MECHANICAL ENGINEERING | PHYSICS | PRESERVATION OF THE ARCHITECTURAL HERITAGE | STRUCTURAL, SEISMIC AND GEOTECHNICAL ENGINEERING | URBAN PLANNING, DESIGN AND POLICY | AEROSPACE ENGINEERING | ARCHITECTURE, BUILT ENVIRONMENT AND CONSTRUCTION ENGINEERING | ARCHITECTURAL, URBAN AND INTERIOR DESIGN | BIOENGINEERING | DATA ANALYTICS AND DECISION SCIENCES | DESIGN | ELECTRICAL ENGINEERING | ENERGY AND NUCLEAR SCIENCE AND TECHNOLOGY | ENVIRONMENTAL AND INFRASTRUCTURE ENGINEERING | INDUSTRIAL CHEMISTRY AND CHEMICAL ENGINEERING | INFORMATION TECHNOLOGY | MANAGEMENT ENGINEERING | MATERIALS ENGINEERING | MATHEMATICAL MODELS AND METHODS IN ENGINEERING

PhD Yearbook | 2024

Chair:
Prof. Gabriele Dubini

# DOCTORAL PROGRAM IN BIOENGINEERING

The main objective of the PhD Programme in Bioengineering is to prepare the PhD candidates to develop high level engineering problem-solving abilities in biomedical, healthcare and life sciences, within research groups or in private/public industrial contexts, through a strong interdisciplinary training bridging engineering and medical/biological knowledge.

During the PhD, the candidates develop a scientific research project dealing with a complex problem, which can be at different scales - from the molecular and the cellular levels to living organisms up to biomedical systems. They investigate original methods, devices, and systems with different purposes: increasing knowledge, proposing innovative methods for diagnosis and therapy as well as improving healthcare and daily life structures and services. At the end of the PhD programme, the candidate are expected to be able to carry out innovative projects and research and development in the field of Bioengineering, by proposing new methodological and technological solutions and properly evaluating the technology impact on healthcare, life sciences and biomedical industry.

During the three years of the PhD Programme, the candidates perform their research through theoretical and experimental activities in four major areas: biomimetic engineering and micro-nano technologies; rehabilitation engineering and technology; technologies for therapy; physiological modelling and non-invasive diagnostics.
More specific areas include, but are not limited to: molecular and cellular engineering, biomaterials, tissue engineering, bio-artificial interfaces and devices, neuro-prostheses, movement analysis, cardiovascular and respiratory system bioengineering, central nervous system signal and image processing for rehabilitation, biomechanics, computational fluid-dynamics, computer assisted surgery and radiotherapy, robotics, artificial organs, implantable devices, microfluidics and lab-on-a-chip systems, biomedical signal and image processing, e-health, bioinformatics, functional genomics and molecular medicine, artificial intelligence in medicine.

The PhD Programme in Bioengineering is organized with an inter-departmental structure. Faculty members of the PhD Board belong to two Departments of the Politecnico di Milano, namely DEIB (Department of Electronics, Information and Bioengineering) and CMIC (Department of Chemistry, Materials and Chemical Engineering "G. Natta").

PhD candidates (on average 20 per year) may carry out their research programs in experimental laboratories located at the Politecnico di Milano or outside, typically in biomedical research centers, hospitals and industries.

When the research is performed within the Politecnico, the PhD candidates are usually assigned to one of the following laboratories belonging to the DEIB and CMIC Departments: the Laboratory of Biological Structure Mechanics (LaBS, CMIC), the Laboratory of movement analysis "Luigi Divieti" (DEIB), the Medical Informatics Laboratory (DEIB), the Neuroengineering and Medical Robotics Laboratory (NearLab, DEIB), the Biosignals, Bioimaging and Bioinformatics Lab (B3 lab, DEIB), the Biomaterials Laboratory (CMIC), the Biomedical Technology Lab (TBMLab, DEIB), the Experimental Micro and Biofluid Dynamics (μBS Lab, DEIB), the Computational Biomechanics Lab (DEIB), the Biocompatibility and Cell Culture Lab (BioCell, CMIC), the Bioreactors Laboratory (CMIC). The Istituto di Elettronica, Ingegneria dell'Informazione e delle Telecomunicazioni (IEIIT) of the Consiglio Nazionale delle Ricerche (CNR, the Italian National Research Council), located at DEIB, represents another possible option.

Stage periods in distinguished research institutes in Italy and abroad are an essential feature of the PhD candidate training. The candidates are encouraged to carry out part of their research activities in contact with other research groups, preferably abroad, for at least three months, in laboratories where the candidates can acquire further skills to develop their research work and thesis.

Collaborations that can involve PhD students are presently active with several national and international research and academic Institutions.

The involvement of companies and clinical partners very often facilitates the technological transfer of applied research into industry and clinical applications.

The educational syllabus includes *ad hoc* advanced courses specifically designed for the PhD students in Bioengineering. The syllabus also includes the School of the National Bioengineering Group, which is held yearly for one week in Bressanone-Brixen (BZ). Every year, the School is focused on a different subject. The themes in the last few years were: Neuro-informatics (2011), Biomedical devices from research to market (2012), Regenerative medicine (2013), From functional recovery to artificial organs (2014), Experimental models for development methods for 3R (2015), Bioengineering for active ageing (2016), E-health and digital medicine (2017), Biomedical images (2018), Technologies and tools in surgery and therapy (2019), AI-enabled health care (2020), Biofabrication: An integrated bioengineering approach for the automated fabrication of biological structures for clinical and research applications (2021), Biomedical engineering for sustainable development (2022).

The PhD Board of professors is made up of highly qualified and active researchers in Bioengineering, belonging to DEIB and CMIC departments. The PhD Board is responsible of all the candidates' activities. The expertise of faculty members covers a wide spectrum of research fields. This allows a continuous updating of the PhD Programme and ensures that the PhD candidates are involved in innovative work.

The PhD Programme in Bioengineering also relies on an Advisory Board, made up of distinguished experts coming from R&D industries, research and clinical centers. The Advisory Board ensures that the goals of the PhD Programme are also aligned with the needs of non-academic world.

