Poster abstracts

DTU Health Tech

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Contents

Bioinformatics

P01. Enabling Isoform-level Enrichment Analysis

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Group	Isoform Analysis
Tag	Pathway Enrichment, Transcriptome Analysis, Single-Sample GSEA

Alternative splicing allows genes to produce protein isoforms with distinct functions, localizations, and interactions. These isoforms play critical roles in cellular processes and disease mechanisms, influencing conditions such as cancer, Alzheimer's disease, type 2 diabetes, and obesity. Some isoforms contribute to drug resistance or drive disease progression, making them potential targets for isoform-specific therapies. Despite their biological significance, current systems biology approaches remain gene-centric and struggle to capture isoform-level changes due to limited annotation and the lack of specialized tools.

This project aims to bridge this gap by integrating isoform-level data into gene-set enrichment analysis (GSEA), a widely used tool for molecular disease studies. We will curate a data-driven isoform-set database and develop computational methods to incorporate isoform-specific changes into enrichment analysis. By leveraging single-sample GSEA, our approach will enable the identification of isoform-associated pathways and networks, offering novel insights into molecular mechanisms.

To support isoform-level research, we will also develop a bioinformatics tool that facilitates isoform-enrichment analysis with an intuitive interface and visualization modules. Benchmarking against gene-level tools will validate its accuracy and effectiveness. The project's key deliverables include a publicly accessible isoform-set database, an isoform-GSEA tool, and scientific publications demonstrating its applications. By shifting from gene-level to isoform-level enrichment analysis, this work will enhance our ability to study alternative splicing in health and disease, ultimately contributing to the identification of novel therapeutic targets.

P02. The Human Response Atlas

Kanakoglou, Dimitrios S. [1]; Vitting-Seerup, Kristoffer[1]; and Olsen, Lars Rønn[2]

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Group	Isoform Analysis Group
Tag	Bioinformatics

Alternative splicing enables a single gene to produce multiple mRNA isoforms, each encoding distinct protein variants that critically shape cellular function and tissue specificity. Despite estimates that over 90% of human genes undergo alternative splicing [1-3], most RNA-seq studies still focus primarily on gene-level analyses. To address this gap, we introduce the Human Response Atlas—a large-scale re-analysis of over 10,000 human RNA-seq datasets at isoform resolution. Inspired by landmark initiatives such as the Expression Atlas [4], the Human Protein Atlas [5], and The Cancer Genome Atlas [6], we are constructing a comprehensive resource that systematically identifies differentially expressed genes, differential isoform usage, and critical isoform switches, while evaluating their functional relevance in diverse biological states. By unifying these data, this project will provide a comprehensive framework for understanding how isoform-level regulation shapes both physiological and pathological processes.

Initially, supervised and unsupervised clustering methods will be employed to group studies by shared transcriptional responses, followed by meta-analyses within each cluster to uncover core expression patterns and isoform switches. By systematically identifying and evaluating these isoform events, we seek to clarify their roles in diverse physiological and disease contexts—ranging from neurodegenerative disorders to cancer. Ultimately, this work will yield: (1) a refined toolkit for large-scale isoform analysis, (2) an integrated atlas of human transcriptional responses, and (3) novel insights into how isoforms influence health and disease states.

Through close collaborations with experts across biomedical fields, the Human Response Atlas stands to advance the understanding of isoform-level regulation and hopefully inspire new diagnostic and therapeutic avenues. We envision that this resource will not only fill a critical knowledge gap but also empower researchers to integrate isoform analyses more routinely into their investigations, thus driving innovations in the field of precision medicine and more.

References

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P03. Can self-resolving canine neoplasia provide a breakthrough in cancer immunotherapy?

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Group	Immunoinformatics and Machine Learning
Tag	Bioinformatics, Immunotherapy, Immunology

Canine histiocytomas are rapidly proliferating tumors of Langerhans cells that predominantly affect young dogs. Although locally invasive, these tumors consistently undergo spontaneous regression through a T-cell-mediated immune response, presenting a valuable natural model for studying immunotherapy. However, three major challenges hinder this research. First, Dog Leukocyte Antigen (DLA) alleles exhibit high polymorphism within the same gene and high sequence similarity among different DLA genes, complicating allele identification. Second, the repertoire of peptides presented by DLA molecules remains poorly characterized, limiting the ability to determine which peptides are associated with specific DLAs. Third, tumor-specific antigens (TSAs) in canine histiocytomas have not yet been identified. This PhD project aims to address these challenges through the integration of genomics, transcriptomics, and immunopeptidomics. First, we will develop the first DLA typing method based on partial order graphs to represent allele diversity and incorporate novel variants, potentially identifying previously unrecognized DLA alleles. Second, we will construct an antigen presentation predictor to characterize peptide presentation in healthy and tumor tissues. Third, we will investigate the mutational landscape of histiocytomas to identify TSAs and employ scRNA-Seq to analyze T-cell responses and tumor antigen expression. Finally, we will assess the presence of circulating antibodies following tumor regression to evaluate long-term immune protection. By leveraging histiocytomas as a spontaneous cancer immunotherapy model, this research has the potential to advance targeted cancer treatments, including therapeutic vaccines and engineered T-cell therapies. Additionally, it will establish essential immunopeptidomics tools for canine cancer research, providing translational insights that bridge veterinary and human oncology.

P04. AbEpiTope-1.0: Improved antibody target prediction by use of AlphaFold and inverse folding

Joakim, Clifford

Group	
Tag	Immunology, Bioinformatics, Immunotherapy

B-cell epitope prediction tools are crucial for designing vaccines and disease diagnostics. However, predicting which antigens a specific antibody will bind to and their exact binding sites (epitopes) remains challenging. Here, we present AbEpiTope-1.0, a tool for antibody-specific B-cell epitope prediction, utilising AlphaFold-2.3 for structural modeling and inverse folding for machine learning models. On a dataset of 1,730 antibody-antigen complexes, AbEpiTope-1.0 outperforms AlphaFold in predicting modelled antibody-antigen interface accuracy. Importantly, by creating swapped antibody-antigen complexes per antibody-antigen complex using incorrect antibodies, we show that predicted accuracies are sensitive to antibody input. Furthermore, a model variant trained specifically for the task of differentiating between true and swapped antibody-antigen complexes shows enhanced performance favoring structures constructed with the correct antibody-antigen pair. The tool evaluates hundreds of structures in minutes, providing researchers with a valuable resource for selecting antibodies most likely to target given antigens. AbEpiTope-1.0 is freely available as a web server and software at https://services.healthtech.dtu.dk/services/AbEpiTope-1.0.

