscopy for advanced live cell and live animal imaging

3) Research Unit IIT – Fondazione Centro San Raffaele del Monte Tabor (HSR)

WP1 – Multiplicity of exocytoses: role of specific forms in physiology and pathology

WP2 – Cellular and molecular imaging of neuron-astrocyte signaling in physiological and pathological conditions

WP3 – Intelligent drug delivery by viral- like particles

WP4 – New tools for modulating endothelial barrier function and drug delivery

WP5 – Optical approaches to the study of neuronal plasticity

WP6 – Dynamics of single molecule and single bioevent in living cells revealed by fluorescence fluctuation and time resolved fluorescence spectroscopy

WP7 – Haptics in neuroscience and robotics

4) Research Unit IIT – Scuola Internazionale Superiore di Studi Avanzati (SISSA)

WP1 – Molecular simulation for biological sciences

WP2 – Neurotelemetry: Remote acquisition and manipulation of neuronal signals

WP3 – Development of new nanodevices for neurobiological applications

***5)* Research Unit IIT – Scuola Normale Superiore (SNS)**

WP1 – Advanced techniques for characterisation and imaging

WP2 – Drug, nanoreporter and nanoactuator delivery

WP3 – Tissue engineering technologies

6) Research Unit IIT – Scuola Superiore di Studi Universitari e di Perfezionamento Sant'Anna (SSSA)

WP1 – Micro- and Nano-technologies for endoluminal and cellular surgery

WP2 – Micro- and Nano-technologies for biorobotic components and systems

7) Research Unit IIT – European Brain Research Institute (EBRI)

WP1 – Neurogenomics and functional proteomics of cholinergic eurons and of cortical interneurons

8) Research Unit IIT – Università degli Studi di Napoli Federico II - CRIB

WP1 - Development and optimization of novel technologies to modulate material structure and properties at micro and nano-levels

WP2 – Bioactivated scaffolds design and production

WP3 – Determinants of metabolic microenvironment in 3D scaffolds and process condition optimization

9) Research Unit IIT – National Nanotechnology Laboratory (NNL)-INFM-CNR

WP1 - Cells to chips and chips to cells

WP2 - Advanced characterization tools and imaging

WP3 - Soft lithography on functional molecules

WP4 - Functionalized nanocrystals for cancer therapy

WP5 - Biodevices and biosensors arrays for electrochemical sensing and redox activity monitoring in cells

The MRN has a strong cross-disciplinar character and it covers a large part of the IIT research program. All Research Units have already well established connections with major industries in different fields, and outstanding PhD educational programs. The MRN is meant to boost the start-up of IIT meanwhile the central research laboratories at Genova Morego are built and made operative. To date, 5 patents and about a hundred of publications on international journals have already appeared with IIT affiliation, thus contributing substantially to the international visibility of IIT since the very beginning of its start-up phase.

5. *POLICY FOR THE INTELLECTUAL PROPERTY*

The IP policy (of IIT) is developed according to the guidelines described in the following. The IP policy for external collaborations with public and private partners and for the internal research staff are treated separately.

In the former case, the general criterion is that IIT equally shares the intellectual property with the partner, and eventually gives exclusive exploitation license to industrial partner(s), only for the applications of the invention which are relevant to the core activity of the joint project. Other agreements are eventually possible, depending on the funding and on the resources invested by the partners. Work is currently in progress in collaboration with the patent offices of the Multidisciplinary Research network to produce a general frame agreement.

For the latter case (IP policy for the staff members) IIT follows a scheme similar to that currently implemented at all Campuses of the University of California, with a few modifications ensuring compatibility with the Italian legislation. Such policy tends on one hand to preserve the rights of IIT and, on the other, to stimulate and award the IIT researchers, namely:

A: Documentation of patentable inventions.
IIT investigators and staff are obligated to appropriately document all experimental results that might lead to patentable inventions. To fulfil this obligation, a standard documenting procedure will be implemented, which will require the use of experimental log-books that are bound, numbered, written in indelible ink, and signed by both the investigator and a knowledgeable witness, who is not an inventor, on each numbered page.

B: Disclosure of patentable inventions.
All IIT investigators and staff are obligated to disclose to the Institute any possible patentable inventions developed within the scope of their employment. To fulfil this obligation, a standard disclosure of invention will be implemented (preliminary forms already available from e.g. UC).

C: Assessment of patentability.

The IIT will conduct a patentability analysis on all disclosures of invention filed and, based on this analysis, will determine whether it will be able to successfully commercialize the invention. This analysis will be conducted in a timely manner and will not exceed a maximal duration of 45 days. At the end of this period, the IIT will inform the inventor(s) of the decision and, if patenting is recommended, will work diligently with the inventor(s) to draft a patent application. If patenting is not recommended, the IIT may release the invention back to the inventor(s).

D: Assignment of patentable inventions.

All IIT investigators and staff are obligated to assign to the IIT their sole and joint rights in any possible patentable inventions developed within the scope of their IIT employment.

E: Royalty sharing.

All IIT investigators and staff will share in net fees and royalties received from licensed intellectual property. 50% of the net proceeds will go to the IIT to cover for reimbursement costs and to support research; 30%-40% of the proceeds will go to the inventor(s); and 10%-20% of the proceeds will go to the inventor's Department.

6. *THE TECHNICAL AND SCIENTIFIC COMMITTEE*

The following guidelines describe the role and operation of the Technical and Scientific Committee of IIT, which is the evaluation and technical advice organism of the Institute.

A: The role of the TSC is:

- Evaluation of the research activity and of the directors of the central research lab of Genova-Morego and of the research carried out in the Research Units of the Multidisciplinary Research Network. The evaluation includes the assessment of the accomplishment of the milestones and deliverables of the directors and of the Research Units of the network.
- Update of the IIT scientific program and check of the overall consistency of the with the program, including technology transfer activities and industrial R&D activities;
- Strategical scientific advice (identification of new research lines,.identification of new Research Units and Departments, selection of new directors, set up of new labs/research infrastructures).

B: Operation of the TSC:

The activity of the TSC will be supported by interactive on-line tools. It is reasonable to assume that the TSC will have yearly meeting, possibly with on-site visits of the main research labs of IIT. Each TSC member will have personal web sites where all reports and information will be accessible on-line. Specific evaluation forms will be made available for interactive evaluation procedures.

C: Composition

So far the following scientists have accepted to serve the IIT Technical and Scientific Committee:

E. Bizzi - MIT spokes person-scientific secretariat, USA;
Y. Arakawa - Director RCAST , Tokyo University, Japan;
P. Greengard - Rockefeller University, USA;
U. Veronesi - IEO Italy;
P. Alivisatos - University Berkeley, Ca-USA;
H. R. Horvitz - MIT Boston, USA;

O. Khatib - Stanford University, USA.

R. Dillmann - University of Karlsruhe, Germany

7. THE TECHNOLOGY TRANSFER PLAN

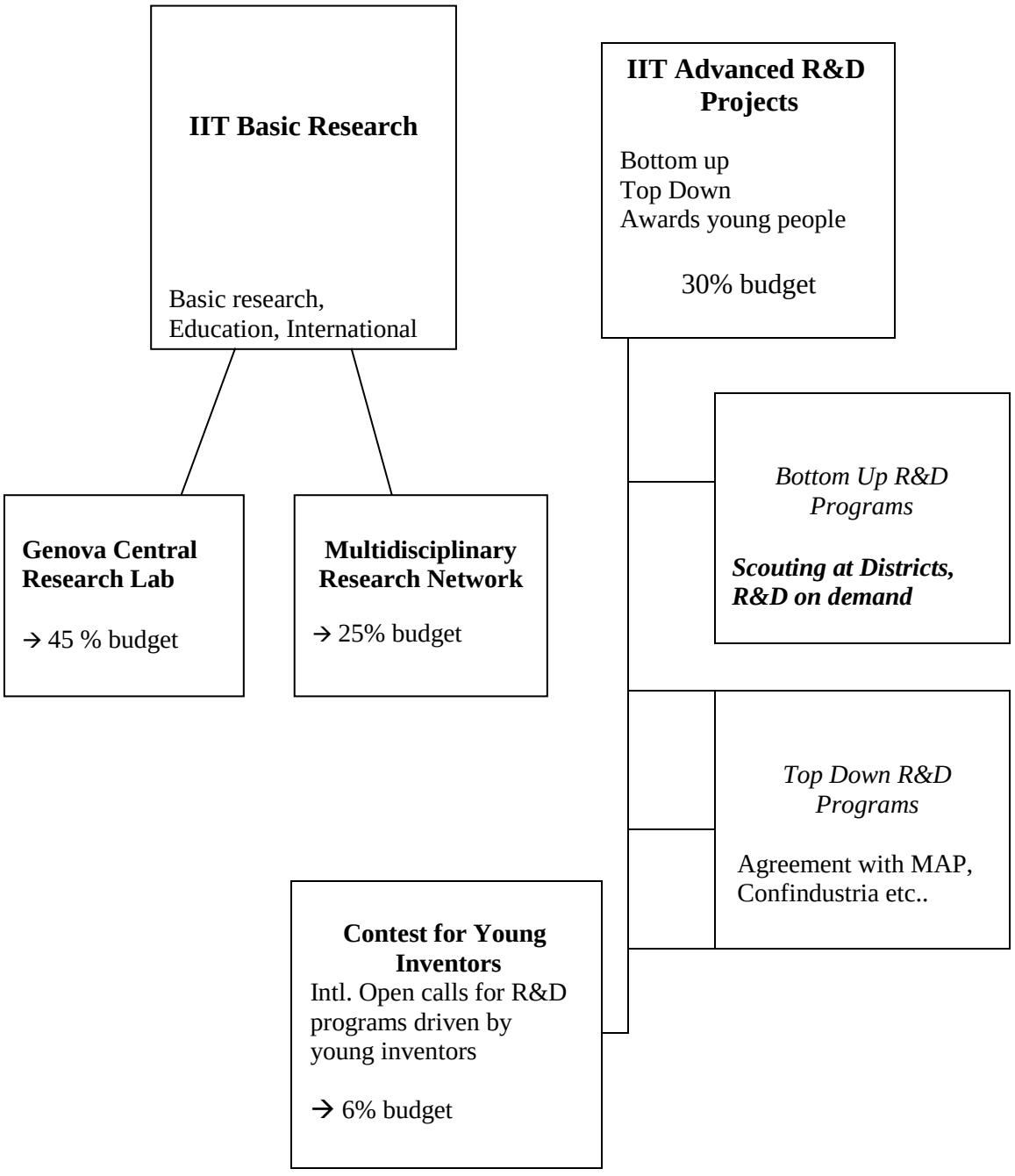


Fig.3

Fig.3 displays a possible strategy for the technology transfer plan of IIT. A Basic Research branch based on the synergy of the central research lab and of the multidisciplinary research network, creates the knowledge basis for any IIT activity. At steady state it is reasonable to

envisage a cost of the order of 70% of the budget for the basic research branch. This will be distributed approximately 45% on the central research lab and 25% on the network. The basic research branch is supposed to raise funds through national and international project calls, from research agencies/ministries and from companies.

An applied research branch should also be created, with the target of developing joint R&D activities with industrial partners. The budget for such a branch could be in the range of 30% of the total budget, at least in the early stage. The main role of the applied research branch is to seed high-risk technology research, R&D program and spin-off actions.

We envisage three main mechanisms for such activities, in most cases based on a co-funding scheme in collaboration with external industrial partners:

1) Bottom-up program, i.e. R&D on demand, based on the scouting actions performed by IIT in the industrial community (Confindustria, industrial sectors, industrial districts, etc.). In this case IIT makes available all the know how existing in the basic research branch, and supports joint R&D activities with companies, with specific agreements for the IP.

2) Top-down program, i.e. call for R&D joint proposals on technologies/productions agreed with major partners such as Ministero Attività Produttive and Confindustria. In this case specific R&D areas as well as specific industrial partners could be selected for targeted investment, once again after having agreed the IP policy, the mutual benefits and contributions.

3) Call for young inventors ideas, i.e. seeding new ideas for new products by young inventors/entrepreneurs, with public calls.

It is worth noting, that the applied research branch could easily evolve into a venture initiative, in which IIT not only has the financial control, but also contributes substantially to the R&D. This benefits from the high flexibility of the basic research branch, which allows IIT to activate in very short time new Research Units or to set up new high-tech lab hiring new scientists from all over the world with specific profiles. Finally it is very important that both the applied and the basic research branches operate under the direct control and evaluation of the technical and scientific committee, to make sure that high-tech R&D activity is actually performed as opposed to bare service and/or product development.

8. *EDUCATIONAL PLAN*

IIT has to invest substantial resources in high education. This is crucial to develop high quality research and attract top professionals in the team. Coherently, IIT launched in 2005 an Education Program, in partnership with a number of high-education institutions country wise, aimed at educating young researchers and at producing new key-competences. Partners of the Foundation in this initiative are 6 of the most recognized Italian schools with doctoral programs in one or more of the relevant disciplines.

The partner schools and IIT have established new PhD programs focused on the research areas specified in the Foundation Scientific Plan. Joint committees are responsible for coordinating the programs and for supervising the research plan, in order to ensure their alignment with the IIT scientific focus. The Partner School is responsible for all activities concerning the selection, assessment and supervision of the students, based on their internationally acknowledged practices, and awards the PhD title. All publications and documents, including the PhD Thesis, explicitly mention the connection with IIT, while the intellectual property is managed according to individual agreements, on a case by case basis and ensuring transparency and fairness. All programs began in 2005 and last 3-4 years. IIT provides all financial support, in terms of student grant and other expenses.

In the longer term, however, IIT aims at establishing its own PhD school, entitled to autonomously issue PhD-equivalent diploma. Such capacity requires that the Foundation is accredited as eligible by the Ministry of Education, University and Research, complying with the following criteria (D.M. April 30th 1999 nb.224/199):

- presence of an adequate number of professors and researchers in the relative scientific field, with proven competence and scientific production in the previous five years;
- availability of adequate infrastructures and financial resources for the education and research activities;
- appointment of a coordinator responsible for the courses;
- network of partner institutions to enable students to carry out experiences in a working environment;
- the establishment of higher education programs at universities, public bodies or private organizations;

- setting up of an evaluation system for the above criteria and for the coherence between the programs and the objectives.

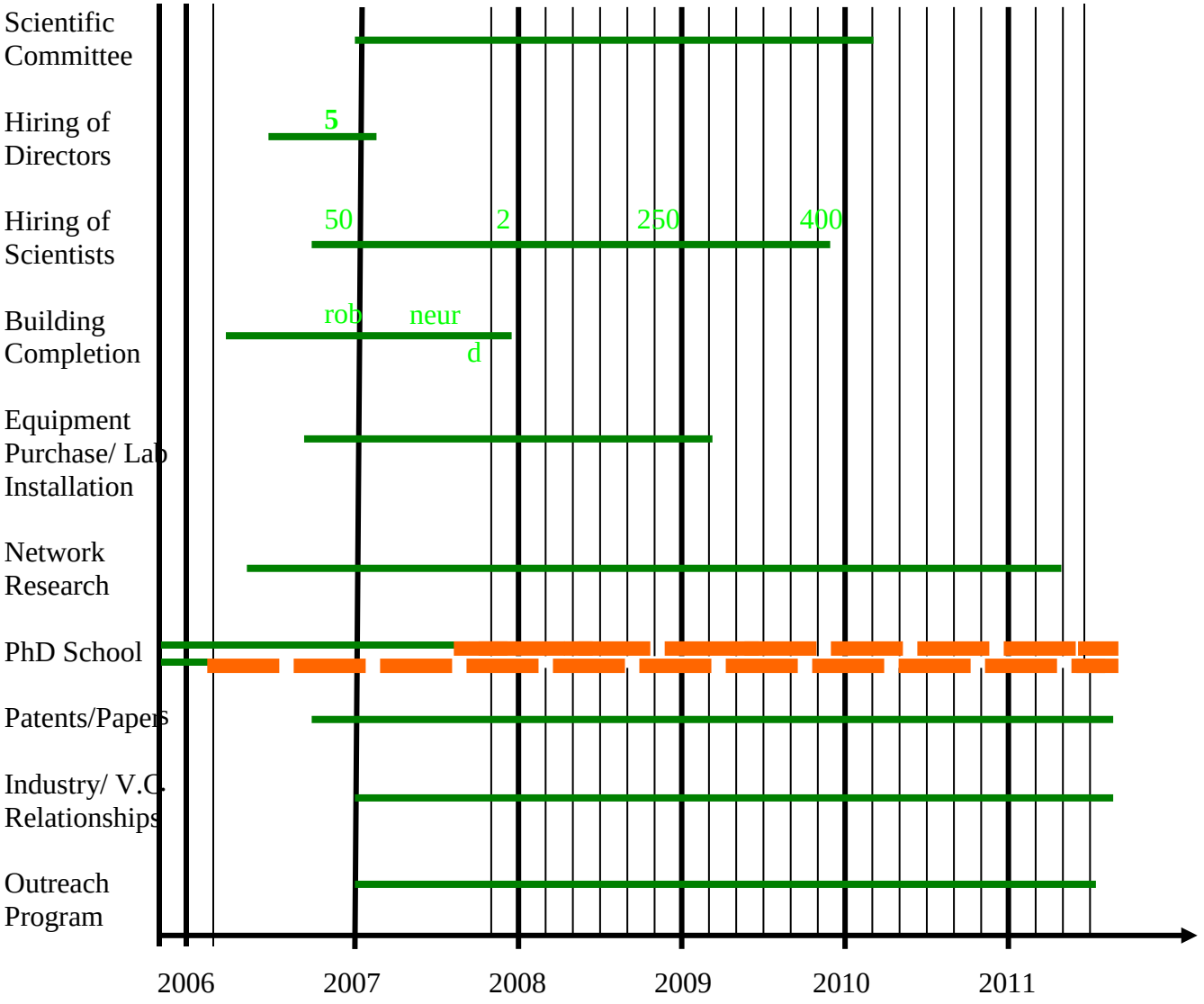
At steady state IIT is expected to have about 100 PhD students operating in the Genova central research Laboratory and another 100 distributed in the network. This needs the institution of an international Course on Advanced Technologies, with specific features:

a) 3 to 4 years duration;
b) strongly multidisciplinary (profiles for physicists, engineers, chemists, biologists, medical doctors) ;
c) 3-4 yearly intake procedures to have project-ready hiring capability;
d) additional research budget for each PhD student;
e) international agreements for PhD exchange;
f) allocated number of positions for foreigner (minimum 30%).

Such a school could be envisaged like an experimental Scuola Superiore di Tecnologie which is made equivalent to an Academic PhD by a specific decree of the Ministry of Education, University and Research provided a special evaluation procedure is carried out at Ministerial level (art.74, law 382 1980).

iit
istituto italiano di tecnologia

EXPECTED TIME TABLE OF IIT



ANNEXES TO EXECUTIVE SUMMARY

iit
istituto italiano di tecnologia

ANNEX 1

THE STARTING SCIENTIFIC PROGRAM OF IIT

The Italian Institute of Technology (IIT) scientific plan

This document presents a possible scientific vision for IIT, meanwhile providing some background material for the search and hiring of leading international scientists who will actually build the body of the IIT research program. The document identifies three technology platforms addressing a few broad and non-exclusive research areas onto which the three platforms should be built. This leaves room and freedom to the future IIT key-scientists to propose and develop their own research activities in the frame of the present vision. Human and financial resources should be allocated according to the scientists' proposals in such a way that a balanced and synergistic development of the three platforms will be ensured.

The IIT vision

IIT has been conceived as a high-tech national infrastructure, developing a cross-disciplinary research strategy of international standard, with high novel content, high competitiveness, and strong potential impact on technology and industry. The research program must be technology-oriented, though exploratory and visionary in the long term, to boost advanced research in a way complementary to the existing Italian research system. Despite the long term character of the initiative, IIT must be set up and launched immediately, with immediate accomplishment of international visibility through patents and scientific publications.

The main boundary conditions in the start-up phase are:

1. the start- up yearly budget is initially provided by a total IIT endowment of 1,050 Meu, secured for 10 years;
2. the site of IIT is Genova, in a 20,000 sqm facility made available by Regione Liguria, to be renewed. This means that a realistic lap of time is needed to renew the infrastructure and build the laboratories (24-36 months). To ensure the immediate start of research and training activities, the Comune di Genova has temporarily offered a 3,000 sqm facility in the centre of Genova (Magazzini del Cotone), available from January 2005.
3. The composition of the board and the provisional work done so far suggest that the mission of IIT should focus on life science (mostly in the field of brain studies and neurosciences) in combination with other high-tech fields such as nanotechnologies, information technologies, automatics and robotics.

With such a picture in mind, we will try to build a workplan based on the parallel development of a few high-tech platforms, synergetic to the development of the IIT vision (with a clear long term strategy and international visibility).
With this intention, a flexible scenario is designed, where important technologies are being developed in parallel, under a coordinated IIT-strategy targeting at some long term achievement. In this case the novel content of the activity stands in its long term vision and in the multidisciplinary approach merging the activities of the platforms.

In this document we follow this approach. We depict a scenario which matches the constraints mentioned above in a unified IIT vision.

In March 2004 the World Scientific Publishing Company started a new research journal, the International Journal of Humanoid Robotics. The editorial to the first issue reports:

"*A humanoid robot typically works in human environments. It requires the synergetic integration of mechanics, electronics, control, communication, perception, cognition, decision-making, artificial psychology, machine intelligence and many other areas. The complexity of a humanoid robot has greatly expanded the scope of investigation in traditional robotics. For instance, it is a challenge to model, analyse and control, in real-time, a highly bifurcated robot body, which is kinematically redundant and may require dynamic compliance for constrained motions. Further, complex, unpredictable, uncontrolled human environments pose very difficult perceptual, cognitive, behavioural and other mental challenges to humanoid robots. Potentially, a humanoid robot will be a platform to test and validate theories in neuroscience and psychology.*
Humanoid robot research is a fascinating and fast growing field. Although there are multiple needs that are propelling the investigation of humanoid robot research, a typical motivation behind this endeavour is the natural desire or ambition of human beings to extend their physical capability (i.e. the ability to act) as well as their mental capability (i.e. the ability to perceive, reason and decide). Undoubtedly, with the companion and assistance of humanoid robots for physically and mentally challenging tasks in manufacturing, service industry, entertainment, security, rescue, health care, medical intervention, defence and space exploration, we will improve our quality of life, and achieve new breakthroughs which are otherwise impossible with less developed robots or machines."

Clearly, Humanoid Technologies will play an ever increasing role in a wide cross-section of crucial activities. We propose that the research strategy of the IIT focuses on an interdisciplinary approach to the development of these technologies. Our aim must be to establish a unique, exciting research environment in which the contributions of science and technology enhance each other in a seamless way. This will require the integration of cutting-edge technologies over a wide spectrum of fields (bionanotechnology, genomics and post- genomics, brain science, robotics and automation) into a unique ambitious program. The IIT strategy will be implemented through three highly interconnected platforms:

P1- Bionanotechnologies
P2- Neural sciences
P3- Automation and robotics

Strong emphasis will be placed on the design and fabrication of new devices for probing and studying living systems (photonics, electronics, nanoelectrode interconnection, nanoparticle markers, tissue engineering in P1), which will be used to generate large sets of molecular and electrophysiological data from selected neural systems (P2). This will provide the background knowledge for computer simulations and, in the long term, for automation and robotics inspired to humanoid systems (P3). The co-existence and strict interplay of these platforms will be a distinguishing feature of the IIT. This will result in an interdisciplinary program that, on the one hand, will provide new analytical tools to the neurosciences, spanning from the molecular to the computational, and, on the other, use the knowledge gained from neural systems to design novel humanoid systems and devices.

The three platforms will develop individual research lines, with their own staff. A set of interconnected core facilities will be established such as advanced nanofabrication (nanotechnologies), parallel multidimensional analysis and manipulation of cellular structures and their molecular constituents (including functional genomics), computation, and automatics and robotics. In addition to resident IIT groups, the IIT core facilities will be made available to collaborating groups and visiting scientists. This structure will create the multidisciplinary knowledge basis for the start-up of the IIT, based on a blend of completely different disciplines

(biology, medicine, nanotechnology, electronic engineering, chemistry, physics). Clearly, the strategy of each platform will be coordinated by the IIT steering committee to ensure that results, know-how and expertise converge onto the final targets.

The Technology Platforms of IIT

Platform 1: Bionanotechnologies

The application of nanotechnologies to brain and life sciences is a challenge for the next twenty years. This includes the *development of new technologies, resulting in new concepts of interconnection of biological matter to physical probes*, and *novel approaches to study cellular functions at the micro and nano-scale, aiming at a better understanding of the physiological behaviour and of the mechanisms causing misfunction and disease, new tools for diagnostics and imaging, new methods for intelligent drug delivery.* The nanobiotechnology platform develops the enabling technologies needed for the accomplishment of such challenging results. In order to exemplify the platform activity, in what follows we briefly address a few broad and non-exclusive research topics which could be strategic:

1 *Cells to chips and Chip-to cells technologies*:
The capability of producing micrometer patterns of biological macromolecules and living cells is of paramount importance for several applications, including advanced medical diagnostics, biological assays, miniaturized biosensors, hybrid molecular electronics and rectifying devices. In the last few years, substantial progress has been made in understanding the interactions between cells and their surroundings. For instance extracellular matrix (ECM) proteins with nanopatterned templates can be used to drive the topology of cells in a culture. *Patterned in vitro* cell cultures should be able to recreate a physiological environment both from the topological and the biochemical point of view, in order to simulate *in vivo* cellular functions. This demand is not fully satisfied by traditional cell cultures, which produce uniform distributions of ECM proteins and cells on the culture surface, and consequently do not allow the control of cell shape and behaviour. This might result in morphological and functional differences with respect to *in vivo* cellular phenotypes.

The "*cells to chips*" concept relies on specific nanotech approaches to reproduce the *in vivo* functions of cells onto patterned devices, thus allowing to monitor and investigate effectively their mechanical and electrical behaviour. Patterning ECM proteins and structured surfaces enable the controlled and selective deposition and growth of cells onto chips, and the realisation and investigation of neuronal networks of controlled complexity and geometry, to study the excitation and the transmission of signals in normal and abnormal cells.

A natural evolution of such concepts stands in the realisation of *patterned serial sequences of excitable cells that mimic physiological circuits.* For instance, neuronal cells can be connected to muscle cells (myocytes) by exploiting their sensitivity to different, previously patterned ECM proteins. The muscle cells in turn can be connected to free-standing silicon micromachines to realise neuron-controlled MEMS devices. The use of the neuronal electrical signals (based on the transmembrane exchange of sodium and potassium ions and typically in the 0.1V range) to stimulate and activate biological molecular motors (kinesins, myosins, etc.) and hybrid polymeric artificial microlimbs can also be investigated in view of controlled molecular motors aiming , in the long term, at the neuronal control of complex robotic devices.

In the long term, physiological circuits realised on-chip may also include sensory functions, as provided by the interconnections of neurons to retina cells (to be addressed by light) for vision, and to stereocilia of the hair cells of the inner ear (to be addressed by mechanical excitation with

scanning probe tips) for hearing. This is a route to replicate on chip the whole range of sensory functions and transmission, enabling an unprecedented approach to the study of cellular physiology.

The "*chips to cells*" approach pursues the strategy of restoring damaged brain and sensory functions with specific implantable chips, designed on the basis of the knowledge provided by the "*cells to chips*" stage. Implanted biochips that allow to turn off brain activity causing the uncontrolled movements associated with diseases such as Parkinson's have been recently demonstrated, resulting in a chip-controlled reversible thalamotomy (Medtronic, Inc.). For example, by using multiple microelectrode systems, one can simultaneously monitor the extra-cellular activity of over a hundred single neurons in awake, behaving animals. These techniques might be crucial in the future to restore sensory functions, by chips including artificial sensing components (photoreceptors, vibrating elements, etc.) and subsequent controlled electrical stimulation of transmitting nerve cells.

The basic technologies needed for the above goals are based on nanofabrication processes allowing electrical interconnections with living matter (such as selective deposition and bio-compatible soft-patterning for cell cultures), advanced fluid-dynamics knowledge, and on the quantitative investigation of cellular dynamics. Experience in the following areas would therefore be extremely important to make the nanobiotech platform mature in short time, and to quickly provide technology support to the other platforms:

a) Nanofabrication technologies to enable masurements of *in vivo* systems, beyond the standard patch-clamp methods, combining high resolution electron-beam lithography and nanofabrication by electrochemical methods, which are suitable for single molecule interconnections. Multi nano-electrode arrays probing single cells and single ion channels on the membrane surface by electrical contact will become key elements for the direct investigation and stimulation of cellular activity. The natural evolution of such methods will be the creation of three dimensional arrays (eg realised by two-photon and multilevel lithographies) to produce complex free-standing circuits and components in biological environments. Novel methods, such as direct writing with alignment over pre-patterned bio-substrates, deep reactive ion etching, low pressure chemical vapour deposition, have to be developed to become compatible with living matter.

b) The deposition and organization of living cells onto suitably structured surfaces is required in order to allow them to work under conditions as physiological as possib le, and effectively mimic their *in-vivo* functions. Among the different nanofabrication approaches, soft contact lithography (replica molding, contact printing, imprinting, capillary lithographies, microtransfers) and functionalization techniques will be strategic for the controlled delivering and patterning of the molecules and living cells, by virtue of their unequalled chemical flexibility and compatibility.

c) Fluidic technologies: Large-scale integrated circuits onto microfluidic chips are a feasible and powerful techniques for handling, processing and analysing biofluids (recently microfluidics was mentioned among the ten key emerging technologies that will change the world, see the MIT Technology Review, January 19, 2004). The development of next-gene ration fluidic technologies at the micro- and nano-scale will be important both for realising new efficient analysis tools and for investigating the fluid dynamics within the living cells with unprecedented accuracy. Key areas of research are: (i) the basic understanding of the fluid dynamics, and of the diffusion and turbulence phenomena within microchannels by spontaneous capillarity and applied driving forces; optimization of channels-array systems to improve the efficiency of hybridization and/or of biomolecular recognition; realization of

diffusive-based immunoassays, investigation of the dependence of performance on the viscosity, interfacial free energies, flow rates, and geometries; (ii) the integration of functional devices within microfluidics circuits (*microfluidic integrated circuits*): conventional and conjugated polymers actuators, electrodes for capillary electrophoresis, electrochromatography, single-use valves, electromechanical pumps and valves, reservoirs, mixers based on both the geometry of the microfluidic channels and on the integration of MEMS to improve binding/hybridization efficiency, resistive elements providing the heat source for microfluidic-based chemical reactions; integration of optical detection (fiber optics,…) within working microfluidic chips.

2 Advanced characterization tools andimaging

The morphology, functionality, and chemical contrast achieved in self- assembled films, few-molecule systems and living cells greatly benefits of advanced scanning probe methods [scanning tunnelling (STM), electric force (EFM), lateral force (LFM) and atomic force microscopy (AFM)]. In this context, the application of nanotechnology investigation tools provides unprecedented and powerful means for the characterisation of biological systems. For instance, surface potential imaging of neuronal cells can be obtained with nm resolution by STM spectroscopy, and ionic currents can be determined through the local density of states of the cellular ion channels. Similarly, force measurements with sub-pN precision can be carried out by optical tweezers as well as by AFM force-distance spectroscopy, exploiting suitably functionalised tips. Surface X-ray microanalysis can be used to discriminate the compositional differences of biological tissues of different ages and functions, whereas X-ray tomography could be used to image tissue engineered constructs, tissue growths, and live animals in 3D.

Advanced molecular imaging is another broad and important area of research for the future. Single-molecule imaging by total- internal reflection microscopy, combined with temporally-resolved spectroscopy can be employed to monitor the dynamics of fluids and drugs within living cells with nm resolution. The development of imaging technologies with high spatial and temporal resolution, includes: i.) application of advanced microscopy techniques, such as multiphoton excitation microscopy and second harmonic imaging. ii) the development of multidimensional data analysis (e.g. by spectral deconvolution), iii) single molecule spectroscopy techniques; iv) implementation of molecular biosensors to probe the environment of specific cellular components (e.g. membrane channels, protein kinases, etc.) by exploiting optical phenomena at the nanometer range (evanescent fields, FRET, FLIM).

The common features to be optimised in such techniques are their high spatial resolution and low impact on cellular metabolism, their high sensitivity to the fine structural (conformational) changes, or signalling events (typical of most neuronal processes), and their fine temporal control, allowing on/off switching with minimal latency.

The combination of the tools and data generated by this area and by the functional genomics approach will be essential to enable the investigation of complex neuronal processes, such as the differential synthesis and distribution of key proteins (channels, signal transducers, enzymes) in critical neuronal areas (synapses); their traffic along multiple pathways; their interaction in intricate but highly regulated complexes; their relevance to brain disorders.

An additional key area of research will be the design of near- infrared optical probes and non-optical probes suitable for PET (Positron emission tomography) and MRI (magnetic resonance imaging), which would allow to extend these analysis *in vivo*, with highly significant medical implications.

3 Intelligent drug delivery

A third strategic area of research for the platform deals with the so called “intelligent drug delivery (IDD)” in combination with several other complementary techniques such as three dimensional (3D) tumour simulation, imaging and drug delivery, nanoparticle intrusion effects on brain tumours..etc . The concept of *intelligent drug delivery* implies the spatially and chemically controlled delivery of a certain drug (or of combinations of drugs) directly into the site of origin of the disease. Several methods are presently under investigation world-wide, such as IDD by swelling, implantation, diffusion, based on nanoparticle carriers and/or nanoporous systems.

Design and development of advanced in-vitro models for monitoring drug activity, in controlled, multiparametric, intact cell-based systems are thus necessary as the first step for the development of such technologies, in order to monitor drug dissolution rate, drug uptake and leakage, MDR, binding affinities, and cellular localization simultaneously with on line monitoring of drug effects. The overall information achieved through these methodologies, enables the investigation of cellular and mo lecular targets, as well as of the mechanisms involved in the therapeutic action of specific drugs. The use of cellular and tissue specimens derived from animal models or human tissues could also facilitate the diagnosis and the drug activity measurements, allowing personalized drug treatment protocols and microchips for gene expression analysis.

A further step into these technologies could combine imaging and drug delivery in brain. To date it is believed that implantable lab on a chip devices based on silicon (either nanoporous or micromachined) in combination with polymeric carriers (such as nanocapsules) can only partially face cancer treatment in the brain. For deep tumours, the possibility of having a “smart” material acting like the drug carrier and the contrast material, would open the possibility of combining *in situ* drug delivery treatment, including gene therapy, and imaging. Emerging technologies combining phase contrast TAC and ultrasounds make use of micro bubbles as the “smart” material (carrier and image contrast agent). The microbubbles support drug delivery by acting as “cavitation nuclei” and as agents to carry drugs for site•specific treatment. The ultrasound field enhances the drug delivery by creating transient non•lethal perforations in cell membranes to aid penetration of large molecules and particles into the cells (“sonoporating”). In general this requires high acoustic power, substantially beyond that permitted for imaging. However, such a power is greatly reduced when microbubbles are present, due to lower amount of energy required for cavitation (the process in which the extreme oscillations induced by ultrasound pulses lead to microbubble collapse).

In addition, the use of microbubbles in treatments may also be very important for diagnostic uses. Microbubbles indeed increase the intensity of Doppler signals from blood for several minutes after their injection thus improving Doppler examination by raising the intensity of weak signals to a detectable level. This improves detection of flow in the intracranial arteries by transcranial Doppler in adults (where the skull greatly attenuates the ultrasound signal) and in smaller vessels, such as in the circulation of malignant tumours.

Finally, another relevant research area in this field deals with the implantable wireless devices for continuous measurement/actions . For example micro *lab on a chip* devices for intracranial pressure measurements can be fabricated on inorganic substrates (typically silicon nitride membranes) containing the miniaturized circuitry including the pressure transducer and the RF telemetry. Particular attention has to be paid to the environment in which the system operates, e.g. to possible problems of interference between the telemetry subsystem and other medical instrumentation (e.g. NMR). Another relevant example is that of a fully integrated *lab on a chip* device for in vitro and in vivo cancer therapy by photactivated porphyrins (porphyrins accumulate selectively in tumoural cells and they are activated by the red light). In the long term, such a *lab on a chip* might include

the electrophoretic section to detect the cell and DNA fragments produced by the photocleavage reaction, the VCSEL (Vertical Cavity Surface Emitting Laser) for the red optical excitation and the microfluidics and sensor array technology.

Platform 2: Neural sciences

The main focus of this platform will be the development of new technologies for the study of brain function. An essential element of these technologies will be their ability to link the different hierarchical levels of the brain organization into one unified conceptual framework, from molecules to higher functions. A major determinant of sophisticated behavior and psychic activity is neural plasticity, which may be defined as the shaping of neural functions induced primarily by experience. At present, the study of neural plasticity is mostly focused on the identification, at various levels of integration, of the mechanisms by which the mammalian brain responds to environmental signa ls with purposeful strategies. Advanced studies of neural plasticity are already being carried out. However, they are based on approaches that, even when using cutting-edge technologies, are often specific to individual disciplines. We propose that the IIT should develop a multidisciplinary approach whereby collaborating scientists will employ distinct, but converging tools including molecular and cellular biology, electrophysiology, system analysis, bioengineering and nanotechnologies in order to investigate individual research tasks. This will provide the projects with the extra power necessary for their success.

Interest in neural plasticity is not limited to basic research but extends to medicine as well. Investigations in this area have already led to significant progress, with the understanding of the mechanisms altered or defective in many brain diseases. The development of new strategies aimed at the prevention, the arrest or the slowing down of human diseases, and at the recovery of lost functions, are among the most important recent successes of medical research, some of which have already found their way to application. These developments have attracted not only attention but also great hopes. Medical neuroscience, in fact, faces the greatest social problems, as also recognized last year at EU level. Neurological and psychiatric diseases are more frequent than cancer and cardiovascular diseases, and presently affect over 4 million patients in Italy. Moreover, the impact of neurodegenerative diseases, such as Alzheimer's and Parkinson's, is constantly increasing in Western countries, in parallel with the aging of the population. The corresponding social costs, already high at present, are expected to become unbearable in the next several years, unless medical breakthroughs are achieved.

Based on all these considerations, the present platform has been articulated into several interconnected areas of basic and applied research related to the neurosciences, to be considered for the elaboration of operational projects based on the collaborative combination with the other platforms.

1. Functional genomics

Biomedical sciences are undergoing a fundamental change with the sequencing of the genomes of man and other organisms. This information will accelerate the rate of discovery in all areas of biology and medicine, including brain research, and, most importantly, will expand the range of questions that can be attacked experimentally. The complete sequence of genes and chromosomes will allow substantial new lines of research concerning genetic influences on the brain. Technology based on whole- genome analysis - from DNA chips to detect the expression of thousands of individual genes to high throughput methods to analyse the complex interactions among gene products and their functional consequences - will make it possible to correlate the molecular phenotype of individual neurons in different brain structures with their physiological features. This will substantially add to our limited knowledge of the basic principles subserving neural functions, and permit the study of brain circuitry at a new and more fundamental level.

Moreover, recent studies have clearly shown that pathological processes can be investigated in parallel with, and with experimental approaches similar to those employed for the normal brain.

A few broad and non exclusive research areas relevant to the platform development are described in the following:

a) the search for genes controlling the expression of specific cellular programs. Several such genes have been identified in seminal studies in model organisms (e.g. *C. elegans* and *D. melanogaster*). However, the genetic control of coordinate differentiation at the cellular level in higher organisms is still largely unknown. This area is of interest not only from the scientific point of view but also because it may change basic perspectives in the molecular and cellular therapy of brain diseases.

b) linkage analysis. The identification of a tight association between a molecular marker and the susceptibility to a disease allows the development of superior diagnostic tools and the validation of candidate genes. Knowledge of the molecular basis of the susceptibility to brain disease could play a major role in the identification of targets for drug discovery and in the formulation of etiological hypotheses testable in animal models.

c) gene expression studies by differential display procedures. These should be carried out at high resolution to investigate events taking place in individual neurons, and possibly also in individual synapses. The aim is to correlate patterns of gene expression in specific brain areas or brain cells to processes such as learning and memory. These studies should be extended at the proteomic level, with the development of novel technologies to assess protein-protein interactions and their relevance to physiological and pathological processes. A fundamental technology to be developed consists in the adaptation of current microarray-based technology to enable analysis of single cells. High throughput systems must be developed that enable the isolation of single cells/nerve terminals (e.g. by cell sorting or laser-capture microdissection), the extraction of macromolecular components (e.g., mRNA and proteins) through protocols that maximise recovery and minimize degradation, and their quantitative detection by methods with sensitivities down to few molecules per sample (e.g. arrays, MS). Alternative technologies must also be developed, such as high throughput *in situ* analysis, based on arrayed probes (e.g. evanescent wave scanning array technology for the analysis of cell surface events, or bar-coded DNA/RNA/peptide probes to be detected via their spectroscopic signatures). This will require the design of novel classes of probes, particularly for proteins and their post-translational modifications.

2. Molecular structure and function of synapses

Synapses are small structures (diameter < 1 µm in diameter) where connections between neurons form, operate and are continuously modulated by a variety of processes such as long-tem potentiation and depression, the initial steps of “elementary” learning and memory. Studies of the last decades have already deciphered various aspects of synaptic organization and function. The general picture that emerges from these studies, carried out by combinations of techniques such as patch clamp electrophysiology, bioimaging, molecular biology, genomics, proteomics and biochemistry, is that of fast (µsec timescale), complex and highly regulated structures working by a variety of coordinate processes including the exocytosis and recycling of their vesicles (named kiss-and-run), the release of neurotransmitters, the activation of receptors at pre-and post-synaptic membranes, the activation of multiple, highly integrated and variously regulated signaling cascades, which either remain restricted to single synapses or extend to others, to the whole neuron or

possibly to other neurons. At a first glance knowledge in this area, which is the target of most CNS drugs, might appear well established. However the information available is largely restricted to classical neurotransmitters (especially glutamate, acetylcholine and amines). Knowledge about other neurotransmitters and neuromodulators, including many important ones such as the opioids, is mostly limited to the receptors and their pharmacology, with no comprehensive understanding of the overall processes. What is often being done at present is to generalize to all neurotransmitters the concepts experimentally proven for a few neurotransmitters only.

Even for the best known synapses, however, fundamental aspects of function remain poorly known. Among these are the dynamic changes of synaptic structure, based largely on the regulated traffic and controlled intermixing of internal and surface membranes. These changes are known to play key roles in tuning, not only during synaptic growth, but also during functioning and regeneration; the bi-directional synaptic signaling operated not only by transmitters but also by growth factors and other released and surface molecules; the role of astrocytes (synapses are more and more defined as tripartite structures, constituted by the pre-synaptic bouton, the postsynaptic structure, often a spine, and the associate astrocyte!) which might be either synergistic or toxic depending on the physiological/pathological conditions, and so on.

Because of the unique properties of synapses, in particular their fast functional response; the specificity and multiplicity of their receptors and transmitters; the intricate structural and functional interconnections of their proteins, both at pre- and post-synaptic sites; the uninterrupted, bi-directional exchange of signals among the participating structures, synapses may appear as the ideal target of high tech approaches in which advanced techniques are developed to reinvestigate at a higher resolution events still poorly known, and to open the investigation of others, that so far have been overlooked or have remained inaccessible to the existing approaches.

3. From intercellular communication to cognitive functions.

This research area, strictly connected to that described above, intends to expand the synaptic investigations to a higher level of integration. In particular, starting from the processes of elementary learning and memory based on long-term synaptic plasticity, these studies could target the information flow and processing induced by epigenetic factors that eventually lead to integrated and finally “mature” forms of learning and memory and be integrated by cognitive approaches. The observation of the human brain at work, under normal conditions and in disease, has allowed the identification of the neurons that lie at the core of cognitive processes and the study of their representation. From this, still largely descriptive level, the work could move on to the investigation of the specialized neuronal signals underlying perceptual decisions, motor plans, emotion, reward, and of how these signals can be sculpted by experience.

These studies will require advanced methods of electrophysiology with multiple electrodes, to monitor simultaneously the extra-cellular activity of over a hundred single neurons in awake, behaving animals. This new recording paradigm is changing the face of systems neuroscience by allowing, for the first time, the visualization of the functions of neural circuits at work. Its great advantage is to track the activity of individual neurons for days, thus making it possible to follow the process of memory consolidation. The electrical activity of populations of neurons can be used for the development of computational models aimed at predicting some aspect of animal behavior. In addition, the multiple microelectrode system could also be utilized as input to a comp utational interface that generates signal for mechanical devices to be used in robotics. The complex peculiarities of neurons, which require investigation *in situ*, and the small size of synapses pose formidable problems to this type of studies, which however could be solved by the new

technologies to be developed at the IIT. Particular interest in these studies comes from the fact that synapses are precociously altered in degenerative diseases of the central nervous system (see below).

Recording from large-scale neuronal assemblies will have to be integrated with the molecular characterization of the participating neurons, with special interest for the process of gene expression at the synaptic level, and with approaches based on conditional gene manipulation.

4. Medical implications

During the last decade knowledge of many brain diseases and in particular our understanding of the molecular mechanisms of disease has grown at astonishing rates, paving the way to a new era of medical studies. So far, however, a proportional fall-out to the applicative level has not occurred, because specific aspects of pathology remain still to be discovered. Advanced molecular and cellular investigations of adequate disease models, to be related eventually to clinical studies carried out in external Institutes, could therefore lead to significant progress in the prevention, early diagnosis and therapy of brain diseases. This approach appears particularly timely based on the recent recognition that pathological processes do not require distinct experimental approaches but can be largely investigated in parallel with, and with the same technologies of molecular, cellular and physiological investigations used for normal brain. The studies described especially in sections 2 and 3 could therefore be also applied to models of brain disease, in particular to those implying neurodegeneration, with an aim not only to understand the pathogenesis, but also to investigate the mechanisms and to generate tools of diagnostic and therapeutic interest. This conclusion is strengthened by the emerging importance of synapses and synaptic transmission as early targets of degenerative processes. Synaptic lesions are typical of the early stages of pathology, when the unspecific cellular damage has no t yet been established. The study of these structures and processes will greatly benefit from the development of new technologies, necessary to characterize the local neuronal lesions at a high level of resolution. Finally, by providing new insights into the affected neurobiological processes, neuropathological studies (in collaboration with medical specialists) could also lead to the development of novel techniques for neurorehabilitation, which so far have been mostly based on empirical criteria.

5. Drug discovery

The results and the tools developed by the IIT neural sciences platform, in particular the identification of new molecular mechanisms and targets, could form the basis for an additional IIT activity in an important area of applied research, i.e. drug discovery. An established policy of several major multinational pharmaceutical companies is to resort increasingly to small companies and to basic research institutions for the early stages of the drug discovery process. A new, competitive Institute, characterized by a unique combination of neuroscience and technological know-how, including an advanced branch specifically expert in the identification and study of drugs, could be fully qualified to enter the game, thus filling a long-term gap in pharmaceutical research in Italy. The initiative, which could be started with no delay, would open opportunities of fruitful industrial interactions not only at the international but also, and especially, at the national level, and address issues of high medical, social and economical relevance. The potential of Italy to support the establishment and further expansion of this research/application effort at IIT could be considerable. In particular adequate expertise and human resources exist in the country that could establish scientific and operational interactions with the core system of IIT. Moreover, the standard of clinical research in neuroscience, carried out at present in many Italian institutions, is high and expected to be available for studies and trials in innovative fields, such as those where the IIT work

will be developed. The role of the drug discovery branch with respect to the other IIT branches should consist first in a critical evaluation of results potentially relevant to drug discovery, and then in the development of these results by the most advanced approaches: molecular design structurally guided by interactive modeling and selection of lead compounds by combinatorial chemistry, bioorganic chemistry, high-throughput screening, physicochemical and computational analysis of interactions with biological substrates. The subsequent in depth physical, biological and pharmacological investigations of selected molecules could be carried out in collaboration with the other IIT groups and with external laboratories.

6. Networks sciences and biological computation

An exciting and rapidly developing area of research on complex systems focuses on the understanding of complex man- made systems (such as communication networks) and biological systems. Altho ugh much of this field is in its infancy, efforts and progress in these directions seem to be growing rapidly. The question of how networks are organized has become central to many areas of scientific and technological interest: telecommunications networks, neural networks and brain function, genetic and biochemical networks, and social networks. An exciting new area of research is to understand the common engineering design principles that underlie networks in these widely divergent domains. In particular, biology provides remarkable examples of network designs for computation and communication that are self-organizing and adaptive, are amenable to improvement through genetic and selective pressures, and are highly robust to circuit defects and failures. Similarly an understanding of the universal principles and algorithms underlying neural computation will have profound scientific and technological implications.

The disciplinary subjects that relate to this area are traditional engineering theories such as information theory, feedback control theory for dynamical systems, and other theoretical frameworks that bear on the principles involved in designing complex engineering systems, including communication networks, that perform their tasks reliably even under unpredictable circumstances. Future communication networks in fact will have to be highly fault tolerant and adaptive. The relation to biological systems, which have such characteristics, is straightforward. Organisms have to perform tasks or functions under trying circumstances, and through the process of evolution, have been able to perform at least a partial search of the available design space. A hypothesis could therefore be made that by understanding the underlying design or engineering principles (if any) of such biological networks, one would able to make progress in understanding complex biological systems. This is in contrast with a reductionist approach that treats biological systems simply as physical and chemical systems. In turn from the study of such biological networks one could learn new design principle or design strategies for man- made networks. Cells use complex signal transduction and genetic networks to process information about the environment and produce complex behaviors. These networks are highly robust over a wide range of network parameters, and yet are rapidly adaptable in response to the local environment. Furthermore, these networks are amenable to evolution resulting from selection pressure. Concerning neural systems it is widely believed that that they are only partially specified genetically, and that much of the development neural circuits is controlled by experience-dependent plasticity in neural connections. Such plasticity is thought to occur as a result of highly local learning rules. An exciting new idea in theoretical neuroscience is that one can control the dynamics and structure of a network purely through local information by the application of different specific learning rules at the synapses. This idea has implications for self-assembling telecommunications and ad-hoc wireless networks, as well as computational systems.

It should also be pointed out that that the body of knowledge about complex biological and manmade systems is itself complex, as it deals with terabytes to petabytes of information and thus requires systematic management and the development of powerful data mining techniques. This is in of itself an exciting area of research. ITT could definitely complement its activity by building a world-class fundamental theoretical and experimental research in the structure and dynamics of biological and man made networks/systems. While the focus of this effort should be on high- impact science, these areas of research clearly relate to long-term advances in networks for telecommunications and computation. A group of highly qualified theoretical physicists, applied mathematicians, and computational biologists, should be a central part of this effort. This would be very helpful in establishing synergistic activities between the information technology and biological research areas of IIT.

Platform 3: Automation and robotics

The advent of electronics and the developments of Control Theory during the first half of the last century gave a tremendous acceleration to robotics, especially to the branch called Industrial Robotics which is nowadays regarded as a mature and consolidated scientific field. Factory automation, by example, is one of the direct results of the advances in control theory, electronics and computer science in the last 60 years. Yet it was long ago acknowledged that robotic applications could go much further than factory automation. Modern robotic technologies include:

- exploration of harsh environments, including space, marine and underwater, volcanoes, artic or desert regions;
- medical applications, including surgery, diagnostics and rehabilitation;
- service robotics, including cleaning and housekeeping, education and entertainment (i.e. edutainment), demining, agricultural and harvesting, lawn mowing, surveillance, inspection, mining, construction, fire fighting, search and rescue, tour guides and office applications.

In perspective, robotic research will face issues such as the use of biologically compatible materials, and of methods and operation principles approaching human capabilities. The co-existence of strong interdisciplinary research programs in nanotechnologies and the neurosciences will provide the ideal foundations for the third IIT program, which will be devoted to the study, design and fabrication of robotic systems based on biological mechanisms (such as learning, vision, hearing), materials and interconnections.

