



DOCTORAL PROGRAM IN BIOENGINEERING

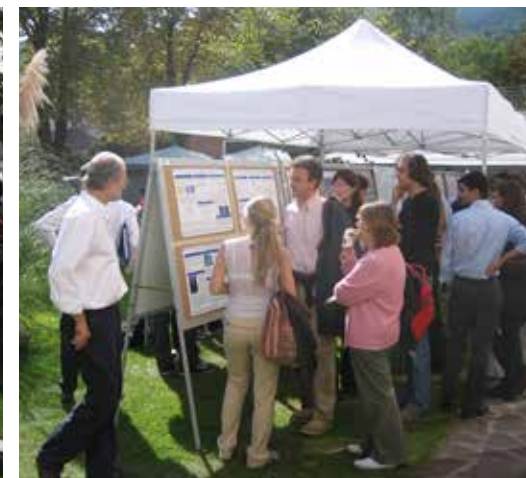
Chair:
**Prof. Maria Gabriella
Signorini**

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 bioelectromagnetism
- 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



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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:

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Istituto di Ingegneria Biomedica ISIB e Istituto di Tecnologie Industriali e Automazione ITIA, CNR, Milano.
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Fondation Leducq, France.
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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).

COMPUTATIONAL MODELS OF THE HEMODYNAMICS IN STAGE 1 PALLIATIONS FOR THE TREATMENT OF SINGLE VENTRICLE HEART DISEASES

Chiara Corsini - Supervisor: Giancarlo Pennati

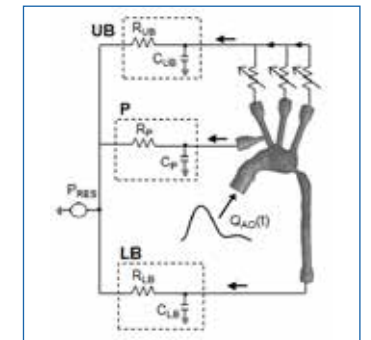
Single ventricle heart diseases include a wide range of cardiovascular abnormalities leading to only one functioning ventricle, which supports both systemic and pulmonary circulations. Although they represent a small percentage of congenital cardiovascular defects, they are certainly fatal if left untreated. In the last decades, various surgical procedures have been developed to treat univentricular patients in a multi-staged fashion, with the ultimate goal of bypassing the nonfunctional ventricle. This dissertation focuses on the first stage (Norwood) palliation and its surgical variants, which are characterized by a shunt interposition between the systemic and pulmonary arterial circulations, that, as a consequence, work in parallel. If properly sized, the shunt allows to regulate the pulmonary flow for maturation of the pulmonary vasculature without overloading the single ventricle, until the next stage will be performed. Sharing the same objectives of traditional procedures, a more recent 'hybrid' approach combining a surgical technique, i.e. branch pulmonary artery banding (PAB), and interventional cardiology techniques, e.g. stenting of the ductus arteriosus (DA), has been introduced to reduce surgical invasiveness.

The literature on shunt modeling is very limited if compared to modeling of the other palliations. This is mainly due to the facts that geometry of shunt and surroundings is very individual, and the associated fluid dynamics is complex, with significant pressure gradients and velocities. Accurate post-operative data are hardly available to build and validate customized models, as sophisticated measurements are rarely performed after surgery. Moreover, Norwood patients are often affected by concomitant vascular defects, e.g. aortic coarctation (AC), whose interaction with the shunt makes mathematical description and numerical simulations even more challenging. The aim of the present work is to study the hemodynamics in different *stage 1* palliations, also combined with AC, adopting various computational approaches with both idealized and patient-specific models. First, multidomain modeling was used to investigate the effects of PAB, DA stenting and retrograde aortic arch obstruction (RAAO) on the local and global hemodynamics of the 'hybrid' procedure, to provide surgeons with general pre-operative guidance for clinical decision support. Idealized models of the hybrid Norwood circulation were constructed by coupling three-

dimensional (3D) models with a lumped parameter model (LPM) in a closed-loop configuration to avoid arbitrary prescription of boundary conditions. Simulation results highlighted that, differently from DA stenting, small changes in PAB diameter can lead to great variations in systemic oxygen delivery and ventricular performance. They also showed that severe RAAO can result in cerebral and coronary malperfusion without affecting other variables. To analyze the impact of different vascular arrangements, the best performing model was then compared with analogous models of conventional surgical palliations, showing poorer hemodynamics. Secondly, a multiscale model and a pure LPM of a patient-specific aortic arch with AC, included in a generic *stage 1* circulation, were developed. The first one was based on an analogous in vitro model, and modified to enable validation against experimental data (Fig. 1). Results showed that disregarding the in vitro manifold, used to reduce circuitual encumbrance downstream the 3D model, would lead to significant discordance between experimental and computational signals. Exploiting computational flexibility, a more realistic scenario was investigated using constant resistances to mimic

the in vivo peripheral circulation independence of flow, and one separate impedance for each outlet of the 3D model. Results demonstrated that, while allowing compactness of the mock system, the in vitro use of non-linear resistances or connections not representative of the in vivo anatomy cannot reproduce realistic hemodynamics in a complex geometry with several outlets and sources of localized energy dissipations (i.e. AC and shunt). As multidomain simulations are computationally expensive, a pure LPM was built using the above in silico results and mathematical literature models. It was validated against in vitro data in different AC scenarios, showing its suitability for a clinical environment, as providing fast and reliable information for clinical care of single ventricle palliations. In the last part of the present work, three 3D models of customized shunt geometries including the anastomoses were reconstructed. Numerical sensitivity analyses of the models were performed, varying the mesh density, the assumptions related to flow regime and those related to boundary conditions. The models were then used to evaluate consistency of the clinical data available for each patient, in view of a pre-operative surgical planning

of *stage 2* palliation. Finally, pressure drops and energy dissipations were thoroughly investigated to compare the hydraulic features of different shunts, and possibly derive a mathematical expression that can reasonably adapt to all shunt models. The obtained results emphasized the hemodynamics complexity of in vivo shunts. Conduits of the same class exhibited different hydraulic behaviors. Kinetic contributions may play an important role in determining the total energy dissipations, depending on the shunt class. Given the inter-patient variability of shunt shapes, anastomoses locations, and their irregular diameters due to narrowing of the internal lumen, modeling customized shunts with a generic mathematical description e.g. LPM would not be trivial. However, in silico simulations allow to obtain pressure drop-flow relationships that could be used for LPM representations of the specific 3D models, potentially included in pure LPM of *stage 1* circulations. A limitation of this study is the impossibility of providing any results associated with narrowing of the shunt lumen due to thrombus formation or neo-intimal growth. These effects were not considered in the present study. Future work will lie in modeling patient-



1. Multidomain model including the 3D geometry, the upper body (UB), lower body (LB) and pulmonary (P) vascular impedances. The manifold merging the UB branches is also included to enable validation against experimental data. $Q_{AO}(t)$ = aortic inlet flow; PRES = reservoir pressure

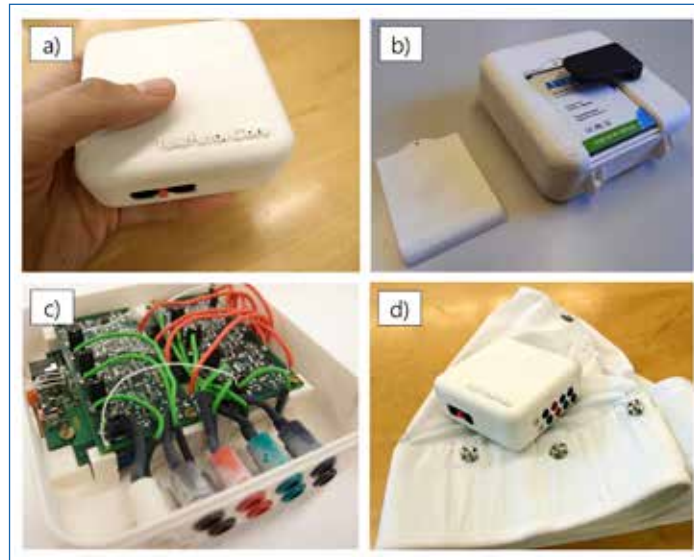
specific shunts immediately after implantation, by modifying the investigated models in order to have a constant diameter equal to the original one. Then, the hemodynamics of non-occluded shunts would be simulated and compared with that of the occluded geometries to understand what might be responsible for narrowing.

TELEFETAL CARE: DEVELOPMENT OF A WEARABLE SYSTEM FOR FETAL MONITORING DURING PREGNANCY

Andrea Fanelli - Supervisor: Giovanni Magenes

Fetal monitoring is extremely important to quantify fetal well-being during pregnancy. The recording and the analysis of fetal heart rate (FHR) allows the identification of risky conditions for the fetus, the selection of the optimal timing for the delivery and, thus, it can contribute to reduce fetal mortality and perinatal morbidity. Signal processing methods have demonstrated their usefulness in quantifying fetal well-being and detecting intra uterine growth restrictions (IUGR) while analyzing heart rate variability (HRV) series.

Intra uterine growth restriction is one of the most severe causes of perinatal morbidity and mortality: it consists of a pathological inhibition of fetal growth, with a consequent failure of the fetus to attain its growth potential. Its incidence is approximately 5% of all pregnancies, and it arises to 30% in twins pregnancies. Particular patterns in fetal heart rate (FHR), that are not detectable with simple eye-inspection of HRV traces, can be measured through quantitative parameters from time and frequency domain. Thanks to smart analysis of FHR, undesired events can be identified before they manifest themselves during gestation. Cardiotocography (CTG) is the most common antepartum



1. Some images of the last prototype of TeleFetal Care

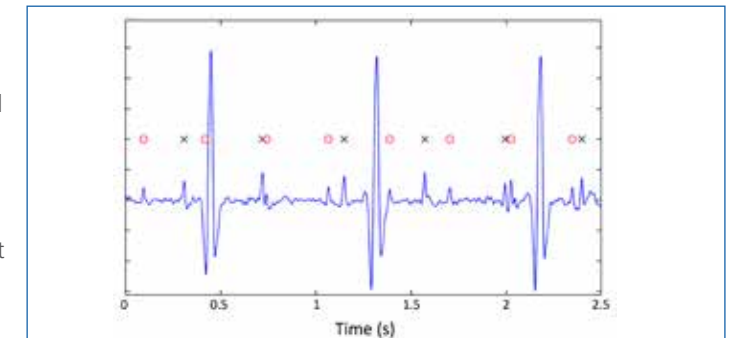
monitoring technique and combines FHR measurements with uterine contraction recordings. Fetal heart rate is obtained thanks to an ultrasound probe that is placed on the maternal abdomen by expert personnel, while uterine contractions are recorded thanks to a sensor pressure placed on the belly. Unfortunately, CTG is only obtained during hospital visits, as it requires an expert nurse for the skillful placement of the ultrasound probe. For this reason, during the third trimester, every pregnant woman is required to visit the hospital in order to receive routine prenatal screenings. These visits might be

cause of stress and anxiety for the mother. Furthermore, they only give a very brief snapshot of fetal well-being, and important events might be missed while the mother is outside the clinic. The goal of the thesis was the development of a wearable system to monitor fetal well-being during pregnancy. The device, named TeleFetal Care, is a home wearable ECG monitor, with improved fetal state quantification capabilities, in particular aimed at IUGR detection. The device might help the clinicians in the detection of growth restricted fetuses, with a continuous and remote monitoring of fetal well-being.

The use of electrocardiography in place of CTG allows a long-term and domiciliary monitoring of pregnancy, preventing risky conditions to be undetected. TeleFetal Care measures maternal and fetal ECG by 8 leads embedded in a panty girdle easy to wear and comfortable. Electrodes are made of silver yarns and are sewed in the textile, thus allowing absolute un-invasiveness and usability. They are placed around the navel with a fixed radial symmetry, thus detecting fetal heart rate independently from the position of the fetus in the uterus. Figure 1 shows some pictures of the last prototype of the device. Pregnant women don't have to worry about electrodes positioning and can use the device without any support. Abdominal recordings are preprocessed by a custom analog front-end, in order to improve signal to noise ratio. Data are then transmitted to a computer through a Bluetooth connection. The device can be used at home, and relevant information about fetal health state is extracted and transmitted wirelessly to the hospital. The thesis doesn't aim just at the technological development of the device. The target is the realization of an entire ecosystem, which also allows fetal well-being quantification. This implies that data recorded by TeleFetal Care are processed and analyzed, in order to extract the signal of interest. Since we are dealing with abdominal ECG recordings, the development of an algorithm for the extraction of FECG signals and for the detection of fetal QRS was an important milestone of the work, in order to construct

FHR variability series. A new algorithm was developed at this purpose. The algorithm was tested on different databases of abdominal ECG recordings, including data acquired through TeleFetal Care, showing excellent performance in FECG extraction and fetal QRS detection. The

entire procedure. The research behind the thesis was broad and variegated: it spanned from signal processing to hardware design, from data analysis to fashion design. It was necessary to develop expertise in circuit design, soldering, manufacturing, CAD design,



2. Detection of fetal QRS in twins' abdominal recordings. The fetal QRS of the two fetuses are detected and distinguished correctly. Red circles highlights the fetal QRS of the first fetal component, black crosses the fetal QRS generated by the other twin

algorithm was also modified in order to be compatible with FECG extraction from twins' abdominal recordings (Figure 2). The thesis also aimed at quantifying fetal well-being from extracted data. In particular, a set of parameters computed on FHR series was computed to provide doctors with an overview of fetal health state. A new index, the Acceleration Phase Rectified Slope, was introduced for the classification of healthy and IUGR fetuses. The index introduced in the analysis was compared to other parameters normally used in the clinical practice. The new index performed better than any other parameter considered in the study. These indices are fundamental to reduce the diffused inter and intra-observer variability in the interpretation of HRV series and standardize the

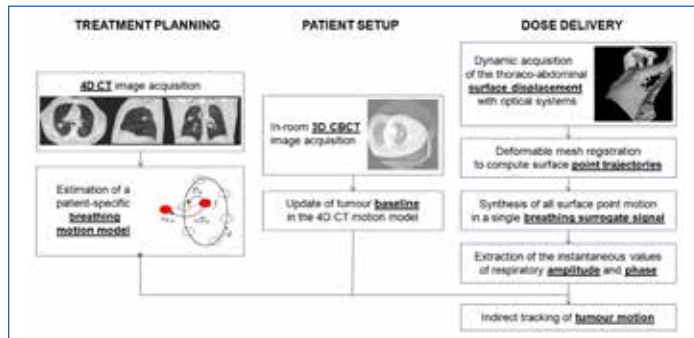
3-D printing, data acquisition, statistics, software design, data processing and analysis. TeleFetal Care represents an innovation in pregnancy monitoring. It would allow an improvement of fetal screening standards, by increasing the number of monitoring sessions. The possibility to use the system directly at home would enhance the quality of care. The objective evaluation of fetal well-being thanks to quantitative parameters would reduce inter and intra-observer variability in reading heart rate variability recordings. Moreover, the reduction of hospital visits would significantly reduce costs in fetal monitoring.

