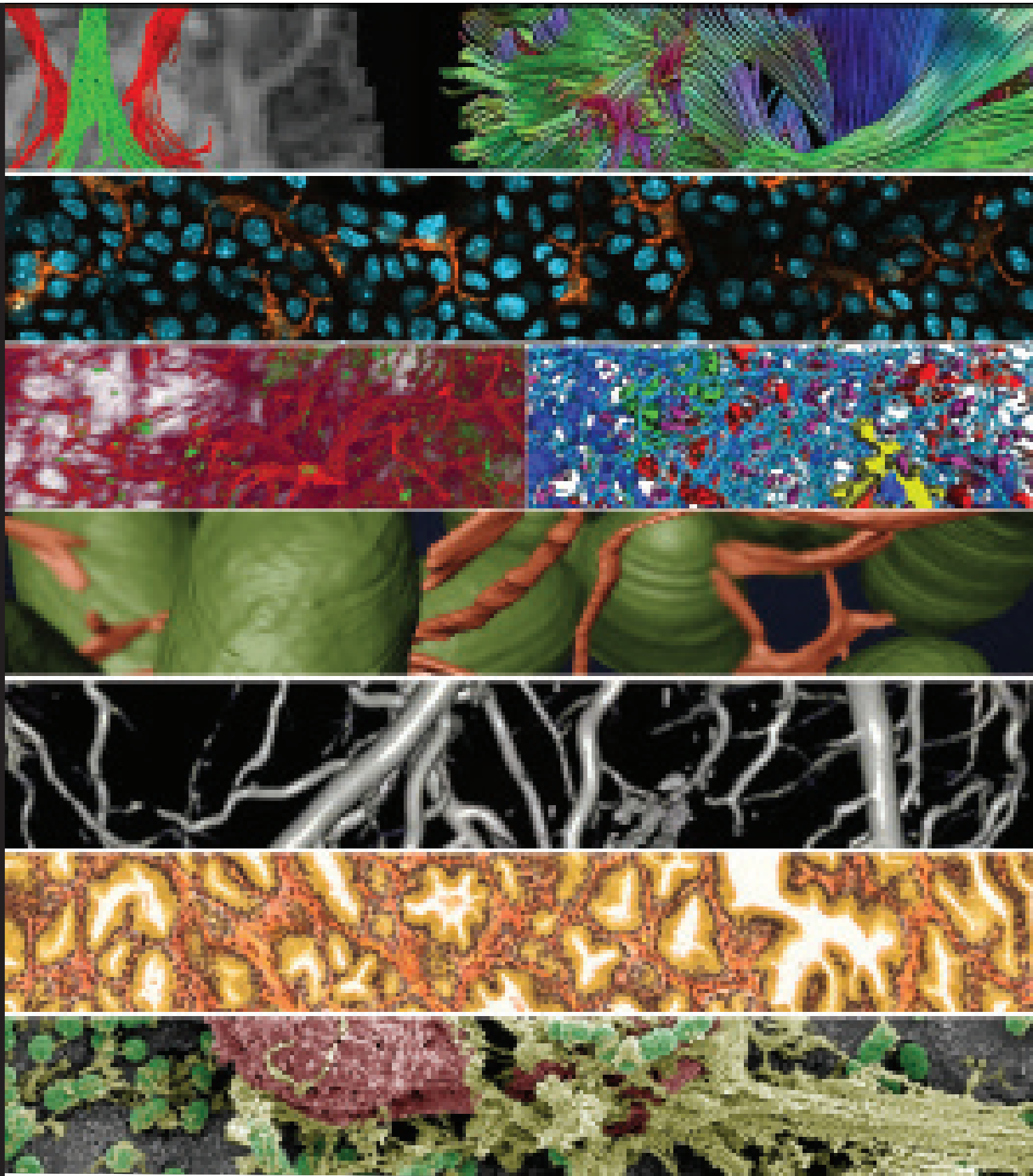