## COMPOSITION OF THE PHD BOARD

| | |
|---|---|
| Aliverti Andrea | DEIB |
| Andreoni Giuseppe | DESIGN |
| Bianchi Anna Maria | DEIB |
| Candiani Gabriele | CMIC |
| Cerveri Pietro | DEIB |
| Cimolin Veronica | DEIB |
| Corino Valentina | DEIB |
| Dellacà Raffaele | DEIB |
| De Momi Elena | DEIB |
| Draghi Lorenza | CMIC |
| Dubini Gabriele (Chair) | CMIC |
| Farè Silvia | CMIC |
| Ferrario Manuela | DEIB |
| Fiore Gianfranco Beniamino | DEIB |
| Gastaldi Dario | CMIC |
| Guazzoni Chiara | DEIB |
| La Barbera Luigi | CMIC |
| Mantero Sara | CMIC |
| Pattini Linda | DEIB |
| Pedrocchi Alessandra Laura Giulia | DEIB |
| Petrini Paola | CMIC |
| Pozzi Giuseppe | DEIB |
| Rasponi Marco | DEIB |
| Ravazzani Paolo | CNR |
| Rodríguez Matas José Félix | CMIC |
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| Soncini Monica | DEIB |
| Vergara Christian | CMIC |
| Villa Tomaso | CMIC |
| Votta Emiliano | DEIB |

# IN SILICO HIGH-FIDELITY AND SURROGATE MODELS OF THE THROMBECTOMY PROCEDURE

**Sara Bridio** - Supervisor: Prof. Francesco Migliavacca

Acute ischemic stroke (AIS) is a pathology caused by an occlusion in a cerebral artery, which prevents the blood perfusion of brain tissues, and if not treated in a short time can cause severe neurological damage to the patient. Stroke is currently the second cause of death worldwide, and large research efforts are made for the improvement of its treatments. After recent successful clinical trials, mechanical thrombectomy (MT) has become the standard of care for AIS due to a large vessel occlusion. MT is a minimally-invasive treatment aiming at removing the occluding thrombus (or blood clot) by means of a stent-retriever and/or aspiration catheters. Despite currently being the best treatment option, the patients' outcomes can be improved by optimizing the devices or the procedures, to reduce the risk of losing the clot inside the patient's vasculature or of fragmenting the clot, causing embolizations. The PhD thesis aimed at developing in silico models of the MT procedure with stent-retriever, both with a high-fidelity and a surrogate modeling approach, with the objective of providing tools for studying the clinical procedure, for supporting the pre-operative planning and for running in silico stroke trials. The high-fidelity modeling consists in creating patient-specific finite-elements (FE) models of the cerebral vasculature, the thrombus and the devices used for the MT, and accurately simulating the clinical procedure (Fig. 1A). This kind of models have great potential for understanding the biomechanics of the procedure, and in particular of its causes of failure. After a validation with in vitro experiments (Fig. 1B) and with a first patient-specific case, the high-fidelity MT model was used to study the impact of vascular anatomy on the procedure outcome, demonstrating for the first time the association of geometric parameters of the carotid anatomy with an unsuccessful thrombus removal. The thesis also proposed the first proof-of-concept in silico stroke trials. In a first approach, the high-fidelity FE model was used to simulate the MT procedure on a cohort of 100 virtual patients, to compare the recanalization outcomes in subpopulations with different types of occlusion and to assess the performance of commercially-available stent-retrievers (Fig. 1C). This study highlighted the increased difficulty of removing stiff fibrin-rich thrombi with respect to soft red blood cell-rich thrombi. Additionally, a double-cage device design proved more successful in the integration and removal of the thrombus, compared to a single-cell device. A second approach relied on the development of a thrombectomy surrogate model, built with machine-learning techniques, to be included in a framework for running in silico trials on large virtual populations. To this aim, the hundreds of high-fidelity simulations were used to train a binary classification model which, based on patient-specific vascular anatomy parameters and the clot characteristics, provided predictions on the probability of successful recanalization. The in silico trial was successfully validated, compared with a real clinical trial. The surrogate modeling and machine-learning techniques were further investigated for the creation of fast predictive models of thrombectomy outcomes, trained on realizations of the high-fidelity FE model, which could be used to support the clinical decision-making for the treatment of stroke patients. In particular, a surrogate model was created using a dimensionality reduction technique in association with a Kriging model, to obtain real-time predictions of the evolution of the strain in the thrombus during the whole MT procedure, which can be used to evaluate the risk of thrombus fragmentation due to the interaction with the stent-retriever. In another application, a combination of a level set technique and a kernel-optimization algorithm was used to create a classification model able to predict a successful or unsuccessful thrombus removal. In conclusion, this doctoral thesis explored different techniques for modeling the MT procedure, which can contribute in different ways to improve the stroke treatments, from the use of high-fidelity models for the optimization of devices and procedures, to the implementation of in silico trials which can reduce the time and cost of the clinical trials, facilitating the research and adoption of new and better treatments for stroke.

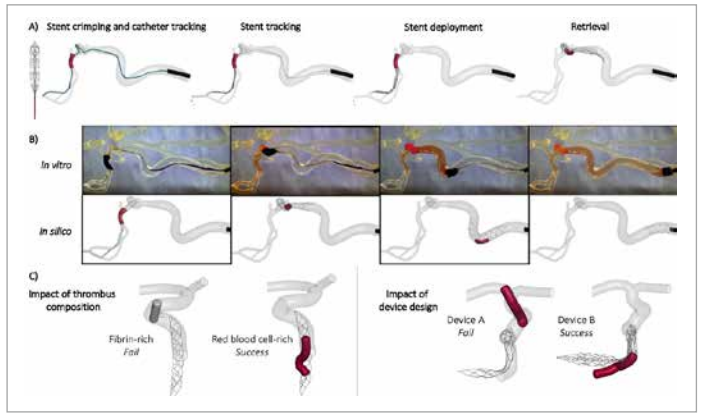


**Fig. 1 - A) Steps of the in silico simulation of the thrombectomy procedure. B) In vitro-in silico comparison. C) Impact of thrombus composition and device design on thrombectomy outcome.**

# METHODS FOR UNCERTAINTY ASSESSMENT AND INTERPRETABILITY OF BRAIN CONNECTIVITY BIOMARKERS IN LOCALIZED AND WIDESPREAD DEGENERATION

**Davide Coluzzi** - Supervisors: Prof. Giuseppe Baselli, Prof. Anna Maria Bianchi

A number of different pathologies, ranging from mental disorder to neurodegeneration, share the characteristics of having both widespread effect throughout the whole brain and in specific sub-networks or regions. The analysis of the connectivity on different levels is a powerful tool to investigate impaired regions and global deficits, study cause and effects of the pathologies, support diagnoses and tailor the rehabilitative treatments. Nevertheless, conducting a multi-level assessment of brain connectivity outside the research settings is not a simple process due to three primary concerns: i) many tools are available, but a user-friendly, interactive and flexible environment allowing automatic qualitative and quantitative assessment at all levels is missing; ii) both structural and functional connectivity measures for edge-weighting lack gold-standard methodologies, with a number of uncertainty sources, resulting in noisy data; iii) the possible biomarkers which can be highlighted from huge amount of data, reducing uncertainty and using artificial intelligence (AI) methodologies, are not always adherent to domain knowledge and difficult to be interpreted. In this PhD work, methods for improving usability of the brain connectivity biomarkers were proposed.

