MECHANICAL ENGINEERING | PHYSICS | PRESERVATION OF THE ARCHITECTURAL HERITAGE | STRUCTURAL, SEISMIC AND GEOTECHNICAL ENGINEERING | SCIENCE, TECHNOLOGY, AND POLICY FOR SUSTAINABLE CHANGE | 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 | 2026

DOCTORAL PROGRAM IN BIOENGINEERING

Chair:
Prof. Raffaele Dellacà

The main objective of the PhD Programme in Bioengineering is to provide candidates with high-level engineering problem-solving skills in biomedical, healthcare, and life sciences. This is achieved through intense transdisciplinary training, bridging engineering with medical and biological knowledge.

During the PhD, the candidates undertake a scientific research project addressing a complex problem at various scales, ranging from the molecular and cellular levels to living organisms and biomedical systems. They explore original methods, devices, and systems with different purposes, including expanding scientific knowledge, developing innovative diagnostic and therapeutic methods and techniques as well as improving healthcare and daily life structures and services. At the end of the programme, candidates are expected to be capable of leading innovative research and development projects in Bioengineering, proposing new methodological and technological solutions, and assessing their impact on healthcare, life sciences, and the biomedical industry.

The PhD candidates conduct their research through both theoretical and experimental activities in four major areas: biomimetic engineering and micro-nano technologies; rehabilitation engineering and technology; technologies for therapy; and 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, biomedical signal and image processing, e-health, bioinformatics, functional genomics and molecular medicine.

The PhD Programme in Bioengineering is structured across multiple departments. Faculty members of the PhD Board belong to two departments at Politecnico di Milano: the Department of Electronics, Information, and Bioengineering (DEIB) and the Department of Chemistry, Materials, and Chemical Engineering (CMIC).

Each year, around 50 PhD candidates carry out their research in experimental laboratories at Politecnico di Milano or in collaboration with prestigious national and international universities, biomedical research centers, hospitals, and industries. At Politecnico di Milano, PhD candidates are usually developing their research in one of the following laboratories: the Laboratory of Biological Structure Mechanics, the Laboratory of movement analysis "Luigi Divieti", the Medical Informatics Laboratory, the Neuroengineering and Medical Robotics Laboratory, the Biosignals, Bioimaging and Bioinformatics Laboratory, the Biomaterials Laboratory, the Biomedical Technology Laboratory (comprising CartCas Lab, Lares Lab and TechRes Lab), the Experimental Micro and Biofluid Dynamics Laboratory, the Computational Biomechanics Laboratory, the Biocompatibility and Cell Culture Lab, and the Bioreactors Laboratory. Additionally, the Institute of Electronics, Computer and Telecommunication Engineering (IEIIT) of the National Research Council (CNR), located within DEIB, offers further research opportunities.

International research stays are a key component of PhD training. Candidates are encouraged to spend at least three months conducting research in collaboration with internationally recognized institutions, particularly abroad, in laboratories that complement and enhance their research projects. The programme already has strong collaborations with numerous national and international academic institutions, research centers, companies, and clinical partners, ensuring effective technology transfer from applied research to biomedical devices and clinical applications.

The educational syllabus includes tailored advanced courses specifically designed for Bioengineering PhD students, aimed at deepening methodological and technical expertise while also fostering essential soft skills for researchers. The programme also includes participation in the annual one-week School of the Italian National Group of Bioengineering (GNB).

Additionally, the PhD Programme in Bioengineering benefits from an Advisory Board made up of distinguished experts from R&D industries, research centers, and clinical institutions. This board ensures that the programme aligns with the needs of both academia and the broader professional world.

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AI-DRIVEN METHODS IN MAXILLOFACIAL EVALUATION AND THERAPY PLANNING

Benedetta Baldini - Supervisor: Giuseppe Baselli, Gianluca Martino Tartaglia

Introduction

Oral health is a key component of overall wellbeing, with significant medical, social, and economic implications. **Oral and maxillofacial radiology** (OMFR) plays a crucial role in early diagnosis and treatment planning, particularly as oral diseases affect nearly half of the global population. Advances in imaging technologies and analysis methods are therefore essential to improve outcomes and reduce disease burden.

The introduction of 3D imaging, especially Cone Beam CT (CBCT), has enhanced diagnostics by providing high-resolution volumetric data. Intraoral scanners (IOS) and stereophotogrammetry further support digital dentistry by non-invasively capturing accurate dental and facial surface information. Meanwhile, digital workflows and Artificial Intelligence (AI) are improving diagnostic accuracy, efficiency, and accessibility, although many image analysis tasks remain manual, time-consuming, and operator-dependent.

This PhD thesis investigates the application of AI in OMFR to enhance diagnosis and treatment planning. It focuses on developing automated pipeline for advanced head analysis by integrating CBCT, IOS, and stereophotogrammetry into a unified virtual patient model. The work includes automated cephalometric landmark detection, tooth segmentation and classification, multimodal data fusion, and clinical validation approaches.

Methods

Cephalometric analysis is a diagnostic tool in orthodontics and maxillofacial practice, used to assess skeletal and dental relationships. To automate this task, multicentric dataset of 350 CBCTs was collected. Sixteen anatomical landmarks were manually annotated by an expert. A 3D V-Net network performed voxel-wise landmark detection. Performance was evaluated using Mean Radial Error (MRE).

Multimodal image fusion combines multiple imaging datasets into a single composite to maximize information content. In CBCT/IOS fusion, CBCT captures detailed 3D dental roots, while IOS accurately represents crowns and occlusion, enabling virtual tooth modeling and enhancing diagnosis, treatment planning in dental applications. This task employed a framework combining CBCT-based tooth segmentation, IOS-based crown segmentation, and CBCT/IOS registration.

The **CBCT segmentation** dataset included 194 retrospective and 104 public volumes. Each tooth was manually segmented, producing up to 52 labels per subject. The dataset was used to train and test a custom 3D U-Net with spatial-channel attention, evaluated via Dice Similarity Coefficient (DSC).

The **IOS segmentation** dataset combined 272 retrospective with 900 public IOS. The dataset was used to train and test two-stream graph convolutional network, and evaluated using overall accuracy (OA).

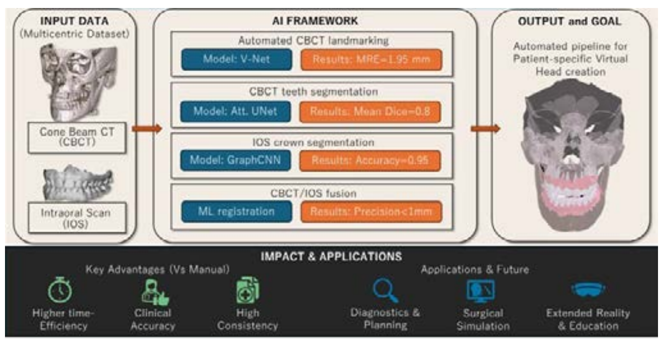

Fig. 1

For **multimodal fusion**, CBCT and IOS segmentations were converted to point clouds, globally aligned via RANSAC registration and refined with Iterative Closest Point (ICP). CBCT-derived roots were merged with IOS-derived crowns, 3D models were reconstructed with Poisson surface reconstruction, smoothed, and assessed using Hausdorff distance, RMSD, and median surface distance.

Clinical validation was conducted during a research period at KU Leuven, developing two clinical validation pipelines for AI-driven multimodal image fusion: CBCT/IOS and facial scan/CBCT fusion for virtual patient creation. In the **CBCT/IOS** study, AI-based accuracy was assessed on 30 clinical cases through surface distance metrics between fused and CBCT-derived tooth segmentation model. Automated and manual methods were compared in terms of reproducibility and processing time. In the **facial scan/CBCT** pipeline, 40 full and reduced field-of-view scans were combined with facial scans and processed via AI and semi-automated methods. Accuracy, consistency, processing time, and radiation dose reduction were evaluated, to evaluate the effectiveness of AI-based workflows.

Results

For automated 3D **landmark detection**, the overall MRE was 1.77 ± 0.97 mm, with inference time under 32 seconds and results within clinical thresholds.

For **tooth segmentation**, the mean DSC was 0.80 (0.93 binary), while IOS crown segmentation reached OA of 0.94–0.95. CBCT/IOS fusion produced accurate 3D dental models (HD of 1.3 mm) in 167 seconds.

For **clinical validation** work, automated CBCT/IOS-fusion showed clinically acceptable accuracy (<300 µm), robustness across ages, genders, and devices, and minimal impact from metal artifacts. It was 32x faster and more consistent than manual. For facial scan/CBCT study, simulated reduced FOV scans did not significantly affect virtual patient creation, underlining the feasibility of reduced field of view scans and radiation dose reduction by up to 33% for efficient and safe virtual patient creation.

Discussion and Conclusions

This PhD thesis contributes to the advancement of personalized digital medicine through the development of AI-based tools for patient-specific virtual head creation in the maxillofacial field. Major strength is the creation of a multicentric, annotated dataset for robust model training and validation. The integration of automated approaches for landmark detection, CBCT and IOS segmentation, and multimodal data fusion demonstrates the feasibility of a unified and reproducible digital workflow. From a clinical perspective, the framework significantly improves efficiency and reproducibility while reducing operator dependency. It also enhances diagnostic support and treatment planning, with potential applications in education and surgical simulation. Future developments will focus on validation on larger datasets to ensure generalizability, standards development for virtual head generation, integration with extended reality technologies. Overall, this research lays the foundation for a scalable, interoperable, and fully automated 3D digital patient ecosystem.

BEYOND SINGLE MODALITIES: UNCOVERING NEURAL MECHANISMS THROUGH MULTIMODAL EEG, FMRI, AND TMS INTEGRATION IN HEALTH AND PSYCHIATRY

Elena Bondi - Supervisor: Anna Maria Bianchi

Co-Supervisor: Eleonora Maggioni

Multimodal neuroimaging, neurosignaling, and neurostimulation integration offers a powerful approach to investigate brain function by combining complementary techniques to overcome the inherent limitations of individual modalities. While electroencephalography (EEG) provides excellent temporal resolution for capturing rapid neural dynamics, functional magnetic resonance imaging (fMRI) offers superior spatial precision for localizing brain activity. Similarly, transcranial magnetic stimulation (TMS) enables direct probing of cortical excitability and connectivity when combined with EEG. This thesis explores how integrating these complementary approaches - specifically EEG-fMRI and TMS-EEG - can reveal subtle neural mechanisms underlying cognitive and emotional processes in both healthy individuals and psychiatric populations, providing insights that would remain hidden when using single modalities alone.