P05. Explainable AI for cancer classification of whole slide images

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Group	Precision Medicine
Tag	Personalised Healthcare, Precision Diagnostics, Bioinformatics

This study addresses a gap in computational pathology by developing an explainable and computationally efficient approach for lung cancer classification of two subtypes. We processed 14 WSI from two lung cancer subtypes using a workflow that integrates data preprocessing with explainable classification methods. By leveraging feature extraction techniques from specific (HistoEncoder) and non-specific (VGG16, InceptionV3 and ResNet50) pretrained models, our method combines unsupervised and supervised approaches to enhance interpretability. This research lays the groundwork for future exploration of feature extraction and model optimization, to enhance clinical decisions. Dimensionality reduction techniques, including PCA and t-SNE, were employed to analyze histopathological features, effectively distinguishing tile types and enhancing clustering accuracy with K-means, which identified two clusters corresponding to cancer subtypes. We trained and evaluated four supervised machine learning models: XGBoost, LightGBM, Artificial Neural Network and Random Forest. All models exhibited moderate to high predictive power and consistent classification decisions. Evaluation on an independent test set provided a thorough assessment of performance, generalizability and resistance to overfitting. We conducted explainability analyses of the supervised methods to elucidate the decision-making processes of each model. Finally, we compared the supervised models with unsupervised methods, aligning our findings with existing literature to highlight the strengths and limitations of each approach.

P06. Pangenome graphs for human health

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Group	Modern and Ancient Genomes
Tag	Personalised Healthcare, Precision Diagnostics, Bioinformatics

Different population groups exhibit distinct genetic traits and varying levels of genetic diversity. By analyzing mapped genetic variations within specific populations, it is possible to estimate the genetic origins of previously unidentified DNA. Identifying an individual's genetic ancestry can provide insight into potential health risks associated with their genetic background and contribute to understanding population movements throughout history. Traditional ancestry inference methods often rely on whole genome sequencing or large-scale genotype data, which can be computationally expensive and require high-quality genomic coverage.

Pangenome graphs provide a powerful framework for modeling genetic diversity across populations by representing multiple genomic sequences as a connected structure rather than a single reference genome. This approach captures population-specific variants, structural variations, and complex haplotype relationships, enabling more accurate ancestry inference. By aligning sequence reads to a pangenome graph, it is possible to trace the most likely genetic origin of observed variations. This method improves inference accuracy by accounting for diverse ancestries without relying on a linear reference genome, reducing reference bias.

P07. An Integrative structure-based approach to link diseases associated mutations to pathogenicity

Matteo Arnaudi

Group	
Tag	Bioinformatics, Personalised Healthcare, Digital Health

Pathogenicity annotations of germline mutations obtained through NGS techniques represent a significant advancement for healthcare and personalized medicine, in particular for disease treatment, genetic counseling, prevention, and monitoring. However, most part of them are Variants of Unknown Significance (VUS), which hinder patient care and therapeutic development due to challenges in determining their pathogenicity. Advances in Artificial Intelligence have led to the development of highly accurate pathogenicity classifiers for VUS classification. Nevertheless, these classifiers provide only probability scores, lacking the molecular mechanisms underlying mutation pathogenicity and limiting their clinical utility. This emphasizes the need for comprehensive interpretations essential for clinicians and molecular biologists.

To address this gap, we are developing a structure-based framework that integrates computational and experimental methodologies to predict and validate the effect of missense germline mutations associated with various diseases on protein structural features. We retrieve target protein structures through AlphaFold and/or the Protein-DataBank (PDB) database. We trim predicted structures using disorder propensity information obtained through a combined approach involving AF pLDDT score and CAID. We employ experimental databases of manually curated complexes for the selection of potential interactors and protein sequence analysis for the Short Linear Motifs (SLiMs) annotations. We combine the information to generate the complexes through AF-multimer in the absence of experimental structures.

We obtain structural ensembles through Molecular Dynamics simulations, to include protein-flexibility and improve prediction accuracy. We integrate free and binding energy calculations, sequence analysis, and Structure-Based-Statistical-Mechanical-Models to predict the effects of mutation collected from three different databases on protein stability, protein-protein interactions, changes in post-translational modification sites, and allosteric changes on protein pockets. These predictions guide experimental validation through a platform currently under development that integrates biochemical and microscopy techniques.

In our preliminary experiments we validated the destabilizing effect of the P263H variant on ARID3A, a transcriptional factor highly mutated in leukemia, and its interaction with the DNA damage sensor TP3BP1 through CHX assay and microscopy techniques. Additionally, we validated sequence analysis predictions identifying a SH3-mediated interaction between Lyn tyrosine kinase and P14arf, suggesting further research avenues in B-cell cellular pathways. These results, along with the computational workflow, not only provide a robust method for investigating mutation effects on protein structural features but also demonstrate potential for exploring intrinsically disordered proteins (IDPs), particularly SLiM interactions.

Overall, this framework supports molecular biologists in both basic and translational research, aiding in the understanding of disease mechanisms and the development of new drugs and treatments.

P08. Cross-Platform Colorectal Cancer Subtyping with SpaceRAT

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Group	Health Bioinformatics and Personalised Medicine
Tag	Personalised Healthcare, Precision Diagnostics, Bioinformatics

In recent years, molecular subtyping has emerged as a promising path towards precision oncology, as specific cancer subtypes are increasingly associated with variations in treatment response and patient outcomes. In the field of colorectal cancer, various efforts to classify tumors based on gene-expression data have led to the establishment of the consensus molecular subtypes (CMS). The four CMS labels offer valuable insights into colorectal cancer heterogeneity and are associated with patient prognoses, therapeutic responses, and the underlying molecular pathways. The CMS framework holds clear potential for clinical use. However, CMS subtyping remains largely unutilized in routine clinical care. This gap exists partially due to technical and data compatibility challenges in current CMS prediction methods, which limit the wider adoption of the CMS system. To address these challenges, we adapted a generalized framework for ranked analysis of transcriptomics (SpaceRAT). Specifically, we created a PCA scaffold for CMS subtyping that can be used across various transcriptomics platforms, including RNA-seq and NanoString nCounter. By offering an adaptable and accurate subtyping approach, this scaffold advances the reliability and robustness of CMS subtyping, paving the way for its integration into clinical practice. With this tool, we aim to facilitate a future where CMS subtyping is utilized more in clinical trials, such that it can inform personalized treatment plans and enhance patient care.

P09. Sub-gene level analysis of proteomics data

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Group	Isoform Analysis Group
Tag	Alternative splicing, Isoform, Proteomics, Mass Spectrometry Bioinformatics

Alternative splicing is a fundamental cellular mechanism that enhances genome functionality by enabling a single gene to produce multiple mRNA variants, each encoding distinct proteins. A recent study by Dam et al. demonstrated that splicing accounts for 48.2% of the biological signal at the transcript level, underscoring the need to investigate isoforms at the protein level, where their functional significance is more directly linked to biological activity. However, proteomics research has struggled to capture isoform-level changes due to peptide-level ambiguity, noise, and a lack of robust analytical tools. To address this, we leverage IsoBayes, a novel Bayesian statistical method that enhances isoform inference by integrating mass spectrometry proteomics with transcriptomic data. IsoBayes overcomes key limitations in peptide-level identification, enabling accurate detection and quantification of isoform-specific signals. This project will conduct a large-scale proteomic isoform analysis, reanalyzing over 100 proteomics datasets to systematically assess the prevalence and functional impact of isoforms at the protein level. The study will begin with careful dataset curation, ensuring sufficient annotation, sample size, and replicates for reliable downstream analysis. By bridging transcriptomic and proteomic perspectives, this research will provide new insights into isoform-level biological functions and disease mechanisms. The findings will not only enhance our understanding of proteomic complexity but also guide the development of improved computational tools and workflows, ultimately advancing proteomics toward a more isoform-centric paradigm.