More specifically, the aforementioned general aspects were addressed in three studies. First, an interactive and user-friendly tool called SPIDER-NET to allow qualitative and quantitative analysis of brain networks and sub-networks was developed. The tool was validated on the structural connectivity of 2 hemorrhagic stroke case studies and 17 healthy controls (HC). Second, a multi-level bootstrapping approach was applied to enable robust abnormalities detection. This approach was experimented on the functional connectivity of 12 schizophrenic patients and 15 HC. Finally, convolutional neural networks employing structural connectivity data and 3D T1-weighted volumes were developed and analyzed by Explainable Artificial Intelligence (XAI). The last study addressed the classification of Alzheimer's disease subjects (135 Magnetic Resonance sessions) and HCs (557 sessions).

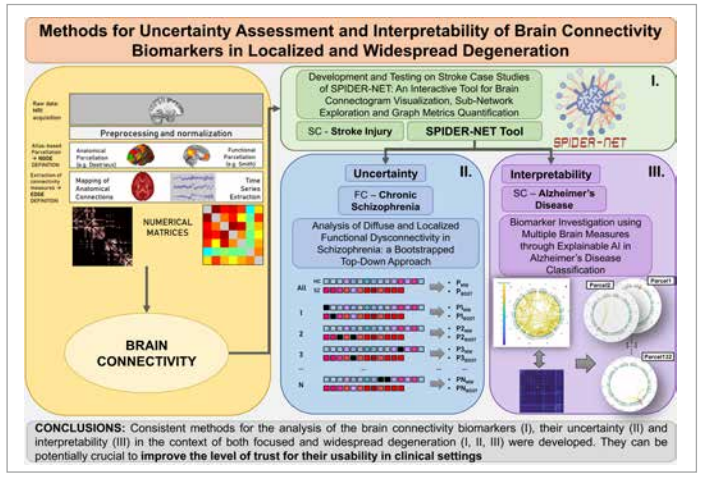


**Fig. 1 - Graphical abstract of the PhD Thesis summarizing steps and studies which were carried out. The procedures to obtain brain structural (SC) and functional connectivity (FC) networks from MRI acquisition are reported in the yellow box. The first study (green box) consisted of developing a new tool called SPIDER-NET (freely accessible online at http://caditer.dongnocchi.it/spidernet) to visualize and analyze connectivity data, which was validated on the SC of two stroke case studies. A second work extending the functionalities of this tool with a new pipeline to analyze connectivity at different scales, on FC data (of healthy and schizophrenic subjects) and assessing the robustness of the detected brain connectivity biomarkers is summarized in the blue box. By using SPIDER-NET, the interpretability of Artificial Intelligence methods finalized to the classification of the Alzheimer's Disease was assessed on a wide population. Main results and conclusions of the PhD project are reported in the grey box.**

First, SPIDER-NET resulted an effective tool to represent the expected (dis)connectivity pattern due to a stroke lesion, in testing a-priori hypothesis by extracting a sub-network of interest and in investigating graph-based topological indexes. Furthermore, it allowed to better interpret complex networks and compare the results from two processing pipelines (Diffusion Tensor Imaging - DTI vs Constrained Spherical Deconvolution - CSD), having different uncertainty causes. Second, the bootstrapped top-down approach revealed different abnormalities of the schizophrenic group on different levels, which resulted to be more stable and robust compared to direct testing and having a trend towards results with greater number of data and subjects. Third, as evaluated through a statistical test ($p < 0.05$) and ranking of the most relevant parcels (first 15%), XAI analysis of interpretability revealed the involvement of target brain areas for both models employing 3D T1-weighted volumes and structural connectivity. These anatomical targets were the medial temporal lobe and the default mode network, respectively. Although the obtained findings had limitations, results suggested that combining different imaging modalities may lead to increased model performance, interpretability, and, thus, reliability.

Although the great potential of measures extracted from brain connectivity, the open issues of uncertainty and interpretability limited their trust and, thus, their usability within clinical settings. Consistent methods to address these issues have a direct connection to the understanding of the relationships between localized affection and widespread degeneration. Improved reliability and interpretation are fundamental in the study of the brain both in health, to map the nervous system and comprehend the mechanisms underlying the brain processes, and in disease, to support clinicians in the early detection and during rehabilitation.

# NOVEL ICT SOLUTIONS TO SUPPORT THE EARLY SCREENING OF SPECIFIC LEARNING DISABILITIES AND THE CHARACTERIZATION OF HANDWRITING EVOLUTION

**Linda Greta Dui** – Supervisor: Prof. Simona Ferrante

**Introduction**

Specific learning disabilities (SLDs) prevent students from acquiring basic academic skills, and cause frustration and low self-esteem. In Italy, Law 170/2010 charged schools to start the screening process by observing and treating learning difficulties early, as in the IndiPote(dn)S project. However, paper-based methods and subjective assessments may mine the effectiveness of the intervention. Technology can streamline this process by enabling systematic data collection and analysis. Considering dysgraphia as a use case, technology can characterize handwriting and anticipate difficulties early detection through drawings characterization. This work aims to provide e-health solutions for early screening of SLDs, specifically dysgraphia. Specific objectives include (1) developing a web-app for screening, (2) creating a serious game to assess graphical alterations, and (3) digitizing a dysgraphia test to inform clinicians.

**Aim 1: supporting the screening of specific learning disabilities at school**

A co-design process involving biomedical engineers, teachers, and the Varese school office was leveraged to develop a web-app to support the early screening of SLDs within the IndiPote(dn)S project, aiding in systematic data collection on student weaknesses in various learning domains. Field testing involved over 30,000 students in Lombardy. Data analysis revealed trends in weaknesses and training effectiveness, with linguistic improvements notable in kindergartners. The web-app's role in supporting observation garnered recognition from local health authorities, prompting an agreement with the health service to prioritize intervention for children resistant to training. Regional authorities have now officially adopted the project.