The first part of this thesis develops and validates two complementary analytical frameworks for EEG-fMRI integration. The first framework utilizes event-related potentials to drive fMRI analysis, enabling the distinction between cognitive sub-processes during inhibitory control tasks. This approach is applied to healthy participants, demonstrating its capability to differentiate N2 and P3 components with high precision, and then extended to patients with major depressive disorder (MDD), revealing depression-specific alterations in frontal, parietal, and temporal regions. The methodology is further applied to investigate auditory processing in early-course schizophrenia (SCZ), where an EEG-driven fMRI connectivity analysis reveals altered network dynamics between the left insula and both prefrontal regions and the default mode network in SCZ. The second framework employs a data-driven approach based on EEG time-frequency representations to investigate motor execution and imagery without prior assumptions about electrode locations or task timing. This analysis reveals an inverse relationship between alpha power suppression and fMRI signal increases in sensorimotor regions and other areas such as the cerebellum, successfully differentiating between right and left-hand movements in both execution and imagery conditions. The second part of this thesis examines TMS-EEG integration to investigate cortical excitability during emotional processing, specifically focused on awe, a complex emotion characterized by perceived vastness and the need for accommodation. Within the SUBRAIN project, virtual reality, EEG, and TMS are integrated to examine the neural correlates of awe in controlled laboratory settings. Through parameter tuning studies, the TMS-EEG preprocessing pipeline is refined, and subsequently, awe-specific modulations in cortical excitability across frontal and central regions are identified. Throughout this thesis, significant technical challenges in multimodal integration are addressed, including artifact correction, parameter refinement, and data integration approaches. The methodologies presented reveal neural mechanisms that would remain hidden when using single modalities alone, with particular emphasis on their application in psychiatric populations. The EEG-fMRI studies highlight altered neurovascular coupling in MDD during inhibitory control and in early-course SCZ during auditory processing, while the TMS-EEG work establishes a foundation for investigating potential therapeutic applications of induced emotional states.

This work contributes to the field by developing robust analytical frameworks for multimodal neuroimaging that can be applied across diverse research questions while maintaining sensitivity to disorder-specific neural alterations. The findings underscore the value of integrated approaches in advancing our understanding of brain function and potentially improving diagnostic and therapeutic strategies in psychiatry.

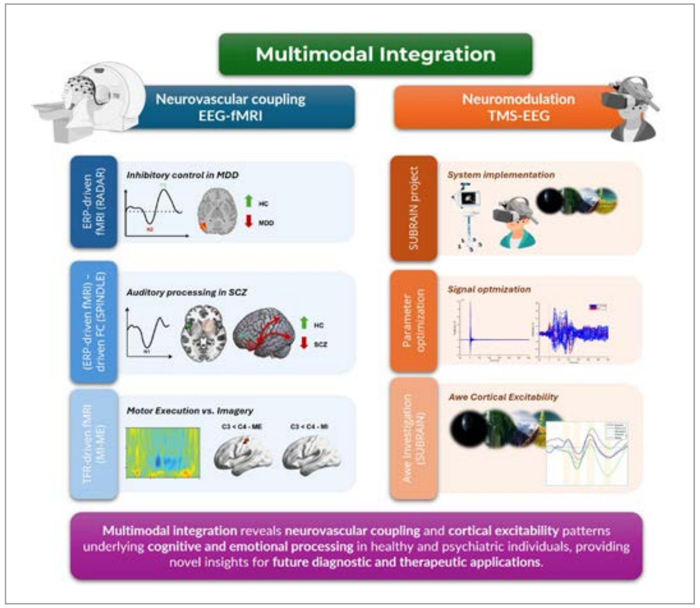

Fig. 1 - Graphical Abstract. EEG: electroencephalography; fMRI: functional magnetic resonance imaging; TMS: transcranial magnetic stimulation; ERP: event-related potentials; TFR: time-frequency representation; MI: motor imagery; ME: motor execution.

FROM RAW DATA TO CLINICAL STRATEGY: AI-BASED DIGITAL TWINS FOR PATIENT-SPECIFIC VASCULAR AND PARENCHYMAL MODELING IN HPB SURGERY

Matteo Cavicchioli - Supervisor: Pietro Cerveri

Co-Supervisors: Giacomo Pugliese, Andrea Moglia, Raffaele Pugliese

Hepato-Pancreato-Biliary (HPB) interventions are among the most demanding fields in abdominal medicine, due to high anatomical variability, dense vascular networks, and the late presentation of malignancies such as pancreatic ductal adenocarcinoma and hepatocellular carcinoma. In this context, accurate three-dimensional understanding of patient-specific anatomy is essential to support clinical reasoning, anatomical inspection, and education. While 2D contrast-enhanced CT scans remain the standard imaging modality, they often fall short in conveying the spatial complexity required for advanced anatomical interpretation.

This thesis addresses such limitations through a research path explicitly shaped by the needs and feedback of HPB clinicians, developing AI-based digital twins, namely task-specific deep learning solutions designed to deliver reliable 3D reconstructions of pancreatic parenchyma and hepatic vasculature, validated through a clinically guided methodology. The work was conducted in collaboration with AIMS Academy and structured along a translational roadmap composed of three progressive phases (Figure 1), each one built upon real clinical demands collected from the network of HPB key opinion leaders. The first phase consisted of a systematic review of 130 peer-reviewed studies on deep learning for pancreas segmentation, identifying recurring methodological gaps such as the absence of subregional anatomical analysis, limited large-scale validation, and the lack of clinically meaningful evaluation metrics. These findings directly informed the design of the subsequent technical contributions. Building on these insights, the second phase introduced a 2.5D deep learning model trained on the AIMS-1300 dataset, comprising over 1,300 manually annotated CT volumes, focused on the segmentation of the pancreatic parenchyma. The proposed architecture outperformed state-of-the-art models on the public AMOS dataset, achieving a Dice Similarity Coefficient (DSC) of 0.87 (HD = 12.73 mm, ASSD = 0.66 mm). Beyond accuracy, this phase formalized two underexplored aspects of medical AI, motivated by concrete clinical concerns: a quantitative analysis of the human effort required to curate clinically valid datasets (estimated at about 900 hours for 1,300 cases) and the relationship between dataset cardinality and segmentation performance. Results showed a logarithmic improvement plateauing around 440 annotated cases, providing a reproducible reference for resource allocation in future projects. A novel slice-based error metric was also introduced to quantify the manual correction effort required by radiologists, complementing standard global metrics with a measure that mirrors real clinical workflows.

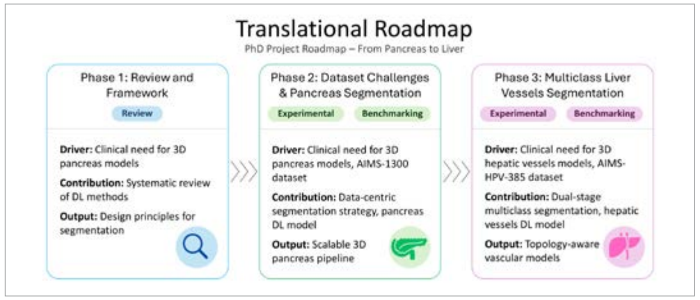

Fig.1 - A structured timeline outlining the three core phases of the doctoral research, each driven by specific clinical needs and datasets.

The third phase extended the methodology to the hepatic vasculature, addressing the multiclass segmentation of portal and hepatic veins on a dataset of 385 CTA scans (AIMS-HPV-385). This effort responded to the clinical demand for detailed multivessel liver reconstructions capable of supporting anatomical exploration in highly variable and pathologically distorted scenarios. The proposed framework, named D^2-RD-UNet, integrates a data-driven preprocessing strategy, vesselness enhancement filters (Sato, Frangi, RORPO, OOF), and a hybrid encoder combining residual and dense blocks. A 4D configuration enriches the input with multi-channel vesselness representations, while a dedicated Vessel Connectivity Correction (VCC) algorithm restores topological consistency by reassigning misclassified vascular segments through centerline-based reasoning. The framework outperformed state-of-the-art models including nnU-Net, VNet, UNet-AG, and SwinUNETR, both on internal and external benchmarks. On the public 3D-IRCADb-01 dataset, it achieved a DSC of 0.62 (HD95 = 15.63 mm, ASSD = 2.90 mm) for hepatic veins and 0.68 (HD95 = 16.72 mm, ASSD = 2.57 mm) for portal veins. A radius-based evaluation metric was also introduced to stratify segmentation quality across vessel calibers, translating technical performance into clinically interpretable insights aligned with the priorities of the multidisciplinary teams involved. Beyond model development, the thesis includes complementary contributions aimed at clinical transferability, such as a tubular rendering pipeline for smooth 3D vessel visualization and a published survey on generalist deep learning models in medical imaging that contextualizes the work within the broader landscape of foundation models. Figure 2 illustrates representative patient-specific 3D reconstructions, highlighting the two target abdominal regions addressed by this thesis.

Overall, this doctoral research bridges algorithmic innovation and clinical applicability, fostering a transition from a generalized protocol-based approach to a more personalized, anatomy-driven understanding of each patient. By transforming raw CT data into navigable, clinically valuable 3D digital twins, the proposed framework contributes to enriching anatomical inspection, supporting clinical education, and enhancing multidisciplinary dialogue in HPB medicine, while providing methodological foundations transferable to other anatomical domains.

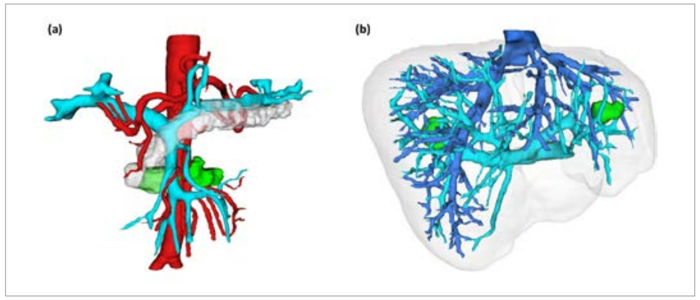

Fig.2 - 3D reconstructions: (a) pancreatic parenchyma from the 2.5D model; (b) hepatic vasculature from the D^2-RD-UNet framework, showing portal (cyan) and hepatic (blue) veins. Lesions in green.

Keywords: 3D Segmentation, Deep Learning, Digital Twins, Pancreas, Hepatic Vessels, HPB Surgery, Patient-Specific Care, Clinical Metrics.