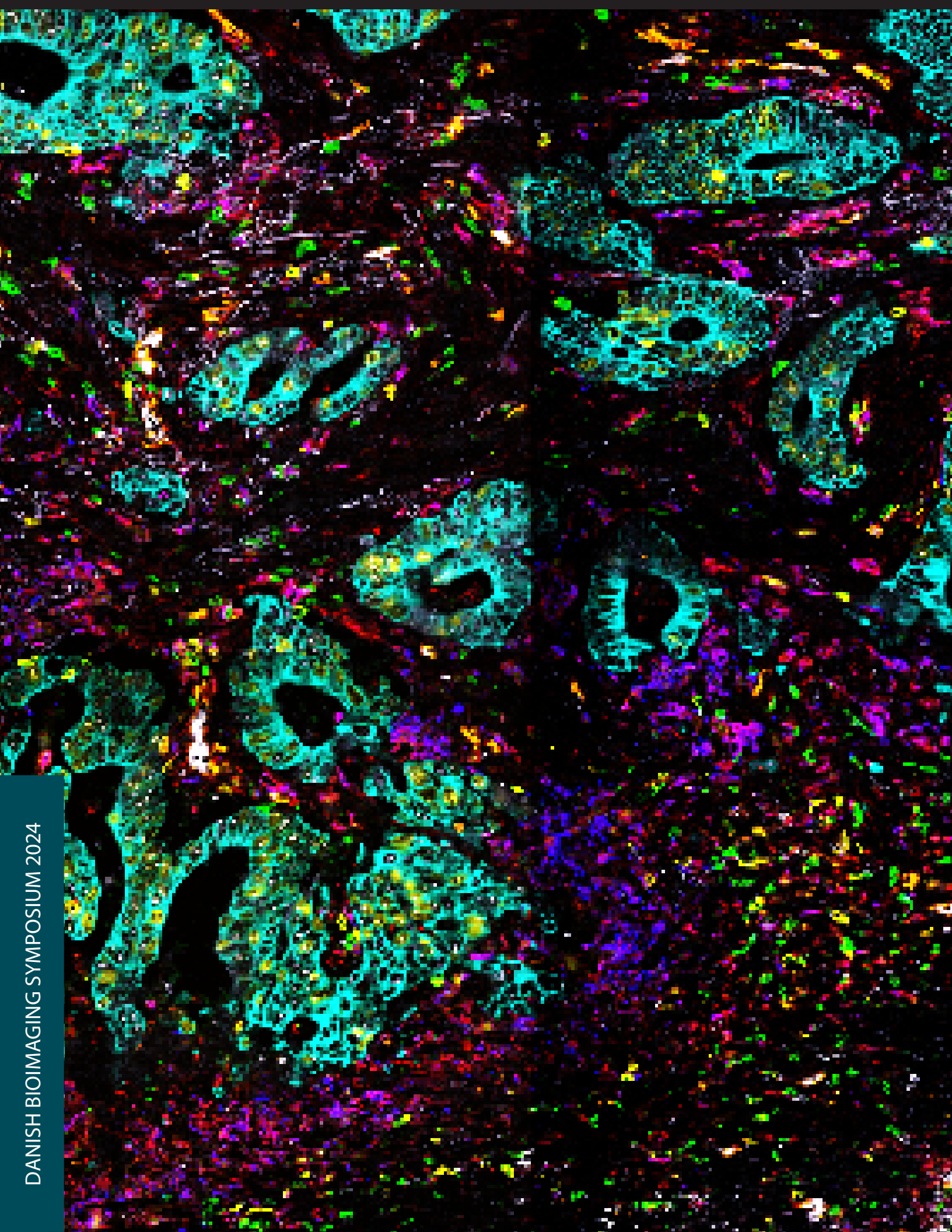
DANISH BIOIMAGING SYMPOSIUM 2024