1 Autonomous Robotics

A fundamental key feature of future robots that will have a relevant impact on the great majority of the above mentioned application domains is autonomy. In spite of the lack of a formal and rigorous definition of autonomy in robotics, it is generally agreed that an autonomous robot should be able to correctly perform its tasks in an unstructured and dynamically changing environment. It should also be able to communicate with a human operator at a high level, i.e. with natural language, and to adapt. As to the question of what the basic ingredients of autonomy should be for a robot, this is by and large open. Much can be learned from brain and behavioral sciences. Autonomy is of particular relevance in service robotic applications where the robot-human interaction is closer. Indeed designing robotic systems that can be naturally integrated in an unstructured “human” environment, as a house, is an issue of paramount importance. In this respect the recent interest towards humanoid robotics comes as no surprise.

2 Humanoid Robotics

The research on robotic systems has often been inspired by biology as proven by the many existing Biomimetic Robots (e.g. insect – like walking machines, robotic snakes, fishes etc.). Yet the interest in humanoid robotics is continuously increasing. Japan and the USA have been investing in this direction for many years, whereas in Europe there are currently only isolated research efforts in some countries (e.g. Germany, Italy and Sweden).

The research initiatives related to the development of humanoid robotics are numerous: one of the best known is RoboCup. It started in Japan in 1997 and it aims to foster artificial intelligence and robotics research applied to a standard problem (the game of soccer), where a wide range of technologies can be examined and integrated. Its popularity, both scientific and at the general public level, israpidly increasing. The forthcoming European championship event in Lisbon (Portugal) next July will host teams from 30 countries with approximately eleven hundred researchers. The

presence at IIT of leading research in brain science, cognition and learning, bionanotechnology, genomics and post-genomics, will offer a unique opportunity for challenging fundamental issues related to autonomous robotics. The interplay among the cited disciplines could foster advanced robotics related research on:

- The development of novel, low power consumption, miniaturized and real-time sensors and computational units based on bionanotechnology (labs on chip) and its advances in the area of new materials for electronic circuits.
- The study of advanced control architectures for distributed control in robotic applications.
- Modular robotics, addressing the fundamental issue of how to interface and integrate simple low- level robot controllers to achieve a complex behavior.
- Navigation, guidance and control of autonomous robots.
- Vision sensors and vision-based control of robots.
- Multi robot systems. Coordinated control.
- Human – robot interactions.
- Robot learning and performance analysis.
- Modeling of complex systems for control system design, and model-based fault diagnosis.
- Advanced, biologically inspired motion and task planning.
- Motion control.
- Signal processing and sensor fusion techniques, for example for simultaneous robotic localization and map building (SLAM) in unstructured environments.
- The study and development of new materials to be used in advanced robotics, including eventually new solutions for prosthetics.
- Artificial muscles and biologically inspired actuation systems.
- Humanoid robots (all the above are relevant).

The advances in brain science and cognition research sho uld be exploited to design biologically inspired control architectures taking into account the current state of the art in Controls, Systems Theory and Artificial Intelligence (AI). The interaction of consolidated and mature methods of control theory and AI with the experimental analysis and theoretical findings of brain science can make IIT a unique opportunity for advanced research in autonomous robotics. Indeed the technological success of Neuromorphic Engineering as experienced at Caltech CNSE Center for Neuromorphic Systems Engineering (http://www.cnse.caltech.edu/) and at INI, the Institute of Neuroinformatics (http://www.ini.ethz.ch/) at the University and ETH Zurich (Switzerland), suggest that the interplay between biology and engineering can be extremely effective for scientific advances in the area of robotics and computer science.

3. *Tissue Engineering*:

Tissue Engineering is one of the major focus of biotechno logical research today, with the expectation that this type of technique will ultimately transform the practice of medicine. The most ambitious tissue engineering schemes assume that specific tissues and organs will be restored, repaired or replaced by homologous tissues created *in vitro*. However, many pre-clinical and also clinical reports demonstrate that poor scaffold design and inadequate tissue culture condition are currently the major problems in tissue engineering, that may prevent its successful clinical applications. To overcome these limitations, novel *biomaterials* and better *processes* are needed.

Materials Better designed materials are required to sustain and guide tissue regeneration. The project will seek to implement and integrate novel bio-hybrid synthetic techniques, nanotechnologies and advanced material processing technologies to obtain scaffolds able to guide

and control tissue growth, differentiation and proliferation. In particular, novel materials both of synthetic and natural origin have to be designed and processed to meet specific physical, chemical, morphological and functional requirements tailored to specific applications.

Process. The development of a functional tissue *in vitro* from cell- seeded scaffolds depends on the process conditions. Novel and sophisticated bioreactor systems are required to monitor and control the biochemical signals and biophysical stimuli and to enhance tissue growth and remodeling. A bioreactor should be able to generate suitable biological environments for the growth of specific tissues, meanwhile monitoring cell functions and viability throughout a 3-D construct, and properly adjusting the environmental conditions. Relevant research topics in this area could be:

- Development of novel technologies and processes for the engineering of polymeric scaffolds, having: (a) well defined and tunable degradation rates; (b) highly structured and organized macro- and micro-porosity networks to guide cell and tissue colonisation as well as the trafficking of microsolutes; (c) specific molecular signals for cell recognition and guidance.
- Development of practical and reproducible *in vitro* experimental models to quantify the effects of mechanotransduction. on cellular functions and on the production of ECM proteins.
- Study of the transport mechanisms that regulate fluid, macromolecular and cellular trafficking in cellular constructs, and of the experimental methods to determine the efficiency of movement of large molecules and fluids through the cellular constructs.
- Develop of “smart” bioreactors for neo-tissue growth, e.g. a prototype of integrated bioreactor with feedback mechanisms, to reproduce the appropriate biological environment for tissue growth.

ANNEX 2

BACKGROUND MATERIAL

iit

Italian Institute of Technology

Technical Annex to the scientific plan and background material

Contents

10th March, 2005

iit

A. INTRODUCTION

This document is meant to provide a technical description of the IIT research plan for those who are interested in joining the IIT research team. The technical annex develops the mainstream lines and the interactions among the three technology platforms defined in the general scientific plan approved by the Board of Trustees on November 8th, 2004 (namely: bionanotechnologies, neurosciences and robotics).

The merge of the three platforms enables a multidisciplinary approach to robotics, in which the study of intelligence and cognitive processes, in combination with state of the art material and nanofabrication technologies, should lead in the long term to the accomplishment of a humanoid robot. The distinctive mission of IIT in its start-up phase is thus to create the arena where neuroscientists, engineers, material scientists work together to realize the intelligent robots of the future. Such a mission does not only imply the development of "soft" (*mental*) technologies (just like in classical artificial intelligence research) but requires the parallel, synergic development of "hardware" technologies in fields such as micro-mechatronics, solid state sensors, electric, hydraulic, pseudo-elastic actuators and nanoactuatuors, advanced materials, computational architectures etc. Stemming from this multidisciplinary and multi-technological requirements IIT will address simultaneously "hard" and "soft" humanoid technologies trying to play a role in the technological and industrial development of the country in the short term (0 to 5 five years) as well as producing new frontier knowledge in the long term (10 to 15 years).

The IIT strategy is outlined in the IIT flowchart research program (Fig.1) and can essentially be condensed in the following points (Fig.2):

1. Investigate the human "hardware" at the molecular/nano level. This will include: i.) the link between the molecular phenotypes of individual neurons and their physiological features; ii.) the genetic/epigenetic factors at the basis of learning, memory, adaptation and development; iii.) the dynamics of neural plasticity.
2. Design and build artificial systems (humanoids) that can perceive, act and learn autonomously, whilst also being able to interact with humans in a natural way (see next point). This will require the use of sophisticated materials and fabrication technologies at the nanoscale to produce the required components (bodyware). Since it is unrealistic to build highly complex structures only by manipulating individual molecules, ways must be found to allow function to shape physical growth. Moreover, software tools will need to be developed to allow the integration and central management of all components (mindware)
3. Interface these systems to humans through teleoperation, tele-presence, natural language and gesture, and ultimately direct connection to the nervous system.

iit

Figure 1 : IIT flow chart research program

BASIC RESEARCH ACTIVITIES

Human Research/ Nano-Micro scale research

Advanced characterisation tools and imaging
- Single molecule, single bioevent monitoring in live cells
- Active nanoprobes as intracellular reporter nanorobots
- Scanning probe characterisations

Intelligent drug delivery
- Cell-penetrating peptides as nanorobots
- Active nanoprobes as intracellular actuator nanorobots

Study human behaviour for cognition and development
- Plasticity of neural circuits (learning/adaptation)
- Perception, representations and cross-modal interaction
- Motor control and learning (locomotion, grasping, grazing)
- Sensory-motor memory and motor learning
- Visual attention

Interaction/Technologies

Study the sense of presence
- Robots as remote bodies
- Adaptive Interfaces
- Motor and cognitive controllers

Interaction spaces
- Simultaneous presence
- Telepresence

Neural Interfaces and smart electrodes
- Brain-machine interface
- Smart Electrodes and connectors
- Neural probes for in-vitro applications

Humanoid Research/Micro-Milli scale research

Cell to chip and chip to cell technologies
- Patterned 3D cell cultures by microfabrication lithography (top down approach)
- Patterned 3D cell cultures by polymeric self assembly (bottom-up approach)
- New strategies for biomolecules immobilisation onto polymeric surfaces
- Fluidics

Build the bodyware
- Sensors and actuators
- Intelligent Microsystems
- Artificial tissues
- Tactile sensors
- Micro- and nano-muscles
- Acoustic/inertial sensors
- Optical-fiber based sensors

Build the mindware
- Head hand coordination, Manipulation , grasping
- Bimanual coordination
- Interaction and affordance
- Interaction and imitation
- Interaction and communication

Humanoid's benchmark
- Technology watch and assessment
- Open-humanoid action
- Training

Nanobio platform

Neurosci platform

Robotics platform

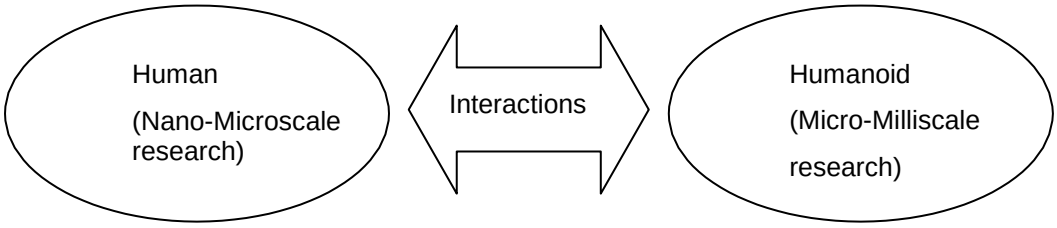


Fig. 2. Humanoid technologies

The outcome of this multidisciplinary basic and targeted research efforts, besides being integrated into humanoid robots and advancing our knowledge, will contribute to many industrially relevant areas of science and technology including microelectronics and micromechatronics, computer science and artificial intelligence, telecommunication, biomedical engineering, computational architecture and networking. To fully appreciate these possibilities, a partial list of technological fall-out of humanoid research that can be exploited in different application areas is explicitly presented below.

1. Learning. The implementation of learning algorithms in humanoids will be directly transferable to artifacts that need to adapt to different environmental conditions and learn from past experience. Examples are devices learning the user's habits and adapting their parameters to the user's predicted intentions or devices learning to adapt to changes in the environmental conditions such as a surveillance system learning to recognize common, non-threatening events and persons in specific environments (a person running is a common event in a field but less so inside a bank). Besides the computational aspects of learning, which represent a key technology in many areas of ICT, the implementation of learning abilities in artifacts may require the realization and use of dedicated microelectronic components such as associative memories, neural network chips, processing units designed for highly parallel architecture.
2. Sensors. Smart, miniaturized, power-efficient sensors probing the environment in an efficient and reproducible way are essential components for all devices designed to act in the real world. Miniaturization of smart sensors with embedded intelligence and low power consumption is a very active area of research in microelectronic and nanotechnology in fields such as automotive and wireless communication just to mention a few. Minimally invasive surgery requires the use of sensorized probes that share the same technologies developed for robots. Procedures requiring precise positioning of surgical instruments with respect to anatomical reference points (e.g. neurosurgery) can take advantage of the sensory and motor technologies developed for autonomous robots.
3. Actuators and microcontrollers. Not only humanoids and robots but any motorized device that has to work in close vicinity or in cooperation with humans must be designed with intrinsic safety. In biological systems (including humans) this is obtained by exploiting the intrinsic elastic properties of the muscles. For artificial systems it is of fundamental importance, therefore, to develop actuators embedding some kind of elasticity in order to reduce the consequences of accidental collision and to allow the system to learn from mistakes. Besides the possibility of realizing true elastic actuators (a research field still very active), an alternative is in the use of "traditional" solutions (such as electric motors) coupled to microcontrollers that, via embedded firmware, control stiffness transforming stiff motors into elastic actuators.

It is worth stressing the consequences that a multi-technological solution based on the combination of (micro)mechanics, microelectronics, computer and neuroscience may have in shaping the know-how of the industry in this field.

4. Biomedical Instrumentation. Mechanical systems used to test the motor performance of patients during the execution of stereotyped tasks share a good deal of technology with humanoids. Intelligent rehabilitation devices are, in all respect, robots interacting with the patient in a controlled, supportive way, to help restoring motor abilities. Finally, recent clinical studies on the interaction of humans and artificial systems have shown promising results in using robots to stimulate social responsiveness in autistic children.
5. Growing and "living" materials. The study of learning, sensorimotor and cognitive development in humans by means of humanoid robots must take into consideration the morphological changes that occur in the human body during its entire life. A major difference between an artificial and a biological system in this respect is the fact that while the materials constituting the former do not evolve in time, human tissues are shaped and grow while the system is learning and interacting. It has been demonstrated that this possibility is a source of great simplifications in the optimization of an intelligent system. It has thus been possible to argue that morphology and morphological changes are an essential part of intelligence. The technology required to manufacture materials that physically grow and change morphology during the lifetime of an artifact (in the sense that the functioning itself contributes to the growing process) is still largely lacking. Once more the study of these manufacturing processes finds its natural environment in research centers where not only materials but also intelligence, learning and cognitive development are studied concurrently.
6. Artificial skin and sensing materials. In spite of the great technological advancements in many areas of artificial sensors, a device with the mechanical and sensing properties of the human skin is still missing. What we lack it is not only the richness of human haptic sensors allowing us to measure roughness, temperature etc., but also, the mechanical properties of human skin and flesh. The absolute need of an integrated approach is also in this case evident. Aspects such as the mechanical and sensory properties of the skin need to be addressed in close relation with the properties of the actuators, the requirements of the processing units, the goals of the action, the performance of the learning and adaptation algorithms. A particularly promising technology for the skin may derive from that of advanced (or technical) textiles where, if the softness and mechanical adaptability of textile materials could be augmented with embedded sensors and connectors, it may give rise to a new generation of so-called "wearable" devices.
7. Visual, haptic and auditory perception. The processing of perceptual data up to the level of "understanding" is still far behind our expectations. Object and event recognition is still done in rather constrained situations and generalization from few examples is a subject of increasing research activity. Developing humanoids able to learn from the richness of their own perceptual experience certainly requires the study and implementation of systems possessing a high degree of understanding of sensory data including their relationship with sensorimotor coordination and learning. Studying such aspects in a "humanoid" environment will force, once more, to attack the problems in a multidisciplinary and multi-technological framework. The implementation not only requires the understanding of aspects of human perceptual abilities but also to find the optimal compromise between hardware and software solutions, between dedicated microelectronic chips and "general purpose"

computational devices, between software based control and morphology of the sensorimotor plant. All these aspects address very relevant and strategic areas of ICT technologies.

8. Real-time control and planning. A physical system like a humanoid requires real-time control not only because it is desirable to have fast reacting systems but, more importantly, because without such abilities, the system will not "survive" in the real world. By a real-time system we do not only mean a system able to react in a fraction of a second but also able to "predict" the effects of its actions and to act accordingly. Once more this ability is learned in humans and other animals as a result of the system's interaction with objects and other systems up to a level that an "adult human brain", more than interpreting perceptual data, is indeed anticipating the sensory consequences of performed and seen actions. This ability is deeply rooted in the way the brain processes sensorimotor data: recent studies have shown that motor areas issuing the commands to move, for example, the right hand are active not only when hand is moved but also when the person is only imagining to move the hand. Real-time operation in these systems, therefore, is not necessarily based on feedback loops with short, predictable delays but a more interesting (and certainly new) interplay between what is known from past experience, the goals of the action, the programming of motor commands and the concurrent processing of perceptual data. Once more only through interdisciplinary efforts it may be possible to propose innovative technological solution by integrating control theory and motor learning principles into special purpose embedded systems.
9. Network sciences and parallel computation. Understanding how a massively parallel system like the nervous system computes has given, and most probably will give, interesting suggestions on how to implement the parallel architecture of an artificial system. The plasticity of neural connections and the highly distributed, reconfigurable structure of the nervous system are working models of distributed networks and, as such, can suggest the theoretical basis and the engineering tricks for how to maintain and expand large distributed networks which are inherently impossible to control in a centralized way (e.g. power distribution, telephone and cellular communication networks). Implementing a humanoid's mind using the principle of neural computation will certainly affect the way we think about processors and networks, providing new insights for telecommunication as well as microprocessor and microcontroller's architecture.
10. Fault-tolerant and redundant systems. Possibly the most relevant aspects of human neural processing is the robustness to even dramatic changes in the physical structure of the system. For example patients with even massive damage to the central nervous system are capable of surprising recovery of lost functions. This is a consequence of the architecture of the human nervous system, which shows a remarkable ability in re-routing its distributed processing units. This ability is still very much lacking in artificial systems where the most widely applied solution is that of duplicating (and in some cases even triplicating) all critical components of the system. From research carried out with the goal of building complex systems with anthropomorphic computational architectures new insights may arise for the realization of distributed, redundant computing architectures (e.g. GRID computing).
11. Distributed cooperating systems. The interaction between multiple robots can be studied and implemented in the framework of distributed intelligence. Examples of such studies based on the use and coordination of many robots, besides being an artificial implementation of the social behaviours in natural systems, can offer novel solutions to the problem of coordinating and integrating the information coming from

loosely coupled multiple sources such as networks of visual sensors. Group behaviour, such as that exhibited by social insects like ants and bees, offer great advantages in tasks such as exploration and patrolling where a high number of simple, cheap individuals, with low bandwidth communication have better performance than a small number of "individually intelligent", expensive systems. Extensions of these studies to, for example, environmental monitoring, surveillance, traffic control systems may be very significant.

12. Human-robot interaction. Today one of the still largely unsolved problems is to design devices that can be activated, used and programmed in a natural way. The improvement in performance of, for example, video recorders, in the last few years has been enormous in relation to image quality, speed of access, efficiency of recording media but virtually non existent in relation to their ease of use. This is mostly due to the fact that, in order to interact in a natural human-like way, some degree of human-like intelligence has to be used including learning and, possibly, gesture and vocal interface. The study and realization of humanoids that use interaction with humans as a way to learn and communicate is a new direction of research that will hopefully suggest new, more efficient solutions. Once more it is worth stressing that these solutions will be based in the combined advancement of knowledge and technologies in many fields including communication, computer science, artificial intelligence, neurosciences.
13. Brain-Machine Interface. Interfacing artefacts to biological systems through direct and indirect connection will be an important technology, for example, in the field of advanced prosthesis but also in application areas based on teleoperation and telepresence. Interfacing an artefact to the nervous system requires, not only the technology of the "connector", but also the "technology of the pre-processor", translating artificial signals into commands suitable for neural processing and vice versa. This means not only choosing the proper modality and adapting the amplitude of the input signals to the requirements of living tissues, but also understating the "communication language" used by the nervous system to code and transfer information between clusters of neurons.

B .BASIC RESEARCH ACTIVITIES

1. Human Research/Nano-Micro Scale Research

The first box in the diagram of Fig.1 deals with research on human systems. This will involve a fully interdisciplinary approach in which nanobiotechnologies and neurosciences are synergistically developed to study perception, cognition, motor performance, learning and the adaptive capabilities of human beings. This in turn requires a combination of advanced technologies to study at the molecular and cellular level signal transmission and processing, and, at macroscopic level, the behaviour of humans. Though far in the future, the fallout of these studies will be crucial to develop new generations of robots with increasing humanoid characteristics and to design artifacts interacting and interfaced with humans. In addition, a byproduct of these activities will be the use of molecular systems as carriers and smart motors for the accomplishment of specific actions, such as intelligent drug delivery and nanoscale actuators.

1.1 ADVANCED CHARACTERISATION TOOLS AND IMAGING

1.1.1 Single molecule, single-bioevent monitoring in live cells

Ultimate resolution in detection experiments will be pursued through the development of novel fluorescent probes in order to minimize the perturbation of the systems under study. This capability will enable a number of biomolecular investigations required by the long-term goals of the IIT project, meanwhile being of immediate interest for patent/commercial applications. Different classes of molecules will be considered, including GFP-class fluorophores, nanocrystals and synthetic dyes. Computer simulations will be extensively used in order to rationalize probe design and a host of biotechnology and synthetic chemistry approaches will be exploited. These probes will be used with single and multi-photon confocal set-ups suitable for the study of living cells and tissues. All state-of-the-art techniques such as FRET, FRAP, FLIM and second harmonic generation (SHG) will be exploited. New imaging techniques are envisioned that take advantage of the reactivable or photochromic markers that will be developed.

The final goal is that of monitoring gene expression, protein localization and protein trafficking in real time in live cells at the single molecule level. Somewhat more ambitious, but within reach, is the goal of monitoring interactions (protein-protein, receptor-ligand, DNA-protein etc.) again at the single-event resolution, in real time and in live cells and tissues.

1.1.2. Active nanoprobes as intracellular reporter nanorobots

Controlled actions and signal generation in live cells and tissues will be pursued eg by means of the design and production of fusion proteins. The principle of operation of these tools is inspired by allosteric enzymes. These reporter nanomachines will change their structures as a result of the presence of a specific signal, namely a protein in a particular state or a specific ion. This change of conformation will produce a detectable signal. An example is

that of a change in optical signaling. The change of conformation would for instance suppress (or favour) FRET between two fluorophores, or would change the optical efficiency leading to a detectable signal. It is of particular interest to implement probes capable of detecting not only the existence of a given species, but the status of a biopolymer (e.g., phosphorylation, sumoilation, a specific conformation etc.). Specific interaction partners should be identified and labeled with complementary FRET fluorophores in order to monitor in vivo post-translational modifications of interest.

Particular attention should be paid to the development of membrane-linked protein biosensors, since most physiological activities of neural cells involve changes at the membrane level. For instance, selected protein probes may be fused to membrane ion-channels or antiporters, in order to monitor ion exchange activity between the extracellular matrix and the cytoplasm. In this perspective, SHG on membrane-anchored GFP can be effectively used to report on the changes in cell-membrane potential, since the large induced dipole of the GFP chromophore upon excitation is strongly affected by the transmembrane electric field.

1.1.3. Scanning probe characterizations

Scanning probe microscopy (SPM) and in particular AFM and SNOM are ideal techniques for the high-spatial-resolution study of biological interactions at surfaces and, most importantly, allow these studies both in air and liquids.

SPM equipped with functionalized tips can be used to map cellular functions in vitro. For example pulse mapping of myocytes can be realized by positioning the probe of the AFM over different areas of the cells and recording the movements of the cantilever induced by the cell pulses. AFM tips functionalised with suitable ligands can be used to map transmembrane receptor activity. Moreover, the nanomanipulation capabilities of an SPM system can be exploited to mechanically probe the stereocilia of the hair cells of the inner ear on a biochip in order to probe their functionality. Haptic feedback and virtual reality can be implemented in order to improve the user sensitivity in manipulation.

Scanning ion conductance microscope (SICM) will also be employed to perform imaging on single ion channels in the cell membrane (exploiting the ionic current variation) and directly delivering to it Ca, K, Na ions or proteins, in order to stimulate single-cell activity. As an example GFP-functionalized probes should be implemented to allow far-field or near-field probing of ion-channel activity trough the fluorescence properties of the GFP cluster, sensitive to the ionic gradient itself. Hybrid SICM-SNOM, where the probe is built as a hybrid electrolyte/near-field-light “dispenser” in order to achieve at the same time topographic, ionic, and optical imaging at high resolution in the single ion channel can be developed.

Finally, FIR and MIR near-field microscopy should be developed yielding nanometer-scale resolution in order to perform imaging on cell membrane samples with biomolecules such as proteins, DNA and polycarbohydrates. Quantum cascade lasers (QCLs) in the 1-4 THz range will be employed as radiation sources and pyroelectric elements and bolometers will be utilized for detection in far field. The possibility to tune the frequency of the incident radiation will also be exploited in order to obtain local spectroscopic information. SPMs with metal-coated tips will be employed for the apertureless SNOM approach. Spatially-resolved label-free diagnostic techniques on biological samples will be developed as new spectroscopic applications.

1.2 INTELLIGENT DRUG DELIVERY

Molecules whose conformation or activity can be changed through the application of light and/or specific chemical reactions can act as nanorobots transporting and delivering active drug at specific sites. Though far from the conventional concept of robotics, this can be considered as an interesting field for IIT, descending from the background knowledge gained in the previous research line (1.1). Possible research activities in this field are addressed in the following.

1.2.1 Cell-penetrating peptides as vectors for the intracellular delivery of drugs and nanorobots

Cell-penetrating peptides or protein-transduction domains (PTDs) are peptides that can translocate through the cell membrane into the cytoplasm in an energy-independent, receptor-less manner. Among PTDs the transactivating protein of HIV (Tat) is known to cross not only the cytoplasmic membrane but also to penetrate into the nucleus, thus representing the molecular basis for new drug-delivery, or even gene-delivery strategies. New short peptides (based on Tat and other PTDs) will be synthesized as cell-penetrating vectors (CPVs) for the delivery of therapeutic molecules such as oligonucleotides or proteins that lack inherent membrane penetrating capabilities but are susceptible to acidic denaturation and enzymatic degradation into lysosomes. CPVs will be designed by means of computer-aided structure analysis of known PTDs or by molecular modeling of surface receptor ligands. They will be synthesized by large-scale peptide synthesis. Trafficking and molecular interactions of CPV-conjugated drugs and prodrugs will be monitored by advanced imaging techniques such as FRET, FRAP, FLIM in order to establish the cellular distribution and activity of drug complexes. CPVs will also be used as a way to introduce and distribute within the cell light-driven nanodevices/nanostructures and light emission biosensitizers, i.e. molecules that emit light when coupled with metabolic processes, as a tool for physiological monitoring.

1.2.2. Active nanoprobes as intracellular actuator nanorobots

Molecular nanorobots can either generate signals or actuate movements to control the delivery of a molecule or carry out a specific action. Upon recognition of an activation signal the change of conformation of a molecule may be used, for instance, to deliver a specific molecule or to activate a site for therapeutic purposes. In this context the computational capabilities of DNA may play an important role and should be evaluated.

In addition to these autonomous nanorobots, light-controlled operation can also be implemented. Long-lifetime fluorescence (bio)molecules (LLFM) possess a wealth of unexploited photophysical characteristics. Among these, we mention the capability of interacting and promoting reactions (energy transfer and electron transfer) with the surrounding molecules upon light excitation. Thus, LLFMs represent a promising approach to mediate and/or carry out desired reactions intracellularly by external light stimulation (“light absorption sensitizers”). Much attention must be given in particular to the development of LLFMs with absorption and emission in the 700-900 nm range. Such near-infrared photons have rather high tissue penetration and allow the monitoring and activation of the probes in complex 3D cell cultures and even in living organisms. In the intelligent drug delivery field, we can think of a designed molecular complex made up of an organic and/or peptide prodrug in a reductively or oxidatively activatable molecular form linked to a LLFM with tuned light

characteristics. The complex should be administered to living cells in culture or in the body and freely internalized; then, upon light excitation of LLFM in selected region of the cells (or the cell culture, or the body), the prodrug is reduced or oxidized to the active therapeutical form.

More, we can think also of LLFM molecular complexes ("light nanorobots") capable to convert light energy into mechanical energy within the cell and thereby performing nanoscale activities such as stimulation, modification, or restoration of biochemical motors (kinesins, myosins, etc). For instance, upon light exposure cells may be driven to migrate into selected region of a cell culture (light chemiotaxis) and create an extended cellular interplay network or perform muscle-like activities.

1.3 STUDY HUMAN BEHAVIOUR FOR COGNITION AND DEVELOPMENT

Any acting artifact (like a robot) has to find a balance between efficiency (task-specific capabilities) and biological compatibility (in the sense of being evolving and versatile). The former leads to the realization of automatic systems, very fast and precise in their operations. Limitations of automatic systems are purely technological (e.g. miniaturization). The latter is what we consider to be a humanoid: a biological-like system that takes decisions and acts in the environment, learns how to behave in new situations (adapts), invents new solutions on the basis of past experience.

An additional degree of freedom of the humanoid is the possibility to interact with the environment, namely demonstrate, communicate etc.. The "biological compatibility" does not represent an intellectual exercise but is prompted by the idea that a humanoid interacting with human beings must share with them representations, motor behaviours and perhaps, even kinematics and degrees of freedom. This representational/procedural sharing provides the possibility of reciprocal understanding of actions and intentions.

To interact, a humanoid must first act (and not simply move), perceive, categorize and therefore, understand. These capabilities cannot arise from pre-compiled software routines. On the contrary, they must be accomplished through an ontogenetic pathway, simulating what happens in developing infants. In other words, humanoids must act in the environment to know it. Knowledge of the environment does not mean the ability to categorize an assembly of static structures and objects, but rather requires an understanding of the consequences of generated actions (e.g. a glass breaks when falls on the ground). During this knowledge acquisition, trials and errors are fundamental because they increase the exploration field. This is the main difference between a humanoid and an automatic system: for the latter, errors are not allowed by definition. The developmental process leading to a 'mature' humanoid requires a continuous study of its human counterpart. This study only partially overlaps with traditional neuroscience, because of its peculiar interdisciplinarity. In other words, the synergy between neuroscience (particularly neurophysiology) and robotics gives origin to a new discipline in which bi-directional benefits are expected. In fact, this knowledge-sharing will reward not only robotics: the developing (learning) humanoid will form a behaving model to test neuroscientific hypotheses by simplifying some extremely complex problems. Particularly, it will allow what is not conceivable in human neuroscience: to investigate the effects of experimental manipulations on developmental processes. To this purpose, various experimental approaches are suitable.

On the human side, motor representations have to be studied by using brain-mapping techniques, such as: functional magnetic resonance imaging (fMRI) associated with high

resolution electroencephalography (the association of the two approaches largely improves temporal resolution); transcranial magnetic stimulation (TMS, to instantaneously measure the excitability of the human motor system, during various modulation conditions); near infrared spectroscopy (NIRS), a very recent technique which allows to monitor cortical metabolism non-invasively; the measurement of movement parameters during normal behaviour, such as eye-movements, limbs 3D kinematics, high resolution electromyography, etc.

On the robotics side, the implementation of motor representations and, more interestingly, of visual-motor maps (for object-recognition and for the understanding of others' actions), will be pursued by continuously referring to human studies. The exchange of information between the two IIT branches represents indeed the peculiar characteristic of the project. In agreement with this strategy, robotics will not only take, but will also pose questions to neuroscience, which will be addressed by appropriately designed experiments.

1.3.1. Plasticity of neural circuits (learning/adaptation)

A very ambitious target of IIT is the understanding of the rules and cellular mechanisms underlining the modification of neuronal connectivity triggered by environmental changes. These adaptive responses are under the control of experience, and unfold during development and aging under the influence of the living environment (enriched or deprived environment). This research should lead to the design of humanoids with the capability to learn and adapt to the environment. The investigation of these processes in neuropathology models will provide qualitative and quantitative information on the specific elements of the neuronal activity altered in the pathology. These data will be useful for the development of better bioelectronic prosthesis directed at rescuing damaged circuitry. This complex line of research will require an extended behavioural and neurochemical characterisation. Furthermore, it will be crucial to monitor neuronal activity in different cerebral areas (with optical and electrophysiological tools and with brain imaging), to determine the flux of information and of plastic changes in correlation with behavioural tasks. Visualization of plastic phenomena at the molecular (i.e. changes of expression or activity of specific molecules) or structural levels (modifications of synaptic contacts, dendritic spines) will be important for the comprehension of the mechanisms involved in the experience-dependent modifications of neural connectivity.

The knowledge of the interaction of different brain areas during specific cognitive tasks, and, in particular, the specification of the feedforward and feedback flow of information between primary sensory areas and multimodal associative areas and between associative areas and motor areas, is essential to understand the rules governing plasticity of these neural circuits. Moreover, an integrated strategy is essential to dissect the interactions between different functional modules during formation and consolidation of a memory trace and during its retrieval. This approach will provide essential clues for the development of a sensible process of rehabilitation in the presence of a neuronal deficit (cognitive ergonomics). The study of neuronal activity and of the cellular mechanisms at the basis of adaptive responsiveness requires the detailed analysis of processes occurring in a large variety of space-time domains. For example, the coordinated responses occurring in the cortex cover areas of several millimetres and occupy a time interval ranging from seconds to hours. However, the molecular reactions at the base of the large-scale response occur on a smaller spatio-temporal scale. Therefore the comprehension of the adaptive responsiveness of the nervous system requires the integration of different techniques of investigation that are apt to cover diverse phenomenology and the use of different models, from in vitro preparations to in vivo analysis in rodents, non human primates and humans. As an integral part of the research plan, innovative probes for the in vivo study of the molecular mechanisms of learning and in

vivo two-photon imaging technologies to monitor structural plastic changes in the developing and adult nervous system should be developed.

1.3.2. Perception, representations and cross-modal interactions

This task basically consists of three research lines, namely:

1. Analysis of the computational and coding properties of single neurons and of neuronal assemblies at different levels along visual pathways, from the retina to the cortex, either in adult or developing subjects.
2. Object recognition and generalization in different positions and orientations of the object. This is a typical example of a task that is routinely performed by the nervous system, yet having a tremendous amount of underlying computational complexity. Many cerebral areas are involved, from visual associative cortices to areas involved in learning and memory. It is therefore mandatory to understand how these regions interact during the execution of this task and how they weight previous experiences to generate the progressive improvement in object recognition. This knowledge would be fundamental to design future humanoids reproducing these essential capabilities. Furthermore, experimental evidence indicates that the motor system plays a central role in object categorization. This 'perceptual' role of the premotor cortex demonstrates that objects are recognized not only because of their visual properties, but also because they are potential targets for actions. The "motor categorization" approach may be implemented in acting/perceiving artifacts as already shown by some practical applications, and deserves further investigations in humans by using brain imaging and transcranial magnetic stimulation (TMS) techniques.
3. Investigation of the interactions between different sensory modalities (cross-modal interactions). This research line is important for the design of humanoids able to interact multisensorially with the environment, integrating information coming from the different sensory modalities. The study of the presence and the strength of cross-modal interactions is also essential for the optimization of the clinical outcome of the implantation of neuroprostheses. As demonstrated by the example of cochlear implants, these therapeutic devices are not always able to promote functional recovery. Understanding the plastic processes that occur in the brain after a new function is restored by means of neuroprostheses would greatly facilitate the design of new neuroprostheses and the optimization of the patient treatment after implantation. These studies should exploit the possibility offered by animal models (rodents and non human primates) of a simultaneous monitoring of neuronal activity in sensory areas that are deprived of their original input and in sensory areas that receive sensory information, during different strategies of rehabilitation or experimental therapies promoting functional recovery. These data should be correlated with the behavioural response to treatment and to the underlying molecular changes.

1.3.3. Motor Control and Learning (locomotion, grasping)

The study of motor control mechanisms in humans and in animal models is important to understand how the central nervous system creates and updates its internal representations, allowing a great movement precision under varying environmental conditions. Such a continuous modifiability requires the activation of motor learning mechanisms to create a

"vocabulary" of action representations, which constitutes a common "motor database" allowing reciprocal recognition of actions between different individuals. For instance the study of mirror neurons, which are a category of premotor neurons discharging during the execution of grasping movements and during the observation of similar movements performed by other individuals, could elucidate many important aspects of locomotion and grasping. The presence of a neural system that automatically links observer's and agent's motor representations provides the brain with a powerful tool to understand other's intentions. While observing another individual acting, the observer may predict the action outcome by using different cues. The observed/executed action, together with available visual information from the environment (e.g. the presence of motivating objects), are shared by both the agent and the observer, and represent informative cues for the prediction of the outcome of the other's actions.

The dissection of these mechanisms, mainly ascribable to plastic changes in the frontal cortex circuitry, wouldrepresents a milestone in our understanding of how the motor system can continuously adapt to the environment whilst keeping motor precision constant. The implementation of these mechanisms in humanoids would endow them with the adaptive abilities of the human motor system and would be a huge step forward in the field.

Another important aspect of motor control stems from the organization of the motor system in hierarchically organized modules located at different levels of the nervous system. Understanding how the activity of the different modules is combined to create a smooth motor control is of great interest in view of the design of a humanoid endowed with locomotion or grasp, both a precision grasp and a strength grip, efficient and adaptable.

It is also important to underline that solving these problems requires a multidisciplinary approach, combining brain imaging studies in behaving animals with computer modelling of neural architectures and with an analysis of the effects of localized micro stimulations in specific areas of the nervous system.

1.3.4. Sensory-motor memories and motor learning

Memory is represented in the brain in a multicentric fashion. In addition to few brain structures known to be specifically involved in mnesic trace formation/consolidation (e.g. hippocampus), cellular plasticity (LTD/LTP), probably accompanied by peculiar patterns of gene expression, plays a fundamental role in "specific memories" (e.g. visual and motor memory) that are located in the same areas where particular functions are accomplished.

The recent discovery that RNA molecules can regulate the expression of genes, independent of their protein-coding function, opens new scenarios on the possible mechanisms used by cells to form permanent traces of conditioning events. The investigation of these mechanisms first requires the identification of a systematic baseline gene expression profile of specific cortical and subcortical areas. This initial set of data would provide the necessary background for subsequent studies of the mechanisms of memory and learning as well as for a series of investigations focused on possible ways to alter cortical plasticity. Experimental activity should investigate those regions that are active during the establishment of short and long-term memories, as well as the processes underlying consolidation of visual memory in rodents and motor memories in the monkey (primary motor area, dorsal premotor area, and supplementary motor cortex). Note that the processes leading to the formation of short- and long-term memories involve the development of new sensory-motor maps. The possibility that learning of new motor skills exert effects on cellular plasticity in motor/premotor cortices finds its basis on psychophysical results showing that learning of a

motor skill in humans and monkeys activates cortical short and long-term neural processes that continue to evolve after practice has ended – a phenomenon known as consolidation.

An objective of IIT will be to identify the gene/protein expression profiles during the acquisition of new sensory (including vision) or motor skills in monkeys and the transformations from short to long-term memory and consolidation. A thorough bioinformatics analysis will allow prioritizing the set of candidate genes for further evaluation as candidate memory genes. Priority for further study will be given to proteins of the dendritic and synaptic compartments of neurons, where phenomena such as synapse to nucleus communication, synaptically controlled local protein synthesis, RNA dendritic transport, receptor relocalization etc. are crucially involved in different phases of the synaptic plasticity phenomena underlying all learning and memory processes in the CNS. The phenomenon of local protein synthesis in the post synaptic dendritic compartment will represent a major focus of study, and in particular the mechanisms whereby a neuron controls independently the protein composition of its thousands of synapses, according to their activation state, and the mechanisms whereby a given neuron can participate in a combinatorial fashion in different "maps".

1.3.5. Visual Attention

It is well known that the nervous system is capable to explore the world not only by moving the eyes but also by allocating (moving) visual attention. Visual attention causes a specific enhancement of the sensitivity of determinate regions of the visual field. It is likely that this enhancement is achieved by the voluntary (or automatic, as in the case of the sudden appearance of a visual stimulus) backward activation of the same circuits serving for the sensorimotor coupling at the basis of saccades generation. The study of the brain mechanisms at the basis of attentional orienting is fundamental to provide a humanoid with new capabilities: prediction and expectation. Experiments should be carried out by monitoring attentional performances during classical psychophysical paradigms, and by measuring the effects of attentional orienting on eye movements (eye tracking) and on cortical metabolism (fMRI-EEG, NIRS). The perturbation of attentional orienting will be explored by applying TMS on various parietal and frontal areas involved in oculomotion.

The mammals' foveal eye forces the nervous system to tightly associate motor activity with vision. Indeed, to visually perceive the external world, the active exploration of the environment through saccadic eye movements is continuously required. How can the brain form a stable and homogeneous representation of the visual space through this 'pin-hole' exploring system? Answering this question will provide new insights into the way by which the brain represents peripersonal and extrapersonal spaces, how it creates a unitary spatial representation, how the various motor effectors (eyes, hand, locomotion, etc.) are involved in building our experiential space. From the robotics point of view, the study of foveated vision will provide new methods for data compression.

A direct application of these studies will be at the basis of a new concept of tele-presence.

2. Humanoids Research/ Micro-Milli scale research

The construction of intelligent robots will be pursued by addressing explicitly and in parallel the implementation of the humanoid physical body and the implementation of its cognitive abilities including the interface with humans.

This will be achieved through a programme of experimental research, drawing on IIT's broad multidisciplinary backgrounds in human developmental psychology, physiology, cognitive robotics, mechatronics, and perceptual science, and will be based on IIT's research infrastructure grouping (micro)mechanical, (micro)electronic laboratories with the more advanced micro and nanotechnology tools.

To make this objective grounded on realistic issues the research target is an embodied system able to learn:

i) how to interact with humans and the environment in various ways, including by complex manipulation and through gesture production/interpretation;

ii) how to develop its perceptual, motor and communication capabilities for the purpose of performing goal-directed tasks

iii) how to interact naturally with a human operator.

The objective, therefore, will be achieved by jointly designing the mindware and the bodyware of the humanoid and using it as a research platform and as a tool to assess the feasibility of industrially and socially relevant applications of humanoid technologies.

It is worth stressing once more that an intelligent humanoid platform is relevant not only as a complete, integrated system but also as a collector of individually-relevant technologies.

2.1. CELL-TO-CHIP AND CHIP-TO-CELL TECHNOLOGIES

Traditionally cell cultures are performed on uniform distributions of extracellular matrix (ECM) proteins and do not allow the detailed control of cell shape and behaviour. This can result in morphological and functional differences with respect to *in vivo* cellular phenotypes. Conversely, an artificial 3D scaffold for tissue regrowth could promote cell adhesion and spreading, cell polarization, reconstitution of the autologous ECM, cell communication and interplay. All these processes are mediated by the interaction of the cell membrane with the biocompatible support. Therefore patterned cell cultures should be able to recreate a physiological environment both from the topological and the biochemical points of view and simulate *in vivo* cellular functions. 3D artificial polymeric structures appear as the best choice to mimic many living tissues, and consequently to control the cellular behaviour within the embedding structure. The scaffold must be interfaced to microfluidic circuits to allow the probing of the physiological cellular response upon stimulation with soluble molecular effectors.

2.1.1. Patterned 3D-cell cultures by microfabrication/lithography (top-down approach)

Specific attention will be paid to the development and the implementation of the most advanced three-dimensional microfabrication approaches, including gray-scale electron-beam and two-photon irradiation of polymeric resists. The realisation of elastomeric elements

starting from non-planar masters will be investigated. Synthesis of gel-like patterned 3D structures mimicking the viscoelastic component of the extracellular matrix will be achieved through the development of specific soft-lithographic methodologies with high resolution.

2.1.2. Patterned 3D-cell cultures by polymeric self-assembly (bottom-up approach)

A new scaffold for cell culture will be realized by exploiting the self-assembly characteristics of block copolymers and their organization into nanometer-scale features. The multiblock structure shall be made up of: 1) hydrophobic chains, possibly comprising functional units capable to report on some cell membrane characteristics; 2) hydrophilic, biocompatible chains provided with biological ligands capable to interact with the cell membrane and promote cell adhesion, contact guidance, and cell polarization. In this case, the overall architecture will self-assemble into a 2D or 3D nanopatterned scaffold where cells can organize in a tissue-like fashion and able to provide information on cell activity at the nanometer scale.

2.1.3. New strategies for biomolecules immobilization onto polymeric surfaces

New methods for biomolecule immobilization onto polymeric surfaces at the nanoscale will be developed to improve biocompatibility of the fabricated and engineered cell scaffold. This method will be compatible with high resolution nanopatterning techniques such as dip-pen, e-beam, and nanoimprint lithography. Research will focus on: 1) chemical functionalisation methods of lithographically or SPM-patterned molecules; 2) surface self-assembly of polymeric materials provided with functional groups capable to recognize and bind selectively biomolecules of interest; 3) development of a surface immobilization method entirely based on molecular evolution of simple living systems, such as viruses. These activities will also feedback onto the realisation of 3D channel systems with complex topologies and geometries, including microfluidic systems formed by connecting several substructures on separate wafers.

2.1.4. Fluidics

Micro- and nanofluidic technologies are the enabling factor for lab-on-chip devices allowing reduced consumption and waste of reagents and samples, and increased operational speed. Microfluidic mixers will be obtained by optimized patterning of channel walls in terms of shape surface characteristics (e.g. patterning with alternating bands of positive and negative charge density and applying an electric field along the channel in order to generate electroosmotic flows). Laminar streams in 3D structures will be controlled by changing the aspect ratio of the elastomeric microchannels, and the lateral position of the streams within the channels. Using a microfluidic approach, spatially and temporally constant gradients will be obtained.

A different fluidic approach based on surface acoustic waves (SAWs) will also be tested. SAWs were already shown to drive liquid droplets (with volumes down to 50 nl) on a flat chip. The waves, generated on a piezo-electric chip material by applying radio-frequency signal to specially-designed interdigital electrodes, propagate across the substrate transporting fluids and solid matter on a chip surface. The force acting on liquid droplets is sufficient to yield a macroscopic effect: low-power SAWs induce an internal streaming inside reagent volumes, SAWs of larger amplitudes moves the droplets as a whole on a surface. This novel

microfluidic technology allows biochips to be programmed so that different biological assays and different paths can be performed with only one chip layout. Importantly such SAW-based nanopumps have no moving parts and can be manufactured by standard e-beam lithographic process or by imprint nanolithography. Within the scope of this technology is the exploitation of SAWs to locally heat the liquid droplets, implementing a T-jump technique on nl-range liquid volumes.

The final goal will be to utilize these schemes to drive liquids on a chemically-functionalised chip surface in standard microfluidic devices, to improve the performance of size-exclusion chromatographic devices, to uniformly stretch or align polymers and DNA coils in nanochannels. The liquid miniaturization also results in enhanced precision by providing more homogeneous reaction conditions and shorter times for diffusion driven reactions.

2.2 BUILD THE BODYWARE

This task deals with the hardware and material-related technologies needed to implement a humanoid robot: the bodyware. The multidisciplinary environment of IIT, where all components and technologies are available “under the same roof”, is meant to favor the global optimization of a complex robotic system, instead of optimizing the sub-parts individually. Furthermore the implementation of a full-body humanoid and its use to test all cognitive abilities is a powerful, ineludible, plan assuring that the various research components integrate coherently.

A major goal of IIT will be to develop an integrated full-body humanoid to be used as a research and development test-bed and as a platform for the implementation of the mindware. The technological challenge here is to build a very articulated (more than 50 degrees of freedom) and sensorially equipped robot of a relatively small size and low cost (in the 50-90 K€ range) so that it could be used for research and training purposes. To allow the investigation of relevant aspects of manipulation, the design will be aimed at maximizing the number of degrees of freedom of the upper part of the body (head, torso, arms, and hands). The lower body (legs) will be designed not only to walk but also to crawl “on four legs” and sitting on the ground in a stable position. The overall body will be designed so that it will be possible for the system to smoothly change from walking to sitting to crawling autonomously. This will allow the robot to explore the environment and to grasp and manipulate objects on the floor. The size will be that of a two-year-old child and the total number of degrees of freedom for the body will be approximately 55. (7 for each arm, 8 for each hand, 7 for the head, 4 for the torso and spine, 7 for each leg). The sensory system will include vision (a binocular system), touch, audition and inertial sensors. Functionally the system will be able to coordinate the movement of the eyes and hands, grasp and manipulate lightweight objects of reasonable size and appearance, walk on two legs, crawl on four legs and sit.

It will be essential to follow a “holistic” design approach based on a wide variety of different technologies as well as on the implementation of novel solutions deriving from the integration of materials, actuators, sensors and computational architectures.

A very rough (although unnatural) subdivision of the bodyware components is into its skeletal, actuator and sensorial components.

In the holistic, bio-mimetic, approach proposed here the design and realization of the skeletal part of the body does not only mean to implement the physical support for the sensors and actuators but, may represent, if correctly exploited, an essential component and a source

of interesting technological solutions. For example recent advances in material sciences are opening up the possibility of using lightweight and highly flexible structures in the limbs and joints of robots. These technologies, associated to well matched control approaches, will speed-up the implementation of more human-like bodies emulating the 'soft' physically compliant structure of muscle, bone, tendons and skin. This trend is particularly relevant in some of IIT's targeted application areas where features like lightness and resilience to damage are essential for the robot abilities to learn and interact safely with humans.

For the same reason, it will be important to investigate the use of composite materials with the goal of duplicating the functional (but not exact anatomical structure) of the lower limbs and spine. The use of composites will provide low mass, resilience to impact and jarring, and yet stiffness.

2.2.1. Sensors and actuators

Organic muscle provides power for motion on land, in water and in the air, in highly variable climatic conditions and in creatures ranging in size from the whales to microorganisms. Further, in their antagonistic pair configuration, muscles can: i) modulate stiffness while controlling position (a vital property for safe interaction activities), ii) give more natural motion and control and iii) enable energy conservation through the spring elements of the actuators. Unfortunately organic muscle is still not an engineering technology and, for the purpose of initial implementation, the solution adopted will be based on more traditional actuators taking, whenever possible, a "soft" biomimetic approach to the actuation structure, possibly devising new mechanical configurations for available technologies. However the fundamental technological issue of elastic actuators composed of fibres with mechanical properties similar to those of biological muscles will be investigated in depth (as detailed in section 2.2.5). Form the performance point of view we see as fundamental requirements power/weight and power/volume performance, compliance and stiffness regulation, robustness, control behaviours and more biological issues such as self-repair and tolerance to injury and adaptability during the repair process. Issues relating to the control during these repair stages will be important as the overall behaviour will be modified during the healing period, and learning and updating control strategies will be essential at this time, as with a child or human.

The sensory components of the bodyware are also very crucial to give the robot the necessary ability to sense the surrounding environment as well as the status of its internal organs. By this we mean the ability to sense exteroceptive and proprioceptive visual, acoustic, tactile, force, and chemical modalities as well as multimodal integration. From the hardware point of view we see as particularly relevant the possibility of designing intelligent sensors integrating, as in biological systems, physical support, sensing and processing (see 2.2.2). In the medium term particularly strategic, because of the current lack of acceptable technological solutions, is the development of haptic sensors (see 2.2.3 and 2.2.4) measuring, in a flexible structure like skin and tendons, touch, force, torque, temperature with sufficient accuracy and reliability.

In the short-term three priority activities should aim at implementing different anatomical components and the related low-level primitives:

- The design, fabrication and test of a head-eye system including basic visual processing primitives as well as low-level oculomotor control, visual, inertial and proprioceptive sensors.

- The design and implementation of a two-arm system with the motor skills and sensory components allowing reaching and grasping primitives as well as primitives to acquire tactile and proprioceptive information.
- The design and implementation of a locomotion system allowing the system to explore the environment not only by manipulating objects but through locomotion as well.

2.2.2. Intelligent microsystems

The use of intelligent/smart systems has become common practice in many engineering applications. The availability of low-cost standard CMOS technology (and foreseeable advances in the future) has created enormous potential applications. The next breakthrough will be the design and development of “smart adaptive systems on silicon”, i.e. high-power and surface area efficient also devices that implement an entire system (i.e. sensing, computing and “actuating” actions) on a single silicon die.

