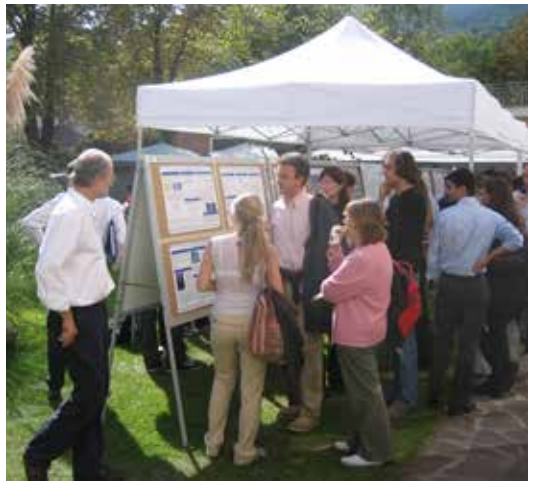
DOCTORAL PROGRAM IN BIOENGINEERING

Chair:
Prof. Maria Gabriella Signorini

The Doctoral Programme in Bioengineering trains graduate students through a strong interdisciplinary education on engineering, mathematics, medical and biological knowledge to develop high level engineering problem-solving abilities in life sciences inside a research group or in private or public industrial context. Students are involved in research works in fields currently ongoing at the Bioengineering Department of Politecnico di Milano which organizes the PhD track.PhD students in Bioengineering are about 20 per year, around 60 in the three year course. Research themes include modelling and analysis of physiological data, signals and systems; biomedical imaging processing and technologies; technologies and instrumentation for movement analysis, rehabilitation, ergonomics and sports; therapeutic devices and life support systems in cardiology, cardio/surgery and pneumology; design and assessment of prostheses; computer aided surgery and surgery optimization through modelling; cardiovascular fluid dynamics; molecular, cellular and tissue engineering for biomaterials and prostheses; neuro-engineering and nanobiosystems; genomic and proteomic data analysis; bioinformatics. Stage periods in distinguished research institutes in Italy and abroad are an essential feature of the student training.

The educational offer includes ad hoc advanced courses specifically projected for the Ph.D. Among them, the school of the National Bioengineering Group is held every year since 1981 for one week in Bressanone (BZ). The content of the School is focused on themes of the bioengineering research and knowledge and it is organised with the support of national and international qualified teachers in the specific field coming both from academic and industrial research. The school is also a unique opportunity to put together students from different Doctoral Programs coming from the entire country. This allows exchanging ideas and experiences also representing a very useful educational event.

Some themes of the recent editions:
2006 Neuro-Robotics. Neuroscience e robotics for the development of intelligent machines
2007 Computational Genomics & Proteomics
2008 Wearable Intelligent Devices for Human Health and Protection
2009 Bioengineering for Cognitive Neurosciences
2010 Synthetic Biology
2011 Neuroinformatics



Scientific and research Ph.D activities receive a strong support by Laboratories located inside and outside the Department in cooperation with other research bodies and university hospitals:

- Laboratory of 2D-3D analysis and modelling of neural and sensory systems and bioelecromagnetism
- Biomaterials Laboratory
- Laboratory of biocompatibility and cell culture -BioCell
- Laboratory of Biological Structure Mechanics – LABS
- Laboratory of Computational Biomechanics
- The "Luigi Divieti Posture and Movement Analysis Laboratory
- Laboratory of micro and bio fluid dynamics
- Biomedical Signal Processing Laboratory
- Medical Informatics Laboratory
- Biomedical Technologies Laboratories

The PhD in Bioengineering has an **Advisory Board** which has in charge all the student activities

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The External **Reference Committee** is a fundamental link toward the industrial research, the clinical applications with an european and international perspective.

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The interest toward the activities of the Ph.D in Bioengineering is demonstrated also by the external financing of 3 years PhD Fellowships. Some recent supporters, besides the Bioengineering Department, of our PhD are:

SCHOLARSHIP SPONSORS
Istituto di Ingegneria Biomedica ISIB e Istituto di Tecnologie Industriali e Automazione ITIA, CNR, Milano.
Fresenius Medical Care, Italy
Fondation Leducq, France.
IRCCS San Raffaele, Milano, Italy

In 2010 and in 2011 new PhD positions as Executive PhD's have been created. They consist of a special PhD path organized in 4 years and dedicated to PhD candidates that already work in a company/ society.
The Bioengineering PhD opened 3 positions in 2010 (Fraunhofer Institute, Erlangen, Germany; Istituti Ortopedici Rizzoli, Bologna; SKE S.r.l. Milano) and in 2011 (Medtronic SpA, IRCCS Besta, Milano).

GRANGER CAUSALITY ANALYSIS IN DEPRESSED BAROREFLEX REGULATION CONDITIONS

Tito Bassani

Introduction

The baroreflex sensitivity (BS), derived as the variation of heart period (RR) per unit change of systolic arterial pressure (SAP), represents a fundamental clinical parameter valuable e.g. to better predict the risk of death in subjects after myocardial infarction. Recently, several non-invasive techniques based on the analysis of spontaneous beat-to-beat RR and SAP variability have been proposed for the estimation of BS in more physiological conditions, without the need of perturbing cardiovascular regulation with a pharmacological stimulus. These techniques are helpful in assessing BS only when causality is along baroreflex (i.e. SAP changes contribute to RR variations). Unfortunately, this prerequisite is not properly tested. Usually, assessing the significance of squared coherence function (K^2) between RR and SAP series is considered to be sufficient. Unluckily, K^2 can be high even when RR variations contribute to SAP changes along the reverse causal direction imposed by the mechanical feedforward pathway. Furthermore, the estimate of causality between two signals might be biased by the presence of a third one affecting both. When considering RR and SAP, respiration (Resp) can act on both, thus imposing to account for Resp when assessing causality between RR and SAP. The purpose of this Doctoral dissertation is to assess the degree of correlation between RR and SAP along baroreflex during experimental protocols known to be able to depress the baroreflex function. In addition, the aim of this dissertation is to assess the effect of an exogenous source such as Resp on the causal relation from SAP to RR. The causal relation from SAP to RR was assessed using Granger causality (GC) approach according to two traditional tests in the time domain. Spontaneous BS was assessed evaluating the square root of the ratio of the power of RR to that of SAP calculated in low and high frequency bands (i.e. LF and HF, ranging from 0.03 to 0.15 Hz and from 0.15 to 0.5 Hz respectively) for all subjects with K^2 in the respective band greater than 0.5. BS values were then compared with that derived from subjects with a significant SAP-RR causal relation to check whether causality analysis provides information helpful to refine the BS estimate.

Methods

According to GC definition, given two signals, y_i and y_k, y_i is said to Granger-cause y_k if past values of y_i contain information on y_k above and beyond the information contained in past values of y_k. Given the set of M signals $\Omega_y=\{y_1,y_2,\ldots,y_M\}$ the causal interactions among the M series are described according to a linear time invariant multivariate autoregressive (MAR) model. The F-test checks whether the goodness of fit of the MAR model accounting for y_i is significantly larger than that of the MAR model excluding y_i. At difference with the F-test, the Wald test, carried out directly on the parameters that weight the contribution of y_i towards y_k in the predicted MAR model, checks whether all the predicted parameters representing the coefficients of the linear regression of y_k on y_i are not significantly different from zero.

Simulations

A large number of simulations were performed:
i) to evaluate the ability of GC tests to identify uncoupled series;
ii) to study the dependence of GC detection on the dynamical features of coupled series operating in closed loop;
iii) to assess the bias on GC between coupled series operating in closed loop due to the presence of exogenous sources.

Experimental protocols

Four experimental protocols were considered:

1. 8 juvenile pigs were intravenously anesthetized with propofol as anaesthetic agent. Four mechanical ventilated modes were considered: pressure controlled ventilation, pressure supported ventilation, and the random variable implementation of the previous two strategies;
2. 37 subjects, scheduled for craniotomy for supratentorial lesion, were anesthetized with propofol and mechanically controlled ventilated. Anaesthesia was then maintained according to two different strategies involving the volatile administration of sevoflurane (18 subjects) and the intravenous administration of propofol (19 subjects);
3. 22 patients in sinus rhythm with dilated cardiomyopathy were recorded at rest, while breathing at a selected paced breathing frequency, tilted at 70°;
4. 16 healthy subjects were recorded: at rest, tilted at 45° and 90° carried out in random order, during recovery sessions following each tilt.