DEVELOPMENT AND TESTING OF EXTRA-CRANIAL TUMOUR TRACKING METHODS FOR INTRA-FRACTION ORGAN MOTION COMPENSATION IN PARTICLE RADIATION THERAPY

Aurora Fassi - Supervisor: Guido Baroni

Introduction

Particle radiation therapy is an emerging technique in the field of radiotherapy for cancer treatments, due to the favourable depth-dose profile of charged particles. Dose conformity to the target volume can be further improved with the advanced methods of dynamic dose delivery, based on the active scanning of particle beams through steering magnets. However, active scanning treatments feature a high sensitivity to intra-fraction tumour motion, caused by physiological organ movements in the thoraco-abdominal sites mainly due to breathing. For these reasons, only targets with a negligible intra-fraction motion, like head and neck cancers, have been treated with active scanning particle therapy up to now. The opportunity to extend these advantageous techniques to the treatment of extra-cranial moving tumours necessarily requires the application of adequate strategies for organ motion compensation. The aim of this thesis was to develop and evaluate a surface-based tumour tracking method for the dynamic localization of extra-cranial targets during radiotherapy treatments, providing the real-time continuous monitoring of breathing-induced intra-fraction organ motion. The proposed



1. Flowchart of the proposed surface-based tumour tracking method

approach is based on external surface surrogates estimated from non-invasive optical devices, and on patient-specific adaptive motion models derived from time-resolved planning images, such as 4D Computed Tomography (CT), and in-room X-ray imaging systems.

Methods

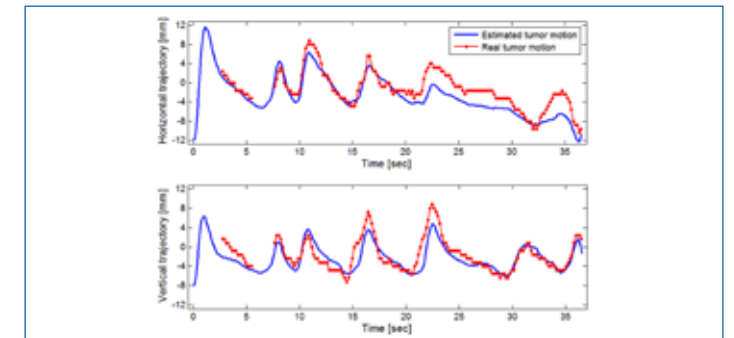
A schematic representation of the developed tumour tracking method is reported in Figure 1. The 4D CT images acquired during treatment planning are used to estimate a patient-specific respiratory motion model, describing the tumour movement during all phases of the breathing cycle. The model, obtained through deformable image registration, is parameterized as a function of the respiratory tumour baseline, amplitude and phase. The model's parameters are adapted

at each treatment session according to the inter- and intra-fraction variations in the patient's anatomy. The tumour baseline is updated according to the daily information on target localization derived from volumetric Cone Beam CT (CBCT) images. The breathing amplitude and phase parameters are retrieved from the motion of the patient's thoraco-abdominal surface, acquired with 3D surface imaging systems during dose delivery. Deformable mesh registration algorithms are applied to derive the spatial correspondence between markerless optical surfaces. The obtained 3D trajectories of all thoraco-abdominal surface points are summarized in a single respiratory surrogate signal through k-means clustering techniques. The instantaneous values of the respiratory phase

are extracted from the surface surrogate using the Hilbert transform. The amplitude scaling factor is obtained by comparing surface motion amplitudes during treatment planning and delivery. The adapted breathing parameters are integrated into the 4D CT motion model to estimate the intra-fraction 3D tumour motion.

Results

The developed tumour tracking method was tested on a clinical database of 7 lung cancer patients, including the synchronized information on the external surface and internal tumour breathing motion during rotational CBCT scans. About 30 seconds of synchronized acquisition of CBCT projections and optical surfaces, captured with the VisionRT system, were analyzed for each patient. An image-based approach was developed to synchronize CBCT and VisionRT data acquisitions, obtaining a median temporal accuracy below 20 msec. A novel automatic method for markerless tracking of lung tumours in X-ray images was implemented, exploiting the anatomical information derived from 4D CT planning volumes to increase target region visibility and reduce the overlapping effect of the nearby anatomy. The real tumour trajectories identified on CBCT images



2. Comparison between real and estimated tumour trajectories obtained for a tested lung cancer patient, showing strong breathing irregularities both for amplitude and phase parameters

with the developed contrast-enhancement algorithm were compared to the target trajectories estimated from surface displacement combined with the a priori 4D CT motion model. The resulting absolute differences between real and estimated tumour motion ranged between 0.7 and 2.4 mm, with median values of 1.5 mm both along the horizontal and vertical image dimensions. The measured phase shifts did not exceed the 7% of the breathing cycle length, corresponding to a maximum of 170 msec of delay.

Conclusion

The developed tumour tracking method proved to be effective in estimating tumour motion from the external surface displacement even in presence of breathing irregularities, as depicted in Figure 2. The

innovative methodological aspects, related to the use of patient-specific adaptive motion models and to the redundancy of markerless surface data, are put forward to improve the accuracy and robustness of tumour targeting techniques. The investigated approach is expected to represent an effective tool for the non-invasive compensation of intra-fraction organ motion, potentially allowing the extension of the advanced active scanning particle therapy techniques to the treatment of extra-cranial tumours.

EXPOSURE ASSESSMENT OF ELECTROMAGNETIC FIELDS ON CHILDREN, NEWBORNS AND FETUSES DUE TO RFID DEVICES

Serena Fiocchi - Supervisors: Paolo Ravazzani, Marta Parazzini

The surging growth of exposure to Electromagnetic Fields (EMF) represents one of the main factors of people concern about possible negative biological effects generated by environmental stressors. In view of the increasing rate of exposure due to the development of new technologies which make use of EMF, the "Health Risk Assessment", defined as the process aimed to estimate the nature and probability of adverse health effects in humans who may be exposed to potential environmental stressors, addressing precisely EMF, become unavoidable. That process, among the others steps, include the specific assessment of the levels of EMF to whom people are exposed, also known as "exposure assessment", which is the goal of this dissertation.

The high rate of increase of EMF exposure is speeded up by the nonstop proliferation of wireless technologies, which make use of EMF at frequencies still unexplored. That is the case of RadioFrequency IDentification (RFID) technology, a generic term for technologies that make use of EMF to automatically identify people or objects. It is substantially based on the communication of information via EMF between a label, named "RFID tag", which identifies

the person/object to which is attached, and an interrogator, named "RFID reader", able to transmit to the tag, to receive from the tag and to decode the EMF signal. RFID applications can be found everywhere: asset tracking, manufacturing, supply chain management, payment systems, security and access control, transport systems, electronic passports and ID cards, animal identifications, medical applications, sporting events, and any other application that can take advantages by an automatic identification of people and objects.

However, if the RFID great diffusion is surely justified by the advantages that it offers in terms of automatism, reliability, safety, versatility, speed of reading and relatively low costs, the lack of accurate exposure assessment studies remains unmotivated and should be urgently addressed. This is even more true considering the lack of selectivity of the RFID exposure, which can involve subjects who are considered more vulnerable to EMF exposure, and the lack of studies which address the peculiarity of their exposure. To this purpose, children, newborn and fetuses have been for a long recognized as possibly more susceptible to EMF, either because of the precocity of their exposure, and because the

time of the exposure coincides with the development and maturation of their system. Therefore, this doctoral dissertation addresses the exposure assessment of EMF generated by the two main classes of RFID devices, distinguished on the basis of the frequency for the physical coupling between reader and tag:

- High-Frequency or HF (13.56 MHz);
 - Ultra-High-Frequency or UHF (around 880 MHz in Europe);
- on children, newborns and fetuses (pregnant women). That means to identify, by solving Maxwell equations, the three-dimensional distribution of EMF and related dosimetric quantities inside tissues and organs.

To this purpose the International Commission on Non-Ionizing Radiation Protection (ICNIRP), provided, within the process of exposure assessment, guidelines for limiting EMF exposure that would guarantee protection against known adverse health effects. In particular, at the frequencies of RFID devices, i.e. radiofrequencies (RF), dosimetric studies and exposure guidelines usually refer to the Specific Absorption Rate (SAR) as the metric used to account the rate at which energy is absorbed by the body when exposed to RF EMF and to the temperature

increase to directly quantify the heating produced by that absorption.

The great advances in computers technology of the last years, have made particularly valuable and reliable to perform that assessment through computational techniques on computers-based models of the human body. That allowed to define specific exposure scenarios for both the classes of RFIDs considering realistic applications.

The first one is a specific application for identity check based on RFID HF system, used for newborn-mother identity reconfirmation. On the basis of the variations of RFID technical specifications, including the Read-Range (RR) distance, that is the maximum distance at which the reader is capable to activate the tag and the average threshold level for the magnetic field (H_{th}) necessary to activate the tag, the adherence of the EMF levels with ICNIRP limits was computed as a function of the time of use of the system close to the body. The results show that attention should be paid in the identification of the optimal reader-tag technical specifications to be used in this type of application in particular for what concern newborn exposure, being the compliance with ICNIRP guidelines strongly affected by the choice of the H_{th}

and the time of use of the device close to the body. Moreover, an uncertainty budget was also estimated to investigate to what extent change in parameters would affect the results. Those comprehend the influence of the scaling to model newborn and the use of adult dielectric parameters to newborn tissues, disregarding their possible variation with age due to the changes in body tissues composition.

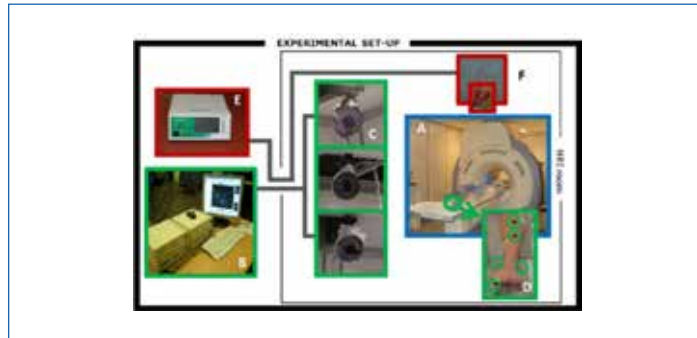
The second class of RFID system investigated (i.e. UHF RFID), is particularly suitable to be used in the great range of applications that require longer read ranges and hence their exposure can involve people that occasionally and accidentally pass close to the RFID system itself. In particular accidental head exposure is identified to represent the worst case exposure scenario for children (5-, 6-, 8-, 14- years old); abdomen exposure as the worst case for pregnant woman (both 7- and 9-months pregnant). The reader consists of a circularly polarized patch antenna and the results of the computation in terms of SAR normalized to 1 W radiated power of the RFID antenna are as such to indicate a localized overexposure on pregnant women and their fetuses, who have a limited capability to dissipated the heat produced by power absorption.

In particular, considering the existing link between SAR and the raise in temperature in the body organs, the temperature raise assessment in the previous worst case scenarios was also performed through the numerical solution of the Bio-heat equation. However, the temperature exposure assessment performed in those critic exposure scenarios resulted in negligible average temperature increase in the fetal body core (i.e., 0.004 °C at the steady-state) and maximum temperature raises at the steady-state of about 0.2 °C and 0.6 °C over 7- and 9- months fetuses, respectively. The great spread of the temperature raise distributions and the practical short time of exposure assures that those values represent a great conservative estimate of maximum temperature increase and, therefore, UHF RFID are unlikely to be a potential harmful source for fetus. This dissertation represents the first attempt to provide information about the exposure of children, newborns and fetuses to RFID device at the level of organs and tissues, contributing to fill some of the gaps in knowledge related to children exposure to electromagnetic fields.

FUNCTIONAL ANATOMY OF SENSORIMOTOR INTEGRATION IN THE HUMAN BRAIN AND PERSPECTIVES IN NEUROREHABILITATION

Marta Gandolla - Supervisors: Alessandra Pedrocchi, Simona Ferrante, Nick Ward

The correct execution of a voluntary movement is shaped by the integration of sensory feedback. Manipulation of proprioceptive input influences motor cortex excitability, and conversely, the response of somatosensory cortex neurons to proprioception is modified by the nature of the motor task. In this work, the effect of proprioceptive manipulation during an active motor task has been investigated. Functional Electrical Stimulation (FES) provides an ideal experimental model to explore sensorimotor integration because it can provide externally driven proprioceptive information during movement execution. FES stimulation of a mixed nerve elicit both efferent fibres stimulation directly inducing a muscular contraction, and afferent fibres stimulation eliciting a sensory volley sent to the central nervous system concurrent with the proprioceptive reafference that contributes an additional component to proprioceptive feedback. Some hemiplegic patients treated with FES for foot drop correction during walking have shown a beneficial effect that outlasts the period of stimulation, known in literature as 'carryover effect'. This supports the hypothesis that FES induces some plasticity mechanisms leading to central



1. Experimental set-up. A) MRI scanner CV/I 1.5 T; B) motion capture system Smart μ g with C) three cameras and D) reflective markers; E) current-controlled stimulator RehaStim and F) stimulation electrodes

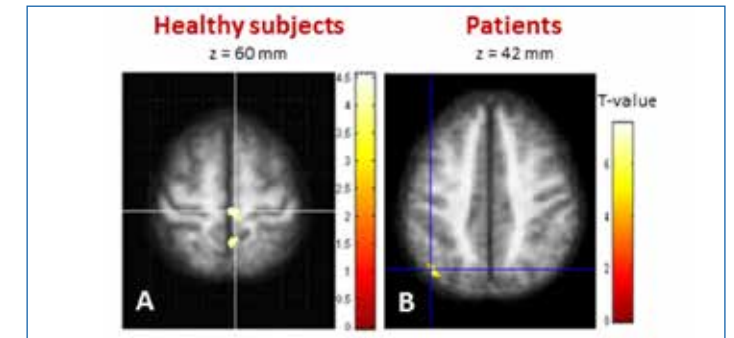
nervous system reorganization and therefore maintenance of improvements in motor control that is worthy to be investigated. An experimental set-up that is suited to measure movement related cortical correlates during functional electrical stimulation was defined, tested and validated. The feasibility of a proposed multi-modal experimental set-up (Fig. 1) so as to functionally electrically stimulate the subject while undergoing fMRI scanner and simultaneously record the effectively executed movement was demonstrated. The kinematic measure of the executed movement under the scanner allows to record multi-segment movement without any constraint. Moreover it allows to control for all movement parameters that cannot be kept for granted when patients are performing an experimental

protocol. The reliability of the acquired images in the integrated set-up has been demonstrated, with particular attention to the detectability of the BOLD signal. An experimental protocol that is suited to study sensorimotor integration during an active motor task was designed. A 2x2 event-related fMRI protocol with voluntary effort [V: with the levels volitional and passive] and FES [F: with the levels present and absent] as factors was performed using right ankle dorsiflexion. The positive interaction defined as (FV-V)-(FP-P) identifies regions where the FES augmented proprioception in the context of volitional intent (i.e. FV-V) produced a higher activation than FES augmented proprioception in the absence of volitional movement (i.e. FP-P). The interaction contrast directly

represents the sensorimotor integration during an active motor task.