**Aim 2: supporting the early detection of handwriting weaknesses**

Employing co-design principles, a serious game was developed to assess handwriting characteristics via symbol drawing, targeting laws of motion known to be altered in dysgraphia: isochrony, homothety, and speed-accuracy trade-off (SAT). Isochrony, which predicts increased writing speed with larger size to maintain constant execution time, was tested by drawing symbols at varying sizes. Homothety, predicting a constant ratio of time spent on each symbol relative to total word time regardless of size, was examined by drawing symbol sequences at different sizes. SAT, which implies slowing for complex actions, was assessed through tasks requiring varying levels of accuracy. Additionally, a free drawing game was included to explore handwriting characteristics without mimicking handwriting directly. Fig. 1 shows the final screens.

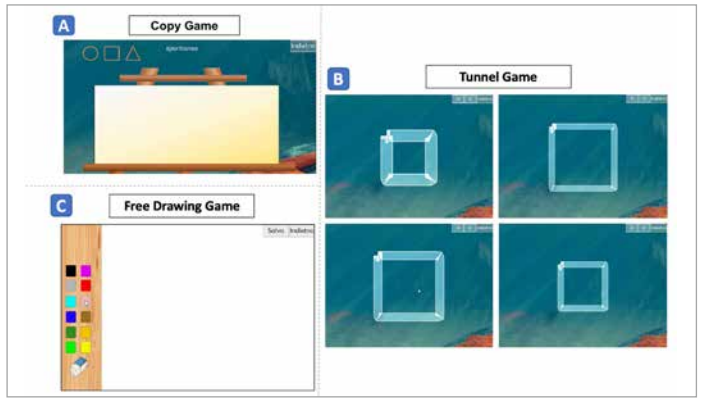


Fig. 1 - Interfaces of the serious games to test isochrony and homothety (A), SAT (B), and free drawing (C)

The game was validated involving typically developing third graders and kindergartners, confirming that the laws of motion were evident in symbols drawing, even before handwriting is learned. Following successful pilot testing, a longitudinal field study involving 250 kindergartners over three years was conducted. The games were administered at each time point, alongside observational grids from the IndiPote(dn)S protocol to identify potential weaknesses, and a handwriting fluency test in second grade to assess dysgraphia risk. Usability and satisfaction assessments received positive feedback (median system usability score = 82.5).

Features extracted from the games were analyzed to characterize weaknesses, and alterations were quantified in different domains. Various artificial intelligence techniques, including classical machine learning models and deep learning models, were employed to predict weaknesses or dysgraphia risk. Results demonstrated promising predictive capabilities, with the app providing a classification starting from symbol drawing, allowing for early screening. E.g., dysgraphia risk in second grade could be predicted by free drawing performed in kindergarten with an area under the precision-recall curve of 0.72. The results are a significant advancement in early screening for handwriting problems, potentially offering a universal screening tool independent of language or culture.

**Aim 3: supporting the characterization of handwriting for a better diagnosis**

The digital version of a dysgraphia diagnosis test was designed, as fostered by national guidelines. Following co-design principles, an iPad app replicating a handwriting fluency test was developed and tested with 52 primary school children, alongside with its paper version. Paper-tablet correlation was significant ($p<0.001$), but with consistently lower fluency on tablet. Statistical differences in handwriting features between school classes showed that the tool was effective in assessing proficiency.

**Discussion**

The research project introduces a technology-based screening ecosystem for schools, focusing on specific learning disabilities from a pre-clinical stage. The tools include:

1) A web platform for structured data collection to enhance screening of learning delays.
2) Serious games using drawings to anticipate screening for graphomotor weaknesses associated with dysgraphia.
3) A digital dysgraphia test to support diagnosis and understanding of handwriting difficulties.

In case of weakness, personalized training should be designed. Then, in case of training inefficacy, the complete learning path should be evaluated to reserve a technology-enhanced visit only for those who need it.

# BIOFABRICATION OF A 3D IN VITRO MODEL OF HUMAN SKELETAL MUSCLE FOR FIBROSIS MODELING AND DRUG DEVELOPMENT

**Riccardo Francescato** - Supervisor: Prof. Marco Rasponi

Tutor: Prof. Matteo Moretti, Prof. Simone Bersini

Skeletal muscle fibrosis is a pathological condition associated with various muscle diseases such as dystrophies, laminopathies, injuries, and aging. Its most prominent outcome is the excessive accumulation of extracellular matrix within muscles, ultimately leading to functional failure. Due to the concurrence with the main pathology and the involvement of numerous biochemical and biophysical factors, muscle fibrosis poses a significant challenge in musculoskeletal research. Moreover, the absence of specific therapeutic options emphasizes the urgent need for a better understanding of the pathological mechanisms. Recognizing the great advantages offered by three-dimensional in vitro modeling, the development of more reliable and comprehensive in vitro skeletal muscle models could provide new insights into this pathology. This thesis focuses on the development of an advanced three-dimensional in vitro model of human skeletal muscle.

In this work, biofabrication techniques were employed to realize an advanced and effective skeletal muscle model. A meso-scale functional myobundle based on human myotubes was generated on a custom-made platform (Fig. 1). Notably, stromal cells, including fibroblasts, endothelial cells, and macrophages, were integrated to form a stromal compartment, which is crucial for investigating muscle fibrosis. An electrical stimulation system was developed to achieve active contraction of the myobundle. Additionally, the myobundle anchoring system was mechanically characterized to non-invasively quantify the force generated by the construct, allowing for functional assessments. The engineered modular setup resulted in a robust model with the potential for scalability.

To validate the system as an effective tool for investigating muscle fibrosis mechanisms, two studies were conducted. First, a Duchenne fibrosis model was created by introducing fibroblasts derived from Duchenne dystrophic patients. This model was used to study the impact of fibrotic fibroblasts accumulation on muscle contraction. The results demonstrated a direct reduction in muscle force with an increase in collagen production by fibrotic fibroblasts. Furthermore, the model was extended to study Endothelial-Mesenchymal Transition (EndoMT) in the context of fibrosis and inflammation. Models of Duchenne fibrosis and Inflammation, the latter based on M1 macrophages, were developed. These models included microvascular endothelial cells to observe the relationship between EndoMT and the surrounding environment. Early signs of EndoMT were detected in both conditions.

This research aims to enhance our understanding of muscle fibrosis and inspire novel therapeutic approaches based on 3D in vitro modeling, a powerful method for recreating architectural, structural, and functional muscle properties in an engineered and informative setting for the understanding of specific mechanisms.