Results

F-test and Wald test performed similarly in terms of detections of GC. Simulations proved:
i) the reliability of the statistical tests to detect the absence of GC relation on uncoupled series;
ii) the dependency of the detection of GC relations on the dynamical features characterizing closed loop interacting series;
iii) that the presence of an exogenous source affecting the series operating in closed loop is able to bias the detection of GC relation. Assessing GC by accounting for the exogenous source allowed the cancellation of this biasing effect. Experimental protocols results showed the presence of a significant GC relation from SAP to RR in a large percentage of humans or animals. When accounting for Resp baroreflex control was found less involved in animals undergoing controlled ventilation in comparison to assisted ventilation, in humans undergoing deep anaesthesia during both volatile and intravenous strategies and in healthy subjects during recovery sessions. Animals undergoing deep anaesthesia showed significantly smaller values of BS during controlled ventilation in comparison to assisted one. In the human subjects during deep anaesthesia, intravenous strategy significantly decreased BS in HF band with respect to volatile strategy. Heart failure patients had very low values of BS in LF during paced breathing and tilt with respect to rest. In healthy subjects, with respect to rest BS significantly decreased during tilt and slightly increased during recovery.

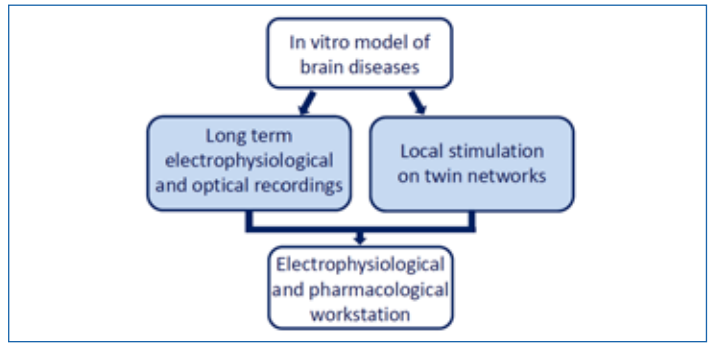
Conclusions

The Doctoral dissertation demonstrated over simulated and real data the importance of accounting for an exogenous source contaminating closed loop interactions in GC studies. During deep anesthesia in controlled ventilation and in healthy subjects during a recovery from a sympathetic activation maneuver (i.e. tilt), disregarding Resp leads to erroneously attribute to baroreflex the direct influences of Resp on RR. Even though BS was depressed during anaesthesia both in animals and humans, in heart failure population at rest and during tilt both in healthy and heart failure population, analysis of GC from SAP to RR suggested that a relevant percentage of subjects preserved a significant causal relation along baroreflex. This results demonstrated that baroreflex control is still present and working both during deep anesthesia and in heart failure population. Notable exception is under deep anesthesia in humans using a volatile administration strategy, thus providing a tool to assess the different abilities of anesthesiological treatments in preserving an active cardiovascular control. GC analysis suggested also differences during deep anesthesia between different ventilatory strategies in animals, thus making available a tool to assess the performances of different ventilation strategies. In all the considered experimental protocols the estimate of BS computed after the exclusion of the subjects without a significant causal relation from SAP to RR along baroreflex was similar to that derived from the group of subjects with a significant K^2 correlation. This result was disappointing and largely unexpected since causality from SAP to RR is a prerequisite for a reliable assessment of BS. This finding indicates that the estimate of BS from spontaneous RR and SAP variability, even when causality from SAP to RR is verified, might be still affected by important biases and unaccounted influences.

DEVELOPMENT OF INNOVATIVE DEVICES FOR RELIABLE STUDIES OF *IN VITRO* MODELS OF CENTRAL NERVOUS SYSTEM PATHOLOGIES

Emilia Biffi

Neurodegenerative diseases are chronic pathologies of the Central Nervous System (CNS) that cause brain functional derangement. These brain diseases are growing in incidence due to increase in life expectancy, but still most of their causes and mechanisms are not well known. This raises the interest in studying brain alterations as well as developing and testing new molecules that could re-establish brain functionalities. Often, researchers take advantage of using animal models of diseases with the purpose of better understanding these neuropathologies. Given the complexity of the adult tissue, these studies take advantage of *in vitro* neuronal networks, which are widely used as a model for acquiring a basic understanding of the network functionality. These in vitro models can be investigated by means of Micro Electrode Arrays (MEAs) which offer a simple approach to examine the activities of neuronal cells on a large scale and provide new insights into the dynamics of in vitro networks.
Within this framework, it is of great interest to use MEA technologies to characterize the spontaneous electrophysiological activity of in vitro models of pathologies and to pharmacologically modulate it in order to regain a physiological network activity. This goal gives rise to many technological challenges concerning the ability of studying the electrical activity during network maturation and of stimulating neurons locally with neuroactive molecules. Indeed, applications involving long-term recordings of neuronal activity suffer from fluctuations in temperature, pH and osmolarity as well as mechanical perturbations occurring during experiments in the laboratory environment. These cause a gradual decline in the health of cell cultures and a decrease of data reproducibility, for which the environmental stability is critical. Moreover, standard techniques to chemically stimulate networks of neurons make use of pipettes and require partial or complete change of the cell culture medium. Disadvantages of these protocols are the unknown kinetic of the interaction between molecules and cells and the rapid changes in the cellular environment. Moreover, these stimulation protocols often lack of defined spatio-temporal control.
Within this framework, the goal of this thesis is to improve the reproducibility and reliability of in vitro electrophysiological data. In this work, the scientific importance of MEA technology in the neurodegenerative field was stressed by means of an electrophysiological and pharmacological study on the experimental autoimmune encephalomyelitis (EAE) model, the mice model of multiple sclerosis. This study suggested that chronic inflammatory stimulation of neuronal networks leads to profound alteration of inhibitory transmission. These results should drive the search of drugs for patient treatments able to control this alteration. During this project, many restrictions related to the experimental equipments and techniques were evidenced. First, it emerged the impossibility of recording neuronal activity continuously for many hours, because of the size of data and the huge memory space required. Furthermore, repeated acquisitions of the same neuronal culture were limited since they caused electrophysiological changes and cellular viability decline. Finally, the standard biochemical stimulation technique with pipettes showed its drawbacks by causing uncontrolled neuronal response and electrode saturation. Consequently, several technological challenges have been tackled. First, a custom chamber for neuronal growth and long term recording of network activity was developed and tested. This chamber solves the problem of MEA long term acquisitions by growing cells and recording their activity since the beginning of their maturation. This reduces thermal, osmotic and mechanic shocks, which neurons are usually subjected to, and thus improves data reproducibility. It was demonstrated that this chamber can grow neuronal networks with morphology and viability comparable to cell cultures grown in standard incubators. Furthermore, it allowed to repeatedly record neuronal electrical activity reducing mechanical disturbances and cellular stress and thus increasing experimental duration. Another strength of the system is the possibility of housing multiple MEAs inside the chamber, which guarantees parallel tests in the same controlled environment. Then, dedicated electronic boards for signal conditioning and post-processing (i.e. spike detection and sorting) were designed to handle the huge amount of data. Their development will make the chamber portable and independent not only from incubators but also from standard recording equipments. In addition, a single comprehensive and high sensitive descriptor, focused specifically on neuropharmacology, was developed and validated to provide a comprehensive functional evaluation of neuronal network communication properties. Indeed, the ability of extracting parameters which describe correlated network activity enhances the compactness and the automation of the device. Finally, a microfluidic device, coupled to an array of microelectrodes, was developed and validated for neuronal extracellular electrophysiology and for local controlled biochemical stimulation. This solves the drawbacks of standard stimulation techniques, enables to work with twin networks and allows to stimulate selectively only one of them. Once more, this approach reduces the variability which characterizes experiments with different MEAs and provides, on the same device, both the treated and the control trial. Finally, it allows to grow and stimulate on a single device both physiological and pathological animal models, comparing their electrophysiology in a more reliable manner.
To conclude, it is appealing the perspective of an integration of these devices into a single one. As Figure 1 shows, the system would permit long-term electrophysiological and optical examination of in vitro brain models during selective and controlled stimulation of cell activity, while maintaining environmental conditions. This integrated system could have a high impact on the screening of neuroactive molecules promoting a better understanding of brain pathologies and neurodegenerative diseases. Hence, this thesis work provides the elements that establish an integrated electrophysiological and pharmacological workstation for brain studies and offers great advantages in central nervous system research.