The functional anatomy of sensorimotor integration in the human brain was investigated. Sixteen healthy volunteers with no neurological or orthopaedic impairment were studied (mean age 36 ± 14 years, range 22-61). Primary sensorimotor areas (i.e. M1 and S1) exhibit a profound interaction between artificially enhanced sensory feedback and volitional movement. In other words, the effect of augmented proprioception depends on the presence of concurrent motor signals in both M1 and S1 (Fig. 2A). Using dynamic causal modelling, it has been shown that augmented proprioception preferentially increased the influence of M1 on S1, while attenuating the excitability of S1 (increasing self-inhibition). Crucially, the fact that augmented proprioception selectively increased input to S1 from M1 suggests that effect of stimulation depends upon the presence of top-down volitional or intentional signals. In this context, one can regard the modulation of the M1 to S1 connection as mediating the interaction between augmented proprioception and volitional movement (as encoded by M1 activity).

How functional anatomy of sensorimotor integration is



2. Interaction contrast. Statistical parametric maps (thresholded at $p < 0.001$, uncorrected) showing regions activated for the positive interaction contrast. A) Healthy subjects - clusters located anatomically in M1 (anterior cluster) and S1 (posterior cluster) leg areas; B) Patients - cluster located anatomically in postcentral and angular gyrus

affected by a brain lesion was investigated. Eleven stroke patients were recruited (mean age 53 ± 14 years, range 28-72). All patients had suffered from first-ever stroke > 6 months previously. Sensorimotor integration in chronic stroke patients has been investigated demonstrating the feasibility and the reliability of FES cortical correlates in patients, highlighting a clear network of activation related to FES-induced motor task. Sensorimotor integration was observed in secondary areas (Fig. 2B). Angular gyrus is a recipient of proprioceptive information encoded in the postcentral gyrus. These results suggest that sensorimotor integration takes place in a more widely distributed network of brain areas after stroke, and that

patients may take advantage of secondary areas to support motor learning. The results of this work support FES as a tool to promote improvement in motor function after central nervous system injury. This functional architecture and the underlying synaptic mechanisms may be important for future studies in patients during rehabilitation, by correlating changes in FES and interaction cortical correlates in a longitudinal design. Indeed the brain correlates that has been shown to have a correlation between sensorimotor integration (i.e. interaction contrast) and the degree of impairment might be important in the investigation of the carryover effect brain signature.

4D PET-BASED TREATMENT VERIFICATION IN ION BEAM THERAPY: RECONSTRUCTION AND OPTIMIZATION STRATEGIES

Chiara Gianoli - Supervisor: Guido Baroni

Radiotherapy is an important and effective therapy used for curative and palliative management of patients with cancer. The ideal concept of radiotherapy is to selectively deliver the radiation dose to the tumor while sparing the surrounding healthy tissues. Ion beam therapy is an emerging technique in radiotherapy, better approaching this ideal concept compared to conventional photon therapy. The physical selectivity of ion beam therapy implies an intrinsic sensitivity to inconsistencies of the treatment situation with respect to treatment planning. Geometrical uncertainties, less critical for conventional photon therapy, correspond to important variations of the prescribed dose in ion beam therapy.

The improved dose conformality of ion beam therapy demands methods to verify the dose delivery, in order to assure the consistency of the treatment or act the proper solution in case of inconsistency (i.e., re-planning the treatment for subsequent fractions). The aim pursued by the different dose delivery verification strategies is to detect the unpredictable deviations that vanish the advantages of a highly conformal and effective radiotherapy treatment. The Positron Emission Tomography (PET) imaging is the unique, clinically validated, strategy that

currently allow for treatment verification in ion beam therapy. Positron emitters such as ^{11}C , ^{15}O and ^{10}C are produced via nuclear interaction along the particle beam path and can be imaged during or shortly after treatment, carrying indirect information about the dose delivery. PET-based treatment verification is based on the comparison of the spatial distribution of the measured β^+ activity with the β^+ activity calculated from the treatment plan.

When envisioning the clinical implementation of the 4D treatment delivery in presence of moving targets, the effectiveness of the 4D PET-based treatment verification depends on 4D PET count statistics optimization and 4D PET motion compensation strategies. The feasibility of 4D PET-based treatment verification is strongly limited by the extremely low count statistics characterizing the PET image, orders of magnitude below that in conventional nuclear medicine applications. Typically, the problem of 4D PET low count statistics has been tackled with the application of motion models, calculated by means of deformable image registration tools, aiming at the reconstruction of motion compensated 4D PET images at full count statistics. The availability of co-registered 4D anatomical images represent

the key feature to address the feasibility of the 4D PET based treatment verification in ion beam therapy for moving targets. Robust 4D PET count statistics optimization and 4D PET motion compensation strategies are therefore required to overcome the typical drawbacks of conventional strategies (i.e., the post-reconstruction and the intra-reconstruction warping). Technological and methodological improvements of PET-based treatment verification in ion beam therapy represent the rationale of this PhD project. The basic layouts for PET imaging in ion beam therapy have been analyzed. Limitations are pointed out and a proof of concept of what is proposed as the optimal solution is outlined, according to advantages and drawbacks. To delineate the optimal solution, the availability of co-registered anatomical imaging is assumed, providing important advantages concerning the localization of the induced PET activity on the patient anatomy and the possibility to explore inter-fractional anatomical variations during the fractionated treatment with respect to the treatment planning CT. As proof of concept of the optimal solution for PET-based treatment verification in ion beam therapy, an in-room PET imaging system

combined to the CBCT robotic system is envisioned. The robot enables the transportation of the PET system in the treatment position, thus implementing a combined in-beam and in-room PET imaging. The optimization of PET information acquired are considered the key point, enabling to minimize the PET acquisition time and increase the patient throughput, being this latter the unique drawback of this in-room PET concept. In this framework, the PhD project is presented as a technological and methodological contribution towards the developments of quantitative image based treatment verification in ion beam therapy, focused on the optimization and the reconstruction of PET-CT images. The tomographic information provided by the integrated 4D PET-CT imaging system (in perspective of 4D PET-CBCT) are exploited to tackle with the issue of target motion, as reconstruction and optimization of 4D PET-CT images. In this PhD project the application of a regional MLEM reconstruction algorithm is proposed as a novel quantification tool in PET-based treatment verification, for a robust, three-dimensional, region-based comparison between the measured and expected PET. The region-based MLEM reconstruction strategy is a noise robust reconstruction algorithm designed to quantify the consistency between the PET measurement induced by treatment and the PET prediction derived by the treatment plan. Two different strategies for 4D PET count statistics optimization and motion compensation are

proposed, relying on motion models based on 4D CT dataset: the virtual 4D PET strategy and the pre-reconstruction sinogram warping strategy. Both are based on co-registered anatomical imaging, provided with the multiple points 4D CT sorting method to increase the robustness of the motion model in presence of breathing irregularities or incorrect breathing phase identification, and are specifically designed for low count statistics PET imaging. They are thought as specific strategies for the 4D PET-based treatment verification in ion beam therapy, able to overcome the typical drawbacks of the post-reconstruction and intra-reconstruction warping. In conclusion, a framework of optimization and reconstruction strategies in combined PET-CT imaging is presented, in the context of the clinical implementation of PET-based treatment verification in ion beam therapy. Specifically, the strategies developed for off-line PET imaging but generally extensible to other PET layouts (i.e., in-beam PET and in-room PET). The application of the proposed strategies on commercial PET-CT scanners relies on the reverse engineering procedures developed for the proper use of PET raw data extracted from the scanners, aiming at the definition of the geometrical correspondence between the PET raw data and the dicom PET-CT image. The know-how gained on commercial PET-CT scanners for off-line PET can be exploited in the developments of the envisioned in-room PET-CBCT robotic system. In the emerging context

of ion beam therapy PET imaging is the elective imaging technique: the well-known dose-correlated PET signal is directly induced by treatment and can be easily localized in the three-dimensional space. The developments of dedicated PET systems can make use of well-founded knowledge coming from nuclear medicine applications. Moreover, advanced technologies in PET hardware are available and further improvements are expected with the envisioned technologies described in this PhD project. Finally, the possibility to treat moving targets relies on the implementation of motion mitigation techniques. Developments towards 4D treatment delivery require quantitative, three-dimensional and time-resolved (i.e., four-dimensional) dose delivery control strategy, to assess the effectiveness of motion compensation. PET imaging potentially meets these requirements but issues related to reduced count statistics are implied. The methods developed and presented in this PhD project support PET imaging combined with anatomical information as quantitative and four-dimensional technique for research in 4D dose delivery, in perspective of 4D treatment verification for moving targets.

IN-STENT RESTENOSIS AND CORONARY CURVATURE: TRANSLATIONAL APPROACH TO COMPUTATIONAL FLUID DYNAMICS

Brandis Keller - Supervisor: Gabriele Dubini

The thesis at hand is a smaller contribution into an innovative, multi-disciplinary and multi-centre project entitled MeDDiCA: Medical Device Design in Cardiovascular Applications (www.meddica.eu), which is part of the European Union Framework 7 Health Programme, and a Marie Curie Initial Training Network initiative. As cardiovascular disease is yet one of the main causes of illness and premature death in the EU and other developed nations, identification of key impact areas for medical device technology innovation and contribution to patient care not only improves quality of life, but also relieves the economic burden due to lengthy hospital stays. The coronary stent is a rigid mesh scaffold used to support open severely occluded blood vessels due to atherosclerosis or other diseases which narrow the passage for blood flow. Coronary in-stent restenosis (ISR) is a pathology afflicting the stented coronary following the procedure due to neointimal hyperplasia and re-occlusion. The mechanisms leading to the formation of ISR are still conjecture. However, hypotheses relating to fluid mechanical influences (e.g. wall shear stress (WSS), flow patterns) and geometric changes due to stent placement, in conjunction with species involvement (e.g.

hypoxic conditions), have been identified as potential components of inflammation. Contemporary numerical models strive to gain insight into ISR through qualitative comparison of image-based models with clinical data. However, the geometric simplifications may fail to provide appropriate flow conditions. Through utilization of a realistic 3D reconstruction geometry of implanted coronary stents within a porcine model as base for the computational fluid dynamic (CFD) simulations, in addition to collaborative works to involve other mechanisms such as wall injury scoring and lowered oxygen flux due to the presence of the stent, local influences of these mechanisms can be identified and correlated to the corresponding histology or alternative modalities for appreciation of tissue growth following stent implantation. The present study has been performed to investigate hemodynamic parameters (e.g. WSS, etc.) and/or oxygen mass transport in a realistic configuration of the stented coronary vessel, which is characterized by asymmetries and non-uniform geometry (Figure 1). To this end, stented porcine right coronary arteries have been analyzed, whose in vivo geometry was reconstructed by combining data from micro-CT (stent) and by finite element

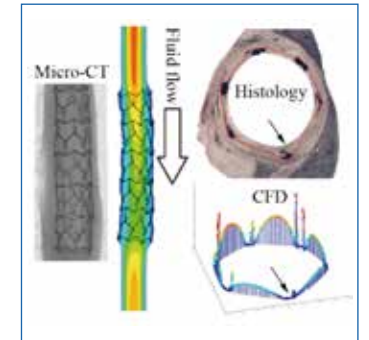
analysis (vascular wall). The availability of corresponding histological images allows a direct correlation with CFD and mass transport results (Figure 1). Additional histomorphometric measurements were performed where necessary. Following establishment of the workflow, a series of computational application studies were undertaken to evaluate the influence of the fluid and/or mass transport within the stented coronary. The following is a brief description of each application study undertaken:

Application study 1. Geometric alteration of fluid domain due to coronary curvature: the radius of curvature for animal coronaries was chosen to range from 20 to 36 mm, as based on measurements taken from 2D qualitative coronary angiographic images of similar porcine models. The centerline of the vessel is assumed to be a plane curve, positioned according to a visible residual curvature in the micro-CT images. Both steady and transient simulations were performed. It was found that the greater radius of curvature greatly influenced the WSS at the entry region of the stent due to skewing of the velocity profile; yet the localization was notable throughout the entire stent. This impact may

be pertinent when considering implant conditions; An additional comparison of deep injury to fluid mechanisms was incorporated into the first application study: CFD is performed in two stented geometries with different measures of injury according to an established scoring system. The first is a stretch injury study and correlates regions with NIH to the hemodynamic parameters under evaluation. The second is a deep injury study and evaluates the fluid dynamics in a model presented with tissue laceration. Results indicate that the presence of deep injury has greater influence over the tissue response in terms of NIH than hemodynamic indices (e.g. WSS);

Application study 2. Localization of lower oxygen mass transfer within straight and curved stented domains: diffusional mass transport is analyzed in the same geometry by two methods. The first utilized only the lumen layer as a wall-free model. The second incorporates the adventitial boundary layer for analysis of the fluid-wall model on arterial oxygen transport in the presence of a stent. Results clearly display the complex stented coronary geometry has a great effect on convection dominated mass transport species, such as oxygen. These evaluations are

instrumental in characterizing the localization of NIH due to the presence of the device within coronary artery through CFD simulations; Better elucidation of the overall effects of the fluid environment, especially in relation to geometry curvature, on the effective delivery of renewed flow and oxygen to the arterial wall following stent placement may lead to improved options to reduce ISR through arterial patency and device longevity.



1. Translational approach to construct the image-based (micro-CT) stented coronary for use in geometric computational fluid dynamic (CFD) and mass transport simulations for comparison with in vivo tissue response (histology)

FRACTAL BEHAVIOR AT CENTRAL SYNAPSES: CHARACTERIZATION OF QUANTAL RELEASE AFTER LONG-TERM POTENTIATION

Jacopo Lamanna - Supervisor: Maria Gabriella Signorini

The integration of synaptic activity mediated by a neuron and the effects that it produces on the activity of a neuronal network is one of the most cutting-edge topics in neuroscience. A real comprehension of this process is hindered by our limited knowledge about the mechanisms underlying the generation of synaptic signals, either evoked by action potentials or spontaneous. Chemical synapses of both the central and the peripheral nervous systems are able to transmit information thanks to the release of discrete packets of neurotransmitter, also known as synaptic quanta, whose anatomical counterpart is represented by the synaptic vesicles. Both the action potential evoked and spontaneous release processes share a common pool of synaptic vesicles and this duality of transmission based on the same source of quanta explains the complex interactions between these two processes. With these clear ideas in mind, I started the study of spontaneous synaptic transmission. The application of non-linear analysis methods to the series of spontaneous releases generated clear experimental and analytic evidence for the presence of short-and long-range dependencies (fractal behavior). The emergence of this behavior

was also studied in relation to other experimental treatments, known to act on the vesicle release machinery: the induction of long-term potentiation (LTP) and the administration of the black widow spider toxin (α -LTX).