CDT - Cell and Drug Technologies

P10. To the Core of Core-Shell Nanoparticles as Gel Crosslinkers

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Group	Nanosmithery
Tag	Biomaterials, Rheology

Viscoelastic materials and yield stress fluids are central in many applications such as adhesives, 3D printing, foods, injectables, etc. Hydrogels are a commonly used material type in the mentioned applications; however, to control the hydrogels' yielding and viscoelastic properties, the material's microstructure needs to be fully understood. Previously, a biocompatible nanocomposite hydrogel platform (PNP gel), consisting of modified cellulose and polymeric core-shell nanoparticles, has been reported to have extensibility up to 2000% strain, yielding points >500 Pa, and self-healing properties. The PNP gel platform has been utilized in various applications such as drug depot formulations, postoperative adhesive material, and wildfire prevention. However, only four different core-shell nanoparticles have been successfully incorporated in PNP gel platform, limiting its expansion to new applications. We present a further investigation of the relationship between the core-shell nanoparticles and the yielding and viscoelastic properties of the hydrogels. A method for fast and agile synthesis of block-copolymers allows for the introduction of several new variants of core-shell nanoparticles into the PNP gels. The independent contribution of the shell and core of the core-shell nanoparticles on the yield stress, viscoelastic properties, and self-healing kinetics of the hydrogel are investigated by shear rheology. This will provide fundamental knowledge to rationally tune the properties of the gel by controlling the core, shell, and size of the overall nanoparticles.

P11. 3D muscle tissues

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Group	Tailored materials and tissues
Tag	Personalised Healthcare, Tissue Engineering

Living tissue is three-dimensional, soft, and mechanically active. Indeed, mechanical strain plays a fundamental role in the development and maturation of e.g. human striated muscle, as well as in the development of diseases. State-of-the-art in vitro models of human muscle are engineered 3D muscle tissue strips anchored to elastomer beams or pillars. However, current miniaturized platforms lack the ability to apply mechanical strain or compression within the tissue, which is crucial for replicating native physiology and injuries.

We aim to overcome limitations in current in vitro tissue models and mechanical stimulation platforms by finding new ways to impose and evaluate the effect of mechanical strain and compression in human tissue. In this workflow we will develop new functional materials for fabrication using micro-extrusion 3D printing, and new bioinks containing live cells for embedded 3D bioprinting to facilitate the incorporation of live tissues within the platforms.

Using multi-material 3D printing, we design and fabricate devices that allow for attaching and straining of 3D printed engineered muscle tissues. We use custom build laboratory setups to evaluate the laboratory models and characterize the tissues.

P12. The secret recipe for making polymer-protein coacervates

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Group	Nanosmithery
Tag	Drug Delivery, Polymer Chemistry, High Throughput Synthesis

Complex coacervation has shown its potential as a drug delivery system where you pair a biologic of choice with a polymer.[1] To choose a suitable polymer you need to know what parameters affect coacervation. In this study, bovine serum albumin (BSA) and lysozyme and the parameters molar mass, chosen monomers, composition of monomers, concentration of polymer, concentration of salts, concentration of protein and polymer charge are investigated to scratch the surface of complex coacervation mechanics.

A library consisting of 1.856 polymers was created using a liquid handling robot. The main backbone of each polymer consists of *N*-hydroxyethyl acrylamide for water solubility and *N*-isopropylacrylamide – a monomer known to create coacervating polymers.[1] Seven other monomers were used to modify the backbone in various combinations with up to five different monomers in one polymer.

The polymers were synthesized using PET-RAFT. Each polymer was tested in 18 wells in Lower Critical Solution Temperature (LCST) assays. Each assay was measured 17 times in the temperature interval 26 to 42 °C.

Early analysis indicates significant differences between the blank, BSA and lysozyme measurements. This is indicative of the importance of choosing the right macromolecules for coacervates and that the structure of the synthetic polymer becomes more important when a protein is added. Further results of analyses will be presented in the future. 1. Vandewalle, S. *Eur Polym J* **98**, 468–474 (2018).

P13. Rapid 3D Bioprinting for Autologous Surgical Procedures

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Group	Tailored Materials and Tissues
Tag	Tissue Engineering, Personalised Healthcare

Urogenital reconstructive surgery is severely challenged by the availability of native tissue, particularly in pediatric patients. Tissue engineering approaches have traditionally relied on enzymatic digestion and in vitro expansion of autologous biopsies before seeding onto a biodegradable scaffold. These methods remain costly, time-consuming, and full of regulatory challenges. To address these limitations, we aimed to develop a rapid bioprinting approach for creating a tubular autologous scaffold within a single surgical procedure. Specifically, we investigated the viability and proliferative potential of mechanically dissociated porcine urothelial micrografts incorporated into a biocompatible hydrogel and extruded via embedded bioprinting. We optimized the composition of a gelatin-based scaffold with embedded micrografts and assessed its suitability for surgical handling. A simplified patch-like geometry modeled a cross-section of a tubular construct and was evaluated for cellular viability in an in vitro model. Additionally, mechanical properties were determined through suture retention testing. Our findings demonstrated that micrografts remained viable post-processing and in vitro culture in gelatin-based environments, but did not remain viable within the printed construct. This supports the feasibility of rapid cell dissociation and material integration but demonstrates a need for additional optimization of bioprinting settings. The data also highlights that additional modifications to the material system are required to withstand surgical handling. Finally, our results confirm the possibility of integrating both rapid dissociation and bioprinting within the time constraints of a standard surgical procedure, offering a potential alternative for personalized urogenital reconstruction that requires less complexity and resource dependence.

Core Facility, CfD, BioF, Hevesy, Dosimetry, TIC

P14. A Quantitative Approach to CT Quality Assessment

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Section	Department of Oncology
Group	The Radiotherapy Research Unit
Tag	Nuclear Medicine, Optical Bioimaging

Introduction: High-quality imaging is essential for accurate tumor and organs-at-risk (OARs) delineation, directly impacting radiotherapy (RT) effectiveness. Qualitative CT image assessments by clinicians can be subjective and time-consuming. This study investigates whether contrast-to-noise ratio (CNR) can serve as a quantitative surrogate for clinical image quality assessment in photon-counting CT (PCCT).