The goal is to have smart adaptive systems on silicon able to “adapt” independently and autonomously to the changing environment. Instead of being programmed using traditional techniques, the systems will infer (operative) knowledge directly from the environment and from raw sensed data. In other words, they will be able to act in an “intelligent” manner, carrying out both perceptual and cognitive tasks. In the end, they may communicate through wireless channels, be battery or remote powered (via inductive coupling) and become truly ubiquitous in our daily lives. The “smart adaptive systems on silicon” will be able to operate on a micro/nano scale and in hostile and/or unexpected environments in a robust manner: e.g. plants, engines, the human body, satellites, etc. From this perspective, only a few (or no) human actions will be required to calibrate, program, repair, reconfigure, etc. Since CMOS silicon technology is already mature and generates significant and reliable results, the research efforts will be focused on creating feasible and reliable systems by exploiting the systematic and synergic integration of silicon micro/nano technologies, soft computing algorithms and On-Chip Systems.

On the technological point of view, the facility will not be addressed to the fabrication of standard CMOS integrated circuits because of their high cost, but rather to the post processing steps necessary to add to a CMOS integrated circuit (obtained from a commercial source) micro sensors and actuators, microinductors for wireless links, etc. in order to develop energy and area efficient smart adaptive systems on silicon.

As to the sensing elements the most relevant topics to consider are sensors to measure proprioceptive signals and sensors supporting tactile, visual and acoustic perception. In this respect a possibility is to study the implementation of Microelectromechanical Systems (MEMS) measuring inertia (through miniature accelerometers, or gyroscopes) and other exteroceptive components (e.g. deformation, touch, temperature) as well as proprioceptive components (e.g. muscle stretch, joint position, tendon tension, etc.). An exciting new possibility is to explicitly address the issue of merging sensing, actuation and processing into microelectromechanical perceptual engines implemented by means of distributed MEMS structures (MEMS dust) able to deal with signals in space and time. Multilayer systems of such perceptual engines will mimic cortical layers and interactions among cortical areas. Microfluidic techniques could be employed to implement interlayer interactions.

The effective development of Microelectromechanical Systems and Nanoelectromechanical Systems (NEMS) components requires the synergism of advanced computer-aided design (CAD), multi-physics computer-aided analysis and engineering

(CAE), computer-aided manufacturing (CAM) and fabrication methodologies, materials science and technology, and also of effective quantitative testing methodologies for characterizing the performance, reliability, and integrity of MEMS and NEMS at the different levels.

2.2.3. Artificial tissues

Tissue Engineering is one of the major focus of biotechnological research today. The most ambitious tissue engineering schemes assume that specific tissues and organs can be restored, repaired or replaced by homologous tissues created *in vitro*. However, many pre-clinical and also clinical reports demonstrate that poor scaffold design and inadequate tissue culture conditions are currently the major problems in tissue engineering that may prevent its successful applications. To overcome these limitations, novel biomaterials and better processes are needed.

Materials Better designed materials are required to sustain and guide tissue regeneration. A specific area of research could seek to implement and integrate novel bio-hybrid synthetic techniques, nanotechnologies and advanced material processing technologies to obtain scaffolds able to guide and control tissue growth, differentiation and proliferation. In particular, novel materials both of synthetic and natural origin have to be designed and processed to meet physical, chemical, morphological and functional requirements tailored to specific applications.

Process. The development of a functional tissue *in vitro* from cell-seeded scaffolds depends on the process conditions. Novel and sophisticated bioreactor systems are required to monitor and control the biochemical signals and biophysical stimuli and to enhance tissue growth and remodeling. A bioreactor should be able to generate suitable biological environments for the growth of specific tissues, meanwhile monitoring cell functions and viability throughout a 3-D construct, and properly adjusting the environmental conditions. Relevant research topics in this area are:

- Development of novel technologies and processes for the engineering of polymeric scaffolds, having: (a) well defined and tunable degradation rates; (b) highly structured and organized macro- and micro-porosity networks to guide cell and tissue colonisation as well as the trafficking of microsolutes; (c) specific molecular signals for cell recognition and guidance.
- Development of practical and reproducible *in vitro* experimental models to quantify the effects of mechanotransduction on cellular functions and on the production of ECM proteins.
- Study of the transport mechanisms that regulate fluid, macromolecular and cellular trafficking in cellular constructs, and of the experimental methods to determine the efficiency of movement of large molecules and fluids through the cellular constructs.
- Develop of “smart” bioreactors for neo-tissue growth, e.g. a prototype of integrated bioreactor with feedback mechanisms, to reproduce the appropriate biological environment for tissue growth.

2.2.4. Tactile sensors

Haptic sensing in humans provides non-visual information about the 3D shape of objects, the material they are made of and their surface characteristics by measuring pressure, force, force distribution, slippage, roughness, temperature and temperature gradients. As such, it is of fundamental importance for systems designed to interact manually with the environment and learn from experience. Biological sensors are characterized by a very high degree of integration of different sensors matched to optimal surface characteristics of the sensing surface. For example distinguishing between metal and wood by touch is possible by measuring the temperature gradient of the fingers in contact with the surface. In spite of the intrinsic relevance of this sensory modality, the technology of, for example, tactile sensing is still largely unsatisfactory (possibly because of the need of an integrated sensing/material solution required to realize an "artificial skin").

Of course the type of sensing element chosen for a tactile sensor (fibreoptics, piezoelectric polymer, optical, capacitive, magnetic, piezoresistive) has a major impact on the ultimate performance and size of the sensor. Piezoresistive materials have been used in many tactile sensor designs, and are usually based on a conductive polymer film called FSR (Force Sensitive Resistor). To understand the technological difficulties that have to be solved, it is sufficient to consider that an optimal tactile sensors should have: a) the ability to measure the magnitude, direction and point of action of the external force applied, b) Low weight, to avoid sources of errors during fine control of finger joints. c) High sensitivity and robustness, to endure external forces (including normal and shear forces) of 0.5- 10g, d) Good dynamic range, acceptable spatial resolution (1-2 mm) and fast response (at least 100Hz), e) Low hysteresis and temperature drift, f) Small size, enough to be housed in a finger body, g) Linearity is desirable, but some non-linearity may be tolerated, h) Low power consumption. Ideally it should also be easy to fabricate and cheap. One open problem for the implementation of tactile sensors is how to "interface" the sensing element to the external environment (e.g. by means of an elastic sheet) and in which material to embed the sensing element (possibly a deformable material to facilitate stable grasping). Solving such problems would require accurate investigations of the contact mechanics between the external surface (the skin), the underlying material (the flesh) and the touched object, the estimation of the stress fields and their coupling to the real sensor array. Such calculations should obviously take into account the structural and morphological properties of typical solid objects and provide a general guidance to improve sensor sensitivity. Calculations could be coupled to micro/nanoindentation measurements on FSR surfaces to test tactile capability and sensitivity as a function of the morphological and structural parameters of the polymer. The experimental measurements could also lead to the identification of important classes of conductive polymeric systems suitable for future FSR macro/micro tactile sensors¸ with superior sensitivity and easy manufacturing.

Another important problem is the capability to measure the local distribution of forces. The FSR provides an average force information, where the average is on the sensor active area. To improve performace it may be interesting to exploit optical/ionic lithographic techniques to reduce FSR size, by using micrometric interdigitated electrodes and developing micro-patterned FSR areas capable to provide the spatial stress distribution with micrometric lateral resolution. Developing a novel class of micrometric-sensitive tactile sensors would also represent a first step towards robots miniaturization. In the range of millimetre to micrometre scale, the adhesion mechanisms of small living organisms with hairy attachment pads (beetle, spider, gecko) offer a paradigm for the development of efficient tactile systems. In fact gecko and many insects have evolved fibrillar structures on their feet to achieve

extraordinary adhesion on vertical walls. These biological attachment systems consist of finely structured protruding hairs with a size ranging from a few hundred nanometers to a few micrometers, depending on the animal species. The systematic development of surfaces mimicking the gecko footprint has been recently reported in the scientific literature but considerable problems still persist in their mechanical stability. Nevertheless it represents one of the most promising routes for the comprehension of tactile interaction in small living objects and for the development of miniaturized tactile sensors.

2.2.5. Micro- and nano-muscles

The human muscle is a phenomenal example of engineering. As simple as this physical system may be, it has yet to be duplicated by artificial means. In the late 1940s, Israeli scientists found that bundles of fibers made from polymer gels can shrink or expand, mimicking the action of natural muscle tissue. Much of the current research involving synthetic muscle technology is attempting to increase the degree to which the material responds to electrical or chemical stimulation. Some research work has focused on a poly(vinylidene fluoride-trifluoroethylene) copolymer, which was developed originally for thin-film blood-storage bags. It has been found that bombarding the material with electrons alters its molecular conformation and creates new chemical bonds. The electron bombardment inserts defects into the material, making it more compliant and flexible. To date the time required for a polymer material to respond to the application of electric fields has been reduced to about 100 milliseconds. This is a significant advance in the development of artificial muscles. Carbon single-wall nanotube (SWNT) sheets are presently being used to develop artificial muscles which are considerably stronger and more durable than human muscles or currently available materials. In principle, artificial muscles can be reduced in size to connect micrometer arms and legs. To achieve this goal it is necessary to develop micro- and nano- manipulation facilities and appropriate tools for material machining such as Focused Ion Beam systems.

2.2.6. Acoustic/inertial sensors

A robot interacting with humans in a natural way must be equipped with efficient acoustic sensors. In this respect the mammalian inner ear provides inspiration for the development of a new class of sensors. The mammalian inner ear is a sensory organ that has specialized hair cells that detect *sound*, as well as *orientation* and *movement* of the head. The 'hair' bundle on the apical surface of these cells is a mechanosensitive organelle that consists of precisely organized actin-filled projections known as stereocilia. The sensitivity of the stereocilia is such that signals arising from thermal fluctuations can be detected, as well as acoustic signals of intensity 12 orders of magnitude higher.
Such a complex organ can be artificially constructed if appropriate mechanical resonators can be organized in an array. To do that, two different ways may be explored:

a) Carbon nanotube arrays synthesized on a patterned substrate by pyrolisis of acetylene on ordered arrays of Co(Ni) nanoparticles. The nanotubes are nano-mechanical resonators and their mechanical properties (stiffness, resonance frequency etc.) depend in a critical way on their length, on their diameter, on the fact that they grow as single or multiple walled. All these parameters can be controlled by the growth conditions inside the reactor. The best scheme for detection seems to be the use of a laser beam which shines

on the whole array and in a parallel detection of the optical speckles reflected by the oscillating array of nanotubes;

b) FIB fabricated stereocilia on materials of variable mechanical stiffness. Synthetic stereocilia can be made with a focussed ion beam (FIB) and the mechanical response of the resonator determined as a function of the size of the synthetic cilia. The candidate material is a piezoresistive substrate that will provide direct electrical coupling. Determination of the mechanical properties of the sythetic stereocilia will be performed by using AFM as a force sensor. As applications of the Synthetic stereocilia sensors may find application in miniature microphone/hydrophone, miniature acoustic imaging arrays, micro-flow detectors and shear-stress micro-sensors.

2.2.7. Optical-fiber based sensors

The past two decades have seen a rapidly growing interest in the field of fiber-optic sensors, mainly caused by the advances made in related fields like opto-electronics and optical signal processing. Some of the principal reasons for the popularity of optical fiber based sensor systems are small size, light weight, immunity to electromagnetic interference (EMI), passive (all dielectric) composition, high temperature performance, large bandwidth, higher sensitivity as compared to existing techniques, and multiplexing capabilities. Moreover, the widespread use of optical fiber communication devices in the telecommunication industry has resulted in a substantial reduction in optical fiber sensor cost. As a result, optical fiber sensors have been developed for a variety of applications in industry, medicine, defense and research. Some of these applications include gyroscopes for automotive navigation systems, strain sensors for smart structures and for the measurement of various physical and electrical parameters like temperature, pressure, liquid level, acceleration, voltage and current in process control applications. In all optical fiber sensors, information about the measure is primarily conveyed by a change in either polarization, phase, frequency, intensity or a combination of the above. Optical fiber sensors can be broadly classified on the basis of their operating principle as intensity based or interferometric type. The latter ones have greater sensitivity and are of most interest in smart sensor applications. The Mach-Zehnder interferometer, the Michelson interferometer and the Fabry-Perot interferometer are some of the fiber optic interferometers in use today. Fiber optics sensors can be well integrated in small robots, offering a simple solution to the development of multi-sensorial approach.

2.3. BUILD THE MINDWARE

The mindware refers to the cognitive/intelligence aspects of humanoid research and focuses specifically on the issues of how to exploit the interaction of the humanoid's body with the environment in the framework of manipulation and locomotion. The goal of the interaction may be, for example, learning/understanding the properties and affordance of an object, the purposive use of goal-directed manipulation, the meaning of gestures and actions, how to exploit the body (self or someone else's) to communicate and how to understand communications, events, and contexts. Obviously a full integration of cognitive aspects, perception components and sensorimotor coordination behaviours is required to accomplish a full control of the robot. It is therefore necessary to implement the mindware by following, as much as possible, the human cognitive development, i.e. to implement cognitive skills by

building a system that can learn incrementally through interaction with the environment and other agents just like a human baby does.

This means that cognitive skills cannot be implemented without considering action and embodiment and how perception and cognition are intertwined with development.

An exemplar experimental roadmap is:

i.) Discovering the manipulation abilities of the humanoid's body:

- Learning to control one's upper and lower body (crawling, bending the torso) to reach for targets.
- Learning to reach for static and dynamic targets.
- Learning to balance in order to perform stable object manipulations when crawling or sitting.

ii.) Discovering and representing the shape of objects:

- Learning to recognize and track visually static and moving targets.
- Discovering and representing object affordances (e.g. the use of "tools").

iii.) Recognizing manipulation abilities of others and relating those to one's own manipulation abilities:

- Learning to interpret and predict the gestures of others.
- Learning new motor skills and new object affordances by imitating manipulation tasks performed by others.
- Learning what to imitate and when to imitate others' gestures.

iv.) Learning how to regulate interaction dynamics:

- Approach, avoidance, turn-taking, and social spaces.
- Learning to use gesture as a means of communication.

v.) Developing robot "personalities" via autobiographic memory based on interaction histories:

- Learning about meaningful events in the lifetime of the robot.
- Sharing memory (events) during interaction.

These general targets should be accomplished through the development of specific tasks, such as:

2.3.1 Head-hand Coordination, Manipulation, Grasping

The sensorimotor primitives at the basis of eye-head-hand coordination have to be studied in detail. The need to include this level stems from the fact that the implementation of cognitive skills, particularly in an embodied system, has to take into account the peculiarities of the body and its sensorimotor coordination. For example, a control system able to adapt to lengthening and shortening of muscles, growing and aging of sensors and actuators, etc., forms the basis of the adaptability that is a crucial aspect of cognition. Cognitive abilities required by visual tracking, goal-directed reaching, hand shaping and pre-shaping have to be

implemented as well as more general interactive behaviours based on contingent sensory perception.

2.3.2. Bimanual Coordination

Another relevant aspect will be the study of how two hands can cooperate to achieve higher-level manipulation skills and the implementation of cognitive skills required for Bimanual Cooperation. By this we mean for example the ability to use the two arms/hands as a scaled up version of a single hand (as during *grasping* of larger objects); the ability to use the two hands in a cooperative and similar way (such as grasping the two ends of a rope or lifting and extending soft materials); the ability to use two hands in a cooperative but dissimilar way (for example holding a glass with one hand and pouring water from a bottle with the other). These abilities are virtually unknown to current state-of-art robotic systems.

2.3.3. Interaction and Affordance

Interaction and Affordance will address how it is possible, by interacting with the environment, to discover/learn the use of objects. By this we mean the ability to manipulate objects according to shape as well as use. An example is the grasping of a rod either with a *palm grasp* when it is used like a hammer or with a *pinch grasp* when it is used like a pen. These aspects address abstract properties of objects, i.e. properties that cannot be derived by contingent sensory perception alone. The acquisition of the "affordant" use of objects can occur through self experimentation as well as through imitation and social interaction with other animate agents. The implementation of these abilities to generate artificial systems able to understand autonomously the use of tools could extend considerably the potential applications of robots.

2.3.4. Interaction and Imitation

Within this area Interaction and Imitation is one of the most crucial objectives. The concept of imitation is, in fact, intimately linked to that of *understanding.* Imitation is seen not only as a "kinematical" playback aimed at reproducing the temporal evolution of an action but it implies two essential cognitive abilities: i) learning how to act upon objects (with all the implications due to the need of a unified sensori/motor/affordant representation of *tools*); ii) the ability to communicate through imitation. Data coming from neurophysiological investigations as well as from brain imaging and electrophysiological studies in humans will be used in defining the framework for imitation and in guiding its implementation. It is worth stressing the fact that developing a system with the ability to learn by imitation will open up the use of robots to non-expert operators.

2.3.5. Interaction and Communication

The activity addressing Interaction and Communication subsumes all previous activities in the sense of requiring the system to learn how to use knowledge to communicate through gestures and to interact socially with other agents. To some extent this is the core problem of "cognitive interface". In order to be able to interact socially, the system has to understand how to exploit the physical structure of the body, its manipulation skills, the affordances of objects,

the difference between animate agents and inanimate objects, the self from the others, how to imitate and when it is being imitated.

2.4 HUMANOID'S BENCHMARK

One of the main goals of IIT is to establish itself as an international research center investigating all aspects of humanoid technologies and finding industry and socially relevant applications of these technologies. To this aim three different strategies will be pursued.

2.4.1. Technology Watch and Assessment

A continuous technology watch and assessment of the more strategic aspects of humanoid technologies will be carried out. Many research centers and a few industries in the world are developing humanoid systems based on a wide variety of customized solutions. The most promising will be tested and, if considered strategic, acquired through joint programs of research and development. This activity will have strong links particularly with the targeted research aspects of IIT research. Examples include assessing the advantages of different kinds of actuators and sensors (hydraulic vs. electric vs. pneumatic) in different application areas or the level of integration reached in low-cost entertainment systems and their applicability to other robotics applications. In the short term we consider particularly strategic to assess:

The usability of high-complexity robotic systems implemented using hydraulic actuators. One of the world leaders in this field (possibly the only one with experience in building humanoids) is SARCOS in the USA.

The ease-of-use and actual performance of full humanoid systems developed by multinational companies such as: Sony, Honda, Fujitsu, Toyota. What we intend to do here is not "reverse engineer" what has been done by others but to assess how different solutions are suited to different application areas.

2.4.2. Open-humanoid action

This will consist in the realization and maintenance of a full humanoid system that can be shared, used, copied by anybody interested in studying/implementing basic research aspects of humanoid cognition (we call it Cognitive Universal Body or CUB). Robot intelligence is by far the most required technology for the implementation of robots interacting and cooperating with humans. Implementing systems with human-like intelligence is a long and challenging task that will require many generations of young researchers. The CUB will be an essential common tool that on one side will provide a system of sufficient complexity to scientists not expert in robotics and on the other will serve as an accumulator of research results (much like the Linux operating system has proved to be a very effective common tool for the development of software applications).

2.4.3. Training

This will be realised with the promotion of joint activities aimed at starting international research projects and of high level training activities on humanoid technologies based on the

CUB system. A Research and Training Site (RTS) will be established at IIT consisting of a fully functional and equipped laboratory where several fully functional copies of the CUB are maintained. The institutional activity of the RTS is expected in the following fields:

Open-system maintenance: this includes the preparation of suitable server computers, www access, updates, and management of releases. Also, the RTS will act as a gathering place where new software or upgrades could be attached to the system and tested before being released.

Training facility: a system of the complexity of the CUB (we expect the CUB to have more than 50 degrees of freedom) will not be usable (for research) without a minimum of training. This applies both to experienced researchers willing to use the CUB for their research and development activities (including the implementation of new CUB components) and to PhD students participating in IIT programs.

Research activities: the RTS will be also a site of considerable research effort in a multi-disciplinary environment. The RTS will be open to any researcher with a sound research program in robotics and cognition (access policies to be defined through a competitive call mechanism). We expect this to be a very powerful attractor of bright young students and researchers.

3. Interaction Technologies

Besides working on the study and implementation of a new generation of high performance intelligent robots and contributing to the definition of innovative design principles, methods and technologies, a goal of IIT will be to study, develop and test the technologies required to create real and virtual environments where humans and robots can work together with different degrees of coupling and integration.

The degree of integration will span from tele-operated devices to systems interfaced to the human nervous system aiming at what the Future and Emerging Technologies (FET) Unit of the European Commission calls Hybrid Bionic Systems. HBS are defined as systems that would support human capabilities such as perception of the environment, motion, interaction with other humans. Aiming at these systems would involve smooth integration of sophisticated robotic and information processing systems with human perception-action systems using bi-directional interfaces (invasive or non-invasive) with the human nervous system.

3.1 STUDY THE SENSE OF PRESENCE

3.1.1. Robots as remote bodies

A hot contemporary topic is that of “robots as remote bodies”, meant to be remotely acting systems linked to humans by bi-directional links and giving the human controller “the sense of being there”. In this framework it becomes essential not only to remotely transmit motor commands (like in basic teleoperation), but also to provide the human controller with realistic sensory feedback about the environment and the ongoing actions in all relevant modalities (i.e. proprioceptive, tactile, acoustic and visual sensations). This two-way link challenges engineers with high-impact technological problems, mainly related to the necessity to implement a very fast acting-reacting system with bidirectional flow of real-time sensory data but it also challenges neuroscientists investigating the sensorial requirements supporting exploratory behaviours and giving rise to subjective experience. The main challenge is not how to transmit sensory information in the form of external sensorial displays but how to transmit sensory information to the user giving rise to a sense of presence. To obtain human-humanoid “immersive” interfaces of this kind the operator must not be seen as a simple controller of a remote mechanical device but as the central part of a control loop where perceptions and sensations are bidirectionally exchanged.

3.1.2. Adaptive interface

Another important requirement is to study how to design humanoids that can adapt to (learn) the operator’s abilities and the operating environment. The motivation is twofold. First, each user has his/her own past experience and "style" and may have different motor, sensory and cognitive abilities (the robot must be able to adapt to the operator’s skills and abilities, not the reverse). Second, the robot must have the ability to adapt to (and exploit) the peculiarities of the environmental conditions in which it needs to operate (the robot must be able to adapt to the environmental conditions). This is the artificial counterpart of the incremental increase of human sensorimotor and cognitive performance occurring during familiarization with a new situation and/or environment. This mechanism of reciprocal

learning whereby the operator and the robot learn from each other is a fundamental requirement for future human-robot interactions.

Stressing the multidisciplinarity of the IIT environment, it will be possible, for example, to investigate a developmental approach by studying how sensorimotor coordination develops in humans and by designing systems that, equipped with a limited vocabulary of "innate actions" expressed through motor synergies, evolve more personalized, tailored actions and procedures. Even more challenging will be to explore this process in the case of non-anthropomorphic configurations.

3.1.3. Motor and cognitive controllers

Considering that a “human brain” is always present and in charge of the overall control of the robotic system, adaptation and control could be operating at the motor or at the cognitive level. At the motor level the brain controls the robot via direct commands issued in different ways. For example through non invasive controllers and exoskeltons, or through mioelectric activity recorded by surface electrodes, or through the efferent signals acquired at the peripheral nerve fibers, or, finally, through the activity of the motor cortex recorded by implanted electrodes. In this case the interface is a direct mapping between a motor signal of biological origin and a command to the robotics system, and the controller has to learn the way the user actually drives the controllable degrees of freedom and implement new synergies.

Adapting and controlling the system at the cognitive level requires that a significant part of the interface has the ability to understand the intentions of the operator and to transform them into appropriate motor commands and actions. In the case of grasping, this predictive ability can be obtained, for example, on the basis of the shape and the use of the object to be grasped (its affordances) and what the system has learned from being used before in the same situation (learning from past experience). This "cognitive controller" has to be able to adapt to some extent to the environment and its user, and should also serve the goal of monitoring what is going on (i.e. predicting what a hand should be doing).

As a final remark it is worth mentioning that this mechanism of reciprocal learning may require, in its initial stages, the use of ad-hoc devices serving the function of transitory scaffolding supporting the adaptation process. For example a passive exoskeleton on the left arm can be used to "teach" a prosthesis on the right arm how to perform a grasping action or a two-arm action.

3.2 INTERACTION SPACES

Research activities related to human-humanoid interaction can be structured according to the particular type of functional context where the interaction takes place. The main hypothesis is that the robotics system works in a real operational space (or workspace) while the human operator works in the so-called control space. In case the control and the operational spaces are co-located (they physically coincide), the human(s) and the humanoid(s) are in a so-called situation of simultaneous presence. On the other hand, whenever the operational and control space are different, the interaction is defined as teleoperation or, if the sensory feedback of the interface is almost complete, as telepresence.

3.2.1. Simultaneous presence

In this functional context human(s) and humanoid(s) are operating together on real objects, they share the same workspace. This situation generates a number of interesting issues from safety (the operator and the robot are in close contact) to the level of intelligence of the artificial system. Performing a task in a condition of simultaneous presence requires a certain degree of autonomy of the robotic system: an effective cooperation implies that the robotic system is not only sensorially and motorically skilled, but also has the ability to interpret commands issued by the human operator. Ultimately one would like to have a co-worker with a certain level of understanding to be able to predict the next operational phase. It is worth stressing here the very tight link between these aspects of the interaction and some of the key research areas of investigation in the neurosciences and in cognitive robotics.

Going back to simultaneous presence, different interaction paradigms between humans and robots can be defined according to the degree of autonomy and versatility of the robot and the kind of contact, direct or mediated, between robot's parts and humans. Possible paradigms are related to interactions between:

- Humans and humanoids cooperating to jointly carry out manipulation tasks. For example, during assembly operations or maintenance the humanoid may cooperate by taking care of the heavy aspects of the task or by providing the human with the correct tool.
- The robot is in contact with the human and supports/corrects her/his movements during rehabilitation. The most classical example of this kind of robots is the so-called *manipolandum* which, in its simplest implementation, consists of a two degrees of freedom robot arm equipped with torque sensors at each joint. This kind of device has been invented and used to study motor learning in humans and has offered the possibility of directly transferring results of basic research studies to clinical use.
- Humans and body extenders such as exoskeletons used to augment the motor capabilities of humans while exploiting dexterity and precision of motor control in *out-of-scale* actions such as lifting heavy objects or manipulating micro-objects.
- In building interactions of this kind, besides the obvious electronic, mechanical and computational components, specific research activities are foreseen which, as said before, are in strict relationship with the neuroscientific and cognitive robotics aspects detailed in other parts of this document. Among them the most relevant are:
- Development of exoskeleton systems or wearable robots and robots operating in contact with humans.
- Study and implementation of interfaces allowing the understanding of the emotional states and of the motor reactions of the human operator during the interaction, and, conversely of robots able to express emotional states through human-like behaviour (the so-called Kansei robots)
- Study and implementation of control algorithms and physical interfaces for task execution during human-robot cooperation (the so-called Cobots);
- Study and implementation of control algorithms and physical interfaces to acquire and transfer knowledge between humans and robots during task execution (the concept of reciprocal learning described before)

The results of these research activities are expected to have a strong impact on the targeted aspects of IIT research also in relation to the design and development of interactive and immersive environments.

3.2.2. Telepresence

In a telepresence functional context the human operator controls the execution of a task that is being performed remotely by a robot. Telepresence conditions are obtained through interfaces between humans and robots conveying not only rich sensory information from the robot itself but also very realistic data about the environment. Systems of this kind are said to give the operator the "sense of being there" (or the sense of presence). Also in this case a strong link is evident with the study of human perception on one side and the implementation of the humanoid sensorimotor system on the other.

Depending upon the level of autonomy left to the robotic system, different kinds of action transfer can be adopted to drive task execution in the remote environment:

- Teleoperation. In this condition the sensorial feedback from the remote site to the human operator is limited to visual feedback (direct or indirect) with the addition, at most, of force feedback to support manipulative actions. This is the simplest level of interaction and is usually adopted because of its low cost and the limited bandwidth required for the sensorial links.
- Telerobotics. This term usually defines an interaction at the level of task execution. In this case the operator controls the remote robot by giving task-level commands such as "go ahead two meters" or "grasp the hammer". Also in this case the research topics are strictly related to other basic as well as targeted research themes of IIT. However, the most relevant and specific to telepresence are:
    a) Development of interfaces integrating different sensory modalities (tactile, visual, kinesthetic, acoustic, etc.). This is far from trivial if, as in this case, the goal is not simply to "display" information to the human operator but to give her/him the sense of being there. It may involve technological issues not only related to how to measure and present sensorial information at a sufficient level of spatial and temporal resolution but also how to compensate/adjust for the intrinsic delays between different sensory modalities and between afferent and efferent signals.
    b) Development of whole-body interface systems replicating inertial effects and, at the same time, simulating the locomotion of the remote system (Whole Body Motion Haptic Interface). This is again particularly relevant for robots operating in largely unpredictable situations (such as moving on rough terrain) or having to react to unpredictable forces acting on the entire body (strong wind, currents, or simply running up/down steep slopes).
    c) Development of systems recording body part movements to directly drive a robot link (body tracking). Some of these devices are already available but, as far as telepresence is concerned, there is still a wide range of opportunities for both technological development and scientific investigation.
    d) Development of algorithms to translate high level commands into sequences of elementary actions and complex behaviours.

e) Development of control spaces conceived as immersive environments in which the human operator can directly foresee the behaviour of teleoperated robots by exploiting a co-located paradigm of interaction with a synthetic model of a humanoid robot.

f) Development of training procedures of humanoid robots built on coexistent digital and real workspaces.

3.3 NEURAL INTERFACES AND SMART ELECTRODES

A key aspect of interaction technologies is to obtain stable, long-term recordings of the activity of neurons of the central nervous system (CNS) in awake, behaving subjects. This technological challenge is shared by engineers studying how to interface prosthetic devices to the human nervous system and by neuroscientists investigating how to correlate defined sensory-motor or information-processing tasks with the functional activity of neurons. In the past few years it has become evident that single neuron recording is not sufficient to describe higher brain functions (e.g. perception, cognition, learning and memory) resulting from intricate, dynamic interactions in very large cortical networks. Therefore the investigation of these functions and the control of directly interfaced devices both in normal and disease states requires the ability to record the activity of neuronal populations with a high spatial and temporal resolution (at the micron/millisecond level).

Traditional extra- or intra-cellular (single- or multi-unit) electrophysiological recordings, whilst providing much valuable information, suffer from a number of limitations. One general goal at IIT is to explore novel technologies to enable the recording of neural activity from the CNS over extended periods of time, with minimal invasiveness and ultimately in a highly parallel manner. IIT efforts will be directed at the study and implementation of brain interfaces on the basis of the results of biological experiments performed in conjunction and interplay with neuro-physiology (particularly those working with non-human primates) and neuro-surgery centers (those with experience in electrophysiological monitoring of awake patients during surgery). The field of application of brain interfaces will obviously depend on their invasivity. If the adoption of penetrating multielectrodes or smart-electrode interfaces is acceptable to give motion to tetraplegic patients, the study of surface electrodes, possibly carrying out sophisticated electronic on-board processing, will extend our knowledge of the brain correlates of normal behaviour.

3.3.1. Brain-machine interface

Recent developments in the technology of electronic implants and in the understanding of neural functions has enabled the construction of interfaces that work by exchanging information between the nervous system and artificial devices (brain-machine interface). Preliminary results obtained in the United States by various researchers have demonstrated the feasibility of this approach and its great promises. The efficiency of these systems is however directly related to the number of single neurons recorded at the same time and has led to the attempt to build three-dimensional arrays of recording electrodes, in some cases integrated with signal-processing electronics. These new devices do not blindly stimulate regions of the brain, but they sense their neurophysiological surroundings and utilize this information to react on demand. They will pave the way to completely new and efficient closed-loop control and therapy systems for both intrinsically acting (back to the brain source)

and extrinsically acting working (robotic output) devices (bi-directionally neuroprostheses). The concept of bi-directionality is fundamental. It is known from amputee patients, that the absence of feedback signals represents one of the major problems to achieve the sense-of-self of the prosthetic limb.

A further effort in electrode design will be aimed at improving the technology of surface, non-invasive electrodes. It has been shown, indeed, that mathematical processing of EEG signals (through fuzzy-logic and laplacian routines) has largely improved the signal-to-noise ratio of surface recordings. This might lead, in principle, to a new generation of surface (or, at least, epidural) electrodes allowing to extract on-line information from the brain.

3.3.2. Smart electrodes and peripheral connectors

We define as "smart" electrodes for extracellular recording or sensors capable, upon injection into, or surface application onto CNS tissue, to dock to, or near, a specific cell target forming a stable electrode/tissue junction. This will be achieved by functionalizing the distal end of the electrode with a targeting/docking molecule (e.g. recombinant scFv, scaffolded peptide, aptamer) selected/designed to bind with high affinity to a specific component of the extracellular matrix or of the neuronal cell surface (e.g. a neuroreceptor). The latter option would in principle allow the selective targeting of specific cell types expressing a unique marker of choice.

An additional field of investigation is represented by peripheral electrodes connected to traumatically (or surgically) sectioned nerves, as in the case of amputees. These electrodes could in principle work bi-directionally, receiving motor commands from the nerve ending, in order to drive external actuators, and sending gross proprioceptive and tactile information to the brain trough the sensory axons of the same nerve.

3.3.3. Neural probes for *in-vitro* applications

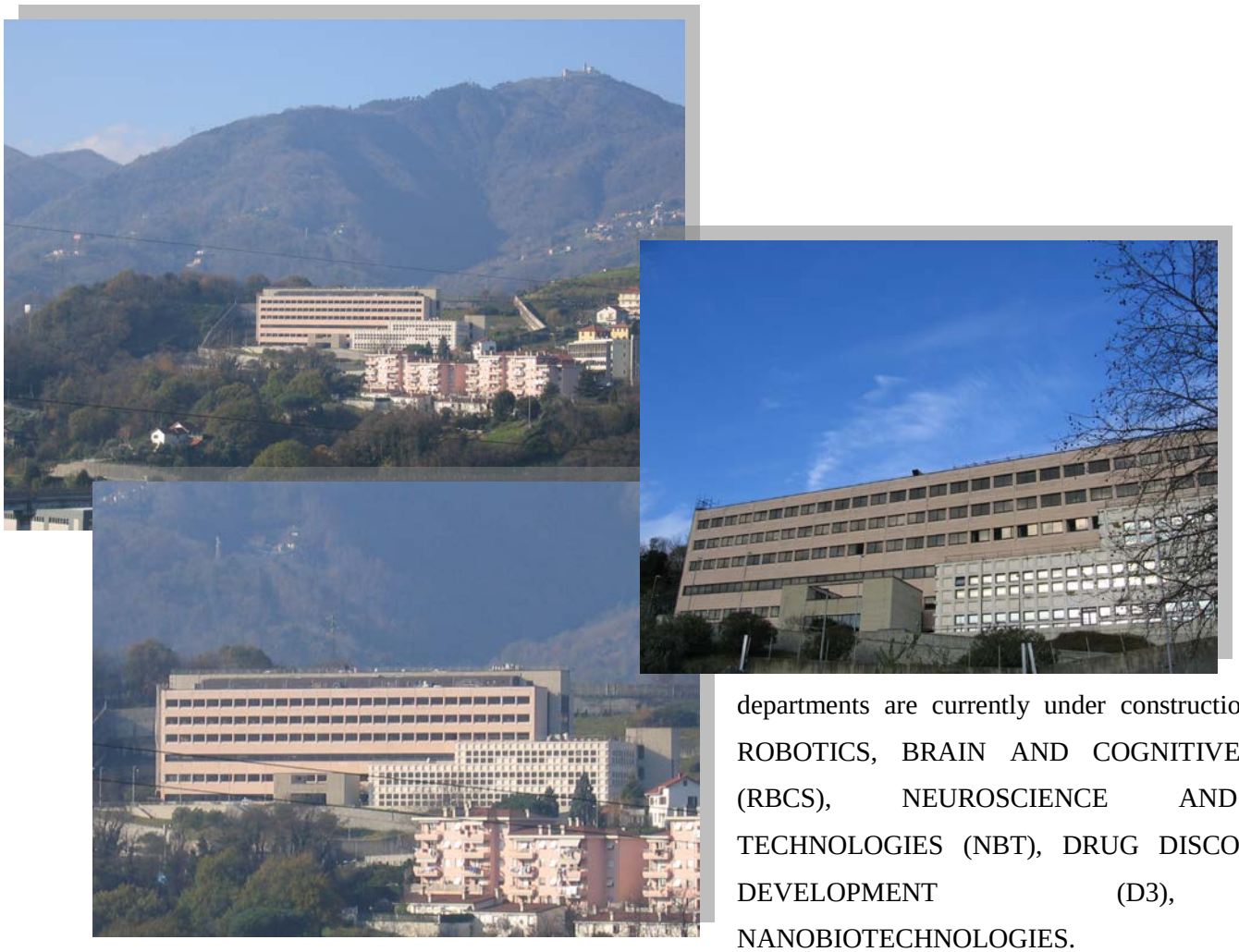
Using planar or three dimensional microtransducer arrays (MTAs) to which neuronal cultures or brain slices are acutely or chronically coupled, we can monitor their electrophysiological activity (i.e. networks dynamics) on a short-/long-term basis. This approach has opened new opportunities for studying the spatio temporal dynamics of a neuronal system in living condition, greatly contributing to the neuroscientific and biomedical field but with far reaching implications also for the information technology field. The state of the art of MTAs is constituted by 60-100 planar or 3D electrode arrays on a silicon or glass (quartz) substrate and with micromachined structures for network patterning. Many techniques are available for building such devices and commercially available systems have promoted the widespread use of these tools. Micro and nanotechnology advances would further improve these studies. An increase number of electrodes is needed (multi-addressable, large-modular micro-nano electrode arrays) with the possibility of scaling down to the sub-cellular levels (multi-patch, high-density devices). Moreover, a detailed comprehension of the coding capabilities of a natural system coupled to artificial devices depends on the possibility of electrical/chemical interactions at the cellular and sub-cellular level. We are thinking of nano-actuators capable of delivering quanta of neurotransmitters (mimicking the vesicular release) or microbiosensors that can detect small amount of neurotransmitters and/or neuromodulators.

ANNEX 3

THE CENTRAL RESEARCH LABORATORY AT GENOVA-MOREGO

THE CENTRAL RESEARCH LABORATORY AT GENOVA-MOREGO

The IIT Central Research Lab in Morego is designed to be one of the largest multidisciplinary research laboratories in the world. The location of the institute is close to Genova airport, about 15 minutes by car from the city centre. The building is a facility of about 30000 sqm (including external space) distributed over 5 floors and the underground level, fully equipped with power stations, air conditioning, continuity groups, services, etc. The building host a few research departments directed by internationally acknowledged scientists. The central research lab provide a common infrastructure consisting of dozen of advanced research laboratories, mechanical and electronic facilities, meeting and seminar rooms and restaurant. Up to about 400 people are expected to operate in the central research lab in the next three years.



Four departments are currently under construction at the IIT: ROBOTICS, BRAIN AND COGNITIVE SCIENCES (RBCS), NEUROSCIENCE AND BRAIN TECHNOLOGIES (NBT), DRUG DISCOVERY AND DEVELOPMENT (D3), and NANOBIOTECHNOLOGIES.

The main technological goal of the **ROBOTICS, BRAIN AND COGNITIVE SCIENCES (RBCS)** department is to move ahead from traditional humanoid robots with mechanical hands and legs (hard-bodied systems) toward next generation hybrid systems realized with soft materials (soft-bodied systems) and with cognitive abilities allowing the interaction with

humans in a natural way. This goal will be pursued through multidisciplinary research spanning from mechatronics, to advanced material, to the study of how humans learn, perceive, and act in a natural environment. The RBCS is structured into

five main labs:

Humanoid platform, directed by Prof. Giulio Sandini (formerly at the University of Genova, Italy) and Prof. Darwin Caldwell (formerly at the University of Salford, UK), designing new generation, epigenetic and interacting platforms.

Human behaviour laboratory, directed by Prof. Giulio Sandini, exploring the brain mechanisms at the basis of motor behavior, learning and sensorimotor integration. New generation imaging systems will be developed for this purpose.

Interaction Laboratory, directed by Prof. Darwin Caldwell focusing on the study, development and testing of technologies required to create real, virtual or augmented human-robot interaction environments such as whole body haptic interfaces, exoskeletons and wearable systems.

Biotechnology laboratory, directed by Prof. G. Sandini, studying new technologies to produce brain probes and integrate artificial materials with the human body.

Targeted Robotics directed by Prof. Jean-Guy Fontaine (formerly at the University of Paris, France) and Prof. Darwin Caldwell fostering the development of industrially and socially relevant technologies through joint projects and labs with industries in all research areas of the department and addressing specifically the area of micro and macro teleoperation, telepresence and telexistence (tele 3x).

The five branches functionally integrate to form a unique interdisciplinary research infrastructure putting together a complete hardware-software strategy for humanoid robotics with the study of the brain mechanisms underlying motor control, learning and cognition. Brain-machine interfaces and advanced neuro-prosthetics will be investigated by interactively merging engineering and neurophysiology competencies, in synergy with the NBT.

The **NEUROSCIENCE AND BRAIN TECHNOLOGIES (NBT)** department, led by Prof. Fabio Benfenati (formerly at the University of Genova, Italy), has the objective to apply new technologies to the study of the central nervous system. The mission of the NBT is to elucidate the genetic and epigenetic factors at the basis of synaptic transmission and plasticity, the learning and adaptation strategies of the nervous tissue and the relationships between neural molecules and information coding and processing in the brain. The following research themes will be investigated:

at various levels of brain complexity from individual synapses to neural networks to the live animal by using advanced techniques coupling mouse genetics, patch-clamp/multi-electrode recordings and functional imaging of live neurons;

in experimental models of brain diseases (including epilepsy, addiction and neurodegenerative diseases) in synergy with the D3 department;

in bio-hybrid systems by generating neuro-electronic and bidirectional neuro-robotic interfaces. These neuron-to-chip

systems will allow the study of the basic properties of simplified neuronal networks and the implementation of new neuron-based biosensors and neuroprosthetic interfaces. These topics will be investigated by interactively merging nanotechnology, engineering and neurophysiology competencies, in synergy with the NANOBIO and RBCS departments.

The laboratories and research infrastructures of the NBT are currently under construction.

The Department of **DRUG DISCOVERY AND DEVELOPMENT (D3)**, directed by Prof. Daniele Piomelli (from the University of California, Irvine, USA), was launched on January 2007. Its mission is to discover and develop innovative medicines in the areas of brain disorders and inflammatory diseases. The D3 will accomplish this mission by creating an internal research engine, constituted by a multidisciplinary group of talented and creative scientists, and fostering the development of public-private partnerships aimed at accelerating the drug discovery process. It will closely collaborate with other Departments within the IIT in the areas of neuropsychiatric disorders, intelligent nanomaterial-based drug delivery and drug discovery technology. The research laboratories of the D3 are currently under construction.

State of the art facilities will provide hardware and characterization support to the Lab's scientific activities.

The hardware facilities include:

- Mechanical workshop with advanced design and manufacturing capacities
- Electronic workshop with advanced design and fabrication capacities
- Animal facility with pharma test area, surgical area, in-vivo experiment area, etc.

The **NANOBIOTECHNOLOGIES FACILITIES** department will provide:

- Clean room with micro- and nano-fabrication and material processing
- Transmission electron microscopy facility
- Scanning electron microscopy facility
- Scanning probe (AFM/STM) microscopy facility
- Optical spectroscopy facility with fs, ps, ns and cw spectroscopy
- Polymer laboratory for composite systems
- Basic chemistry and characterization laboratory
- Colloidal Chemistry laboratory for synthesis of nanoparticles
- Super computer facility

FLOOR MAPS

- **Undergroud floor**

  Nanobio Labs (Clean room, Microscopy Lab, Optical Labs) and Animal Facility

- **Ground floor**

  Seminar room, Restaurant, Administration, Offices

- **First floor:**

  Administration, 3D Labs, Offices

- **Second floor:**

  NBT Labs, Offices

- **Third floor:**

  RBCS Labs, Offices

- **Fourth floor:**

  RBCS labs, Offices

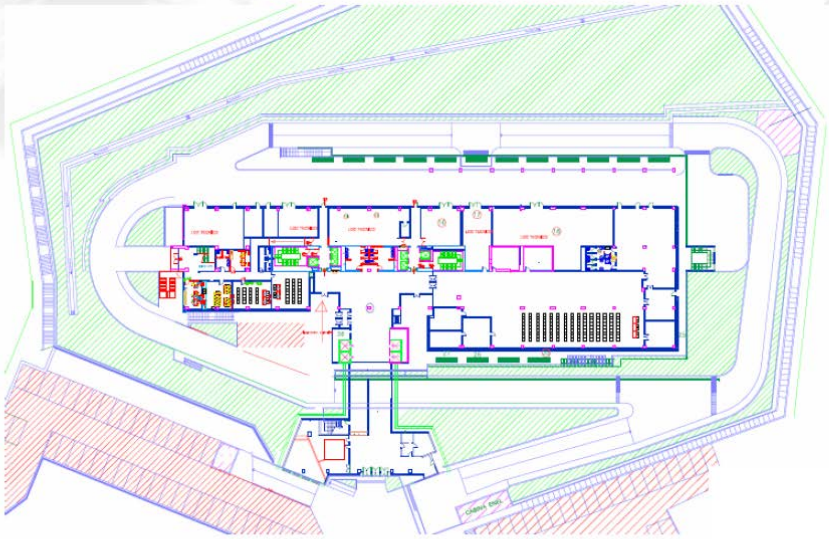
- **Fifth floor:**

  Nanobio Labs (Chemistry, Biology)

Level 0

Fondazione Istituto Italiano di Tecnologia iit

Administration, Meeting rooms, Training rooms, Facilities

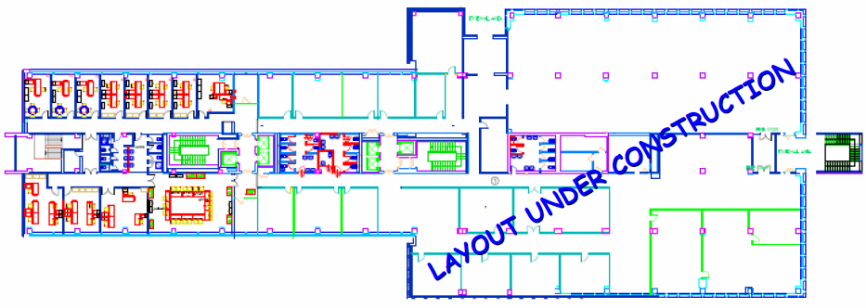


IIT Administration

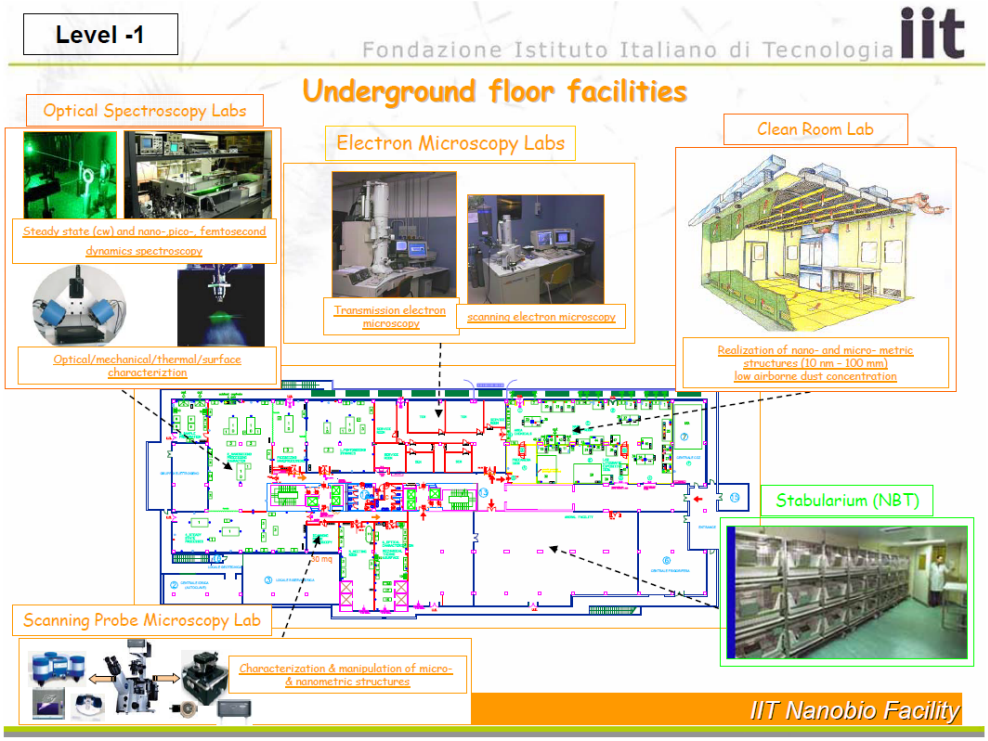
Level 1

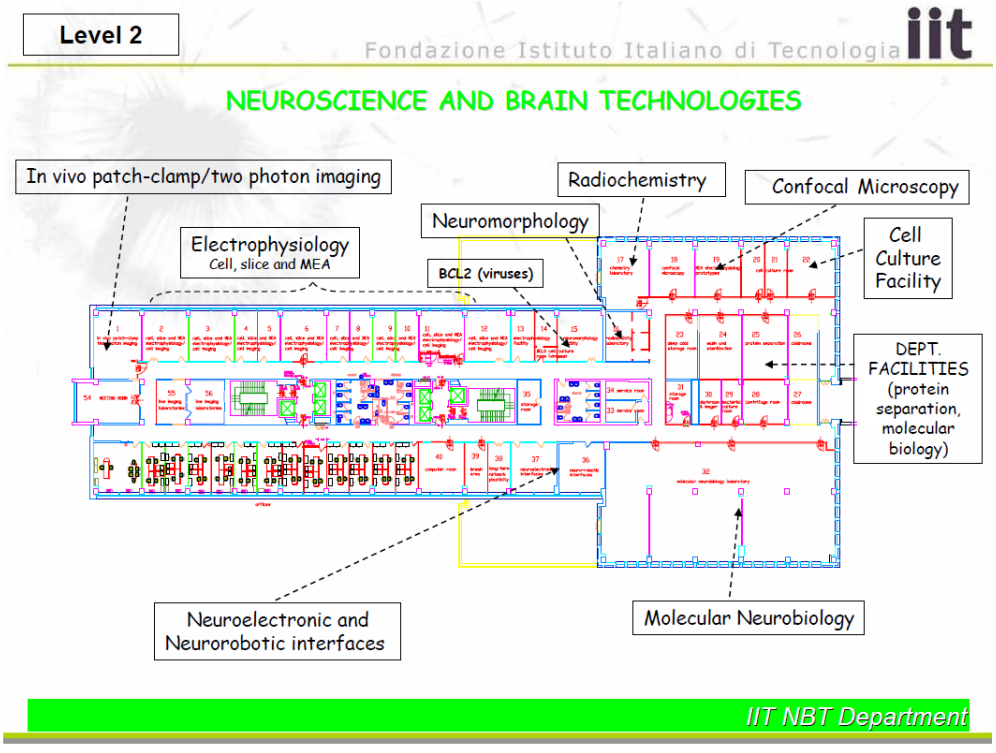
Fondazione Istituto Italiano di Tecnologia iit