Fractal analysis of spontaneous release in resting conditions

During the experimental stages, several series of quantal releases were recorded by means of whole-cell patch clamping of the post-synaptic neuron, in conditions where the action potential generation was suppressed by tetrodotoxin (TTX). Another kind of recording used was the loose-patch on a single synaptic bouton, which assures that the recorded minis are only the ones produced by that synaptic site. All the recordings were performed on primary cultures of dissociated hippocampal neurons obtained from the hippocampus of post-natal rats. In this system, in the absence of action potentials, only single quanta releases are detected. By means of classical statistical methods, the main features of the series of releases obtained in physiological conditions were depicted. Furthermore, I evaluated the presence of 1/f (or fractal) behavior and described its dynamics, by applying established methods

for the quantification of the fractal exponent α in fractal point processes. The presence of a slowly-decaying correlation in these series, proved by the power-law or fractal behavior, is an evidence that these signals, albeit being spontaneous, can actually possess a "long-term memory", i.e. the present fusion events can be influenced by other events that are really far in time.

Numerical validation of the methods

Starting from the observations made on the data of synaptic quantal release, a computational study was faced aimed at evaluating, based on simulated series of quanta, the reliability of the selected and implemented analysis methods for the quantification of the fractal exponent. For achieving this result, a custom algorithm was developed for simulating realizations of the release process showing crucial features that were evidenced in real data. Eventually, I obtained a reliability evaluation for the selected methods.

Effects of LTP

Several experiments were performed to evaluate the behavior of synapses following the induction of long-term potentiation (LTP). We chose a protocol that, at least

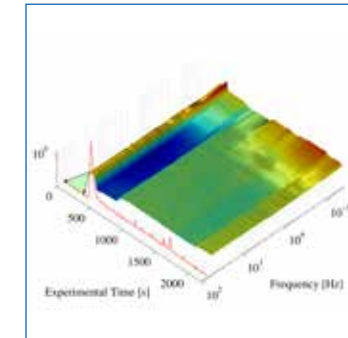
ideally, could induce LTP on every synaptic unit belonging to a post-synaptic neuron. Furthermore, as a control experiment, I applied the very same protocol in the presence of a cocktail of well-known blockers of LTP. By comparing the results obtained in these two conditions, I demonstrated that the 1/f structure of the release process is specifically modulated by LTP (Fig. 1). Eventually, it was verified an increased degree of asymmetry in the interval distributions after LTP, from the mono-exponential profile to the bi-exponential profile.

Effects of α -LTX

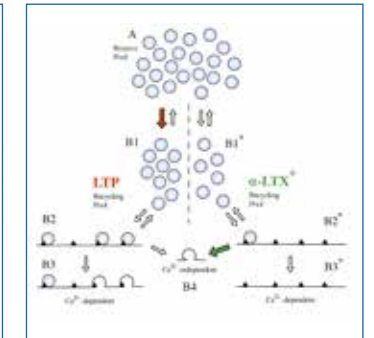
α -LTX is a neuroactive toxin that massively increases quantal release rate. In these conditions, although the exponential nature of the interval histograms was unchanged, the 1/f behavior was increased (the α values found in the presence of the toxin were shown to be different, on average, than in the LTP case). Taken together, these observations let me conclude that the modulation of fractal behavior can be totally independent from the average rate of the process, pinpointing to different sites of action for the toxin and LTP.

Development of an interpretative model of fractal quantal release

The model can be summarized as follows (Fig. 2): i) A non-Brownian, subdiffusive random process (1/f in nature) recruits the vesicles from a cytosolic pool, causing vesicles to be docked to the membrane's active zone (AZ). ii) After docking, the vesicle can undertake fusion by means of a calcium



1. 3D representation of the time-frequency analysis performed on the quantal release series of a LTP experiment. After LTP is induced, significant 1/f behavior is observed, together with bursting epochs



2. LTP (left side) causes an increase in the rate of transition of vesicles from the Reserve Pool (A) to the Recycling Pool. The transition B2 to B3 is calcium-dependent and explains bursts occurrence. α -LTX (right side) strongly promotes the calcium-independent release of vesicles, with consequent reduction of docked vesicles (B2*). Both the experiments help revealing the fractal nature (1/f) of the underlying fractal-like vesicle motion

independent mechanism, which spontaneously occurs at very low probability in resting conditions, thus blurring the 1/f recruitment process. The probability of this release mechanism is increased many orders of magnitude by α -LTX, presumably with a depletion of the docked vesicle pool, thus revealing the upstream 1/f recruitment process, altered by the depletion. No short-term correlations can emerge with this fusion mechanism. iii) If the vesicle is not spontaneously fused, it can undertake a different maturation and fusion process, which is calcium dependent. When the synapse modifies the size of its docking and recycling pools (as well as release probability), as with LTP, the calcium-dependent

spontaneous release displays both short-term and long-term correlations (bursts and 1/f, strongly modulated).

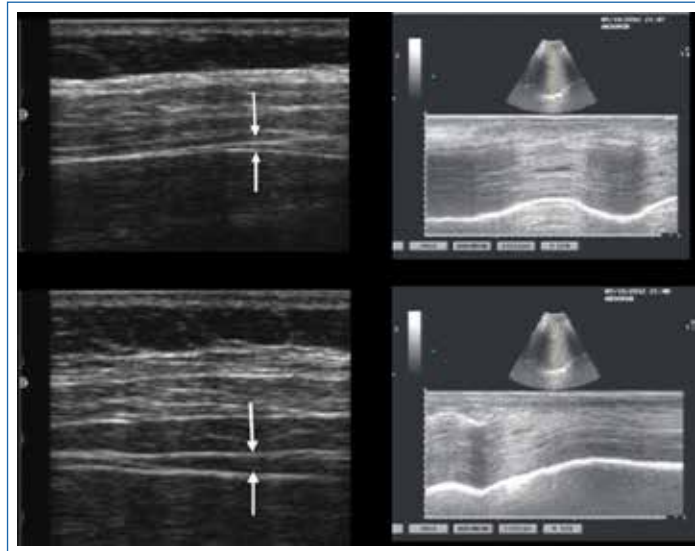
Conclusions and remarks

In conclusion, the results of my study, besides confirming the ubiquity of fractal behavior at all the levels of the nervous system and promote these non-linear features of neural signals as key elements in facing the study of learning and memory, provides new insights for understanding the real functioning of the vesicle fusion machinery as well as the pre-synaptic mechanism supporting neural plasticity.

DEVELOPMENT OF NEW METHODS FOR NONINVASIVE FUNCTIONAL ASSESSMENT OF THE DIAPHRAGM

Marianna Laviola - Supervisor: Andrea Aliverti

Respiratory muscles are the only skeletal muscles vital to life and breathing is borne mainly by the inspiratory muscles, especially the diaphragm. The alteration of the diaphragm, the main inspiratory muscle, can have dramatic effects on ventilation. Consequently, the complete evaluation of the function of the respiratory muscles requires to determine their length, velocity of shortening (estimated by lung volume) and developed force (estimated by pressure). The purpose of this thesis is to develop and define a noninvasive approach to the functional evaluation of the diaphragm based on two different techniques: ultrasonography (US) and Opto-Electronic Plethysmography (OEP). US allows to study the geometry and kinematics of the diaphragm, OEP provides data on displacement and volume variations of thoraco-abdominal wall and its compartments thus indirectly on the different respiratory muscle groups acting on them, including the diaphragm. The approach allows to overcome the limits of several methods currently used, i.e. the need to perform invasive measures and/or the necessity of the collaboration of the patients. It has been applied to different situations of clinical interest, namely to evaluate patients with Duchenne Muscular Dystrophy (DMD) at different stages of the disease (with both US and OEP) and to study



1. Left: B-mode US images taken during spontaneous quiet breathing at end-expiration (top) and at maximal inspiratory pressure (bottom) to determine diaphragm thickness (DT) as the distance between pleural and peritoneal membranes of the diaphragm (arrows). Right: B and M-mode images performed during quiet breathing (top) and full inspiration at TLC (bottom) to determine diaphragm excursion (DE)

different populations of patients undergoing different kinds of thoracic surgery (with OEP). To develop new methods for non-invasive assessment of respiratory muscles by US, a system able to measure **diaphragmatic thickness (DT) and excursion (DE)** has been developed and validated. The system is composed by commercial echographs and dedicated software for image acquisition and processing. An ad-hoc software allows to acquire synchronously US images with analog signals, such as flow and pressure at the mouth,

and another ad-hoc software is able to process off-line US images and extract thickness or excursion. Using several region growing algorithms and a set of morphological operators, the software allows to semi-automatically extract from the US images pleural and peritoneal membranes of the diaphragm to calculate DT and the tracing representing DE (Fig 1). In order to determine measurement characteristics in terms of **reproducibility and reference values of DT and DE** US data, several

experiments were conducted on healthy subjects in different postures, during different maneuvers and with different operators. To determine inter-observer variability, DT and DE measurements were obtained by two different operators on two different occasions and to determine intra-observer variability, DT measurements were performed by the same operator on two different occasions. The obtained results showed that DT and DE measurements are not influenced by operator, time and posture and they are therefore highly reproducible. In the **DMD patients**, DT and DE were evaluated under different conditions. OEP analysis allowed to evaluate, during the same conditions performed by US, the corresponding changes in abdominal volume, which recently were proved to be a good index of changes in diaphragmatic geometry. In older DMD patients we found that, despite an increase of DT during the maneuver, the maximum generated pressure decreases. This result shows that in older patients a dissociation occurs between the drive to the muscle and the development of muscle strength. A significant linear correlation was found between the excursion of the diaphragm measured by US and the variation of abdominal volume measured by OEP, suggesting that abdominal volume measurements can be considered as an optimal index of diaphragm length and changing length. Three studies have been conducted on different kinds of thoracic surgery. The first one was performed on patients

with cystic fibrosis before and after an intervention of bilateral **lung transplantation (LTx)** by OEP during QB and slow vital capacity. After LTx, the volume changes analysed with OEP revealed a significant decrease of TLC as well as the functional residual capacity (FRC) and RV when the chest wall volume was considered. We found that the decrease of total chest wall volume was mainly due to the abdominal compartment, suggesting a less flattened diaphragm; the lower rib cage becomes slightly larger, probably due to the re-accommodation of the diaphragm; the pulmonary rib cage also decreases, probably due to the lower degree of lung hyperinflation. These findings were corroborated by CT analysis performed in one subject. The second study was conducted on patients with lung cancer who underwent **pulmonary lobectomy** in order to evaluate the effects of two different protocols of physical therapy, i.e. CPAP and cycling exercise. Both CPAP and cycling increased tidal volume but cycling was more effective. During the application of CPAP, end-expiratory chest wall volume increased significantly, particularly in the abdominal compartment, suggesting lung distension, particularly in the diaphragmatic regions. During cycling exercise, end-expiratory chest wall decreased significantly, particularly in the abdominal compartment, suggesting that and increased tidal motion of the lung due to the action of the expiratory abdominal muscles. The third study was conducted on patients with **congenital**

diaphragmatic hernia, a malformation consisting in the lack of or incomplete formation of the diaphragm. When there is enough diaphragmatic tissue, the surgery consists in suture of flaps to avoid a straight hemidiaphragm. In case of a large defect of diaphragm, it is necessary to make use of diaphragmatic patch, which could reduce the contractility of the diaphragm because it does not grow in parallel to the diaphragm. The aim of the study was to assess diaphragmatic function by OEP, which obviates the problem of patient cooperation. We found that patients who underwent surgical repair by patch show lower abdominal volume variations than those who underwent suture. These results suggest that in the former the mobility of the diaphragm is reduced and inspiratory rib cage muscle must be used to compensate this reduced mobility. In conclusion, the set of proposed methods has shown to provide useful information regarding the altered function of the diaphragm in several clinical situations and can be considered for the non-invasive evaluation of diaphragm even when patient collaboration is not possible.

STRUCTURAL AND FLUID DYNAMIC ASSESSMENT OF STENTING PROCEDURES FOR CORONARY BIFURCATIONS

Stefano Morlacchi - Supervisor: Francesco Migliavacca

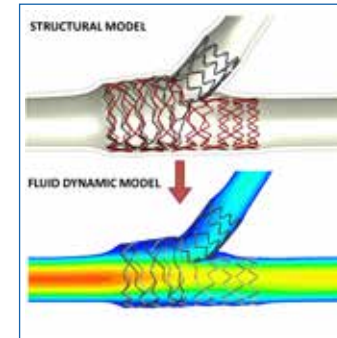
This project has been developed at the Laboratory of Biological Structure Mechanics (LaBS) of Politecnico di Milano within the Bioengineering PhD program. Its main scope is the implementation of both structural and fluid dynamic engineering methods able to provide a better understanding of the biomechanical alterations induced by stenting procedures for coronary bifurcation. The contents of this thesis are divided in six main chapters that are presented according to the following outline.

Chapter 1 introduces the clinical problem of atherosclerosis in coronary bifurcations and underlines the importance of better investigating the biomechanical effect of stenting procedures on coronary arteries. Coronary bifurcations are a very atheroprone environment mainly due to their specific hemodynamic conditions characterized by recirculation and stagnation zones. Moreover, the role of biomechanics is also relevant in the development of post-stenting clinical drawbacks such as in-stent restenosis or stent thrombosis. Many conventional and dedicated approaches involving one or two stents are proposed in the literature and reviewed in this chapter to highlight their main features and limits. At present, none of them can be considered as the gold standard technique

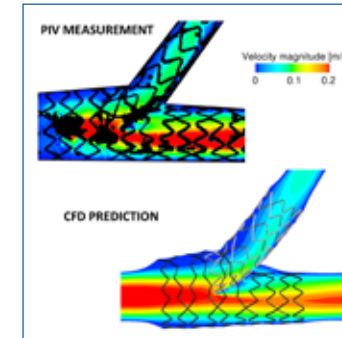
proving the necessity of further research in this area. Chapter 2 reviews the current state of the art of numerical modelling of stenting procedures, highlighting the main advantages, current applications and potential future developments. In particular, numerical methods have the potential to complement the results obtained with traditional clinical studies with advantages in terms of reduced costs, high flexibility and ability to assess important biomechanical quantities. Deeper attention is here paid to those studies involving complex geometries such as curved vessels or coronary bifurcations that are characterized by lower clinical success rates and still represent an open clinical issue. Structural, fluid dynamics and drug elution models are described separately even if most of the newest studies involve sequential implementations of these numerical models responding to the accepted evidence of the multifactorial nature of this topic. In this light, the main scope of this work is the implementation of a sequential structural and fluid dynamic numerical method (Fig. 1) able to simulate stenting procedures (Chapter 3). The commercial finite element code Abaqus is used to simulate the stent deployment in atherosclerotic coronary arteries. Preliminary analyses are

implemented to simulate the stent insertion in complex and tortuous patient specific geometries as well. Subsequently, the final geometrical configurations are used to define realistic fluid domains and perform transient fluid dynamic simulations with the commercial software Ansys Fluent. For sake of clarity, a stenting expansion in a simplified curved atherosclerotic coronary artery with no branches is taken as an example while three case studies involving coronary bifurcations are discussed in Chapter 4.