ASSESSMENT OF NEW TECHNOLOGIES AND SOLUTIONS FOR TELE-REHABILITATION AND TELE-MONITORING FOR INDIVIDUALS WITH MOTOR DISABILITIES

Serena Cerfoglio - Supervisor: Veronica Cimolin

Background. This thesis explores the integration of emerging technologies into tele-rehabilitation and tele-monitoring frameworks for individuals with motor disabilities. The research addresses key challenges in modern healthcare, including the rising prevalence of chronic and motor impairments and the need for scalable, cost-effective, patient-centred rehabilitation solutions. Traditional in-clinic rehabilitation, while effective, is resource-intensive and often inaccessible to patients facing geographical, financial, or health-related barriers. In this context, tele-rehabilitation and tele-monitoring offer the potential to transform care through digital technologies that enable remote, patient-centred interventions. Through a series of methodologically rigorous studies, this thesis evaluates the technical accuracy, clinical relevance, and feasibility of innovative motion analysis tools and their application in remote and hybrid care models. It is structured around four primary studies, each contributing to advancing knowledge in remote rehabilitation for individuals with conditions such as hemiplegia and long COVID-19.

Technological Validation. The first part of this work evaluates the technical accuracy and clinical relevance of innovative motion analysis tools against gold standard motion capture (MoCap) systems. The first study assessed the accuracy of kinematic measurements from a wearable device based on a single inertial measurement unit (IMU) across rehabilitation tasks targeting trunk and lower limbs (i.e., anterior trunk flexion, lateral bending, hip abduction, flexion and extension, semi-squat, and seated knee extension). High accuracy and strong correlations were achieved for most tasks, with resilience to sensor misplacement, supporting its suitability for remote and unsupervised rehabilitation. The second and third studies focused on optical sensors, specifically the Microsoft Kinect® series, for gait-related metrics. The second study, a narrative review of Kinect-based approaches for gait analysis in post-stroke individuals with hemiplegia, highlighted its potential as a low-cost, reliable, and feasible clinical tool. However, limitations were identified in tracking complex kinematics and inconsistencies in methodologies, making comparisons across studies challenging.
Building on this, the third study evaluated the latest model, Microsoft Azure Kinect (MAK), against MoCap during gait assessment in both healthy and post-stroke populations. MAK showed strong agreement with MoCap for most spatio-temporal parameters and center of mass displacements, especially in hemiplegic gait. However, challenges in capturing subtle biomechanical details (e.g., some gait sub-phases) highlight the need for further algorithm refinement to improve precision in remote applications.

Clinical Implementation. The last study included in this thesis implemented and assessed the effectiveness and the feasibility of a hybrid tele-rehabilitation program targeting motor and respiratory impairments in long COVID-19 patients. This program combined in-hospital care with home-based interventions supported by digital tools. Upon discharge, patients were provided with easy-to-use equipment, including a respiratory muscle training device (SpiroTiger, MVM Italia, Lainate, MI) and a motor rehabilitation device (euleria home, Euleria Health, Rovereto, TN). Clinically significant improvements were observed in functional mobility (e.g., > 73 m increase in the distance covered during the Six-Minute Walking Test), also with enhanced respiratory function and cardiovascular fitness. In addition to clinical outcomes, high levels of patient satisfaction and adherence reflected the feasibility and the potential of the proposed approach both as long-term recovery solution and to alleviate healthcare system burdens while ensuring continuity of care.

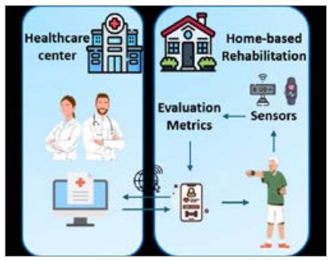

Fig.1 - Integrated tele-rehabilitation and monitoring framework: from home-based sensor data collection to remote clinical evaluation and treatment adjustment.

Conclusions and Future Research. Overall, the included studies highlight the transformative potential of tele-rehabilitation and tele-monitoring technologies in addressing the growing demand for accessible, scalable, cost-effective motor rehabilitation solutions in modern healthcare. Following technological validation of IMU-based systems and optical sensors, such as the Microsoft Kinect series, the research demonstrates their capability to serve as reliable alternatives to traditional MoCap systems in settings where these are not feasible. These tools enable continuous and accessible assessments in both remote and clinical environments, supporting personalized interventions and enhancing rehabilitation outcomes. The integration of these technologies into tele-rehabilitation platforms offers additional benefits, such as improved patient engagement through biofeedback and gamification. Moreover, the proposed hybrid tele-rehabilitation model exemplifies how digital tools can help alleviate the socio-economic burden on healthcare systems by extending care to underserved populations. Ensuring the accuracy and reliability of these technologies, especially for complex kinematic analyses, remains critical. Rigorous validation, standardized clinical protocols, and interdisciplinary collaboration are essential to bridge the gap between technological innovation and practical application. In conclusion, this thesis underscores the role of new technologies in innovating rehabilitation for motor impairments. By addressing challenges in validation and access, tele-rehabilitation can complement traditional methods and set new benchmarks for sustainability in healthcare systems worldwide.

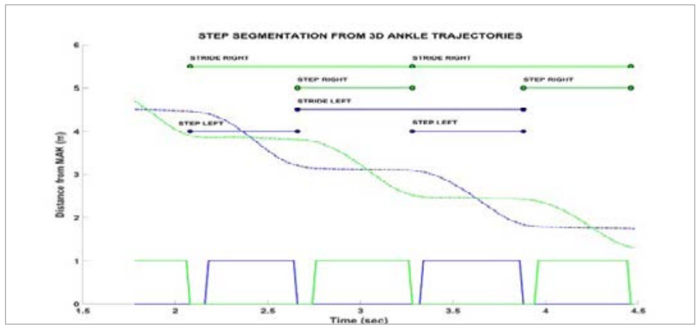

Fig.2 - MAK ankle trajectory segmentation. Dashed lines indicate right (green) and left (blue) ankles. Bottom plot: 0 = stance, 1 = swing. Top plot: estimated steps/strides. Spatial information is calculated from joint z-coordinates (depth)

ON THE ASSESSMENT OF KNEE JOINT BIOMECHANICS AND LIGAMENT TENSIONS THROUGH A TAILORED MUSCULOSKELETAL MODEL

Lucia Donno - Supervisor: Carlo Albino Frigo

Daily activities, such as walking or climbing stairs, despite their apparent simplicity, involve complex interactions at the knee joint level. In this intricate anatomical structure, the ligaments play a crucial role in ensuring the knee stability and functionality: they are rigid enough to prevent joint dislocation under high loads, but at the same time they are arranged in such a way as to ensure a wide range of motion in the sagittal plane. Understanding the loads supported by knee ligaments during daily activities and how these loads are influenced by muscular enhances the knowledge of knee biomechanics and injury mechanisms, with implications for prevention, treatment, and prosthetic design.
Experimental studies present limitations: cadaveric analyses allow investigation of real anatomy but cannot replicate physiological loads, while in vivo measurements face ethical and technical constraints, often quantifying ligament strains rather than tensions. In this context, musculoskeletal modelling emerges as a key tool for estimating ligament tension under dynamic conditions. However, relatively few studies have focused on dynamic tasks such as walking and stair climbing, and results are often inconsistent.
Thus, this thesis aims to implement a three-dimensional musculoskeletal model of the knee joint to quantify ligament tensions, analyse their relationship with muscle forces, and predict the effects of single-element modifications on joint biomechanics. The study focuses on walking and stair climbing.
The first step was to implement a three-dimensional model of an intact knee joint, then applied all along the research process and adapted from time to time to the different simulated conditions.
The knee model, implemented in SimWise4D (DST, Canton, MI, USA), includes femur, tibia, fibula and patella obtained from magnetic resonance images. The ligamentous structure, including capsule, cruciate and collateral ligaments, was simulated by means of 13 straight springs with nonlinear behaviour (viscoelastic elements) and each ligament was modelled as consisting of several fascicles which could be recruited differently under different conditions. Each muscle acting on the knee joint was modelled as a force actuator controlled by input functions estimated by the "minimization of the maximum force" criterion.
The model reproduces key knee joint mechanisms, including the screw-home mechanism, femoral rollback, tibiofemoral contact force patterns, and ligament loading/unloading due to muscle activity.
In the first application of the implemented model, a gait cycle was simulated animating the intact knee model through motion capture data. Ligament tensions were quantified and the role of muscles in determining the ligament loads was examined. This study showed that the Quadriceps contraction is the main responsible for the Anterior Cruciate Ligament (ACL) loading, while the Hamstrings muscle group is the primary contributor to the high tension on the Posterior Cruciate Ligament (PCL), specifically during the swing phase. The collateral ligaments, consistently with their longitudinal arrangement along the femur and the tibia, showed a reduced sensitivity to muscle contractions.
The next step in the research aimed to fill a gap in the literature regarding the impact of ACL sacrifice on knee kinematics during walking. Thus, the ACL was removed from the knee model, and a dynamic simulation of the gait cycle was performed. It was found that the superficial and deep bundles of the Medial Collateral Ligament (MCL) become the main passive stabilizers to the tibial anterior translation when the ACL is missing. The reduction in the Quadriceps activity, leading to partial atrophy over time, finds its effectiveness in reducing the loads of all the remaining ligaments except for the PCL.
A further study was designed to bridge another scientific gap: due to the rarity of isolated PCL injuries, the literature still lacks a comprehensive understanding of the changes in knee joint biomechanics during walking. Hence, many simulations of the gait cycle were performed exploring different severities of PCL injury and the redistribution of internal loads among the remaining ligaments. It resulted that in case of PCL lesion, MCL becomes the primary stabiliser against the posterior tibial translation, supported by the Lateral Collateral Ligament (LCL). Despite this adaptive mechanism, the tibial-femoral contact force appeared increased compared to the intact knee condition and the tibia still experienced posterior displacement, resulting in a forward shift of the tibial-femoral contact point. This phenomenon clarifies the common occurrence of tibial cartilage degeneration in PCL-injured knees. Moreover, it emerged that reducing Hamstring activity could relieve the remaining ligaments compensating for the PCL deficit, thereby reducing the tibial-femoral contact force.
Then, the prosthetic knee was examined: although many studies have compared different implant designs, no reference was found to the changes induced with respect to the natural knee joint. A posterior-stabilized prosthesis was virtually implanted in the musculoskeletal model, and a dynamic simulation of the gait cycle was run. Among the remaining ligaments, the MCL superficial and deep bundles supported the highest load, consistently with the fact that in case of cruciate ligaments deficiency the medial ligamentous structures become the primary stabilizers to the anterior-posterior tibial translation.
Once the walking task was extensively explored, the research shifted to climbing stairs. To the author's best knowledge, this is the first study trying to quantify the load supported by the knee ligaments during this task.
The PCL and deep fibres of MCL supported the highest load, specifically during the mid-swing phase when the Hamstrings contract to counteract the forward limb acceleration.
The ACL and fibrous capsule were mainly recruited during the stance phase, consistently with Gastrocnemii contraction. Conversely, LCL and superficial MCL bundles stabilized the knee joint during the swing phase.
Overall, this thesis highlights the potential of musculoskeletal modelling to investigate knee biomechanics under dynamic conditions, providing information difficult to obtain in vivo and relevant for clinical applications.