1. Hypothesis of the final integration of the developed devices.

FORCE SENSING AND DISPLAY IN ROBOTIC DRIVEN NEEDLES FOR MINIMALLY INVASIVE SURGERY

Danilo De Lorenzo

Introduction

During standard manual needle insertion procedures, the operator inserts straight needles inside the tissue in order to perform diagnosis (e.g. biopsy) or treatment (e.g. drug delivery, electrode placement). Surgeons can perform the intervention through minimally invasive keyhole incisions (typically 5-10 mm in diameter), thus allowing shorter recovery time, less pain and less complications (such as infection) for the patient. However, in this procedures, surgeon visual inspection is drastically reduced, therefore he/she must rely on his/her limited sense of touch or on a planned needle path on preoperative medical images in order to perform the intervention. This decreases performances during surgical task execution and increases its duration. In keyhole neurosurgical interventions, for example, where a straight needle is inserted from a small opening on the skull, the possibility to detect unexpected situations, like touching a vessel, could prevent vessel rupture and consequently bleeding inside the brain parenchyma.
Force feedback in robotics for minimally invasive surgery (MIS) allows the human operator to manipulate tissues as if his/her hands were in contact with the patient organs. Whether force feedback increases or not the surgical precision and outcome in MIS robotic procedures is still under debate, but recent studies confirm that the haptic feedback can increase the performances in tissue discrimination and can reduce tissue damage and surgical task duration. Most of the robotic devices for MIS or needle insertion do not provide any force feedback and the outer control loop between the operator and the robot relies only on surgeon hand–eye coordination. In order to display the forces to the hand of the operator, specific sensors need to be designed in order to sense the small interaction forces between the tissue and the surgical instrument. For needle insertion tasks, this small variation is easily masked by the relatively large friction force between the needle shaft and the surrounding tissue, which significantly complicates manual discrimination of the change in tissue property. However, force sensors design and implementation is a balance between miniaturization, sterilization possibility, cost and equipment encumbrance.

Materials and methods

The goal of the thesis was to improve surgeon performances during MIS, adding force feedback information during needle insertion procedure as the paradigmatic clinical scenario, focusing on neurosurgical interventions.
The first part of the work was realized at the NearLab, Politecnico di Milano, Italy, within the European research project ROBOCAST – FP7-ICT-2007-215190 (Robot and Sensors Integration for Computer Assisted Surgery and Therapy) and was aimed at investigating force sensing modalities to detect the small interaction forces between the needle and the brain tissue, in order to display them through an haptic interface on the hand of the surgeon in a tele-manipulation scenario. In this framework, we evaluated the ability of a slave tele-operation system prototype for biopsy probe insertion in estimating the resistance to the advancement (force) experienced by a standard probe for brain biopsies within a brain-like material. The biopsy probe is inserted by a piezoelectric actuated inchworm device that is driven by the surgeon through an Omega haptic device (Force Dimension, CH). The objective of the study was to allow the neurosurgeon detecting the interfaces between tissues with different mechanical impedance (e.g. due to the presence of membranes or to vessels walls) in order to stop the procedure if an unsafe situation was encountered.
Force estimation without using sensors was compared to actual force sensing. Sensors were included in the piezoelectric actuated device, realized in collaboration with the Medical Robotics Lab at the Technion, Israel Institute of Technology. Sensors were not directly placed on the needles, avoiding any sterilization issues. Force display and control loop were realized in collaboration with the Sirs Lab at the University of Siena, using an Omega (Force Dimension, Switzerland) haptic interface, able to reproduce on the hand of the operator the sensed forces.
System accuracy proved to potentially satisfy the neurosurgery application requirements since the maximum error was 0.16N as average value. Using the gelatine 8%, which proved to replicate brain tissue mechanical properties, the system resolution (worst case) was around 20% of the puncturing force, independently from the insertion velocity. This result showed that the system can convey to the operator the information on tissue discontinuities with a Signal to Noise Ratio (> 15dB) close to the Just Noticeable Difference (JND).
The second part of this work was implemented at the Haptics Labs at the Johns Hopkins University in Baltimore, MD, USA and involved also the collaboration with the Surgical Assist Technology Group of the AIST Institute (Tsukuba, Ibaraki, Japan). The goal was to enhance force perception during needle insertions, giving to the surgeon the possibility to better feel the presence of discontinuities within the tissue (like membranes). The use of a coaxial needle allowed to separately sense the forces at the tip of the needle and the forces along the shaft, even if the sensors are not directly placed on the needle tip/shaft. The sensed forces were then enhanced and fed back to the operator using a robotic assistant in a co-manipulation approach, where operator and robot share the control. Subject experiments showed that the coaxial needle assistant facilitated perception of thin membranes during needle insertion when an amplified version of the tip force at the needle tip is displayed to the user. Results showed that this control method performs better than displaying the overall interaction forces between the needle and the tissue (tip and friction forces together). The new force feedback type significantly decreases false positive membrane detection (close to zero), which is desirable to be as small as possible since an high ratio of false positive increases the procedure duration in the clinical practice, due to repeated double check with other type of detection modalities (e.g. clinical imaging). The system proved to be able to increases detection of thin membranes inside soft material, thus increasing the safety of the procedure.

Conclusions and future works

Together with costs and safety issues, one of the reasons that blocks the proliferation of robotic devices inside the operating room is the change of procedures modalities and instrument required by the surgeon in order to use the new technology. In this work, we showed that for needle insertion procedures it is possible to integrate force sensing with robotic devices, without changing the standard surgical needle and surgical workflow modality, thus increasing safety and performances with relative low costs.
We showed that enhanced force feedback helps the surgeon in a variety of different experimental conditions using different control modalities and force sensing principles.
Tele-operation allows to perform surgery with a remote control, providing movements and force scaling, thus increasing precision in the execution of the task.
Co-manipulation allows force scaling, direct inspection of the patient, since the surgeon is sitting close by, but it suffered of equivalent small residual inertia and friction. Enhance tip force perception using a co-manipulated assistant device proved to be suitable for detecting small tissue property changes, thus increasing procedure accuracy and safety.
The developed systems should be carefully tested in preclinical conditions, such as ex vivo biological tissues, to better evaluate clinical relevance.

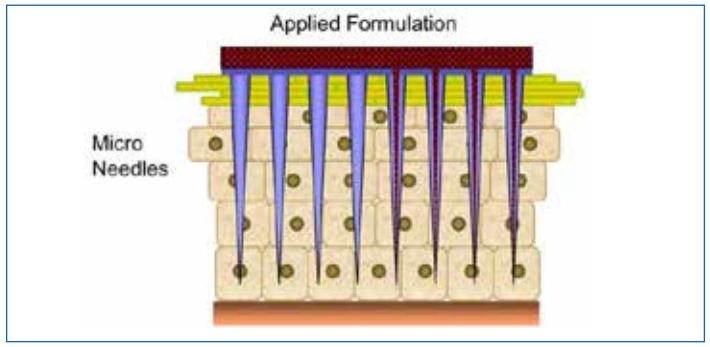
SMART TRANSDERMAL MICRODEVICES FOR BIOMEDICAL APPLICATIONS

Elena Forvi

Skin is one of the most extended organ of the human body: due to its dimensions, skin satisfies several different functions, the most important of which is to isolate and protect the human body from the surrounding world and its exogenous factors. In particular its upper layer, called stratum corneum, represents the real interface with the external environment: thanks to its well organised structure, this layer acts as a chemo-physical and mechanical barrier.

In order to improve the communication between the body and the external world, through the skin interface, systems have to be studied to overcome the stratum corneum barrier.

In the past, syringes equipped with needles have been adopted to bypass the skin barrier, proving an effective modality to enhance the delivery of drugs into the body. Although the possibility to administer each kind of drugs, this approach is highly invasive and uncomfortable: standard needles are some centimetres long in order to deliver medicines in deeper tissues, which are rich in pain receptors and nerve endings. The invasiveness of this method is responsible for increasing anxiety and needle phobia in patients, in particular in paediatric ones.



1. Microneedles technique – The application of microneedles onto skin generates direct pathways into skin layers, when piercing occurs. The open channels allow the delivery of drugs into deeper tissue.

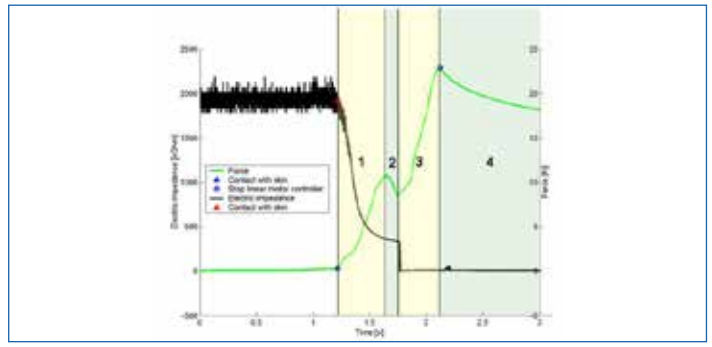
For these reasons, in the last century, other administration routes have been found to encompass the invasiveness of injections and to increase the compliance and comfort of patients: the **transdermal route**, in particular, appears to be the most promising.

Delivering drugs through the skin, i.e. transdermally, is a safe and non or minimally invasive delivery method, which allows for a rapid and direct absorption of the drug into the systemic circulation.