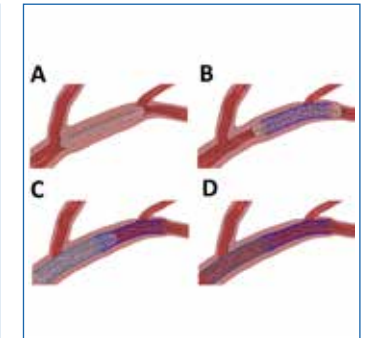
In the first study, a new tapered balloon is proposed to improve the final kissing balloon inflation within the provisional side branch approach. This novel device aims at reducing the overexpansion provoked by the simultaneous inflation of two angioplasty balloons in the proximal part of the bifurcation and its negative biomechanical influence. In the second study, the most common singleor double-stenting procedure involving conventional devices are simulated and compared in terms of several structural and hemodynamic quantities. Lastly, a new dedicated device designed to facilitate the double-stenting culotte technique is investigated highlighting its main advantages and drawbacks. This example shows how dedicated devices may be the next revolution for



1. Example of sequential structural and fluid dynamic numerical model implemented to investigate a double stenting procedure for coronary bifurcations



2. Velocity magnitude fields in m/s in the transversal plane after the T-stenting technique with protrusion (T-PR). The experimental DPIV measurements are shown on the left, while the CFD results are reported on the right



3. Image-based finite element simulation of a real clinical case. Steps of the final numerical simulation: A) Angioplasty procedure with a 2.5 mm balloon expanded at 13atm; B) Expansion of an Endeavor stent across the distal bifurcation with a 2.75 mm balloon inflated at 12 atm; C) Deployment of a second Endeavor stent across the proximal bifurcation with a 3.0 mm balloon at 14 atm; D) Final geometrical configuration at the end of the stenting procedure

stenting procedures in coronary bifurcations.

Since lack of accurate verification and validation processes is the current major limit of numerical methods, a comparison between digital particle image velocimetry measurements and structuralbased CFD predictions of blood flows is presented in Chapter 5. Both stented and non-stented coronary bifurcation models are examined to highlight the influence of different bifurcation angles and stenting procedures (Fig. 2). This work is carried out in collaboration with Aether Lab of Virginia Tech where I spent five months of PhD program. Beside the intrinsic differences and modelling assumptions of the two approaches, both CFD

and DPIV analyses proved to capture the main features of the fluid flows. Nonetheless, both approaches still require a challenging direct validation against in vivo measurements to fully demonstrate their ability to describe the existent hemodynamic of coronary flows. Lastly, the use of highly idealized geometries is the main cause that currently restricts the routine application of computational models in the clinical field. In this light, a feasibility study where two real clinical cases are simulated by means of finite element models is presented in Chapter 6. Image-based reconstructions are created by the Universitat Pompeu Fabra in Barcelona combining the information from conventional coronary

angiography and computed tomography angiography while structural finite element models are implemented by means of the ABAQUS commercial code following the main modelling techniques presented in Chapter 3 (Fig. 3). In summary, this work proved the feasibility of patient-specific numerical models that uses image-based reconstructions of coronary bifurcations and is able to replicate real cases of stenting procedures. These results may be considered a step forward towards the routine application of such models in the clinical field.

DESIGN OF MICROFLUIDIC-BASED MICROARRAYS FOR RAPID, EFFICIENT SINGLE-CELL ISOLATION

Francesca Nason - Supervisor: Federica Boschetti

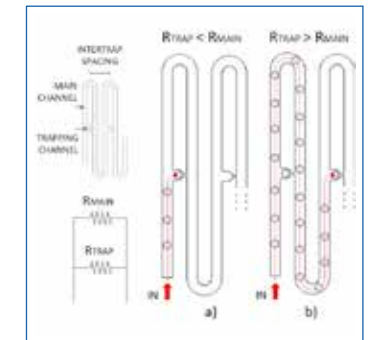
Stem cells have a prominent position in the regenerative medicine because of their unique ability to undergo self-renewal combined with the capability to give origin to numerous differentiated cell types, including progenitor and effector cell populations. In fact, upon necessity, stem cells can be stimulated to expand and to differentiate into a specific cell type, thus tissue-engineered surrogate parts can then be constructed and used surgically for the purpose of repair. However, the lack of an intrinsic capability in traditional cell culture protocols for high throughput assessment of changes in the properties of individual cells within a population, in the absence of experimental robotic culture systems and integrated Flow Assisted Cell Sorting, represents a significant limitation in the field of stem cells, where researchers and industry require knowledge of the definitive status of cell phenotypes during each stage of expansion, maturation, or differentiation. To understand the impact of a stem cell initial phenotype on subsequent fate decisions, including proliferation, self-renewal, differentiation, and apoptosis, and hence, potentially, their ultimate therapeutic potential, studies at the single cell level are necessary. In this work, the design, prototyping

and characterization of a microfluidic device for single cell isolation is proposed. Numerical modeling in combination with an experimental campaign was adopted to systematically optimize the device architecture, a microfluidic chip with enhanced single cell trapping and on-chip culture performance. The design concept requires the establishment of differential flow resistances along a section of the serpentine (main) channel that encourages fluid to preferentially flow through a connecting channel between two adjacent curved sections of the main channel, but only in the absence of a cell being lodged in this connecting channel. A particle or cell being transported along the main channel is thus preferentially forced to enter the trap and thereafter the dominant flow path is that of the main channel, until the next open connecting channel is reached (Fig. 1). This approach proved to be really successful since does not require the use of sophisticated machinery for the operation and standard microfabrication techniques can be adopted for prototypes realization. 2D numerical simulations of flow through the device were carried out aiming to confirm the hydrodynamic conditions, determine the geometrical parameters that impact the flow,

and pinpoint a velocity regime at which the design fails. The prediction of the stresses acting on cells when flowing into microfluidic devices would allow the optimization of the design of the geometry configuration and the experimental conditions for cell trapping experiments that ultimately maximize cell viability and capture efficiency, minimizing at the same time the duration the isolation process. Since this is a critical key point for the device application with cells, the numerical modeling of a deformable cell flowing in the microdevice with was carried out. In particular, to study the evolution of liquid capsules in micro channels a hybrid boundary integral/immersed boundary method coupled with a spectral discretization of the membrane surface was adopted. This numerical model was implemented within NEKTON, an open-source computational fluid dynamics solver written in Fortran77/C. Several parameters were identified in the literature according to the capsule model adopted, the shear elastic and the bending modulus. Unfortunately, it was possible to simulate only the first part of the path followed by the capsule; in particular limiting the analysis to the point where the capsule stops within the trap. However, almost the entire path followed by the cell in the

microdevice was considered. Simulation of the cell trapping process confirmed that the path followed by the capsule is directed to the trapping channel without deflection towards the main channel. Moreover, the velocity regime chosen allows for a safe handling of cell samples and ensures that cells are not distressed due to the flowing along complex geometries with L-bends and changing of section of the microchannels. In fact, with regard to the portion simulated, the maximum pressure applied to the cell is around 0.1 Pa which can be assumed as acceptable when handling living cells. Finally, prototypes microfabrication, characterization and the experimental tests were carried out. A soft-lithography technique was adopted for microfabrication. For experiments, purified human bone marrow derived mesenchymal stem cells (MSCs) were adopted. To validate the fluid dynamic field of the prototypes against the results of computational simulations, the microchannels fluid flow profile was obtained using micro Particle Image Velocimetry, an optical method of flow visualization used to obtain instantaneous velocity measurements and related properties in fluids. Experimental tests to assess the efficiency

and the selectivity of single cell trapping on 100 trap microfluidic chips were carried out. From these analyses it was possible to confirm the fluid dynamic field calculated by CFD simulation: the hydrodynamic condition designed was verified to be favorable to the trapping process. Results of trapping experiments with rigid polystyrene microbeads and MSCs showed that trapping efficiency may vary depending on the trap geometry (from 20% to 100% of filling). The composition of the filled traps was also analyzed at the end of the capture process. Given the computational and experimental results accomplished, it was possible to identify the most efficient trap shape among the ones tested. Moreover, selectivity tests demonstrated the possibility to adopt such devices also for applications where a sample contains two different cell populations, given that they have different sizes and/or mechanical properties. Finally, the working principle of the complete trapping/shunting device was demonstrated with an experiment one trap unit using microbeads, being able to shunt a rigid particle off trap after the capture and culture it in a separate well. However, the devices must be optimized for each cell type and designed in accordance with the trap



1. Working principle of the hydrodynamic trapping of single cells: (a) trapping mode and (b) bypassing mode

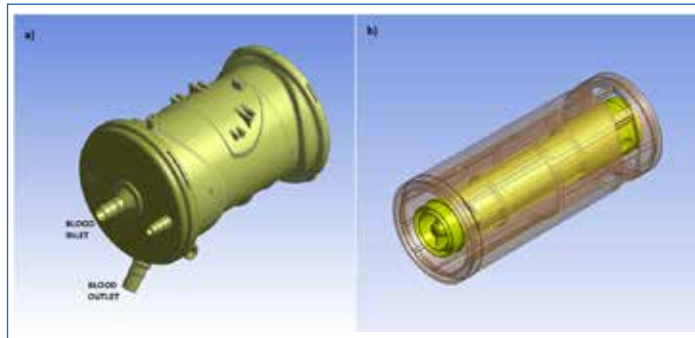
size to cell size ratio. This innovative research is among the first to couple numerical simulations and experiments to accurately investigate cell behavior in flow in a complex geometry in order to optimize the design of effective microdevices for cell isolation. This would help optimizing the operating conditions as well as the geometrical parameters for cell manipulation/culture micro-devices, to ensure the cell viability and ultimately improve such high-throughput micro-devices, which still represents of the most significant challenges in the field of stem cells.

DESIGN OPTIMIZATION AND THROMBOGENICITY EVALUATION OF BLOOD RECIRCULATING DEVICES FOR CARDIOPULMONARY BYPASS

Alessandra Pelosi - Supervisor: Alberto Redaelli

The present work describes a comprehensive multiscale computational fluid dynamics (CFD) approach for the design optimization and blood damage minimization of cardiopulmonary bypass (CPB) devices, with specific application to a prototypical hollow-fiber membrane oxygenator with an integrated polymeric heat exchanger.

Blood oxygenators (OXY) and heat exchangers (HE) are commonly used in CPB during open heart surgery to regulate the proper arterial blood concentrations of O₂ and CO₂, and to shorten the time required to cool the blood prior to surgery and to rewarm it after surgery. The most common type of OXY and HE in use are composed by microporous polymeric hollow-fibers. The flow of blood within the interstitial space between the fibers, together with the presence of vortices and blood recirculating areas, induce non-physiological flow patterns, that may lead to blood damage (hemolysis and thrombosis). The evaluation of the thrombotic risk associated with extracorporeal membrane oxygenators should play a critical role into their preclinical design process. The two main flow-related factors inducing blood damage have been experimentally shown to be shear stress and exposure time.



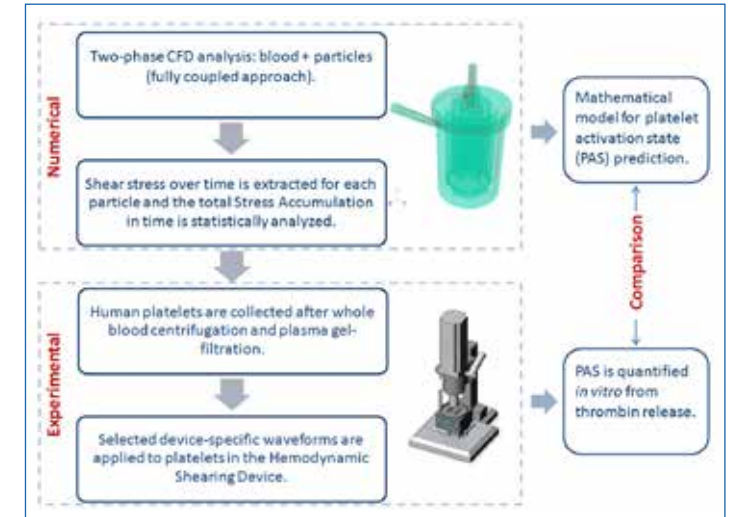
1. a) 3D CAD drawing of the device. b) Internal core and external shell surrounding the heat exchanger fiber bundle

The design process of CPB devices should account for both macroscopic aspects (e.g. minimization of the pressure drops and stagnation areas) and microscopic aspects (time history of the stress acting on blood particles) affecting their behavior. The present work highlights the importance of computational modeling in the early stages of the design optimization of CPB devices as an effective tool to get fast, accurate and complete results as opposed to the classical empirical approach. A CFD based approach, supported by experimental validation, was applied to the following purposes: (i) to quantify pressure drops, heat exchange efficiency, blood recirculating areas, velocity pattern and stress distribution for different working conditions and/or for different design solutions, with increased time/

cost efficiency; (ii) to quantify the flow-induced thrombotic risk of the device; (iii) to obtain concise indices that synthesize the trade-off between risks and benefits, that could accelerate the manufacturing process. The macroscale performances of a prototypical hollow-fiber OXY with integrated polymeric HE (fig. 1) were analyzed through CFD, focusing on hemodynamic (i.e. pressure drops and vortex formation) and thermodynamic (i.e. heat exchange efficiency) parameters affecting the performances of the device during CPB. CFD simulations were coupled with experimental tests for results validation. New design solutions for the heating module were introduced to improve the device performances and blood stagnation area were minimized. Flow-induced platelet activation

was investigated to assess the thrombotic risk of the device. A fully-coupled Lagrangian approach was adopted to infer the trajectory and the shear stress loading history experienced by platelet-like particles in the entire device and in a periodic elementary subunit of the fiber bundles. The loading history was incorporated into a damage accumulation model in order to estimate the platelet activation state (PAS) associated to repeated passes of the blood within the device.