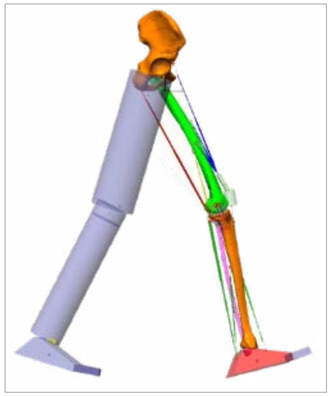

Fig.1 - The walking model.

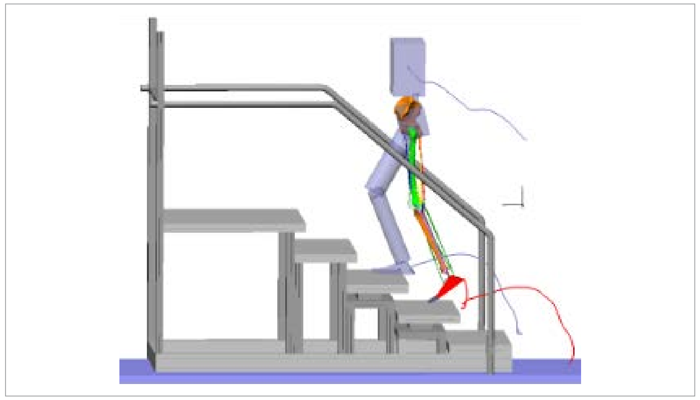

Fig.2 - The driving model in climbing stairs.

MODELING AND OPTIMIZATION OF A RIGHT VENTRICULAR ASSIST DEVICE: A HEMOCOMPATIBILITY-DRIVEN APPROACH

Ilaria Guidetti - Supervisors: Maria Laura Costantino, Francesco De Gaetano

Right ventricular failure (RVF) is a complex syndrome in which the right ventricle is no longer able to sustain the pulmonary circulation. It affects up to 40% of the patients who received a left ventricular assist device (LVAD) and is associated with high in-hospital mortality.
At present, there are no mechanical circulatory support devices specifically designed to treat RVF. The only durable options involve off-label use of LVADs as right ventricular assist devices (RVADs). However, these devices are optimized for the systemic circulation, which operates at higher pressures than the pulmonary circuit. As a result, when used as RVADs, they work off-design, compromising their efficiency and increasing the risk of blood damage. This research project aimed to develop and optimize a rotary RVAD specifically intended for the pulmonary circulation, with the goal of achieving both high hydraulic efficiency and low hemolysis.
To assess hemocompatibility, a numerical hemolysis model was implemented based on a Lagrangian strain-based formulation. This model was validated using the Food and Drug Administration (FDA) nozzle benchmark, which served as a reference geometry for blood damage studies.
First, a Reynolds-averaged Navier-Stokes (RANS) turbulence model was optimized to improve flow predictions in the FDA nozzle while keeping a low computational cost.
The optimization process involved tuning the model's free parameters to minimize discrepancies between experimental and numerical velocity measurements.
The optimized RANS model was then used to simulate blood flow in the FDA nozzle, providing the input required for hemolysis analysis. To address the limitations of traditional stress-based models, a new strain-based approach was developed. The results showed improved predictive capabilities, though further refinement is still needed.
The study then focused on the development of a parametric design for the initial RVAD prototype. Based on classical pump theory, the prototype features a mixed-flow impeller.
The RVAD was optimized using a surrogate-based framework to maximize efficiency and minimize hemolysis. Three surrogate models were trained on computational fluid dynamics (CFD) results obtained from a set of design points generated via Latin Hypercube Sampling. The CFD simulations were validated against the RVAD experimental characteristic curve. The surrogate models enables rapid prediction of pressure drop, efficiency and hemolysis across a wide design space. A genetic algorithm was then used to identify the optimal geometry, shown in Figure 1.
To evaluate the device's performance under pathological conditions, surrogate models were coupled with a lumped parameter model of the cardiovascular system simulating isolated RVF. This enabled the assessment of the RVAD functioning under two possible placements and provided insight into the effects of different operating strategies. The placements studied are illustrated in Figure 2.
The results demonstrated that the RVAD prototype can provide effective right heart support, maintaining both efficiency and hemocompatibility across a broad range of operating conditions.
Figure 3 shows a comparison between the pressure waveforms obtained with the two placements and the improvements in the pressure-volume loops with respect to pathological conditions.
Overall, the findings of this thesis represent a step forward in the development of a dedicated, durable solution for RVF patients.

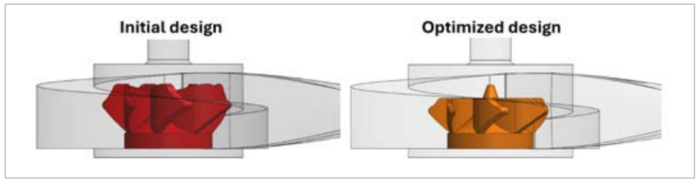

Fig.1 - Comparison between initial and optimized RVAD design.

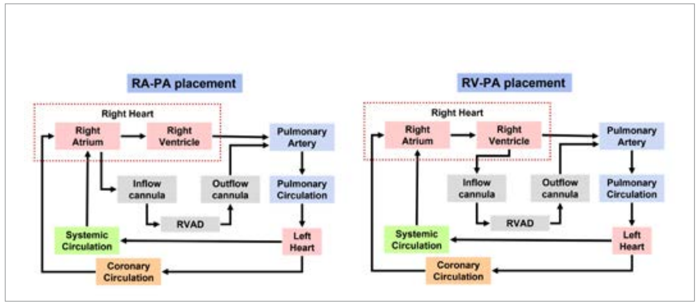

Fig.2- Diagrams of the two configurations implemented for LPM-RVAD coupling: RVAD implanted between the right atrium and the pulmonary artery (RA-PA placement) and RVAD implanted between the right ventricle and the pulmonary artery (RV-PA placement).

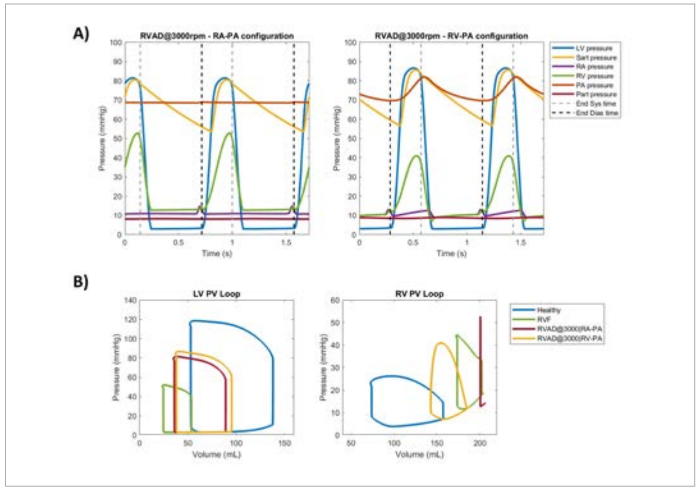

Fig.3 - A) Comparison of pressure waveforms under constant support at 3000 rpm with the two RVAD placements. B) Pressure-volume loops for the left and the right ventricles under healthy, RVF and RVF with RVAD at 3000 rpm in both implantation configurations.

DIGITAL HOLOGRAPHIC MICROSCOPY: ALGORITHMS FOR PHYSICS-BASED SIMULATION AND AI-DRIVEN RECOGNITION

Alessandro Molani - Supervisor: Andrea Aliverti

Co-Supervisor: Francesca Pennati

Digital holographic microscopy (DHM) is a non-invasive and quantitative imaging technique that has attracted growing interest for studying transparent microscopic biological samples without the need for labelling. The hologram, formed by the interference between light scattered by the specimen and the unperturbed reference light, encodes not only intensity but also phase, which is related to the sample's optical properties and morphology. The DHM imaging capabilities combined with its potential for compact implementation has made it attractive for biosafety, environmental monitoring and biohazard detection, an area still lacking reliable, rapid and field-deployable tools.

However, DHM development is fragmented due to missing standardization for acquisition protocols and hardware configurations, a scarcity of publicly available annotated holographic datasets and the absence of reproducible approaches to system optimization and image analysis. Computer simulations play a relevant role in addressing these limitations, offering the possibility to systematically study how system parameters influence holographic features, guiding hardware design decisions and supporting the generation of synthetic, fully annotated datasets for algorithm training and testing. Equally important for the reliable analysis of DHM is to tailor the approaches to the inherent variability introduced by sample preparation and hardware configuration.

This PhD research explored this application by addressing the entire development process, from simulation-based system design and synthetic dataset generation to sample preparation and image analysis using both traditional and artificial intelligence (AI) approaches.

The first part of the research focused on developing simulation tools to model light-particle interactions and analyse how particle and system parameters influence the resulting holographic signals. Numerical simulations based on Lorenz-Mie theory were implemented to systematically explore the effects of variables such as particle size, refractive index, axial position and medium properties. Although based on ideal assumptions of spherical and non-absorbing particles, these simulations provided an efficient and theoretically grounded investigation of detection limits. The results showed that refractive index contrast plays a significant role in detectability. For example, smaller particles suspended in air produce stronger holographic signals compared to those in water. Moreover, despite increased hologram complexity in denser samples, statistical analyses of overlapping patterns suggested that even simple features could help distinguish particle classes, highlighting the possibility of bypassing reconstruction and feeding lightweight machine learning (ML) models for classification.

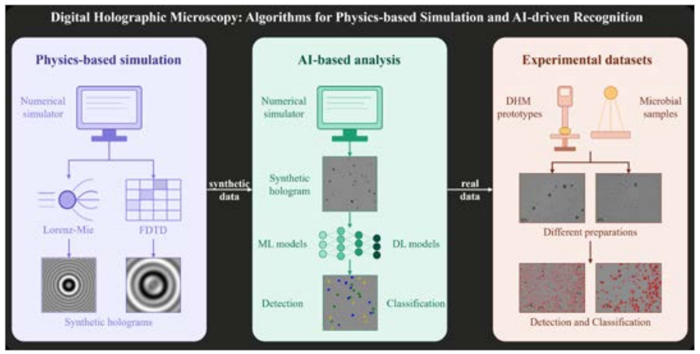

Fig.1 - Overview of the research workflow. From left to right: physics-based simulation via Lorenz-Mie theory and FDTD method; AI-based detection and classification on synthetic holographic datasets; experimental acquisition and analysis of real holograms from microbial samples using custom DHM prototypes.