LUNDSGAARD AUDITORIUM - UNIVERSITY OF COPENHAGEN - 24-25 JUNE





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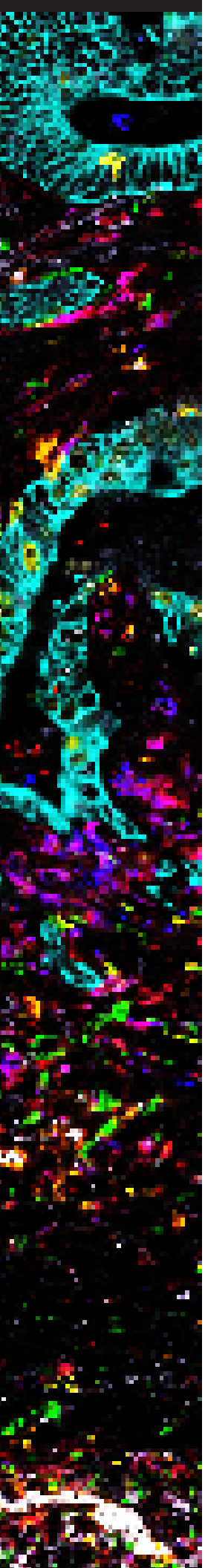

DANISH BIOIMAGING SYMPOSIUM 2024

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Winner of the Danish BioImaging Microscopy Image Contest 2024
Multiplex staining of colon cancer on a challenging membrane slide, highlighting the tumor epithelium and immune cells in the tumor microenvironment. The image was obtained using MACSima imaging plateform at the Biotech Research & Innovation Centre (BRIC), University of Copenhagen. Imaged by Xiang Zheng, NNF Center for Protein Research, University of Copenhagen

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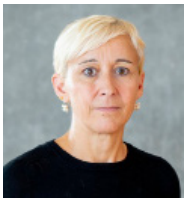

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Faculty of Health and Medical Sciences

DANISH BIOIMAGING NETWORK

PROGRAM 24TH JUNE

08:30-09:00 **REGISTRATION**
09:00-10:20 **SESSION 1**

09:00-09:30 **Welcome to SUND and the Danish BioImaging Scientific Symposium**
by Vice Dean Hans Bräuner and Clara Prats, University of Copenhagen

09:30-10-00 **The Metrics for Building the Human Organ Atlas**
by Claire Walsh, University College London

10:00-10:30 **Clinical AI imaging from a Radiologist perspective: The practical approach**
by Ole Graumann, Aarhus University

10:30 - 10:45 **3D ultrasound to improve surgical precision during oral cancer treatment**
by Fatemeh Makouei, Rigshospitalet

10:45-11:00 **Seeing is believing: abundance and function of G-quadruplex DNA in Staphylococcal biofilms**
by Gabriel Antonio Minero, Aarhus University

11:00-11:15 **BREAK**
11:15-12:30 **SESSION 2**

11:15-11:45 **Quantitative imaging of behavioural and mechanical heterogeneities underpinning mouse anterior patterning**
by Shankar Srinivas, University of Oxford

11:45-12:00 **Drug Distribution Analysis: A Comprehensive Approach Using Whole Mouse Organ Clearing and Lightsheet Microscopy**
by Sara Elgaard Jager, University of Copenhagen

12:00-12:15 **Non-invasive label-free assessment of cellular growth and lipid storage in a free-living dinoflagellate Symbiodinium**
by Walter Dellisanti, University of Copenhagen