**Fig. 1 - Brightfield image of the differentiated myobundle at day 7.**

# INNOVATIVE MODELS FOR COGNITIVE LOAD ASSESSMENT AND MITIGATION THROUGH PERSONALISED ROBOT INTERVENTION

**Marta Lagomarsino** - Supervisors: Prof. Elena De Momi, Dr. Arash Ajoudani

The advent of Industry 4.0 has revolutionised ordinary workplaces, profoundly reshaping the role of the workers in the production chain and resulting in new occupational safety and health challenges. Research on ergonomics in industrial settings mainly focuses on reducing the operator's physical fatigue to improve throughput and avoid safety hazards. However, as the production complexity increases, even the cognitive resources demand and induced psycho-physical stress could compromise the operator's performance and the efficiency of the shop floor workplace. The escalating global prevalence of work-related Common Mental Disorders (CMDs) underscores the urgency of addressing this issue. However, state-of-the-art methods for estimating cognitive load often operate offline and involve unwieldy equipment, disrupting the natural flow of work activities and rendering them impractical for deployment in industrial settings. Furthermore, complex laboratory-based approaches tend to lack personalisation and overlook the interconnections among various human and environmental factors, as well as subject-specific attitudes and perceptions of other elements within the system.

The scientific objective of this thesis is to rethink cognitive ergonomics in industrial settings by exploiting artificial intelligence techniques and collaborative robotics to anticipate and mitigate excessive mental demands and acute stress. Specifically, the idea of comprehending human cognitive load through *mind-induced motor behaviours* (i.e. body-language cues such as hyperactivity and attention diversion) is introduced and online, personalisable assessment methods grounded in *mind-body connection theory* are pioneered. By integrating this valuable data about human workers in the control loop, Collaborative Robots (CoBots) gain the ability to adapt, learn and provide personalised and contextually relevant support, addressing human distress and individual needs. CoBots, equipped with reduced-complexity models of the human psycho-physical state derived from practical sensor data and adaptive motion planning, form the cornerstone for a comprehensive understanding and optimization of hybrid environments. In this direction, the thesis introduces a first approach that integrates physical, cognitive, and robot-related factors within a unified learning-based framework, mirroring human behaviour in social contexts. This multi-dimensional perspective not only mitigates ergonomic risks but also has the potential to significantly improve overall workplace efficiency and productivity.

The first part of the thesis focuses on defining and detecting *mind-induced motor behaviours*. Monitoring workers' head pose and upper-body kinematics with suitable external sensors such as cameras allows to estimate their attention distribution and detect hyperactivity and other body-language cues. The integration of these aspects enables the development of the cognitive load assessment system, a personalisable model for online estimation of individuals' exposure to work-related stress and mental effort during occupational activities, accounting for multiple ergonomic risk factors. Next, the study delves into variables arising from the interaction between humans and robots in shared environments, exploring intuitive inputs and practical visual feedback interfaces that offer warnings about excessive cognitive exposure or provide updates on the CoBot state and the task progress. The assessment system is here extended to include factors reflecting humans' lack of confidence and trust in the robotic partner, inspired by research on gaze tracking and interpretation. Human awareness of the CoBot motion is also estimated by monitoring the attention level toward a moving area around the robot end-effector. Additionally, assessing synchronisation with the CoBot movements provides insights into the planning required by the human in conjoint actions. This thorough analysis contributes to a deeper understanding of how cognitive load evolves during interactions.

The central part of the thesis is dedicated to developing ergonomic Human-Robot Collaboration (eHRC) strategies aimed at mitigating potential mental health risks in the workplace. It proposes the adaptation of the proximity level and reactive behaviour of the CoBot based on accurate perception and monitoring of human attention and psycho-physical state. Exploiting estimated human awareness and mental effort, the CoBot adapts its path on the fly to ensure safety zones around the human, considering both physical aspects like collision prevention and socio-cognitive factors such as enhancing perceived safety. This is achieved while maintaining the continuity and smoothness of the planned trajectory through astute exploitation of the intrinsic properties of B-spline curves. Moreover, the implementation of a multi-objective optimisation problem and a human-aware decision-making method is proposed to optimise the total execution time and smoothness of the CoBot trajectory, ultimately maximising productivity without perilously increasing workers' psycho-physical stress.

Lastly, the thesis presents a pioneering approach towards a more comprehensive and unified framework, considering human cognitive comfort, physical ergonomics, and internal robot costs. This introduces a paradigm shift in human-robot interactions, enabling CoBots to make *co-efficient* decisions, i.e. maximise the human partner's benefits while being sensitive to their own expenses, mirroring human behaviour in social contexts. The concept of *co-efficiency* is inspired by neuroscientific and experimental psychology studies and conceived through a multi-disciplinary exploration of human-robot interactions. Human-robot *co-efficiency* is modelled by online capturing cues pertaining to both human and CoBot needs and employed as the reward of a reinforcement learning problem to shape the CoBot behaviour.

Results of multi-subject experiments and extensive statistical analysis on both qualitative and quantitative data suggest that the system, based on *mind-body connection theory*, provides effective assessments of cognitive ergonomics in occupational settings, and its integration in the CoBot control loop shows promise for achieving trustworthy and empowered collaboration.