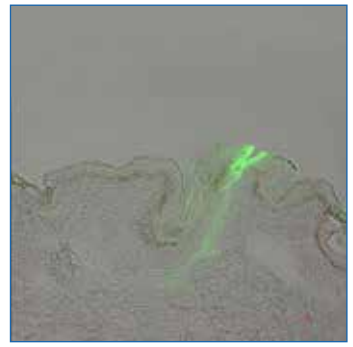
Among the transdermal methods available, **microneedles**, which consist of arrays of micrometer sized needles, seem to be a promising solution to cross the stratum corneum with a minimal pain perception (**Figure 1**).

Microneedles, applied onto skin surface, are able to penetrate and disrupt the stratum corneum barrier, creating real and direct pathways of micron dimensions: the generated holes are order of magnitudes larger than molecular dimensions, thus microneedles potentially allow the releasing of every type of drug, independently by chemo-physical nature and molecular weight in underling tissues.

Drugs, by means of microneedles, can be released locally in the epidermis layers (for vaccines application), or systematically: after having reached the dermis microcirculation, in fact, drugs can diffuse directly towards the systemic circulation, with a potential reduction of drug dosing and consequently of side effects. Microneedles, coupled with pumps, allow a controlled delivery of drugs, which can be administered at constant rate for longer periods of time, thus avoiding a frequent dosage, useful for the therapeutical treatment of chronic and degenerative disesases.



2. Penetration tests on ex vivo human skin samples - A successful skin failure due to the piercing and the insertion of a silicon microneedles array is observable in both the electric impedance (black) and the skin reaction force (green) signals (a).



3. Histological analysis of ex vivo human skin sample after continuous delivery of insulin. An histological section of ex vivo human skin shows a successful penetration of the skin by means of hollow silicon microneedles and consequently a successful active infusion of green insulin.

Microneedles, before to be introduced into the clinical practice, have to satisfy both **technical** and **functional requirements**.

Technical specifications concern: i) skin penetration, i.e. the ability of microneedles to penetrate skin tissue (**Figure 2**), ii) solidity, i.e. the ability of microneedles to be inserted into skin without breaking for buckling, and iii) biocompatibility, i.e. the ability of microneedles to not induce skin adverse reactions.

Functional requirements, instead, concern in the ability of microneedles to infuse drug at specific skin depth in a proper manner, according the typology and dose of the therapeutic treatment (Figure 3).

Finally, microneedles can also be used to improve the communication from the "in" to the "out" of the body, such as in the monitoring of biopotentials: for these reasons, also microneedles-based **dry electrodes** have been considered in this study as a secondary biomedical application.

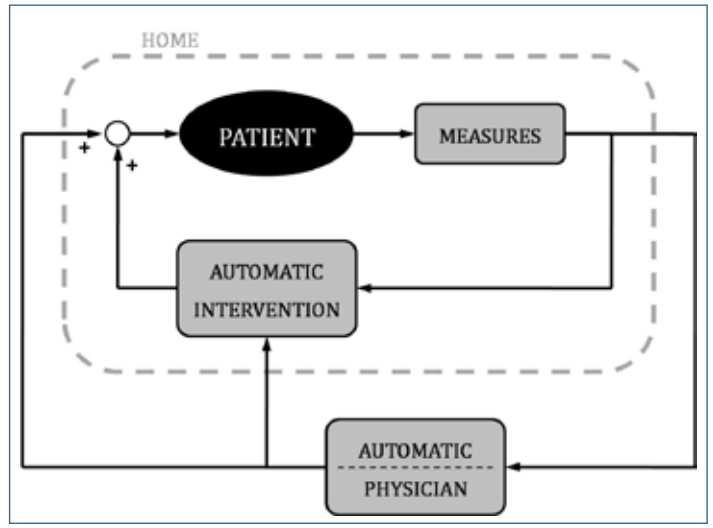
In this work some of these aspects have been addressed: ex vivo and in vivo experimental tests have been performed and encouraging results have been obtained as in the drug delivery field as in that of biopotentials monitoring.

In conclusions, some regulatory aspects, such as pain perception, and some methods of applications for microneedles have been considered and two patented ideas are reported.

DEVELOPMENT OF NEW TECHNOLOGIES FOR THE HOME MONITORING AND TREATMENT OF PATIENTS WITH CHRONIC RESPIRATORY DISORDERS

Leonardo Govoni

Telemedicine has been defined as the use of information and communications technologies (ICTs) to deliver health services and transmit health information at distance for the purpose of improving patient's care and education. Thanks to the clinical effectiveness and cost savings of telemedicine, it has become a standard methodology for monitoring and treating patients directly at home, especially those with chronic pathologies. Chronic diseases are the main cause of death in almost every developed country, and deaths from chronic respiratory diseases are second only to those from cardiovascular diseases. According to which structure of the respiratory system is injured, alteration or inflammation of lung parenchyma, disease of the pleura, chest wall, or neuromuscular apparatus, it is possible to distinguish different chronic pathologies like asthma, chronic obstructive pulmonary disease (COPD), obstructive sleep apnoea syndrome (OSAS), neuromuscular pathologies, etc. Chronic pathologies determine a serious burden on patients and health care systems because of low quality of life and frequent and expensive hospitalizations. The need to reduce this burden brought health care providers to rely on telemedicine services, which perform a better follow up of the patient at home and provide health services at home in order to prevent acute events that can lead to the hospitalization of the patient. The architecture of the conventional telemedicine systems is based on a complex informatics structure managed by external providers and centralized computer servers which operates through a call center. This structure requires complex and expensive agreements among the hospitals, the telemedicine service providers and the communication line providers and is the main obstacle to the wide application of the ICT in the medical routine.
The purpose of the work here presented is to design, realize and test an easy to use new telemedicine system based on a simple and reliable architecture, able provide constant monitoring of patients affected by chronic diseases of the respiratory system, such as COPD and OSAS, using new technologies designed expressly for home care, and able to provide the physician a tool to optimize at distance the treatment at patient's home in real-time.New, small and easy-to-use sensors based on old and edge-cutting techniques and methods have been realized to simply acquire specific physiological parameters, moreover broad and efficient communication services have been implemented. Taking advantage of the use and the combination of these technologies it is possible to support the realization of a tool that is complex and articulate but also practical and easy to use, and that is able to assist the physician managing, monitoring and treating chronic patients, and that provides easy access to all patient's clinical information. The proposed model accounts on the use of an approach on three different levels, tightly bonded among them by a feedback structure.The new model is based on one side on the development and use of new technologies to measure patient's significant parameters in a domestic environment. The acquired data are fused and computed by an artificial intelligence in order to perform the automatic intervention on therapy adjustments directly at patient's home. On the other side the new model comprehends the possibility of a remote intervention by an automatic central decision server. The server is a concentrator that is able to collect, cross-analyse and evaluate all the data coming both from patient's house instruments, and from results of clinical exams and visits, and from previous patient's clinical history. Moreover the server can intervene in modifying



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the provided therapy to the patient with automatic and autonomous decision tools. Finally the architecture modelled allows the simple and quick connection between the patient and the physician, thus to permit the clinical supervision and the possible remote intervention of the physician for therapy optimization.The acquisition of the physiological significant parameters is done by devices designed expressly for domestic use by the patient himself and without physician supervision (mechanical ventilator, etc). These instruments are able to gain the necessary information in a non-invasive way and with the minimal patient cooperation. They're easy to use and adaptable to different conditions of the patient involved.All the information collected is catalogued, synchronized and conveniently stored by a device that acts as a centralizer. The information is analysed through the use of intelligent functions implemented inside the systems that provide the therapy, and inside the centralizer, in order to perform the continuous monitoring of patient's conditions and to perform the automatic intervention on the therapy delivered to automatically optimize it according to the conditions and necessities of the patient. The monitoring function can be oriented to the identification of sudden alterations of particular parameters or life threatening conditions in order to alert the physician at the right time.
The designed architecture allows the physician to quickly access to previously acquired data of a single patient and to real time traces of particular parameters, and permits complete monitoring of patient's conditions. The physician is also provided with tools that allow him to modify the patient's therapy at distance in order to optimize the treatment directly at patent's house from the hospital.Finally, thanks to an Internet connection all the information acquired at patient's house are collected and stored by a remote central server, which also concentrate information and results of clinical exams originating from the hospital, about test and clinical history of the patient.The central remote system is endowed of an intelligence for the evaluation of the acquired information, and performs a deeper and more detailed analysis crossing and fusing the available information thanks to use of new procedures, data mining techniques and new developed clinical guidelines. As the central system is able to perform such detailed analysis, very wide in terms of time and parameter's diversity, it is provided with decisional intelligence able to connect to the devices at patient's house in order to intervene and, automatically and conveniently, modify the provided therapy with the purpose of the optimization of the therapy itself.The new Telemedicine system designed and realized during the Doctorate program is thought to be composed of an architecture simple but robust, realized on different levels. It is a complex system but simple to be applied and easy to be used, and it is able to provide a valid support to the health care professionals managing a growing number of chronic respiratory patients.