However, biological particles such as bacteria, spores and viruses deviate from idealized spherical geometries and have internal structural heterogeneity. Recognizing the limitations of Lorenz-Mie theory for modelling more realistic objects, the second part of the research introduced a simulation framework using the finite difference time domain (FDTD) method. This simulator featured a modular approach to form complex microorganisms, assembling subcomponents with distinct geometrical and optical properties, the latter stored in an implemented database of refractive indices and absorption coefficients. The FDTD simulator represents a flexible and expandable tool for exploring DHM applications in constrained scenarios, where direct data acquisition may be limited by biosafety or ethical concerns.

Building on the simulation outputs, the next phase of the work involved the generation of synthetic datasets to support the development and evaluation of ML and deep learning (DL) analysis pipelines for object detection and classification under a wide range of conditions. An evaluation framework was introduced to assess algorithm performance across varying levels of particle concentration, micrometric and sub-micrometric particle sizes and acquisition parameters. The results illustrated the potential and limits of AI-based image analysis for DHM, especially in scenarios involving high-density particle distributions and overlapping interference fringes. The synthetic datasets and evaluation tools can contribute to the ongoing efforts toward standardization in DHM, offering a reproducible baseline for future algorithm development and pre-testing.

While simulation studies were informative, transitioning to real data is essential to address the full complexity of DHM systems. Experimental datasets were acquired using multiple custom DHM prototypes developed as part of the European project HoloZcan. Real datasets highlighted the variability introduced by hardware configurations and sample preparation protocols, significantly affecting the characteristics of the recorded holograms. Sample aggregation emerged as a primary obstacle in dried samples, with dilutions, low spotting volumes and non-ionic surfactants offering only partial mitigation. Imaging in liquid media helped reduce aggregation but introduced new challenges such as motion blur and defocusing. These observations underlined the importance of context-aware image analysis pipelines for successful deployment of DHM in real-world settings, which should consider and adapt to highly heterogeneous experimental conditions and resulting holograms. Preliminary analyses of the real datasets confirmed that performance is sensitive to variations in acquisition settings, sample preparation and hologram quality. These early results serve as a proof of concept and provide a foundation for the development of more robust, generalizable analysis frameworks.

Together, the results highlight the need to integrate accurate simulations, standardized protocols and effective algorithmic analysis for DHM systems to advance in environmental monitoring and biosafety applications.

QUANTITATIVE IMAGING AND MULTI-SCALE MODELLING IN PERSONALIZED RADIOTHERAPY

Letizia Morelli - Supervisors: Guido Baroni, Chiara Paganelli

Quantitative imaging (Q-imaging) represents a powerful tool for non-invasive tumor characterization, providing quantitative imaging biomarkers (QIBs) that extend beyond conventional anatomical assessment. When integrated with computational modeling approaches, these methods enable the characterization of tissue properties across multiple spatial scales, from the macroscopic (voxel-level) to the microscopic (sub-voxel) level, allowing insights into tumor heterogeneity, aggressiveness, and response to therapy. Diffusion-Weighted Magnetic Resonance Imaging (DWI) represents one of the most relevant Q-imaging modalities, as it enables the non-invasive quantification of water molecule mobility, which reflects underlying variations in tissue composition and microstructural heterogeneity. The integration of Q-imaging with multiscale modeling is particularly relevant in the context of radiotherapy (RT) and particle therapy (PT), where spatial and biological heterogeneity significantly impact treatment response.
This PhD thesis investigates the integration of Q-imaging and multiscale modeling for biologically informed and personalized approaches in PT.
A comprehensive computational framework was developed, combining advanced QIBs, Monte Carlo simulations, and validation strategies encompassing both macroscopic and microscopic scales.
At the macroscopic level, radiomics and dosiomics analyses were performed to extract quantitative features from DWI and both biological dose maps and dose-averaged linear energy transfer (LET_d) maps, enabling the development of models for tumor characterization and treatment outcome prediction.
At the microscopic level, biophysical modeling based on Monte Carlo simulations of water diffusion within synthetic cellular substrates allowed estimation of tissue-specific microstructural parameters from routine DWI data. These models were applied to investigate biological aggressiveness, recurrence risk, and to map intratumoral subregions (habitats) associated with unfavorable outcomes (Figure 1). In addition, a dedicated framework for histological validation was implemented. Combining *in vivo* MRI and *ex vivo* imaging with patient-specific 3D-printed molds, this approach enabled accurate alignment between DWI-derived parameters and histological cell density, providing insight into the biological accuracy of different DWI models (Figure 2). Finally, a simulation tool based on the Geant4 Monte Carlo codes was developed to model microscale radiation-matter interactions in realistic multicellular geometries derived from microscopy, providing a framework for future integration between imaging and radiobiological modeling.
Overall, this thesis provides a comprehensive framework integrating Q-imaging and multiscale modeling to enhance tumor characterization and guide biologically informed, personalized strategies in particle therapy.

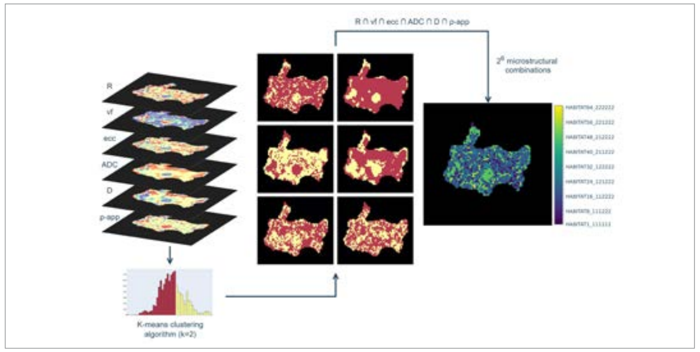

Fig.1 - Schematic workflow of the microstructural habitat identification process. Tumor voxel-wise maps of microstructural parameters (i.e., R, vf, ecc, ADC, D, and ρ-app) were clustered into binary classes (low=1, high=2) using K-Means (k=2), and combined to generate a comprehensive set of 64 microstructural habitats (26), each representing a unique combination of features.

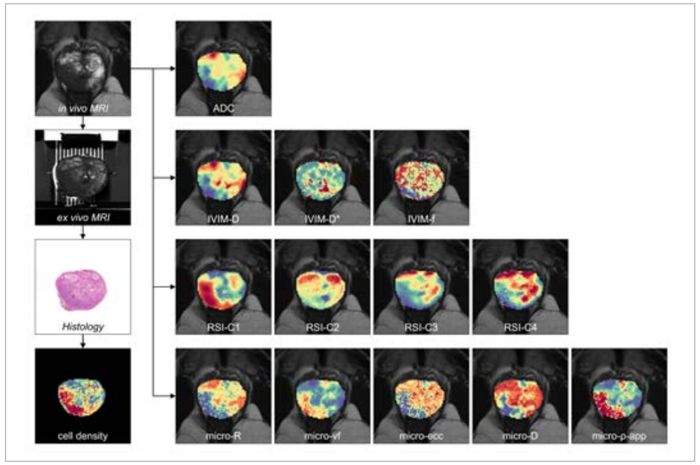

Fig.2 - Representative parametric maps extracted from in vivo DWI using ADC (Apparent Diffusion Coefficient, row-1), IVIM (Intravoxel Incoherent Motion, row-2), RSI (Restriction Spectrum Imaging, row-3), and the microstructural model (row-4). Each column shows the voxel-wise distribution of a specific parameter. The histology-derived cell density map is shown for comparison, after co-registration and resampling to the MRI space.

DESIGN, DEVELOPMENT AND IN VIVO VALIDATION OF THE “OBJECTIVE-ON-A-CHIP”:
AN IMPLANTABLE MINIATURIZED IMAGING WINDOW FOR LONGITUDINAL STUDIES OF FOREIGN BODY REACTIONS

Alessandra Nardini - Supervisor: Manuela Teresa Raimondi

In the framework of biomaterial, small-molecule, and drug testing, the ethical concerns surrounding the use of animal models have driven the development of advanced in vivo imaging techniques aimed at reducing the number of laboratory animals required for statistically significant validation. The first step in achieving this is to assess the foreign body reaction (FBR), which is the immune response that occurs naturally when the host identifies implanted materials as “foreign objects”. This process involves complex inflammatory signaling, sometimes leading to fibrosis and collagen encapsulation, which can impair the functionality of the implant. Current methods for assessing the FBR to implants are limited by invasive procedures and the need for multiple animal sacrifices. Intravital fluorescence microscopy has emerged as a critical tool in this domain, enabling real-time, high-resolution imaging of immune responses within living organisms. However, despite significant advancements in non-linear microscopy technologies, in vivo fluorescence microscopy faces persistent challenges, particularly in terms of limited tissue penetration due to light scattering and optical aberrations induced by surrounding animal tissues. These limitations hinder the ability to achieve deep-tissue imaging, which is essential for the comprehensive evaluation of biomaterial biocompatibility and drug efficacy.
This Ph.D. research contributes to address these challenges by the development and validation of a novel implantable imaging window that integrates an array of microlenses onto 3D microscaffolds for intravital imaging enhancement. The device is fabricated by the two-photon polymerization (2PP) of a biocompatible photoresist called SZ2080 and it is engineered to function as an advanced in-situ microscope objective. The microlenses are designed to act as in vivo low-magnification microscope objectives. They will serve for optical aberration correction and penetration depth deepening, thereby overcoming the traditional limitations of in vivo imaging. The microscaffolds provide a precise spatial framework that will allow for consistent repositioning of the field of view (FOV) during longitudinal studies on living organisms8.
Most of my Ph.D. work is part of the IN2SIGHT project, an EU-funded initiative aimed at revolutionizing biocompatibility testing through innovative imaging technologies. By enabling detailed, longitudinal imaging of immune responses in situ, the developed device is expected to significantly reduce the number of animals required for biomaterial testing, lower associated costs, and improve the reliability and quality of biocompatibility assessments.
Herein, my research involved several key steps. First, a robust protocol for micro-optics prototyping was developed and optimized for non-linear excitation microscopy. The microlenses were fabricated with precise geometries and were preliminarily tested in vitro on living cells to validate their optical properties. These tests demonstrated that the microlenses effectively mitigated optical aberrations and efficiently excite fluorescence cells by non-linear excitation providing high-quality imaging.
Following successful functionality in vitro validation, the microlenses were integrated into a microstructured imaging window. The design and the prototyping process of the whole device were improved in accordance with the experimental conditions aiming at high optical quality, stable and high resolution microlenses and microscaffolds. This refinement significantly improved manufacturing efficiency and precision. Following, the integrated device was characterized in vitro showing improved fluorescence imaging of live cells with high signal-to-noise ratios (SNRs) and providing the capacity to track the behavior of cells over time and space. These remarkable results led to the in vivo validation of the device through its implantation in living chicken embryo models to assess the device’s biological integration and imaging capabilities.
The system’s ability to perform high-resolution imaging through the microlenses sharpening the acquisitions and improving the SNR demonstrated the greater efficiency of the optical system in sample excitation and light collection. This ex vivo validation is pivotal in providing a useful tool for the further real-time observation of angiogenesis and for instance the effects of vascular drugs, like Doxorubicin, on an in ovo model of chicken embryo chosen for its cost-effectiveness, ethical advantages, and rapid tissue development.
To this purpose, in the final part of my Ph. D. activity, my work was dedicated to the development of an alternative in ovo model for drug testing, with a primary focus on defining and optimizing protocols to increase the survival rate of chicken embryos. This involved assessing drug administration procedures and dosage to determine the mean lethal dose (LD50), thereby identifying an effective concentration window that allows observation of drug effects without causing embryo mortality. Hence, I exploited the Doxorubicin administration onto the chicken embryo CAM to assess the drug acute and systemic toxicity by first analyzing the weight of the embryos and organs, then histological sections of the target organs. Furthermore, considering the toxic effects of Doxorubicin on vascular integrity, this model holds significant potential for future integration with the developed implantable device. Such a combination would facilitate the intravital investigation of Doxorubicin’s impact on vascularization within a living system using the proposed optical system.
In conclusion, the research presented in this thesis offers a significant advancement in the field of biomedical optics and tissue engineering. The implantable micro-optical system developed herein provides a powerful tool for real-time, high-resolution imaging of the host’s response to implanted materials, paving the way for more ethical, efficient, and accurate biocompatibility testing. In future, the final phase of the research will involve the implantation of the device into mouse models to evaluate its long-term performance in a mammalian system. This included assessing the fibrotic reaction, vascularization, and immune cell recruitment at the implant site, providing critical insights into the device’s biocompatibility and effectiveness in real-world applications.