# CONVERGING CHEMICAL AND CELL-BASED APPROACHES FOR IMPROVED NON-VIRAL GENE DELIVERY

**Federica Ponti** - Supervisors: Prof. Gabriele Candiani, Prof. Diego Mantovani

In the past decades, the remarkable progress made in biotechnology and genetic engineering enabled the rise of a novel way to develop therapeutic strategies relying on the delivery of nucleic acids (NAs) as drugs. While this approach, commonly referred to as gene therapy, could have revolutionized the way to treat a wide variety of pathologies, its use in clinical practices is still far from routine. In addition, it is worth noting that the use of genes, and delivery approaches, is extensively exploited in basic and applied research to study target cell mechanisms underpinning some pathologies as well as for recombinant protein production.
From a historical perspective, the development of gene delivery systems relying on viral particles as the NA carriers has set the rules in the field. Nevertheless, research has been focusing on safer options, such that the recent approval of Pfizer/BioNTech's and Moderna's COVID-19 vaccines has flipped the side of the coin in this respect. The molecules used in these vaccines are now at the center of every research focused on the delivery of NAs into cells. Non-viral gene delivery strategies have attracted significant interest in the development of novel therapeutic approaches as well as for basic and applied research in vitro. Compared to popular viral vectors, the class of non-viral carriers, namely cationic lipids (CLs) and polymers (CPs) able to spontaneously interact with negatively charged NAs to give nanoparticles called complexes, is now witnessing a surge of interest within the scientific community because they are relatively safe, cost-effective, and they can be easily produced and functionalized even at large scale. However, their efficiency in achieving the delivery tasks is still too low to outperform their viral counterparts. The efficacy of non-viral vectors is a tradeoff between their ability to drive NAs into cells, thus allowing/inhibiting their expression, their inherent toxicity, and their ability to deliver genes to target cells (i.e., transfection). Extensive research effort has thus been put into developing novel ways to improve the efficiency of such a class of delivery systems.
In this context, my Ph.D. aimed at developing innovative strategies to improve non-viral vector effectiveness. To this purpose, we dealt with the delivery issue from two different perspectives: on one hand, the modulation of the vector chemistry was disclosed as a way to develop multifunctional carriers with improved effectiveness; on the other hand, we sought to improve cell-(nano)particles' interactions through the mechanical modulation of the cell behavior in response to the delivery of non-viral vectors.
First, this PhD work was thus aimed at highlighting the importance of vector chemistry on the structure-function relationship of such kinds of materials, with a focus on lipid-based carriers.
As a proof-of-concept study, I first dealt with the characterization of a novel class of multifunctional lipid carriers that allowed me to investigate the role of specific functional moieties through a structure-activity relationship study. Specifically, we built a novel class of lipid NA carriers based on a triazine moiety linking i) an aminoglycoside headgroup, namely neomycin, to exert both NA condensation and antibacterial activity, and ii) different hydrophobic tails and assessed their ability to transfect mammalian cells while exerting antibacterial activity. Broadly speaking, the presence of a neomycin-based cationic headgroup eased the assembly of the vector with the anionic pDNA, while the different lipophilic moieties impacted the ability of the resulting nanoparticles to effectively deliver it into the cells. Of note, one-tailed steroid derivatives and symmetric two-tailed aliphatic ones (both saturated stearyl and unsaturated oleyl tails) were the most effective in transfection, in agreement with previous literature data.
From this proof-of-concept study, the very core of my Ph.D. project relied on developing smart strategies to improve transfection through the design of effective vectors, and the control of the cell behavior. To this purpose, I dug into polymer-based vectors, that is, the other class of materials used as NA carriers, to carry out the experimental activities of my work. Polymer-based gene delivery, also referred to as polyfection, has been often overlooked with respect to lipid-based transfection.
From a material perspective, however, polymer vectors can be easily synthesized, modified, and produced on large scale.
In this light, my Ph.D. work focused on the use of the renowned polymeric vector polyethyleneimine (PEI), and explored new ways to improve its effectiveness.
A thorough investigation of all the experimental variables affecting the performances of PEI-based polyplexes was carried out to disclose the best working conditions of PEI-based carriers and improve the standardization of in vitro screening protocols.
Next, I focused on the development of a vector-based approach to functionalize branched PEI (bPEI) with targeting moieties to improve the vector's selectivity towards a specific cell type. We thus synthesized a series of bPEI conjugates incorporating targeting peptides to selectively deliver genes to vascular smooth muscle cells (vSMCs). Moreover, the targeting vectors were incorporated into a polyplex releasing matrix to enable their local and controlled release for cardiovascular-related approaches. Through the conjugation of an elastin-derived peptide sequence to the bPEI structure, we were able to improve the polymer's effectiveness on target vSMCs while leaving off-target cells unaffected, a fact that is especially relevant for the translation of nonviral gene delivery approaches in vivo.
On the other side, the second pillar of my Ph.D. dealt with a novel strategy based on the regulation of cell response to the delivery of nanoparticles. Indeed, cells in vivo are constantly subjected to different environmental cues that govern some key cell functions. We thus investigated the application of an exogenous mechanical stimulus to cells undergoing transfection. We developed a device that allowed the mechanical stimulation of cells in culture in the form of micro-to-nano vibrational loading. By applying short mechanical stimuli to cells, we were able to trigger temporary and reversible cell responses that ultimately led to a significant improvement of the performances of both PEI-based polyplexes. Moreover, no cytotoxic effects given by the combination of non-viral vectors and mechanical stimulation were observed. Through a thorough investigation of the mechanisms underlying this increase in transfection efficiency, we showed that the mechanical stimulus applied to cells temporarily activated specific cell pathways, such as clathrin-mediated endocytosis at the basis of polyplex internalization. This strategy outlined the importance of cell responses to exogenous cues on the ultimate internalization and expression of a gene of interest and set the stage for a novel way to deal with the non-viral delivery issue.
Overall, the big picture drawn by this Ph.D. project highlighted the suitability of chemical-based approaches and cell-based approaches as promising ways to improve non-viral vector effectiveness. Further improvement in non-viral gene delivery research might be achieved by combining the strategies devised in this project. The development of multidisciplinary approaches taking into account both the delivery vector, the environment in which the delivery of genes takes place, and the cell response may thus pave the way to ever more effective strategies, and expedite the translation from the bench to the bedside of these materials.

# PERSONALIZED VALVULAR TISSUE ENGINEERING: A SCALED UP RECELLULARIZATION PROCEDURE TO PRODUCE FUNCTIONAL TISSUE FOR LIVING AORTIC VALVE SUBSTITUTES