A NEW LASER SELF-MIXING INTERFEROMETER SYSTEM FOR THE ASSESSMENT OF THE CHEST-WALL MECHANICS

Ilaria Milesi

The respiratory system is a complex system which accomplishes gas exchange by means of the synergy among functions and structures which have specifically developed at this aim. Impairment at only one of them can yield to a general worsening in the life condition of the subjects. Diseases affecting the respiratory system, such as ARDS (acute respiratory distress syndrome), restrictive diseases and lung oedema, produce alterations and inhomogeneities in the lung structure and in the chest wall which cause changings in the mechanical properties. Up to now, the diagnosis and the management of these kinds of pathologies have been carried out mainly by chest radiography and CT or by laboratories analysis which present some intrinsic limitations: they are invasive or required long time to be carried out; therefor they can't be used to monitor in a continuative way the pathology evolution. On the contrary several published data suggest that chest wall mechanical properties are parameters that might be useful for estimating the possible impact of both restrictive and obstructive diseases on respiration. Although the rich informative content of this parameter, it has been almost disregarded because of the intrinsic difficulties in its measurement, therefore a new approach is needed. The more promising and inspiring one has been reported by Dellacà et al. which suggest the union of FOT (Forced Oscillation Technique) and OEP (Optoelectronic Plethysmography) to estimate the impact of low amplitude pressure stimuli given at the mouth at the level of vibration induced at the chest-wall. This study opens interesting insight into the comprehension of chest wall mechanics but it is limited by the complexity in the methodological approach and costs connected. The already uncovered point in this approach is the measurement of chest wall vibration in a reliable and resolute way by means of low costs and not invasive devices.The objective of this work is to develop and to validate a new method that allow the estimation of the local mechanical properties of the chest-wall based on FOT and optical devices in a non-invasive, low cost and compact way. This new approach is based on the possibility to have reliable and resolute measurements of the local displacement of the chest-wall. To achieve these results an optimum candidate is laser self-mixing interferometry which allows the measurement of very little relative displacement (less than $1\mu m$) of diffusive target, with a compact and economic approach. The introduction of this technology allows fixing the main problem for chest-wall mechanics estimation, that is the measurement of its displacement. The only limitation is the presence of speckles which required the design of a new method for estimating the movement also in the low-injection regime, since traditional algorithms are not reliable in this application. To validate the algorithm in vivo e in vitro measurements have been carried out. Interferometer was carefully aligned with a piston of a linear servo motor and its signal was recorder during the motion of the motor which is controlled to produce a sinusoidal displacement. High linearity correlation, r^2=0.99, and the absence of any biased errors demonstrates the algorithm skills to reconstruct displacement from interferometric signals. Then the interfeometer is used directly on skin during a FOT measurement on eight subjects. Eight normal healthy subjects in supine position during quiet breathing while submitted to a sinusoidal pressure forcing at the mouth with components at 5, 11 and 19 Hz. Displacements of 9 chest wall points were measured by laser self-mixing interferometers. The phase shift among the velocities of these points and the pressure at the mouth was measured by interferometer. The results show spatial inhomogeneities strongly dependent on position and frequency. The results are in good agreement with previous data measured by OEP. In conclusion laser interferometry may be an attractive technique for the assessment of local CW motion. The basic set up proposed here allows to validate the algorithm and to estimate the phase shift of some points on skin but it can't be proposed to acquire more signals and to generate an impedance maps because of the long time required to move manually the interferometer and because the lack of knowledge about the distances between the points measured.
For this reason a new complex system laser scanning system has been design: distantiometry have been added to measure absolute displacement and a stepper motor is used to drive five interferometers. The realized system has been carefully design to fulfill the design requirements of: 1) high spatial resolution, 2) localizing point on the chest-wall, 3) non-invasiveness, 4) easy methodological approach, 5) short measurement time, 6) contactless, 7) modularity. By means of this new device the mechanical properties of patients undergone to anesthesia have been measured. Five patients were studied in the supine position while ventilated in pressure support mode. Just before surgery measurements of oscillatory mechanics were performed at different stages measuring input impedance and local transfer function at 5-11-19 Hz. The patients were connected to the mechanical ventilator and the laser scanning system has been adopted to measure chest wall movements. The results are very promising, in fact the data are very reliable and reproducible, moreover, although patients don't present any atelectasis, as confirmed by the contemporary measurement of the input impedance, the phase reconstructed shift map shows some interesting features, In more details, although five patients are not enough to have a statistical analysis, it is possible to state that these data are comparable with results obtained by Dellacà et al, the maps allows to separate the ribcage compartment from the abdomen one and to detect a slightly little differences according to the frequency and the condition at whom the patient is undergone. In particular all the trends seem to show that the behavior of the chest-wall is more uniform as the frequency increase, while at low frequency, such as 5 Hz, the abdomens and the ribcage are very heterogeneous.

In conclusion with this thesis we reach the aim of proposing a new approach to measure local transfer impedance based on interferometer and we validate both the reconstructive algorithm both the scanning system realized; in this way the first stone in order to study how mechanical chest-wall is influenced by pathophysiological alteration has been set.
Future development will be the acquisition of more patients to verify which kind of parameter may be extrapolated by the chest-wall mechanics to identify the respiratory system status.

DEVELOPMENT OF METHODS FOR THE EMPLOYMENT OF NEAR INFRARED SPECTROSCOPY (NIRS) DEVICES IN CLINICS AND RESEARCH

Erika Molteni

This PhD work explored the applicability of Near Infrared Spectroscopy (NIRS) in the neurophysiologic field. Functional NIRS is a low-cost and easy-to-use tool to non-invasively monitor changes of oxy- and deoxy- haemoglobin (Hb) concentration in the human tissues. We aimed at integrating hemodynamic information captured by time-domain NIRS devices with electroencephalographic, electrocardiographic and electromiographic data. A set of methods was applied for NIRS data processing, among which General Linear Model proved to be the most valuable. Effort was done for highlighting the potential advantage deriving from the combined use of optical information from the brain with the other biological signals. Some major "open issues" in physiology, which seem to be interested by the use of NIRS devices, have been highlighted: neurovascular coupling, the identification of cognitive pattern and the study of cognitive load. A broader extension of NIRS employment to a large part of the neurological field has also been discussed, providing examples for Myotonic Distrophy Type 1 and Unverricht-Lundborg epilepsy.

Method

The interest of our work has been the analysis of the mean level of brain activation. The General Linear Model (GLM), a statistical method for analyzing time- series data, was applied. Inferential procedures at a second level (e.g. about activated channels over all subjects) were then performed.
Contrast matrixes were designed for a direct comparison of:

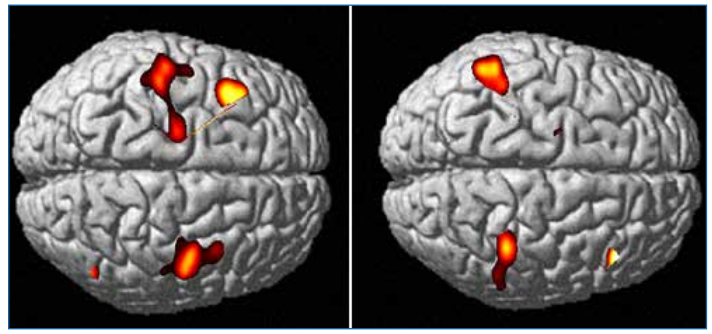
1. activation during the task periods and rest condition with respect to the reference (whole test), and
2. activation during each cognitive load and rest condition with respect to the load/rest immediately harder/ easier or with respect to the reference.

Cerebral activation was indicated by positive t-values in the changes in hemoglobin concentration. The results from second level analyses were plotted as statistical parametric maps, which illustrate the brain regions where increased or decreased concentrations correlate with the stimulation protocol over time.
A second group of contrast matrixes, including both the task design and some preprocessed biological signals was also constructed for data integration. Biological data included inside the design matrix could be either:

1. Electrocardiographic dynamics
2. Electroencephalographic power in specific frequency bands (alpha, beta, gamma, etc.)
3. Electromyographic power

Data integration

Data integration was performed by applying "data informed" GLM for the identification of areas in the brain in which hemodynamic activation is coupled with biological dynamics of different origin, such as those of data capturing the electrical activity of the brain, the heart and the muscles. It was thus possible to investigate the functional role of the brain regions involved in neurovascular coupling, also highlighting the **regional specificity**, due to the difference in data coupling. For example, the two regional hemodynamic patterns shown in fig.1 displayed different coupling with alpha and beta EEG rhythm respectively.
Moreover, "informed" GLM allowed the investigation of **regional growth** due to the increasing difficulty of the task. In fig 2 a right-handed subject performed a right handgrip (with the dominant hand – left panel), a left handgrip (with the non-dominant hand – middle panel), and the most complicated task, alternating right and left handgrip (using both dominant and non-dominant hands – right panel). In this application, GLM proved to be sensitive to the enlargement of the region(s) involved in neurovascular coupling in the beta EEG range. This result is in line with the theories of neuronal recruiting which explain the regional growth of activation with a gradual involvement of neurons supporting a "minimal" neural network during the performance of difficult tasks.