ADVANCED MODELING TOWARDSPERSONALIZED PARTICLE THERAPY

Giovanni Parrella - Supervisor: Guido Baroni

Particle therapy (PT) provides unique physical and biological advantages for treating radioresistant and deep-seated tumors, offering highly conformal dose delivery through the Bragg peak and enhanced biological effectiveness via high linear energy transfer (LET). Despite these benefits, patient treatment response remains variable, highlighting the need for advanced personalization strategies in PT. This thesis aims to explore and develop complementary approaches to improve treatment personalization by integrating anatomical adaptation, predictive modelling, and biologically targeted planning. Accurate adaptation to inter-fractional anatomical changes was investigated through MRI only workflows, where synthetic CT images generated via a conditional generative adversarial network enabled reliable dose recalculations for abdominal cancer patients, supporting adaptive planning without additional CT scans. To better predict clinical outcomes, advanced dosiomics and survival models were developed using quantitative features extracted from RBE-weighted dose and dose-averaged LET (LETd) maps in patients with sacral and skull base chordomas treated with carbon ions radiotherapy (CIRT). While LETd emerged as a potential predictive biomarker (though requiring validation on larger and independent cohorts to confirm generalizability) further improvements in treatment outcome predictions was achieved by personalizing tumor control probability (TCP) models with clonogenic cell counts estimated from diffusion MRI rather than assuming a uniform distribution. Biologically targeted treatment planning approaches were then explored including LETd optimization and dose painting based on voxel-wise cell count estimates. LETd optimization enhanced LET coverage on the GTV without compromising dose robustness or sparing of organs at risk, demonstrating clinical feasibility and potential for improved tumor control (Figure 1). Similarly, dose painting allowed to escalate dose in more aggressive regions, while enhancing LET in the GTV and sparing OARs (Figure 2). Despite robust evaluation of such plan is required, the presented work showed a great potential for biologically targeted treatments towards an increased therapeutic effect. Overall, this work establishes a comprehensive framework that integrates MRI-guided anatomical adaptation, treatment outcome modelling, and biologically targeted planning, towards an enhanced personalization of CIRT treatments.

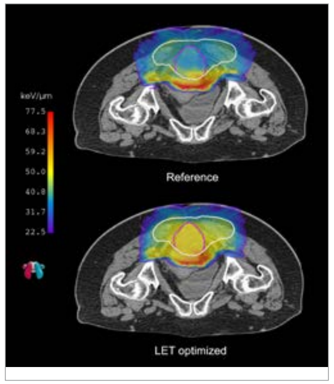

Fig.1 - Comparison of dose-averaged linear energy transfer (LETd) maps from carbon ions treatment plans of sacral chordomas. A conventional plan obtained with dose-based objectives only (top) is compared against a plan obtained with LETd optimization (bottom). Overlayed in white the clinical target volume, in red the gross tumor volume.

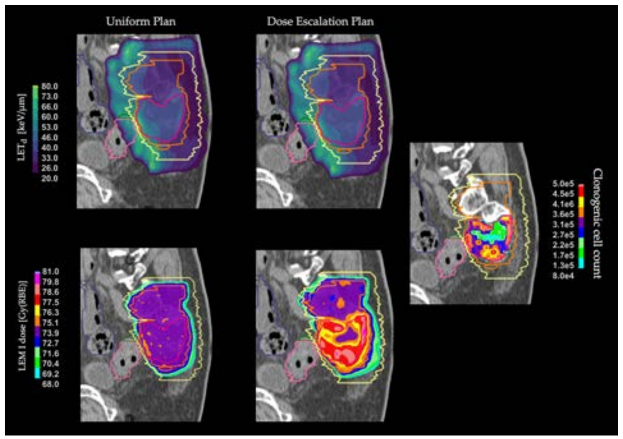

Fig.2 - Comparison of effective dose and dose-averaged linear energy tranfer maps from a conventional uniform-dose plan (left), and a biologically-guided dose escalation plan (center). On the right, the clonogenic cell count map estimated from diffusion-weighted magnetic resonance imaging. Overlayed in yellow and orange the low- and high- risk clinical target volumes, in red the gross tumor volume, in pink the sigma. LEM-I = Local effect model.

DESIGN OF 3D *IN VITRO* MODELS MIMICKING HEALTHY AND TUMORAL BREAST TISSUES

Matteo Pitton - Supervisors: Silvia Farè, Lorenza Draghi

Breast cancer is nowadays the most diagnosed cancer pathologies in the Western World. For instance, there is an urgent need to understand the complex intra- and inter-cellular mechanisms which are involved in the pathology origin and progression. During breast cancer progression, alterations in the surrounding adipocytic extracellular matrix (ECM) occur, inducing disequilibrium in the physiological homeostasis.
A cascade of intracellular pathways is activated in epithelial breast cells which release chemical compounds which trigger the increased/decreased accumulation of ECM components, e.g. collagens and glycosaminoglycans (GAGs). Consequently, the stiffness of the surrounding ECM increases. Additionally, some chemical compounds trigger the accumulation of hypoxia-inducing factors, whereas new tumoral vascularization is formed which appear leakier and more fenestrated. In these fenestrations, breast cancer cells detach from the primary tumor site and consequently migrate in other anatomical districts forming metastasis.
The possibility to study the origin and the progression of the breast cancer pathology has been investigated by using 3D *in vitro* models. A review of the most popular approaches for the Biofabrication of 3D *in vitro* models for breast tumor microenvironment (TME) have been carried out. Spheroids allowed the possibility to generate 3D models of the TME, due to their morphology, characterized by an inner necrotic core and a surrounding proliferative shell. Alternatively, organ-on-chips (OoCs) emerged as possible solutions for the studying of anticancer drugs or novel therapies, by culturing breast cancer cells in microchambers connected by microchannels. However, limitations emerged from these technologies. In this context, engineered 3D constructs emerged as promising solution to bypass mentioned drawbacks and address the possibility to resemble the *in vivo* breast TME, co-culture pathological and healthy cells, and test novel anticancer drug therapies. Among these engineered constructs, natural hydrogels resulted the most suitable solution to build a versatile *in vitro* microenvironment which surrounds cells with a biocompatible matrix with tunable features.
Hydrogels can be designed and prepared with different crosslinking strategies. Light photocrosslinking offers the possibility to fabricate natural hydrogels with photoinitiator inclusion in the polymeric mixture, ensuring the direct encapsulation of cells. However, the selection of adequate parameters requires a huge work of optimization, since different parameters play an important role in tuning hydrogels properties, e.g. exposure time, light source, distance to light, photoinitiator concentration, polymer concentration. For instance, we carried out a comparison between traditional UV technique and a more innovative visible light approach to obtain gelatin-based hydrogels. In addition, a deep characterization of the obtained hydrogels has been performed by varying different parameters for each considered approach. A library of hydrogels with various properties has been obtained, evaluating their *in vitro* stability, mechanical properties and *in vitro* cytotoxic effects.
To target the breast adipocytic ECM, natural biomaterials need to be selected to faithfully mimic the chemical composition of the tissue. One of the main advantages of natural hydrogels resides in the possibility to build functional substrates aiming to resemble the chemical, physical, mechanical, and biological composition of the targeted tissue. Photocrosslinked hydrogels have been designed and developed to resemble the features of breast adipose ECM, by involving gelatin and hyaluronic acid as natural biomaterials (Figure 1). Gelatin mimics the proteinaceous collagen part of adipocytic ECM, whereas hyaluronic acid recapitulates the presence of GAGs. Gelatin and hyaluronic acid have been photocrosslinked in pristine or hybrid (gelatin/hyaluronic acid) hydrogels by involving visible light exposure to trigger the photocrosslinking process. A library of versatile hydrogels has been obtained, with a wide range of physical, mechanical and biological properties that suit those exploited by adipocytic *in vivo* ECM, from healthy ECM to pathological TME. Healthy 3T3-L1 preadipocytes have been encapsulated and their proliferation rate and differentiation ability in mature adipocytes have been investigated, depending on the photoinitiator concentration and polymeric combination of the obtained hydrogels.
The hydrogel-based models offer a versatile and tunable platform to address the mimesis of the adipocytic ECM in different stages. However, the degree of complexity of the model can be enhanced, aiming to build a promising platform to investigate the origin and progression of the breast cancer pathology, as metastasis occurrence. For this purpose, a core-shell model has been designed with an inner portion, made of alginate, and an outer layer formed by crosslinked gelatin (Figure 2). The alginate core serves as non-adhesive portion to allow the migration of MCF7 breast cancer cells, previously encapsulated in the alginate polymeric solution, whereas the crosslinked gelatin outer shell offers adherent RGD motifs that allow the possibility for cancer cells to anchorage and form tumor-like aggregations in the shell layer. The ability of breast cancer cells to proliferate and migrate has been evaluated. The optimized core-shell model offers a versatile platform to model the progression of the pathology and represents a valuable and promising tool to test anticancer drugs and novel therapies against breast cancer.
The increased degree of complexity of hydrogel-based 3D *in vitro* models requires the incorporation of an efficient vasculature-like network to promote a dynamic flux of nutrients, oxygen, and metabolites into the whole polymeric construct. Two different methods of vascularization have been implemented and characterized. The first vascularization approach requires the possibility to incorporate in gelatin-based hydrogels decellularized vegetal leaves with microchannels which resemble the dimensions of human capillaries and microcirculation. Contrarily, the second approach provides the embedded 3D printing of sacrificial Pluronic vasculature-like channels into a crosslinked gelatin-based supportive bath. Both approaches have allowed the obtainment of open internal channels in an adipocytic-like ECM, which have been manually perfused to ensure the patency of the network.