**Stefano Rizzi** – Supervisor: Prof. Federica Boschetti

Tutor: Prof. Sara Mantero

Nowadays, cardiovascular diseases represent one of the most relevant causes of death worldwide, even more than the sum of all forms of cancer. Their treatment and prevention involve high direct and indirect costs for society. In the case of heart valve diseases specifically, current stented biological heart valve implants still report a reoperation rate of up to almost 40%, while innovative minimally invasive procedures (such as the transcatheter valve implant, TAVI) still suffer from structural degradation due to calcification over time and are associated with high pacemaker implantation. Deterioration of the biological implants is caused primarily by chronic inflammatory reactions due to the failure to detoxify the fixative remnants in the tissue and/or the incomplete removal of major xenoantigens (α-Gal). Such problems appear to be even heavier burdens if considered for pediatric VHDs, where, in addition, the prosthetic devices should also guarantee growth over time. At the same time, mechanical heart valves still require lifelong blood-thinning therapy for the patient. A promising approach to circumvent the mentioned shortcomings was introduced by Langer and Vacanti in 1993 under the definition of Tissue Engineering, which involves the application of "the principles of engineering and the life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function". Nevertheless, contradictory results obtained in the attempt to engineer complex tissues still relegate TE to a purely theoretical discipline. In addition, given the intrinsic complexity and heterogeneity of advanced cell-based therapy products, to date, no detailed standard or guidance is covering their manufacturing, and thus Good Manufacturing Practice (GMP) is intended to demonstrate whether or not tissue-engineered products are produced according to predefined manufacturing criteria, concerning both production and quality control, thus being suitable for in vivo trials. With the aim of being GMP compliant, a clinically relevant tissue for heart valve tissue engineering must meet numerous criteria, among others: non-immunogenicity, non-thrombogenicity, promotion of cellular proliferation and viability, and functionality in physiological conditions. In this perspective, one of the most crucial tissues to be repaired in the case of disease is the aortic valve. Ideally, a tissue-engineered heart valve (TEHV), but more in general a heart valve substitute, should include the capacity for self-repair, adaptive remodeling, growth, and resistance to infections and thrombogenicity, as stated by one of the pioneers of heart valve surgery, Emary Harken. Unfortunately, so far no currently available heart valve prosthesis possesses all these features. Among the strategies developed to create a functional TEHV, one promising option remains the possibility to exploit decellularized matrices as substrate, given their favorable characteristics in terms of biocompatibility. In fact, the decellularization process aims to provide an extracellular matrix-based scaffold with low immunogenicity and retained regenerative potential, and the availability of large livestock. Indeed, for example, previous interesting contributions showed the suitability of a decellularization procedure with ionic/non-ionic detergents to maintain the mechanical properties and reduce the immunogenicity of porcine pericardium. Besides drastically reducing the content of xenoantigens, the treatment also increased the permeability of the tissue, thus making possible the employment of a perfusion bioreactor to enable mass transport through the pericardial matrix and promote stable cellularization. Despite their different structural, mechanical, and morphological characteristics, in the last decades, bovine and porcine pericardia have alternatively been used as suitable matrices for heart valve fabrication. But in the perspective of TEHV manufacturing where the strategy is to reintroduce cells into the scaffold matrix, the more loosened microstructure of the porcine pericardium appears, indeed, to be more appropriate. Ideally, the best option as a cell source to fabricate a TEHV would be autologous heart valve cells, but these types of cells are non-sacrificial. Given the fibroblast-like phenotype of valvular cells and their mesenchymal origin, human adipose-derived stem cells (hASCs) have been shown to be a promising alternative. Indeed, besides their potentially suitable resemblance to valve cells, they are also readily available cell sources. Confined perfusion bioreactors allow cell seeding and culture of cells in three-dimensional constructs, fostering cell growth and viability by enhancing the supply of nutrients to the cells and allowing efficient removal of waste. Compared to other recellularization techniques to seed cells in valve-competent scaffolds based on static culture, the employment of this system indeed enables higher penetration of cells inside the scaffold. The present project aims to produce recellularized tissue by seeding ASCs into a xenoantigens and aldehydic residue-free decellularized animal matrix exploiting confined perfusion bioreactor systems, capable of representing the first personalized approach to aortic valve TE in the framework of the Ozaki technique. This surgical procedure relies on the possibility for the surgeon to shape the autologous pericardium, after mild glutaraldehyde treatment, directly in the surgical setting, to obtain an aortic valve tailored to the patient's needs, with optimal fluid dynamics. In an attempt to engineer the process of in vivo self-remodeling toward functional tissue rather than providing a fully engineered tissue, our recellularized tissue could be used as a "living" alternative to autologous pericardium during the Ozaki technique. With the advantage of eliminating glutaraldehyde treatment, which is a well-known cause of possible calcium influx into biological heart valve prostheses, and of higher biocompatibility.
To achieve the overarching aim of the project, we developed a rational step-wise recellularization procedure suitable for producing pericardial patches of the proper dimensions to be used during the Ozaki procedure for both pediatric and adult procedures.

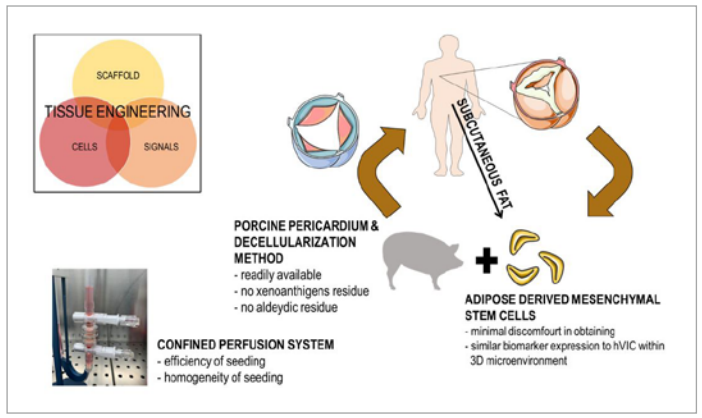


**Fig. 1 - The idea behind the project is to exploit a readily available cell source, hADSC; porcine decellularized pericardium, which is nowadays one of the elective materials for biological valve construction; and tailored bioreactors that can guarantee high seeding efficiency and culture. This can represent, in the framework of TE, a personalized therapy for the aortic valve capable, at least on paper, of solving problems related to the Ozaki procedure in the long run of the follow-up.**

# HIERARCHICAL CONTROL FOR OPTIMAL HUMAN-ROBOT COLLABORATION

**Francesco Tassi** - Supervisor: Prof. Elena De Momi

A crucial challenge in modern control is to endow robots with complex human skills such as multi-tasking and human-like dynamic movements. This is of great interest in Human-Robot Collaboration (HRC), where the human expects a similar behavior from the robot, to establish a reliable collaboration. However, tasks that are perceived by humans as simple, might often prove highly complex to be implemented on a robot.
In Fig.1 are some of the most common interaction modalities typical of HRC, each requiring different control types.