1. Neurovascular coupling between alpha EEG rhythm and total hemoglobin (HbT) is shown on the left for one healthy subject. Neurovascular coupling between beta EEG rhythm and HbT is shown on the right for the same subject. The comparison between the two images highlights two foci with different regional location and different functional role. In this case the EEG-driven GLM helps in functionally distinguishing the areas in which neurovascular coupling is present.

2. Neurovascular coupling between beta EEG rhythm and oxygenated hemoglobin (HbO) is shown for one right-handed healthy subject performing handgrip with the dominant hand (left panel), left with the non-dominant (middle panel) and dominant vs. non-dominant hand (right panel). The brain area involved in the task grows with the increase of the task demand.

Clinical Applications

Some effort has been focused on the assessment of the feasibility of fNIRS cognitive investigations in Myotonic Dystrophy type 1 (DM1), Unverricht-Lundborg patients and photosensible patients.

Results highlighted a much larger hemodynamic activation in DM1 patients with respect to healthy subject in two different attentive tests: Continuous Performance Test and Conners' Test. These findings confirmed previous results obtained by PET and fMRI, and thus support the belief that fNIRS could help in assessing the neurological outcome of DM1 subjects in clinics, also allowing a comfortable, uninvasive and relatively cheap assessment of some neurofunctional correlates of myotonic dystrophy. Further encouraging research on cohorts affected by Unverricht-Lundborg epilepsy and photosensitivity supports broader extension of NIRS employment to a large part of the neurological field.

TIME-FREQUENCY ANALYSIS FOR THE DYNAMIC QUANTIFICATION OF THE INTERACTIONS BETWEEN SIGNALS RELATED TO THE CARDIOVASCULAR SYSTEM

Michele Orini

In this dissertation, some advanced methodologies for the study of non-stationary signals in the joint time-frequency (TF) domain are presented, with the purpose of characterizing the dynamic interactions between cardiovascular signals. This study is motivated by the necessity of improving the understanding of the autonomic control of the cardiovascular system, whose impairment is related with several pathologies. The dissertation is articulated in three parts: An introduction in which relevant physiological and methodological aspects are described; a methodological part in which TF synthesis as well as TF spectral, coherence and phase difference analysis are described; and a part in which the proposed methodologies are applied to physiological studies.In the introduction, the control of sympathetic and parasympathetic nervous systems on the cardiovascular regulation as well as the interactions between cardiovascular parameters and respiration are described. In particular, the physiological mechanisms that are still unclear or that are currently matter of debate are highlighted. To better contextualize the work proposed in the dissertation, a description of the most recent time-varying techniques of analysis is also given.The second part is composed of four chapters, §2–§5, which face the following issues: simulation of non-stationary signals, spectral analysis, coherence analysis and phase analysis in the TF domain.

In chapter §2, a method to generate non-stationary stochastic processes which mimic the dynamics of cardiovascular signals is described. These processes are characterized by a predetermined and controlled TF structure: the design parameters that are used as input of the model are either the instantaneous frequency and power or the instantaneous frequency and spectral amplitude of each spectral component and the output is the stochastic process associated to them. The accuracy and robustness of the method are evaluated in simulation studies which aim at simulating heart rate variability during exercise stress test and listening to different music excerpts.

In chapter §3, the TF distributions belonging to the Cohen's class are introduced. In particular, the smoothed pseudo Wigner-Ville distribution (SPWVD) is described.Owing to the possibility of performing an independent smoothing in time and frequency, the SPWVD is considered one of the best options to analyze non-stationary signals. A method to quantify the TF resolution of these distributions is proposed and it is used throughout the entire dissertation. A simulation study based on signals generated by means of the method presented in chapter §2 is carried out to evaluate the accuracy of the SPWVD in conditions characterized by different degree of non-stationarity. Finally, a method that performs a parametric decomposition of the SPWVD is described. The advantage of this method, which will be used in a physiological study in chapter §6, is that it allows separating relevant signal components from noise, thus offering the possibility of reducing the interference terms that usually appear in the distributions of the Cohen's class.

Chapter §4 is about the estimation of time-frequency coherence between non-stationary signals. Time-frequency coherence has the advantage of allowing the simultaneous localization of temporal intervals and spectral bands in which two signals are locally correlated, thus providing robust and accurate tracking of local correlation changes.Coherence estimates depend on the TF resolution of the distribution used in the estimation. To give a correct interpretation of the results, two methods based on surrogate data are proposed to assess whether the coherence estimates are statistically significant. Two algorithms to automatically determine signal-dependent kernels which allow estimating TF coherence by SPWVD are proposed. In a comparative study which involve both simulated and physiological recorded data, the SPWVD is shown to localize with higher accuracy than other distributions, such as the multitaper spectrogram (MTSP) and the continuous wavelet transform, the TF regions in which signals are locally correlated. Finally, an example of application of TF coherence analysis on cardiovascular signals, such as heart period variability, systolic arterial pressure variability and respiration, is given.

Chapter §5 is about the estimation of phase differences between cardiovascular signals in the TF domain. Time-frequency phase difference analysis allows a fast tracking of the variation of the degree of synchronization between the spectral components of two signals. Moreover, phase difference information can be used to establish, to a certain degree, causal relationships between non-stationary spectral components. The use of the SPWVD to estimate TF phase differences is particularly suited because TF phase difference estimates are reliable only around well localized time-varying spectral band in which spectral components are locally correlated. The proposed methodology is evaluated in different simulation studies based on both computer generated and recorded physiological data.In the second part of the dissertation, composed of chapters §6–§8, three physiological studies are described.

In chapter §6, the effect that musical excerpts characterized by different emotional valence has on HRV and respiration is studied. The characterization of the influence of music on cardiovascular parameters has both physiological and clinical relevance, since the use of music for therapeutic purposes is a matter of increasing interest. In this study, it is shown that the emotional valence of music specifically affects the respiratory frequency and the respiratory oscillations in HRV. It is shown that the transition from a musical stimulus to another provokes variations characterized by a first rapid response, which lasts about 10-20 seconds, and a second slower phase, which last more than one minute. The cardio-respiratory interactions are also studied.It is shown that musical excerpts characterized by different emotional valence do not provoke different pattern of response in the coherence and phase differences between HRV and respiration.

In chapter §7, the degree of similarity between the TF structure of HRV and the pulse rate variability (PRV) obtained from the photoplethysmography (PPG) signal, during tilt table test, is studied. The aim of the study is to assess whether PRV can be used as a surrogate for HRV during non-stationary conditions. The use of PRV to indirectly estimate HRV is interesting since the device used to estimate the PPG signal is not cumbersome is cheap and widely used in the clinical environment. Time-frequency and TF coherence analysis suggest that PRV can be used as alternative measurement of the HRV, at least during tilt table test. The study also reveals that some differences between HRV and PRV also exist, especially in the oscillations related with respiration.However, in the analyzed signals, these differences, which are due to variations in the pulse transit time, are not sufficient to modify the conclusions of the physiological study.

In chapter §8, the cross TF analysis presented in chapters §4–§5 is applied to the study of the dynamic interactions between RRV and systolic arterial pressure variability (SAPV). The study of these interactions is interesting because they are still partially unclear, and because of the clinical relevance of baroreflex sensitivity, which has both diagnostic and prognostic value. This study shows that during tilt table test, postural changes provoke a fast decrease in the baroreflex sensitivity and phase changes between RRV and SAPV. In another data base, the indices obtained by TF analysis allow discriminating between healthy subjects and subjects with autonomic dysfunctions.