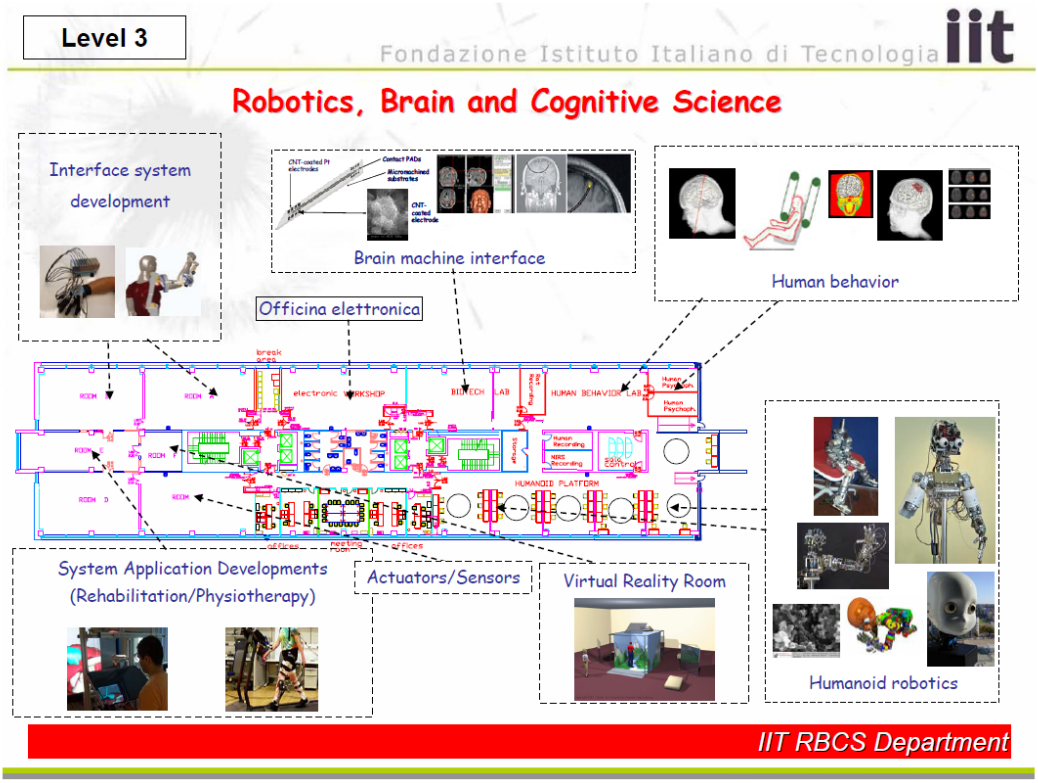
Administration Offices, DRUG DISCOVERY AND DEVELOPMENT

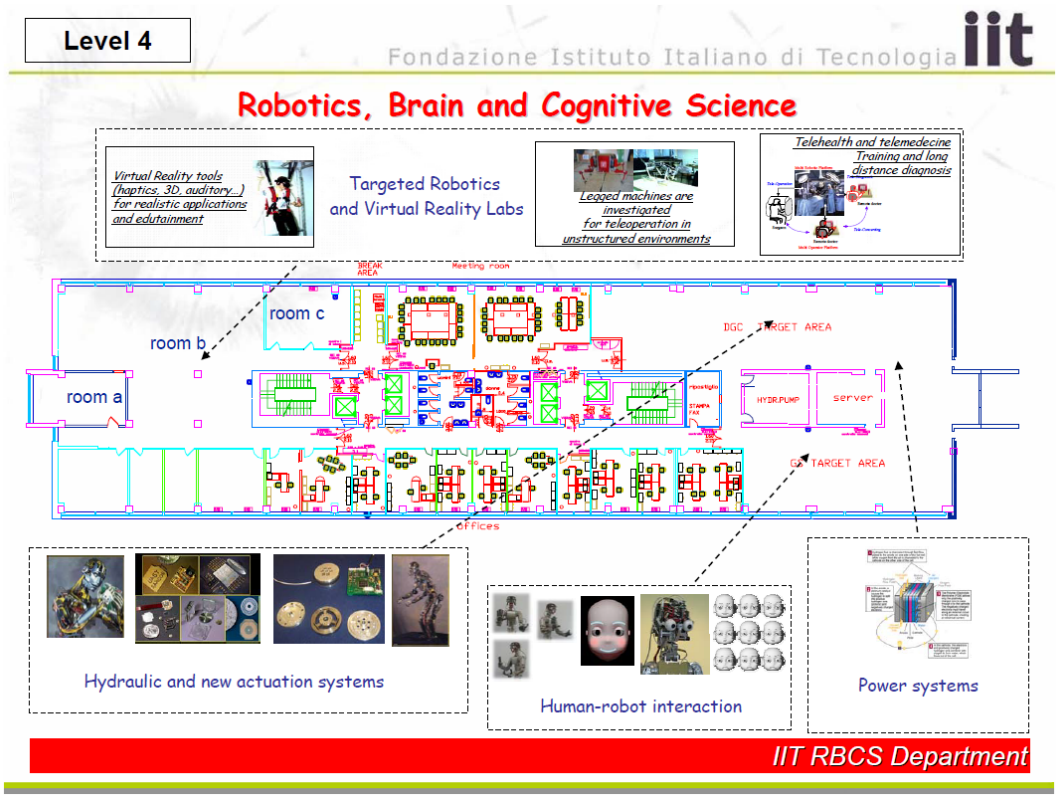


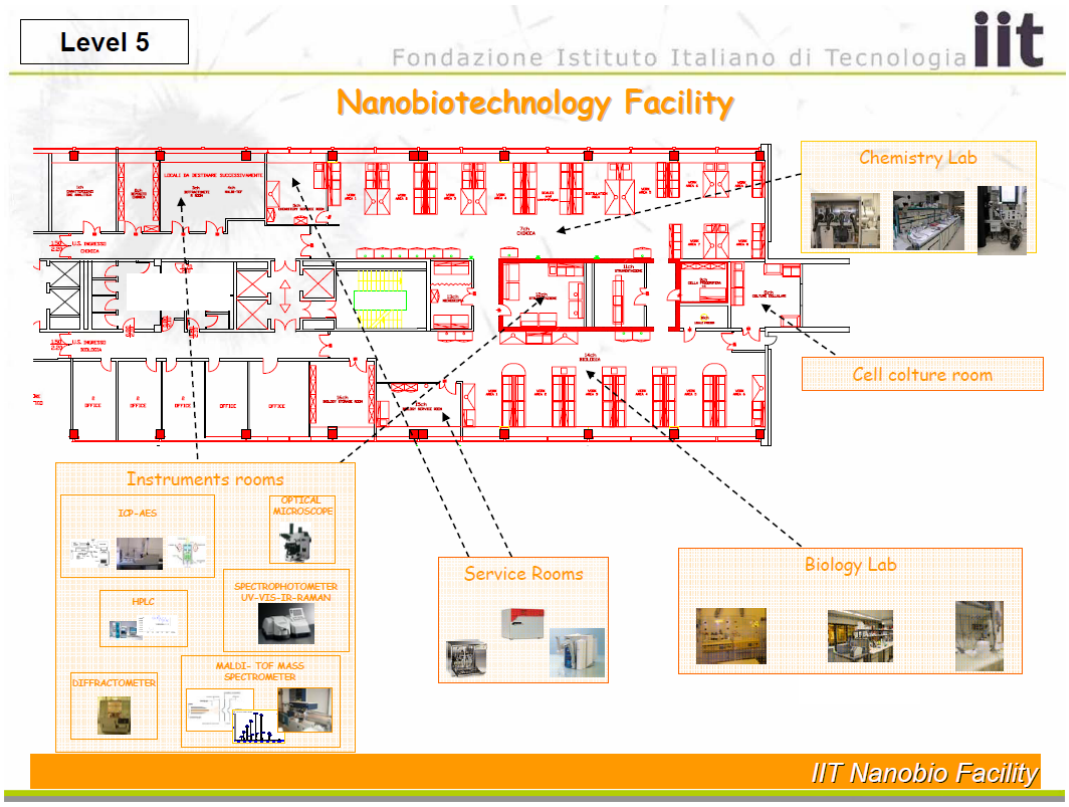
IIT D3 Department


Level -1
Fondazione Istituto Italiano di Tecnologia
Underground floor facilities
Optical Spectroscopy Labs
Steady state (cw) and nano-,pico-, femtosecond dynamics spectroscopy
Optical/mechanical/thermal/surface characteriztion
Electron Microscopy Labs
Transmission electron microscopy
scanning electron microscopy
Clean Room Lab
Realization of nano- and micro- metric structures (10 nm - 100 mm)
low airborne dust concentration
Stabularium (NBT)
Scanning Probe Microscopy Lab
Characterization & manipulation of micro- & nanometric structures
IIT Nanobio Facility


Level 2
Fondazione Istituto Italiano di Tecnologia
NEUROSCIENCE AND BRAIN TECHNOLOGIES
In vivo patch-clamp/two photon imaging
Radiochemistry
Confocal Microscopy
Neuromorphology
Electrophysiology
Cell, slice and MEA
BCL2 (viruses)
Cell Culture Facility
DEPT. FACILITIES (protein separation, molecular biology)
Neuroelectronic and Neurorobotic interfaces
Molecular Neurobiology
IIT NBT Department

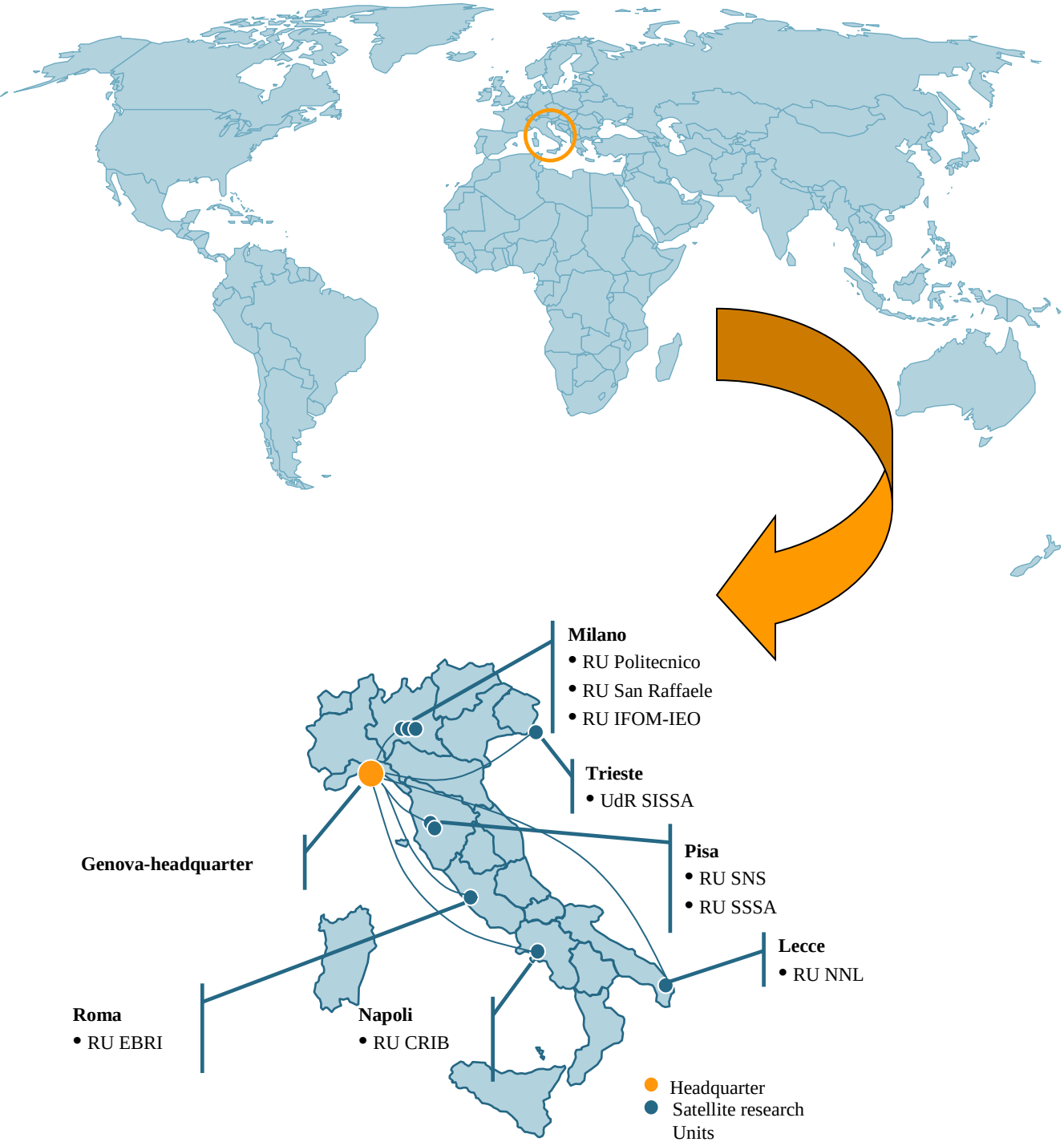

Level 3
Fondazione Istituto Italiano di Tecnologia
iit
Robotics, Brain and Cognitive Science
Interface system development
Brain machine interface
Human behavior
Officina elettronica
System Application Developments (Rehabilitation/Physiotherapy)
Actuators/Sensors
Virtual Reality Room
Humanoid robotics
IIT RBCS Department


Level 4
Fondazione Istituto Italiano di Tecnologia
iit
Robotics, Brain and Cognitive Science
Virtual Reality tools (haptics, 3D, auditory...) for realistic applications and edutainment
Targeted Robotics and Virtual Reality Labs
Legged machines are investigated for teleoperation in unstructured environments
Telehealth and telemedecine
Training and long distance diagnosis
room a
room b
room c
Meeting room
BREAK AREA
DGC TARGET AREA
HYDR.PUMP
server
Offices
Hydraulic and new actuation systems
Human-robot interaction
Power systems
IIT RBCS Department


Level 5
Fondazione Istituto Italiano di Tecnologia iit
Nanobiotechnology Facility
Chemistry Lab
Cell colture room
Instruments rooms
ICP-AES
OPTICAL MICROSCOPE
SPECTROPHOTOMETER UV-VIS-IR-RAMAN
HPLC
DIFFRACTOMETER
MALDI- TOF MASS SPECTROMETER
Service Rooms
Biology Lab
IIT Nanobio Facility

ANNEX 4

THE MULTIDISCIPLINARY RESEARCH NETWORK OF IIT


Milano
• RU Politecnico
• RU San Raffaele
• RU IFOM-IEO
Trieste
• UdR SISSA
Genova-headquarter
Pisa
• RU SNS
• RU SSSA
Lecce
• RU NNL
Roma
• RU EBRI
Napoli
• RU CRIB
Headquarter
Satellite research Units

www.polimi.it

Research Unit IIT – Politecnico di Milano

Scientific coordinator of the RU : Prof. Rinaldo Cubeddu

SCIENTIFIC OBJECTIVES

NanoBiotechnology

- ***WP1 Bioelectronics and biophotonic interfaces between cells and artificial systems.***
  P.I. Prof. A. Lacaita, Dipartimento di Elettronica e Informazione
- ***WP2 Functional surfaces***
  P.I. Prof. A. Cigada, Dipartimento di Chimica, Ingegneria Chimica e Materiali
- ***WP3 Organic materials for artificial bio-systems***
  P.I. Prof. G. Lanzani, Dipartimento di Fisica
- ***WP4 Molecular imaging***
  P.I. Prof. C.E. Bottani, Dipartimento di Ingegneria Nucleare
- ***WP5 Models and methods for local drug delivery from nano/micro structured materials***
  P.I. Prof. A. Quarteroni, Dipartimento di Matematica

Rehabilitation

- ***WP1 Multi source neurophysiological information processing for innovative and personalized rehabilitation protocols***
  P.I. Prof. S. Cerutti, Dipartimento di Bioingegneria
- ***WP2 Human machine interface for recovery of lost functions***
  P.I. Prof. A. Pedotti, Dipartimento di Bioingegneria

- ***WP3 Robotic companion exploiting affective feedback for modeling emotional state of the patient and adapting the rehabilitation treatment***

  P.I. Prof. A. Bonarini, Dipartimento di Elettronica e Informazione

Being a Technical University, Politecnico di Milano has a large variety of competences in both basic and engineering sciences. This allows the integration among different disciplines which is mandatory for the development of interdisciplinary research fields.

The research activity to be carried out at Politecnico di Milano is structured in two main research lines. The former refers to NanoBiotechnology and the latter to Rehabilitation. The whole project is structured in eight Work Packages, five of them belonging to NanoBiotechnology and three to Rehabilitation.

WORKPACKAGES

NANOBIOTECHNOLOGY

1. ***WP1 - Bioelectronics and biophotonic interfaces between cells and artificial systems***

   P.I.: Prof. A. Lacaita

   Cooperating units: Department of Electronics and Information (DEI), Department of Bioengineering (BIO), Department of Physics (FIS)

5 year planning

The development of cell-based biosensors for pharmaceutical, environmental, toxicological, scientific and other applications has received growing interest in recent years. These devices utilize in vitro cells for the detection, and convert the cellular signal into an electronic one that can be processed and analyzed. Traditionally, electrogenic cells have been preferred, as they produce an electrical signal that can be directly measured, and microelectrode (typically in the tens of micrometers range each) arrays have been largely adopted for sensing the action potentials. Indeed, this technology has been commercially exploited for drug profiling in the fall of 2004 by Multichannel Systems GmbH, Germany.

The current project aims at the development of improved tools for interfacing cells and chips along two distinct paths: microelectronics and integrated optics. The former will take advantage of the integration capabilities of modern microelectronics to develop a compact system integrating a high-resolution array and the processing circuitry, in order to build a truly portable, low-cost device that could be adopted in routine analysis. The latter targets the development of integrated photonic devices, from the caged neurotransmitters and from the voltage-sensitive dyes, in order to realize a neuro chemo-photonic communication between biological and artificial parts. These technologies allow the microscale stimulation input to meet with the net macroscale output.

Furthermore, in vivo experiments will be conducted, with the aim of directly recording and processing the

brain signal for the development of a so-called brain-machine interface (BMI), i.e., an instrument able to translate the brain activity into defined tasks, for example controlling mechanical devices to restore lost ability. In more details, the activity will be carried out with the characterization and development of both microelectronic devices and integrated photonic devices.

For the microelectronic devices the following research lines will be developed: a) Characterization of electrodes realized in standard CMOS technology and their impedance in contact with the electrolytes; evaluation of possible non-lithographic methods for improved electrical contact and cell positioning; b) Design of integrated amplifiers for cell signal detection; extension to arrays of microelectrodes; possible integration of the A/D converter; realization and test of the integrated circuits; c) Realization of the setup for in-vivo experiments for BMI; analysis of the correlation with movements; development of integrated circuits for compact BMI.

The integrated photonic devices will be developed according to the following lines: a) Characterization of caged neurotransmitters in terms of quantum efficiency and neuronal response depending on the optical stimuli parameters (amplitude, pulse width and repetition rate); characterization of voltage-sensitive dyes in terms of spectral response (intensity and shifting) and life-time (FLIM, with the facility of the Physics Department); test of methods for protein patterning. b) Development of chips for field stimulation, of systems for optical stimulation by integrated waveguides and of systems for fluorescence (High-speed camera) and decay-time (FLIM) recording. c) Test of the devices for stimulation and imaging and on the cellular differentiation induced by field stimulation; application of photonic technologies for BMI and hybrid information processing.

Implementation: first 24 months

In the first 24 months, the activity related to microelectronics will cover the characterization of the chip electrodes and the test of different coating procedures in order to achieve a good biocompatibility. Integrated amplifiers in CMOS technology will also be designed and adopted for neuronal firing rate measurements. The setup for in-vivo experiments for the BMI will be also realized and data will be collected.

From the biophotonic standpoint, the quantum efficiency and neuronal response of two neurotransmitters will be evaluated, and voltage-sensitive dyes will be characterized with different techniques. The optimal parameters of the electrode array will be defined, and a prototype will be built.

Objectives

1. Development of integrated CMOS amplifiers and small arrays for recording of neuronal signals.
2. Analysis of correlation between neuronal data collected in-vivo and simple movements for BMI.
3. Full characterization of caged glutamate and VSDs.
4. Design, development and realization of a chip prototype for field stimulation.

Tasks

Task 1 - *Microelectronic Devices.*

This task will be performed according to the following research lines:

- Characterization of electrodes realized in CMOS technology; test of coating procedures for standard electrodes;
- Design of CMOS low-power integrated amplifiers based on linear feedback and on switched-capacitors; realization and performance evaluation; applications to neuronal firing rate measurements.
- Realization of the setup for in-vivo experiments for BMI; collection of experimental data; analysis of the correlations with simple movements; design of integrated circuits for firing rate processing.

Partner DEI.

Task 2 - Integrated Photonic Devices.

This task will be performed according to the following research lines:

- Characterization of quantum efficiency and neuronal response of two different forms of caged glutamate, MNI-Caged and CNB-Caged, using a UV Laser Diode (373nm, 10mW) coupled to a single-mode optical fiber that is moved by a micromanipulator. Quantum efficiency will be evaluated using a fluorescence probe that binds the glutamate where it is caged by the caging group. Also, neuronal response will be evaluated using microelectrodes that will record the evoked responses of neurons excited with the laser diode in a medium containing caged glutamate;
- Characterization of voltage-sensitive dyes by spectrophotometry, FLIM and field stimulation of neuronal cultures;
- Definition of the optimal geometric parameters and the optimal substrates of the electrode array and the best parameters for the stimulation (current/voltage amplitude, pulse width, repetition rate);
- Realization of a prototype using photolithographic and galvanic methods.

Partners BIO and FIS.

Milestones (12 months)

- Definition of the optimum architecture for the integrated amplifier; preliminary screening of coating possibilities
- Definition of field stimulator parameters

Deliverables (24 month)

- Performance of the integrated system for cellular signal readout and results of the data analysis for development of the BMI.
- Characterization of the caged compound neuronal response and of the voltage-sensitive dyes.

2. WP2 - Functional surfaces

P.I.: Prof. Alberto Cigada

Cooperating Units: Department of Chemistry, Chemical and Materials Engineering (CMIC), Department of Nuclear Engineering (DIN), Department of Bioengineering (BIO)

5 year planning

The Work Package "Functional Surfaces" aims to develop and verify new strategies for the production of micro- and nanostructured surfaces with tailored functional properties, to use for biosensor devices. Different chemical, electrochemical and physical modification techniques will be developed, applied and studied to produce surfaces with specific features (morphology, surface roughness, porosity, chemical composition, functionalization capability). The developed surfaces will be used for drug and bio-molecules immobilization (e.g. oligonucleotides, DNA, protein and oligosaccharides), in order to stimulate specific cell responses. The development of such engineered surfaces, together with the ability to create specific patterns, will be relevant for the fabrication of a new generation of biosensors, to be used for *in vitro* and *in vivo* analysis, gene or drug delivery systems, scaffolds and for long term implantable devices.

Among other biomaterials, titanium will be preferentially considered as substrate for modification treatments, thanks to the possibility to conjugate its excellent biocompatibility to different surface modification and functionalization possibilities.

The CMIC and BIO groups will study and test chemical and physical modification techniques on titanium substrate, in order to design new surfaces, which morphology and chemistry will be particularly suitable to trigger specific positive interactions with biologic systems (cells and living tissues).

CMIC group will use low voltage anodization and Anodic Spark Deposition (ASD) techniques to modify the morphology and composition of titanium, and to properly dope the surface oxide layer with different ions and chemical functional groups. Such electrochemical techniques, performed in various solutions and conditions, followed by or associated to other physical and chemical treatments, are intended to achieve a strong and permanent surface anchoring of selected bio-molecules. The binding will be obtained by proper bridge/spacer molecules interposition, or, preferably, directly on the titanium surface. The strong long lasting grafting of the bio-molecules will be especially suitable for the manufacturing of permanently implanted biosensors.

The DIN group research will be committed to the development of new patterning strategies. Surface deposition techniques, such as pulsed laser deposition (PLD) and physical vapour deposition (PVD), will be considered to create nanostructured surfaces with different chemical composition and tailored properties. The capability of the PLD technique to produce a wide range of surface morphologies and structures and finely predictable chemical composition, is well known. The DIN group research will focus especially on the development of nanostructured surfaces of TiO_2, hydroxyapatite etc., for the enhancement of cell-material interactions. The functionalization and immobilization of selected bio-molecule on smooth surfaces (e.g. Au), will be considered as well.

The development of such innovative surface pattering techniques will open the possibility to produce nanostructured surfaces, capable to operate the modification of the surface properties at a sub-micrometric level, in specific single locations.

Implementation: first 24 months

objectives

Evaluate and test the best surface modification technologies in order to immobilize drugs and biomolecules on the surface of titanium and gold, to use for long term implantable biosensors.

Tasks

Task 1 - *Surface modification of titanium for bio-molecular functionalization.*

Modification processes involving electrochemical techniques will be applied to titanium to modify its surface properties. Low voltage anodization and Anodic Spark Deposition treatments will be developed and applied. Micro-structural, chemical and in vitro studies will be used to assess and to verify the effectiveness of the developed modification techniques, in order to evocate specific biological response.

New titanium functionalized surfaces, perhaps enriched by –OH groups, will be developed and tested.

The immobilization on the titanium surface of different molecules, such as oligonucleotides, oligosaccharides, proteins, will be verified and tested. The possibility to bind drugs such as antibiotics, potentially useful with implantable devices, will be considered as well.

The research will aim to achieve a long term immobilization of the bio-molecules on titanium surface.

Partners CMIC and BIO.

Task 2 - *Synthesis of nanostructured and patterned surfaces for biological functionalization and cell growth.*

PLD, thermal evaporation and sputtering deposition will be used for the synthesis of nanostructured surfaces (e.g. TiO_2, Hydroxyapatite). The developed materials will be studied for cell biocompatibility and interactions. Smooth surfaces (e.g. Au) will be functionalized for bio-molecule immobilization. Chemical and biological functionalization will be operated and verified. New patterning strategies will be explored in order to achieve modifications at a micrometer and submicrometer level, useful to manufacture micro biosensors devices. Several patterning routes will be considered: micro masking techniques, such as micro-grids and nanostencils, nanoparticle coating by self-assembled layers, plasma ablation techniques and post-deposition nanolithography techniques.

The use of such patterning techniques will permit to achieve surface modification at a single dot basis, suitable, in principle, for the immobilization of different bio-molecules on different locations of the same device.

Partner DIN.

Milestones (12 months)

- Development and application of modification processes, involving low voltage anodization and ASD techniques, capable to properly modify the titanium surface chemistry and morphology. In vitro analyses and testing of the developed treatments. Ranking and selection of the best treatments.
- Development of PLD techniques for the synthesis of nanostructured TiO_2 and gold surfaces, and their characterization. Development of a controlled surface capable to enhance cell compatibility and interactions, and to obtain bio-molecules (oligonucleotides, DNA, proteins) immobilization.

Deliverables (24 months)

- Synthesis and characterization of porous surfaces, particularly suitable for cell colonization. Development of patterning routes to create patterned surfaces with functionally graded properties. Test of the obtained materials for the biological compatibility and selectivity.
- Development, characterization and testing of grafting techniques capable to achieve a strong and long lasting immobilization of selected bio-molecules. Characterization and in vitro study of the interactions between the functionalized material and cells.

3. WP3 - Organic materials for artificial bio-systems

P.I.: Prof. G. Lanzani

Cooperating Units: Department of Chemistry (DCM), Department of Physics (FIS), Department of Electronics and Information (DEI)

5 year planning

Organic materials with π-electron delocalization are the subjects of intense research since 1980, when a renewed interest arose following the discovery of conjugated polymers. Our long-term strategy focuses on organic materials as new tools for:

1. Building artificial systems that mimics biological ones in their structures or working principle.
2. Building electronic, opto-electronic or photonics devices based on molecular units or nanostructures.

The activity comprises basic research towards the understanding of properties and natural functions of conjugated systems and applied research, which investigates how molecular mechanisms could be exploited in devices. The construction of artificial systems such as sensors for humanoid technology is one of the possible outputs of this activity, which includes also advances in fundamental knowledge and identification of new functioning mechanisms.

Regarding the first point, a very challenging and fascinating project is the realization of a human-like visual system.

This is indeed a mammoth task, which involves several disciplines, like physics, chemistry, engineering, brain-science, neuro-physiology and psychology at least. We are concerned with a part of it, related to the construction of

a detector operating like retina. Our approach is based on organic materials, which have some advantages with respect to inorganic ones: They allow for a more human-like colour response (the chemical class of natural pigments consists of chains of conjugated carbon atoms, oligomers and polymers, and share similar features in the electronic structure). They can operate at low power and have reduced dissipation. They can be made fully flexible, easily adaptable to geometrical constrains and compatible with biological environment.

A parallel work, complementary to the former, will aim at the understanding on molecular level of the mechanism of vision by relating the optical vs structural properties of retinal and carotenoids in general. These are the collectors of the photons in the retina and the transducers to generate the nervous stimuli in the mechanism of vision. This activity will be implemented by FIS and DCM.

A second topic regards probing the electrical response of biological materials. It is developed within a project for the realization of olfactory sensors that mimic nature by using the olfactory receptors (7 folded transmembrane protein family) effectively present in mammal nose but output a direct electrical signal. However the activity might bring to other applications as well. The artificial nose idea has recently come into a concrete stage after biologists have evidenced the possibility of expressing nanosomes containing OR1740, one of these receptors. Before coming to a working device, whose sensitivity and selectivity might be exceptional, steps are still necessary in the direction of proper functionalisation of substrates with specific antibodies and of the design of specific integrated electronics that senses the tiny signals produced by single bio-molecules. This latter aspect will be specifically addressed by designing unconventional transimpedance amplifiers to probe the electrical response of the proteins. The amplifier will make possible a vaste range of electrical measurements at the molecule level like static I-V curves, impedance spectroscopy up to the MHz range and direct noise spectroscopy with correlated signal suppression, in presence of conductive electrolite. The electronic system will be designed in a single chip with standard CMOS technology and will contain the nanoelectrodes for nanosome grasping, with the aim of producing a disposable lab-on-a-chip. This project will be developed by DEI.

The second point concerns a broad range of studies of new organic materials for a variety of applications in optics, optoelectronics and photonics. One is the study of reversible photochromic organic materials (originally discovered and produced at POLIMI). The change in colour implies relevant changes of the refractive index. The aim is to have smart optical components supporting the development of vision as bio-event. Then there is the production, by electrospinning technologies, of nanowires of suitably functionalized organic materials with specific optical and electrical properties as nano-components in bio-nano devices. Other activities regards new bio-mimetics conjugated systems which bears characteristics of semiconductors and some biological functionalty, such as self assembling capabilities.

Implementation: first 24 months

Objectives

1. The realization of prototype devices as organic based colorimeter and detectors for artificial retina.
2. Enhance fundamental knowledge onto organic materials properties as components in artificial systems for humanoid technology.
3. The synthesis and realization of new organic functional materials for nano-components.
4. Develop lab-on-a-chip prototype to sense the electrical response of biological recetors.

Tasks

Task 1 – Artficial retina and the molecular mechanism of vision.

Artificial retina systems will be approached by first realizing a proof of concept experiment: to build and test a colorimeter, based on the tri-stimuli approach, made by organic photodiodes. A selection of appropriate conjugated molecules will be used for building photodiodes whose spectral response can satisfactorily mimics colour matching function in colorimetry and later the S-, M- and L- response of natural photoreceptors. The molecular mechanism of vision will be investigated by means of advanced spectroscopy techniques, which include time resolved spectroscopy from the fs to the ms and s time domain and in-situ local probes (Raman) that can monitor the bioevent and its relation to the brain stimulus. (FIS and DCM).

Task 2 - Realization of integrated electronics that senses the tiny signals produced by single bio-molecules.

The design of specific integrated electronics that senses the tiny signals produced by single bio-molecules will be addressed by designing unconventional transimpedance amplifiers to probe the electrical response of proteins. The amplifier will make possible a vast range of electrical measurements at the molecule level like static I-V curves, impedance spectroscopy up to the MHz range and direct noise spectroscopy with correlated signal suppression, in presence of conductive electrolite. The electronic system will be designed in a single chip with standard CMOS technology and will contain the nanoelectrodes for nanosome grasping, with the aim of producing a disposable lab-on-a-chip. (DEI)

Task 3 – New organic functional materials.

Synthesis and characterization of new photochromic materials for tuneable light filters and tuneable focal length micro lenses. Realization of nanowires of suitably functionalized organic materials by electrospinning technologies.

Studies of electronic properties of bio-mimetics organics compounds in solution and aggregated state. (DCM, FIS).

Milestones (12 months)

- Knowledge on photophysics of chromophores involved in visual systems.
- Selection of organic photodiodes with response function approaching colour-matching functions.
- First set of experiments on filters with photochromic properties.

- Study of the morphological properties of molecules on the surface e and in the bulk of organic nanowires selected for electrical conductivity. Optimization of the production procedures.
- Design and realization of a first prototype of integrated measuring system to sense the electrical response of biological receptors.
- Testing of the prototype and design of an optimized lab-on-a-chip version ready to be interfaced with biological receptors.

Deliverables (24 month)

- Knowledge on the molecular mechanism of vision.
- A working colorimeter with human like spectral response function.
- Lab-on-a-chip prototype to sense the electrical response of biological recetors.
- Reversible photochromic organic materials with variable refractive index.
- Electrically conducting organic nanowires. and non woven conducting tissues.

4. WP4 - Molecular imaging

P.I.: Prof. C. Bottani

Cooperating Units: Department of Nuclear Engineering (DIN), Department of Physics (FIS), Department of Electronics and Information (DEI), Department of Mathematics (MAT), Department of Chemistry, Chemical and Materials Engineering (CMIC)

5 year planning

Molecular imaging is a growing research discipline aimed at the characterization and measurement of biological processes at the cellular and molecular level. Recent advances in molecular and cell biology and the ability to decode the entire genome give the chance to develop smart nano-probes that address the molecular bases of many diseases and biological functions. The field of view spans from the single molecule, to the single cell, from the small animal up to the human being, with the common mark to track molecular pathways. WP4 will be focussed on two activities belonging to the framework of molecular imaging. This framework well fits with the Research Topic 2 of the Scientific Plan of the IIT ("Advanced characterization tools and imaging").

The first activity will be focused on the development of advanced direct space imaging techniques based on scanning probe microscopies (SPM): atomic force microscopy (AFM) and scanning tunneling microscopy (STM). Biological molecules, supported on micro and nanostructured patterned surfaces (WP2), need advanced characterization tools in order to study their structure, morphology, behavior. The research aims at employing and improving existing scanning probe techniques to better understand the structure and interactions of biological macromolecules and living cells on different surfaces. In particular AFM requires no specific surface preparation and allows morphology investigation at the nanoscale. It is particularly suitable for mesostructural investigations

(e.g. fibrils) of bio-molecules (e.g. protein, DNA) even in a liquid ambient (enabling in vivo analysis). Moreover, AFM permits to manipulate and to test the nanomechanical properties of isolated nanostructures. STM, though requiring a conductive substrate, attains atomic resolution in ideal conditions. So far STM has been used to study mainly DNA fragments, but it opens the possibility to observe the internal structure of single proteins. Significant and complementary single molecule information can also be obtained by spectroscopic techniques such as surface enhanced Raman scattering (SERS) on nanostructured surfaces (WP2) and Raman spectroscopy.

This activity will be carried out as a collaboration of DIN with CMIC.

The second activity will be focussed on the development of instrumentation, algorithms, and methodologies for molecular imaging on small animals. Using suitable imaging systems capable of detecting such probes in living animals and, potentially, in the human body, it is possible to reveal the onset of a disease since the early molecular modifications, or to follow the molecular changes related to a biological function. However, the localization and characterization of a fluorescent probe within a living animal requires to solve relevant problems related to: i) the strong light attenuation of biological tissues, ii) the high scattering of tissues causing the blurring of the fluorescent signal, iii) the poor knowledge of the optical properties of different tissues in-vivo, iv) the high heterogeneity of the medium to be traversed, and v) the presence of an aspecific endogenous fluorescence background overlapped to the target signal.

These problems will be faced combining the know-how on the development of time-resolved optical systems and the competence on the issues related to photon migration applications in clinical diagnosis (e.g. optical mammography, tissue oximetry, ..). This activity will be ruled at different level of complexity, starting from reflectance imaging providing 2D information on sub-surface structures, then moving to a projection geometry carrying information of deep structures, and finally to a complete 3D tomography. Alongside with the technical development, the proposed approaches will be tested on animal models in vivo and applied on sound biological problems with the aim of exploiting the high potential of in vivo molecular imaging since the beginning. In the long term, it is possible to envisage the application of these techniques also to the human being, provided that smart-probes suitable for clinical use will be made available.

This activity will be carried out as a collaboration of FIS with DEI and MAT.

Implementation: first 24 months

Objectives

1. Development of SPM techniques to study biosystems at the nano- and mesoscale.
2. Development of optical techniques for imaging smart probes in small animals (mice).

Tasks

Task 1 - Imaging of biological systems by scanning probe microscopy techniques.

Scanning probe techniques and methodologies (based on AFM and STM Microscopy/Spectroscopy) will be

developed (DIN) in order to study both the functional/functionalized surfaces produced in WP2 (surface roughness, morphology and micro/nanopatterning structure) and to characterize classes of bio-molecules of particular interest. In particular the formation of amyloid aggregates in systems of proteins such as human ataxin, thought as related to neurological diseases, will be investigated. The problem of protein denaturation will be addressed also by AFM measurements in liquid environment using a liquid micro-cell. In parallel other surface characterization techniques will be employed along with scanning probe microscopies for the study of surfaces and biomolecules: high-resolution Scanning Electron Microscopy (DIN), vibrational spectroscopies (e.g. Raman or Surface Enhanced Raman Scattering, SERS) for chemical and structural microanalysis (DIN,CMIC), fluorescence (FIS). Home-prepared micropatterned surfaces (WP2) will be used to evaluate the capabilities of spectroscopy techniques (e.g. Raman, SERS) for biodetection.

Task 2 - Development of imaging workstations for small animals.

Two different imaging modalities will be developed by FIS. A first system will be based on a low-noise, high dynamic range camera combined with a wide-field illumination of the animal. The addition of a gated intensifier combined with pulsed laser sources will permit to exploit the potentialities of the FLIM technique for discrimination of the probe fluorescence from the endogenous background fluorescence. A second workstation will be based on a time-correlated single-photon counting system (TCSPC) equipped with compact photomultipliers, coupled to a translation stage scanning the whole mouse in a projection geometry. Single-photon avalanche diodes (SPAD), produced as technological development at DEI, will be also tested as single detectors or arrays, in view of the high quantum efficiency and good temporal resolution. Finally, the use of a gated camera will be considered as a possible step towards the construction of a tomographic system (FIS).

Task 3 - Development of algorithms for the localization and quantification of fluorescent probes within highly scattering media.

Different measurement strategies and algorithms derived from theoretical models of photon migration will be proposed and tested for the reconstruction of the probe localization and concentration within the biological tissue. These methodologies will be first tested against phantom measurements (FIS). Also, a dedicated FEM code developed by MAT will permit to investigate the efficacy of the proposed algorithms in more complex geometries and structures mimicking the in vivo situation, and to investigate possible novel approaches.

Task 4 - Applications on animal models in vivo.

The systems will be applied to investigate two major issues in experimental oncology (FIS) in collaboration with clinical partners.

a) The study of the effect of new vascular damaging agents using an infrared emitting marker that tracks the blood flow in living animals

b) The study, on the same animal "in vivo", of the dynamic growth of experimental tumors induced by

cancerous cells transfected with genes expressing fluorescent proteins, like Green Fluorescent Protein (GFP) or Red Fluorescent Protein (RFP).

Milestones (12 months)

- Identification of surfaces for optimal AFM/STM imaging of proteins and development of AFM measurements in liquid cell. Study of the formation of amyloid aggregates in human and rat ataxin with different polyQ expansions. Imaging of surfaces prepared in WP2.
- Choice of the optimal technical approaches for the imaging workstations on small animals.

Deliverables (24 month)

- Definitions of protocols for maximum SPM imaging resolution of cells and proteins on patterned surfaces.
- Development of two state of the art workstations for Molecular Imaging.
- Definitions of protocols to evaluate - by optical means - the growth of experimental tumors and the effectiveness of new drugs (e.g. vascular damaging agents) on specific tumor models.

5. *WP5 - Models and methods for local drug delivery from nano/micro structured materials*

P.I.: Prof. Alfio Quarteroni

Cooperating Units: Department of Mathematics (MAT), Department of Structural Engineering (DIS), Department of Bioengineering (BIO)

5 year planning

Micro-structured materials can be designed to store a drug and deliver it in a desired way after implantation in a biological tissue. This can be used in different contexts, ranging from coating of prostheses and bone inserts for reducing the inflammatory reaction after the implantation up to drug delivery in brain or contact lenses. An accurate mathematical modeling of the drug transport accounting for interactions at both the nano and the micro scales with the materials or the biological tissue is still missing. This is a crucial point, since accurate drug delivery and absorption are mandatory for the effectiveness of this technology. The goal of this research is to devise mathematical models of intelligent delivery able to treat different release devices, drugs and drug carriers and targeted biological tissues. Moreover we will set up numerical methods for the reliable and affordable numerical simulation of these processes.

This is a challenging task both for physics and mathematics. Indeed, drug delivery from either polymeric or ceramic matrices involves complex phenomena at the nanoscopic and microscopic scales as brownian ratchet effects, hydrophobic/hydorphilic transitions, phase transitions (solid/liquid) and material erosion.

The objective is to tailor at the nano and micro-scale the ceramic or polymeric material by combining the desired release rate with various other properties needed to ensure processability, durability and multiple end-uses.

This purpose can not be achieved without the application of numerical simulation tools, whose development and tuning for the specific problem at hand represents the core of this project.

We plan to extend models and methods previously developed for the specific case of vascular prostheses to different applicative fields. In fact, the partners of the present proposal are already working on drug delivery from endovascular stents within the framework of a project funded by Fondazione Cariplo (Milan, Italy). Moreover, the approach will be integrated at the nanoscale by using molecular modeling methods to evaluate, and prospectively tailoring, permeability properties from free volume data and small molecule mobility in the bulk, according to techniques which have been developed within the framework of a FP6 European STREP project .

Implementation: first 24 months

In the first two years we will investigate specific features of different problems concerning drug release, in order to identify a benchmark case. Accurate methods and numerical simulations for this case will be carried out. New methods and more accurate models will be developed in the second part of the project. In particular, among the major challenges that arise when the numerical simulation of such phenomena is addressed, we mention the difficulty of accounting for multiple space and time scales and the necessity to develop numerical schemes that respect the conservation principles which stand at the basis of the governing equations. In this regard, we plan to investigate “multi-scale” methodologies, in which different models with a different level of detail are coupled together at the numerical level in order to have accurate solutions when needed, with acceptable computational costs. As a matter of fact, there are preferential directions of drug delivery that can possibly require more accurate description than the others. Geometrical accuracy and molecular structure verisimilitude will be pursued, as well. Realistic 3-D models of the drug release material bulk/surface, drug release device and its implantation site (vascular vessel, bone, etc.) will be developed so as to allow the simulation of the operational release conditions for the nano/micro-structured materials/devices.

Objectives

1. Mathematical models of drug release from nano/micro structured materials in realistic geometries.
2. Numerical methods accounting for multiple time-space scales.
3. Extensive numerical simulations and validation.

Tasks

Task 1 - Identification of the relevant physical scales and corresponding multi-scale models.

We aim at evaluating which space and time scales play a role in the local drug release form nano/micro structured materials. On this ground, suitable models to describe the phenomena at hand should be identified. This will lead to the definition of a few benchmark problems, which allow a validation study by means of available results (from literature or experiments). To describe phenomena at very different physical scales, models with

heterogeneous mathematical structure will be needed. In this perspective, suitable multi-scale coupling methods should be proposed.

Partners MAT, DIS, DIB

Task 2 - *Efficient and accurate (possibly multi-scale) numerical simulation methods.*

Moving from the models identified by task 1 we will focus on the development of numerical methods. Because of the deep complexity of the phenomena at hand, the key point will be to develop numerical schemes that represent a satisfactory trade-off between accuracy and computational efficiency. Model reduction techniques and multi-scale methods will be the core to achieve this goal.

Partner MAT.

Task 3 - *Extensive simulation and validation.*

We will extensively test the numerical methods developed in task 2 on the benchmark problems of task 1. These numerical simulations will involve the benchmark cases identified in Task 1 and other realistic cases.

Partners MAT, DIS, DIB.

Milestones (12 months)

- Identification of a benchmark problem and the relevant models at different scales for drug delivery from a nano/micro-structured material (task 1).
- Definition of the requirements for satisfactory numerical simulations (part of taks 2).
- Analysis and validation of preliminary numerical results (part of task 3).
- Preliminary CAD modeling of drug release materials and devices (part of task 3).

Deliverables (24 month)

- Step by step definition of the methodology to simulate the benchmark problem and to validate the results (tasks 1 & 3)
- Simulation software for a class of problems concerning drug delivery from nano/micro-structured materials (task 2),
- Consolidation of a simulation tool and a problem database shared between the project partners (task 3).

REHABILITATION

1. ***WP1 - Multisource neurophysiological information processing for innovative and personalized rehabilitation protocols***

   P.I.: Prof. Sergio Cerutti

   Cooperating Units: Department of Bioengineering (BIO), Department of Physics (FIS), Department of Electronics and Information (DEI)

5 year planning

Rehabilitation involves the recovery of some lost functions (i.e. movement, speaking, cognitive processes) that are compromised after traumatic events, or strokes or by congenital or degenerative pathologies. The approach is generally focused on the evaluation of how the lost function is restored through the natural way, through the involvement of other different functionalities or both. A quantification of how much the restored function differs from the normal one is a measure of the efficiency of the rehabilitation therapy. Minor attention is generally dedicated to the central nervous system (CNS) behaviour and to the mechanisms through which the brain can readapt to a modified peripheral situation, thus involving brain plasticity.

The functional investigation of the CNS can be carried out by many different techniques and modalities ranging from functional imaging (fMRI, PET, SPECT, NIRS), to multichannel signal recording (EEG, MEG). All of them are characterized by different spatial and temporal resolutions, by different levels of invasivity, by the different capabilities of investigating specific brain regions and all of them take advantage from the growing digital processing capabilities. During the rehabilitation process the study of brain functionalities can provide great advantages at different levels: 1) quantitative and objective evaluation of the CNS damage and of the residual functions that can be used and empowered during the rehabilitation process; 2) monitoring of the CNS reorganization during the therapy: this is useful for obtaining a continuous, focused and personalized adjustment of the proper rehabilitation actions to be taken on the patient; 3) quantitative evaluation of the entire rehabilitation protocol, improvement obtained in comparison with the initial situation of the patient, estimation of the brain plasticity response due to the rehabilitation in each single subject and estimation of statistical inter-subject variability.

Aim of the present research is the employment of proper technologies and methodologies for monitoring the CNS functionality, brain plasticity and the adopted strategies during the rehabilitation process. At this purpose different tools for the functional investigation of the CNS will be designed and applied on selected groups of patients as well as on normal subjects. In particular, integration among different signal-imaging modalities will be introduced, in order to develop efficient and innovative tools able to provide a more complete information with the minimal invasivity and discomfort for the patient.

Near infrared spectroscopy (NIRS) will be proposed as a novel noninvasive technique which employs diffuse light in the spectral region between 600 and 1100 nm to non-invasively monitor hemodynamics (i.e. evolution of blood distribution within a biological tissue in terms of concentration of oxygenated and deoxygenated hemoglobin) and oxidative metabolism (i.e. oxygenation status) in the muscle or in the brain. In the muscle, this can help in studying mechanisms related to normal physiology under exercise, but also to pathology caused by myopathies and/or peripheral circulation diseases. In the brain, it can lead to the monitoring of cerebral activity in response to some stimuli (motor, visual, cognitive), and thus it can help in the study of cognitive processes, diagnosis of mental diseases, localization of brain injuries.

The project aims at a deeper understanding of the potentiality of NIRS for noninvasive studies of human behaviour for cognition and development. Main activities will be: the development and optimization of advanced compact and flexible NIRS instrumentation with imaging and spectroscopic capabilities for muscle and brain studies based on novel strategies of data acquisition in the time-domain, broadband and compact laser sources, photodetectors with high temporal resolution and enhanced sensitivity; the integration of NIRS with standard technique for muscle and brain studies (e.g. EEG, fMRI) for multimodality imaging; the application of NIRS for extensive clinical and pre-clinical studies on muscle and brain in particular for rehabilitation and development paradigms. Dedicated signal processing tools will be designed to enhance useful clinical information from NIRS signals.

The project will use a dedicated framework based on soft computing techniques for the analysis and intelligent classification of biological signal features extracted from different sources during a rehabilitation session. The aim of this framework is to provide robust knowledge representation and on-line modeling of the physiological response of a patient to a specific rehabilitation protocol. Models should be adapted to the single user to provide information useful to customize and fine-tune the rehabilitation activities.

The clinical use of multimodal monitoring of the CNS is intended to open and suggest new rehabilitation strategies based on a deeper knowledge of the physiological mechanisms adopted by the whole system for restoring or for substitute lost functions. Applications are foreseen in three areas of rehabilitation: 1) *cognitive rehabilitation,* in both post-trauma patients and in cognitive pathologies (i.e. dyslexia); 2) *motor rehabilitation,* for the investigation of the central response to therapeutic interventions such as surgical operation to restore paraphysiological motor functions and the evaluation of muscular metabolic mechanisms during rehabilitation phases; 3) *neurophysiological follow-up*, in the monitoring of Parkinsonian patients undergoing deep brain stimulation (DBS) therapy, to be associated to the traditional clinical findings. Cooperation with medical and clinical units on neuroscience and rehabilitation are already active: in particular with IRCCS Medea Foundation, Bosisio Parini (LC), Villa Beretta, Fondazione Valduce, Costamasnaga (LC) and Department of Neuroscience, Policlinico in Milan, University of Milan. A cooperation with Drexel University, Philadelphia, USA is actually on course about NIRS technology aspects and signal-image processing methods of fusion.

Implementation: first 24 months

Objectives

1. Definition of testing and experimental protocols for rehabilitation
2. Development of algorithms of biomedical signal and image processing and software platform for integration
3. Development of NIRS prototype equipment for rehabilitation

4. Information fusion from multisource data

Tasks

Task 1 - Development of algorithms for multimodal integration of different signal-imaging techniques.

Various approaches will be investigated using linear and non linear algorithm of signal and image fusion, as well as through artificial intelligence methods (BIO) (DEI).

Task 2 - Development of innovative NIRS instrumentation.

A novel optical system will be developed, based on time-resolved near infrared reflectance spectroscopy (NIRS-TR), with high-temporal resolution and fast data analysis (FIS).

Task 3 - Definition of testing and clinical protocols suited for the investigation of rehabilitation processes.

Simple test protocols (motor, visual, cognitive, etc.) capable to provoke the highest, most consistent, reproducible and well located cortical oxygenation responses, will be identified and tested on NIRS prototype. Clinical protocols for cognitive and motor rehabilitation as well as in monitoring Parkinsonian patients with Deep Brain Stimulation therapy will be designed and activated in cooperation with the clinical units (BIO) (FIS).

Task 4 - NIRS for muscle oximetry and brain functional imaging studies.

Muscle exercise protocols on healthy volunteers will be defined to non invasively investigate de-oxygenation at the highest workload and heterogeneity in de-oxygenation among the different parts of the muscle. These results will be used to interpret data from patients affected by muscle impairment and to monitor the effects of rehabilitation paradigms (FIS) (BIO).

Task 5 - Acquisition of selected groups of patients and normal subjects, data analysis. In cooperation with the clinical cooperating units, normal as well as pathological patients will be recorded and the data analysis will be carried out (BIO).

Task 6 - Feature analysis, model selection and design.

Selection of already available biological signals. Statistical characterization and pre-processing of biological features and parameters. Methods of feature selection and reduction targeted on the rehabilitation process. Selection and definition of models to classify and cluster patient response before, during and after rehabilitation treatment. This models should foster the definition of feedbacks for the rehabilitation management (Outcome Assessment) exploiting classification and prediction (DEI) (BIO).

Task 7 - Preliminary framework design and prototyping.

Software platform is designed and prototyped on the basis of techniques devised in previous tasks. Selected models and procedures are going to be integrated in a common tool to be validated and tested on real cases in following phases of the project (DEI).