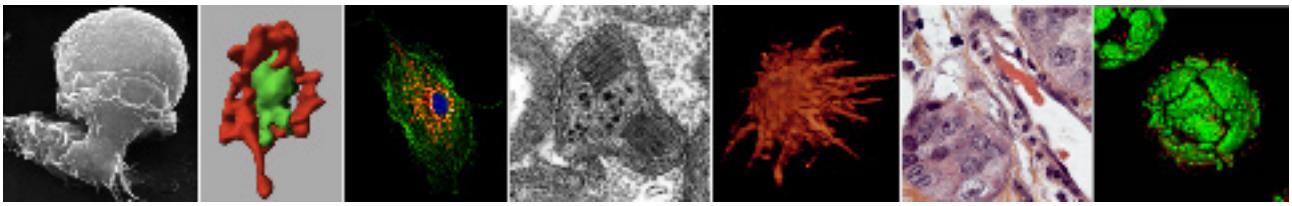
12:15-12:30 **Deciphering functional tumor-immune crosstalk through highly multiplexed imaging and deep visual proteomics**
by Xiang Zheng, University of Copenhagen

12:30-13:30 **LUNCH**
13:30-16:30 **WORKSHOPS**

13:30-15:00 **Image to data with Cellpose segmentation** - Room- 15.2.26
Light Sheet Microscopy- CFIM

15:00 - 16:30 **Python for 3D Bioimage analysis -** Room- 15.2.26
Zeiss Lattice Lightsheet and SIM, Dynamic Profiler and Arivis - Room- 15.2.18

16:30-18:00 **POSTER SESSION- NETWORKING**
18:00-20:00 **FINGER FOOD & DRINKS**



DANISH BIOIMAGING NETWORK

PROGRAM 25TH JUNE

09:00-10:20 SESSION 3

09:00-09:15 **Euro-BioImaging - Opportunities for the Danish BioImaging Community**
by John Eriksson- Director General Euro- BioImaging

09:15-09-45 **Resolving the molecular mechanisms governing 3D chromatin organisation**
by Fena Ochs, BRIC, University of Copenhagen

09:45-10:15 **Deciphering the cell fate of developing mucociliary epithelium by single-cell phenomics**
by Jakub Sedzinski, NNF ReNEW, University of Copenhagen

10:15- 10:30 **Label free Raman based microscopy for studies in skin physiology and pharmacology**
by Jonathan Brewer, University of Southern Denmark

10:30-10:45 **Ultrastructural alterations in the Slitrk5-/- Mouse Model using SBF-SEM and vEM**
by Ole Borup Svendsen, Center for Molecular Morphology, Aarhus University

10:45-11:00 BREAK

11:00-12:35 SESSION 4

11:00-11:30 **From Methods to Tools to Image Analysis as a Service: our experiences with Content-Aware Image Restoration and Semantic Unmixing of Microscopy Data**
by Florian Jug, Computational biology, Human Technopole

11:30-11:50 **Holistic Imaging of Microsized Axons: From Morphology to Topological Organization in the Brain Network**
by Tim Dyrby, Danish Research Centre for Magnetic Resonance, Hvidovre Hospital

11:50-12:05 **High-throughput classification of S. cerevisiae tetrads using deep learning**
by Balint Szücs, Dep. Of Biology, University of Copenhagen

12:05-12:20 **Combining Light Sheet Microscopy and Large Data Analysis to Uncover Intricacies of Tumor Immunotherapy**
by Serhii Kostrikov, Technical University of Denmark

12:20-12:35 **3D Image Analysis with qim3d: An Open-Source Python Library**
by Felipe Delestro, QIM, Technical University of Denmark

12:35-13:30 LUNCH

13:15-16:30 WORKSHOPS

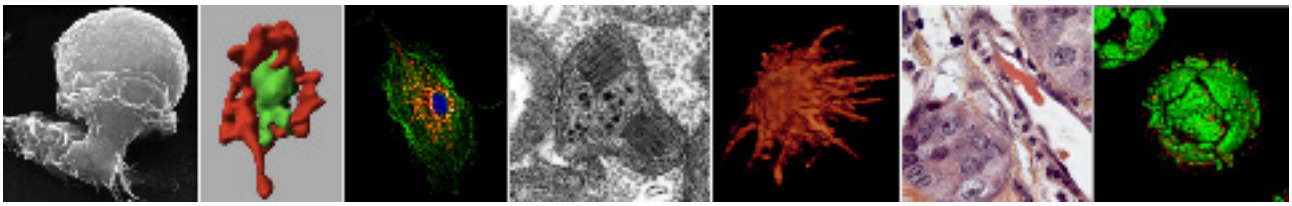
13.15-16.00 **AI in biodata: Industry insights - Alexandra Instituttet -** Holst Auditorium