TECHNOLOGIES AND METHODS FOR TREATMENT GEOMETRY OPTIMIZATION IN RADIATION THERAPY WITH ACCELERATED PARTICLES

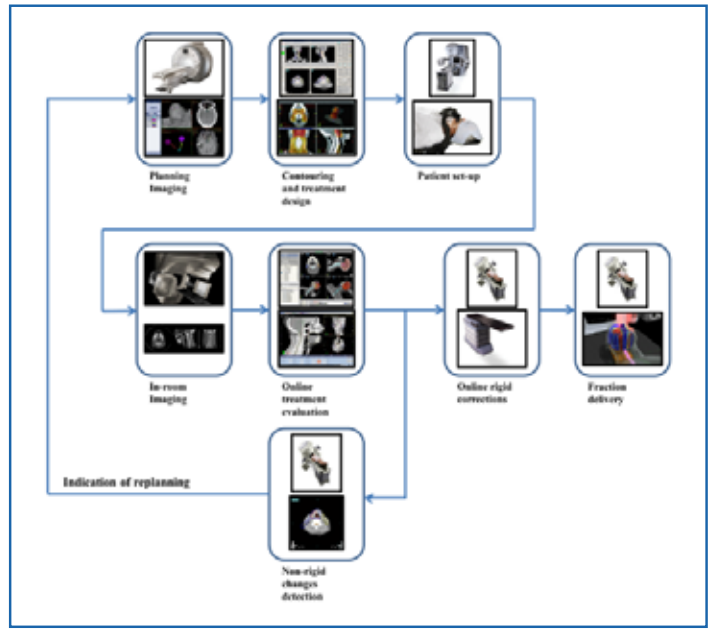
Andrea Pella

Protons and carbon ions are proved to be a valuable technique in cancer treatments. Potential advantages are represented by a high local control of the tumor and lower unwanted side effects. Moreover, this treatment is the only therapeutic solution for several types of tumors (slow progression, hypoxic deep seated tumors). The number of facilities able to offer a treatment with accelerated particles is nowadays worldwide increasing. Technology in this field is growing due to improvements in control and tools for setup verification before and during irradiation. High costs are the principal reason why this type of treatment is not largely diffused. Actually, in Europe only two facilities can offer this treatment: the first one is in Germany and the second in Italy (Centro Nazionale di Adroterapia Oncologica, CNAO).Dose distribution in depth requires an high accuracy and control in patient positioning and setup before and during irradiation. CNAO, the only facility in Italy able to perform radiotherapy with particles and carbon ions aims to provide a state of the art treatment. In this facility, our team has been involved in the selection of appropriate systems for patient positioning and in room setup control. The solutions adopted are described in the thesis. They represent the result of a selection process between different possibilities. Today, CNAO is starting its clinical activity. Results of commissioning activities and measurements carried out during acceptance protocols are reported in the manuscript, supported by statistical analysis. They demonstrate that is possible to obtain sub-millimetric accuracy in patient repositioning. Integration of different systems was also successfully proved. In between different treatment sessions, as during irradiation, the position of a tumor mass may change because of organ motion effects. When the position of a tumor mass changes because of physiological movements, the setup control is more challenging. Several methods are available to manage organ motion, ranging from immobilization tools, to the use of online imaging during treatments (image guidance), to algorithms able to estimate the position of the tumor in respect to fiducial points and\ or surrogates. The combination of such models and active beam scanning, usual in the newest proton and carbon ions facilities, allows in principle to perform tumor tracking, that is, to follow the tumor mass with an always active treatment beam. Even if difficulties in treatment planning optimization remain, dose delivery systems in radiotherapy with particles could offer enormous potentialities for tumor tracking when organ motion is not negligible. Organ motion management requires not only an accurate setup procedure, but also a description of the trajectory (magnitude of movements and phase) of the target volume. This is usually performed by means of correlation models between internal target and external surrogates.Different approaches, all based on a patient by patient basis, are described in the thesis. It is important to point out that the final result concerning correlation models should be intended as a feasibility study of different methodologies that could be applied in radiotherapy with particles aimed at tumor tracking. Firstly, a benchmark of alternative methods (based on: linear \ quadratic correlation, artificial neural networks and fuzzy logic) vs. a commercial device (Cyberknife® Synchrony®) was performed. Results put forward these models as a potentially valuable tool for tumor position estimation, given patient's surface surrogate information. The state model was built as a linear/ quadratic correlation between the principal component of external marker motion and the internal tumor trajectory. The ANN-based approach resulted in comparable results with respect to Cyberknife® Synchrony®, pointing out similar generalization capabilities. The implemented fuzzy logic algorithm proved to be superior to Cyberknife® Synchrony® in the analyzed patient database. Due to the intrinsic variability of breathing motion, a fuzzy environment may be optimal for tumor motion estimation. Results are promising, but again these models are far away from clinical trials in radiotherapy with particles. Main limitations consisted for example in the lack of a proper optimization. Moreover, since no evidences lead researchers to claim that one model is better than others, different approaches should not be discarded a priori. Alternative predictors or finite element modeling could be in principle utilized. First trials were conducted using a Cyberknife® database, thus processing three external surrogates and variable numbers of X-rays acquisitions. Further investigations should be devoted to the search for an optimum setup for models validation (more reference points, or placed in an optimized configuration), keeping in mind that a patient's specific approach is mandatory. Last, the restricted number of database does not allowed a proper validation of the proposed solutions.A first step towards optimization of neural networks and fuzzy logic approaches was performed. The aim in this case was to obtain better performances (in terms of 3D errors reduction) and to understand if it was possible to design an application able to run in CNAO hardware. All these studies suffered for the lack of large database. In order to increase data for model testing and validation, it was implicitly required to build a custom phantom. This phantom, deeply described in the thesis, is able to mimic a realistic breathing and it features an internal target that moves along an hysteretic trajectory in phase with the external ribcage. Preliminary studies were conducted with ANN approach integrated in CNAO hardware. Results on phantom study demonstrate that neural networks can be not only integrated in CNAO hardware, but that they can be trained and updated with a clinical compatible time consumption.At this time there are no phantoms in commerce able to mimic the breathing and in the same time to provide an internal trace of a moving target visible by an optical localizer. Obviously the design of our phantom can be improved, as the range of motions, that is adequate only for preliminary studies. It is evident that the motion patterns are too simplified if compared with a human like motion. Moreover, it would be interesting to add a functionality that allows to load a real respiratory pattern and to move consequently the phantom.The use of an integrate system (phantom, optical tracking system with predictive capabilities) allowed us to test our method in GSI, Germany. There, it was possible to test the potential integration of our systems in the steering logic of dose delivery. In this respect, result was successfully reached. Even if we observed remarkable performance of predictors during the second experimental session, retraining capabilities remain at this time a major issue. More experimental sessions will be dedicated to the search of a reliable estimator, and different methodologies have to be investigated. But the main goal of this experimental activity, that was the active control of a carbon ion beam using a signal processed by a predicting model, was reached.

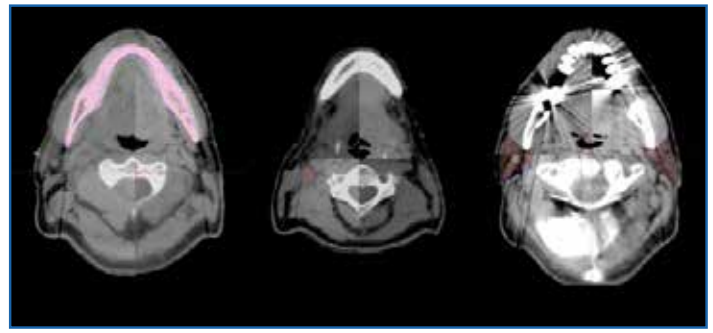
METHODS AND ALGORITHMS FOR IMAGE GUIDED ADAPTIVE RADIO- AND HADRON-THERAPY

Marta Peroni

In Radiation Therapy (RT), the main goal of achieving a high local selectivity implies the necessity of accurately locate and monitor the lesion. The current strategy for Image Guided Adaptive RT is illustrated in Figure 1. Offline phase includes initial images acquisition, structures of interest delineation and irradiation scheme definition. Online procedures instead encompass one or more imaging sessions both for positioning and changes tracking prior to proper treatment delivery. Non-rigid modifications are generally detected with a combination of macroscopic observations and in-room imaging, such as Cone Beam Computed Tomography (CBCT). If changes are beyond an empirically established threshold, a completely manual replanning is done on a new Computed Tomography (CT). Therefore, in the classical strategy, no online adaptation of the treatment is foreseen but rather an offline manual update. My PhD project aimed at producing a reliable initial segmentation of simulation CT (CTsim) and at sparing replanning CT (CTrepl) scans to the patient by modeling the inter-fractional deformations, thus avoiding any delay in the therapy as well as reducing the clinical workload. We concentrated on Head and Neck patients treated at European Institute of Oncology (Milan, Italy). At the basis of my work is Deformable Image Registration (DIR), which models deformations occurring at a different time points of the treatment (intra-subject registration) or between different patients (inter-subject registration), and is not restricted to same modality registration. The performance of a non parametric (demons) and of a parametric (B-Splines) were compared. B-Splines demonstrated to be more flexible, faster and less sensitive to initialization than Demons algorithm, but also more prone to discontinuous deformation field. A stopping criteria investigation was carried out applying synthetic non-rigid deformation fields to phantom and patient CT. Analyzed metrics include registration metric, harmonic energy (i.e. degree of smoothness), jacobian (i.e. number of discontinuities) and quantity of update (i.e. speed of convergence) of the velocity field. A set of possible reference values, to compare the current stopping condition value with, was also examined. Both for patients and phantom, the most efficient condition is based on harmonic energy and a combination of last iteration condition value.In the present work, I developed a fully automatic multi-atlas based segmentation algorithm, for Head and Neck CT datasets. First of all, we ranked a database of representative patients on the basis of a pre-alignment of database subjects with a reference image by means of affine registration. The group of subjects to be actually used for image segmentation is than selected in a fixed number or NMI thresholding fashion. The selected atlases and corresponding structures are than deformed onto the patient image by means of DIR. The tentative segmentations are then recombined according to mean, majority voting and Gaussian Weighted (GW) fusion, in order to select the most robust and accurate for RT planning. Winning strategies were the percentile based selection and the fusion strategy based on GW. A reduction in segmentation accuracy can be seen as the number of patients is reduced to selecting just the most similar one. The algorithm accuracy is comparable to inter-observer variability, thus it can be adopted for clinical use. I developed and validated an efficient and automatic strategy to generate on-line virtualCT scans for HN ART cancer treatment on the basis of a new CBCT volume, thus dropping the need of a new CTrepl (Figure 2). The virtualCT