Methods: 140 kVp PCCT scans of a (semi-)anthropomorphic abdomen phantom and two prostate cancer patients were acquired. CNR was calculated between an iodine insert (2 mg/cm³) and solid water for the phantom, and between the prostate and bladder for the patients. Two regions of interest (ROIs) were manually positioned, and CNR was determined as

$$CNR = \frac{|HU_1 - HU_2|}{\sqrt{SD_1^2 + SD_2^2}}$$

where $HU_{1/2}$ and $SD_{1/2}$ are the mean and standard deviation of the two ROIs. Identical ROIs were used across reconstructions to eliminate variability.
A blinded, multidisciplinary expert assessment had oncologists, radiologists, and radiographers (5-7 in total) evaluating key structures, and selecting the best kernel and reconstruction in a “choose best” setting. The correlation between CNR and expert preferences was analyzed using Spearman’s test.

Results and Conclusion: Softer kernels (Qr/Br ≤ 48) and VMIs ≤ 70 keV resulted in higher CNR, aligning with expert preferences for kernels but not for VMI. Experts were divided between low VMIs and VMI 70, which resembles standard CT. The Spearman test confirmed a strong correlation between CNR and kernel preferences (r=0.83, p=0.01) but not between CNR and VMI preferences (r=0.8, p=0.2). This suggests that while CNR can guide kernel selection, additional image quality metrics may be necessary for VMI optimization.

P15. Monte Carlo Simulations of Electrostatic - Field Effects on Dose Distributions

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Section	Medical Isotopes and Dosimetry
Group	Dosimetry

Background: Electron beam irradiation is frequently used for sterilization of medical devices. During this process, electrons can become immobilized in non-conductive materials like polymethyl methacrylate (PMMA), resulting in a stored charge distribution. High doses used in industrial sterilization can thereby generate significant electrostatic fields in the material, potentially affecting the dose distribution.

Methodology: This study employs TOPAS MC/Geant4 simulations to examine the impact on dose distributions of these electrostatic fields within a PMMA cylinder. We developed a framework to calculate the electrostatic field based on simulated electron deposition, and implemented this non-homogeneous field in TOPAS. The absorbed doses within simulated dosimeters on the cylinder surface were analyzed under varying electrostatic field strengths. Computational results were validated against experiments conducted at an industrial irradiation facility.

Results: The results from the simulations showed good agreement with dosimetric measurements. The results indicate that electrostatic fields within a PMMA cylinder significantly impact the dose to surface-mounted alanine pellets, particularly those positioned from 45 to 105 degrees around the cylinder as field strength increases.

Conclusion: This study indicates that electrostatic fields arising from stored charges can have a significant influence on the dose distributions during e-beam sterilization of medical devices. This framework could form the basis for a tool for predicting the effects of stored charge distributions in polymer-based products.

Digital Health

P16. Frequency-Aware Masked Autoencoders for Human Activity Recognition using Accelerometers

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Section	Digital Health, Bioinformatics
Tag	Digital Health, Precision Diagnostics, Wearables

Wearable accelerometers are widely used for continuous physical activity monitoring. Supervised learning has long been used to extract meaningful activity information from raw accelerometry data, but scarcity and small sizes of publicly available labeled datasets limits the potential of supervised methods. Exploiting large unlabeled datasets via self-supervised pretraining is a relatively new approach in human activity recognition (HAR).

We used a time-series transformer masked autoencoder (MAE) for self-supervised pretraining and propose a novel spectrogram-based loss function, the log-scale mean magnitude (LMM) loss. We compared MAE models pretrained with LMM to one trained with mean squared error (MSE) loss. Leveraging the large unlabeled UK Biobank accelerometry dataset (n = 109k), we evaluated downstream HAR performance using a linear classifier in a smaller labeled dataset.

Pretraining with LMM loss improved performance over MSE loss, with balanced accuracies of 0.848 and 0.709, respectively. Better convergence of LMM loss, but not MSE loss, significantly correlated with improved downstream performance ($r=-0.61$, $p=0.04$). Finally, we compared our MAE models to the HAR state-of-the-art, also pretrained on UK Biobank data using a different self-supervised approach. Our LMM-pretrained models performed better with a linear classifier and comparably with an LSTM classifier, while MSE-pretrained models underperformed.

Our findings demonstrate LMM loss is a robust, effective method for pretraining MAE models on accelerometer data for HAR. Future work should optimize loss function combinations and extend this approach to other tasks.

Hearing Systems

P17. Reducing motion sickness symptoms by doing the Valsalva maneuver: a pilot study

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Group	Vestibular research
Tag	Personalised Healthcare, Hearing Technology

Background

Motion sickness is a common complaint during most modes of travel. Several medications can be used to treat motion sickness but are often accompanied by side-effects. Numerous non-pharmaceutical methods have also been suggested, here we address the possibility whether performing the Valsalva maneuver can influence the subjective feeling of motion sickness in healthy individuals.

Method

16 healthy participants (4 female) underwent two sessions of motion sickness induction through off-vertical axis rotation (OVAR) using the Epley Omniax Chair. Participants were randomized to do the Valsalva maneuver during either their first or second rotation. We assessed motion sickness symptoms using the Motion Sickness Severity Scale (MSSS) and Motion Sickness Assessment Questionnaire (MSAQ) at baseline (pre-rotation) and at 0-, 10- and 30-minutes post-rotation.

Results

Motion sickness symptoms were significantly lower in participants who did the Valsalva maneuver during their first rotation when measured on the MSAQ ($p = 0{,}0024$) and MSSS ($p = 0{,}0027$). This effect was not present if the Valsalva maneuver was done on the second rotation.

Conclusion

The Valsalva maneuver demonstrates potential in mitigating the development of motion sickness symptoms induced by OVAR.

P18. Modeling the effects of energetic and modulation masking on the intelligibility of non-vocoded and vocoded speech

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Group	Computational Auditory Modeling
Tag	Hearing Technology

Vocoded speech can be used to simulate aspects of cochlear implant (CI) listening. Modeling the intelligibility of such stimuli in normal-hearing (NH) listeners may lead to valuable insights into the auditory processing of CI listeners. The present study replicated and extended a dataset collected by Oxenham and Kreft (2014; Trends in Hearing **18**, 1-14) using two types of vocoders, a tone vocoder and a tone vocoder that simulates the effects of current spread in CIs. In order to evaluate the relative contributions of energetic and modulation masking across vocoder-types, three maskers were considered: speech-shaped noise, pure-tone complexes (PT) and noise-modulated tone complexes. In addition, the present study used a speech intelligibility model to simulate and predict NH listeners' performance obtained with these stimuli. The model includes a temporal processing stage accounting for modulation masking. Model simulations accounted for the increased speech intelligibility in the PT condition as compared to the performance obtained with maskers exhibiting inherent modulations, both in the unprocessed and vocoded conditions. Additionally, the model predicted similar speech intelligibility performance for all three maskers when using the vocoder with current spread, in line with the data. The model simulations presented here support the hypothesis that overall masker energy, and not modulation energy, determines speech intelligibility in CI listening. However, overall prediction offsets for the vocoder with current spread indicate that the effects limiting the speech perception in CI-simulated listening are not yet fully captured by the modulation-based model.