13:30-15:00 **High Content Screening -** Room- 15.2.26

Pre-clinical/animal bioimaging facities in Denmark - where ae we now? - Room- 15.2.18

15:00-16:30 **Zeiss Lattice Lightsheet and SIM, Dynamic Profiler and Arivis -** Room- 15.2.18

16:30 END of the SYMPOSIUM



DANISH BIOIMAGING NETWORK

INVITED SPEAKERS

The Metrics for Building the Human Organ Atlas

Claire Walsh
Director, Senior Post Doctoral Research Fellow
Faculty of Engineering Sciences
University College London

L. Walsh*, J. Brunet, M. Berg, P. Tafforeau, P. D. Lee
*c.walsh.11@ucl.ac.uk

Hierarchical Phase Contrast Tomography (HiP-CT) is an ex vivo X-ray imaging technique developed at the European Synchrotron Radiation Facility in Grenoble, capable of multi-resolution imaging of intact human organs. With HiP-CT, we can image whole human organs with 25 µm voxels, then zoom down to near single-cell resolution anywhere within the organ without physically cutting the sample (bit.ly/HiP-CT-videos, mecheng.ucl.ac.uk/HiP-CT). We have begun to create the Human Organ Atlas (HOA) using these data (www.human-organ-atlas.esrf.eu).

To provide quantitative biomedical insights from HiP-CT data, we must segment structures of interest across multiple organs routinely on datasets of approximately 1 TB. These structures can then be used in a variety of ways, including direct morphological analysis, as landmarks for multi-modal registration, or as inputs for biophysical modeling of, for example, blood flow.

Segmentation is increasingly performed using machine learning frameworks in various ways, such as supervised, unsupervised, and active learning. A common theme across all these workflows is the need to assess the end segmentation using metrics. Common metrics such as DICE, accuracy, precision, recall, and F1-score are ubiquitous in the ML field; however, their application without understanding the downstream use of the segmented data poses significant challenges for projects such as the HOA.

This talk will show how ML is applied in different contexts to HiP-CT data and how the application and use of different metrics in these situations lead not only to different segmentation outputs but can also lead to substantively different downstream outputs from a biological viewpoint.

We use this to motivate discussion and development around what and how metrics should be tailored to the intended downstream use of segmented image data. In particular, this should be applied to large-scale projects such as the HOA, which aim to provide such segmented data in a FAIR manner to the scientific community at large.

INVITED SPEAKERS

Clinical AI Imaging from a Radiologist's Perspective: A Practical Approach

Ole Graumann
Professor
Aarhus University

Clinical AI imaging is a transformative approach in radiology that utilizes advanced machine learning and AI techniques to enhance diagnostic capabilities. This presentation focuses on the practical applications and implications of AI in radiology, emphasizing the integration of these technologies into clinical practice

AI imaging systems have revolutionized the interpretation of radiological data by providing highly accurate and consistent automated analysis. These systems employ various machine learning frameworks, including supervised, unsupervised, and reinforcement learning, to segment and analyze medical images across multiple modalities, such as MRI, CT, and X-ray.

A key challenge in clinical AI imaging is ensuring the reliability and interpretability of AI-generated results. The presentation will explore how AI models are trained and validated using large, diverse datasets to ensure robustness across different patient populations. Additionally, the integration of AI tools into the radiologist's workflow is examined, showcasing practical examples of AI assisting in diagnostic decision-making and improving efficiency.