1. Current Clinical Workflow for Image Guided Adaptive Radiation Therapy. Offline phases include planning images acquisition as well as treatment (contours and beam geometry) definition. Patient setup adjustment as well as non-rigid changes detection happens online, right before daily fraction delivery. If significant modifications are detected, the daily treatment is suspended, while offline corrections are computed.
This adaptation implies the acquisition of a new Computed Tomography.



2. Checkerboard rendering of three axial slices of patient 19a, comparing virtualCT and CBCT scans. Manually outlined (red) and automatic (blue) mandible, nGTV and parotids are presented in panels (a), (b) and (c) respectively. In green, we report the contour that would have been obtained by rigid registration of CTsim on CBCT.
nGTV contour highlights the variability of the obtained results.

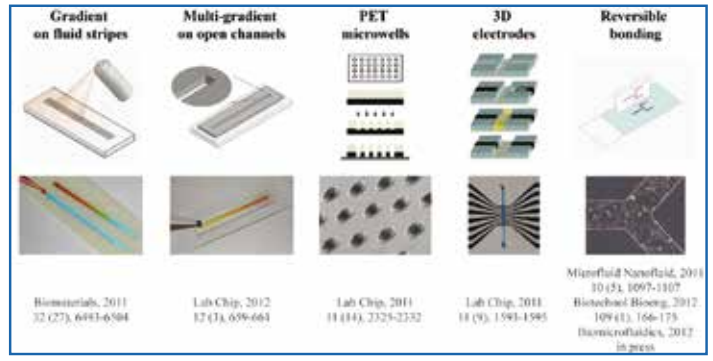
is generated using a multistage B-Spline deformable registration between CTsim and CBCT. Median COM distance and RMSE of contours propagated on CBCT from CTsim and from CTrepl were comparable with image resolution. Critical issues raised in some patients, such as 6 and 7, mainly because of either sub-optimal registration performances induced by external features like immobilization mask or to macroscopic modifications between CBCT and CTrepl (i.e. different jaw position). Dosimetric evaluation will be needed to compare virtualCT and CTrepl distributions and introduction of virtualCT concept into the clinic. I also worked on alternative approach to evaluate DR performance is to compare the position of anatomic or external landmarks after registration has been performed. Scale Invariant Features Transform (SIFT) features extracted on original CBCT, simulation CT rigidly registered on CBCT, virtualCT and replanning CT, were compared with the corresponding CBCT landmarks in terms of residual point distance and the accuracy of the associations is compared with other indices proposed in literature based on contours (DSC, COM and RMSE). The extracted features are located both on bony structures and in soft tissues and therefore provide a great alternative to manual point clicking.

REGULATING CHEMICAL, PHYSICAL AND MECHANICAL CUES IN THE IN VITRO CELLULAR MICROENVIRONMENT USING MICROENGINEERING TECHNOLOGY

Francesco Piraino

There are many biomedical applications where it is necessary to handle cells outside of the body. The most common of such applications are cell biological research, drug screening and cell-based therapies. A frequent problem in these types of *in vitro* cell manipulations is that one can study, test, or use the exact same cells, but get different responses from them because the microenvironment they are subjected to *in vitro* is different from what the cells experience physiologically *in vivo*. This difference may cause the cell researchers to make scientific conflicting conclusions. Biologists have identified and made available many bimolecular components that comprise the physiologic microenvironment for *in vitro* culture. However, the typical culture process of adding excessive volumes of culture media that contain a variety of these biomolecules together in a dish with a thin layer of cells attached often only goes so far in closing the microenvironment gap to produce physiological cellular responses.
The cellular microenvironment is a specific set of physical, chemical, and biological conditions in the surrounding area of cells within a distance where those conditions can have an effect on or be sensed by the cells. The cellular microenvironment provides cells with physical architecture, fluid and solid mechanical stimulation, adhesive signals, growth factors and cytokines, nutrients, inhibitor chemicals, and cell-cell interactions. The *in vivo* cellular microenvironment is composed of an intricate mixture of extracellular matrix proteins, immobilized protein factors, proteoglycans, mineralized tissue, soluble protein factors, small molecule signals, and various adjacent cell types, which vary in space and time due to material deformation, fluid flow, chemical reactions, or cell proliferation, differentiation, death, or migration. Cells continually sense these inputs, process the information through signal transduction and genetic regulation, and execute a cell behavior. Therefore, to study cell biology under well-controlled and physiologically relevant conditions *in vitro* it is important to recreate the physical characteristics of the tissue.
The use of microfluidics makes intuitive sense for cellular microenvironment engineering. The physiological microenvironments in living systems are largely microfluidic in nature. Just a look at the vascular network, pulmonary system, liver sinusoids, other tissues and organs of animals and humans reveals a wealth of physiologic microfluidic structures.
The aim of my research was the development of various microfluidic platforms and techniques to control the cellular microenvironment. The following are specific examples of how microfluidic cultures can be used to simulate *in vivo* conditions and the specific aims for my study.

During tissue morphogenesis and homeostasis, cells experience various signals in their environments, including gradients of physical and chemical cues. Spatial and temporal gradients regulate various cell behaviors such as proliferation, migration, and differentiation during development, inflammation and cancer. One of the goals of functional tissue engineering is to create microenvironments that mimic the tissue complexity found *in vivo* by incorporating physical, chemical, temporal, and spatial gradients within engineered three-dimensional (3D) scaffolds. Hydrogels are ideal materials for 3D tissue scaffolds that mimic the extracellular matrix (ECM). Various techniques are used to synthesize biomimetic hydrogels with encapsulated cells and tailored microenvironments. In particular, a host of methods exist to incorporate micrometer to centimeter scale chemical and physical gradients within hydrogels to mimic the cellular cues found *in vivo*. Considering that *in vivo* microenvironments have more complex gradients



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such as pulsed gradients, exponential gradients or other non-linear gradients of multiple factors simultaneously, one can expect many more enhancements and innovations in this area.

In vivo, cells have interactions with one another and their surrounding ECM. The ECM acts as a support network containing proteins which gives the tissues their mechanical properties and promotes communication between cells embedded in the matrix. Receptors on the surface of the cells anchor to the ECM and attract biochemical cues from the immediate surroundings. This complex mechanical and biochemical interplay is not reproduced when cells are cultured in 2D. 3D cell cultures re-establish cell-cell and cell-ECM interactions and can mimic real tissue better than conventional 2D cultures.

Directed embryonic stem (ES) cell differentiation is a powerful approach for generating a renewable source of cells for regenerative medicine. Typical *in vitro* ES cell differentiation protocols involve the formation of ES cell aggregate called embryoid bodies (EBs). Recently, it was demonstrated the use of microwells as templates for directing the formation of these aggregates, offering control over geometrical parameters.

Microscale engineering approaches in medicine have the potential to recreate physiologically relevant cell microenvironments to enhance our understanding of cell behavior and bring cell therapy closer to fruition. The realization of such advancements will impact a number of therapeutic applications. Despite intense advances in creating physiologically relevant *in vivo* cell microenvironment through the control of biochemical regulatory factors, further synergism of innovative techniques promise to elucidate the impact of a number of physical cues such as stem cell differentiation into cardiac cells and the electromechanical coupling among these cells.
Reversible sealing of two different functional layers is an advancing and effective technique for the fabrication of microdevices. Reversible sealing enables microdevices to be dismountable and reusable, which are promising features for the high-throughput analysis by means of spatial, temporal, and parallel process. It is therefore reversible sealing is potentially being used for various research fields such as flow analysis at microscale, biomolecule analysis, cell analysis and other related fields.