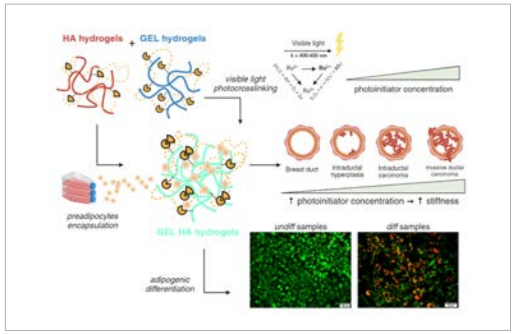

Fig.1 - Functionalized hyaluronic acid and gelatin were combined to obtain photocrosslinkable hydrogels to sustain 3T3-L1 preadipocytes growth and differentiation. The properties of gelatin/hyaluronic acid hydrogels were tailored by varying photoinitiator concentration and polymer composition. Adipocytic functionality seemed to be influenced by the properties of the hydrogels.

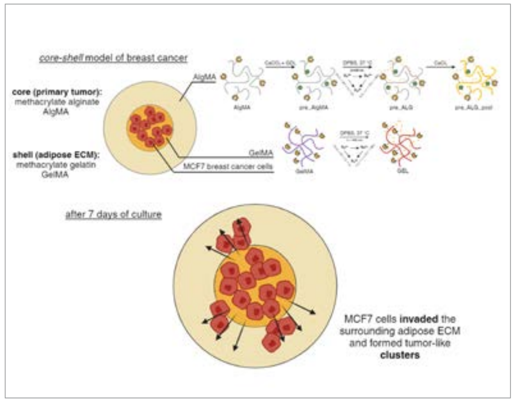

Fig.2 - Core-shell model for the investigation of the invasiveness of breast cancer cells in a surrounding matrix mimicking breast tissue.

EARLY DETECTION AND DISEASE MONITORING: TECHNICAL CHALLENGES AND CLINICAL APPLICATIONS OF POINT-OF-CARE (BIO)SENSOR TECHNOLOGIES

Andrea Rescalli - Supervisor: Pietro Cerveri

Co-Supervisor: Francesco Cellesi

Point-of-care testing technologies are considered a crucial tool that enables accessible, decentralized healthcare. Their potential to deliver timely and actionable information makes them especially valuable in the contexts of early diagnosis and chronic disease monitoring. However, translating sensing technologies from the laboratory to the clinic remains challenging, requiring not only analytical accuracy but also robustness, simplicity, and system-level integration.

This doctoral thesis presents two complementary approaches to advancing point-of-care diagnostics through innovative sensing strategies and data-driven analysis. The first focuses on diabetes monitoring via glycated albumin (GA), a mid-term biomarker offering insight into glycemic control over 2–3 weeks. To address the limitations of current assays, in particular their reliance on enzymatic digestion and multi-step workflows, a single-step electrochemical sensor was developed using a redox probe-labeled aptamer capable of binding both glycated (GHSA) and non-glycated albumin (HSA). The method exploits the distinct binding kinetics of the two analytes: a novel analytical parameter, termed evolution, captures signal changes over time and enables accurate determination of clinically relevant glycation ratios (10%, 20%, and 40%) across physiological albumin concentrations, without the need for enzymatic steps or multiple assays (Fig. 1).

The second part of the work targets early disease detection through non-invasive analysis of volatile organic compounds (VOCs) in exhaled breath. A temperature-modulated electronic nose (eNose) was developed using commercially available metal oxide semiconductor sensors. Temperature modulation, in particular a square-triangular waveform pattern, was shown to significantly enhance the sensors selectivity and sensitivity when exposed to individual analytes such as methane, carbon dioxide, and butanone. Building on this foundation, the system was then applied to ternary mixtures of clinically relevant organic compounds: acetone, isopropanol, and toluene. Two machine learning approaches were implemented to classify and quantify components in the mixtures. A model trained on all mixture classes achieved 91% accuracy. A re-mapping strategy based on single-analyte data reached 84% accuracy, demonstrating how the system can leverage what it learns from simple conditions to understand complex ones (Fig. 2).

The work addresses the design of point-of-care platforms for disease monitoring and early diagnosis, contributing to the development of compact, reliable, and versatile diagnostic tools ready for real-world application.

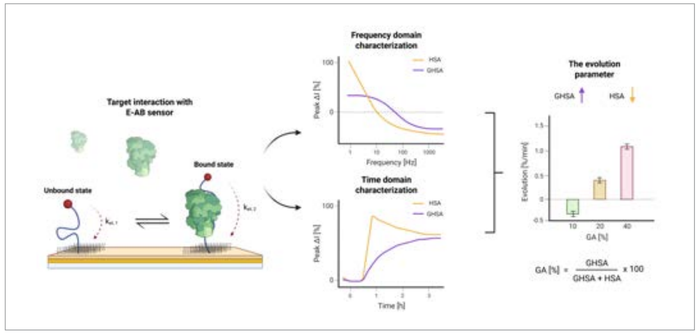

Fig.1 - Schematic representation of the proposed electrochemical aptamer-based (E-AB) sensor and methodology. Binding-induced conformational changes of the aptamer alter the electron transfer rate (ket) of the redox probe attached to it. These variations can be monitored across different frequencies, and in time. HSA and GHSA produce different responses. The evolution parameter accounts for these differences by tracking how the signal evolves in time at certain frequencies, and allows to distinguish between different percent GA ratios.

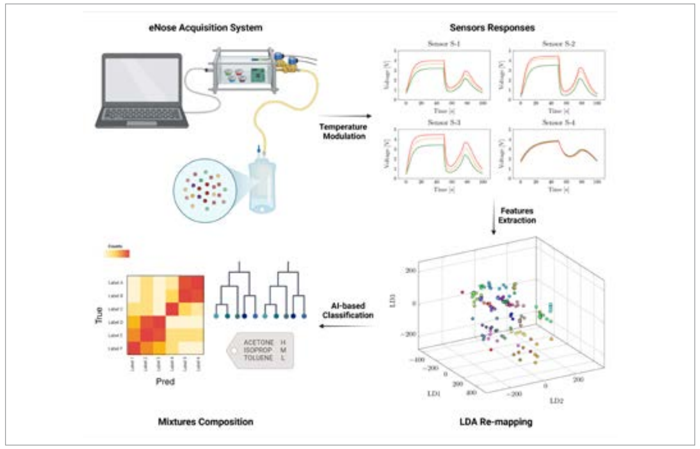

Fig.2 - Overview of the AI-based eNose pipeline for VOC mixture analysis. Gas samples containing VOCs at varying concentrations are introduced into a custom eNose acquisition system equipped with four temperature-modulated MOS sensors. Features extracted from each sensor response are projected into a reduced 3D space via linear discriminant analysis (LDA), and an AI-based classifier identifies the composition and concentration range of each compound within the mixture.

PATIENT-SPECIFIC SOLUTIONS FOR MAXILLOFACIAL AND ORAL SURGERY: IMAGE-BASED MODELLING AND DESIGN FOR 3D-PRINTED DEVICES

Ilaria Rota - Supervisor: Dario Gastaldi

Orthognathic surgery is a well-established clinical procedure aimed at correcting maxillofacial deformities and malocclusions in order to restore proper anatomical relationships and functional balance of the craniofacial system. During the surgical procedure, the maxilla is carefully resected and repositioned to achieve an optimal occlusion tailored to the patient's specific anatomy. Once the desired position is obtained, titanium plates and screws are commonly used to stabilize and fix the maxilla, ensuring mechanical support during the healing phase.
In standard orthognathic practice, fixation devices such as plates and screws are often left in place after surgery to avoid subjecting the patient to an additional removal procedure, which could cause discomfort and increase clinical risks. However, once sufficient bone regeneration has occurred and the stability of the skeletal segments is restored, these devices may no longer be necessary. In some cases, removal becomes mandatory due to post-operative complications such as infection, irritation, or hardware failure. Furthermore, aesthetic concerns may arise over time, particularly in patients experiencing aging or significant weight loss, as the implanted devices can become palpable or visible beneath the skin.
The first part of this research focuses on the design and numerical optimization of patient-specific, 3D-printed titanium plates for orthognathic applications. A detailed finite element model (FEM) was developed based on pre-operative computed tomography (CT) scans of a clinical case. The model incorporated bone segments, fixation screws, and customized titanium plates designed to match the patient's anatomy. The biomechanical performance of the system was evaluated under both static and cyclic loading conditions. Fatigue behavior was assessed using established criteria, including the Matake and Fatemi–Socie approaches, to account for multiaxial stress states. The fatigue life of the plates was estimated through a ad-hoc MATLAB implementation. Key geometric parameters, such as plate thickness and fillet radius, were iteratively modified to enhance fatigue resistance while preserving anatomical compatibility and surgical feasibility, as shown in Figure 1. The optimized designs demonstrated improved mechanical reliability and sufficient fatigue life compared to the initial configurations.
Guided bone regeneration (GBR) is another widely used surgical technique aimed at augmenting bone volume in cases of alveolar ridge defects. The core principle of GBR involves the use of a barrier membrane to isolate the bone defect from surrounding soft tissues. This prevents the rapid ingrowth of connective tissue into the defect site, thereby allowing osteogenic cells to proliferate and form new bone. Titanium membranes are typically employed in the presence of large defects due to their superior mechanical properties, which help maintain the necessary space for bone regeneration. These membranes also stabilize the graft material, usually composed of a combination of deproteinized bovine bone and autologous bone, promoting effective healing.
Despite their effectiveness, GBR procedures using titanium membranes require a second surgical intervention for device removal prior to dental implant placement. Additionally, in both orthognathic surgery and GBR applications, the radiopacity of titanium can interfere with post-operative imaging, complicating the evaluation of bone healing through X-ray or CT examinations. For these reasons, there is a growing interest in developing biodegradable alternatives capable of providing adequate mechanical performance without the need for removal.
The second part of this work investigates the feasibility of using biodegradable composites based on polylactic acid (PLA) reinforced with hydroxyapatite (HA) as a substitute for titanium in the fabrication of fixation screws and GBR membranes. These devices were manufactured using fused deposition modelling (FDM), a widely accessible additive manufacturing technique. Samples with varying HA content (0%, 20%, and 35% by weight) were produced and analyzed in terms of printing fidelity, surface quality, and micro-mechanical properties. The influence of processing parameters, such as extrusion temperature and filler content, was systematically evaluated to determine their effect on both printability and mechanical performance.
In addition, the study explored the fabrication of patient-specific membranes and fixation screws using FDM, including surface roughness measurements and physical characterization of the composite material. The degradation behavior of the PLA-based materials was also investigated over a period of 24 days in phosphate-buffered saline (PBS), simulating physiological conditions. Changes in mass, molecular weight, and mechanical properties were monitored to assess the stability of the material over time. The results indicated that PLA/HA composites with 20% HA content represent a promising candidate for temporary, patient-specific devices in maxillofacial surgery, offering a balance between mechanical strength and controlled biodegradability.
The final part of the work presents a comprehensive digital workflow (Figure 2) for image-based, patient-specific modelling, aimed at supporting the biomechanical evaluation of 3D-printed devices and their interaction with bone tissue. It includes the calibration of bone density using cone-beam computed tomography (CBCT) data, segmentation of anatomical structures, and assignment of heterogeneous, patient-specific material properties. Finite element analysis (FEA) was then performed to simulate the mechanical response of the bone–device system. By accounting for the anisotropic and non-homogeneous nature of bone tissue, the model provides a more accurate prediction of stress distribution, displacement, and strain, ultimately contributing to improved design and clinical outcomes.