Milestones (12 months)

- Quantitative comparison of the behaviour of different functional imaging modalities, in order to clearly identify their relevant properties and the degree of applicability in the present research context;
- Definition of the clinical protocols to be adopted for the different groups of patients;
- Implementation and testing of algorithms of integration among different signal-imaging modalities, to improve the information achievable and to minimize the discomfort of the patient;
- Development and characterisation of a multichannel time-resolved NIRS instrument for muscle and brain studies;
- Acquisition of testing cases for the setup of the analysis procedure and the algorithms, preliminary analysis of the recorded data;
- Report on the outcome of *Tasks 6 and 7*, including data analysis for the features extracted for real cases acquired during the rehabilitation protocol and developed software tools.

Deliverables (24 months)

- Neurophysiological parameters for rehabilitation.
- Optimisation of NIRS for brain and muscle studies.
- Software platform for data/image treatment and fusion.

2. WP2 - Human machine interface for recovery of lost functions

P.I.: Prof. Antonio Pedotti

Cooperating Units: Department of Bioengineering (BIO), Department of Phisycs (FIS), Department of Electronics and Information (DEI), Department of Mathematics (MAT)

5 year planning

This WP deals with an interdisciplinary approach which includes cutting-edge technologies over a wide spectrum of fields (micro-technology, sensors, neuro-information technology, neuroscience, complex system analysis, computational models, optoelectronics, robotics and automation) aimed to design human-like devices able to interact bi-directionally with the patient to restore lost functions. The neurological diseases here considered are among the most common and disabling in European countries: stroke, spinal cord injuries and neurodegenerative

and neuromuscular diseases like amyotrophic lateral sclerosis. Thank to the recent progress in medical treatments the expectance of life of most of these patients has reached very high values which in some cases do not differ to much from normality. However they are in a dramatic condition in which can not communicate, move and even breath. The restoration and the optimisation of these functions can strongly benefits from an innovative approach in which natural and artificial systems are strictly interacting and complexity is a common paradigm. This approach implies a deep knowledge by computational models of biological functions and motor control, the design and developments of adaptive human-like machine with sensors and actuators implementing a bi-directional communication to cooperate for performing the lost function in an optimal way compatible with the patient injury. Such approach will be applied for recovery of complex movements, respiratory function and communication .

To perform any motor task the CNS process in parallel a great amount of information on the internal state of the body deriving from proprioceptive sensors and on the external environment (visual, tactile etc) in order to fulfil multiple objectives by controlling multiple and redundant actuators. The research will be focused on the mechanisms controlling complex movements of pointing in standing posture where precise movements of the arms are integrated with balance components in a system with many degrees of freedom including also grasping and hand movements. A deep understanding of such functions will be used in rehabilitation of neurological patients where the integration of these aspects is affected by the pathology. Multifactor analysis of movement including kinematics, forces and electrophysiological data will be combined with computational models and simulation in collaboration with MAT. Motor learning will be explored by investigating adaptive strategies adopted by normal subjects in micro gravity. Such knowledge constitutes the basis to design and develop humanoid robotics in collaboration with DEI and novel rehabilitation strategies by functional electrical stimulation. The specific aim of the latter is to improve muscle tone, peripheral and cardiac blood circulation, sensory motor integration, to prevent sores, joint rigidity and to learn new motor strategies exploiting the residual capabilities of the patients .Normal clinical practice in the last few years started to use open loop controller, able only to provide the a priori rigid sequence of stimulation pattern for a pure stereotyped function. One of the main limit of such approach is the rapid occurrence of muscle fatigue preventing a wide adoption of these techniques. Closed loop controllers based on neural network, online adaptive, for human-like strategies of control will be studied and developed. Neural networks will be designed to learn from healthy subjects closed loop controllers able also to map muscle fatigue phenomenon by NIRS in collaboration with FIS and customise the stimulation parameters over the single patient.

The ventilatory mechanisms controlling acid-base balance, oxygen delivery and CO2 elimination and which involve complex interactions between receptors, spinal, brainstem and cortical neural controllers and pathways, respiratory muscles, lungs and chest wall is a complex system functioning close to, but not in, equilibrium, capable of beneficial adaptations to changing environmental conditions. In neurodegenerative and neuro-muscular diseases like Amyotrophic Lateral Sclerosis (ALS) and spinal cord injury (SCI) these mechanisms are profoundly altered

and therefore breathing problems are the leading cause of mortality. Several factors including respiratory muscle weakness, alteration of respiratory system mechanics and impaired central control of respiration play a crucial role. Pulmonary rehabilitation is increasingly recognized as an important component of the comprehensive management of these patients. Increased knowledge of the interaction between respiratory muscles, respiratory mechanics and the mechanical ventilator has led to the development of new technologies aimed at improving ventilatory treatment. Biologically variable (or fractal) ventilation represents a new, volume-targeted, controlled ventilation mode aimed at improving oxygenation; it incorporates the breath-to-breath variability that characterizes a natural breathing pattern. All these advances are aimed to improve the patient–ventilator interaction by matching the ventilator support with the needs of the patient, simulating and interacting with the patient's respiratory control centres. Up to now, the technology used to assess chest wall kinematics was able to provide only a qualitative description of asynchronies and/or paradoxical motion. The recent introduction of the Opto-Electronic Plethysmography (OEP), developed by our research group, in the mechanically ventilated patients allows to obtain accurate measurements of the volume changes of the total chest wall, and of its different compartments (rib cage and abdomen) to develop a bi-directional human-like ventilation.

In the fundamental research about subject's intention detection and communication recovery, we propose a research line on BCI systems,i.e. those "communication systems that do not depend on the brain's normal output pathways of peripheral nerves and muscles": the aim is to provide a direct communication between the brain and a machine (in general a PC) through the on-line detection, classification and transduction of the cortical activity of people suffering of very severe pathologies. The actual generation of BCI system are EEG-based: this means that the base technology used is electroencephalography or electro-corticography, but several criticism in these systems still exist both on the hardware side and on the software one. The main problems in actual BCI systems are: 1) cost of device (today they require an 'open' EEGraph); 2) the possibility to measure and classify in real time specific cortical events; 3) the low communication rate; 4) the accuracy of the classification. For example noninvasive functional human brain mapping by diffuse optical methods is a novel technique that employs near infrared light to probe the brain for changes in parameters relating to brain activity and it could be applied to re-enforce the EEG-BCI to obtain a more reliable and efficient BCI. The multimodal detection of the subject's intention could lead to a new understanding of the brain physiology but also to the realisation of a next-gen BCI platform, that now is still at the beginning and needs new technologies and the study of "ad-hoc" experimental protocols as of data acquisition and processing campaigns on a larger numbers of subjects.

Implementation: first 24 months

Objectives

1. Design of experimental protocols and collection of data on selected motor performances in healthy subjects .
2. Design and developments of suitable computational models and software implementation.

3. Design and development of experimental prototypes.
4. Preliminary validation on patients and adaptation of technology and experimental set-up.

Tasks

Task 1 - Simulation and computational modelling purposely for the design of technological devices for neuroprostheses and functional electrical therapy and for the analysis of subject-environment interaction.

Task 2 - First experimental campaign on healthy subjects and neurological patients, results analysis and revision of technological devices and set-up.

Task 3 - Development of computational model and experimental activity to characterize respiratory control systems, with special attention to the control of the end expiratory lung volumes, by developing new methods for studying short and long-term correlation in end expiratory lung volume (based on OEP) and how this parameter is affected by alterations in mechanical properties and blood gasses.

Task 4 - Design and prototype development of new modality of ventilatory support in which the ventilator adapts its activity on the basis of the neural output of the respiratory centers. We will apply the newest results in brain-computer interface to develop and evaluate neurally-driven ventilators, in which the mechanical ventilator will act as required by the patient's respiratory centres in an optimized coordination with the respiratory muscles

Task 5 - The main goal of this task will be the design and the integration of BCI EEG-Based and fNIRS-based in one portable devices (BCI Next-gen) to offer an absolutely innovative opto-electric-functional device for the brain investigation and to explore the possibility to increase BCI accuracy and speed of communication through the integration of those cerebral signals. Furthermore, the technological and economical analysis for the transfer of the devices small scale production will be carried out.

Task 6 - Next-gen BCI preliminary validation and clinical testing: The prototype will be technologically and metrologically validated on a preliminary sample of healthy subjects and patients. Algorithms and software optimization will be carried out to design new integrated BCI setup and experimental protocols. A continuous literature survey and update will be the background activity.

Milestones (12 months)

- Development and testing in simulation of closed loop controllers for neuroprostheses and functional electrical therapy.
- Design of experiments for subject-environment interaction.
- Design of experiments for computational model validation.
- Definition of specifications.
- Next-gen BCI system specifications.
- Next-gen BCI experimental protocols and algorithms.

Deliverables (24 months)

- Prototype for cycling by FES with closed loop control and preliminary results on clinical trials.
- Publications on international journals and conferences of the results of the experimental campaign on healthy subjects-environment interaction. Final definition of technology for functional electrical therapy and neuroprostheses.
- Publications on international journals of the computational model and experimental validation.
- Document describing the final design.
- The Next-gen BCI prototype and the document describing technical specifications and preliminary validation.
- Next-gen BCI system protocols, Publications on international journals and conferences of the results.

3. *WP3 - Robotic companion exploiting affective feedback for modeling emotional state of the patient and adapting the rehabilitation treatment*

P.I.: Prof. Andrea Bonarini

Cooperating Units: Department of Electronics and Information (DEI), Department of Bioengineering (BIO)

5 year planning

The application of robotics and automation technology can serve to assist, enhance, evaluate and document the rehabilitation activity. Among the possible applications of this personal therapist is the rehabilitation of people suffering from neurological or orthopedic lesions. Currently, physical therapy takes advantage of brain ability to adapt, change and take over (some of) the functionalities from the part affected by such limitations. When these limitations affect movements, therapists can exercise the corresponding part to make the patient regaining its control. From the point where the patient regains some movement, he/she could perform exercises without physical assistance from the therapist. The use of robots, to support the activity of the therapist, and play some of her/his roles, seems almost logical given the repetitive nature of these exercises.

When limitations concern the cognitive and emotional aspects, the therapist has a fundamental role to obtain a successful treatment through the interaction with the patient. In this case, a robotic therapist should be able to recognize the patient needs and adapt its behavior to them.

Some research activities have approached robotic rehabilitation from different directions. Some Japanese researchers have developed affective robots that can interact with people stimulating emotions and have tested them on patients showing cognitive problems. Another interesting class of applications concerns direct involvement in parallel tasks (such as playing a sort of videogame, as in a MIT research project) to divert attention from the specific (possibly tedious and negatively perceived) exercise. The therapeutic activity automatically adapts its characteristics to the performance of the patient as perceived through the parallel task execution itself. Other applications get the information from sensors like EMG, ECG and others to detect the patient performance and

eventually adapt the therapy based on the physical response.

A really effective robot therapist needs to have a more complete and deep feedback from the patient, to optimize and customize the treatment. At present, there are few applications where this feedback concerns mainly the physical performance of the exercise. To improve its performance and widen the range of possible applications, the robot should have more information about the patient, including his/her emotional state and attitude w.r.t. the exercise. This can be obtained by an intelligent patient modeling activity performed on data coming from different sensors that acquire physiological and physical parameters. The patient should also receive an appropriate feedback both for therapeutic reasons and for acceptance of the specific treatment.

The project is aimed at designing and developing methods, devices and techniques to implement rehabilitation robotic companions able to interact with the patient by adapting the rehabilitation protocol to the patient reaction both from the physical and emotional points of view. In particular, this robotic companion should be able to interact also with the affective sphere of the patient to influence positively its relationship with the rehabilitation management. The success of a rehabilitation therapy strongly depends on the trusted interaction with the therapist, and his/her capability to catch the specific needs of the single patient, the level of stress, and limits in following the rehabilitation protocol. This robotic companion will implement the concept of personal therapist, which supports, and partially substitutes, the work of the real therapist in everyday life, also outside the rehabilitation center, exploiting an affective feedback from the user.

Implementation: first 24 months

Objectives

1. Definition of signal potentially suitable to get emotional information.
2. Development of models of the emotional aspects of the patient involved in rehabilitation.
3. Development of a prototype rehabilitation system.

Tasks

Task1 – Task identification and test cases definition/analysis.

This task is aimed at the selection of some rehabilitation tasks to focus on. Test cases will be identified by considering their significance to assess the proposed technology. Test cases will be analyzed and modeled, to put in evidence both the emotional and physical aspects to be considered (DEI).

Task2 – Identification of potentially available signals.

This task is aimed at the identification of the biological and physical signals that can be acquired from the patient and that are useful to build suitable performance and emotional models. The evaluation of the different alternatives will take into account the reduced grade of invasivity, and the reliability of the signals, together with their significance for the given task (DEI) (BIO).

Task3 – User modeling.

The results of tasks 1 and 2, also in their preliminary phases, will be used to model the application and identify relationships between the data potentially available and the features of the model, by using soft computing techniques such as Neural Networks, Fuzzy Rules and others (DEI).

Task4 – Prototype design and integration with user models.

A first, preliminary prototype will be designed and implemented to evaluate the possibilities of the proposed approach in some of the defined case studies (DEI) (BIO).

Milestones (12 months)

- Report on the outcome of *Task 1* and *Task 2* including the case study definitions, signal descriptions and preliminary patient modeling.

Deliverables (24 months)

- Emotional model of the patient.
- Definition of test cases and evaluation criteria.
- Prototype of affective rehabilitation system.

QUALIFICATION OF THE RESEARCH UNIT

The "Politecnico di Milano" Technical University is a **science and technology university** producing engineers, architects and industrial designers through a variety of innovative specialising courses, with great attention being devoted to all sides of education.

The "Politecnico di Milano" Technical University has always been based on quality and innovation in teaching and research, resulting in a prolific relationship with the economic and manufacturing worlds through experimental research and the transfer of technology.

Today, **research is increasingly and more closely connected to teaching** and represents a priority commitment which makes it possible for us to attain high level results at international level. Research work goes hand in hand with cooperation and alliances with the industrial system. Knowing the world where one will work is a fundamental requirement of students' training. Being confronted with the needs of manufacturing, industrial and public administration sectors helps research to approach new terrain and to meet the need for constant and rapid innovation. Such an alliance with the industrial sector not only permits the university to continue along its traditional areas but also acts as a stimulus for their development.

There are several teaching and research areas in which the Politecnico has distinguished itself in the past, which have fuelled a **tradition of excellence** that has been progressively updated: developing excellence and at the same time striking alliances with other Italian and foreign universities and research centres makes it possible for the

university to fully carry out its teaching function, improving its offer to the students, and to carry out its role of stimulating innovation and therefore Italy's development. This alliance is becoming increasingly important in **Europe**, where the Politecnico takes part in many research and training projects with the best qualified European universities, as well as expanding to other countries: from North America to South East Asia.

In this way, the students of "Politecnico di Milano" Technical University become citizens of the world, offering companies the opportunity to hire skilled people to compete with their colleagues from other nations, while also facilitating international relationships of Italian companies.

In order for this challenge to be successful, we have started a "hospitality" policy, which is progressively making accommodation, internships and apprenticeships available to students, **in collaboration with local businesses**.

Year by year this challenge becomes increasingly more important in a world where globalisation and competition are becoming essential reasons to improve and ultimately survive. The awareness of this challenge, by professors, technical and administrative staff, students, together with the quality of education and research, are the assets of the "Politecnico di Milano" Technical University.

The Politecnico di Milano Technical University is **strongly oriented towards scientific and technological research**. Its involvement in the continuous development of the forefront sectors of research wishes to assure an international visibility of the Institution. The participation in international research programmes is one of the priorities of the Politecnico di Milano.

The Politecnico di Milano T.U. plays an important role also in the panorama of the international research thanks to the close connection with the productive and economic community, and towards the wide development of research activities (basic research and applied research), technological transfer and industrial exploitation of the results.

The **alliances formed with other universities, centres of research and industries all over the world** allow the Institution to improve constantly his performance in the domain of research and to contribute to the development of the European innovation.

Furthermore, the constant involvement of the academic staff in research activities allows them to transfer the results of both technological evolution and scientific progress to their students in real time, improving the educational offer of the Institution.

Research activities take place mainly at the Departments, where they are developed by a large number of research groups. Starting from Giulio Natta, Nobel Prize for Chemistry in 1963, many scientists working at the Politecnico di Milano T.U. received very distinguished **awards** and recognition by the scientific community.

Many research contracts are signed and established every year. They are supported both by national and international public funds and by the European Commission (e.g. Framework Programme for R&TD). Most Departments count also active research contracts signed up with public bodies or private companies.

The Institution strongly supports multidisciplinary research activities, in order to improve the co-operation between the Departments.

The links developed with the business community, led the Politecnico di Milano T.U. to participate in associations and consortia (specific structures oriented to applied research, to technological transfer or to specific educational objectives) and to support the establishment of new companies during their start-up through the activities of the Acceleratore d'Impresa.

A significant hinge role with the outside world of institutions and enterprises has been entrusted to the **Fondazione Politecnico di Milano**, established in 2002. It has among its objectives the acquisition of research projects, which could involve the Departments of the Politecnico di Milano T.U..

www.ifom-ieo-campus.it

IFOM FIRC INSTITUTE OF MOLECULAR ONCOLOGY FOUNDATION

European Institute of Oncology

Research Unit IIT – Institute of Molecular Oncology Foundation-European Institute of Oncology (IFOM-IEO)

Scientific coordinator of the RU : Prof. Andrea Musacchio

SCIENTIFIC OBJECTIVES

- ***WP1 Protein interaction dynamics and systems biology of macromolecular networks***

  P.I.: Prof. Andrea Musacchio

  IFOM-IEO Campus Scientists involved: dr. Marco Simonetta, dr. Mario Faretta, dr. Marina Mapelli, dr. Dario Parazzoli, dr. Alberto Diaspro, dr. Andrea Ciliberto, dr. Martin Vink

  P.I. Prof. A. Lacaita, Dipartimento di Elettronica e Informazione

- ***WP2 Light microscopy for advanced live cell and live animal imaging***

  P.I.: Prof. Andrea Musacchio

IFOM-IEO Campus Scientists involved: dr. Mario Faretta, dr. Dario Parazzoli, dr. Alberto Diaspro, Prof. Andrea Musacchio, dr. Massimiliano Garrè

The "3I" program (the IIT program at the IFOM-IEO campus) will focus on the fields of i) advanced imaging approaches for phenotypic screening and other applications (Workpackages WP1 and WP2), and ii) nano-biotechnology and tissue engineering (Workpackage WP3). Extending our research lines in these two fields will complement the very solid genomics and proteomics platforms existing at the IFOM-IEO campus and will create a fertile ground to develop a continued tight interactions with the IIT. The Director of the IIT Unit at the IFOM-IEO campus is Dr. Andrea Musacchio, a tenured scientist and a member of the Executive Committee, the principal organ of governance of the IFOM-IEO Campus. The direction of Dr. Musacchio will insure a smooth integration of the "3I" program within the IFOM-IEO campus, and will create and maintain the necessary links with the management of the IIT. Administrative and organizational help required to run the "3I" program will be offered using the available resources of the IFOM-IEO campus, on the basis of an agreement with the management of the IIT. The "3I" program will be subdivided in 3 workpackages (WP), for each of which we provide here a brief scientific description, the main milestones, and the associated budget.

WORKPACKAGES

1. WP1 – Protein interaction dynamics and systems biology of macromolecular networks

P.I.: Prof. Andrea Musacchio

IFOM-IEO Campus Scientists involved: dr. Marco Simonetta, dr. Mario Faretta, dr. Marina Mapelli, dr. Dario Parazzoli, dr. Alberto Diaspro, dr. Andrea Ciliberto, dr. Martin Vink

WP 1 is concerned with identifying new approaches to measure protein dynamics using fluorescence microscopy. Modeling macromolecular reactions in biological systems requires an understanding of the kinetic parameters involved. The group of Dr. Musacchio is interested in modeling complex protein networks involved in the regulation of the mammalian cell division cycle using realistic, experimentally determined, kinetic parameters. For this, biochemical reconstitution of complex protein networks using purified components is required. The Biacore® instrument is the most commonly used device for measuring protein interaction dynamics. This instrument exploits a property known as surface plasmon resonance, a mass-dependent change in the refraction index of a metal-coated chip on which the binding reactions are simulated. The time-dependency of the change provides information on the kinetic parameters k_{on} and k_{off} of a reaction occurring at the surface of the chip. The goal of WP1 is to create an alternative to the Biacore® instrument using fluorescently labeled proteins analyzed by confocal or TIRF microscopy. In particular, the group of Musacchio is developing approaches to correlate protein dynamics *in vitro* and *in vivo*, the best example of which is *in vitro* FRAP. The WP involves biochemical reconstitution, the development of chips for protein immobilization, the development of specific protein labeling strategies, the integration of chips in microfluidics cells, the development of modeling strategies based on realistic kinetic parameters.

2. *WP2 – Light microscopy for advanced live cell and live animal imaging*

P.I.: Prof. Andrea Musacchio

IFOM-IEO Campus Scientists involved: dr. Mario Faretta, dr. Dario Parazzoli, dr. Alberto Diaspro, Prof. Andrea Musacchio, dr. Massimiliano Garrè

WP2 aims at the creation of innovative imaging strategies and implies the hiring of a new IIT group leader operating at the IFOM-IEO campus. We envision that the successful candidate will be a relatively young person with a competitive CV and several years of postdoctoral research experience at his/her first appointment as an independent group leader. He/she will be hired on a 5-year basis according to a consolidated scheme that includes a competitive salary and startup package and a review at 3 and 5 years that might eventually result in a rolling tenure. This scheme is already been used at the IFOM-IEO campus and we envision that salary scales and startup packages similar to those available for JAPs (Junior Assistant Professors) at the IFOM-IEO Campus should be made available. The new group leader is expected to start a successful research program involving innovative imaging technologies. These might include for instance the use of techniques such as FLIP, FRET/FLIM or FCS to study macromolecular dynamics and interaction in living cells, the development of innovative labeling schemes using quantum dots, the design of low- and high-content phenotypic screenings using fluorescently tagged proteins in live cell assays, the acquisition of new technology capable of overcoming Abbe's resolution limits (nanoscopes), the developments of approaches in molecular imaging aimed at developing and testing novel tools, reagents, and methods to image specific molecular pathways in vivo, particularly those that are key targets in disease processes. The IIT group leader will be flanked by the existing imaging facility at the IFOM-IEO Campus, directed by Dr. Mario Faretta. This facility is well equipped to do high-resolution work on a variety of live and fixed biological samples using wide-field and confocal microscopes, and is also developing a research program making use of evanescent wave illumination both for biological samples and for single molecule work on substrate. The start up should include the purchase of machinery to extend the assets of the imaging facility. The IFOM-IEO Campus will also contribute financially to the creation of this laboratory.

Milestones

YEAR 1

- Hiring of one group leader working in the field of imaging in the frame of "3I" program. Set up of the laboratory, purchase of necessary equipment, hiring of collaborators.
- Evaluation of strategies for cultivation of stem cells, dendritic cells, and melanocytes on nano-structured surfaces. Cell properties such as cell division, will be monitored.
- Immobilization of retroviruses on nanostructured surfaces for RNAi-based or overexpression screening programs.
- Improvement of *in vitro* FRAP approaches.

- Immobilization of proteins on nano-structured surfaces.
- ***Beginning of creation of several stable cell lines for live-cell imaging using cDNAs or BACs.***

YEAR 3

- Third year evaluation of imaging group hired in the frame of 3I program. The review will concentrate on the scientific productivity of the group, its contributions to the goals, its collaborative spirit, and its competitiveness in raising research funds.
- ***Setup of phenotypic screenings exploiting nano-structured surfaces for gene or siRNA delivery.***
- Application of *in vitro* FRAP approach to a number of components of selected biochemical networks such as the spindle assembly checkpoint.
- First FRET, FLIM and TIRF applications tested on pilot projects.
- Innovative strategies being developed.

YEAR 5

- Fifth year evaluation of IIT's imaging group with possibility of offering rolling tenure at the IFOM-IEO campus.
- Analysis of genes delivered by low- and high-content phenotype screening efforts.
- Patenting of new technologies or applications developed within the 3I program.
- Routine use of sophisticated imaging techniques to study protein interactions, cell gradients, protein dynamics in living cells.
- Modeling of spindle assembly checkpoint with experimentally derived kinetics constants and concentrations.

QUALIFICATION OF THE RESEARCH UNIT

The Department of Experimental Oncology of the European Institute of Oncology and the Institute for Molecular Oncology of the Italian Foundation for Cancer Research are undergoing a major expansion and integration of their scientific activities. These two leading italian institutions in Cancer research, located in Milan, have a recent but distinguished history. The IEO was created in 1994 as a comprehensive cancer centre. The IFOM was founded in 1999 to serve as a common house for many excellent institutions in Milan for advanced studies in post-genomics applied to oncology. The interests of the two institutions include cell cycle regulation, signal transduction, cancer genetics, cancer immunology, angiogenesis, and structural biology. IFOM and IEO adopt an open structure model that inspires communication and collaboration between research groups. Their success relies on competitiveness in fund raising, at both national and international levels. This allowed the hiring of group leaders with competitive start-up packages and salaries, an internal fellowship program for PhD students and post-docs, and the creation of state-of-the-art, shared facilities and resouces for imaging, DNA and tumor micro-array,

mass spectrometry, DNA sequencing, bioinformatics, protein expression and model organisms (from C. Elegans to mouse).

New jobs

IFOM and IEO are undergoing a major expansion, which will be completed in september 2006. Two adjacent structures for over 22,000 m^2 will host up to 600 researchers in a shared campus. A strong integration of research and training activities is planned, which will result in the creation of one of the widest and most innovative cancer research programmes in Europe. The developments will include hiring of several new group leaders, aiming at young excellent scientists at their first group leader experience. Hiring of already established researchers with innovative programs in cancer biology is also expressely contemplated. Finally, the new campus will offer space for start-up biotechnology activities, creating a technology park of unique strength in the Italian scenario. For inquiries, contact Pier Paolo Di Fiore (director of IFOM) and/or Pier Giuseppe Pelicci (Chairman of IEO's Department of Experimental Oncology).

PhD and Postdoctoral Opportunities

The IFOM-IEO campus has been hosting the PhD program of the European School of Molecular Medicine (SEMM), a collaborative program with the University of Milan and the University of Naples as PhD-awarding institutions. PhD students admitted into the SEMM will be working with IFOM and IEO group leaders at the IFOM-IEO campus, and will receive theoretical and practical training from lecturers recruited inside and outside the campus. SEMM aims at creating a most competitive PhD School open to students worldwide. www.fondazionesanraffaele.it

Research Unit IIT – Fondazione Centro San Raffaele del Monte Tabor (HSR)

Scientific coordinator of the RU : Prof. Jacopo Meldolesi

SCIENTIFIC OBJECTIVES

- ***WP 1 Multiplicity of exocytoses: role of specific forms in physiology and pathology.***
  P.I. Jacopo Meldolesi
- ***WP 2 Cellular and molecular imaging of neuron-astrocyte signaling in physiological and pathological conditions.***
  P.I. Fabio Grohovaz

- ***WP 3 Intelligent drug delivery by viral-like particles.***
  P.I. Fulvio Mavilio
- ***WP 4 New tools for modulating endothelial barrier function and drug delivery.***
  P.I. Angelo Corti
- ***WP 5 Optical approaches to the study of neuronal plasticity.***
  P.I. Flavia Valtorta
- ***WP 6 Dynamics of single molecule and single bioevent in living cells revealed by fluorescence fluctuation and time resolved fluorescence spectroscopy.***
  P.I. Valeria R. Caiolfa
- ***WP 7 Haptics in neuroscience and robotics.***
  P.I. Gabriel Baud-Bovy

The program of this Unit **offers a comprehensive overview of the scientific and technological developments envisaged at the San Raffaele Institute (HSR) in various fields of IIT competence, i.e. neuroscience, drug development and robotics.** The groups have an established tradition of excellence, documented by patents and publications, widely recognized and appreciated at the international level. In addition, they have participated in the organization and operation of technological initiatives aimed at the generation of new research tools and the development of scientific results towards application. Therefore, their participation in the Unit does not change their activity but offers them the possibility to expand their perspectives. Collaborations with groups active in other Units and with groups destined to join the IIT in the near future are already going on. In the future we expect them to be further expanded by the participation of other groups, and also qualified by the increased technological potential and impact of the IIT enterprise.

In order to introduce the role that the HSR Unit intends to play in the IIT Network program let's first briefly summarize **a few important aspects of the whole HSR**. Collaboration among research groups in terms of both scientific and technological research is continuous and extremely fruitful. Along this line, HSR has developed **advanced facilities** in several important research fields, from protein sequencing to transgenic and knock-out mice; from in vivo imaging to microchip RNA analysis, that have proven to be of key importance not only for the advancement of the work but also for the widening of the technological know-how. In addition, the recent contract with IBM for the **installment of the megacomputer Blue GeneP** is expected to result in a real explosion of bioinformatics, with ensuing major and precious fall-out in all fields of bio-science and bio-technology. Finally, **HSR includes a famous hospital**, leader in the world in a number of important areas, including in particular medical neuroscience, where research is carried out at very high level. The vast opportunities offered by this environment, which so far have been essential for the development of the internal groups, including those of the HSR Unit, will now become open to the entire IIT network.

All the 7 workpackages of the HSR Unit have distinct scientific profiles, illustrated in detail in the following presentations. WPs 1, 2 and 5 have strong background in **cellular neuroscience**, oriented however to distinct technological and applicative developments. WPs 3 and 4 have long and innovative experience in the field of innovative drug development. WP 6 is specialist in **fluorescence spectroscopy** while WP 7 is active in the **contact area between neuroscience and robotics**. All groups have research support from external sources for studies adjacent to, or coinciding with those of the IIT Unit. Therefore support to the studies presented in this document **can be envisaged as a real co-funding**. For the first few months the Unit will begin its activity in the laboratories already occupied by the participating groups. Soon, however, **additional space, dedicated specifically to the IIT Unit**, will be assigned in the new research building of the HSR area. We envisage this as an important step to strengthen our coordinate activity and to facilitate external collaborations. Finally, **a new branch of the PhD**

School in Molecular Medicine, oriented specifically to Biotechnology of Neuroscience, has been recently opened for two 4year cycles, sponsored by both the IIT and the San Raffaele University. The Unit and the School will work in strict collaboration, assuring the students continuous contacts with IIT and introducing them to qualified research work oriented towards technology.

WORKPACKAGES

1. WP1 – Multiplicity of exocytoses: role of specific forms in physiology and pathology

P.I.: Prof. Jacopo Meldolesi

Senior scientists: dr. Evelina Chieregatti, dr. Emanuele Cocucci

Graduate student: dr. Carlotta Giorgi

This project refers to the IIT program, B section, points: 1.1.1.: single molecule, single bio-event monitoring; 1.2.1.: delivery of nanoprobes to cells.

Introduction

Regulated exocytosis is the process by which, upon stimulation, cytoplasmic organelles fuse with the plasma membrane, opening their lumen to the extracellular space. For decades regulated exocytosis has been identified with quantal release of secretion products (neurotransmitters, hormones etc.), unique for timing (from min to 0.1 msec), specificity and discrete localization. Recently, however, it has become clear that many forms of exocytosis do not serve (or do not serve only) to discharge products but to modify the surface expression of important molecules (i.e. receptors, transporters, pumps) or to expand the surface membrane. The latter can be necessary for important functions: wound healing, surface enlargement, differentiation, cell movement. Therefore exocytoses have been divided in two groups, defined secretory and non-secretory (Chieregatti & Meldolesi, Nature Rev. in Mol. Cell. Biol. 6:181-7, 2005). The organelles of non-secretory exocytoses are still unknown except for a few, including one identified in our laboratory, the enlargeosome (Borgonovo *et al*., Nature Cell Biol. 4:955-62, 2002). Non-secretory exocytoses play key roles not only in physiology but also in pathology. Their control, therefore, might become important for the therapy of various diseases. In this project the study of exocytosis will be developed along three lines investigated in parallel: new tools to study regulation; role of enlargeosomes and other exocytic organelles; neurodegenerative diseases.

Research plan

1st year

Our laboratory has a long experience in trans-membrane signalling investigation by fluorescent probes. So far our studies have been focused primarily on Ca2+ (see Pozzan & Meldolesi, J. Cell Biol. 142:1395-8, 1998).

Recent developments have extended the study to the array of signals regulating the exocytic process. A collaboration has been established with Acquotec, a biotec spin-off of the Universities of Ferrara and Padova, for

the production of new fluorescent probes specific for second messengers and transduction molecules. By these probes we will investigate factors regulating the exocytic process, first in neuroendocrine PC12 cells and then in neurons.

Concomitantly we intend to start investigating the role of enlargeosomes in diseases. The organelles have been characterized and are now in the process of being isolated. Whether they are involved only in the processes recognized so far or also in others remains to be established. We have already accumulated cells and tools to carry out these studies. Moreover, we will start the investigation of exocytosis in neurodegenerative diseases, that we intend to develop in the 2nd year.

2nd year

The study of new probes revealing in particular protein kinases C, B and A as well as various AKAPs, that dictate the intracellular localization of active kinases, will be continued. The work on enlargeosomes (and other exocytic organelles) will be developed towards the identification of drugs blocking specifically their exocytosis, investigated in collaboration with Tom Kirchhausen, University of Harvard, and Sienabiotec,a majorcompany of the Siena Science Park. Except for chlostridial toxins, the only agents blocking single regulated exocytoses are vacuolins, developed in collaboration with the Kirchhausen's lab (Cerny *et al*., EMBO Rep. 5:883-888, 2004). The new drugs will be tested in relation to cell growth, cell migration and cell death. It is predicted that an inhibition of the first two and stimulation of the third function might be ultimately important in the treatment of specific tumors. In neurodegeneration our aim will be the identification of the exocytic vesicles specific for □ □amyloid and the establishment of their role in pathology. For these studies we will rely on our specific experience in the isolation and characterization of organelles and in the dissection of the various steps of the exocytic process (see Chieregatti *et al*., Mol. Biol. Cell 15:1918-1930, 2004). The possible action of single proteins (i.e., synuclein, mutated in some forms of Parkinson's disease) will also be investigated.

Milestones

YEAR 1

- Selection and characterization of new fluorescent probes revealing protein kinase activation.
- Isolation of enlargeosomes and comparison with other non-secretory exocytic organelles.

YEAR 2

- Identification and characterization of new fluorescent probes.
- Isolation by high throughput screening of the first drugs blocking exocytosis of enlargeomes and other exocytic organelles, and initial analysis of their effects.
- Identification of the vesicles positive for â□ amyloid and characterization of their role in neurodegeneration.

YEAR 3-5

- Generation and characterization of new fluorescence probes, which might be made commercially available.

- Role of enlargeosomes in tumor cell growth and migration.
- Pharmacological and toxicological characterization of drugs blocking enlargeosome exocytosis.
- Pharmacology of βamyloid intracellular transport. Effects of synuclein and its mutants on various forms of exocytosis. Role of these mechanisms in various models of Parkinson's disease.

2. *WP2 – Cellular and molecular imaging of neuron-astrocyte signaling in physiological and pathological conditions*

P.I.: Prof. Fabio Grohovaz

Senior scientists: dr. Franca Codazzi, dr. Daniele Zacchetti

PhD student :dr. Ilaria Pelizzoni

This project refers to the IIT program B section, points: 1.1.1. Single molecule, single bio-event monitoring; 1.1.2. Active nanoprobes as intracellular nanorobots; and 1.3.1.Plasticity of neural circuits (learning/adaptation).

Introduction

The activity of glutamatergic synapses is recognized since many years to play a key role in the central nervous system (CNS), however the present knowledge is still fragmentary, and several aspects remain elusive, concerning in particular the integration between electrical and chemical signals triggered at each synapse by glutamate receptor activation with those generated in the close cellular environment. These aspects are important not only in itself but also for their close applicative perspectives, concerning in particular the pathogenesis of neurodegeneration.

The present project pivots on the idea that synapses perform an electro-chemical computation of both local and environmental signals they receive. In the environment the key role is likely to be played by the astrocytes, the close partners of neurons which, upon stimulation, release a number of neurotransmitters and signalling molecules (see Volterra & Meldolesi, Nat. Rev. Neurosci. 6:626-40, 2005). The integration between astrocytes and synapses would be particularly important during mild synaptic activity, when chemical signals from the first might contribute to the development of long term synaptic plasticity states, i.e. LTP or LTD. However, the interplay between glutamate and the other agents might become dangerous under condition of hyperstimulation and oxidative stress, when excess signal transduction can ultimately trigger neurodegenerative events and the ensuing diseases. We intend to investigate the development of these processes by developing and exploiting innovative molecular imaging approaches.

Research plan

The general approach anticipated in the introduction will be applied to various issues, all related to neurodegenerative processes. Their investigation will be carried out in a coordinate fashion, concentrated however in specific time windows.

1st year

The complex interplay of signalling proteins and second messengers, activated by neurotransmitters, local

mediators and/or toxic molecules, will be investigated by a coordinate approach based on the combination of both molecular/cellular biology and innovative optical imaging methods. In particular, proteins or minimal domains fused with GFP (used as readouts for signalling molecules and second messengers) will be investigated by a total internal reflection microscope setup (TIRM, Codazzi *et al*., Curr. Biol. 11:1089-97, 2001) which is being improved in our laboratory. The narrow illumination depth (< 100 nm) makes possible a very high discrimination of fluorescence changes, effective even at the synaptic level.

2nd year

In this year the study of neuron, synapses and astrocytes will be extended to investigate the role of iron in the CNS oxidative damage, a process common to various neurodegenerative disorders including Alzheimer's and Parkinson's diseases, to be carried out in collaborations with Dr Levi (UHSR) and Dr. Cabantchik (Hebrew University). New derivatives of the fluorescent probes employed so far to measure the labile iron pool (Esposito *et al*., Anal. Biochem. 304:1-18, 2002) will be identified and characterized to reveal changes of the metal in neurons and astrocytes expressing ferritin variants. Mapping of iron under conditions of altered homeostasis will be extended to the microanalytic level in single cells by the use of electron spectroscopic imaging, a high-resolution ultrastructural elemental localization procedure greatly improved in the lab several years ago (Grohovaz *et al*., Proc. Natl. Acad. Sci. USA 93:4799-803, 1996). This study will include the analysis, to be carried out also by the use of primary neurons and glial cells from knock-in mice, of cells expressing mutations of proteins, such as ferritin, participating in the regulation of iron metabolism and known to be involved in neurodegeneration. Functioning of these cells will be investigated by the imaging techniques developed in the first year.

3rd year

The study by the imaging techniques discussed so far will focus on the interplay between signalling and accumulation of toxic molecules. Special attention will be devoted to amyloid βpeptides accumulated in the CNS during Alzheimer's disease, and on BACE-1, the neuronal enzyme responsible for their generation.

TIRM will be used to investigate the translocation of specific proteins.

4th to 5th year

Application of the advanced imaging/molecular-cellular biology approach to investigate the activity-dependent de-novo protein synthesis during synaptic activity. Development of new fluorescent tools to detect transcript-specific modulation of translation in subcellular compartments of single live neurons.

Milestones

YEAR 1

- Setup of the approaches used in the project.
- Characterization of signalling in astrocytes and neuron/astrocyte cultures, with focus on prostaglandins and on stimulation by sphingosyl-phosphorylcholine.

YEAR 2

- Definition of signaling processes under conditions of iron dismetabolism.

YEAR 3

- Set up of the local assay of BACE-1 activity by fluorescent reporter genes.
- Changes in BACE-1 expression/activity in primary cultures of neurons upon environmental stress.

3. WP3 – Intelligent drug delivery by viral- like particles

P.I.: Prof. Fulvio Mavilio

Senior scientists: dr. Alessandra Recchia, dr. Catia Traversari

This project refers to the IIT Program B section, point 1.2.1. delivery of nanoprobes to cells.

Introduction

The aim of this workpackage is the design and construction of new systems of intelligent drug delivery to human cells in vitro and in vivo for the therapy of cancer and infectious diseases. The delivery strategy is based on genome-less viral-like particles (VLPs) assembled by specifically engineered cell lines, and loaded with peptides, proteins or nucleic acids (DNA or small RNAs). VLPs will be designed to be used as targeted drug-delivery vehicles and as recombinant or genetic vaccines for the immunotherapy of cancer, or for the therapy of infectious diseases refractory to conventional vaccination strategies, such as AIDS or hepatitis C.

"Intelligent" VLPs are based on the HIV virion core pseudotyped with recombinant envelope proteins (RD114-TR) capable of high-efficiency targeting of professional antigen-presenting cells in vitro and in vivo, and loaded with peptides or proteins (e.g., vascular targeting proteins, tumor antigens, viral antigens) fused to the HIVNef7 protein, incorporated at high efficiency into HIV-based recombinant virions.

VLPs will be also used to deliver small RNA molecules (miRNAs or iRNAs) with gene regulation properties under the form of non-integrating RNA pseudo-genomes, or DNA integration cassettes coupled to Nef7-fused viral integrases. The San Raffaele academic group and/or its spin-off biotechnology company MolMed S.p.A. has already protected or in-licensed part of the relevant intellectual property. Future patents generated by the IIT/HSR co-funded research will be shared by the two Institutions. On a five-year basis, the aim of this workpackage is to provide preclinical proof of principle in vitro and in vivo of the efficacy of VLPs as drug-delivery agents and vaccines, and to translate know-how, technology and to lead products into specifically designed clinical investigation.

Research plan

1st year

The basic technology for the construction of VLPs will be established, including the development of a suitable

cell line for the packaging of HIV-based VLPs. Proof of principle of the targeting capacity of VLPs will be provided by pseudotyping with the complement-resistant, chimeric RD114-TR envelope protein, which targets a neutral amino acid receptor (RDR) expressed at high levels on hematopoietic cells and particularly on professional antigen-presenting cells (APCs: dendritic cells, macrophages) (Sandrin *et al.*, Blood 100:823-32, 2002). VLPs will be loaded with different types of molecules to establish their relative delivering efficiency (small peptide drugs, tumor antigens, nucleic acids) in vitro and in murine models in vivo. Proteins will be loaded in the forms of hybrid molecules with a variant of the HIV Nef protein (Nef7), which is incorporated at extremely high efficiency into HIV-based recombinant virions (Peretti *et al.*, Mol. Ther. 12:1185-96, 2005). A number of Nef7 fusions will be tested for their efficiency of incorporations into VLPs and their biological activity upon delivery to their specific targets. Nucleic acids will be loaded as non-integrating, pseudo RNA genomes in VLPs carrying integration-deficient mutants of the HIV reverse transcriptase/integrase for high-efficiency, transient expression in the target cells. Finally, integration cassettes will be loaded under the form of RNA pseudo-genomes co-packaged with Nef7-fused viral integrases, e.g., the Rep replicase/integrase of the adeno-associated virus (Recchia *et al.*, Mol. Ther. 10:660-70, 2004).

2nd year

A number of biological assays will be developed to test the targeted delivery capacity and efficiency of VLPs, and the capacity of the Nef7 fusions in eliciting appropriate biological responses. RD114-TR-pseudotyped VLPs will be delivered to APCs in vivo by injection into the blood or lymphatic circulation, and tested for their capacity to deliver effective protein doses. In particular, viral-specific (EBV, CMV) or tumor-specific (MAGE-3) antigens will be delivered as Nef7 fusions, and tested for their efficacy in eliciting anti-viral and anti-tumor responses in established cell and animal models. Small RNA-containing VLPs will be tested as gene expression-modulating agents in vitro and in vivo models. Finally, VLPs containing integrating pseudo-genomes and Nef7-fused Rep78 will be tested for their ability to direct site-specific genomic integration into specific cell targets (APCs).

3rd to 5th year

Depending on the results obtained in the first two years, VLPs will be modified and adapted for targeting to different cell types, and particularly to the endothelial component of normal and tumor vasculature, for targeted delivery of antitumor and anti-angiogenic drugs. Furthermore, VLP production technology will be adapted from small laboratory scale to mid-size, semi-industrial scale under GMP/GLP standards.

Milestones

YEAR 1

- Development of cell lines and molecular tools for RD114-TR-pseudotyped VLP packaging.
- Production of VLPs containing Nelf7- fused drugs and tumor-specific antigens.
- Production of VLPs containing RNA and DNA pseudo-genomes.

YEAR 2

- Delivery of RD114-TR-pseudotyped VLPs to APCs in vitro and in vivo.
- Generation of viral- and tumor-specific immune responses by VLPs in animal models.
- Modulation of gene expression and integration of gene expression cassettes in APCs in vitro and in vivo.

4. WP4 – New tools for modulating endothelial barrier function and drug delivery

P.I.: dr. Angelo Corti

Senior scientists: dr. Luca Crippa

This project refers to the IIT Program B, point 1.2.1. delivery of nanoprobes.

Introduction

The penetration of drugs in tissues is often a critical step that limits their therapeutic potential. Drug resistance can arise not only due to genetic modifications, but also to lack of penetration of drugs into tumor cells distant from vessels (Curnis *et al*., Nat. Biotechnol. 18:1185-90, 2000; Curnis *et al*., J. Clin. Invest. 110:475-82, 2002). The same applies to delivery of active nanoprobes or molecular nanorobots to target cells in tissues. To reach target cells, molecules must enter blood vessels, cross the endothelium and migrate through the interstitium, all these steps representing penetration barriers. Thus, studies on the mechanisms that regulate transport of macromolecules from blood to tissues, and strategies aimed at reducing these barriers are of great experimental and clinical interest.

Previous studies had shown that chromogranin A (CgA), a protein released to circulation by neuroendocrine cells and neurons, play an important role in the regulation of vascular permeability. After stimulation, CgA is released to the extracellular environment, together with co-resident hormones, and reaches the blood stream via capillaries or lymphatic vessels (Corti & Ferrero, Curr. Med. Chem. - Imm. Endoc. & Metab. Agents 4:161-7, 2004). CgA is believed to carry out extracellular functions as a precursor of regulatory peptides with endocrine, paracrine and autocrine effects on the vascular compartment. Elevated levels of CgA have been detected in the blood of patients with neuroendocrine tumors, hepatic failure, or renal failure (Corti & Ferrero, as above). We have reported that circulating CgA is markedly increased also in patients with heart failure, depending on the severity of the disease, that it correlates with TNF and soluble TNF-receptors (Ceconi *et al*., 2002), and that increased levels of CgA can inhibit TNF-induced leakage from liver vessels (Ferrero *et al*., FASEB J. 20:20, 2004). Moreover, CgA fragments can regulate TNF-, thrombin- and VEGF-induced changes of endothelial cell shape and barrier function (Ferrero *et al*., FASEB J. 20:20, 2004); and regulate fibroblast and smooth muscle cell adhesion and spreading (Colombo *et al*., J Biol Chem 277:45911- 19, 2002b; Ratti *et al.*, J. Biol. Chem. 275:29257-63, 2000). Finally, CgA can inhibit tumor growth and morphogenesis by affecting the stromal compartment of the tumor (Colombo *et al*., Cancer Res. 62:941-6, 2002a). These findings suggest that CgA contributes to the regulation of vessel barrier function in pathological conditions. Interestingly, in patients with lung cancer a increased blood levels of CgA correlate with reduced responses to chemotherapy. These observations, suggest that CgA could be an important

regulator of the stromal and vascular compartment of tumors and, consequently, it could affect tumor physiology and response to therapy. The molecular mechanisms underlying these effects are poorly understood. Very recently we have provided evidence suggesting that CgA interacts with membrane phosphatidylserine (Blois *et al.*, Regul Pept. in press 2006).

The aim of this project is to clarify many properties of the CgA action and explore its use as a drug carrier. In particular we intend a) to understand its pathophysiological role, b) to characterize its molecular mechanisms, c) to assess whether CgA and or/its fragments can be exploited to generate nanoprobes for in vitro and in vivo imaging of activated endothelial cells (exposing phosphatidylserine) and apoptotic cells in tumors and inflammed tissues, d) to study CgA fragments as ligands for the targeted delivery of drugs or particles to tumor vessels, or as compounds modulating vascular permeability and the penetration of drugs in tumors.

Research plan

1st year

We will investigate the CgA action to obtain results necessary for the subsequent steps of our work. In particular we intend to a) characterize the circulating levels of CgA and inflammatory molecules in patients with tumors and inflammatory diseases; b) to produce by recombinant DNA technology and peptide synthesis various CgA N-terminal fragments, and prepare conjugates with fluorescence compounds, gold particles, quantum dot nanoparticles; c) investigate the interaction of these nanoprobes with the plasma membrane of resting, activated and apoptotic endothelial cells (exposing phosphatidylserine) and other cells of the tumor microenvironment (fibroblasts); d) study the biological effects of CgA fragments on endothelial cell permeability, adhesion, proliferation and migration in vitro.

2nd year

We intend to investigate: a) the CgA receptors and the signalling pathways activated in endothelial cells by the protein and its fragments, alone or in combination with factors that increase vascular permeability (VEGF, TNF); b) the effects of CgA and its fragments on tumor growth in vivo, in animal models; c) the structural determinants of the biological effects exerted by CgA; d) the CgA fragments and/or monoclonal antibodies that inhibit the effects of CgA on endothelial cells.

Subsequent years

The research activities will be focused on the development of animal models suitable for testing the CgA-based molecular nanoprobes for their ability to image activated endothelial cells and apoptotic cells in tumors and in inflammatory diseases, in vitro and in vivo, and for assessing whether CgA fragments could be exploited as ligands for targeted delivery of drugs or other particles to tumor vessels. These models will be used also for investigating combination therapies with CgA fragments that modulate vascular permeability.

Milestones

YEAR 1

- Production of active CgA fragments.
- Revelation of their interactions with membranes and their functional effects.

5. *WP5 – Optical approaches to the study of neuronal plasticity*

P.I.: Prof. Flavia Valtorta

Senior scientists: dr. Andrea Menegon, dr. Chiara Albertinazzi

This project refers to the IIT Program B section, points 1.1.1.: Membrane trafficking and protein-protein interactions in living neurons; 1.3.1.: Optoelectronic biosensors for the analysis of neuronal networks.

Introduction

Neurotransmitter release from nerve endings is the main form of information transfer from one neuron to another or to an effector cell. Release occurs by exocytosis from storage organelles, the synaptic vesicles, and is triggered by the action potential-induced depolarization of the nerve terminal.

The features that make synaptic vesicle exocytosis unique among other forms of regulated secretion are the temporal and spatial regulation of the process (which occurs on a hundreds of microsec scale and exclusively at specialized release sites, the active zones) and its high modulability. Under resting conditions a fraction of synaptic vesicles appear to be held apposed to the plasma membrane at release sites, thus maximizing the efficiency of excitation-secretion coupling. The basis for the high modulability of release are less well understood. This feature is of paramount importance for information processing in the central nervous system and for adjusting the efficiency of a synapse to the physiological needs of a neuron. In addition, it represents a safety mechanism which confers to neurons a high resistance to fatigue, and allows a diseased neuron to function for a long time before exhibiting overt failure. Whereas action potentials are stereotyped events characterized by constant amplitude, the amount of neurotransmitter released following each action potential is subjected to modulation depending on both previous stimulations and the release of other neurotransmitters by the same or by neighboring cells.

Our laboratory participates since many years to the study of the molecular and cellular mechanisms that underlie the phenomenon of neurotransmitter release. From a technological point of view, we have a long standing interest in the development and implementation of optical approaches to the in situ study of signal transduction and protein-protein interactions in neurons. We have been the first to utilize FRET coupled to video-enhanced microscopy to dynamically investigate single, living synaptic terminals. We have also implemented a computer program for the acquisition and automated analysis of images which allows to evaluate molecular interactions in single synapses. This procedure has been made available to other laboratories (Dunlap and Valtorta, An Image Pro-Plus Macro for FRET Measurement. Media Cybernetics Application Note, 2003).

Research Plan

1st year

1.1.1 Membrane trafficking and protein-protein interactions in living neurons

Based on our extensive experience with GFP-technology, we will develop novel probes for the analysis of membrane trafficking in living neurons. Particular attention will be devoted to the development of expression systems which induce minimal perturbation of cellular activity. HIV-derived lentiviral vectors will be engineered to express neuronal proteins fused to spectral variants of green fluorescent protein.