P19. AI-driven diagnostics of Benign Paroxysmal Positional Vertigo using the TRV-Chair

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Tag	Precision Diagnostics, Hearing Technology,

Introduction: Benign paroxysmal positional vertigo (BPPV) is the most common cause of peripheral vertigo, with a lifetime prevalence of 2.4% [1]. BPPV is associated with a decline in mental health and self-perceived quality of life, [2], [3] as well as non-benign complications such as an increased risk of falls [4]. Although patient history and questionnaires are often sufficient for diagnosing BPPV, up to 65% of patients undergo potentially unnecessary testing and interventions [5]. The aim of this study is to examine whether AI models could prove effective as an aid in the diagnosis of BPPV.

Method: Objective measurements from 5955 Dix-Hallpike examinations performed in a TRV-chair between 2013-2020 were extracted and labelled with their corresponding diagnosis. The dataset was divided into an 80/20 split for training and testing two AI model.

Results: Following the application of exclusion criteria, 3114 Dix-Hallpike examinations were deemed suitable for training the AI models. The deep learning model demonstrated a high diagnostic accuracy of 88 % for recognising posterior canal BPPV for both the training and test data, with coherence in accuracy between the training and test curve.

Discussion: While the high accuracy of the model suggests that AI models may be viable as a diagnostic aid, the model's prediction error remains high and the discrepancy in prediction error between the training and testing data, indicate low prediction confidence and imperfect generalisation. Further investigations into the factors contributing to the high prediction error are warranted.

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P20. Development of the Everyday Conversational Danish Sentence Test

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Traditional audiological tests often fail to replicate real-world listening environments, resulting in discrepancies between clinical assessments and patient experiences. Tests commonly used in Danish clinical practice involve recorded words or sentences read aloud verbatim, which do not adequately represent real-life communication. This study outlines the development of a speech material for more ecologically valid speech-in-noise testing. Sentences were extracted from recordings of spontaneous dialogues between a female talker and two partners. Each pair communicated both in quiet and noisy conditions to elicit normal and raised vocal efforts. 450 sentences were extracted, half of them with a normal vocal effort and half with a raised vocal effort. The sentences were level-normalized and presented in spectrally matched speech-shaped noise to 40 normal-hearing participants at five signal-to-noise ratios ranging from -10 to 0 dB in 2.5 dB steps. Psychometric functions were fitted to each sentence based on the measured intelligibility scores. The overall psychometric function across all sentences showed a speech reception threshold (SRT50) of -5.3 dB SNR and a slope of 12 %/dB. Sentences were intelligibility normalized to align their individual SRT50 with the mean SRT50 and then divided into balanced lists. The ECO-DAST material can be paired with more realistic background noise to provide assessments that better reflect everyday listening challenges.

P21. Identification of Conversation Partners from Egocentric Video

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Group	Centre for Auditory Neuroscience
Tag	Computer Vision, Egocentric Video, Conversation Analysis

Communicating in noisy, multi-talker environments like busy restaurants can be challenging, especially for individuals with hearing impairments. While most previous works have studied these situations by investigating behavior of one conversation group amidst noise, we propose that it may be relevant to study scenarios with multiple simultaneous conversations. We employ video captured from wearable, egocentric cameras to capture rich information during conversations and enable a novel task in computer vision: identification of conversation partners in egocentric video.

Building on recent advancements in computer vision for analyzing social interactions, we introduce a dataset designed for this task. Our dataset comprises 69 hours of egocentric video of diverse multi-conversation scenarios where each individual was assigned one or more conversation partners, providing the labels for our computer vision task. This dataset supports the development and evaluation of algorithms for identifying conversation partners. Here, we present the dataset and initial baseline results. These demonstrate that simple methods already yield interesting insights and that a diverse dataset like ours is crucial for this task. In the future, this information extracted from egocentric video could be paired with selective acoustic amplification methods to develop a smart hearing device that can help with communicating in noisy environments.

IDUN - Drug Delivery and Sensing

P22. On-demand delivery of antibiotics and modelling of bacterial biofilms

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Group	Pharmaceutical Technology group
Tag	Drug Delivery, Bacterial infections, *In vitro* modelling

Bacterial biofilms pose a major healthcare challenge, especially with rising multidrug-resistant (MDR) clones due to antibiotic overuse. Biofilms are 10–1,000× less susceptible to antibiotics compared to their planktonic counterparts, as their extracellular polymeric substance (EPS) matrix blocks drug penetration. Biofilm bacteria also express efflux pumps, produce antibiotic-degrading enzymes, and form dormant persister cells, further complicating treatment.

Quorum sensing enhances resistance, while the biofilm microenvironment—low oxygen, nutrients, and acidic pH (~5.5)—supports chronic infections like chronic rhinosinusitis (CRS). Systemic antibiotics for CRS are limited by metabolism and toxicity, while localized delivery offers higher drug concentrations but struggles with EPS penetration.

Polymeric nanoparticles provide a solution, encapsulating antibiotics to improve EPS penetration and targeted release. pH-responsive nanoparticles using Eudragit® E PO that dissolve in acidic conditions, enhancing drug delivery. Microfluidic fabrication ensures monodisperse nanoparticles (~100 nm), optimizing efficacy.

Current CRS models rely on static biofilms or complex flow systems. To evaluate the NP drug delivery system, a Bacterial Culture on Disc (BCoD) model including *Pseudomonas aeruginosa* laboratory strain (PAO1) mimics biofilm conditions using centrifugal force.

P23. Enhancing oral drug delivery via microneedles for improved retention and mucopenetration

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Group	IDUN
Tag	Drug Delivery, Personalised Healthcare

The oral delivery of macromolecules, such as proteins and peptides, offers a patient-friendly approach with potential for sustained drug release. However, challenges such as the mucus layer, tight junctions, and enzymatic degradation limit bioavailability. Current strategies, including permeation enhancers, nanotechnology-based formulations, and microdevices, have shown promise but still suffer from limitations, including low absorption and significant interpatient variability. Recent advances in oral drug delivery devices utilize mechanoadhesion principles—such as microneedles—to enhance retention and mucopenetration.

In this study, we investigate the use of 3D features, specifically microneedles, to improve drug retention and penetration within the gastrointestinal (GI) tract. We fabricated and characterized microneedles composed of polyvinyl alcohol (PVA) and biocompatible polydimethylsiloxane (PDMS) as a starting point. Preliminary formulations with PVA (15%) and PDMS resulted in microneedles with an average height of 618 µm. To enhance penetration efficiency, alternative mold designs were explored using a CO_2 laser ablation process, yielding needle heights ranging from approximately 800 to 1200 µm.

Additionally, fluorescence-labeled (FITC dextran, 40 kDa) microneedles were developed as proof of concept for macromolecule incorporation. These were analyzed using scanning electron microscopy (SEM) and fluorescence microscopy. Future work will focus on optimizing the microneedle formulation and fabrication process, followed by in vitro testing using an artificial intestinal model and ex vivo studies. Furthermore, alternative 3D-structured features will be designed and evaluated to improve retention and mucopenetration.