The level of platelet activation was quantified through in vitro experiments performed at the Biofluids Laboratory of the Stony Brook University, NY. Human platelets were exposed repeatedly to selected shear stress loading waveforms obtained from the CFD simulations (fig. 2), using a computer-controlled Hemodynamic Shearing Device. The evaluation of the PAS was performed using an acetylated prothrombin-based assay to quantify the rate of thrombin generation. Experimental results were compared with numerical predictions of PAS, obtained using different damage models, in order to verify their accuracy in the prediction of the thrombotic risk. A parametric analysis was performed to investigate the trade-off between heat transfer efficiency, pressure drops and flow-induced blood damage for different fiber arrangements in periodic computational models of progressively packed elementary fiber beds. Blood damage models, based on a Lagrangian stress-based formulation, were used to predict the extent of platelet



2. Workflow of the coupled numerical and experimental methodology adopted in this study

activation and red blood cell damage. This analysis was conducted varying different geometrical parameters (i.e. inter-array distance, fiber inclination angle, inter-fiber distance) and for different flow conditions (i.e. axial and cross flow). Results highlighted the presence of blood stagnation areas in the inlet section that significantly increased the activation levels in platelets remaining trapped in this region; a new design solution was proposed for the inlet section that consistently reduced the percentage of stagnation areas. A power-law relationship was obtained to determine the order

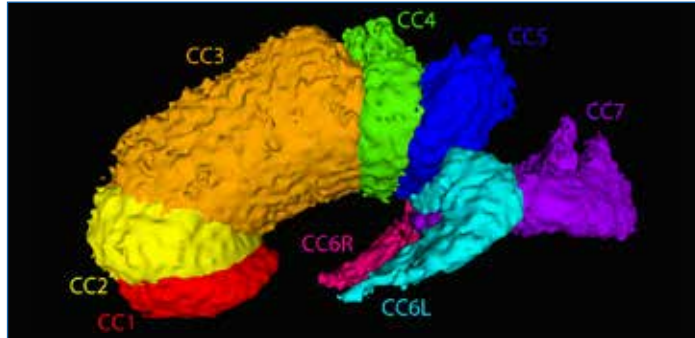
of magnitude of the platelet activation state with respect to the duration of the CPB. A wide set of fiber arrangements, accounting for both fibers density and orientation, allowed to assess the related hemolytic and thrombotic risk, pressure drops and thermal efficiency in different flow configurations. These results represent a valid indication for the primary phase of the design optimization process of blood recirculating devices.

THE STUDY OF WHITE MATTER MICROSCOPIC DAMAGE IN NEURODEGENERATIVE PATHOLOGIES: ATLAS-BASED AND FMRI-GUIDED TRACTOGRAPHY

Maria Giulia Preti - Supervisors: **Maria Marcella Laganà, Nikos Makris**

Background and Aim

Magnetic Resonance Imaging (MRI) allows the in-vivo observation of brain tissues, permitting the evaluation of both gray and white matter damages occurring in neurodegenerative pathologies and other neurological disorders. While macroscopic tissue damage and atrophy are assessable with conventional MRI, advanced MRI techniques, such as Diffusion Tensor Imaging (DTI) and functional MRI (fMRI), are needed for a more accurate analysis of white matter microstructure and brain functional organization, respectively. Allowing the in-vivo virtual reconstruction of white matter tracts, tractography appears particularly promising in the study of pathologies in which brain disconnection plays a major role, such as Multiple Sclerosis (MS), Alzheimer's Disease (AD) and amnesic Mild Cognitive Impairment (aMCI), the transitional stage between normal cognition and AD. The applicability of this technique to pathological brains, however, still represents a matter of active research, due to the higher inter-subject anatomical variability regarding damaged tissues, causing difficulties in localizing and segmenting the structures of interest. Hence, strategies such as the use of reference templates of the



1. Corpus callosum probabilistic atlas of an elderly population, (shown as a mask, probability threshold 0.5). CC1: orbital frontal, CC2: anterior frontal, CC3: superior frontal, CC4: superior parietal, CC5: posterior parietal, CC6L/R: left/right temporal, CC7: occipital

tracts, called tractographic atlases, are employed to assess the diffusion properties along white matter bundles in cases in which a reliable fiber reconstruction is difficult. Moreover, the integration of tractography with fMRI (i.e. fMRI-guided tractography) can help in overcoming tractography known limitations, like manual bundle segmentation, as well as permitting the combined study of anatomical and functional deficits. However, this technique has not been standardized in literature yet, in particular as far as a group analysis is concerned. The aim of this study was to introduce and validate methods of tractography in order to address white matter damages in neurological disorders. To this purpose, we meant to involve both the use of tractographic

atlases and the combination with functional activations from fMRI, in order to overcome the limitations of individual tractography. Specifically, we aimed at: 1) optimizing and clinically validating the procedure of atlas-based tractography with the objective of reducing misregistration artifacts and partial volume effects usually affecting atlas-based methods; 2) introducing and experimenting a novel method of fMRI-guided tractography allowing a group analysis.

Protocols and Results

The first methodological development of this work consisted in the introduction of an optimized method of atlas-based tractography, aimed at overcoming the misregistration

inaccuracies usually concerning this technique. A fine tuning of the techniques of tractographic atlas construction and application was performed, comprehending an analysis of different registrations, averages, thresholding. The optimized method gives as a result tractographic atlases of probabilistic nature, that were constructed for the main white matter bundles and applied to different protocols. First, tractographic templates of the main white matter bundles for an elderly population, not available in literature yet, were created (example shown in fig. 1). Further, the first large-sample study (on 74 healthy subjects) of the Middle Longitudinal Fascicle, only recently discovered and investigated in humans, was performed and an atlas for this bundle was also provided. Two clinical applications of atlas-based tractography were explored, allowing to assess the clinical value of the procedure. The former consisted in the analysis of the corpus callosum in 19 subjects with amnesic aMCI and 37 with AD, and showed the capability of the atlas-based tractography to characterize the damage pattern of patients along this bundle. The latter regarded the analysis of the Cortico-Spinal Tract in 59 patients with MS at different stages and it demonstrated

the capability of the atlas-based method of assessing the specific neuro-motor damage MS patients, also distinguishing between the different disease stages. The second considered methodological issue concerned the integration between fMRI and tractography, aimed at exploring the white matter connections beyond a specific functional activation. First, a preliminary work was performed on a case of cortico-basal degeneration: in this context a proper tuning of fMRI-guided tractography procedure permitted the correct identification of the asymmetric brain damage characterizing this pathology. Then, a completely novel technique of group analysis was proposed, allowing to obtain an fMRI-guided ensemble (group) tract including the 3D trajectories involved in a specific task for a particular population. This method was clinically validated on 15 subjects affected by aMCI, 14 affected by AD and 14 age-matched healthy controls and successfully allowed to describe the different activation patterns for the groups (Fig. 2). In particular, it was possible to highlight the compensation circuits activated in the aMCI condition, both in white and gray matter, allowing to maintain a good brain functionality despite the brain

deficit. Further, an alternative approach of group analysis was proposed, aiming at labeling the cortical areas onto which specific white matter bundles are projected. The novel approach was preliminarily experimented for the Arcuate Fascicle, highlighting the intersections with Broca's and Wernicke's areas, coherently with previous findings.

Conclusion

Special MRI neuro-imaging methods concerning the white matter microscopic fiber structure and the functional response of cortical areas do offer high potentials in neurodegenerative diseases, but require specific tuning and integration tools. The present work has demonstrated and validated both the optimization of known protocols and also novel approaches in basically two directions: 1) construction of normal tractographic atlases and projection onto diseased brains; 2) integration of fMRI and tractography at individual and group level. Discussed results are promising towards an improvement of evidence based diagnosis, follow-up, and prognosis in neurodegenerative diseases.

THORACO-ABDOMINAL ASYNCHRONIES: NEW INSIGHTS IN HEALTH AND DISEASE

Rita Priori - Supervisor: Andrea Aliverti

The respiratory muscles play a vital role, as they guarantee pulmonary ventilation. These muscles are an essential part of the ventilatory pump, which comprises also the chest wall and its three compartments, that is the pulmonary rib cage (RCp), the abdominal rib cage (RCa) and the abdomen (AB). During spontaneous breathing (SB) these compartments move as the respiratory muscles, namely the rib cage muscles and the diaphragm, contract. Respiratory muscle contraction in healthy subject is coordinated and the movement of the chest wall compartments during SB results to be homogeneous. Isolated contraction of a muscle group alone is able to guarantee ventilation, but it may also have undesired effects on the other compartments, such as asynchronous or even paradoxical inspiratory or expiratory movement, which cause ventilation to be less effective or even totally vain. A complete evaluation of the function of the respiratory muscle involves very invasive pressure measurements or requires patients' collaboration, however their severe condition often does not allow them to collaborate in performing demanding maneuvers. The aim of this thesis is the development of new indexes for chest wall kinematics analysis, and

more specifically chest wall asynchronies quantification, for the noninvasive assessment of respiratory muscle function in critical clinical situations whereby it is not possible to perform invasive measurements. For this purpose, optoelectronic plethysmography (OEP), has been extensively used in this work, as this noninvasive technique provides a continuous and accurate measure of chest wall and compartmental volumes (RCp, RCa and AB). Compartmental volumes variations reflect the activity of the main respiratory muscles, which act on the three compartments, therefore the advantage of this technique is that it noninvasively allows the assessment of respiratory muscle function, without particular patients' collaboration. In this sense, the computation of compartmental asynchronies provides a unique information as asynchrony between RCp volume signals and RCa volume signals represents an index of rib cage distortion, while asynchrony between RCp volume signals and the AB volume signals is an index of relative coordination and activation between the rib cage muscles and the diaphragm. First in this work the reliability of the methods traditionally used for the quantification of chest wall asynchronies (Lissajous

figure and cross-correlation) has been extensively evaluated and new methods have been developed. To answer the need of breath-by-breath quantification of chest wall asynchronies a new algorithm based on the assumption that compartmental volume signals can be fitted by a combination of sinusoids on a breath-by-breath basis (by least squares fitting), was developed. The phase shift among the two signals can be calculated as the angle of the transfer function between them. Compared to the traditional methods, least squares fitting resulted in a lower percentage error and has the advantage of the performance of a breath-by-breath measurement. For this reason this method has been extensively used in this work. A major aim of this thesis was to study chest wall asynchronies in some diseases that directly or indirectly affect the respiratory muscles such as chronic obstructive pulmonary disease (COPD) and Duchenne muscular dystrophy (DMD), which in their more severe condition lead to respiratory failure, in order to characterize thoraco-abdominal motion in stable (baseline) conditions and have a well-matched comparison to healthy subjects. Specifically first we studied the effect of posture on asynchronous

chest wall movement in COPD. Measurements of phase shifts between RCp and RCa and between RCp and AB were performed with OEP during SB in the seated and supine position. Changes in diaphragm zone of apposition were measured by ultrasounds and were correlated to compartmental volume variations. In this study we found that in COPD the changes in body posture induces substantial differences in the respiratory muscles' coordination. In seated posture COPD patients show asynchronous rib cage movement, while in supine diaphragmatic contraction always anticipates rib cage muscle contraction and rib cage distortion disappears. This loss of synchronization suggests that in COPD the diaphragm is able to shorten effectively in both postures and that in supine position diaphragm mechanics improves. Thoraco-abdominal asynchronies were then studied during SB in Duchenne muscular dystrophy. Recently it has been demonstrated that chest wall kinematics analysis during SB is an important marker of the progression of the disease and of diaphragm impairment. The supine position was the posture in which it was possible to emphasize early signs of ventilatory problems. One hundred DMD patients aging from 4 to 30 years old were therefore considered to investigate if studying asynchronous chest wall movements could give better insights into progressive alteration of chest wall kinematics and muscle function. It was found that in DMD patients with increasing age

RCp progressively anticipates AB in generating tidal volume and that in older DMD patients there is a high paradoxical motion of AB during expiration. This is a new index of progressive diaphragmatic weakness that not only evaluates the prevalent contribution in generating tidal volume, but shows how inspiration is started. These indicators are complementary to the assessment of respiratory function traditionally provided by spirometry and have the great advantage of not requiring patient's cooperation, motivation and coordination. After having characterized chest wall asynchronies in COPD and DMD in stable conditions, we studied chest wall asynchronies in the same diseases in clinical situations of interest, during which the measure of chest wall kinematics is not obvious, such as during exercise before and after intervention by lung volume reduction surgery (LVRS) in COPD patients and during weaning from mechanical ventilation. In selected patients with COPD, the decrease of lung volume following LVRS leads to several beneficial effects, including the ability of the inspiratory muscles to generate force and exercise performance. We studied COPD patients before LVRS, 1 and 3 months after surgery by OEP at rest and during an incremental test on a treadmill. During SB and the different treadmill speeds, there was an improvement of the synchronism between RCp and AB, with respect to the preoperative condition in both post-operative phases. The paradoxical movements of the chest wall, which characterize

patients with COPD, are reduced in patients that undergo LVRS. The synchrony between RC and AB is improved not only during SB, but also during exercise. Finally we studied if chest wall kinematics can be a useful indicator of successful weaning from mechanical ventilation. Respiratory muscles impairment is an important determinant of the need for mechanical ventilation (MV) in difficult-to-wean patients. The contribution of RCp and AB volume changes to tidal volume were measured by OEP in difficult-to-wean patients, during MV and SB at three weaning stages. We found that at the start of weaning there is a predominant compartment in its percentage contribution to tidal volume, while at the end of weaning all compartments result to be homogeneous. Moreover by analysis of chest wall asynchronies the changes in the timings of activation of the two main inspiratory muscle groups were studied as they improved during weaning, and the information was combined to compartmental contribution to tidal volume. As expected, recovery from diaphragmatic weakness was evident both in its more homogeneous contribution to the generation of tidal volume and in the timing at which it is recruited during spontaneous breathing.

INDEPENDENT COMPONENT ANALYSIS APPLIED TO THE STUDY OF THE CONNECTIVITY NETWORKS IN FUNCTIONAL MAGNETIC RESONANCE IMAGING

Marta Re - Supervisors: **Anna Maria Bianchi, Gianluigi Reni**

Introduction

Although current knowledge of the structure and function of the human brain is substantial and growing fast, there are still many unclear points and functionalities that have to be explored more in details. The study of the brain connectivity is crucial to elucidate how neurons and neural networks process information and thus to have a better explained vision of the brain. Thanks to the functional Magnetic Resonance Imaging (fMRI) technique it is possible to understand how the brain and his connectivity works, in terms of its physiology, functional architecture and dynamics. Independent Component Analysis (ICA) is a signal processing technique to solve the blind source separation (BSS) problem in a data-driven approach. Using the ICA method applied to fMRI, it is possible to record signals related to the neural activity of the human brain, to highlight signal covariations in networks of brain regions and to separate networks. ICA has been used with success in several fMRI studies but some problems in the adoption of ICA and many questions in term of brain functions and connectivity remain to be well explained. Aim of the Work
The aim of this work is to develop a new method that

permits, using ICA method, to identify cerebral networks involved in different fMRI experiments and to do behavioral and statistical tests on selected brain activities. My method not only permits to decompose signals in different contributes, but also to maximize the signal to noise ratio by utilizing a selection of time windows and to focalize the attention on specific cerebral areas. To have a good explanation of the differences that may occur in fMRI studies, I will apply my method on two different fMRI tasks.