These viral vectors, designed for gene therapy protocols, are particularly suitable for efficient and stable transduction of non-dividing cells, such as neurons, without compromising their viability and developmental program. These probes will be employed for the analysis of the molecular mechanisms underlying protein targeting to specific subcellular compartments, and for the FRET analysis of protein-protein interactions aimed at defining the cascade of molecular events at the basis of synaptic transmission and development.

1.3.1 Optoelectronic biosensors for the analysis of neuronal networks

Novel tools and technologies can arise from the use of optical probes for recording and stimulating neuronal activity with high-resolution. For these purposes, in collaboration with the Department of Bioengineering of the Politecnico di Milano, we are planning to develop and build new systems based on optical microtechnologies in order to use caged compounds for the focal stimulation of neurons, and voltagesensitive dyes for neuronal recording (Ghezzi et al., Biosystems, in press). This combines high-resolution stimulation of neuronal activity (by caged neurotransmitters such as caged glutamate) or high-resolution modification of neuronal activity (by intracellular caged probes such as caged Ca2+ or caged Ca2+ chelators) with quite high-resolution imaging of the neuronal activity that flows in the neuronal network (by voltage-sensitive dyes). The Department of Photonics of the European Commission Joint Research Center of Ispra and the small enterprise Optotec will collaborate to implement optical recording technologies with the appropriate temporal resolution to detect fast events.

The ability to follow and clarify the molecular mechanisms at the basis of the in vitro neuronal network behaviour is the first step toward the transfer of these techniques to in vivo systems. The data obtained from the in vitro analysis could also be useful for the development of neuronal-electronic interfaces and bioelectronic prostheses.

3rd to 5th year

In vitro and in vivo FRET analysis of protein-protein interactions in neurotransmitter release; use of optoelectronic biosensors for the analysis of the activity of networks of neurons isolated from various brain areas and screening of the drug's effects (with special reference to antiepileptic drugs) on the network activity.

Milestones

YEAR 1

- Construction of the molecular probes (plasmids, viral vectors).
- Upgrading of the available equipment.

- Construction of a prototype of an optoelectronic biosensor for neural network analysis.

YEAR 2

- Analysis of the molecular determinants for the selective targeting of proteins to neuronal compartments.
- Fine-tuning of the conditions for culturing primary neurons on the opto-electronic biosensor and for the analysis of the global activity of the neuronal network.

6. WP6 – Dynamics of single molecule and single bioevent in living cells revealed by fluorescence fluctuation and time resolved fluorescence spectroscopy

P.I.: dr. Valeria R. Caiolfa

Senior scientists: dr. Moreno Zamai

This project refers to the IIT Program B section, point 1.3.1.: Optoelectronic biosensors for the analysis of neuronal networks.

Introduction

The cell membrane is an active player in molecular signal transmission as well as in cell-to-cell recognition and cross talk. Protein interactions and signalling are guided by an array of coordinate processes including sorting of proteins from intracellular compartments to the cell membrane; segregation and partitioning in membrane domains and molecular arrangement of the cell surface. The spatio-temporal regulation of protein interactions might be a key for understanding their specific activation of signal transduction cascades. To define these processes, we need to follow protein interactions in living cells not just in space but also in time, with introduction of only minimal perturbation and reaching nanometric (quasi-molecular) resolution. Fluorescence fluctuation spectroscopy (FFS) is known since long to detect the intensity of fluctuations resulting from the diffusion of one (or a few) fluorofore in a confocal or multiphoton-excited volume (Magde *et al*., Phys. Rev. Lett. 29:705, 1972). The dynamics of protein interaction (multiple diffusion coefficients, brownian versus restricted diffusion) and interactions of molecules in the cell membrane (Qian & Elson, Proc. Natl. Acad. Sci. USA 101:2828-33, 2004; Chen *et al*., Biophys. J. 82:133-44, 2002) can therefore be followed by this approach in living cells engineered to express labelled proteins (GFP and spectral variants), without artefacts due to protein overexpression. In addition, time resolved fluorescence (FRET, FLIM) can reveal the molecule environment (van Munster & Gadella, Adv. Biochem. Eng. Biotechnol. 95:143-75, 2005; Duncan *et al*., J. Microsc. 215:1-12, 2004).

Detailed and quantitative analyses of the dynamics of spatially and temporally resolved molecular interactions

can therefore be carried out based on these approaches. In this project we intend to apply them to solve a variety of processes concerning sorting and/or internalization of proteins as well as their multiple interactions and partitioning in the cell membrane. Our probes will be both membrane proteins and receptors labelled with EGFP and spectral variants and extracellular ligands labelled with single or multi-photon suitable fluorofores. A novel cell scanning multi-photon and confocal single photon counting spectrophotometer was designed in collaboration with ISS Srl, the european satellite of ISS Inc (www.iss.com), a manufacturer of researchgrade instrumentation for fluorescence spectroscopy with documented record of successful projects in collaboration with academic groups.

Research plan

1st year

Our first objective is to consolidate in our laboratories the platform for multiple wavelength, single photon fluorescence correlation and crosscorrelation spectroscopy in living cells, based on our experience in fluorescence correlation spectroscopy (FCS, FCCS) for quantifying the dynamics of cell membrane receptors and the stoichiometry of the protein complexes (Zamai *et al*., Biophys. J. 88:255A, 2005; Malengo *et al.*, Biophys. J. 88:588A, 2005). A number of conditions for FCS and FCCS experiments will be tested on cells expressing the fluorescent chimera of the GPI-anchored urokinase plasminogen receptor (Blasi & Carmeliet, Nat. Rev. Mol. Cell. Biol. 3:932-43, 2002), and suitable mutants that lack one or more properties of the wt receptor.

2nd year

The following step includes the development of novel approaches for fluorescence lifetime imaging (FLIM) in living cells, to carry out rigorous quantifications of FRET efficiency revealing selected protein-protein interactions. Suitable cell models will be developed also for FLIM, using the newest spectral variants of GFP. Recruitment of proteins in membrane rafts and their oligomerization will be analysed by FRET-FLIM. Fluorescence cross-correlation spectroscopy will be applied to investigate the interaction and dynamics between integral membrane proteins such as integrins and GPI-anchored receptors. The motility of the molecules will be analysed using different physical models (Brownian, sub-diffusions and corallility). We will also study the effect of cholesterol depletion and disruption of the cytoskeleton on these molecular interactions.

3rd to 5th year

Depending on the results obtained in the first two years, we plan to attempt FFS applications to moving or migrating living cells using raster image correlation spectroscopy and/or scanning fluorescence correlation spectroscopy.

Milestones

YEAR 1

- Set up of a multi wavelength FFS platform and development of suitable cellular models based on GFP-labelled GPI-anchored receptors.

YEAR 2

- Combined FCS-FLIM description with nanometric resolution of receptor associations and dynamics in living cells.

Deliverables (Indicators for evaluation)

YEAR 1-5

- Experimental protocols and analysis methods for FCS, FCCS, FLIM in living cells at single molecule resolution.
- Vectors and fluorescently engineered cellular models.

7. WP7 – Haptics in neuroscience and robotics

P.I.: dr. Gabriel Baud-Bovy

This project refers to the IIT program B section, points: 1.3.3., human behaviour.

Introduction

In most human activities, the hand is the organ of choice to interact with the environment. The motor redundancy of the hand and its rich sensorial endowment render the analysis of multi-fingered grasps a challenging task, both at the experimental and theoretical levels (Baud-Bovy & Soechting, J. Neurophysiol. 86:604-615, 2001; Baud-Bovy *et al*., in Barbagli, Prattichizzo, & Salisbury (Eds.)

Multi-point interaction with real and virtual object. Springer Tracts for Advanced Robotics, vol. 18, p. 21-40, 2005). Recent studies have suggested that multifingered grasping involves complex synergies that depend on the internal representation of the task constraints, including the effector's and object's properties.

While it is well acknowledged that a distributed network of interconnected motor and sensory areas controls hand and finger movements, much remains to be understood about the contributions of the individual areas (reviews in Castiello, Nat. Rev. Neurosci. 6:726-36, 2005; Schieber & Santello, J. App. Physiol. 96:2293-300, 2004; Rizzolatti & Lupino, Neuron 31:889-901, 2001). At the technological level, the development of experimentally validated models of the human grasp can contribute to the design of multi-contact haptic interfaces, which are crucial for the development of new applications, such as robot-aided rehabilitation, tele-manipulation, minimallyinvasive surgery, product prototyping and human-computer interaction (Srinivisan, in Stanney (Ed.), Handbook of Virtual Environments: Design, Implementation, and Applications, Lawrence Erlbaum Assoc., 2002). The general objective of this work package is to yield a better comprehension of the synergies, internal models, and brain mechanisms involved in complex manual tasks such as grasping and manipulating an object.

Research Plan

1st – 2nd year

Progress in motor control is often impeded by the need to build ad-hoc hardware to interact with the subject. To study the mechanisms of manual dexterity, we intend to use a recent robotic technology - the haptic interface - that permits the software control and simulation of any type of mechanical interaction with the environment.

This technology is useful to study, for example, how subjects cope with unexpected perturbations by applying short force pulses to the grasped object, or the role of visual and kinaesthetic information by simulating in a virtual environment the reaction force caused by a contact. In addition, this technology has a promising potential for rehabilitating the manual dexterity of patients with sensory or motor deficits.

The initial objective of this workpackage will be to understand how the motor system controls finger forces during and just before an impact. Unlike previous studies that involved a single joint or the pinch grasp, the number and position of the fingers, the type of grasp and direction of impact will be systematically varied. The consequence of a self-initiated vs. external perturbation will also be investigated. The hypothesis is that the motor system is able to implement an optimal trade-off between opposite requirements such as maximizing the stability of the grasp and minimizing energy expenditure. The properties of the grasp will be analyzed using mathematical models developed to control artificial robotic hands.

Transcranial magnetic stimulation (TMS) is the only technique that allows routine non-invasive evaluation of the excitability and conductivity of cortico-spinal motor pathways. This technique will be used to disentangle the roles of the various brain areas contributing to the control of the grasp by either stimulating (low-intensity single pulse TMS) or disrupting (repeated TMS) the normal processing of information. In addition, TMS and other neuro-physiological techniques will be used to monitor changes in cortical and spinal excitability. Pairing EMG files with data recorded by other devices is labor-intensive and error prone when the experimental conditions vary from trial to trial, or when some trials need to be discarded or repeated. These experiments will therefore require the custom integration of EMG amplifiers/filters and digital/analogical conversion cards because the available clinical EMG systems cannot be controlled remotely (except for a trigger signal).

Milestones

YEAR 1 – YEAR 2

- Development of the robotic platform to control the dynamic of the object manipulated while measuring contact forces and EMGs.
- Study of contact forces in complex grasps during the manipulation of an object.

YEAR 3

- Study of complex grasps in patients with sensory and motor deficits and of the underlying brain mechanisms (by TMS).
- Development of a theoretical framework that will provide objective measures of the quality of grasp and/or manual dexterity.

YEAR 5

- Evaluation of the potential of haptic interfaces for clinics and rehabilitation.
- Development of haptic devices and man-machine interfaces based on experimentally validated models of the human grasp.

QUALIFICATION OF THE RESEARCH UNIT

The San Raffaele Institute was established in 1971 and represented one of the first examples of a fully independent private hospital in Italy. Shortly thereafter, the San Raffaele became a sponsoring establishment of the State University of Milan Medical School, and was granted the status of IRCCS, making the hospital a site for clinical research of national interest. As such, the Institute was originally specialized in diabetes and metabolic disorders. In 1992 the San Raffaele expanded further by creating the DIBIT (Department of Biological and Technological Research), a basic science institute with a dedicated research space of about 12,000 square meters, which nowadays employs 270 people, including scientists, technicians and administrative personnel, along with more than 100 fellows, trainees and graduate students. DIBIT is part of the largest biomedical science park in Italy, which includes the San Raffaele Hospital with 1036 beds, the Science Park Raf created to support the Foundation's development objectives, and the University. The University Vita-Salute San Raffaele started its operations in 1996 with a degree course in Psychology, followed by the new faculty of Medicine and Surgery in 1998, and a University Course in Biotechnology, University Courses in Nursing and in Physiotherapy, and the new Faculty of Philosophy in Cesano Maderno in 2002.

The teaching activities also include an international Ph.D. program in Cellular and Molecular Biology, co-sponsored by the Open University of London and the Vita-Salute university San Raffaele, and the more recent International Ph.D. Program in Molecular Medicine.

Much effort and many resources have been invested in basic preclinical and clinical research. The Institute's scientific production experienced quite an impressive progress as regards both the number of publications and the overall quality. In 2002, 537 scientific papers were published, with a total impact factor of 2504. These figures confirm the Institute's leading position in the country and contributed to a significant increase in the extramural financial support, provided by various public and private sources. In 2000, the San Raffaele Institute has invested in research a budget of ITL 50 billions. Italian public sources (Ministry of Health, Superior Institute of Health, Ministry of Education, University and Research, and CNR), and the European Community funded roughly 50% of

such amount. The remaining 50% is funded by grants provided by private charities, including Telethon and the Italian Association for Cancer Research (AIRC). Telethon has funded two research centers at San Raffaele: the San Raffaele-Telethon Institute for Gene Therapy of Genetic Disease (HSR-TIGET) which is pioneering the clinical application of gene transfer technology and the Stem Cell Research Institute (SCRI), which studies the biology and the potential clinical applications of stem cells: a field in which San Raffaele Institute is one of the leaders in the world.

Diabetes and metabolic disorders and biomedical technologies have been the historical areas of the San Raffaele's scientific leadership. In the last years biomedical technologies have undergone an impressive development and diversification, opening the doors to a rapid evolution of clinical medicine: into "molecular medicine". The complete sequencing of the human genome has radically modified the scientific scenario. In the years to come, researchers' efforts will be dedicated to the elucidation of the mechanisms of regulation and expression of genes and the functional characterization of their products, the identification of pre-symptomatic diagnostic tools and the design of appropriated therapeutical approaches. The Scientific Institute San Raffaele claims the merit of having interpreted from the beginning this novel approach to medicine, in which basic and clinical researchers and physicians operate side by side, with the goal of improving the translation of basic research into medical practice. The need to combine basic and clinical research has prompted the reorganization of the Institute in Biomedical Departments, which include research programs of strategic institutional interest.

www.sissa.it

Research Unit IIT – Scuola Internazionale Superiore di Studi Avanzati (SISSA)

Scientific coordinator of the RU : Prof. Paolo Carloni

SCIENTIFIC OBJECTIVES

- ***WP 1 Molecular simulation for biological sciences***

  P.I.: dr. Paolo Carloni, dr. Cristian Micheletti, dr. Stefano Gustincich, dr. Anna Menini

- ***WP 2 Neurotelemetry: Remote acquisition and manipulation of neuronal signals***

  P.I.: dr. Mathew E. Diamond

- ***WP 3 Development of new nanodevices for neurobiological applications***

  P.I.: dr. Vincent Torre

The contribution that SISSA offers to the IIT network project is the combination of cutting-edge theoretical techniques from statistical and computational physics with those from biology and neurogenomics to provide (i) an integrated approach to the understanding of brain function starting from genes and molecules and culminating in behavior, and (ii) a direct link between Neuroscience and Robotics. Within this framework, SISSA will support for the next five years the following new scientific initiatives which will profit from the existence of the IIT network.

WORKPACKAGES

1. WP1 – Molecular simulation for biological sciences

P.I.: dr. Paolo Carloni, dr. Cristian Micheletti, dr. Stefano Gustincich, dr. Anna Menini

We propose an integrated computational and experimental approach based on two experimental activities present at SISSA, i.e the investigation of (1) the molecular mechanism of Parkinson disease, and of (2) the molecular mechanisms of odor molecules recognition. Our work will be of applicative technological interest: Indeed, (1) the development of a structural model for alpha-synuclein, a validated target for Parkinson's disease, will allow **rational drug design**; (2) the knowledge of the molecular mechanisms underlying the high sensitivity and the capability of discriminate among thousands structurally diverse odor molecules are of applicative interest for **developing an artificial nose based on mechanisms similar to those employed by living organisms**.

The investigation of the key molecular recognition events involved in the complex neurobiological processes (1)-(2) requires the structural information of its components. The powerful combination of computational physics tools, statistical mechanics along with molecular biology, electrophysiology and functional genomics will provide such information.

SISSA will also establish collaborations in molecular simulation for biological sciences with other groups in the IIT-network, like for instance the one at SNS.

Structural and functional genomics and drug design

Parkinson's Disease (PD) is the second most common progressive neurodegenerative disorder, affecting 1-2% of all individuals above the age of 65. PD is characterized by muscle rigidity, resting tremor, bradykinesia and gait disturbance with disequilibrium. The neuropathological hallmark in post-mortem brains is the selective degeneration of specific subsets of mesencephalic dopaminergic (DA) cells and the formation of cytoplasmic aggregates called Lewy Bodies (LB). The loss of DA synapses in the striatum is believed to be the primary cause for disruption of the ability to control movements. At present, the dopamine-related motor features of PD can be temporarily improved by the use of L-DOPA or dopamine agonists. The benefit of such treatment gradually disappears after approximately 5 years in parallel to the continuing death of DA neurons. Life expectancy following diagnosis is 20 years. Thus, because the rising average age of the population, the impact of PD is expected to

increase even further. Therefore, there is an urgent requirement to understand the molecular mechanisms of the disease and to develop a therapeutic treatment to halt the progression of neuronal dysfunction.

The identification of genes associated with rare forms of early-onset familial PD has provided novel insights into the molecular mechanisms of pathogenesis. Among the genes and mutations identified so far, our lab focuses on two key and widely investigated proteins, alpha-synuclein (PARK1, OMIM 168601, 4q21-q23; PARK4, OMIM 605543) and DJ-1 (PARK7, OMIM 60324, 1p36) (9-11).

(i) Ligand binding to Alpha-synuclein (AS)

The role of AS for PD has been confirmed by its localization in the Lewy bodies, fibrillar intracytoplasmic inclusions that are the histopathological hallmark of PD. Thus, the derangement of AS metabolism is believed to contribute to the pathogenesis of sporadic PD. Furthermore, it is believed that inhibition of AS activity or expression may be a valid approach to develop new therapeutics for PD. One of the few chemicals that so far have shown to affect AS derangement, is dopamine (Conway et al, 2001; Rochet et al, 2003; Norris et al 2005; Hong-Tao et al, 2005).

In this project, we plan to investigate dopamine-binding to AS along with an investigation of its elastic properties. Subsequently, we will extend our investigation to other molecules, (namely short peptides), which can have beneficial effects by binding to the protein.

AS-Dopamine interactions. Because AS is a natively unstructured protein (Bennet et al 2005; Halbach et al 2005; Bussel et al 2005; Maguire-Zeiss et al 2005), the investigation of ligand-binding to the protein is clearly a very difficult issue. Here we will consider large ensembles of the proteins which stem from the experimental evidence provided by NMR data, which have provided a wealth of distance restraints between pairs of amino acids at large spatial separation. Unlike traditional NOEs, these distance restraints do not identify a unique conformation . The problem of establishing the most probable set of structures compatible with the available distance measurements is an open issue that we plan to address within an already-established collaborative framework with the international groups that have carried out the above-mentioned pioneering structural studies (Dedmon et al 2005; Bertoncini et al 2005, Rasia et al 2005). These groups have followed a computational protocol that has produced putative AS conformers starting from experimental measurements.

In addition to this, models of AS in solution and attached to membrane will be constructed by performing multi-nanosecond MD simulation of the protein from its NMR structure in micelle (Ulmer et al 2004). The analysis of Hess (Berk Hess, 2002) will be carried out to test the degree of convergence of the structural determinants. On such models, by using a computationally unexpensive yet relatively accurate methodology developed in our group, the Beta-Gaussian model (Micheletti et al., 2004), we will identify the slow modes of the various AS representatives and compare them in order to identify (a) common large-scale (functional) movements across the representatives and hence (b) obtain clues about the location of key sites for the modulation of the affinity for

binding substrates. The coarse-grained beta-Gaussian model will also be used to probe the extent to which various amino acids affect the proteins' elastic properties and the related affinity for lipid surfaces. This analysis, previously validated for a number of proteolytic enzymes (Piana et al., 2002; Micheletti et al., 2004; Cascella et al., 2005) may allow to understand better the role played by a number of key sites whose mutation (associated with the onset of Parkinson's disease) have been found to influence the above-mentioned properties (Bussel 2004).

Dopamine will be dock on representatives of this structure by using docking programs such as autodock (Morris et al, 1998; Goodsell and Olson, 1990). The free energy of binding of most stable adducts will be investigated by metadynamics simulations (Laio and Parrinello, 2002; Iannuzzi et al, 2003; Laio et al, 2004; Micheletti et al, 2004; Laio et al, 2005; Gervasio et al, 2005). The model of dopamine/AS complex will be validated by performing mutating those residues that are involved in dopamine binding. We will then characterize the ability of AS mutants to retain their binding to dopamine and the effects of this binding to the fibrillation process. Furthermore, we will construct stable transfectant cell lines in neuroblastoma SH-SY5Y cells to investigate the effects of dopamine treatment on cell viability both in wt over-expressing AS, in cells with PD-linked AS mutants and with dopamine-binding uncompetent AS molecules.

Peptide-AS interactions. We will isolate small peptides (aptamers) that bind AS with high affinity to develop new pharmacological treatment for PD. The strategy of using small aptamers has been widely validated. They selectively interact with target proteins and interfere with protein complexes. These peptides can be isolated as interactors by using a yeast two-hybrid screening: a random peptide library is inserted into the active site of the E. coli Thioredoxin scaffold protein and assayed against selected baits. This approach has been successfully applied to identify peptides able to bind to and block cdk2 activity and E6 oncoprotein (Colas et al 1996; Butz et al. 2000). Most importantly, aptamers provide a platform for the design of pharmacologically active small molecules that target specific proteins and protein-protein interactions involved in diseases (Hoppe-Seyler et al 2001, JSBMB 78:105-111). As a first step in the dissection of protein networks relevant for PD pathogenesis, we are currently isolating aptamers that bind alpha-synuclein in a yeast two-hybrids assay. Using LexA-Synuclein A53T as a bait, we screened 108 yeast colonies of a library containing combinatorial random variants of 16 amino acids cloned into the E. coli Thioredoxin gene fused to the B42 transactivation domain and a nuclear localization signal.

Once isolates, we will investigate the interactions between AS and the Synuclein Binding Aptamers (SBA) via NMR experiments. By titrating the protein with the aptamers, we will be able to see in the spectrum AS's residues mostly affected in these interactions. Thus, we will be able to establish contacts between residues of the protein with those of the peptides. We will then perform MD simulations of the peptides and AS in which distance constraints between these residues will be imposed. These data will be obtained experimentally to map the residues of alpha-synuclein involved in the interaction with each aptamer. To validate the model, we plan to perform pull-down and co-immunoprecipitation experiments. Based on the final MD structural models, we will estimate the

change in free energy of binding by accurate (yet computationally costly) free energy calculations (Jorgensen et al. 1988; Kollman et al 1993), as well as electrostatic calculations based on the Poisson-Boltzman equation (Settanni et al, 2003). These calculations are much faster yet provide only an approximate estimate of the change of electrostatic free energy associated to the mutations. Both approaches will be used to identify eventual peptides with even higher affinity. This project will strongly benefit from the diverse expertise of the applicants and by a plethora of international collaborations, Prof. Dobson (University of Cambridge, UK) and Dr. Fernandez (Max Plank, Gottingen, Germany), with NMR data of alpha-synuclein structures in solution. Dr. Pastore (MRC, London), with NMR data of aptamers binding and as in solution.

(ii) Role of DJ-1 protein for oxidative stress

Genetic data suggest that specific mutations of DJ-1, a dimeric protein involved in the cellular response to oxidative stress, lead to neurodegeneration of dopaminergic neurons of the midbrain and, ultimately, to PD.

We plan to investigate the effects of such mutations for PD structure and function in reduced and oxidized states using molecular dynamics simulations based on DJ-1 structure. Furthermore, we take advantage of a recently discovered DJ-1 partner expressed in the brain, TTRAP, to unveil the molecular mechanisms at the basis of DJ-1 mediated neuroprotection against oxidative stress. TTRAP may act both as a transcriptional regulator and as an endonuclease sharing significant structural homology and biochemical activity with APE1/Ref-1, a major player in cellular oxidative stress response. We will investigate the molecular interactions between DJ'1, its partner TTRAP and TTRAP'related protein APE1/Ref'1 by molecular docking and molecular dynamics simulations (Rodriguez-Lima et al, 2001).

The model will be validated by mutagenesis experiments similarly to previous work (Pantano et al., Proteins 2005). We will then describe the expression of TTRAP and APE1/Ref-1 in PD brain and in mouse models of the disease. Finally, we will assess their functional interaction in the transcriptional regulation of genes involved in the control of apoptosis and survival and in the DNA damage response in the nucleus and mitochondria. To do so we will take advantage of DJ'1 knock-out mice and lymphoblastoid cells of PD patients.

Sudies (i) and (ii) collate different though highly integrated expertise. Stefano Gustincich will provide expertise in molecular and cellular neurobiology. This laboratory presents expertise in biochemical and functional analysis of neurochemical and genetic models of the disease. Furthermore, with a large network of international collaborations, the coordinating center is providing human specimen form post mortem brains and patients. Hystochemical analysis of protein expression in different brain regions are performed at the confocal microscope with identification of specific neuronal subtypes by double labeling confocal microscopy. The group of Paolo Carloni will use simulation techniques to provide insights on DJ-1 oligomeric state and mutants as well as on the interactions with DJ-1 molecular partners. This atomistic approach will be complemented with the expertise available in the group of Cristian Micheletti. The latter will, in fact, build on the established expertise on the

topological modeling of protein folding and functionality to (a) explore new schemes for identifying AS representatives from available NMR data and (b) identify the putative functional movements of AS representatives and related binding sites.

Milestones

YEAR 1

- Identification of AS residues involved in dopamine binding
- Isolation of AS-binding aptamers in yeast
- MD simulation of DJ-1 mutants

YEAR 3

- Functional assessment of non covalent-binding of AS-dopamine
- Validation of AS aptamers
- Assessment of DJ dimer in neuroprotection
- Description of DJ-1-TTRAP interaction

YEAR 5

- Development of new therapeutics in PD

Chemical sensors: from biological olfactory systems to an artificial nose

The knowledge of the molecular mechanisms underlying the sense of smell is very important both for basic research and for building an artificial nose based on biological mechanisms.

Olfactory systems have two remarkable properties: the first is the capability to detect and discriminate among thousands of structurally diverse odorant molecules; the second is an amazingly high sensitivity that allows the detection of very low amounts of specific odorant molecules even in the presence of high noise due to the presence of many other chemicals at high concentrations.

In spite of the fact that our knowledge of the molecular mechanisms underlying olfaction has greatly improved in the past 15 years, the questions of how the olfactory system finely discriminates among similar molecules and how reaches a very high sensitivity are still far from being answered. Several hundred odorant receptor genes have been identified and some of them can now be expressed in cell lines; their selectivity among odorant molecules can be studied with imaging techniques.

The molecular mechanisms of odor discrimination and high sensitivity will be studied by a combined experimental and theoretical approach. Binding of odorants to receptors causes, via G protein activation of adenylyl cyclase, the production of a cyclic nucleotide, cAMP, which directly opens CNG ion channels in the plasma membrane, allowing calcium ions into the cell. Olfactory sensory neurons maintain an unusually high intracellular concentration of Cl ions, and the increase in the internal Ca concentration causes the opening of Ca-activated Cl

channels, whose molecular identity is still unknown, that produce an efflux of Cl ions from the cilia contributing to the olfactory neuron depolarization. The complex calcium-calmodulin exerts a feedback action on the CNG channel by decreasing its open probability and activating a phosphodiesterase that will reduce the concentration of cAMP.

Electrophysiological recordings will be obtained from individual olfactory sensory neurons, where the binding of odorants is transduced into an amplified electrical signal. By selectively activating or blocking the activities of the various proteins involved in the signal amplification the molecular basis of the high sensitivity will be examined. Molecular models of proteins involved in the olfactory process will be obtained. The origin of the selectivity of odorant receptors will be investigated by a combination of computational and experimental approaches. The binding site of odorant receptors will be identified by site-directed mutagenesis of specific amino-acids, as indicated by the computational approach, and the altered functional properties will be assessed by calcium imaging measurements after expression of the mutated receptors in heterologous cell lines. The physiological role of the accessory odorant binding proteins will also be investigated by combining a study of the energetics involved in the binding or unbinding of odors to these proteins with functional experiments.

The understanding of the molecular mechanisms employed by these biological olfactory sensors to achieve their extraordinary properties of discrimination and sensitivity will be of interest for the development of an artificial nose that operates on the same principle used by living organisms.

Milestones

YEAR 1

- Energetics of binding and unbinding of odors to odorant binding proteins.
- First model of the binding site of some odorant receptors to be validated by experiments of site-directed mutagenesis.
- Experimental determination of molecules involved in the adaptation process to odorants.

YEAR 3

- Molecular modeling of the molecules experimentally determined to be involved in adaptation.
- Results of functional experiments of site-directed mutagenesis on odorant receptors: validation or modification of the model develop at year 1.

YEAR 5

- Experimental characterization of the functional olfactory transduction process.
- Calculation of the energetics involved in odor signaling.
- Experimental and computational determination of the selectivity to odor of odorant receptors.
- Evaluation of the applicability to artificial devices for odor recognition.

2. *WP2 – Neurotelemetry: Remote acquisition and manipulation of neuronal signals*

P.I.: dr. Mathew E. Diamond

As systems neuroscience increases its understanding of the neuronal coding – the electrical language used to transmit information within the brain – two intriguing opportunities become realistic: (i) the acquisition and on-line analysis of brain signals to permit experimenters to "decode" the sensory experience of a subject as it evolves, and (ii) the insertion of information into the brain from a remote site, allowing researchers to generate controlled sensations, percepts, or movements in the subject. The on-line exchange of information between brain and external device thus has deep implications for basic research on brain function, for robotics, and for clinical neurology. Together with IIT, SISSA would carry out the final stages in projection and building of a miniature instrument, fixed to the head, which will acquire neuronal signals through microelectrodes, amplify and digitize them and transmit them by radio to a remote receiver. The laboratory will test the applications to tactile sensation in rodents.

The project is at the meeting point of Neuroscience and Robotics. The elimination of transmission cables will allow the subject to move freely. This will open up new frontiers for the study of the neuronal codes underlying sensation, perception, and movement. In the laboratory, the study of the neuronal activity in the experimental animal subjects allows one to investigate the working mechanisms of the brain. In the clinical setting, the same approach offers the possibility to understand the cellular bases of neurological disease and to create innovative therapies. The acquisition of neuronal signals and their wireless transmission is an essential component of *neuroprosthetic* devices, instruments whereby the neuronal activity of a human neurological patient could be used to drive, in real time, the keyboard of a computer, a robotic arm, or potentially the very same subject's arm. Beyond their importance in basic and clinical research, the instrumentation developed within NeuroTelemetry would be expected to have commercial outcomes.

Milestones

YEAR 1

- Testing and use of *Neurotelemetry* in freely moving laboratory rats.

YEAR 3

- Standardization of materials; adaptation to currently available commercial systems.
- Additional miniaturization with next-generation microcontroller chips.

YEAR 5

- Clinical and robotic applications.

3. *WP3 – Development of new nanodevices for neurobiological applications*

P.I.: dr. Vincent Torre

SISSA, in collaboration with nanotechnologists from other SNS and Lecce units, plans to address fundamental questions in Neurobiology by using recent experimental tools of Bionanotechnology. In particular, by combining optical tweezers and two-photon microscopy, SISSA will aim to manipulate single molecules in relevant neurobiological experiments such as (i) synaptic transmission (ii) neuronal growth and regeneration and (iii) signal and sensory transduction.

The interface and collaboration between Bionanotechnology and Neuroscience is a major goal of IIT and in particular the development of new nanodevices for obtaining a better insight of molecular events underlying fundamental neurobiological processes.

Milestones

YEAR 1

- Measurement of the force exerted by filopodia and growth cones during neuronal growth.

YEAR 3

- Manipulation of a single transmitter molecule.

YEAR 5

- Measurement of fluctuations of single residue in ionic channels and other receptors.

The combination of new experimental tools and devices recently developed by Nanotechnology provides an exciting new scientific arena for Neuroscience. In collaboration with nanotechnologists from other IIT centers in Pisa and Lecce, SISSA plans to adress fundamental questions in Neurobiology by using recent experimental tools of Bionanotechnology. In particular

1 - Advanced Spectroscopy for Neurobiology

SISSA will aim at improving the signal to noise ratio, spatial localization and time resolution of several traditional spectroscopical techniques such as Infrared and Raman Spectroscopy to be used in live cells and tissues. These techniques will be used to answer fundamental questions on protein conformational changes in neurobiological processes such as signal and sensory transduction.

2 - Single molecule manipulation, measurement and detection

By combining optical tweezers, atomic force microscopy and two photons microscopy SISSA will aim at manipulating single molecules in relevant neurobiological experiments such as:

synaptic transmission

neuronal growth and regeneration

signal and sensory transduction

3 - Nanofabbrication

SISSA aims at developing several new nanodevices for neurobiological applications. These applications have

an increasing complexity, depending on the time required for the occurrence of the neurobiological event under investigation. Nano-devices will be used for guiding the formation of neuronal networks with a controlled connectivity or wiring and for testing properties of neuronal networks with an unprecedented spatial resolution. SISSA will also attempt to develop new tools for investigating chemical properties at single cell level by using Raman Spectroscopy and nanostructures able to provide the required Surface Enhanced Raman Signal (SERS).

4 - Atomic force microscopy

SISSA will explore how to use atomic force microscopy (AFM) to provide new insight on the molecular structure of biological samples which cannot be crystallized. AFM will be used in combination with other techniques, to obtain significant progress in nanotechnology and and neurobiology.

QUALIFICATION OF THE RESEARCH UNIT

Postgraduate training and leading-edge research in various areas of Physics, Mathematics, and Neurosciences are the objectives of SISSA, the International School for Advanced Studies of Trieste. SISSA was the first Italian university to offer the PhD degree, and has continued to do so with notable success. Since its founding in 1978, SISSA has prepared more than 500 young people for careers in research and teaching: today SISSA represents one of the leading scientific institutions in Italy and a noted center on the international stage.

Each of the shool's eight sctors hosts a staff of internationally recognized professors and junior fellows. The sctors carry out original research projects and organize the post-graduate training of university graduates from throughout the world. In many cases, the training and research projects combine and integrate the expertise of multiple research groups. In this open-minded environment, the graduate students attend courses and conduct research under their advisor's guidance, acquiring the relevant academic background and, more importantly, the capacity to conduct research independently. In short, the students gain the experience necessary for a successful entry into the national and international scenes.

SISSA is part of a scientific campus located near Miramare Castle, about 6 km from downtown Trieste and enjoys its rather picturesque setting on the hills facing the Adriatic sea. Other neighboring institutions are the International Centre for Theoretical Physics (ICTP) and the Department of Theoretical Physics (DFT) of the University of Trieste. The school also hosts permanent staff of the INFN (*Istituto Nazionale di Fisica Nucleare*) and INFM (*Istituto Nazionale di Fisica della Materia*) and enjoys a close collaboration with the International Center for Genetic Engineering and Biotechnology (ICGEB).

Research

The scientific research at SISSA is organized into sectors. At present there are 8 active sectors which cover different branches of pysics, mthemathics and bology. The sectors can autonomously set their own policies concerning their scientific activities, teaching and administration of funds. Each sector can invite visiting scientists

and post-doctoral fellows within the limits of its own funds. These decisions are taken by a Sector Council, consisting of the scientific staff of the sector and two student representatives.

The school has the following established sectors:

- Astrophysics
- Cognitive Neuroscience
- Condensed Matter Theory
- Elementary Particle Theory
- Functional Analysis and Applications
- Mathematical Physics
- Neurobiology
- Statistical and Biological Physics

In addition to the above sectors there is the:

- Interdisciplinary Laboratory for Advanced Studies (ILAS)

SISSA Training and Courses

Ph.D. Courses

The International School for Advanced Studies SISSA is one of the leading scientific institutions in Italy for postgraduate training and high-level research. The students are selected yearly by means of an entrance examination held in Trieste; non-EU students can also be admitted through a preselection based on academic qualifications only. At the end of a three- or four-year course of study and research students are admitted to the final examination to obtain the Ph.D. degree, equivalent to the Italian "Dottore di ricerca". During the course of these studies they benefit from contacts with scientists from all over the world.

Other courses:

Undergraduate fellowships

SISSA offers the possibility to Italian and foreign undergraduate students to obtain fellowships by means of a competition based on academic qualifications only. The activity will last from one up to twelve months. To the admitted candidates a fellowship of the amount of € 500.00 gross per month will be assigned. A monthly contribution towards living expenses of € 300.00 will also be granted to students who are not resident in the Trieste province. Travel expenses will be covered up to € 500.00 depending from the residence of the candidate.

Post-graduate fellowships

SISSA offers the possibility to Italian and foreign graduate students to obtain fellowships by means of a competition based on academic qualifications only. The activity will last from two up to twelve months. To the admitted candidates a fellowship of the amount of € 1000.00 gross per month will be assigned. Travel expenses will be covered up to € 500.00 depending from the residence of the candidate.

Laurea Magistrale in Matematica

An agreement between SISSA and the University of Trieste, started in 2004, created a joint curriculum for students in Mathematics who want a high level training for future research activity.

Master in Science Communication

Among the activities of the Interdisciplinary Laboratory for Advanced Studies (ILAS), noteworthy is the Master in Science Communication, a 2-year part-time degree established in 1993. Its goal is to train scientific communicators in various fields - journalism (radio, television, web based, and printed), institutional and commercial communication, publishing (traditional and multimedia), and museum curatorship. Master Lectures are for Italian speakers only.

Facilities

Library

The SISSA library contains more than 14.000 books, 12.716 bound journals,all SISSA theses (Ph.D. and Magister) and currently subscribes to about 320 scientific journals.

Computing resources

Information about SISSA general computing facilities, calculus servers, workstations, helpdesk and assistance at the disposal of its members.

Laboratories

SISSA's laboratories are equipped with sophisticated instrumentation to ensure the most innovative approaches to important reserach problems.

www.nest.sns.it

Research Unit IIT – Scuola Normale Superiore (SNS)

Scientific coordinator of the RU : Prof. Fabio Beltram

SCIENTIFIC OBJECTIVES

- ***WP1 Advanced techniques for characterisation and imaging***

  P.I. dr. Ranieri Bizzarri
- ***WP2 Drug, nanoreporter and nanoactuator delivery***

  P.I. dr. Michela Serresi
- ***WP3 Tissue engineering technologies***

  P.I. dr. Marco Cecchini

WORKPACKAGES

1. WP1 – Advanced techniques for characterisation and imaging

P.I.: dr. Ranieri Bizzarri

Workpackage 1 consists of three activities.

Activity 1.1 targets the detection of ***protein expression*** and ***post-translational modifications*** in living cells based on the development of high-performance protein tags and high-performance FRET systems. Protein labelling will be optimised by using fluorescent probes that are expressed by the cell itself or are membrane-permeable or are linked to peptides that can promote cellular uptake. The final goal is the development of high-efficiency markers allowing the detection of single molecules or single interaction events in a given sample or live cell.

To reach this goal the chosen tags will be integrated in biosensors capable of visualizing post-translational modifications such as acetylation, sumoylation, or ubiquitination in intact cells. These biosensors will be based on protein domains or peptide substrates that undergo a conformational rearrangement upon modification, thereby determining a change in intramolecular FRET. By means of the developed technology, molecular beacons will be engineered for *in vivo* use, by including peptide sequences capable to penetrate effectively the cell membrane; targets will initially be chosen from amongst viral genomes as well as from tumour markers of high current clinical relevance.

Activity 1.2 within this same WP, ***THz waves*** will be explored ***as probes*** for biomolecules also in the apertureless-SNOM microscopy regime for the imaging of biological specimens. This activity will target spatially-resolved label-free analysis and spectroscopy of biological samples at THz frequencies. The implementation of compact and practical systems will be pursued by taking advantage of SNS long-standing experience with quantum cascade-based sources.

Activity 1.3, also in collaboration with SISSA, Trieste, we shall target the development of experimental and computer-based tools to elucidate the basis of ***molecular recognition*** between protein-protein pairs. Particular attention will be devoted to selective binding/interactions followed by a controlled change in configuration or release of appropriate signals. These tools will impact a number of applications such as immobilization of molecules, the realization of highly specific biosensors, and for the design of nanoactuators, ultimately opening the way to nanorobotic systems.

2. WP2 – Drug, nanoreporter and nanoactuator delivery

P.I.: dr. Michela Serresi

Workpackage 2 consists of one activity.

Cell-penetrating peptides are a class of molecules that are capable of crossing the cell membranes. This property will be exploited within **Activity 2.1** for the intracellular delivery of drugs or more complex molecular

systems (biosensors, nanoactuators). We shall focus our activity on the HIV transactivating protein (Tat) and peptides derived thereof as effective penetrating vectors for drugs and nanostructured systems.

The peptide structure will be engineered in view of

i.) the modulation of the cell (and nucleus) entrance pathway,

ii.) the intracellular targeted delivery of molecular cargoes with therapeutic activity,

iii.) the intracellular targeted delivery of molecules to be used as nanoactuators capable of responding to a specific chemical/physical signal by making available a drug/pro-drug or by making available a detectable signal.

Also in collaboration with NNL, Lecce, we shall explore the intracellular delivery of inorganic molecules (nanocrystals, qdots) to develop new two-photon nanoprobes.

3. WP3 – Tissue engineering technologies

P.I. : dr. Marco Cecchini

Workpackage 3 consists of two activities.

Activity 3.1 will target the development of technological strategies based mostly on the bottom-up approach to realise the biocompatible polymeric and composite scaffolds needed for the recreation of functional tissues from single cells or for lab-on-chip systems. Also in collaboration with NNL, Lecce, we shall focus on surface-modification strategies such as:

i.) chemical functionalisation;

ii.) surface self-assembly of polymeric materials with functional groups capable to selectively recognize and bind biomolecules of interest.

The developed methods will be usable together with high-resolution nanopatterning techniques such as dip-pen, e-beam, and nanoimprinting lithography.

Within **Activity 3.2** a specific effort will be devoted to the development of complex microfluid networks within the biocompatible scaffold, to recreate the essential vascolarisation characteristics of biological tissues. In the context of lab-on-chip systems, we shall focus in particular on surface-acoustic-wave (SAW) based approaches that need no moving parts. This work will exploit our experience with SAWs in nanostructured semiconductors that will be applied in the context of piezoelectric biopatterned, functionalised substrates to manipulate and analyse biological specimens. In collaboration with Scuola Superiore Sant'Anna, Pisa, we shall also target the fabrication of reconfigurable nanofluidics arrays based on grids of crossing nanochannels that integrate check valves at each node. The final aim is the rapid control of flow-paths and droplet temperature.

Milestones

YEAR 1

- ***Commissioning of the TIRF microscope setup***
- ***Design of the fluorescent biosensor for post-translational protein modifications***
- ***Identification of the chosen CPP vector sequence***
- ***Computational protocols for chromophore design optimizing one-/two-photon excitation spectra***
- ***Design, production, and purification of high performance chromophores***

YEAR 3

- ***Fluorescent biosensor to monitor in vitro a selected post-translational protein modification***
- ***Identification of the molecular-beacon structures for in vivo and in vitro applications***
- ***Realisation of a sub-micrometer resolution THz SNOM system***
- ***Demonstration of cell loading of selected biomolecules/nanocrystals driven by the selected CPP***
- ***Demonstration of nanostructured functional protein patterns***
- ***Control of droplet motion and temperature by SAW on a biocompatible substrate***
- ***Computational protocols for the design of recognition peptide sequences***

YEAR 5

- Fluorescent biosensor to monitor *in vivo* a selected post-translational protein modification.
- Sensitivity to single-pair interaction in live cells
- Optimized molecular beacons for *in vivo* and *in vitro* applications
- Label-free and spatially-resolved THz SNOM imaging and spectroscopy of biological specimens
- CPP-driven loading of nanostructured molecules for drug delivery/nanosensing
- Integrated nanofluidic protein-patterned scaffolds

QUALIFICATION OF THE RESEARCH UNIT

The research group at NEST, SNS can offer a long, successful track record of research in nanoscience and nanotechnology in a broad range of fields spanning from nanostructured optoelectronic devices to single-molecule monitoring in live cells. This diversified expertise represents the ideal background for the proposed activities and uniquely matches the objectives of IIT Nanobiotechnology platform. Information on NEST SNS activities can be found in our web site www.nest.sns.it, here we shall only discuss some of the issues most strictly related to the proposed objectives. Preliminary, however, we should like to emphasize the interdisciplinary nature of the research team offered comprising computational physicists and chemists, and experimentalists with very diverse backgrounds in physics, biology, chemistry, engineering.

Advanced methods in quantum chemistry like Density Functional Theory (also in the “time-dependent”

version) and other quantum-chemistry methods are used to investigate relationships between the biomolecule structure and function with a particular emphasis on the tailoring of the optical properties of chromophores in close integration with experimental investigations. The complex photophysics of fluorescent proteins are modelled with molecular dynamics (*ab initio*, force-field based and hybrid QM/MM) and with associated methods for free energy prediction in order to optimise molecule design for biomedical and IST applications. This has already lead to the development and patenting of new biomolecules with unique optical properties that are making it possible to monitor single-molecule expression and trafficking in live cells and to applications to drug design in collaboration with pharmaceutical companies. Advanced microscopy (one and two-photon confocal systems) is used together with these proprietary markers (and others) to study relevant biomedical problems (protein expression, function, interactions). Notably the RU can offer equipment and personnel for recombinant-protein production, purification (double-step liquid chromatography) and structural/functional characterisation.

From the point of view of THz radiation, the RU is a world leader in quantum-cascade THz sources and holds the patent for the unique waveguiding scheme used in these solid-state devices. This together with the experience in Atomic Force Microscopy and nanopositioning represents the basis for the proposed activity in THz sensing.

Cell-penetrating peptides have been for several years an active area of research for the RU and, based on the RU results on the HIV1-Tat protein a number of derived peptides are being evaluated. The single-molecule studies carried out by the group complement together with the molecular modelling capabilities will be the basis for the drug/nanoprobe delivery objectives listed above. Importantly these activities will benefit from the financial resources available to the RU through FIRB programs and industrial grants from pharmaceutical companies.

Finally the state-of-the-art capabilities of the group for nanopatterning, the long experience with surface acoustic waves will be made available for the described tissue engineering objectives. The group has already shown the ability to nanopattern functional proteins and generate and drive acoustic waves on a number of different substrates of interest for the proposed objectives. In this context we should like to stress the facilities and competence in cellular biology and the integrated collaboration with two of other RUs (NNL and Sant'Anna).

The objectives pursued by the RU and listed above ***map exactly*** on the approved IIT activities. In particular and referring to the technical annex the RU addressed items

1.1 (Advanced characterisation tools and Imaging),

1.2 (Intelligent drug delivery),

1.3 (New strategies for biomolecule immobilisation onto polymeric surfaces),

1.4 (Fluidics).

Facilities. The Scuola Normale Superiore (SNS) has a long-time experience in studying the electronic, transport

and optical properties of nanostructures and mesoscopic systems, and, more recently, has been developing an aggressive research program in biological studies, particularly concerning the engineering of fluorescent proteins and their use in confocal microscopy or as a basis for novel bio-photonic devices. This multidisciplinary activity benefits from the integration of computational physicists/chemists and experimental biophysicists, biologists, chemists and engineers.

The SNS will make available from 2006 a newly established laboratory location which features ***300 square meters of clean-room space*** with all necessary facilities for device fabrication. These include e-beam microscopy and lithography (with resolution of about 1 nm and 20 nm respectively), optical lithography, nanoimprinting facilities, wet and dry (reactive-ion) etching systems (also with inductively-coupled plasma sources to allow for low-damage processing of high aspect-ratio structures). Deposition systems are available based on e-beam evaporation for high-purity metals, thermal evaporation for electrical contacts and gates, chemical vapor deposition for oxides and insulators. A ultra-high-vacuum sputtering system for superconductors is also present. Epitaxial chambers will also be located in the facility: a molecular beam system will be installed for III-V semiconductors, while a chemical-beam system is being commissioned for the bottom-up growth of nanostructures. Structural characterization set-ups are based on atomic force microscopy (AFM) and scanning tunneling microscopy (STM), with a state-of-the-art cryogenic STM system now being commissioned. Computational activities take advantage of the SNS computer centre and dedicated parallel machines located in the laboratory itself.

Additional ***200 square meters*** are dedicated to the preparation of biological samples and synthesis of bio-molecules. They feature all the necessary equipment for cell culture (refrigerators, incubators in controlled atmosphere, inverted and normal microscopes, biological hoods, etc.) and for the synthesis, purification, handling, and characterization of biomolecules. These include fast protein and high performance liquid chromatography (FPLC and HPLC), electrophoresis cells, rotary evaporators, polymerase chain reaction (PCR), various centrifuge systems, NMR spectroscopy.

Additional ***400 square meters*** are occupied by various laboratories fully equipped for optical and transport measurements in a variety of experimental conditions. Cryogenic facilities offer temperatures down to 8 mK with two dilution refrigerators (one with optical access), and magnetic fields up to 16 T. Various laser sources are present covering a spectral range that extends from the ultra-violet to the far-infrared (Kr, Ar, Ti:Sa, CO_2, methanol, etc.) with complete electrical and optical characterization systems (including photo-current-voltage, capacitance-voltage, deep-level-transient-spectroscopy, spatially resolved optical analysis, two Fourier-transform infrared spectrometers, state-of-the-art Raman and inelastic light scattering set-ups featuring a triple monochromator with master gratings).

The microscopy lab features fluorescence confocal microscopes including a two-photon microscope (with real-time lifetime analysis capability) fully integrated with Ar/Kr and Ti:Sa laser sources, FCS and FLIM capabilities

are available. A spectrophotometer and a fluorometer offer complete characterization possibilities. P3 facilities for the study of active viral agents and radioactive material handling facilities are available.

Adequate office space, mechanical and electrical workshops are available. In case of need, the laboratory space available will be extended of ***additional 600 sqm***. This expansion in contiguous space is already planned and can be made readily available to IIT activities.

Staff, training of junior IIT staff and graduate school SNS staff included senior computational physicists/chemists that will support the experimental activities and guide data analysis and molecule/nanorobot design, other senior staff include biophysicists, experts in advanced microscopy, molecular and cellular biologists, chemists and bioengineers. Additional junior staff is available together with a number of graduate students attending SNS PhD school (some of them within a joint IIT-SNS PhD program in Molecular biophysics). The current agreement between IIT and SNS foresees the selection of two groups of 8 PhD students on a four-year fellowship program. The present proposal extends this activity with 2 PhD students hired each year from the third. Thanks to IIT funding some staff will be hired and additional junior personnel will be hosted within the SNS facilities. Up to 10 junior scientists will be hosted within the present RU proposal.

Funding. NEST SNS will cover over 50% of the proposed-activity yearly costs thanks to internal and external resources and personnel costs.

www.sssup.it

Research Unit IIT – Scuola Superiore di Studi Universitari e di Perfezionamento Sant'Anna (SSSA)

Scientific coordinator of the RU : Prof. Paolo Dario

SCIENTIFIC OBJECTIVES

- ***WP 1 Micro- and Nano-technologies for endoluminal and cellular surgery***

  P.I. Prof. Paolo Dario

  Senior Scientists: dr. Arianna Menciassi, dr. Alfred Cuschieri, dr. Cesare Stefanini, dr. Oliver Tonet, dr. Anna Eisinberg, dr. Ivano Izzo, dr. Virgilio Mattoli, dr. Vittoria Raffa

- ***WP 2 Micro- and Nano-technologies for biorobotic components and systems***

  P.I.: Prof. Paolo Dario

  Senior Scientists: dr. Maria Chiara Carrozza, dr. Arianna Menciassi, dr. Cesare Stefanini, dr. Oliver Tonet, dr. Barbara Mazzolai, dr. Cecilia Laschi, dr. Lucia Beccai

The objective of this proposal is to investigate and develop theoretical models and technologies to design and fabricate novel micro-robotic systems and micro-systems for robotics components which exploit and go beyond state-of-the-art technologies in micro/nano-engineering and micro/nano-fabrication.