P24. Developing innovative delivery systems for mucosal vaccination against Helicobacter pylori

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Section	IDUN (Drug Delivery and Sensing)
Group	FREJA
Tag	Oral drug delivery device, Mucosal Vaccine, H. pylori infection

Antibiotics have been the primary treatment for *Helicobacter pylori* (commonly known as *H. pylori*) infections in the stomach, but their effectiveness is increasingly hampered by rising antimicrobial resistance [1]. Therefore, developing an efficacious mucosal vaccine presents a promising alternative. This project aims to create innovative oral delivery systems for an H. pylori mucosal vaccine that target the vaccine formulation to mucosal antigen-presenting cells in the gastrointestinal tract, initially focusing on the stomach lining. The focus is on ensuring the vaccine is released in close proximity to the mucus layer and achieves a prolonged release, addressing significant research gaps in safely transporting the vaccine and its additives to the stomach and protecting it in the acidic environment.

To address these challenges, we will utilize self-unfolding foil (SUF) technology, optimizing it for a mouse model and modifying the material to make it hydrophilic to establish proof-of-concept and facilitate ease of application in vaccine studies.The SUF, a proven method with peptide delivery, will be adapted for vaccine delivery [2]. The micro-cavities in the SUF will carry the vaccine, sealed with specific coatings to protect it from gastric juice and ensure mucosal adhesion to reach the stomach lining. The SUF is designed to be thin and small enough to fit inside a mouse capsule, achieving proper encapsulation. Making the SUF hydrophilic, optimizing its size, loading vaccine components, and applying protection and adhesion coatings present interesting challenges in making it an effective encapsulated device. These challenges present opportunities for advancements in oral drug delivery devices and vaccination techniques.

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P25. Biocompatible Carbon-Fiber/ZIF-8 MOF composite-based Supercapacitor for Ingestible Devices

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Group	EMGUT
Tag	Ingestible devices, Energy storage, Supercapacitors

Supercapacitors are a highly attractive research topic for applications in powering ingestible devices, due to their inherent ability to charge/discharge rapidly and high-power density compared with batteries [1]. As conventional supercapacitors are not suitable for *in vivo* applications, research on ingestible systems now focuses on developing biocompatible electrode materials that can provide excellent performance in supercapacitor applications, while ensuring good compatibility with the body's natural functions [2]. Additionally, for ingestible applications, the size of the electrodes must be limited such that the supercapacitor can fit into a pill, while still allowing room for other electronics. Thus, the supercapacitor should be based on efficient electrode materials that maintain excellent performance even when miniaturized [3].

In the first work package of this project, we have used a novel approach for depositing ZIF-8 metal organic framework (MOF) structures onto carbon felt by electrospinning of biocompatible Poly (lactic-co-glycolic acid) (PLGA) nanofibers loaded with the MOF structures. The PLGA/MOF fibers are then carbonized onto the carbon felt and this hybrid composite structure is used as electrodes for ingestible supercapacitors, offering superior interfacial sites with enhanced electrochemical activity. The carbonized polymer fibers offer an increased electrode surface area which, in addition to the added pseudocapacitance from the zinc ions in the MOF, increases the capacitance immensely. Preliminary testing has shown specific capacitances as high as 6.2 F/g indicating that the carbon fiber/MOF composite may serve as an ideal electrode material for supercapacitor applications.

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Magnetic Resonance

P26. Establishment of a co-culture air-liquid-interphase lung model utilizing human macrophages

Alexander Frederick Melanson, Søren Molin and Helle Krogh Johansen

Airways of CF patients often get infected by *Pseudomonas aeruginosa (Pa)*, which initially is antibiotic-susceptible, but over time develops resistance towards several antibiotics. Moreover, CF patients have difficulties regulating the inflammatory response. Much is known about the neutrophil response during Pa infection since polymorpho-nuclear leucocytes can be found in large numbers in the sputum. However, the dysregulation of the innate immune response is not elucidated to the same degree. In recent years researchers have started to investigate the role of other innate immune cells, such as macrophages. Macrophages play key roles as a first line of defense against pathogens, but they also participate in immune regulation. There is some disagreement whether macrophages have a hyper-response to Pa, or if there is a lack of a response. To elucidate the role that macrophages play during Pa airway infection, we have developed an air-liquid-interphase (ALI) infection model, which allows us to investigate how Pa, airway epithelial cells, and macrophages interact. This model closely mimics the infection in CF airways. We have used flow cytometry and confocal microscopy to monitor the differentiation of the macrophages and to visualize the adhesion to the basolateral side of the ALI cultures.

In conclusion, we have successfully established an ALI airway culture model and we have managed to differentiate monocytes to macrophages and get them to adhere to the basolateral side of the ALI culture. The next step will be in depth-characterization of the bacterial immune interaction in the ALI model.

P27. Dynamic Hyperpolarized MRI for Imaging ^{13}C-Ketoisocaproate Metabolism in an Alzheimer's Disease Model

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Group	Metabolic Brain MR (MBMR)
Tag	^{13}C Magnetic Resonance Imaging, Metabolism, Hyperpolarization

Hyperpolarized α-keto-[1-^{13}C]isocaproate (KIC) is a metabolic contrast agent for ^{13}C-MRI, enabling *in vivo* tracking of its conversion to leucine via branched chain amino acid transaminase (BCAT). Prior rodent studies have demonstrated its potential, using single-timepoint or time-averaged acquisitions. Dynamic imaging could enhance real-time metabolic investigations of BCAT activity. This study develops and validates an acquisition strategy for real-time dynamic imaging of cerebral BCAT activity, assessing its feasibility *in vivo* in an animal model for AD.

Male 5xFAD transgenic (TG, n=6) and wild-type (WT, n=5) mice (19-22 weeks old) were anesthetized and scanned with a 3T clinical MR scanner. KIC acid (65 mg) with 30 mM AH111501 trityl radical was hyperpolarized via dDNP (~45 min), dissolved in 5mL 50 mM TRIS buffer, and injected intravenously (200 µL, ~3 s). At start of injection, a dynamic IDEAL spiral CSI was initiated using 5 echoes, 1370 µs echo-spacing, 1 s temporal resolution, FOV/matrix = 30 mm/12, slice thickness = 10 mm, 40 acquired frames. A custom-designed multiband excitation pulse minimized KIC excitation (2°), preserving magnetization, while using a larger flip angle (20°) for leucine to enhance SNR.

Dynamic imaging revealed rapid KIC inflow and decay, followed by delayed leucine accumulation. Time-summed images showed widespread KIC distribution, while leucine signals were localized in the brain, in line with the known distribution of both the mitochondrial and cytosolic BCAT isozymes. Time-summed brain signals also displayed a trend towards higher leucine-to-KIC ratios, suggesting a higher metabolic conversion, in the TG mice compared to controls.