However, most current research focuses on these interaction ports individually, by tailoring controllers to specific applications, which inhibits the inclusion of multiple Human-Robot Interaction (HRI) modes, that characterize instead a real-world environment (Fig.1). For such complex tasks, the controller should acknowledge multiple objectives with different priorities, enable multi-tasking capabilities, and simultaneously manage numerous constraints.
This was the underlying motivation of my PhD studies, in which I focused on the formulation of a hierarchical control structure that could intuitively establish multiple robot behaviors and constraints, by assigning priorities to each sub-task.
In hierarchical control, *strict* hierarchies allow to project a secondary task into the nullspace of the primary one, ensuring its lower priority. Hierarchical Quadratic Programming (HQP) belongs to this category, which sequentially solves a Quadratic Programming (QP) problem for each layer in the Stack of Tasks (SoT). Therefore, depending on the number of degrees of redundancy of the robot, it is possible to achieve multi-tasking and complex behaviors.
One of my goals was to merge planning and control into a unique optimization problem, to generate an optimal and hierarchical solution that could satisfy both aspects. This could prove highly beneficial for HRC, which are a good representative of multi-task and multi-objective problems.
However, due to the challenges in the definition and switching of the related (sub)goals, they have never been adequately addressed.
The core objective of my work is to enable multi-tasking through a controller that can prioritize both robot and human related parameters. In particular, my **first objective (01)** was to analyze practical scenarios, where the human might expect quick changes in the robot's behavior, for example passing from a passive to an interactive controller to collaborate on the manufacturing of an object (Fig.1); or to switch between different levels of compliance.

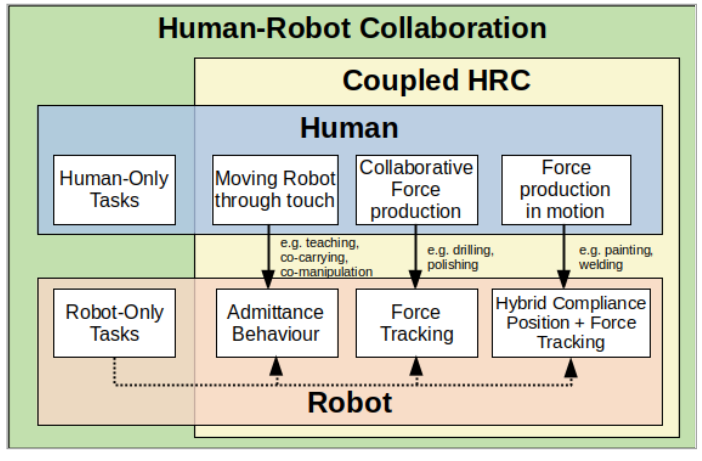


**Fig. 1 - Collaboration modalities between human and robot with interaction ports for different behaviors required by the task.of solving problems related to the Ozaki procedure in the long run of the follow-up.**

Therefore, humans become an integral part of the robot controller, aiming towards a greater synergy in real-world scenarios.
Thus, my **first contribution (C1a)** was to investigate these different interaction modes, analyzing the possible interaction ports and integrating them under a unique framework. This would eliminate the necessity of switching between different control types, that are typically tailored for specific tasks, and would guarantee smoother interaction. I formulate an HQP-based control framework that includes all the different interaction modes explained in Fig.1, and performed multiple experiments for each scenario to prove its validity (Fig.2).
Another crucial aspect are the high impacts resulting from any exchange of forces. Through contribution **(C1b)** I formulated an impact-aware planning strategy, in which an impact model is used to optimally absorb the force on the robot. This was proven useful in practical industrial applications, that currently deal with impact-rich scenarios (Fig.2, top left).
A final contribution related to **(01)** is in field of teleoperation **(C1c)**, with a reconfigurable interface (redundant robotic arm + 3D mouse) used to teleoperate a mobile manipulator for fast-paced manipulation tasks (bottom figures of first (yellow) module in Fig.1).
A **second objective (02)** was to improve robot's adaptability to unstructured environments. With the related **contribution (C2a)**, I formulated the Augmented HQP (AHQP) control scheme that unifies optimal planning and control, avoiding the need for a fixed reference trajectory known a priori. Indeed, we need to evolve from the conventional idea of separately planning and executing, which inherently creates a gap. With the proposed controller the Cartesian reference trajectories become part of the optimization problem. Essentially, we avoid the pre-definition of reference trajectories, rather defining the desired *behaviour* of the robot, which will autonomously calculate the optimal trajectories based on e.g. the points to reach/avoid, human-robot shared workspace, human ergonomics etc.
An additional contribution **(C2b)** is the adaptive compliance of the AHQP-based framework, that can provide a variable compliance controller, useful for safe HRI.
This lays the foundations for my **third objective (03)**, which can now provide deeper emphasis on the human side. Indeed, most studies focus either on human-only or robot-only aspects. Other studies consider instead human-related aspects on the robot-side, however, most of these only act on the reference trajectory planning or on task allocation, never considering robot control.
Therefore, in **contribution (C3a)** I formulate human-related parameters directly at robot control level, such as human ergonomics, with the aim of reducing Musculoskeletal Disorders (MSDs), which are considered the second work-related cause for physical impairment worldwide. I formulate human ergonomics as a Cartesian map using state-of-the-art ergonomics scores and integrate it in the AHQP control framework.
Contribution **(C3b)** extends the ergonomics-aware AHQP framework by integrating the perception of human actions and intentions through vision-based learning techniques (bottom part of Fig.2). This enables the detection of human intentions through the identification of human actions, achieving a quick robot's reaction. The human can now move freely in the workspace while ergonomically engaging in the interaction (bottom part of Fig.2), and the robot will follow accordingly.

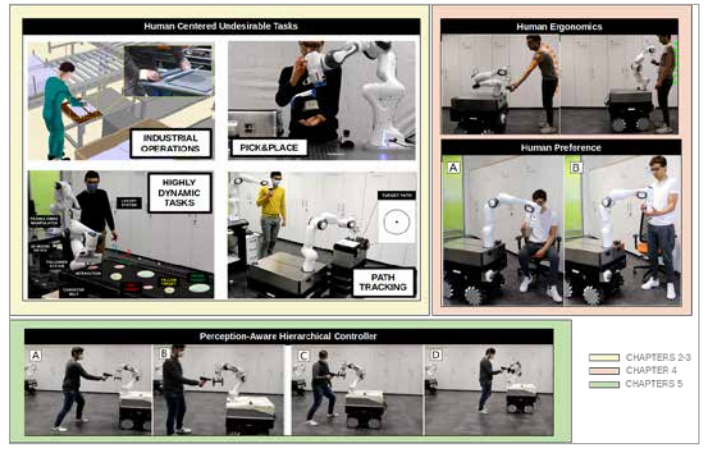


**Fig. 2 - Application domains and their subdivision throughout the thesis' chapters, for each contribution area. Some of the experiments are shown, related to HRC and physical interaction.**