ICA in a Working Memory fMRI Task

Working memory load has marked effects on regional neural activation, but the mechanism through which working memory (WM) load modulates brain connectivity is still unclear. During a working memory task, two of the most involved networks are the default mode network (DMN) and the working memory network (WMN). The aim is to use the time course of the two most involved networks default mode network (DMN) and the working memory network (WMN), selected by means of the ICA, for studying how these networks are involved with the complexity of the task and the phase (encode, maintenance

and retrieval) and how, in these networks, complexity and phase are correlated with reaction times. Fifteen healthy young adult were involved in a 3T MR protocol that consisted of one anatomical sequence and three functional acquisitions. During the fMRI acquisition, the subjects performed a delayed spatial working memory paradigm presented with three levels of difficulty. fMRI images were preprocessed and coregistered with the anatomic data set. From the obtained maps of the total ICA group analysis were selected two Independent Components (ICs) containing the WMN (1 and 2) and one the DMN. The ICs time course was considered in three time windows (for each WM phase) selected taking into account the hemodynamic response (by delaying the windows) and corrected with a baseline value. Mean values of the ICs where examined and the correlation between these and the response time was estimated. A 3x3 two-way ANOVA on Fisher transformed correlation was conducted to test the variation on the complexity of loads, phases and runs. The results show that DMN mean activity is negative while WMN1-2 mean activities have opposite behaviors regarding the phase, but similar concerning with the complexity (WMN1 is

more involved in the first part of the mnemonic phase while the WMN2 is more involved in the last WM phase). DMN shows a reduction of the correlation from encode to retrieval, instead of WM1-2 where it grows. The ANOVA showed significant variation for the phases over all the subjects in WMN1-2, an interaction of the variation of phases and runs in WMN2 and a interaction of phases, runs and loads in DMN. In conclusion, this study shows that, by means of ICA, it is possible to isolate networks of connected regions and relate their time courses to task phases and behavioral performance.

ICA in a Visual Stimulation fMRI Task

Prematurity is a condition of significant emotional stress for both, children and their mothers. Even if the effects of prematurity on mother's emotional reactions have been extensively investigated, no study has yet analyzed the possible neuro-functional associated effects on mothers of preterm infants. The feelings experienced by the infants' (preterm and full-term) mothers could be studied by analyzing the Posterior Cingulate/ Precuneus (PCC) and the Medial Pre-Frontal Cortex (MPFC), the most DMN's areas involved into the maternal care and empathy. The purpose of this study is thus to apply the method developed in the previous chapter to select the MPFC and the PCC regions and, by use of their time course, to study their behavior. Eighteen mothers of full-term and preterm infants were involved. The 3T MR protocol consisted of one functional

sequence, where mothers were shown novel face images of their own and unknown infants in different emotional states (happy, neutral and distressed), and one anatomic sequence. The images were preprocessed and analyzed with the ICA method. Thus, from the DMN IC, two Regions of Interest (ROIs) for PCC and MPFC were drawn both for all the mother and for the two groups of mothers separately. The signal of the ROIs was decorrelated from all the ICs that did not show the DMN and corrected with a baseline value. This signal was also deconvoluted into six signals (types of images shown), corrected for the zero values, averaged and then analyzed. Signals were also tested in two different time windows, taking into account the hemodynamic response. T-tests and a 2x2 two-way ANOVA analysis, both in windows and in averaged signals, of all the subjects was conducted to investigate main and interaction effects. In PCC ROIs there is not difference between groups of mothers while in the MPFC ROIs mothers of preterm infants show a mean signal that is always greater than zero and it is also greater when images of their children are presented, compared with unknown children. This is also significantly higher of the signal of the other mothers in case of view of their children. In mothers of full-term infants the mean signal is always below zero and quite unchanged. In PCC ROIs, differences between own and unknown is more marked than in all the time courses while in MPFC ROIs is less marked. The ANOVA also shows that there is a main effect of the type (own

or unknown child) in MPFC only for the mothers of preterm infants, while in PCC it is for all the mothers. In conclusion, this study shows that, by applying and changing the method developed in the previous work, it is possible to isolate networks of connected brain areas, select ROIs and analyze their time courses.

Conclusions

In my work I showed that this method, by focus in restrict temporal windows, exhibited greater signal and permitted to evaluate trends and correlations with behavioral performances. With this process, I was able to examine both the signal course into the entire networks and in selected ROIs. I have demonstrated that this process has given positive results when applied to different fMRI stimulations and has the capacity of adapt to diverse conditions. Infact, it fits very well both a stimulation with separate trials, and a stimulation with no separation between stimuli. I have thus shown that this method is stable, repeatable and can adapt to different task conditions and can be applied to all the types of fMRI experiments. For all these reasons, it is a powerful method for the study of the cerebral networks.

A STUDY OF CORONARY CLOT REMOVAL BY MEANS OF ASPIRATION CATHETER

Sajjad Soleimaniamiri - Supervisors: Gabriele Dubini, Giancarlo Pennati

The coronary arteries supply blood and oxygen to the heart tissues. If this flow becomes blocked, the downstream part of heart muscles begins to die. This leads to the heart attack and myocardial infarction (MI). The coronary arteries can become blocked by plaques which are the results of a slow process along the years called atherosclerosis. Although a coronary vessel is seldom completely blocked by a plaque, a complete blockage may happen if a clot (a result of hemostasis following tissue injury and abnormal vessel wall repair) lodges on the area. Furthermore, the plaque may also rupture and move and potentially cause a complete blockage.

If the coronary vessel is blocked with a clot, several methods are available to tackle the issue: the coronary vessel could be treated with clot busting medicines or clot removing devices. In clot busting methods pharmacological methods are used to dissolve the clot. In the case of clot removing by means of devices, a very thin tube is guided toward the occluded vessel to mechanically remove the clot. Removal can occur according to different methods, including breaking down the clot and aspirating it. The most reliable method is based on the usage of coronary

aspiration catheters. The aspiration catheters remove the clot only through the aspiration force. This method is the most preferred one as it has high effect and minimal clinical drawbacks.

Although the results of aspiration catheter deployment have been investigated in a number of clinical trial studies, the quantitative prediction and comparison of catheter performances is also important in terms of aspiration ability. The main aim of the current study is to address this issue. More precisely, the purpose is to find out how an aspiration catheter aspirates a clot when used in different scenarios. In fact a wide range of aspiration catheters are available for the percutaneous coronary intervention (PCI) purposes. These catheters mainly differ in their geometry and design. In this thesis the effects of the geometrical differences are studied with regard to a number of catheters. The geometrical differences include size, tip shape, lumen diameter and hole design. The study of catheter geometrical parameters is combined with that of clot mechanical properties, which is a very important factor for the success of PCI for clot removal. To model the clot aspiration via coronary aspiration catheter, the first step was to define

clot rheology. Ideally the blood should be collected from healthy human donor and rheology measurements immediately started. In practice the access to appropriate human blood is restricted and, furthermore, immediate rheology measurements right after blood collection are not feasible. Indeed, in this study the rheology measurements were based on porcine blood. The collected blood was anticoagulated and preserved in room temperature until transferred to the rheology laboratory where, after adding appropriate quantity of coagulation, the rheology of blood during clotting was measured. The choice of relying on properties of porcine blood clot as an alternative for human blood is reckoned reasonable due to the fact that in general porcine blood is similar to human blood in terms of structure, hemostatic properties, coagulation and ADP. However, there are still differences, for example porcine clot is hypercoagulable comparing to human blood.

In order to measure rheological properties of clot, two types of measurements were performed: static measurements and dynamic measurements. In static measurements the blood is exposed to shear strain or shear stress in order to measure

the viscosity. This method is a direct approach to find the clot viscosity, but suffers from several drawbacks. First, the blood is exposed to high shear strain. Second, the in vivo clotting procedure includes the formation of fibrin network, but static measurements may easily rupture this network and cause unreliable measurement. The dynamic rheology measurements, on the contrary, are much more reliable, as they are less likely to rupture the clot structure.

In this study several measurements were done on incipient clots or clots which were initially exposed to mechanical precondition. The mechanical precondition was defined based on the frequency and shear strain as well as time period the clot had been exposed to, before the start of rheology measurements. The results indicated that the clot behavior is shear thinning and that can be described with a power law correlation. In order to model clot aspiration through a coronary aspiration catheter, a generic catheter was considered. Both clot and the aspiration catheter were modeled in a segment of the left coronary artery (LCA) close to the first bifurcation. By applying the pressure difference between the vessel outlets and catheter outlets, the clot position and

movement was studied over time.

To investigate the role of catheter geometry including tip diameter, tip shape, size, location and number of holes as well as clot rheological characteristics, a number of simulations were performed. The results indicated that in many cases part of the clot become trapped between the catheter wall and the vessel. Presence of lateral holes may enhance clot aspiration, in particular by aspirating part of clot which could not be aspirated by the main front hole. However, the presence of lateral hole played a negative role in many cases, due to the fact that holes may enhance aspiration of blood rather than clot. Indeed the hole design (location and size) is an important factor in clot aspiration performance. Only the holes located close to the trapped clot will enhance clot aspiration, while the holes located far from it will weaken clot aspiration performance. Furthermore hole size should be large enough to enhance the aspiration of trapped clot, however preventing aspiration of blood only.

Moreover, the catheter with side holes has better performance for fresh clot (exhibiting lower viscosity) while older clots (with higher viscosity) should be preferentially treated with

catheters without side holes. With regard to the tip design, the simulations indicated that the catheter with a right-angle tip performs better on clot aspiration, although most of the commercial catheters feature a beveled tip, due to the ease of insertion and minimal penetration force.

The study on the role of pressure difference showed that the it actually affects catheter performances depending on the viscosity of the clot.

The catheter internal diameter has a great impact on both clot aspiration speed and success of complete aspiration. The final part of the work was related to the experimental characterization of different models of a commercial manual aspiration catheter provided by Invatec S.r.l., Roncadelle, BS, Italy. The performance of the catheter Diver C.E. MAX is much better than the other two aspiration catheters: Diver C.E. KIT and Diver C.E.. However this difference is more evident when the viscosity of fluid increases.

BLOOD SHIFTS FROM THORAX TO EXTREMITIES: METHODS AND APPLICATIONS

Barbara Uva - Supervisor: **Andrea Aliverti**

The splanchnic vasculature represents a significant reservoir of blood in the human body. It is known that increases in abdominal pressure (Pab) can displace blood from the trunk to the extremities but the effects of tidal breathing and expulsive maneuvers have not been studied and the abdomen has not been modeled as a circulatory pump. Recently it was possible, for the first time, to measure non invasively the volume of blood shifted from the splanchnic vasculature using an innovative technique namely the Double Body Plethysmography (DBP) developed at TBMLab of Politecnico di Milano. This is based on two different techniques: whole body plethysmography (WBP), which measures changes in body volume (ΔV_b), quantifies the volume of air flowing in and out of the lungs, and the volume of gas compression or decompression and Optoelectronic Plethysmography (OEP) measures the same variables as WBP plus any blood shifts between the trunk and the extremities. Using DBP, it was demonstrated that during quiet diaphragmatic breathing 50–75ml of blood were shifted from the splanchnic vascular bed by the increase in abdominal pressure (Pab) with diaphragmatic descent, while the fall in pleural pressure (Ppl)

inflated the lungs. In this case the diaphragm has two functions, decreasing Ppl ventilates the lungs but simultaneously increases Pab shifts blood from splanchnic bed to the extremities. These studies constituted the basis of the present Thesis, where we aimed to verify if the ‘abdominal circulatory pump’ is or can be effectively used in different conditions.

Specific Aim1- To develop new methods to allow DBP to study blood shift during prolonged measurement (e.g. exercise)

The significant thermal drift due to variations in humidity and temperature within the plethysmograph is a common problem in all types of plethysmographs and can't be ignored for prolonged measurements. The purpose of this study was to investigate different possible solutions for conditioning a plethysmograph in order to allow prolonged measurements. Three different air conditioning systems were developed (peltier cells and fans; continuous bias flow produced by a negative pressure source; bias flow and fans) and studied their effects on ΔT and ΔRH in six subjects performing exercise (EX) within a flow-type WBP. The results suggest that combining a cooling device

with air recirculation allows to keep T constant inside the WBP during prolonged measurements, even during EX. None of the 3 considered conditioning systems allows to control RH, however, the repeatability of ΔRH suggests that thermal drift can be corrected by using dedicated software.

Specific Aim2- To study blood shift during exercise by DBP

We have recently demonstrated (Aliverti et al, J Appl Physiol, 2010) that during quiet breathing the diaphragm serves the double function to ventilate the lung and to shift blood from the splanchnic vascular bed to the extremities. We hypothesized that with simultaneous contraction of abdominal muscles, such as occurs during exercise, the circulatory function of the diaphragm can be considerably enhanced. We studied three different types of exercise and we determined how abdominal pressure drives blood shifts from the thorax to the extremities within a WBP measuring V_b s variations using DBP. In all subjects, intra-breath variations of V_b s were bimodal. A precise control mechanism of the diaphragm and abdominal muscles determines intra-breath variations of V_b s during exercise. After 1' of exercise, within breath tidal V_b s significantly increased from rest while abdominal

volume at end-expiration decreased. We believe that these findings have important implications in understanding how blood flow is redistributed and if the respiratory muscles can provide a circulatory function during exercise.

Specific Aim3- to study Blood shift during expulsive maneuvers by DBP

Expulsive maneuvers (EMs) caused by simultaneous contraction of diaphragm and abdominal muscles shift substantial quantities of blood from the splanchnic circulation to the extremities. This suggests that the diaphragm assisted by abdominal muscles might accomplish ventilation and circulation simultaneously by repeated EMs. We tested this hypothesis in normal subjects by using DBP. EMs with Pab $\sim 100\text{cmH}_2\text{O}$ for 1 sec, followed by 2 sec relaxations with a frequency of 20/min produced a global splanchnic output of 6 l/min, that is comparable to resting cardiac output in a mean subject. Refilling during relaxation was complete and the splanchnic bed did not progressively empty. The combined circulatory function of the diaphragm assisted by simultaneous contractions of abdominal muscles have potential for cardio-pulmonary resuscitation. We believe that the abdominal circulatory pump can act as an auxiliary heart.