P28. Correction of time-dependent phase fluctuations in diffusion-weighted MRS at very high b-values with an external phantom reference

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Group	MRI Acquisition
Tag	Magnetic Resonance

Diffusion-weighted MRS (dMRS) provides information on intracellular cell morphology by measuring the mobility of cell-specific metabolites [1, 2]. The strong diffusion encoding gradients needed for dMRS generate eddy currents and field fluctuations that can severely distort spectral line shapes. Therefore, experimental optimization and corrections with water reference scans are needed [3]. However, in experiments with many strong diffusion encoding conditions, water reference scans can be time-consuming and prone to noise [4]. Other correction approaches require special mapping sequences or advanced field camera equipment that is less effective for field oscillations created by diffusion encoding gradients [5].

In this study, we propose a simple phantom measurement that provides high SNR phase reference data as an alternative to a conventional water reference to correct for the spatial characteristics of field fluctuations in a dMRS acquisition on a human 7T system. We employed a high-viscosity silicone oil phantom to map spatial field fluctuations and assessed its potential for phase correction through both simulation and in vivo experiments. The phantom reference was compared to water reference scans, demonstrating that it maintained a stable phase even at b-values up to 37 ms/µm². Subsequently, spectral fitting was performed to extract diffusion properties from the corrected data, confirming the feasibility of the phantom approach.

Our findings indicate that the phantom reference effectively corrects for phase distortions in dMRS acquisitions. This method enhances data quality and accelerates acquisition protocols by reducing the need for time-consuming water reference scans, making dMRS a more practical tool for clinical applications.

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P29. Harmonizing Hyperpolarized MRI: A Replicable Phantom for Multisite Studies

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Group	Metabolic Brain Magnetic Resonance
Tag	Magnetic Resonance

We present a novel phantom designed to validate and harmonize hyperpolarized magnetic resonance imaging (HP-MRI) protocols across multiple clinical sites. HP-MRI is a molecular imaging technique, which allows for non-invasive *in vivo* assessment of changes in metabolic activity – typically using a 13C-labelled metabolite, pyruvate. Variations in system setups across sites hinder large-scale multisite studies which are essential for clinical adoption. Our phantom, made from transparent PVC, serves as a reference for quality assurance and harmonization. It features a Ø200-mm sphere comprised of two half spheres with regions for evaluating *uniformity, geometric distortion, resolution,* and *quantification*. For *quantification* specifically, distinct compartments are filled with stable aqueous solutions of urea, [1-13C]glycine, and [1-13C]propionate doped with Omniscan to allow for imaging within reasonable scan times. These solutions mimic the *in vivo* chemical shift differences of [1-13C]pyruvate and its main metabolites: [1-13C]lactate and 13C bicarbonate.

Initial validation on a clinical 3T scanner (SIGNA Premier, GE Healthcare) confirms the chemical shift differences (10 ppm between urea and [1-13C]glycine, and 12 ppm between [1-13C]glycine and [1-13C]propionate) and demonstrates sensible relaxation properties: T1 values are 42.8 ms, 52.4 ms, and 113.2 ms, and T2 values are 25.5 ms, 28.7 ms, and 45.6 ms for urea, [1-13C]glycine, and [1-13C]propionate, respectively. This chemically stable, cost-effective, and replicable phantom facilitates the harmonization of HP-MRI, supporting broader clinical adoption. Future work involves multi-site testing of the phantom.

P30. Changes of aryl para-position of trityls for SAR study of polarizing agents for Dynamic Nuclear Polarization

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Group	Ardenkjær-Larsen
Tag	Magnetic Resonance

Polarization for Nuclear Magnetic Resonance (NMR) can be enhanced by Dynamic Nuclear Polarization (DNP) [1,2]. DNP uses stable radicals as polarization agents due to the higher magnetism of electrons compared to the nuclei [1]. The polarization of the radicals is transferred to the nuclei, thus enhancing the signal of NMR [1]. This technique increases the sensitivity of NMR, which allows for new types of experiments, such as in vivo ^{13}C tracking [1-4]. One class of polarization agents used for DNP is triarylmethane radicals (TAMs or trityls) [1,5]. However, it is not fully understood why these radicals work well for DNP and how their structure affects their activity towards DNP.

For the more rational design of future trityls as polarizing agents for DNP NMR, several analogs varying at the aryl para-position are synthesized to determine a structure-activity relationship of the trityls. The activity is tested by Electron Spin Resonance (ESR) and DNP. The aim is that such a structure-activity relationship analysis will help with designing new, better, and cheaper trityls for DNP.

Acknowledgments

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P31. Personalized Electric Field Simulations of Deformable, Large TMS Coils based on Automatic Position and Shape Optimization

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Group	Neurophysics
Tag	Sensory and Neural Technology

Transcranial Magnetic Stimulation (TMS) therapies for psychiatric disorders like Major Depressive Disorder and Obsessive-Compulsive Disorder use both focal and unfocal coil designs. Unfocal designs often employ bendable windings and moveable parts, complicating realistic simulations of their electric fields in varying head sizes and shapes. So far, this hinders realistic comparisons of the electric fields in various coil designs and prevents systematic evaluations of their dose-response relationships. We derived accurate models of four coils (Brainsway H1, H4, H7; MagVenture MST-Twin) from computer tomography data and mechanical measurements. Based on accurate coil models, we implemented a principled approach to optimize the coil positions and shapes, with two possible objectives: optimizing the fit of the coil on the scalp or additionally maximizing the field in a ROI. Coil models placed with the new method were closely fitting on N=1100 head models while coil-scalp intersections were avoided. In contrast, simple transformation of coil positions from MNI space to the individual heads regularly led to physically impossible configurations. This also affected the electric fields calculated in the cortex, with peak differences of medians around 15% of the peak field strength. In summary, our novel method enables optimizing the position, shape and electric field of flexible coils while accounting for individual head anatomies. Ignoring shape deformations was demonstrated to considerably affect the simulated electric fields. Consequently, our approach serves as a necessary step for realistic electric field simulations of unfocal coil designs, as it removes the practical hurdles that so far hampered accurate simulations.

P32. Metabolism of Macrophages under Chemical and Mechanical Stimulation

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Group	Magnetic Resonance by Optics
Tag	Magnetic Resonance, Optical Bioimaging

Macrophages are a crucial component of the immune system, playing a key role in the elimination of pathogens through phagocytosis, pro-inflammatory, and anti-inflammatory processes. They are classified into three main groups: resting macrophages (M0), classically activated macrophages (M1), and alternatively activated macrophages (M2). Activation of macrophages occurs in response to chemical (e.g., bacterial endotoxins, interleukins) and physical stimulation (e.g., mechanical properties of tissue environment or biomaterials/implants). In recent studies, the metabolism of macrophages was investigated in relation to their activation states (M0, M1, and M2) after chemical stimulation. However, the impact of mechanical stimulation (e.g., substrate stiffness) on their metabolic status remains unexplored. This study aimed to analyze the metabolic profiles of macrophages in various activation states under chemical and mechanical stimulation using ^{1}H NMR metabolomics and quantum sensing with nanodiamonds via T1-relaxometry to assess changes in free radical concentrations. For experiments, samples were prepared by seeding macrophages (J774A.1) on substrates with different Young modulus (stiffness). The cells were activated using lipopolysaccharide (LPS) for M1 activation and IL-13 and IL-4 for M2 activation. Prior to the T1 measurements, samples were treated with 70 nm fluorescent nanodiamonds with NV centers for 30 minutes before adding chemical stimulants. Based on NMR data, 25 metabolites were identified. We found that M0 and M2 macrophages had similar metabolite profiles, differing from the profile of M1 macrophages. M0 and M2 exhibited similar metabolite profiles only when cultured on stiff substrate. The preliminary T1-relaxometry results show that substrate stiffness affects the concentration of free radicals.