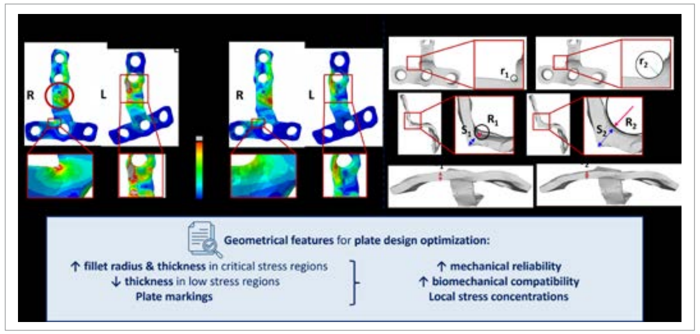

Fig.1 - Pre- and post-optimization results from cyclic analysis of the two plates (left), and corresponding pre- and post-optimization designs (right).

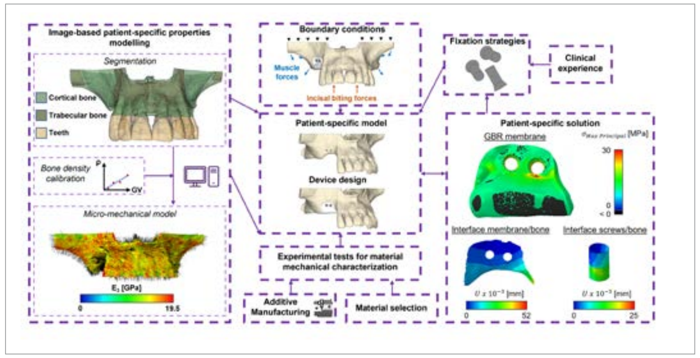

Fig.2 - Workflow of image-based patient-specific modelling in oral surgery.

METHODOLOGICAL ADVANCES IN THE PRECISION PSYCHIATRY ROADMAP: TOPOLOGICAL STRATIFICATION USING GENE -ENVIRONMENT - BRAIN FEATURES

Emma Tassi - Supervisor: Anna Maria Maddalena Bianchi

Psychiatric disorders represent one of the most significant global health challenges, affecting over 970 million people worldwide and imposing a substantial economic burden. Despite advances in neuroimaging and multi-omics technologies, clinical practice still relies heavily on symptom-based diagnostic systems that suffer from diagnostic instability, marked heterogeneity within categories, and limited predictive power for treatment response. In recognition of these limitations, the National Institute of Mental Health (NIMH) Research Domain Criteria (RDoC) framework has emphasized the need for biologically guided approaches that move beyond descriptive symptom clusters toward mechanistically grounded markers. Building on the RDoC foundation, which reconceptualizes mental health and illness as varying degrees of dysfunction across interconnected psychological and biological systems, precision psychiatry has emerged as a transformative paradigm driven by advances in data acquisition and artificial intelligence analysis tools. Aligned with emerging precision psychiatry roadmaps that emphasize continuously evolving, biology-informed diagnostic frameworks, these computational approaches offer unprecedented opportunities to integrate genetic, neuroimaging, behavioral, and environmental information beyond traditional categorical diagnostic systems toward integrated, personalized, biologically informed interventions. The primary goal of the clinical practice is identifying reliable quantitative markers that enhance diagnostic precision, enable earlier detection, facilitate treatment selection, and monitor disease progression. However, despite demonstrated potential, computational psychiatry approaches face major technical challenges and clinical integration barriers. Current methods prioritize classification accuracy over explainability and clinical translation factors, therefore remain fragmented without comprehensive integrated pipelines. Key methodological challenges include site-related variability in multi-site studies, lack of robust multimodal and multi-scale feature integration frameworks, and limited availability of data-driven stratification approaches for translating complex multimodal data into clinically meaningful patient subgroups. To address these technical gaps, this thesis develops and validates the steps of a comprehensive end-to-end analytical framework for quantitative marker extraction in psychiatric disorders, integrating multi-site data harmonization, advanced multimodal feature extraction, and clinically relevant stratification strategies. The research advances three key methodological developments: (i) establishing robust harmonization frameworks for multi-site neuroimaging data using ComBat technique (ComBating batch effect) that effectively remove technical variability while preserving biologically meaningful signals across multiple spatial scales and imaging modalities; (ii) implementing integrated feature extraction strategies that capture both multi-domain relationships, across neuroimaging, genetic, environmental, and clinical variables, and multi-scale patterns within single neuroimaging domains; and (iii) developing data-driven stratification pipelines leveraging Topological Data Analysis (TDA) for unsupervised extraction of multimodal feature combinations and clustering of clinically meaningful subgroups. Two novel complementary TDA-based stratification frameworks were implemented to enable systematic patient subtyping and marker discovery. The proposed methodologies are tested across multiple psychiatric conditions, including mood, developmental and behavioral, brain-gut axis disorders, with applications focusing also on healthy control populations, including both single individuals and twin pairs. Comprehensive analyses demonstrate ComBat's effectiveness in harmonizing structural and functional neuroimaging measures across sites applied on healthy control samples, from voxel-level morphometry to resting-state networks. Advanced multivariate feature extraction approaches, including statistical twin modeling, Partial Least Squares methods, and TDA for Major Depression Disorder (MDD) stratification, reveal complex associations among genetic, environmental, and multimodal and multi-scale brain patterns, identifying transdiagnostic signatures across mood disorders. Furthermore, TDA-based pipelines applied on MDD sample simultaneously identify optimal predictive features and clinical patient subgroups, unifying feature discovery and stratification to bridge statistical modeling and clinical insights. Spatial Analysis of Functional Enrichment applied on TDA graph revealed domain-specific predictive patterns, with environmental, neuroimaging, and functional brain features respectively linked to symptom severity, medical comorbidities, and treatment resistance. In parallel, a community detection framework with cross-cohort validation confirmed these findings, simultaneously extracting predictive features and identifying clinically interpretable patient subgroups with distinct feature-outcome profiles. Taken together, these convergent results demonstrate that multimodal integration of genetic, environmental, and brain features is essential to disentangle psychiatric disorders' heterogeneity, establishing quantitative markers necessary for effective personalized stratification and the transition from symptom-based to biologically informed clinical practice. The developed methodologies provide robust, interpretable, and clinically relevant developments for each stage of a comprehensive computational framework aimed to advance precision psychiatry through integrated marker discovery and patient subtyping. By enabling more accurate patient stratification and marker discovery, this work contributes to moving psychiatry beyond symptom-based diagnostics toward biologically grounded and personalized clinical practice.

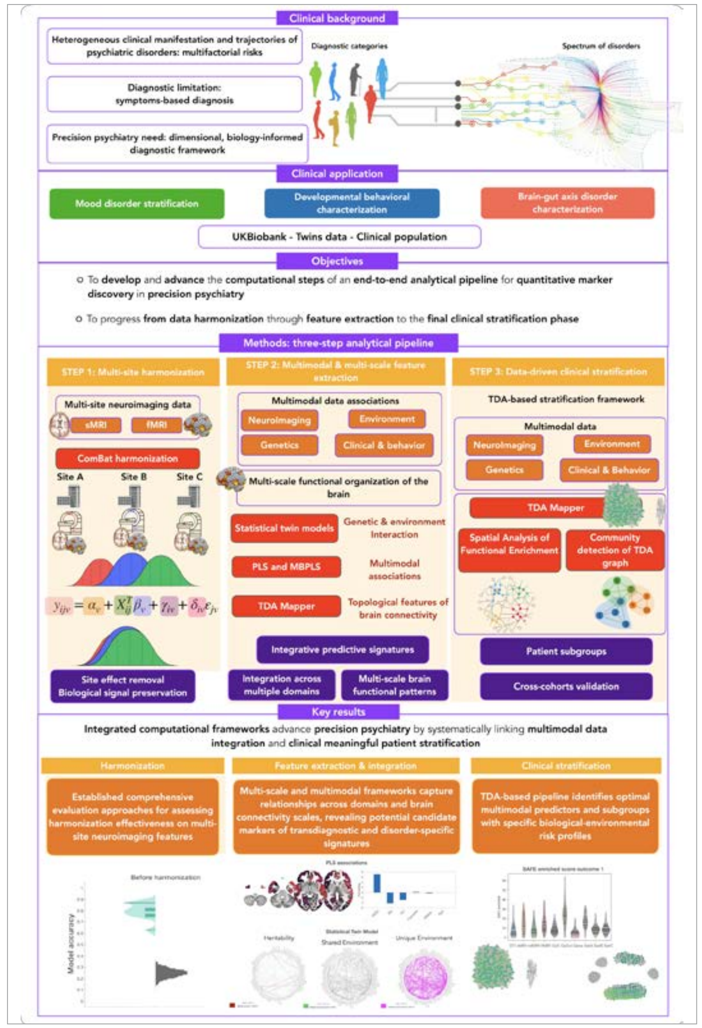

Fig.1 - Graphical Abstract