This talk aims to provide insights into the current state of AI in clinical imaging from a radiologist's perspective, addressing both the opportunities and challenges. By understanding the practical considerations and the impact of AI on clinical practice, we can foster the development of AI tools that enhance patient care and support the radiology community in adopting these advanced technologies.

In conclusion, the presentation will advocate for the continued evolution of AI metrics and methodologies to align with clinical needs, ensuring that AI imaging tools deliver meaningful and actionable insights reasonably to the medical community.

DANISH BIOIMAGING NETWORK

INVITED SPEAKERS

Quantitative imaging of behavioural and mechanical heterogeneities underpinning mouse anterior patterning

Shankar Srinivas
Professor of developmental Biology
University of Oxford

During embryogenesis the anterior-posterior axis must be specified in the epiblast, that forms the foetus. In the mouse embryo, the distal visceral endoderm migrates within an epithelial monolayer over the epiblast and secretes signals essential for patterning.

It is unknown how cells negotiate their way amongst surrounding cells, and how they stop migrating at their destination. To address this, we used lightsheet microscopy to generate a multi-embryo, single-cell resolution, longitudinal dataset of cell behaviour and morphology. We developed a novel machine learning pipeline to segment cells and a data-informed systematic computational framework to analyse morphological, behavioural and molecular cell parameters in a unified coordinate space. Here we show that DVE cells retain regular morphology, don't exchange neighbours, are crowded, and show elevated tension, all hallmarks of the jammed state, while cells ahead deform and undergo neighbour exchanges. DVE cells stop upon reaching a region with matching elevated tension, that is Lefty1-dependent.

Our findings provide novel insights into axis formation, showing that DVE migration is facilitated, but also circumscribed, by heterogeneities in the mechanical properties of surrounding cells and suggests a paradigm by which signalling molecules regulate migration by modulating mechanical properties of tissues cells migrate through.

INVITED SPEAKERS

Euro-BioImaging - Opportunities for the Danish BioImaging Community

John Eriksson
Professor, Turku University
Director General, ERIC Euro-BioImaging

Euro-BioImaging ERIC (European Research Infrastructure Consortium) is a European, publicly funded, nonprofit research infrastructure (https://www.eurobioimaging.eu/). Our mission is to provide open access to biological and biomedical imaging technologies, training, and image data services to all researchers, regardless of research interest or affiliation, in both academia and industry.

Euro-BioImaging empowers researchers with open access to innovative biological and biomedical imaging technologies, expertise, data services, and training. We enable cutting-edge research and contribute to addressing pressing societal challenges. As an ERIC and an ESFRI Landmark Research Infrastructure, Euro-BioImaging contributes to the overall competitiveness of the European Research Area.

To achieve this, we bring together the most prestigious imaging facilities, which make their services, technologies, and expertise available to our users. These facilities, grouped together in entities called Nodes, are based across Europe and the European Molecular Biology Laboratory (EMBL) and offer a broad range of imaging technologies, both for biological and biomedical imaging.

Each facility has a highly unique profile, not only in imaging technology, but also in surrounding infrastructure and multidisciplinary research environment. All have demonstrated their scientific and technical excellence and commitment to user service in a stringent evaluation by the Scientific Advisory Board of Euro-BioImaging.

INVITED SPEAKERS

Resolving the molecular mechanisms governing 3D chromatin organisation

Fena Ochs
Associate Professor
University of Copenhagen

Eukaryotic genomes are organized by loop extrusion and sister chromatid cohesion, both mediated by the multimeric cohesin protein complex. Understanding how cohesin holds sister DNAs together, and how loss of cohesion causes age-related infertility in females, requires knowledge as to cohesin's stoichiometry in vivo.