SSSA has a significant tradition and expertise in micro/nano-engineering and in micro-systems, and it has very advanced facilities for micro/nano-fabrication in the 3D domain. SSSA design and fabrication capabilities in this field are at the level of international state of the art.

This proposal starts from the consideration that these micro/nano-technologies can enable the design and development of radically novel micro-robots as well as of innovative and high performance components for macro-robots.

Based on this consideration, the project is organized in **2 Workpackages**:

- the first Workpackage is devoted to the development of miniaturised biomedical teleoperated machines for remote exploration in the human body, as required for early diagnosis and localized therapy;

- the second Workpackage is devoted to the investigation and development of biomimetic and bio-inspired mini- and micro-components for robotic artefacts which can enable to better understand the locomotion behaviour and working principles both of lower animal species, such as arthropods and simple vertebrates, and humans.

WORKPACKAGES

1. WP1 – Micro- and Nano-technologies for endoluminal and cellular surgery

P.I.: Prof. Paolo Dario

Senior Scientists: dr. Arianna Menciassi, dr. Alfred Cuschieri, dr. Cesare Stefanini, dr. Oliver Tonet, dr. Anna Eisinberg, dr. Ivano Izzo, dr. Virgilio Mattoli, dr. Vittoria Raffa

Innovative concepts will be investigated for the development of microrobots for endoluminal endoscopy and intervention, such as swimming or reconfigurable and assembling microrobots with to ability to reach the accuracy required for cellular surgery.

Miniaturization of components and integration of functions will be pursued according to a truly mechatronic – and interdisciplinary - approach. In fact, when dealing with micromachines which have to reach and operate onto the most remote regions of the human body, many issues arise in terms of material selection, biocompatibility, actuation constraints, power supply limitation, remote control and micro-biomechanics (i.e. interaction between the medical micromachine and the tissue also based on not-conventional phenomena, such as optical forces, electrophoretic forces and so on).

When adequate and useful, bioinspired micro-robots will be considered and investigated for the exploration of the human body, like smart capsules with insect-like locomotion capabilities, on-board camera and miniaturised tools for diagnosis/intervention. In particular, swimming micro-robots able to navigate in the cerebro-spinal fluid or

in the amniotic liquid will be theoretically modelled, designed and implemented using microengineering methods and techniques. These robotic artefacts will be controlled by teleoperation or will be (at least partly) autonomous; bio-inspired swarm behaviours will also be investigated and implemented, by taking inspiration from the emergent behaviour observed in social insect colonies. In this framework, communication algorithms and control strategies for allowing an immersive teleoperation by the human operator (i.e. the medical doctor) onto the robotic miniaturised artefact will be investigated and implemented.

2. *WP2 – Micro- and Nano-technologies for biorobotic components and systems*

P.I.: Prof. Paolo Dario

Senior Scientists: dr. Maria Chiara Carrozza, dr. Arianna Menciassi, dr. Cesare Stefanini, dr. Oliver Tonet, dr. Barbara Mazzolai, dr. Cecilia Laschi, dr. Lucia Beccai

In the area of biorobotics, the proposed research investigates the modelling, design and fabrication of micro/nano components and the integration of these components for the development of biorobotic systems incorporating locomotion, actuation, sensing, control and – possibly - communication capabilities. The main objective of this Workpackage is understanding the locomotion behaviour and the working principle of animals (invertebrates and simple vertebrates, such as the lamprey, but going up to humans) by developing and rigorously testing their robotic counterpart.

For achieving this objective a huge activity on the development of basic components (e.g. bio-inspired effectors and sensory systems) has to be done, by exploiting at the maximum extent micro- and nano-engineering technologies.

In particular, a huge effort will be devoted to the development of muscle-like micro-actuators with the potential to be integrated into biorobotic mini- and micro-machines. A biomimetic skin with proprioceptive and exteroceptive properties will be also developed, that will be used as a high performance tactile sensor for a wide range of applications in biorobotics (swimming systems, locomotion systems, manipulation systems, and also prosthetics hands and humanoids). The sensing skin will incorporate different kinds of micro-sensors, designed and fabricated to mimic the mechanoreceptors of living beings. Special efforts will be devoted to the design and development of an innovative biomimetic vestibular system, consistently with the increasing importance of the role of this organ, in humans and robots, as recognized in neuroscience and robotics.

Education Activity (valid for WP1 and WP2)

In line with the educational model of SSSA, a key component of the proposed activities is education of young, competent, and creative researchers. PhD students will be integrated in the teams and will contribute significantly with focused original research works. The SSSA educational activity, which already includes experimental research works, will be strengthened and improved by the research proposed here. In addition to this general contribution to education at SSSA, specific schools for PhD students will be organized, on the themes of this proposal. They will

be in the form of full-immersion, residential, one-week schools, open to all the RU of the IIT network.

Milestones

YEAR 1

- Assessment, selection and development of micro- and nano-technologies for the design and implementation of microrobots and mechatronic animal-like components; analysis of design issues, actuation, communication, powering. (WP1 and WP2)
- Prototype of swimming microrobot. (WP1)
- Prototype of sensorized artificial skin. (WP2)
- Prototype of vestibular system. (WP2)

YEAR 3

- Study and development of prototype miniaturized robots for extreme operation (e.g. in remote and delicate districts of the human body). In particular prototypes of teleoperated robot for navigation in the cerebro-spinal fluid and in the gastrointestinal tract. (WP1)
- Study and development of prototype animal components, based on the previously identified microtechnologies. In particular, prototypes of muscle-like actuators, skin-like tactile sensors, and artificial vestibular systems. (WP2)

YEAR 5

- Final prototypes (ready for industrialization) of all the above components and systems: microrobots for navigation in the cerebro-spinal fluid and in the amniotic liquid; tactile sensors; artificial muscles; artificial vestibular system. These components will be integrated also in prototypes of biorobotic animal-like systems, artificial hands and humanoids. (WP1 and WP2)

QUALIFICATION OF THE RESEARCH UNIT

The **Scuola Superiore Sant'Anna (SSSA)** is a public University whose explicit mission is to provide excellence education at graduate, doctoral and post-doctoral level, and to perform excellence research, in the sectors of engineering, medicine, agriculture, economics, law and political science. The research laboratories of SSSA involved in the proposal are located at the “Polo Sant'Anna Valdera” (PSV), in Pontedera, a town close to Pisa. PSV is a research park, specifically created to better house the research activities of the SSSA, at the cutting edge of the most important sectors of technological innovation. At Polo Sant’Anna Valdera, dedicated spaces (up to 100 m^2) can be devoted to the research lines and activities carried on in collaboration with IIT. Further information on SSSA can be found at the web address http://www.sssup.it.

The RU SSSA will participate into the project mainly as CRIM Lab (coordinated by Prof. P. Dario). Some activities related to biorobotic components will be carried on in collaboration with the ARTS Lab (coordinated by Prof. M.C. Carrozza).

The **CRIM Lab** (Centre for Research in Microengineering) was established at the SSSA with the specific mission to perform applied research on micro-mechatronics mainly in the biomedical field, and to implement service activities aimed at promoting the industrial take-up and exploitation. At present the CRIM Lab is coordinated by Prof. Paolo Dario, and it has more than 50 full time researchers possessing interdisciplinary expertise; a variable number of undergraduate students are also involved.

The micro-machines that CRIM studies, models, and develops can be bio-applied (this is the case, e.g., of advanced tools for minimally invasive therapy or of micro-sensors for health monitoring), or they can be bio-inspired. As regards bio-applied machines, the CRIM Lab addresses the biomedical field, where “biomedical” is considered at large including not only systems and components for advanced surgery and therapy, but also systems for improving health by monitoring food and environment. The second approach, based on bio-inspiration, is aimed at modelling and developing bio-inspired micro-machines in order to better understand the behaviour of lower animal forms (e.g. invertebrates and simple vertebrates), thus allowing to approach traditional problems in motion generation, control, sensing and communication by exploiting a different – and often more effective – solution. In this sense, bio-inspiration is extraordinarily useful to educate creative researchers.

This twofold approach distinguishing bio-application from bio-inspiration drives also the entire scientific mission of the CRIM Lab. The CRIM research can be generated by (1) very advanced problems, basically in the biomedical or health-related fields, or by (2) imagination-driven and “adventurous” ideas. In both cases, the first outcome of research is the definition of appropriate methodologies and principles and theories, which constitute the main scientific topics of the laboratory. Based on these methodologies and theories, micro- and nano-engineering design technologies and the micro- and nano-fabrication technologies available at CRIM allow to develop the most adequate solution for the devised problem (case 1) or to enter unpredictable classes of problems with a totally innovative approach (case 2).

CRIM developed 10 international patents, and has been or is still involved in more than 10 national projects, more than 20 projects funded by the European Commission, 2 European Competence Centers (on Microfluidics and on Medical Micro Instruments), 3 European Networks of Excellence, and several international projects and cooperation.

The ARTS Lab (Advanced Robotics Technology and Systems Laboratory), founded in 1989, is the advanced robotics laboratory of the SSSA, and it is coordinated by Prof. Maria Chiara Carrozza. The mission of the ARTS Lab is carrying on theoretical and experimental research on bioengineering and robotics, as well as applied research and technology transfer activities, mainly in the biomedical field. Coherently with the strong interdisciplinary

nature of bioengineering and robotics, the ARTS Lab develops its research activities by integrating different technological, scientific and even humanistic knowledge backgrounds in the investigation of theoretical and technological problems related to the development of advanced robotic systems. Multidisciplinary research fields encompass biomechatronics, robot perception, control, and learning, humanoid robotics, neuro-robotics, bionics, and robo-ethics. Applications domains are mainly in rehabilitation and assistance, for which biomechatronic prostheses and ortheses, neuroprostheses, neural interfaces, functional assessment and rehabilitation protocols and devices, mechatronic and robotic aids, assistive robots, domotic systems, gerontechnologies, and telematic services are developed.

The main focus of this proposal fits well with the objectives of IIT on Humanoid Technologies and with the intent to build up the guidelines for designing and developing microrobotic artefacts for *extreme* applications and/or with *extreme* features. In fact, in the strategic vision of IIT, developing Humanoid Technologies means to investigate and develop three parallel platforms in the field of:

- bionanotechnologies;
- neural science;
- automation and robotics.

The three platforms will develop individual research lines, but a set of interconnected facilities and joint projects will be set-up in order to pursue straightforward final systemistic objectives rather than a further specialization in the respective fields. For their nature, microrobotic systems integrate bio-nano-components, thus representing a paradigmatic *merging* of the above disciplines.

Microrobotic technologies are the key factors to address applications of exploration in remote and scaled environments and to introduce disruptive solutions in the medical field (minimally invasive approach to advanced therapy and diagnosis). For its nature, microrobotic is the paradigm of integrated design, because all problems of integration are stressed by the need of extreme miniaturization: size and power constraints, actuation and tribology issues, scaling of sensing signals from the operator to the robot and vice-versa play a fundamental role when designing and developing a microrobot which interact with or is inspired to a biological system.

The RU-SSSA has pursued through the years a “systemic” approach, aimed at not only developing enabling technologies, but also at integrating these technologies into complete (micro)systems, and at exploring their practical applicability and even industrial exploitation. This fits very well the overall strategy of IIT. Furthermore, the proposed research program reflects the specific objective of developing nano/micro-technologies and microrobots. We propose also to find common applications and to explore proactively the applicability of these technologies to problems relevant to other areas of interest of IIT, such as bionanotechnologies and neural science.

Specifically, the proposed research program will address the main areas of application of modern robotics technologies that IIT wishes to explore, such as:

- exploration of harsh environments, using microrobots;
- medical applications. Endoluminal surgery and advanced diagnostics will be explored using microrobots;
- connection between robotics and neuroscience, by developing advanced micro-components (such as sensors and actuators for muscle-like behavior, artificial vestibular system for locomotion, etc.) for the development of biorobotic mini- and micro-machines which will be used to test and advance neuroscientific models.

While pursuing this activity, the RU-SSSA will actively seek collaboration with the main lab of IIT, as well as with the other RUs.

The resources which the RU SSSA can make available for the project are briefly listed below.

Micro- and Nano-fabrication technologies at CRIM Lab. These facilities include precision machines (Precision mechanics machine shop), Ultraprecision Machining (high precision CNC machining, micro-wire and micro-sink Electro Discharge Machining), Nanomachining technologies (Focused Ion Beam), Clean Room Technologies (equipment for lithography, silicon micromachining, thin film deposition – sputtering and ion beam evaporator – and Langmuir Blodgette monomolecular layer deposition), 3D Non-Traditional Technologies (consisting in micro-injection moulding, micro-stereolithography and precision electrodeposition), Silicon Bulk Micromachining, Profilometer and Ellipsometer, Measurement and testing machines, Microscopes (AFM/STM Microscope, Optical fibre microscope, Optical microscope), MEMS characterization equipment (micromechanical test bench with precision load cell, dedicated test-bench for micromotors testing and calibration), surface characterization equipment, electrical characterization equipment, Optical Sensors research equipment (HeNe Laser, Lasers for distance measurements, UV visible power meter), equipment for neural cell culture, Simulation and Design Software (ANSYS, Pro-Engineer, Electronic Laboratory). These microengineering equipments integrate nicely the expertise in modeling, simulation and characterization at component and system level.

EndoCAS Centre (Centre for computer assisted surgery). The EndoCAS Centre is participated by SSSA and is located at the Cisanello Hospital of the University of Pisa. This facility, born to support advanced research and clinical practice in endoluminal surgery and computer assister surgery, could support some activities related to medical microrobotics. EndoCAS is in an autonomous building of 250 m^2, consisting of 2 laboratories equipped with all the instruments needed to carry out research, a replica of a typical operating room, equipped with all the furniture and instrumentation of a real operating room, a meeting room and some other service rooms. While the laboratories are used for the development of systems and prototypes, the replica of the operating room is used to define specification, to evaluate the ergonomics of the developed devices and its integration in the real surgical scenario, and for in-vitro testing of the developed systems.

The facilities of the ARTS Lab include systems for mechanical, electrical and software design and development, robotic and sensorial systems for experimental and educational activities, like for example: artificial hands, integrated robotic platform (robotic head, vision system, anthropomorphic robotic arm, sensorized robotic

hands), domotic house, assistive technologies (HMIs, feeding robots), mobile robots, mechatronic systems for the motor rehabilitation, wearable systems for monitoring some physiological parameters, etc.).

Personnel. The personnel of the RU involved in this research comprises of:

1 Full professor

2 Associate professors

10 Assistant Professors

5 Post-Doc Research Assistants

50 PhD Students

A variable number of graduate students (around 10)

4 administrative staff

4 technicians

Training. SSSA is a public University for education and training in Experimental and Social Sciences, including Biomedical Engineering, Telecommunication Engineering, Information Technology, Medicine, Agriculture, Political Science, Economics, and Law. Courses in the above disciplines are offered by SSSA at undergraduate and master level. SSSA offers high-level post-graduate 'master' courses, on interdisciplinary subjects like peace-keeping, environment management, and others. A relatively large effort is devoted by SSSA to PhD education, in the 6 sectors of SSSA. As for the proposer's areas of interest, 50 PhD students are educated at the ARTS and CRIM labs in biomedical engineering, robotics, micro-systems, and biorobotics.

The educational model of SSSA is deeply based on interdisciplinarity. Students are exposed to a variety of classes on topics spanning different engineering areas and also non-engineering topics, and they especially experience studying and working in teams gathering different backgrounds. The technological paradigm of the SSSA educational model is 'mechatronics In addition to solid building blocks of courses on basic disciplines of engineering, like mechanics, electronics, information engineering, control, and others, the students accomplish hands-on projects which bonds their basic expertise and foster their experience in managing complex problem with a systemistic approach.

Moreover, ARTS and CRIM Labs are carrying on a Marie Curie project of International mobility of human resources, called ASSEMIC, which is devoted to training and research in handling and assembly at the microdimension, involving advanced methods and tools and providing a multidisciplinary, complementary approach (www.assemic.net). This is to be achieved by combining the research competence of R&D centres and universities, with the application oriented view from SMEs and industrial partners.

www.ebri.it

Research Unit IIT – European Brain Research Institute (EBRI)

Scientific coordinator of the RU : Prof. Antonino Cattaneo

SCIENTIFIC OBJECTIVES

- ***WP 1 Neurogenomics and functional proteomics of cholinergic eurons and of cortical interneurons***

The brain is a natural mosaic of a large variety of different cells types: thousands of different classes of neurons, defined by morphology, connectivity, expression of neurotransmitters and receptors, electrical properties, serve as building blocks of brain circuits, each with a specific physiological role in brain function. This cellular diversity represents the most important functional linkage between the molecular and the higher brain function levels.

Understanding how the brain works and goes wrong in diseases, will ultimately depend on our ability to link the different hierarchical levels of the brain organization into one unified conceptual framework, from molecules to behaviour. This is the aim and the mission of the recently established European Brain Research Institute (EBRI), founded by the inspiring scientific vision of Rita Levi Montalcini.

We are witnessing the genomic revolution, that has radically changed the way biological problems are studied. So called “genomic”, or “system biology” approaches provide us with unprecedented amounts of information that will accelerate enormously the rate of discoveries in all areas of biology and expand the range of questions that can be addressed. This technological revolution has also changed the way in which research is organized, calling for large, integrated and interdisciplinary research infrastructures.

For neurosciences, the genomic opportunity represents an additional challenge: in order to take full advantage of the genomic and proteomic information, this needs to be integrated with a large scale, systematic characterization of the electrical signaling properties of neuronal circuits. The frontier for neurosciences is thus to combine cutting edge systems neuroscience with functional genomics and proteomics, in a word “neurogenomics” . This combination will trigger substantial new lines of research concerning the relationship between gene/protein expression, neuronal identity within complex neural circuits and higher brain functions.

The vision implemented at EBRI is that the combination of techniques to record the electrical activity of large neuronal assemblies, with techniques for the large scale genomic screening of specific brain areas, or of single identified neurons, and for the selective modification/interference of genes specific to individual neuronal types, together with the availability of significant experimental models for higher brain functions will push significantly forward our understanding of how the brain works and goes wrong in disease. The unifying overall approach pursued at EBRI will be to identify how the complex electrical activity of cortical circuits responsible for well defined learning and memory tasks are orchestrated at a cellular and molecular level in patterns of cell-type specific gene/protein expression.

At EBRI the full benefit of neurogenomic techniques will be taken up by neurophysiologists involved in the study of individual cells and the larger neural circuits that give rise to behavior. Thus, the unique and salient feature of the EBRI environment will be the focusing on the functions of the cortex through the creative interplay among neurogenomic approaches and the dynamics of large- scale neuronal responses.

EBRI’s approach to reach its long term scientific objectives is to develop new technologies to study brain

function, by linking the different hierarchical levels of the brain into one unified conceptual framework, from molecules to higher functions. EBRI envisages to implement these goals via a multidisciplinary approach whereby collaborating scientists with distinct backgrounds will utilize techniques of molecular and cell biology, electrophysiology, cellular imaging and nanotechnology, towards the common goals of i) investigating how molecular events involved in synaptic plasticity lead to learning and memory, in well defined experimental systems, of ii) understanding the molecular basis of neurodegenerative diseases of high social and medical impact, such as Alzheimer's, Parkinson's and Huntington's Diseases and of exploiting these results to develop new therapeutic approaches to neurodegenerative diseases.

EBRI's long term goals will be approached and reached by an activity subdivided in a number of Projects. Some of the projects exploit the availability to EBRI, thanks to the collaboration with CNR, of a colony of macaque and marmoset monkeys. This project is carried out in collaboration with Emilio Bizzi, at the MIT in Cambridge (Boston, USA). The objective is to identify patterns of gene expression of the non-human primate brain, that are correlated to learning and memory processes in well defined cortical areas involved in sensory motor control. To this end, we shall initially undertake a systematic baseline gene expression profile of specific cortical and subcortical areas at different ages (Neurogenomics of the cortex). Expression profiles will go in parallel with the electrophysiological and immunochemical characterization of cortical cell types. The identification of genes that are expressed in different areas of the monkey's brain and which characterize different cortical cell types will open the way to the study of the molecular events subserving memory and consolidation of learning in well defined regions of the CNS, under well established experimental conditions.

This initial set of data will provide the necessary background for subsequent studies on the mechanisms of memory and learning in motor cortical areas. The goal of the research is to identify gene/protein expression profiles during the acquisition of new motor skills in monkeys and the transformations from short term memory into long term consolidation. We will also investigate monkey brain microRNA gene expression patterns in development, plasticity and aging. MicroRNAs are a new class of regulatory gene products that regulate protein synthesis at a post-transcriptional level in many different organism and whose involvement in neuronal function is an emerging theme of growing interest, also because small RNAs are involved in controlling gene expression in neurons by local protein translation.

We have ongoing investigations focused on ways to modulate cortical plasticity, that may lead to the development of pharmacological interventions of therapeutical relevance. These studies will provide deep and growing insights into the mechanisms of cortical and synaptic plasticity, under well defined experimental conditions, directly linked to measurable specific behaviours and also, importantly, will provide new understanding for the molecular basis of neurodegeneration. In particular, a central focus at EBRI will be to pursue the detailed investigation of the molecular links between neurotrophic factor neurobiology and the onset of neurodegeneration.

This will allow to develop innovative therapeutical approaches for neurodegenerative diseases such as Alzheimer's, Parkinson's and Huntington's disease, where a central involvement of neurotrophic factor signalling and processing deficits is an emerging fact. This will offer the unique opportunity to exploit the longstanding knowledge available in neurotrophic factor neurobiology towards the identification and validation of new molecular targets of therapeutical interest for these devastating diseases, and the development of innovative therapeutical strategies in this direction, with particular regard to strategies based on neurotrophic factors activity modulation and on the direct use of recombinant neurotrophic factors or of their genes.

A specific focus is being dedicated at EBRI on the development of new technologies, as only this will ensure to be able to address new scientific questions in a competitive way. This technology development activity at EBRI is based on existing skills and capabilities, and this will ensure its sustainability and chances of success. In particular, linking the molecular and cellular level to the higher cognitive level of brain function requires the development of new technologies to i) interfere in a spatially controlled and localized way with protein function, ii) to image sites of protein translation in living neurons and iii) to measure neuronal electrical activity in vivo in a non invasive manner. These technological development strategies are aimed at bringing together nanotechnologies and biotechnologies, with particular regard to antibody biotechnologies, towards the development of innovative neuro-nano-biotechnological devices. One of the projects implemented at EBRI is carried out as an established collaboration between EBRI and the National Nanotechnology Laboratory, within the context of a FIRB grant funded by MIUR. In this project, we propose to develop so called "Smart electrodes and peripheral connectors". We define as "smart" electrodes for extracellular recording a sensor capable, upon injection into CNS tissue, to dock to, or near, a specific cell target forming a stable electrode/tissue junction. This will be achieved by functionalizing the distal end of a nanotechnology-build electrode with a targeting/docking molecule (e.g. recombinant antibody domains or scaffolded peptides) selected or designed to bind with high affinity to a specific component of the extracellular matrix or of the neuronal cell surface (e.g. a neuroreceptor) that would allow the elective targeting of specific cell types expressing a unique marker of choice.

WORKPACKAGES

1. WP1 – Neurogenomics and functional proteomics of cholinergic eurons and of cortical interneurons

The general objective of this project is the functional genomics study of the expression profile and expression fingerprinting of pure neuronal populations of i) cholinergic neurons and ii) cortical interneurons. The project aims at bringing the neurogenomic and proteomic approach for large scale, systematic expression studies at the level of purified cellular populations. The project combines advanced transgenic technologies to selectively label neuronal populations, with cell sorting technologies, and genomic and proteomic approaches.

Cholinergic neurons

Cholinergic neurons from nuclei of the basal forebrain provide a widespread and distributed synaptic input to the cortex, and are responsible for the modulation of many higher cognitive functions, linked to attention, learning and memory. Progressive deficits in this cholinergic population are found in Alzheimer's disease patients, and it is thought that preventing their progressive loss represents a major strategy to prevent and treat the effects of Alzheimer's neurodegeneration.

Cortical interneurons

In the mammalian brain, the neocortex is the final destination and site of storage and processing of all sensory information, which is translated into several complex behaviors. Malfunctioning of the neocortex can lead to serious illnesses, such as Alzheimer's disease, schizophrenia and various forms of epilepsy. The anatomical organization of the neocortex is stereotypical among different species and consists of six layers in which specific subtypes of excitatory and inhibitory neurons generate complex intertwined networks. In the neocortex, locally projecting GABAergic neurons (interneurons) make up ~25% of the whole neuronal population, but their activity is crucial, as they provide feedforward and feedback inhibition and prevent development of hyperexcitability and epileptiform activity.

Cortical GABAergic interneurons represent a highly heterogeneous cell population that can be classified by their anatomy, connectivity pattern, electrophysiology and expression of calcium-binding proteins or neuropeptides.

Beyond acting as mere controllers of excitatory neurons, cortical inhibitory interneurons generate, pace and modulate the rhythmic activity of large neuronal populations. This cortical oscillatory activity is associated with several behaviors and cognitive functions, as well as pathological conditions such as epilepsy, schizophrenia and Parkinson's disease. In physiological conditions, the most common cortical rhythms are in the θ (4-12 Hz) and γ (30-80 Hz) frequency bands as well as ultra-fast frequency (>200 Hz) and sharp-wave activity. In particular, γ-frequency oscillations are thought to underlie perception, attention, and sensory representation. Although cortical oscillations depend on the activity of principal glutamatergic cells, the common denominator is the critical involvement of GABAergic inhibitory interneurons. Indeed, it has been shown that cortical interneurons can oscillate in the γ and θ frequency ranges even in the presence of glutamatergic blockers and it is believed that they are the permissive elements in synchronizing large groups of principal cells.

Interneurons shape this cortical activity through their connectivity patterns. GABAergic cells are coupled to each other by electrical and chemical connections, forming functional network entities comprising cells belonging to the same subgroup. In addition to connecting to other interneurons, GABAergic basket cells are self-connected through functional autapses, i.e. synapses that a neuron makes onto itself.

Understanding the function and mechanisms of self-inhibition in the neocortex represents a fundamental

advance of the general knowledge of neocortical physiology in terms of both normal behaviors and pathological activities, such as epilepsy, schizophrenia and Parkinson's disease. The body of evidence supporting crucial functional roles played by GABAergic interneurons in regulating these activities is constantly increasing. For this reason, in this last decade, the cortical interneuron field of study has been tremendously expanding, and it has become an ardent topic in neuroscience.

The overall structure of this project is to exploit available strategies for a

i) selective enrichment of these target cell populations from the mouse brain and in parallel from the brain of mouse models for specific neurological diseases (such as Alzheimer's disease models for the cholinergic neurons) and exploit the enriched neuronal population for
ii) studying systematically the profile of total mRNA expression, and of microRNA expression
iii) further fractionating the enriched neuronal populations for subcellular and protein complexes involved in axonal transport, in dendritic transport and in local protein synthesis.

The overall mRNA expression pattern in the selected and enriched neuronal populations will allow to describe an expression profile fingerprinting, as a function of developmental age, including adult and ageing, that will provide a first basis for further studies, with a more close inspection at selected classes of transcripts (microRNAs) or subcellular proteins (axonal transport system and local translation apparatus in dendrites).

μRNA

In the last few years, it has become evident that small regulatory RNAs plays an important role in regulating gene expression.

As knowledge of microRNAs (miRNA) grows from a compendium of sequences to annotated functional data it has become increasingly clear that a highly significant segment of regulatory biology depends on these 22 nucleotide non coding transcripts. The expression of many miRNAs in the nervous system, some with a high degree of temporal and spatial specificity, suggests that understanding miRNAs in the nervous system will yield rewarding neurobiological insights. High on the list of insights that microRNAs promise is a deeper understanding of the remarkable cellular diversity fould among neurons.

Small RNAs have been observed to act at different levels controlling either the translation and degradation of mRNAs or the transcription by modifying the chromatin structure. Different kinds of small regulatory RNAs are recognized depending on either the source from which they are produced or the mode of action. Eukaryotic cells are able to recognize and process double stranded RNA(dsRNA) in short RNAs 21-25 nucleotides (called short interfering RNA: siRNA). The siRNAs are part of a complex called RISC (RNA –induced Silencing Complex) that uses the siRNA as a guide to induce sequence-specific degradation of mRNAs having a perfect complementary sequence. Natural sources of dsRNA include viruses, transposons and tandemly arranged loci,

suggesting that dsRNA interference plays a role in defense against invading nuclei acids. Small RNAs are also produced by processing of short hairpin RNA encoded in the genome. These endogenous small RNA called miRNA (micro RNA) function to control the expression of endogenous genes by regulating the translation of target mRNAs that show partially complementary sequences at the untranslated 3' end region.

Current models indicate that siRNAs or miRNAs are produced directly from the cleavage of a long double stranded RNA intermediate or a hairpin-like RNA molecule respectively. An RNAse III endoribonuclease, Dicer cleaves the dsRNA to produce characteristic, perfectly matched siRNA duplexes containing 2nt, 3' overhanging hydroxl ends and a 5' phosphate group. Both the Dicer enzyme and the siRNA characteristics are well conserved throughout all organisms that are proficient for RNAi related silencing, suggesting that this method of cleavage is universal. After Dicer cleavage, only one strand of the resulting siRNA duplex is loaded, in an ATP dependent step, onto an RNA induced silencing complex (RISC) to generate an active RISC endonuclease (RISC*). RISC* is able to cleave the targeted mRNA and the specificity of this cleavage is dictated by the sequence of the siRNA; in order for cleavage to occur, near perfect homology is required between the anti-sense strand of the siRNA and sense strand mRNA. The cleavage site is situated at the region covered by the 11^{th} and 12^{th} nucleotide of the siRNA. The RISC is a multi-unit enzyme first isolated in *Drosophila* but has subsequently also been isolated from nematodes and humans. Several of the RISC components have been identified by biochemical purification and the make up of RISC seems to vary somewhat between species. This variation may represent species differences or may reflect developmental- or tissue-specific variations in RISC composition. Nonetheless, the one family of proteins that is common to all forms of RISC that have been analysed so far is the Ago family, and therefore the presence of an Ago protein seems to be a defining feature of RISC. Ago proteins are very tightly bound to single-stranded siRNAs within RISC, asthe RNA–protein interaction persists even under high salt conditions. The PAZ domain of Ago has been implicated in RNA binding, and the PIWI domain seems to furnish RISC with effector-nuclease function. Interestingly, mammalian AGO2-containing complexes associate with siRNAs and miRNAs and show both target-cleavage and translational-inhibition activities, which indicates that a single type of RISC complex could mediate both functions.

The recent large scale cloning efforts in both plants and animals estimate that miRNAs represent more than 0.5% of the total number of genes in the genomes of the species analysed and several of these miRNAs show conservation across related species, highlighting miRNA control as a general mechanism of regulation in the cell. While only relatively few of the cloned miRNAs have so far had their targets or phenotypes validated, the large diversity of cellular processes in which miRNAs function is already apparent, including developmental timing, apoptosis, neuronal differentiation and haematopoesis. In general, the miRNA genes are situated at loci far distant from the genes that they control and as such their transcription is regulated independently.

Furthermore, many miRNA genes are situated in closely packed clusters, and the temporal expression profiles of members of the same cluster are often identical. This has led to the hypothesis that several miRNA genes exhibit polycistronic expression. Another possibility is that a nearby enhancer co-ordinates their expression. Either way, it is clear that the miRNA genes have several levels of complexity in their expression. This complexity, superimposed on the normal transcriptional control of their mRNA targets, is likely to afford the miRNAs a highly refined level of gene regulation. Highlighting the fundamental function of small RNAs in the cell is the recent observation that Dicer, upstream of both the production of siRNAs and miRNAs, is essential for vertebrate development.

Several lines of evidence point out that the gene regulatory networks based on small RNAs may be particularly relevant in neuronal cells. Indeed, modulation of mRNA translation and consequently protein synthesis at specific cellular sites (i.e. synapses) has been proposed to be especially important in neurons for biological processes as memory-related synaptic plasticity and dendridic development. Consistently, RNA binding proteins, like the X mental retardation protein (FMRP), that controls translation of several mRNAs was found to play a pivotal role in correct dendritid spines development . Interestingly, FMRP has been found associated with components of the RISC and with microRNAs. Namely, the Drosophila homolog of FMRP (FXR) and the Vasa intronic gene were both identified as components of RISC. Moreover, the interaction between FMRP and RISC has also been observed in mammals in which FMRP has been found to co−immunoprecipitate with both miRNAs and the components of the miRNA pathways including Dicer and the mammalian ortholog of Argonaute (AGO1). Interestingly, mRNAs that have been found to interact with FMRP were also predicted as target for several human microRNAs. Altogether these findings suggest a model in which the FMRP-mediated and miRNA-mediated translational control pathways may intersect each other.

Studying in a systematic way the expression profile of microRNAs in selected , enriched neuronal population f relevance to physiology and pathology, will allow tgerefore to gain fundamental information on their role in shaping neuronal identity and in regulating neuronal function, also through the process of local protein translation linked to synaptic activity.

Axonal transport

Axonal transport has been at the center of recent advances in the understanding of degenerative diseases such as Alzheimer's and Down's syndrome.

Neurons project axons to target areas that are often far removed from their cell body. Trophic communication between the target and the cell body of neurons has been for a longtime an active subject of research. New molecular biology advances have highlighted some of the mechanisms and the nature of this phenomenon.

A hallmark of Alzheimer's and Down's syndrome is the degeneration and phenotypic decline of basal

forebrain cholinergic neurons. The lack of NGF has been identify has a major player in this phenomena. This has been demonstrated by two approaches. In the first, mice that produce an anti body directed against NGF show degenerative patterns similar to Alzheimer's disease. In the second, NGF treatment of BFCNs has lead to the rescue of their phenotype and recent work in Tuszynski laboratory has shown that in humans the transplantation of cellules producing NGF can rescue BFCNs phenotype. The mechanism by which an NGF deficit can arise remained unexplained until recently. Indeed, NGF is present at normal or higher levels in the centers of production such as the hippocampus. Lately, it has been shown that in a Down syndrome mouse model (a disease that leads ultimately to Alzheimer's disease among others defects) NGF is retrogradely transported at levels that are much lower that in normal animals. In Alzheimer's disease axonal transport is also found to be impaired. Studies in Larry Golstein laboratory in San Diego has shown that in Alzheimer's disease mouse models that involve the mutation of APP and PS1, axons underwent abnormal modifications. Axonal defects consisted of swellings that accumulated abnormal amounts of microtubule-associated and molecular motor proteins, organelles, and vesicles. Impairing axonal transport by reducing the dosage of a kinesin molecular motor protein enhanced the frequency of axonal defects and increased amyloid-beta peptide levels and amyloid deposition.

Nevertheless, the mechanism that lead to defects in axonal transport are not yet well understood. It is clear however that it will involve APP and its processing and therefore also PS1. What is less clear is how the control of axonal transport is implemented. It could involve the regulation of the synthesis of proteins interacting with the transport apparatus. This regulation could be done through the action of micro RNAs in neurons and studying this possibility is one of the aims of this project: Neuronal micro RNAs found in neuron populations affected in Alzheimer's disease could be overexpressed in normal animals. Established paradigms for the study of axonal transport will then be used (Sciatic nerve ligature, sciatic nerve culture and Fimbria fornix monitoring after hippocampal injection). Experiments will also be performed in cell culture systems were we isolate the cell bodies from the axons and terminals (2D and 3D cultures).

Local protein synthesis

It is currently well established that long term synaptic changes involve the expression of newly transcribed genes, that encode for proteins that determine and implement the structural synaptic changes that subserve the function of the facilitated synapses. There is therefore a well documented trafficking in the dendrites between the post synaptic acrivated sites and the neuronal nucleus, where new transcription is regulated. However, not all synapses of a neuron are concurrently activated, and a neuron needs to potentiate only a subset of its thousands of synapse. Thus, a neuronal cell faces the problem of targeting the newly transcribed genes, resulting from signals received from its activated synapses, to those activated synapses, and not to its other synaptic sites. This is a fundamental cell biology problem to be solved, and a growing body of evidence now

demonstrates that this is achieved by a machinery for local protein synthesis, whereby mRNA translation occurs locally in an activity dependent way, where and when synaptic activity dictates it. This direct link between protein synthesis , synaptic activity and mRNA transport to synapses, along dendritres, has become a cross road of fundamental importance to understand at the molecular level synaptic plasticity processes. This is why these phenomena will be studied in detail in the enriched neuronal populations under study, by suitable fractionation procedures, followed by proteomic analysis of the protein complexes, and by new in situ imaging tools that will be developed, based on existing data, results and competitive know how by EBRI scientists.

Specific aim 1 Enrichment of the target neuronal populations from existing mice.

The goal of this aim is to establish the experimental procedures to isolate the relevant target populations from lines of mice that are already available. For cholinergic neurons, this will be done by an antibody mediated procedure, exploiting an antibody against the TrkA eeceptor, that selectively labels cholinergic neurons, followed by cell sorting with available FACS (Fluorescence activated cell sorter) instruments. For cortical interneuron populations, we shall exploit lines on transgenic mice in which fluorescence protein markers are specifically expressed in distinct populations of cortical interneurons, allowing for a direct isolation of fluorescent neurons with FACS. The activities in specific aim 1 will allow establishing and optimizing the experimental procedures and will provide the initial material for subsequent analysis.

It should be noted that the flow cytometry facility available at EBRI is equipped with 1MoFlo HS Cell Sorter DakoCytomation with 3 lasers, 1 becton/Dickinson FACScalibur, 1 F500 Coulter/Beckman, 1 Magnetic beads Sorter Automax. This facility is equipped with the fastest cell sorting technology available, that can detect up to 11 parameters simultaneously on a single cell and can sort up to 100,000 cells/second, more than an order of magnitude faster than standard instruments.

Specific aim 2 New BAC transgenesis and new fluorescent reporter mice

This activity will exploit the possibility to applying transgenic mouse methodologies for reporter gene analysis using precisely modified Bacterial Artificial Chromosomes (BACs), driving the transcription of fluorescent proteins in target neuronal populations. BAC transgenic mice with fluorescent reporter expressed in neuronal populations of interest will be either obtained from existing resources, such as the GENSAT BAC trangenesis project (www.gensat.org), or will be de novo engineered on the basis of tailored specificity, exploiting the in.house transgenic facility at EBRI. The availability of lines of mice with fluoreswcent reporter proteins expressed in cell population of interest, besides providing the material for tha planned molecular analyses, also provides the unique opportunity for in vivo imaging studies following the growth and modifications of identified neurons, with 2-photon confocal microscopy technologies. This part of the work, that does not form part of this project, represents nonetheless an upside potential of the planned work, and an

activity that is part of the medium term scientific plans of EBRI.

In any case, the availability at EBRI of an effectively operating facility with state of the art transgenic technologies, including BAC transgenesis and knock-in by homologous recombination technologies makes sure that the engineering on new generation reporter mice with identified neuronal populations marked with fluorescent markers will be constantly supplied.

Specific aim 3 mRNA and microRNA expression profiles

The isolated neuronal populations will be the source of the material for the expression profiling. The activity for this specific aim will be performed taking advantage of the existing functional neurogenomics facility that has been organized at EBRI, based on the Agilent microarray platform. The deliverable of this specific aim will be a systematic description of gene expression patterns in well defined cortical populations of interneurons and in cholinergic neurons, the latter in comparison between normal mice and mouse models for Alzheimer's disease, that have been obtained by the group of Cattaneo at EBRI.

In the second half of the project the data obtained from the mouse cortex will be transferred to the primate brain, exploiting the availability of the primate colony of Macacque and marmosets.

Specific aim 4 Subcellular fractionation from the enriched neuronal populations: Axonal transport vescicles and complexes and protein synthesis complexes from synaptosomes.

This work package aims at exploiting the preparations of cells derived from the previous analyses for the isolation of protein complexes involved in axonaltransport and in local protein synthesis in dendrites. To this aim, various established fractionation procedures will be pursued, also utilizing specific antibodies being derived by groups at EBRI, for immunopurification and enrichment, in an approach dubbed TAP (tandem affinity purification), followed by mass spectrometric analysis.

The state of the art proteomic facility available at EBRI uses, in addition to approaches such as TAP (see above), 2D_PAGE separation of protein extracts, followed by MALDI-TOF MS for the analysis of tryptic digests for mass fingerprint identification. Protein spot analysis is performed on a Typhoon 8600 Mol Dynamics high resolution image acquisition system, coupled with Imagemaster and PDQuest software. A robotic system is automatically digesting and spotting samples on MALDI-TOF MS target plates (MTP 384 samples from Bruker-Daltonik. MALDI-TOF data are acquired on a Reflex IV (BD) with NCBI database on a computational cluster running MASCOT. Fine micro.characterization of protein primary structure and post translational modifications via fragmentation analysis of proteolytic peptides is achieved by MS/MS experiments on a hybrid ESI-Q_TOF instrument (Q-TOF Micromass Waters) with nanospray ion source or by post source decay (PSD) fragmentation, in MALDI-TOF instr. Nano and micro HPLC systems are coupled to the mass spec.for the investigation of gel plug samples containing multiple proteins. A triple Quadrupole API

365 and other instruments are also available to the facility.

Specific aim 5 Recombinant antibody-based technologies for the large scale isolation of antibodies, for the development of new cellular imaging tools and for spatially localized protein knock out

This is a technological development work package, specifically aimed at exploiting a significant asset available at EBRI, that is represented by the well recognized and unique scientific and technological expertise in the field of recombinant antibodies and their engineering into forms that can be targeted precisely to specific intracellular compartments to achieve selective protein knock down or protein knock out (the so called intrabody technology). This technology, developed over the years by Cattaneo, is based on the ectopic expression of recombinant antibodies against a cellular protein of interest, equipping the intracellular antibody (the intrabody) with targeting sequences that direct the antibody to where the target protein is expressed in the cell. Libraries of intrabodies have been engineered, allowing the fast isolation of functional intrabodies directly from gene sequences, with no need to express the corresponding protein. On one hand, this technology is ideally suited to be used downstream of a functional genomics/proteomics program, such as the one described above, since a natural tool to further validate any protein candidate coming out from those screens is the use of an antibody, and these libraries do indeed allow the large scale isolation of antibodies for downstream uses. But, in addition to their use as standard antibodies, these libraries provide a rich source of intrabodies for functional knock-out studies in cells. We plan to further develop the intrabody technology, in order to exploit it in two new directions:

i) the development of new imaging tools
ii) the use of intrabodies to inactivate, rather than sequester, cellular target proteins.
iii) the development of new imaging tools. We plan to exploit the versatility of the intrabody technology, and in particular the possibility to precisely target the antibodies in neurons to pre-synaptic sites, or to post synaptic sites, such as dendritic arborization or spines , to develop new imaging tools for the assessment of synaptic activity and plasticity and related phenomena. In particular, we plan to develop new sensitive intrabody-based synaptic activity and protein translation reporters in living neurons. We plan to engineer a new generation of hybrid imaging tools, based on hybrid antibody/small RNA molecules.
iv) We plan to develop the use of intrabodies for spatially localized protein knock-out. This is particularly useful in a spatially extended cell such as a neuron. We plan to exploit chromophore-activated light inactivation. The ability of illuminated fluorophore to generate ROS (reactive oxygen species) could be used to inactivate the protein of interest, in a spatially localized manner. To this aim, we plan to develop genetic tags that can operate as the acceptor chromophores, such as GFP itself or the tetracysteine-bound biarsenal dyes FlAsH and ReAsH. The development of this inactivation technology would provide neurobiologists with a unique and universal tool for the fine and precise inactivation of protein function in

a living neuron, with a much better spatio-temporal control than possible with genetic knock-outs or RNA interference.

In summary, the proposed collaboration with IIT builds on topics and objectives that are based on unique strength points of EBRI, as outlined below, on existing capabilities, and is compatible and synergistic with the objectives of IIT scientific plan.

Among EBRI strength points that form the basis for this project are:

i) microRNA is a booming theme in neuroscience, and EBRI scientist Carlo Cogoni is responsible for initiating the whole field, with the initial discovery of the phenomenon of RNA silencing by small RNAs and the elucidation of the main molecular mechanisms

ii) the organization and the role of cortical interneurons is a topics of central importance in present neuroscience, to achieve a better understanding of cortical function, and plasticity, and EBRI scientisit Alberto Bacci has made substantial contributions in this field, and in particular with the study of autaptic synaptic contacts among interneurons.

iii) The availability of lines of transgenic mice with unique properties for imaging and for modelling neurodegenerative diseases, together with the ability to generate new ad hoc mice for the project is a fundamental and competitive prerequisite for this project.

iv) The antibody technologies developed by Antonino Cattaneo constitute an essential elelemt of this proposal, and promise to deliver new substantial improvements in the ways we can image molecular processes in living neurons, and interfere in a spatio-temporally precisely tuned manner with protein function in living neurons.

v) Study of the mechanisms of axonal transport and local protein synthesis is a field were EBRI scientists, including Cattaneo, Delcroix, and Cogoni have made substantial contributions.

Milestones and deliverables

YEAR 1

- Implementation and optimiziation of experimental procedures for:
  i) the isolation of cenriched neuronal populations from mice by immunoaffinity and cell sorting or from fluorescent reporter mice by cell sorting;
  ii) Subcellular fractionation procedures from such pure neuronal populations for proteomic studies.
- Pilot functional genomic mRNA and microRNA study from the target neurons.
- Intrabody technology development and hybrid RNA/intrabody technology.

YEAR 3

- Identification of microRNA specific fingerprinting expression atterns in cortical interneurons and cholinergic neurons.

- Identification of Alzheimer's disease –related and other neurological disease-related genes expressed in cholinergic neurons.
- Development of new reporter transgenic mice
- Proof of concept of intrabody based molecular imaging technology in living cells
- Proof of concept of fluorophore-assisted laser inactivation technology with intracellularly targeted intrabodies
- Large scale, high throughput, isolation of antibodies against cortical interneuron and cholinergic neurons proteins

YEAR 5

- Identification of mRNA and microRNA gene expression profiles in cortical interneurons subpopulations in the mouse and in the primate cortex.
- Systematic and quantitative description of the axonal transport machinery in cholinergic neurons.
- Systematic and quantitative description of the local protein synthesis machinery in cortical neurons.
- New technology for molecular imaging of proteins and of protein-protein interactions in living neurons with intrabodies
- New protein knock out technology in living neurons with fluorophore assisted laser inactivation
- Identification of pharmacological targets for the modulation of synaptic plasticity for therapeutical enhancement of learning and memory.

QUALIFICATION OF THE RESEARCH UNIT

The present organizational structure of EBRI

The organizational structure of EBRI is fashioned according to international standards.

An international scientific advisory board, closely oversees both the hiring of scientists and the scientific programs. Searches are advertised in Science and Nature and the selection of young investigators is accomplished with the help of a specially appointed international search committee.

EBRI is organized in independent **Research Laboratories** and **Core facilities.**

In addition to EBRI core facilities, EBRI scientists have access to (and contribute to) shared facilities by the other institutions on site (CNR and IRCSSS. Lucia)

Research Laboratories and group Leaders

Operative laboratories are the following:

Antonino Cattaneo lab

Neurotrophic factors and neurodegenerative diseases

Alberto Bacci lab

Microcircuits in the cerebral cortex

Carlo Cogoni lab

Gene silencing

Jean Dominique Delcroix lab

Neurodegeneration and Axonal dynamics

Helene Marie lab

Molecular mechanisms of synaptic plasticity

New group leaders have been selected and/or are under evaluation and should join EBRI in 2006.

EBRI core facilities

Disease modelling facility

State of the art transgenic mice technologies

Viral vector technologies for in vivo gene delivery

Functional neurogenomics facility

Microarray expression profiling

Large scale recombinant antibody isolation, directly from gene sequences

Bioinformatics

Shared core facilities

Thanks to the presence of CNR and IRCSS S.Lucia on the site where EBRI is located, other core facilities provided by the other institutions are available to EBRI scientific staff and projects. Some of these facilities are being effectively implemented and run as joint facilities, both financially and organizationally . These include

- Advanced optical imaging
- Fluorescence activated cell sorting
- Proteomics and mass spectrometry

The **scientific programs** of EBRI are organized in

- **Institutional Projects**
  - Cortical neurogenomics
  - Neurotrophic factors and neurodegenerative diseases
  - Nano-biotechnology developments
- **Individual projects** under the direct control of group leaders

EBRI research plans fit with the IIT scientific goals, both in terms of general objectives as well as in its specific research and technological lines.

EBRI aims at addressing ambitious, long term, scientific questions about brain functions, linking molecules to human behaviour, a theme that is central to the IIT scientific plan. In order to pursue and achieve these objectives, the proposed plan envisages the development of new technologies, both at the level of the neurogenomic work, encompassing the new and “hot” class of micorRNA molecules in the systematic functional genomics analysis of cortical neurons, as well as at the level of new molecular imging and interfering technologies

With respect to Figure 1 of the IIT scientific plan, which summarizes the basic research activities of IIT, the EBRI proposed plan touches on the following areas of the plan:

- Human research/nano-microscale research (Advanced characterization tools and imaging, Plasticity of neural circuits, Sensory-motor memory and motor learning)

EBRI advanced molecular and cellular studies would provide the basis for understanding pathology and provide the basis for prevention of brain diseases.

In particular the knowledge acquired on the fundamental process of learning, in well defined experimental models, is likely to have an impact on a broad range of pathological situations, were manipulating the synaptic plasticity processes is of relevance. In addition, the identification of target genes, proteins and expression patterns identifying selected neural cell types will provide the basis for the development of new therapeutic approaches, and for the deeper understanding of the “building” and function of the cortex

EBRI's planned activities are particularly relevant and synergistic to

- Bionanotechnologies: the planned activity with EBRI recombinant antibody technology platform will develop advanced molecular imaging tools and more refined and fine-tuned molecular interference tools. Also, the IIT statement is central for EBRI present project: "*the combination of the tools and data generated in this area and in the functional genomics approach will be essential to enable the investigation of complex neuronal processes, such as the differential synthesis and distribution of key proteins (…) in critical neuronal areas (synapses); their traffic along multiple pathways; their interaction in intricate but highly regulated complexes; their relevance to brain disorders*" (IIT p 6)
- Neural sciences: the EBRI project will contribute to the following areas of this platform:
  - Functional genomics area, and in particular, part c),
  - Medical implications area, and in particular neurodegeneration, neurological diseases, and the increasing importance of synapses as early targets of degenerative processes.
  - Sensory and motor memories and motor learning (1.3.4, page 15), since in that part a big importance is given to the emerging role of small non coding RNAs to memory and learning processes in the cortex, and to the process of local protein synthesis

www.crib.unina.it

Research Unit IIT – Università degli Studi di Napoli Federico II (CRIB)

Scientific coordinator of the RU : Prof. Paolo Antonio Netti

SCIENTIFIC OBJECTIVES

- ***WP 1 Development and optimization of novel technologies to modulate material structure and properties at micro and nano-levels***

  P.I.: Prof. Luigi Nicolais

  Collaborators: Prof. Domenico Acierno, Prof. Francesco Barbato, Prof. Salvatore Iannace, Prof. Fabiana Quaglia, Prof. Roberto De Santis

- ***WP 2 Bioactivated Scaffolds design and production***

  P.I.: Prof. Luigi Ambrosio

  Collaborators:Prof. Giuseppe Mensitieri, Prof. Francesco Branda, Prof. Maria La Rotonda, Prof. Gianfranco Peluso, Prof. Ernesto Di Maio

- ***WP 3 Determinants of Metabolic microenvironment in 3D scaffolds and process condition optimization***

  P.I.: Prof. Paolo Netti

  Collaborators:Prof. Mosè Rossi, Prof. Lucio Pastore, Prof. Francesco Bellucci, Prof.Antonio Cittadini, Prof. Dante Ronca

Tissue and organ failure, produced as a result of injury or other type of damage, is the major health problem accounting for almost half of total expenditure in health care in Europe. Treatment options include transplantation (auto or xenotransplantation) surgical repair, artificial prostheses, mechanical devices, and in few cases, drug therapy.