Optical Sensing and Imaging Systems

P33. Spatially Offset Optical Coherence Tomography for Detection of Tumor Margins

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Group	Biophotonic Imaging Group
Tag	Diagnostic Imaging, Optical Bioimaging

It is currently not possible to verify microscopic tumor boundaries during cancer surgery. Therefore, the dissection area is based on the doctor's experience and well-defined safety margins. Verification of the excised region occurs postoperatively when the removed tumor is sent for testing, with results available days later. Depending on the results, the patient may require additional surgery and/or radiation/chemotherapy.

Intraoperative verification of complete tumor resection could therefore be beneficial to the patient. Optical Coherence Tomography (OCT) has been investigated for accurate identification of microscopic tumour residues, providing similar insights into morphology as the gold standard of histology. However, OCT relies mainly on ballistic scattered light, limiting its penetration depth and potentially overlooking information from multi-scattered light. Spatially offset OCT (SO-OCT) is a new and interesting modality to investigate, with a focus on obtaining better boundary detection. SO-OCT utilizes multi-scattered light by collecting the light at an offset from the emitted beam. It has already been shown that SO-OCT enables better depth penetration for highly scattering samples and might contain different information depending on the chosen offset.

We have thus compared OCT and SO-OCT images of mice tongue samples with tumors. We investigated the difference in contributions from the different offsets and how we can extract information from merging images. Comparing results from the OCT and SO-OCT images to histology shows a better correlation between the SO-OCT images and histology compared to OCT and histology.

P34. Continuous Titration Based Method For Rapid In-solution Analysis Of Non-Covalent Interactions

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Group	Nanofluidics and Bioimaging
Tag	Optical Biomaging

The development of new drugs relies on identifying and validating molecular inhibitors or promoters of biological processes. Traditional methods for screening small-molecule ligands typically provide only qualitative yes/no results and often suffer from high false positive and false negative rates. Quantitative approaches, while more accurate, are time-intensive due to the need for multiple measurements across a dilution series to generate titration curves and determine dissociation constants (K_d). To address these limitations, we present Continuous Titration Based Spectral Related Intensity Change (cSPRING) – a novel technique that integrates flow induced dispersion analysis (FIDA) with ratiometric fluorescence detection to measure K_d in a single experiment. cSPRING is an in-solution method that reduces sample preparation time eight-fold and requires only nanograms of protein. We validate cSPRING by demonstrating good agreement with established quantitative methods for three well-characterized protein-small molecule interactions, spanning low nanomolar to high micromolar affinities. Additionally, we show that cSPRING enables binding affinity measurements in under a minute, underlining its efficiency and potential for high-throughput screening applications.

XTI - Experimental and Translational Immunology

P35. Deciphering the molecular signature of induced pluripotent stem cell to T-cell differentiation using CRISPR activation screens

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Group	T-cell and cancer group
Tag	Immunotherapy, Immunology

Induced pluripotent stem cells (iPSC) are a promising avenue for the production of next-generation adoptive cell therapies (ACT). The differentiation of iPSCs to T cells poses great challenges, mainly associated with the low efficiency of the process and protocol variability. Therefore, decoding the molecular signature of this process is of significant importance for advancing this type of therapy. In this regard, CRISPR activation (a) or interference (i) screens offer a comprehensive and systematic approach to unravel the complexities of gene function and regulate the differentiation process.

We have generated iPS cell lines that express dead Cas9 (dCas9) fused to either the VP64 transactivating domain or the transinhibiting domain KRAB. We are currently working on inducible systems using Doxycycline or Shield-1 as inducers. We have validated this technology in two iPS cell lines, where a robust gene upregulation of CD4, CD8 and CD14 has been achieved in CRISPRa and downregulation of CD11a, CD45 and CD3 has been observed in CRISPRi.

For CRISPRa screens, these iPS cell lines will be transduced with gRNA libraries specifically targeting drugable comdurponents, phosphatases, and signaling pathways. We will then follow the enrichment of gRNAs in differentiated populations to evaluate which genes regulate the differentiation at different stages of the process.

We anticipate that the knowledge obtained from the screens will not only enhance the understanding of T cell development but will also provide opportunities for more precise manipulation of gene expression, which will lead to the generation of more robust protocols for iPSC-derived T cell products.

DTU Biosustain

P36. Investigating Bacterial Interactions in Lung Infections by 3D Bioprinting and Air-Liquid Interface Model

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Group	Infection Microbiology
Tag	3D bioprinting, Polymicrobial interactions, Lung Infections

Chronic lung infections are often comprised of polymicrobial communities shaped by genetic and ecological interactions. Within such infections, *Pseudomonas aeruginosa* (*Pa*) experience intra-clonal diversification, driven by genome-wide mutations and micro-niche-specific selective pressures. On an interspecies level, Pa uses its Type VI secretion system (T6SS) against *Staphylococcus aureus* (Sa), which may influence microbial dominance during infection progression. Nevertheless, the spatial and genetic dynamics underlying these interactions are still not fully understood.

Therefore, to address this we have developed a lung infection model using Air-Liquid Interface (ALI) epithelial cultures, closely mimicking human airways. Additionally, we use 3D bioprinting to precisely position bacterial cells onto ALI cultures at defined microscale distances, allowing controlled studies of bacterial interactions on lung epithelium cells. Different microscale distances are used in between each bacterial strain. This approach allows us to study bacterial complementation, competition, antibiotic tolerance, QS dynamics, and T6SS-mediated antagonism in a spatially controlled manner.

We have successfully optimized 3D bioprinting for *Pa* strains on ALI cultures, ensuring bioink compatibility with both *Pa* and BCi-NS1.1 (Bronchial cell line-nonsmoker) airway epithelial cells. We obtained uniform and reproducible bacterial droplets at micrometre precision. Interaction studies between PAO1 (PAO1, PAO1 Δ*H, PAO1* Δ*H2)* and *Escherichia coli* show increasing antagonistic behaviour at decreasing spatial distances, underlying the role of micro-scale positioning in interspecies interactions. The ongoing work is focusing on *Pa-Sa* interactions to study the genetic and ecological factors conditioning their coexistence/competition in lung infections.