Specific Aim4- To determine venous blood flow during breathing and EM

It is known that increases in Pab may stop inferior vena caval return below the entry of the hepatic vein (Qivc) entirely. We believe this is due to increases in hepatic venous outflow increasing

the pressure at its entry to the inferior vena cava and abolishing the energy gradient producing flow distal to it. This causes blood to pool in the lower extremities increasing venous pressure there (Ple). We hypothesized that as Ple increases Qivc would restart at higher values of Pab than those at which it stopped. We tested this hypothesis by measuring Pab at which femoral venous flow (Qfv) stopped and restarted during quiet breathing and ramp increases and decreases in Pab, keeping Ppl quiet constant. These data suggest that during QB Pab variations produced by diaphragm contraction have a profound modulatory on venous return from the lower limb and this modulation is dependent on Pab dynamics.

Specific Aim5- to determine if electrical or magnetical stimulation can be considered as a tool for CPR by EM

Recent studies of conscious expulsive maneuvers in healthy volunteers have shown that subjects can pump up to 6 l/min above resting cardiac output repeating EMs with particular timing and pressures. We have also hypothesized that EMs can be performed in human subjects by stimulating the phrenic nerves and the nerves innervating the abdominal muscles by activating a magnetic coil over the 7th cervical vertebra and the 10th thoracic vertebra respectively. The results of this study show that abdominal pressures of $50\text{cmH}_2\text{O}$ can be produced via magnetic stimulation of phrenic nerve combined with external abdominal binding and pressurization, while magnetic stimulation of the abdominal muscles results in abdominal pressures of $94\text{cmH}_2\text{O}$.

Synchronous stimulation of both sets of muscles has not yet been tried, but, when it is, increasing Pab by $100\text{cmH}_2\text{O}$ should not prove difficult, at least in healthy subject.

Combining our prior work with the current study suggests that external, non-invasive magnetic stimulation is a feasible method for enhancing the circulation of blood.

Specific Aim6- To develop a new stimulating system in order to reproduce EMs in animal model

It is known that standard sternal compression CPR results in a cardiac output of only 20% of control. Ventilation by positive pressure at the mouth aggravates this situation increasing right atrial pressure and decreasing venous return. We recently have demonstrated that EMs performed at particular pressures, rate and duty cycle might result in circulatory outputs considerably greater than those obtained by standard sternal compression resuscitation techniques. The objective is to develop and to test an innovative stimulating system which implements EMs protocol as new method of CPR. We implemented an electrical stimulator based on the transvenous stimulation of the phrenic nerve, to induce diaphragm contraction, and on the electrical stimulation for abdominal muscle contraction. The device was proven to be an effective artificial abdominal circulatory pump stimulator and therefore we can state that the scenario is now open for future studies to be performed on animal models during cardiac arrest.

MULTIMODAL SENSORS MANAGEMENT IN COMPUTER AND ROBOT ASSISTED SURGERY

Alberto Vaccarella - Supervisor: Elena De Momi

Surgical interventions are among the most challenging activities performed by humans. To facilitate such a complex task, research efforts in biomedical engineering led to the introduction of Computer Assisted Surgery (CAS). CAS is a set of technologies and methods to assist the surgeon from the preoperative planning phase to the intraoperative phase, providing image guidance and, in some cases, robotic assistance to perform the procedure. CAS systems typically use different types of sensors (localization system, force sensors, intra-operative imaging devices). In this work we focus on optical and electromagnetic localization systems. Optical Tracking Systems (OTS), typically using wired infrared light-emitting diodes (IRED) or wireless retro-reflective spheres, are the de facto standard for present navigation system due to their accuracy and cost-effectiveness, though they require line-of-sight between the camera and the tracked object, which is not always reasonable in a crowded OR. Electromagnetic Tracking Systems (EMTS) localize small electromagnetic field sensors (coils) in an electromagnetic field of known geometry. EMTS have the major advantage of tracking object inside patient body (line-of-sight is not required) but their accuracy is lower and

depends on electromagnetic field distortion (e.g. the presence of ferromagnetic objects or electrically powered devices affect localization accuracy). Such limitations of the aforementioned sensing technologies can be overcome by their combined use. To this extent information sources must be first correctly synchronized and then fused together, typically with a probabilistic approach.

Timing aspects are crucial in a multiple sensor scenario since each data needs to be correctly synchronized to the other. Spatial consistency is also required when dealing with different sensors information; spatial transformations between reference systems are obtained with calibration procedures. In the framework of the ROBOCAST we proposed a multi-sensor management system (*Sensor Manager*) for a distributed CAS architecture, which addresses information consistency in both space and time. The software architecture is based on an object-oriented middleware, the Common Object Request Broker Architecture (CORBA) and on the Image-Guided Surgery Toolkit (IGSTK). The Sensor Manager (SM) encompasses two main parts; 1) interfacing the hardware

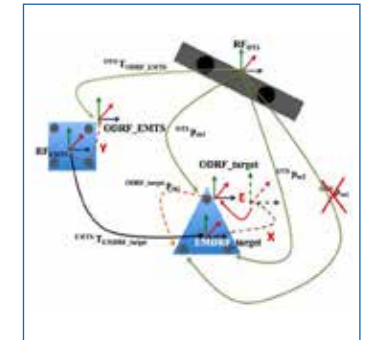
based on the open source libraries IGSTK and OpenCV and 2) acting as server to provide data and services to other modules in a CORBA-based network architecture. Based on IGSTK, the SM also provides a spatial object hierarchy, which facilitates spatial transformation computation between couples of reference frames. This allows clients to ask for the transformation matrix of a reference system associated with an object in the ROBOCAST scenario, with respect to other reference systems. The SM was validated in a modular and distributed CAS system. Experiments proved that the presence of different clocks on an Ethernet network, which does not carry synchronization information, is not acceptable as it leads to time drift due to clock offset and an unpredictable combination of quartz inaccuracies that depend on the operating temperature and manufacturing process; the frequency tolerance for the single quartz is typically in the range of $\pm(10 - 100)$ ppm. We proposed the use of a unique timestamping module for all sensors to avoid inaccuracy due to the offset between different clocks. Response latency was also evaluated resulting in about 2 ms for each tested tracking system. With this latency, the

client can issue up to 500 requests per second -- much faster than data generation rate for the majority of commercial tracking systems (NDI Optotrak Certus is the only tracking system that can reach frame rate higher than 500 Hz if less than seven markers are connected). It is relevant that this result does not depend on the tracking system.

Based on the SM architecture, a robust tracking algorithm of surgical instruments during navigated surgery was designed and implemented using a sensor fusion approach that allows for overcoming the limitations of OTS and EMTS. The proposed algorithm uses the Unscented Kalman Filter (UKF), an extension of the standard Kalman filter, which makes use of a deterministic sampling to estimate the probability distribution of random variables undergoing a non-linear transformation, rather than approximating the non-linear functions.

As shown in Fig. 1, the OTS (\mathbf{RF}_{OTS}), localizes markers (${}^{OTS}\mathbf{p}_{mi}$) on the target object, which define an optical dynamic reference frame ($\mathbf{ODRF_target}$). Optical markers configuration with respect to $\mathbf{ODRF_target}$ is fixed and known (${}^{ODRF_target}\mathbf{p}_{mi}$). OTS also localizes $\mathbf{ODRF_EMTS}$ on the electromagnetic field

generator, which is related to its own reference frame, \mathbf{RF}_{EMTS} , through the calibration matrix \mathbf{Y} . The EMTS localizes a sensing coil on the tracked object, $\mathbf{EMDRF_target}$, which is related to the $\mathbf{ODRF_target}$ through the calibration matrix \mathbf{X} and the error matrix \mathbf{E} . When optical markers are available the predicted pose of the target is updated and corrected comparing predicted markers position with the measured one. In case one or more markers are occluded (as an example in Fig.1 marker 1 is occluded), their position is indirectly derived from the EMTS measurement. The proposed sensor fusion (SF) in case of line-of-sight occlusion was compared with a pure predictive filter relying only on available optical measurements. Robustness to electromagnetic field distortion was also experimentally evaluated. Results showed a significant improvement in translation and rotation error when exploiting EMTS data with the SF method in particular for longer occlusion where the error remains approximately constant (RMSE < 1 mm in case of only two visible markers). The SF algorithm showed good results also in presence of metal distorter (RMSE ranging from 0.5 mm to 3.5, depending on occlusion length), achieving a tracking accuracy up to ten



1. Coordinate systems involved in the fusion algorithm

times higher than the estimated static accuracy of the EMTS in the disturbed environment (RMSE 18.64 mm); results are comparable with the vendor's stated accuracy in a metal free environment (RMSE 1.6 mm). The presented work is relevant not only for enhancing current state of the art navigation systems, but also in the vision of the OR of the future. Next generation ORs are envisaged to involve context-aware systems and smart environments equipped with a number of sensors (e.g. cameras, microphones, tracking systems etc.) to extract information on the operating theatre and human intentions and to react promptly providing proactive services in terms of user-interface and workflow adaptation.

EFFECTIVE BRAIN CONNECTIVITY COMPUTATIONAL METHODS FOR THE LOCALIZATION OF THE EPILEPTOGENIC ZONE

Giulia Varotto - Supervisor: Sergio Cerutti

Focal epilepsies, in which the seizures originate from a region limited to a part of one cerebral hemisphere, are common and account for more than 50% of all epilepsies (Hauser, 2007). Despite the great improvement in pharmacological research, approximately 30% of patients with focal epilepsies experience seizures that are resistant to anti-epileptic drugs (AEDs) (Beleza, 2009). In this case surgical resection of the EZ zone, i.e. the region responsible for the onset, early organisation and propagation of seizures, can be the only way to suppress or reduce seizures (Munari et al., 1994). The EZ represents the minimum amount of cortex that must be resected (inactivated or completely disconnected) in order to achieve seizure freedom (Rosenow and Luders, 2001). The EZ can sometimes be adequately localised by means of non-invasive investigations including a clinical neurological examination, a detailed description of ictal signs and symptoms, imaging findings by means of Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET), and interictal and ictal EEG scalp recordings associated with a video-recording of ictal events. However, when the region of seizure onset cannot be precisely identified non-invasively, or when the obtained

information is insufficient to exclude the involvement of "eloquent cortical areas" in seizure generation, intracranial EEG recordings using depth electrodes or cortical strips and grids can be the gold-standard procedure. After invasive recordings, the EZ is currently identified by visually inspecting the video-SEEG recordings, a time-consuming procedure that requires the involvement of specifically trained neurophysiologists and is inevitably affected by the drawback of subjectivity. Moreover, patients admitted to invasive evaluation, have worse surgical outcomes compared to those admitted to surgery without invasive evaluation. The reason is that in most of the cases the organization of the EZ is quite intricate, corresponding to a complex epileptogenic network of neuronal populations distributed in distinct brain areas (Spencer, 2002; Bartolomei et al., 2008b). The identification of the intricate network connecting different brain areas, and the study of its role in many neurophysiological and cognitive mechanisms, are crucial issues in the field of neuroscience. In fact, there is growing evidence that synchronization plays an important role in brain functioning and dysfunctioning and that certain brain disorders

and cognitive dysfunctions are associated with abnormal neural synchronization (Ulhaas and Singer, 2006). For this reason in the last years, growing efforts have been paid to develop signal processing methodologies to measure synchronization and nowadays a large spectrum of different methods for the investigation of brain networks is available and extensively applied to different invasive (intracranial EEG) and non-invasive recordings (fMRI, EEG, MEG). However, there is no agreement about advantages, disadvantages and performances of the various techniques; most of the approaches have limits and benefits that strongly depend on the type of signal being analysed and on the clinical context under study. Moreover, considering the whole process from signal pre-processing to network estimation, several steps, as the threshold procedure, the choice of the epoch length to be analysed, the number of nodes in the network, still represent open issues that require more theoretical investigation to be further improved. For this reason the first step of the present work was to apply and validate linear and nonlinear methodologies for the estimation of brain connectivity from intracranial or

scalp EEG signals, to evaluate their difference and implement methodological improvements. Among the different pathologies that are recognized as being characterized by an abnormal pattern of connectivity, epilepsy, along with its cardinal symptom, the epileptic seizures, represents a prominent example of altered neural synchronization mechanisms. It's indeed well known that epileptic phenomena are associated with abnormal changes in brain synchrony mechanisms and several studies have shown that seizures in humans are associated with abnormal synchronization of distant structures. In the field of epilepsy, studies of brain connectivity have been performed to address different issues, such as seizure prediction, the identification of specific epileptiform activity, or the identification of the area responsible for seizures generation and propagation, the so called Epileptogenic Zone (EZ). A first general aim of this project is to develop a comprehensive framework for measuring functional and effective connectivity, based on linear and non-linear multivariate time series methods, and for the investigation of the topological properties of complex brain networks by means of graph theoretical approaches. The main applicative aim of this study is to develop and validate a procedure and software tools (developed in Matlab environment) including advanced multivariate linear and nonlinear techniques of signal processing and measures derived from graph-theory to study the intracranial EEG

connectivity and the network characteristics sustaining seizures in the different form of epilepsies. The main purpose is to find quantitative indexes representative of abnormal connectivity characterizing the dynamics between brain areas involved in the seizure generation with the aim of supporting epileptologists and surgeons in the identification of the EZ. The final aim is to have quantitative indexes able to allow an automatic classification, based on clustering and machine intelligence algorithms, of the intracranial electrodes contacts as belonging to the EZ or not, and compare the output of the classifier with that resulting from the clinical evaluation. The result of this work is expected to provide epileptologists and neurosurgeons with new tools and information helpful to improve sensitivity and reduce the time required to identify with more precision the EZ. Moreover, this quantifiable and computer-assisted approach could improve the diagnostic capacity and advance the understanding of epileptogenic mechanisms underlying the mechanisms of seizure generation and spreading. Finally, the developed procedure to analyse scalp EEG connectivity have been applied to study others pathological and physiological brain mechanisms, namely photosensitive epilepsy, disorders of consciousness and mechanisms of perception of musical emotion stimuli, in order to evaluate the appropriateness of the proposed approach and verify other possible applications and usefulness in other clinical and cognitive contexts.

The thesis consists of three main parts. The first part briefly summarizes the context of brain connectivity as basic mechanism of most of physiological and pathological brain activity, with particular attention to the field of epilepsy. In the second chapter of the first part the methods used in the present work for the estimation of functional and effective brain connectivity and the analysis of complex networks, according to graph theory, will be described in details. In the section part, results of the application to intracranial EEG signals recorded in patients with focal epilepsy candidate to epilepsy surgery will be reported. New methodological improvements will also be introduced. In the last part the same approach will be adapted and applied to other clinical or physiological conditions, namely to study mechanisms underlying disorders of consciousness and photosensitive epilepsy.