However, major damage to tissue or organ can neither be repaired nor long-term recovery effected in a truly satisfactory way by these methods.

Tissue Engineering (**TE**) is emerging as a potential alternative or complementary solution, whereby tissue and organ failure is addressed by implanting natural, synthetic, or semi-synthetic material or tissue hybrids which can be either fully functional from the start, or are able to grow into the required functionality. Initial efforts have focused on skin equivalents but an increasing number of tissue types are now being engineered, as well as biomaterials and scaffold used as delivery system.

Notable results include tissue-engineered bone, skin, blood-vessels, muscle, cartilage and nerve conduits.

However, the strategies proposed so far suffer of major draw-back that strongly impairs the successful clinical implementation of TE's high potential.

One of the major limitations in TE is the poor design of appropriate material scaffold that provide adequate biological and biophysical platform to assist, promote and guide growth and regenerative processes. To guide biological events that underline the tissue growth and regenerative processes, novel and more sophisticated biomaterial are required. Biomaterials may guide the regeneration processes in several way: (a) provide the guide for cellular and tissue growth; (b) provide a platform for delivery morphogenic factors able enhance and promote the mechanism of tissue repair; (c) provide the temporary or permanently mechanical support of a damage tissue or organ; (d) provide a platform for the local delivery of cell specialized to the repairing processes. To comply with any or all of these functions material must possess a unique set of structural and molecular properties. In particular, research efforts should be devoted to the control of the structure at the nanometric and micrometric scale and to develop new technologies to encode biological and biophysical signals within the materials which can control and trigger biological events.

The ideal scaffold for TE must combine several structural and functional properties, therefore the aims of this project are:

- **develop novel technologies to modulate material structure at the nano and micro-scale.**
- **achieve a co-ordinated and spatially defined control of molecular and biophysical signals that can promote and guide specific cellular events.**

WORKPACKAGES

1. *WP1 - Development and optimization of novel technologies to modulate material structure and properties at micro and nano-levels*

P.I.: Prof. Luigi Nicolais

Collaborators: Prof. Domenico Acierno, Prof. Francesco Barbato, Prof. Salvatore Iannace, Prof. Fabiana Quaglia, Prof. Roberto De Santis

Novel materials with controlled structural and biophysical properties at micro and nano scale are urgently needed for a successful implementation of tissue engineering approach. The recent advances in material processing techniques hold the promise to engineering material properties at micro and nano scale and this maybe instrumental for fabricating novel scaffolds for tissue engineering. To this aim, novel processes and technologies able to modulate materials properties at micro and nanoscale will be tested and optimized in this workpackage.

An ideal scaffold should mimic the extracellular matrix in terms of chemical-physical and biochemical properties. Therefore it must possess a highly organized structure at the micro and meso-scale and provide matricellular molecular cues distributed in a spatially controlled manner. To produce material with this complex organization novel techniques able to process the material with a highly spatial resolution are needed. The research will be focused on exploiting and optimising technologies to control the following parameters at micro and nano scale: porosity degree and interconnection, pore size and geometry, matricellular cues (protein and peptides) distribution. In particular the workpackage envisages the development of new technologies:

- *to emboss ordered porosity at micro and nano-scale within polymeric matrices,*
- *to modulate materials structure in polymeric fibers at nanometric scale and*
- *to develop innovative techniques to obtain micro and nano particles/spheres.*

Ordered porosity at micro and nano-scale within polymeric matrices

Several techniques will be developed to emboss ordered porous array within polymeric materials both at the micro and nano-scale. These techniques include thermodynamics-based processing of polymeric solutions such as gas foaming, phase separation and freeze drying. By modulating the process condition we aim to finely control the materials structure, in terms of pore dimensions and interconnectivity, at micro and sub-micrometric scale. In particular by exploring the fundamental thermodynamic and kinetics laws that underline the process we seek to emboss highly ordered porosity pattern with tuneable morphological orientation to realize three-dimensional scaffolds with bimodal porosity, highly oriented porosity and desirable pore interconnection.

Moreover, recently has been shown that by reverse casting technology carried out by using a templating agent, it is possible to emboss ordered nanometric array of porosity within polymeric matrices. To this regard, the activity of the current workpackage will exploit: (a) reverse casting by using highly organized polymeric network as template; (b) reverse casting by using self-assembly nano-particles as template; and (c) casting by using co-continuous polymer blends.

The expected outcome of this research activity is to exploit and optimise technologies to design material

scaffolds develop with highly ordered micro and nano patterned porosity network with elevated degree of interconnectivity and of surface/volume ratio and modulable tortuosity.

Microfluidic devices will also used to realize systems able to obtain tuneable distribution of material within complex polymeric matrix. The engineered conditions supply (flux, concentration) of polymer (melt or solution) to microfluidic devices along with a designed pathway of micrometric channels will be studied to encompass gradient of material within multi-component matrix. As an example, monodisperse microgels by emulsifying an aqueous hydrogelic-based phase within an oil phase at the junction of microfluidic channels can be developed to encapsulate bioactive molecules .

Solid free-form fabrication (SFF) technology (3D printing, 3D plotting) will be taken into account to create parts with high reproducible architecture and compositional variation by computer aided design. In particular rapid prototyping offer an unique way to precisely control matrix including size, shape, branching, geometry and orientation The entire process is performed under room temperature, which allows the eventual incorporation of growth factors. As result of this technologies, tissue constructs will be prepared that contain engineered gradient of scaffold with a predicted microstructure. About this, the development of solvent free and aqueous-based systems will be exploited.

Materials structure in polymeric fibers at nanometric scale

Novel technique to modulate materials structure in polymeric fibers at nanometric scale will be also considered. Our attention will be particularly addressed to synthetic polymeric nanofibrers. This activity will be focused on the development of electrospinning technique based on the competition between forces of the static electric field and the liquid’s surface tension to obtain high porous structure (non-woven tissues) composed of tiny fibers. The proposed technique can be also used in combination with other aforementioned technique such as phase separation.

Innovative techniques to obtain micro and nano particles/spheres

Novel fabrication techniques of polymer micro and nanospheres to realize via in situ polymerization within microfluidic channels encapsulation of drug and therapeutic agents will be addressed. The main advantages of the proposed technique consist in the absolute dimensional control of the polymeric spheres and the possibility of encapsulation of drug and therapeutic agents.

The aim of the this research will be to provide and optimize a new scheme for making dimensionally highly controlled devices by microfluidic systems The proposed research project envisages the realization and development of microfluidic systems, the study and prevision of fludynamics regime inside the microchannels, the optimization of flows and geometry conditions to achieve sub-micrometric beads and an improved drug loading.

Nanosized carriers will be also prepared in form of **nanoparticles** (prepared by nanoprecipitation, coacervation and emulsion techniques) and **micelles** (prepared by using amphiphilic block copolymers that undergo

self-assembly in aqueous solutions). Both will be modified to fit a specific application by changing surface charge as well as hydrophilic/hydrophobic characteristics.

Surface nanopatterning

Finally, methods of **substrates patterning** will be optimized to pattern molecular signals on the substrate of the scaffold in controlled way at nano level. A strategy based on microcontact printing of SAMs and a approach using scanning near-field optical microscopy-Atomic force microscopy (SNOM-AFM) will be explored.

2. WP2 – Bioactivated Scaffolds design and production

P.I.: Prof. Luigi Ambrosio

Collaborators:Prof. Giuseppe Mensitieri, Prof. Francesco Branda, Prof. Maria La Rotonda, Prof. Gianfranco Peluso, Prof. Ernesto Di Maio

The aim of this workpackage is to design and produce bioactivated scaffolds able to selectively and specifically guide cellular processes such as adhesion, migration, differentiation and growth. Polymers of natural and synthetic origins will be used. **Natural polymers** such as collagen, hyaluronic and fibronectin have encoded signals for biological recognition in their chemical structure and therefore closely resemble the native cellular milieu. However, large batch-to-batch variations upon isolation from biological tissues, poor mechanical performances, scarce processability, and elevated costs represent the major limitation for the use of these materials. On the other hand **synthetic resorbable polymers** while overcome most of the limitation of the natural polymers do not contain any biological recognition site and therefore maybe considered as inert or passive in this respect. Recently it has been suggested that synthetic polymers can be hybridized with bioactive molecules to obtain biomaterials able to affect cellular function. However, the functional and structural parameters that influence cellular response and the mechanisms underlining the biological recognition of the material are still poorly understood. Therefore, to engineering novel material platforms able to control and guide the process of tissue development a deep understanding of the role of cell-material interaction on cell activity is necessary. In this workpackage the influence of structural and functional properties of the material on specific cell activity (migration, adhesion, growth and differentiation) will be first studied and quantified to establish a guideline for the design of bioactivated scaffolds. Then the information will be integrated to produce 3D scaffolds able to promote and direct the process of cellular and tissue development.

Cell adhesion

Both molecular and structural characteristics of the materials can influence cell adhesion. The presence of the bioactive molecules including biological signals such as appropriate peptides and polymeric moieties on substrate can deeply affect the mechanism of the adhesion. Moreover, surface treatment of material scaffold (SAM, hydrophilic/hydrophobic characteristic of surface) can enhance or hamper molecular events affecting the adhesion. Equally, it is well know that mechanical properties such as flexibility as well as topological characteristics

(roughness) of substrate does influence the cell adhesion.

The activity within this workpackage aims to study and quantify in term of cell shape and focal contacts spatial distribution in response to molecular and structural characteristics of the materials.

In particular, the adhesion of several cell types as function of the density, spatial distribution of molecular signals, surface chemistry and structural parameters will be measured to properly design a scaffold for an improved or depleted adhesion. The sprouting of cell onto substrate will be quantified thought focal contact determination along with the evaluation of cell shape by an appropriate imaging software. Technologies proposed in workpackage 1 will be combined to produce adhesive or nonadhesive properties of the substrate in reproducible and tuneable way.

Migration

Soluble signals as well as topological characteristics of substrates affect the cell migration in 2D as well as 3D scaffolds. The evaluation and quantification of cell migration will be carried out by cell tracking technique as function of the spatial distribution or patterning of biological signals within the substrate. In addition to being influenced by receptor distribution, cells can change their motility by specific recognition of the local stiffness of their environment. The specific aim is to estimate intrinsic cell motility parameters, persistence length and speed, for different cell types in the bioactive synthetic materials with different density of adhesion peptides and different mechanical properties.

The immobilization as well as the spatial gradient of biological molecules will be performed through the techniques optimized in workpackage 1. In particular the role of immobilization of biological signals with a constant gradient will be also investigated during the migration process.

Growth and differentiation

Soluble and insoluble signals represent key factors in cell growth and differentiation. Particularly, insoluble factors such as growth factors are demonstrated as responsible for the guide of the fate for stem cell. The influence of growth factors concentration and/or spatial distribution on cell growth and differentiation is far to be completely understood. Along with biological signals, the structural properties in term of flexibility, pore structure and orientation of the scaffold will be investigated to evaluate their influence on cell activities.

Growth factors release and immobilization as well as morphological properties at micro and nano scale will be obtained by implementing and combining the techniques proposed in workpackage 1.

Several platforms for cell activity regulation will be so developed and produced on the basis of the findings provided in the frame of this workpackage.

Immobilization of molecular signals

A research activity intends to study the adhesion of cells on monolayers prepared from a mixture of alkanethiols terminated in **adhesive peptide** like Gly-Arg-Gly-Asp-Ser (GRGDS) and the tri(ethylene glycol)

group. More specifically, we aim to chemically link specific peptides (such as the adhesive sequence Arg-Gly-Asp, RGD) to polyethylene glycol (PEG) molecules of variable length and then assemble these molecules in three-dimensional matrices by photo-polymerization to achieve a biologically responsive hydrogel. In addition to cell signalling peptides previously described, growth factors are important polypeptides to trigger tissue formation. As an example the most osteogenic growth factor include the members of transforming growth factor-β (TGF-β) superfamily including the bone morphogenic protein (BMP) family. Such growth factors can also be immobilized onto polymeric structure at controlled density.

Encapsulation of molecular signals

With the aim to induce a release in a controlled way, the encapsulation of growth factors also including vascular endothelial growth factor (VEGF) will be exploited by **encapsulation** in biodegradable polymeric carrier like PLGA or PLLA encompassed by several technique including multiple emulsion by microfluidic devices.

Gradient of bioactive molecules

In order to measure the influence of the biomolecules proposed to trigger biological event a system to evaluate the effectiveness of these motifs will be designed. Our approach to the fabrication of gradients of ligands within the polymeric material consist on using laminar flow of fluids in microchannels as discussed above (workpackage 1). Our attention will be particularly addressed to the family of polymeric hydrogels such as poly-2-hydroxyethylmethacrylate (PHEMA), poly acrylic acid (PAA) and polyethylenglycol (PEG).

For instance, gradients that range from pure PEG-RGD to pure PEG may be formed in solution by using a network of microchannels, and then these solutions are allowed to photopolimerizate into hydrogel in presence of living cells. With these systems it is possible to modulate the spatial distribution of bioactive peptides within the polymer gel and therefore to study the role of an imposed gradient of active signal on cellular behaviour.

By using technologies described in the frame of the workpackage 1, scaffolds materials with constant gradient of molecular signals in a matrix of a natural polymer can be prepared. As an example, scaffold of hyaluronic acid (HA) in collagen (type 1) will be realized to first study and then modulate cell activities.

Scaffold with controlled microstructure at micro and nano level

Polymeric scaffold with modulated structural parameter at micro and nano scale can be used to control and guide tissue regeneration. Our attention will be addressed to the influence of **structural** and **topological** characteristics of the scaffold on cell adhesion, migration, growth and differentiation.

As for structural properties the influence of porosity parameters will be taken into to account to design and realize the appropriate materials to modulate cellular events.

Pore size and interconnection as well as porosity degree and orientation may influence cell adhesion, growth and differentiation. Moreover scaffold mechanical properties and flexibility will also play an fundamental role in cell activities.

By processing of polymeric solutions such as gas foaming, phase separation, freeze drying as well as SFF it will be possible to modulate material structure at micro-level with high oriented pattern of porosity, tunable tortuosity as well as pore interconnection. The gas foaming technique can be used to prepare porous devices from synthetics biocompatible polymers such as polyesters (PLLA, PGA, PCL) or natural polymers such as thermoplastics protein (gelatin, zein) and polysaccharides (starch). Using a phase separation technology, scaffolds with large porosities (up to 95%), but small pore sizes (13-35 nm) will be fabricated. Using a technology of electrospinning, nanofibers in biodegradable materials (PLLA, PCL) will be also realized. Moreover, poly-methyl-methacrylate (PMMA) nanoporous structure can be obtained by reverse casting In fact, in the range of 10nm to 200nm reverse casting is a enabling technique to realize high interconnected and porous structure. Reverse casting will be studied by exploiting different templating agents such as colloidal systems, micro-phases separated by block copolymers, Micelle- and stars-like polymers and nanofibers.

By combining different proposed techniques and described polymer it is possible to prepare open porous materials with specific porous network morphology, mechanicals properties, biocompatibility and biodegradation rate.

Finally, topological characteristics such as roughness can modulated though indentation of surface scaffold by technique described in workpackage 1.

3. WP3 – Determinants of Metabolic microenvironment in 3D scaffolds and process condition optimization

P.I.: Prof. Paolo Netti

Collaborators: Prof. Mosè Rossi, Prof. Lucio Pastore, Prof. Francesco Bellucci, Prof.Antonio Cittadini, Prof. Dante Ronca

The generation of a fully functional biohybrid tissue in vitro depends upon the assurance of an adequate control of microenvironmental condition within the 3D scaffold. Cellular constructs maintenance, survival and growth depend upon a delicate balance among cellular activity and metabolism, nutrient transport and scaffold's properties. Pericellular microenvironment controls cellular biosynthetic and proliferative activity and depends upon transport properties of the scaffold and metabolic demand, which, in turn, affect the local microenvironment. Since cellular de-novo synthesised tissue and its assembly relies strongly upon local microenvironment, the aim of this workpackage is to develop technologies to measure the evolution of local microenvironmental condition at cellular level and develop models able to simulate the growth of biohybrid tissue in vitro. This approach will lead to the design of the optimal culture condition to process biological tissue in vitro.

The current workpackage envisage the following four operative steps:

- Evaluation of fluid and macromolecular transport proprieties within 3D cellular scaffold;
- Individuation metabolic model of cellular consumption and biosynthesis within 3D scaffolds;
- Development of mathematical model to simulate tissue growth in vitro

- Design of proper bioreactor for tissue growth;

Parameters and technologies to monitor cell microenvironment

Scaffold physical structure may control cell function by regulating the diffusion of oxygen and other nutrients, as well as removal of waste products from the implanted cells. The biosensor technology is a fundamental instrument to investigate in the cellular environment and provide parameters capable to a better understanding of processes of cell life.

In particular, the PQM (Phosphorescence Quenching Microscopy) will be used and optimized as non invasive technique with high spatial resolution and the ability to follow real time changes in oxygen pressure.

The technology of confocal microscopy conjugated with the optical principle of FCS (Fluorescence Correlation Spectroscopy) give the possibility to measure macroscopic parameters such as oxygen concentration at the level of single molecule. The confocal microscopy assures an observation volume of the order of phantom liter

Macromolecular and fluid transport

The research effort in this area will be devoted to experimentally quantify the evolution of the transport parameters (diffusivity, binding and hydraulic permeability) as tissue forms and neo-synthesised ECM is laid down. The mass transport barrier, presented by different construct designs to metabolically important microsolutes will be established and compared with values using a range of cell seeded scaffolds. Furthermore, the change in mass transport parameters due to the deposition of the novo-synthesised ECM will be monitored and correlated to the amount and distribution of the main component of the tissue (collagen, proteoglycan). The final aim is to obtain a semi-empirical relationship between transport parameters (diffusivity, hydraulic conductivity) and tissue deposition. To quantify this effect it is necessary to measure the evolution of the permeability at each stage of tissue growth. In particular the activities will encompass the following steps: (a) Set-up and validation of non-invasive technique to monitor of molecular diffusion in neo-formed tissue in vitro; (b) Set-up and validation of a mechanical based technique to assess the evolution of fluid transport resistance in neo-formed tissue in vitro; (c) Non-invasive monitoring of spatial and temporal evolution of molecular diffusion in biohybrid constructs; (d) Evolution of fluid transport and mechanical properties of biohybrid constructs

Development of mathematical model to simulate tissue growth in vitro

Neotissue formation could be affected by several factors (mechanical stimuli, transport conditions, metabolic and catabolic activity). The optima processing conditions should not be determined through a trial-and-error approach, but rather should be supported by numerical simulations.

Tissue maturation embraces two individual steps: biosynthesis of extracellular matrix elements and remodelling of individual elements into supra molecular structures. The simulation of this complex process of maturation and growth could be achieved starting from the knowledge of the relationship between culture processing and biosynthetic activity and culture processing and deposition/remodelling mechanisms. Even though

these phenomena could be very difficult to describe, research groups have recently proposed sets of equations which can model the mechanical properties evolution of a growing tissue. In order to improve such equations, and to apply them to tissue engineered biohybrids, specific assays will be undertaken in this workpackage. These assays can be grouped as follow: morphological, radiological and microtomographical analysis to evaluate neotissue assembling and remodelling; biochemical analysis to quantify the matrix elements deposed; macroscopic properties testing of the growing tissue.

A proper constitutive equation would link the macroscopic properties with composition and microstructural related parameters, as a function of tissue culture processing parameters. Such constitutive equation could then be implemented in a bioreactor software. In this way the bioreactor itself would become a smart device, able not only to provide the biohybrid with adequate physical stimuli and nutrient supply, but also able to monitor neotissue properties and to intervene whenever required by correcting the processing conditions in a sort of feedback mechanism.

Design of adequate processing condition

For the understanding and definition of the optimal tissue culturing condition a better understanding of the regulatory role of specific physiochemical culture parameters on tissue development is needed. The development of a successful cell-matrix bioreactor system will depend on a complex interplay between the cells, media, matrix components, design, mechanical stimulation and operation strategy. For this reason we will take an integrated approach in which all of these factors are considered: Cell Seeding on 3D scaffold; Mass transport limitation; Mechanical Conditioning. Novel strategies and protocol to optimize the cell seeding operation within 3D scaffold will be defined and optimized. This will include simple perfusion within the 3D scaffold of a cell suspension and/or multiple and countercurrent flow. Moreover, characterization of fluid-dynamic condition within the 3D porous scaffold will be performed both experimentally and numerically. Experimental evaluation will include both diffusion and convection throughout the cellular constructs and the determination of the transport parameters that control the phenomena. These parameters will be used to predict the evolution of the distribution of microenvironmental condition within the 3D scaffold.

In this activity particular attention will be given in identify the optimal loading stimulation that a bioreactor should deliver to better process the tissue. This will be coupled with quantitative analysis and computational modeling of the physical forces experienced by cells within the engineered tissues, including mechanically induced fluid flows and changes in mass transport. Together with biomechanical characterization and stimulation, a bioreactor should also be equipped in such a way to define when engineered tissues have a sufficient mechanical integrity and biological responsiveness to be implanted.

Milestones

Scaffold structure and design can dramatically affect the final outcome of the overall tissue engineering

approach. The present project aims to develop methodologies and strategies to emboss regular architecture to scaffold in order to modulate material structure at the nano- and micro-scale along with proposing technique to present peptides and proteins from scaffold material. The basic constructs will be processed to obtain defined microstructural features through the application of innovative technologies associated with micro and nanotechnology. Different combinations of materials and processing technology will be used to achieve emboss the desirable micro-structure and to encode gradients of specific biological signals. The porous structure at micro and nano-scale will be controlled by adapting templating, gas-foaming and 3D printing techniques. Novel templating techniques based on self assembly nanoparticles and/or organic nanoclay will be used to modulate and control the porosity structure. Furthermore, nano-fibers of the selected polymer matrix will be realized by electrospinning. At molecular level, material will be designed by encoding specific receptor able to trigger specific biological events such as cell adhesion, migration and differentiation. The local density and the spatial organization of the ligand presentation will be designed by specific microfluidics and nano-printing techniques.

The project will have run for 5 years with the following milestones:

YEAR 1

- Definition and development of novel techniques able to emboss regular porosity pattern at micro and nano-scale within polymeric matrices. Patterns should be modulated in terms of pore size, directionality of pores channels and degree of interconnectivity.

YEAR 3

- Realization of bioactive materials that include multiple biological signals (e.g., peptides) that trigger specific cellular events (adhesion, migration).

YEAR 5

- Realization of novel bioactivated polymeric platforms with well defined structure and with spatially organized distribution of biological signals able to guide specific cellular events (migration, invasion, proliferation and differentiation).

QUALIFICATION OF THE RESEARCH UNIT

The Interdisciplinary Research Centre in Biomedical Materials (**CRIB)** is recognized as a leading European Academic Institution in the field of biomaterials and tissue engineering. The CRIB co-ordinates research in various areas of biomedical applications (materials science; orthopedics, cardiovascular, dental and maxillo-facial surgery; tissue reconstruction; and tissue engineering). The research concerns all aspects of the relationships between structure, processing and properties of macromolecular materials for biomedical application and bioreactor design for processing of tissue engineered constructs. The CRIB include leading research groups within the University of Naples in Engineering, science, medicine and biology. A strong interactive network between the CRIB and other

international institutions such as **Massachusetts Institute of Technology, M .I.T**., Cambridge, MA – USA (Prof. Yannas, Prof. A. Grodzinsky); **Harvard Medical School**, Boston, MA – USA (Prof. R.K. Jain) **University of Cleveland**, Cleveland, USA (Prof. J. Anderson); **University Hospital Basel** (CH) (Prof. I. Martin); **Cambridge University**, UK (Prof. W. Bonfield e Dr. Serena Best; Queen Mary College, **University of London**, London, UK (Prof. D. Bader; Prof. E. Tanner; Dr. J. Shelton; **University of Brighton**, UK (Prof. A. Lloyd; Dr. M. Santin; University of Liverpool, Liverpool, UK (Prof. D. Williams); **University of Ghent**, Ghent, Belgium (Prof. E. Schacht); **Universitad Politecnica de Catalunya**, Barcelona, ES (Prof. J. Planell; **CSIC,** Madrid, ES (Prof. J. SanRoman); **INEB** University of Porto, P, Prof. M. Barbosa; **Lipsig University**, DE, Prof. A. Bader; **Federal Polythechnic of Losanna** EPF, SW (Prof. J. Hubell); **University of ULM**, DE, (Prof. L. Claes Prof. L. Durselen; **University of Twente**, NL (Prof. I. Feijen, Prof. D. Grijmpa); **Università di Torino** (Prof F. Bussolino Dr. G. Serini); **Istituto Ortopedico Rizzoli** di Bologna (Dr. Nicola Baldini Prof. R. Giardino Prof. M. Marcacci); **Università di Catania** (Prof. Giovanni Marletta). and many biomaterials companies exists. The strong collaborative work that the structure carries with other research institution in the medical and biological field as well as with biotechnological companies ensure the effective transfer of technical results to solve clinical problems.
Projects within the CRIB involve both basic science and novel technological development. In the field of bioengineering the center has recently developed novel techniques to define optimal culture conditions within three-dimensional tissue bio-hybrids, novel material to be used as scaffolds and novel techniques to measure and control the process of tissue remodeling and growth in vitro. Current related research: Government and industry-funded projects to develop novel synthetic and semi-synthetic materials as tissue engineering scaffolds, cell and drug delivery systems, tissue engineered constructs and bioreactors. The CRIB is equipped with a whole series of instruments to design, sinthetize and characterize macromolecular materials including proteins as well as equipment for processing and realization of cellular biohybrid constructs. **The CRIB group** offers a interdisciplinary expertise in biomaterials and polymer-based devices for drug delivery and tissue engineering, encompassing material synthesis and processing, device modeling, material and device haracterization along with cell-material interaction analysis and toxicity/biocompatibility assessment.

The proposed project is within the scope of the main research program of IIT and in particular is focused on the activity of the platform ‘HUMANOID RESEARCH’. The development of bioactive platforms able to promote and guide human cell growth, proliferation, biosynthesis and development represent a relevant step towards tissue and organ regeneration *in vitro* and *in vivo*. Furthermore, the understanding of cell-material interaction and the biological mechanisms that underline biological recognition is of fundamental relevance to develop *cell to chip and chip to cell technologies* with the expectation that platforms developed to guide biological process *in vitro* and *in vivo* will at the end be also useful for cell immobilization.
On the broader view, the proposed project has implication in all the three platforms proposed. For instance, the

guidance of tissue growth through bioactive scaffolds has relevance in the intelligent drug delivery system since the sequestration and delivery of morphogenic factors is a fundamental requirement for the appropriate and physiological tissue development. Therefore the bioactive scaffolds require the encoding of intelligent drug delivery system that may control the time and space distribution of biological agents within the system. Furthermore, the study of cell-material interaction will be of interest for the INTERACTION TECHNOLOGIES and in particular for the adaptive interfaces.

The Interdisciplinary Research Centre in Biomedical Materials (CRIB) includes more than 15 Departments of the University of Naples Federico II and over 100 researchers with expertise in different areas such as materials science; orthopedics, general surgery, veterinary, biochemistry, organic chemistry, cardiovascular, dental and maxillo-facial surgery, tissue reconstruction and pathology.

www.nnl.it

Research Unit IIT – National Nanotechnology Laboratory (NNL)-INFM-CNR

Scientific coordinator of the RU : Prof. Ross Rinaldi

SCIENTIFIC OBJECTIVES

- ***WP1 Cells to chips and chips to cells***

  P.I. dr. Franco Calabi
  Collaborators: dr. Dario Pisignano
  External partners: A. Sannino (Dipartimento di ingegneria dell'Innovazione, Lecce), M. Spector and I. Yannas (Dept. Mechanical engineering, MIT, Boston), F.Beltram (SNS Pisa)

- ***WP2 Advanced characterization tools and imaging***

  P.I.: dr. Pier Paolo Pompa
  Collaborators : dr. Teresa Pellegrino and Prof. Giuseppe Gigli
  External partners: Prof. F. Beltram SNS (Pisa)

- ***WP3 Soft lithography on functional molecules***

  P.I.: dr. Andrea Camposeo

  Collaborators: dr. Dario Pisignano, dr. Athanassia Athanassiou, dr. Luana Persano and dr.Liberato Manna

  External partners: Prof. F. Beltram SNS (Pisa)

- ***WP4 Functionalized nanocrystals for cancer therapy***

  P.I.: dr. Teresa Pellegrino

  Collaborators: dr. Franco Calabi

  External Partners: Prof. Jacopo Meldolesi – Scientific Institute San Raffaele (Milano)

- ***WP5 Biodevices and biosensors arrays for electrochemical sensing and redox activity monitoring in cells***

  P.I.: dr. Giuseppe Maruccio

  Collaborators : dr. Franco Calabi

  External partners: Prof. Pelicci IFOM-IEO (Milan)

WORKPACKAGES

1. WP1 - Cells to chips and chips to cells

P.I.: dr. Franco Calabi

Collaborators: dr. D.Pisignano

External partners: A. Sannino (Dipartimento di ingegneria dell'Innovazione, Lecce), M. Spector and I. Yannas (Dept. Mechanical engineering, MIT, Boston), F.Beltram (SNS Pisa)

The purpose of this activity is to produce high-resolution patterns of biomatrices and to investigate their effects on the proliferation and differentiation potential of stem cells. Patterns with submicrometer features will be generated on planar substrates (SiO_2, plastics, organic polymers) by soft lithography or inkjet printing technology, or in 3D by multilevel layer soft lithography or LbL technology. Biomatrices will consist both of natural or synthetic ECM components (eg collagen/elastin fibers, GAGs, nanofibrillogenic peptides), and will be spatially patterned with signalling molecules (morphogens/growth factors). The primary source of stem cells will be rodent and human bone marrow. In parallel, experiments will be conducted on neuronal cell types, in particular neuronal precursors from the developing nervous system. The aim will be to induce selective growth of neural processes along predefined directions, and synapsing between neural processes, and between neural processes and artificial biosensors (eg based on receptors for neuromediators). Analysis of cell behaviour on patterned substrates will be conducted by video lapse microscopy to track cell dynamics, and by quantitative assays of cell markers (from cell size/shape to specific differentiation/proliferation markers), both in vivo and upon fixation, at different time points.

Milestones

YEAR 1

- Production of patterned biomatrices.

YEAR 3

- Analysis of stem cells behaviour in biomatrices.

YEAR 5

- Analysis of on chip tissue regeneration.

2. WP2 - Advanced characterization tools and imaging

P.I.: dr. Pier Paolo Pompa

Collaborators : dr. Teresa Pellegrino and Prof. Giuseppe Gigli

External partners: Prof. F. Beltram SNS (Pisa)

Several lines of investigation will be pursued under this general heading. A first line will address the development of novel probes for cellular imaging in vitro and in vivo based on recently introduced fluorophores (semiconductor nanocrystals, oligothiophenes). These fluorophores offer several potential advantages on existing markers: i.) high efficiency (hence increasing sensitivity); ii.) narrow emission spectra (hence allowing high-level multiplexing); iii.) minimal photobleaching (hence enabling tracking over extended periods of time). In particular the feasibility will be explored to use conjugates of these fluorophores to specific ligands (peptides/aptamers) as exogenous in vivo labels both for membrane-bound and intracellular targets.

A second, strictly related line will investigate the phenomenon of metal-enhanced fluorescence (i.e. the ability to modulate the spectral properties of fluorophores by metal micro- and nano-patterns) and in particular its application to imaging of specific events in living cells.

A third line of investigation will be devoted to the application of scanning probe microscopy (including NSOM) to the study of living cells. This will be pursued through the integration of high-resolution optical and scanning probe methods.

A fourth line will make use of total-internal reflection microscopy, combined with temporally-resolved spectroscopy, to monitor the dynamics of the plasma membrane compartment of living cells with sub-µm resolution.

Milestones

YEAR 1

- Synthesis of novel fluorophore conjugates
- Fabrication of bio-(cell-)compatible micro-, nano-patterned metal substrates

YEAR 3

- Application of fluorophore conjugates to live cell imaging
- MEF on substrate-adherent cells

YEAR 5

- Imaging of single components in living cells by MEF-based techniques
- Implementation of a TIRF microscope with time-resolved spectroscopical capability at the ms scale

3. *WP3 - Soft lithography on functional molecules*

P.I.: dr. Andrea Camposeo

Collaborators: dr. Dario Pisignano, dr. Athanassia Athanassiou, dr. Luana Persano and dr. Liberato Manna

External partners: Prof. F. Beltram SNS (Pisa)

The conventional expositive lithographic approaches *do not offer any degree of chemical flexibility with*

respect to the target material: i.e., they can be applied to a very limited class of radiation-sensitive polymers, being completely incompatible with organic compounds, and especially biomolecules and living matter.

This WP aims at implementing and developing strongly innovative technologies, which are strategic for processing functional, bioactive and nanocomposite materials. We will explore approaches that, being based on low-temperature nanostructuring procedures (hence able to prevent damage of the functional, optical and electronic properties of active molecular compounds and semiconductor nanocrystals), allow us to exploit the features of nanocomposites and functional molecules for building new devices.

(i) First, we will investigate the basic investigation of the glass-transition phenomenology of organic compounds (and especially polymers), of functional molecules and of nanocomposites, to be employed in the fabrication tasks of the IIT network for embossing and imprinting manufacturing. The study of the physical properties of polymers involved in the manufacturing micro- and nanotechnologies, will require measurements of the dependence of the flow characteristics and of the viscosity of such disordered materials on the temperature. The viscosity of polymers will be monitored over the whole range of temperatures interesting for manufacturing processes –from room temperature to 250°C.

(ii) The amorphous character of the employed films, preserved by lithography, favours the irreversible deformations required by the patterning process, as eventual crystallites usually suppress the flow of the material. This means that all the process parameters, i.e. times, temperature, applied pressure, employed elastomeric compounds, pressing areas, etc., have to be assessed for patterning nanocomposites made by polymer matrices (both inert and functional) and nanocrystal particles, and functional molecules. This will be the core of this task, aiming at delivering a complete set of nanofabrication platform, with resolution up to sub-100 nm, for nanocomposites, functional molecules, including photo- and electrochromic compounds, and biomolecular materials.

(iii) By means of the developed soft lithographies, microfluidic prototypes of lab-on-chip will be realized for a full control of the flow and circulation of complex fluids within microfabricated channels, and the final integration of micropatterned cells will complete the device prototypes, for scaffolds and bio-microdyalisis applications. An unprecedented level of flow control within the chips will be allowed by the wettability changes induced by patterned, optically or electrically gated, functional molecules.

Milestones

YEAR 1

- Development and control of embossing and nanoimprinting lithography for polymers and nanocomposites. Measurements of the glass transition behaviour of different polymers for micro/nanofabrication.

YEAR 3

- Soft molding and room temperature-nanoimprinting up to sub-100 nm on composite materials, photo- and electro-chromic molecules, and biological molecules

YEAR 5

- Demonstration of nanopatterned bio-devices for cell-to-chip applications: patterned cell cultures within functionalised microfluidic channels with flow control.

4. WP4 - Functionalized nanocrystals for cancer therapy

P.I.: dr. Teresa Pellegrino

Collaborators: dr. Franco Calabi

External Partners: Prof. Jacopo Meldolesi – Scientific Institute San Raffaele (Milano)

The broad goal of this WP is to achieve the fabrication of a multifunctional tool kit for cancer therapy based on functionalized nanocrystals. These nanoparticles will be capable of performing the combined actions of highly specific targeting, controlled drug release and hyperthermia treatment. In the WP, we will focus our research on the following activities:

i) Synthesis of nanoparticles with enhanced magnetic properties, with a high response to the applied field strength.

ii) Coating the surface of the nanoparticles to increase their circulation time and to facilitate the binding of ligands. The presence of the ligands on the nanoparticle surface will allow the delivery of nanoparticles to tumour cells according to receptor-mediated endocytosis with high specificity.

iii) Nanoparticle-mediated drug delivery. The engineered coating should also be able to act like a scaffold to load hydrophobic drugs and allow a controlled drug release.

The coating should vary its permeability under the effect of a physical stimulus such as temperature increase, pH dependence, and allow the release of the drug in a controlled way.

iv) Exploitation of the magnetic properties of the nanoparticle core in order to provoke hyperthermia. We expect by this approach to destroy specifically primary tumour cells inaccessible to surgery.

Milestones

YEAR 1

- Synthesis of different types of magnetic nanoparticles

YEAR 3

- Design of the appropriate magnetic nanoparticle coating for the controlled drug release
- Identification, purification and validation of the performance of the engineered coating
- Bioconjugation of the magnetic nanoparticles with specific receptors
- Characterization of the performance of the polymer-coated nanoparticle-ligands conjugates in in vitro experiments on primary tumour cells

YEAR 5

- Set up the hyperthermia experiments in in vitro experiments
- Perform the hyperthermia experiments in vivo

5. *WP5 - Biodevices and biosensors arrays for electrochemical sensing and redox activity monitoring in cells*

P.I.: dr. Giuseppe Maruccio

Collaborators : dr. Franco Calabi

External partners: Prof. Pelicci IFOM-IEO (Milan)

This WP aims at: (1) fabrication and optimization of biosensor arrays for electrochemical sensing based on electrodes/substrates modified with macromolecules (proteins, DNA) or cells, (2) their use for specific medical applications, such as the high throughput screening for factors modifying cellular redox proteins involved in apoptosis and for the monitoring of redox reactions in cells (work in collaboration with the group of Prof. Pelicci, IFOM-IEO (Milan)).

The first milestone will consist in demonstrating electrochemical biosensors based on cellular redox proteins (particularly those involved in apoptosis, such as $p66^{shc}$), to be generating fast multivariate response to factors (eg interacting proteins) or drugs. In a second phase of the project, microfluidic technology will be exploited to improve analytical performance by reducing the consumption of reagents and decreasing the analysis time, and to provide automation. A further target will be to design and implement sensors suitable for monitoring the redox state of cellular components within intact cells, which eventually will be interfaced to live cell arrays. These biosensors will be applied to fast, highly parallel screens for factors able to modulate the generation of ROS (Reactive Oxygen Species), which are known to play a central role, amongst others, in the control of apoptosis or tumourigenesis.

Milestones

YEAR 1

- Fabrication of biosensor prototypes for electrochemical sensing based on electrodes/substrates modified with redox proteins and cells.

YEAR 3

- Fabrication and optimization of biodevices and biosensor microarrays for high throughput electrochemical screening
- Design of a biosensor to probe the redox state of cellular components in whole living cells

YEAR 5

- Integrated microfluidic biosensor arrays
- Investigation of the molecular mechanisms underlying apoptosis
- Fast and parallel screening of factors inducing the formation of ROS

QUALIFICATION OF THE RESEARCH UNIT

The National Nanotechnology Laboratory of CNR-INFM is an interdisciplinary center for nanotechnology. Launched in January 2001, the centre at present is one of the largest cross-disciplinary facilities at the European level. It consists of around 170 scientists with a vast representation of different backgrounds (physicists, chemists, biologists, electronic engineers and medical doctors) working in close collaboration to foster exploratory and seeding research in cross-disciplinary areas (such as molecular electronics and nanobiotechnologies), exploiting either the bottom-up or the top-down approach, in the same mainframe.

Different facilities with a large collection of sophisticated scientific equipment are available at NNL:

1. A semiconductor growth and epitaxy facility, which relies on a class 100/class 1000 clean room (~ 120 sqm) containing a double-chamber AIXTRON MOCVD and a Riber MBE machine.

2. A nanofabrication facility, which relies on 4 class 1000/10000 clean rooms equipped with reactive ion etching systems, evaporators, mask aligners and 3 electron beam lithography systems (a Leica Lion FEG system and two Raith systems with point resolution up to 2 nm). The facility includes SEM and TEM for basic characterization.

3. An organic chemistry and molecular devices fabrication facility, for the synthesis and film technology of organic compounds, containing three 3 glove boxes (2 with evaporators), fume-hoods for the nanofabrication of colloidal nanoparticles and soft lithography equipment.

4. A spectroscopy and transport facility for material characterization, based on spatially resolved spectroscopies (micro-photoluminescence, luminescence with 100 nm resolution, and scanning tunneling luminescence with 20 nm resolution, all at 20K), magnetospectroscopy (10 Tesla at 2.2 K), Raman, photoluminescence excitation, tunable pump-probe and optical gain measurements, photocurrent up to 6 micron wavelength, femtosecond and picosecond spectroscopies, femtoamperometric transport systems, parameter and network analysers, etc..

5. A scanning probe lab, including a Digital AFM/STM system, a CP-Research Thermomicroscope AFM /EFM /SCM and liquid AFM system, a biological (Digital Bioscope) AFM system mounted on an inverted microscope, and an Omicron ultra-high vacuum low temperature STM.

6. A biology laboratory including a 50 square meter, class 10,000 clean room equipped with a nanoplotter and a fluorescence scanner, and a dedicated facility for tissue culture.

7. An high-performance computational center, the Center for Advanced Computational Technologies (CACT), a node of the Southern Partnership of Advanced Computational Infrastructures (SPACI), with the following computational resources: Server HP XC6000 with 132 Intel Itanium2; Server HP rx8620 with 8 Intel

Itanium2 shared memory; Server Compaq SC with 16 EV67. The value of these parallel computers is about 1.5M euro. On these servers various computational chemistry tools are installed, such as TURBOMOLE and GAUSSIAN.

The high-level research at NNL, together with its strong interdisciplinary know-how and state of the art equipment, have attracted several multinational companies, and joint R&D activities devoted to high-risk technological research are currently in progress with Agilent Technologies, TechInt and STMicroelectronics (several patents have already been deposited), Alenia, DataLogic, FIAMM, Leuci, IGuzzini.

A modern management structure, including economists, lawyers and webmasters, ensures an international standard in the management of intellectual property issues, in the legal relationships with industries and private R&D activities, and in outreach and dissemination programs. In the last three years, NNL has launched three spin-offs in high-tech areas out of its patents and know-how. Moreover, NNL acts as the training laboratory for the Nanoscience Department of ISUFI, which is a school of excellence for post-graduate education and provides a reservoir of talented young researchers to NNL.

A dense network of international collaboration has been set up since the beginning of the Lab's activity. Bilateral research programs, funded by MIUR, have recently been launched with Japan, University of Tokyo, University of Berkley –CA (USA), and MIT Cambridge (USA).

Recently Prof. R.Rinaldi has been started a collaboration with the activity of the European Technology Platform on Nanomedicine.

One of the major sections of NNL is the Nano-Biomolecular-Electronics and Nanobiotech Division. It is devoted to the exploration of new concepts and systems based on self assembled and/or promoted interactions of biological and molecular entities with nano-scaled and nano-structured inorganic matter. The goal of this research is to create and exploit new concepts, new architectures and novel hybrid devices for fundamental studies and applications in the field of nanoscience and health.

The division operates in a 1200 square meters area divided in four sections:

1) Nanotechnology-clean room lab; 2) Biology lab; 3) Chemistry Lab; 4) Advanced characterization and microscopy lab.

All the sections are equipped with state of the art and advanced instrumentation, among which we highlight:

Electron beam lithography system, microarray spotter, electrochemistry workstation, 4 scanning probe microscopes (one of which is a Bioscope), 4 fully equipped hoods for chemical synthesis and a nitrogen glove box, a TEM, a double grating spectrofluorimeter (equipped with accessories for sample cooling, TIRF and time resolved spectroscopy), tissue-culture facility, 16sqm cold room.

The division's personnel amount to 40 scientists with different background in physics, chemistry biology,

engineering, and medicine.

Initially, the Lecce RU of IIT will share spaces and facilities with the Nano-Biomolecular-Electronics and Nanobiotech division of NNL. Whilst the two units will maintain a close interaction, an additional 600 sqm of dedicated lab space will be made available specifically for IIT activities by the end of 2007.

The Lecce center will also contribute with the Labs of Soft Matter Nanotechnologies, which include a Class 10,000 cleanroom environment dedicated to soft lithography processes (also multilevel), fume hoods and optical benches for the characterization of active molecules. This group includes about 15 units of personnel, with a deep know-how about processing of organic and inorganic compounds, optical characterizations of conjugated molecules, organic-based optoelectronics, and microfluidics, that will be available for the IIT-related activities.

The IIT RU personnel and activities will be integrated in this area and the PhD students will benefit from the association to the ISUFI school.

iit
istituto italiano di tecnologia

ANNEX 5

THE INTELLECTUAL PROPERTY'S POLICY

THE INTELLECTUAL PROPERTY'S POLICY

Two different cases for the IP policy are treated:

- The IP policy for the external collaborations with public and private partners

and

- The IP policy for the internal research staff.

In the former case, the general criterion is that IIT equally shares the intellectual property with the partner, and eventually gives exclusive exploitation license to industrial partner(s), only for the applications of the invention which are relevant to the core activity of the joint project. Other agreements are eventually possible, depending on the funding and on the resources invested by the partners. Work is currently in progress in collaboration with the patent offices of the Multidisciplinar Research network to produce a general frame agreement. The expected release of the final document is scheduled for December 2007.

For the latter case (IP policy for the staff members) IIT follows a scheme similar to that currently implemented at all Campuses of the University of California, with few modifications ensuring compatibility with the Italian legislation. Such policy tends on one hand to preserve the rights of IIT and, on the other, to stimulate and award the IIT researchers. The IP policy document elaborated by the Scientific director and approved by the Comitato Esecutivo is displayed in annex 2. The principles of such an IP Policy are listed in the following:

A: Documentation of patentable inventions.

IIT investigators and staff are obligated to appropriately document all experimental results that might lead to patentable inventions. To fulfil this obligation, a standard documenting procedure will be implemented, which will require the use of experimental log-books that are bound, numbered, written in indelible ink and signed by the investigator(s).

B: Disclosure of patentable inventions.

All IIT investigators and staff are obligated to disclose to the Institute any possible patentable inventions developed within the scope of their employment. To fulfil this obligation, a standard disclosure of invention will be implemented (preliminary forms already available from e.g. UC).

C: Assessment of patentability.

The IIT will conduct a patentability analysis on all disclosures of invention filed and, based on this analysis, will determine whether it will be able to successfully commercialize the invention. This analysis will be conducted in a timely manner and will not exceed a maximal duration of 45 days. At the end of this period, the IIT will inform the inventor(s) of the decision and, if patenting is recommended, will work diligently with the inventor(s) to draft a patent application. If patenting is not recommended, the IIT may release the invention back to the inventor(s).

D: Assignment of patentable inventions.

All IIT investigators and staff are obligated to assign to the IIT their sole and joint rights in any possible patentable inventions

developed within the scope of their IIT employment.

E: Royalty sharing.

All IIT investigators and staff will share in net fees and royalties received from licensed intellectual property. 50% of the net proceeds will go to the IIT to cover for reimbursement costs and to support research; 30%-40% of the proceeds will go to the inventor(s); and 10%-20% of the proceeds will go to the inventor's Department.

For details http://www.iit.it/files/File/pdf/Ip_policy.pdf

ANNEX 6

APPLIED RESEARCH PROGRAM

TECHNOLOGY TRANSFER PROGRAM

Growing interest by IIT's main stakeholders such as the Government and its Ministries' officials have increased expectations on IIT's role within the Italian scientific and economic arena, therefore, an adequate response by IIT is required. We believe that the Technology Transfer Program (TTP) is instrumental at producing an impact in compliance with IIT's key mission statement:"… of contributing to the nation's productivity growth."

The final goal of the TTP is to implement an effective plan that will ensure a direct link between industry and science by matching specific industrial needs with ad hoc technology solutions.

Since the last Board meeting (July 2006), where we presented our vision of the overall TTP strategy, we have started fine-tuning its various components through the following action items:

1. **Bottom up R&D Programs**
2. **Top down R&D Programs**
3. **Contest for Young inventors**

An up-to-date report of the Technology Transfer Program is detailed in Annex B to the "STATUS REPORT June 2007".

1. BOTTOM UP R&D PROGRAMS

In this first action item we have mapped all scientific streamlines carried out by IIT within the National Lab of Morego (NLM) and Network of satellites in order to identify main technology outputs, industrial relevance, and time to market. The second step consisted in an intensive presentation of IIT activity to potential industrial partners.

The **Show-room** set up with together with the Research Directors of the Robotic area in the NLM will be a fundamental tool where IIT's potential industry partners will see and test prototypes of our technology transfer portfolio. The location will be also used to host events aimed at establishing contacts with potential industry partners.

Within the **Network of Satellites** we are in the process of defining a shared Intellectual Property policy in order to launch a joint technology transfer program.

Finally, we are in the process of defining **"promotional activities"** that will include:

- Technology day of IIT, where Research Directors from the NLM and network of satellites will present current and future prospects of R&D activities to government officials and industry representatives
- Participation to technology fairs (i.e. Research to Business of the Fiera di Bologna, May 2007)
- Restyling of IIT's website, that will include a Technology transfer section containing all information on our technology transfer portfolio
- Articles on specialised industry sector journals

2. TOP DOWN R&D PROGRAMS

This action item is aimed at enlarging the current scientific agenda by defining top-down initiatives to be conducted with strategic Italian industry partners. Following this approach, the Technology transfer program, in this action item, will be conceived as a "complementary source of new projects" that will enable a direct link between industry and the global scientific world.

The first step will be to launch calls for proposal aimed at building collaborations with pre-selected industry partners operating in strategic industrial and technology sectors. Selection of industry candidates and related technology sectors will be conducted together with Government officials, and national industry representatives.

We will then plan exploratory actions and meetings aimed at developing jointly:

- Initiatives: exploration of new hi-tech scenarios
- Activities: launch of joint R&D streamlines
- Labs: set-up of common facilities for specific R&D purposes

This activity will be combined with bottom-up action items described above.

3. CONTEST FOR YOUNG INVENTORS

We are in the process of defining an International contest for young inventors. This contest named ***"I2Talent"*** is aimed at selecting most promising projects proposed by young inventors (maximum age is 35) within Nanobiotechnology and Robotics scientific sectors.

The maturity level of new applications on which we are focusing our attention, belong both to the initial and final stages of the technology development process. We are therefore considering the following two different prizes and selection processes:

- **Builders**: in this area we will select projects proposed by young inventors characterized by innovation/breakthrough solutions in approaching the development of new applications. The projects we will select in this area will therefore be at the very early stage of the research process. This consideration will include such factors as: the degree of creativity and innovation embodied in the scientific idea, the technology involved and its viability with a final judgement on its future business potential.

- **Developers**: in this area we will select projects/applications that have already reached a maturity level supported by strong scientific evidence and economic rationale. We will then test the scientific background, industrial feasibility and market potential of all proposed ideas including a buisiness plan to be submitted by contest candidates. The selection will therefore analyse the business idea, the technology involved, the suitability of the strategy for taking advantage of the opportunity, the team's capability to implement the plan, and the rationale of the economics (capital needed, revenue and profit potential, as well as the time line to profitability, and the exit strategy).

In both selections the jury will be composed by members representing the international business and scientific environment

The **"Builders Award"** will consist of a two year Research contract that will be granted to the winner that will also include other worth in kind services such as the hosting within IIT's facilities and all related services. The final aim of this award will be to guide the young inventors through the different stages of the scientific program including possible technology transfer support (i.e. spin-off, venture capital ecc.)

The **"Developers Award"** will consist of a start-up package made of € 200,000 cash plus approximately € 100,000 worth in-kind services such as one year free incubation services, one year free access to IIT's facility labs and other consulting services (fiscal, administrative, IP policy, etc.). In exchange, IIT will own a small percentage of equity of the newco , on the understanding that all operations will have to be established in Italy. Equity owned by IIT could eventually be expanded according to accomplishment of scientific and industrial milestones also in agreement with external financial partners (venture capital, private companies, banks etc.).

Considering the two single prizes (Builders+ Developers) to be awarded both in the Nano-biotechnology sector and Robotics, we are budgeting for two years a total expense (cash + worth in kind expenses) of around € 1.3 million.