We have developed a super-resolution microscopy approach based on Structured Illumination Microscopy (SIM) that allows the single molecule resolution of chromatin processes in the 3D space of intact cells.
Using quantitative 3D-SIM, we identify two discrete populations of chromatin bound cohesin in postreplicative human cells. While most complexes appear dimeric, cohesin localized to sites of sister chromatid cohesion and associated with sororin is exclusively monomeric. The monomeric stoichiometry of sororin:cohesin complexes demonstrates that sister chromatid cohesion is conferred by individual cohesin rings, a key prediction of the proposal that cohesion arises from their co-entrapment of sister DNAs.

INVITED SPEAKERS

Deciphering the cell fate of developing mucociliary epithelium by single-cell phenomics

Jakub Sedzinski
Associate Professor
Novo Nordisk Foundation Center for Stem Cell Medicine
University of Copenhagen

Mari Tolonen, Ziwei Xu, and Jakub Sedzinski, Novo Nordisk Foundation Center for Stem Cell Medicine
University of Copenhagen

As the developing embryo forms, the simple mass of cells becomes more and more complex throughout the morphogenetic shaping of tissues. Morphogenetic processes display an astounding level of tissue self-organization, where an initially unorganized mass of cells rearranges to form a functional tissue. One example of such process is the formation of regularly patterned, multilayered tissue, such as mucociliary epithelia (MCE); however, the formation of the tissue from initially pluripotent cells remains uncharacterized.

The morphogenetic shaping and cell fate choices in the mucociliary epithelium takes place both collectively and individually. Cells exhibit collective movement, but single cells in in the deep cell layer can also migrate individually and remodel their environment. Similarly, with fate choices, neighboring cells end up in differing cell types as intercalating cell types become evenly spaced out, but at the same time cells also signal to each other to regulate their differentiation. To resolve the morphogenetic behaviors across time and across the scale of individualism-collectivity, we quantify morphological and kinetic phenotypes of single cells in the embryonic Xenopus mucociliary epithelium. Using explanted prospective epidermis, aim to can image and quantify developmental dynamics in single-cell resolution.

To achieve this, we have developed a state-of-the-art quantitative imaging pipeline to track cell dynamics in the bottom layer of developing Xenopus epidermal explants. Simultaneously, we trace the positioning of different cell types throughout the development by visualizing the cell type specific marker genes in developmentally critical stages of the explant to link the cell trajectories to cell types. The detailed backtracking of the cell's histories allows us to connect individual cell behaviors to cell fate.

By assaying the embryonic epidermis, we aim to provide a detailed view of the developmental dynamics of a mucociliary epithelium. Understanding these fundamentals of mucociliary differentiation could provide a novel understanding of pathological conditions arising from defective development of airway epithelia.

INVITED SPEAKERS

From Methods to Tools to Image Analysis as a Service: our experiences with Content-Aware Image Restoration and Semantic Unmixing of Microscopy

Florian Jug
Head of Image Analysis Facility, Computational biology
Research Group Leader, Human Technopo

The necessity to analyze scientific images is as old as the ability to acquire such data. While this analysis did initially happen by observation only, modern microscopy techniques enable us to image at unprecedented spatial and temporal resolutions, through the 'eyes' of many and very diverse imaging modalities.

The unfathomable amounts of data acquired in the context of life science research cannot any longer be analyzed by manual observation alone. Instead, algorithmic solutions are helping researchers to study and quantify scientific image data.

In the past years, our abilities to use artificial intelligence (AI) for the automated analysis of scientific image data gained significant traction, and many important analysis problems have now much improved solutions based on ANNs. In this context, I would like to give an update on some of our latest algorithmic developments, i.e. the semantic unmixing of superimposed structures in fluorescence microscopy data.

But the current explosion of AI methods alone is not sufficient to addressing all analysis needs. It will require a community effort to turn useful methods into powerful tools, and even more community efforts to build suitable workflows and teach users how to best benefit from them. In this context, I will also be presenting AI4Life and bioimage.io (the Bioimage Model Zoo), a FAIR and easy to use infrastructure to store, share, and use AI based methods.

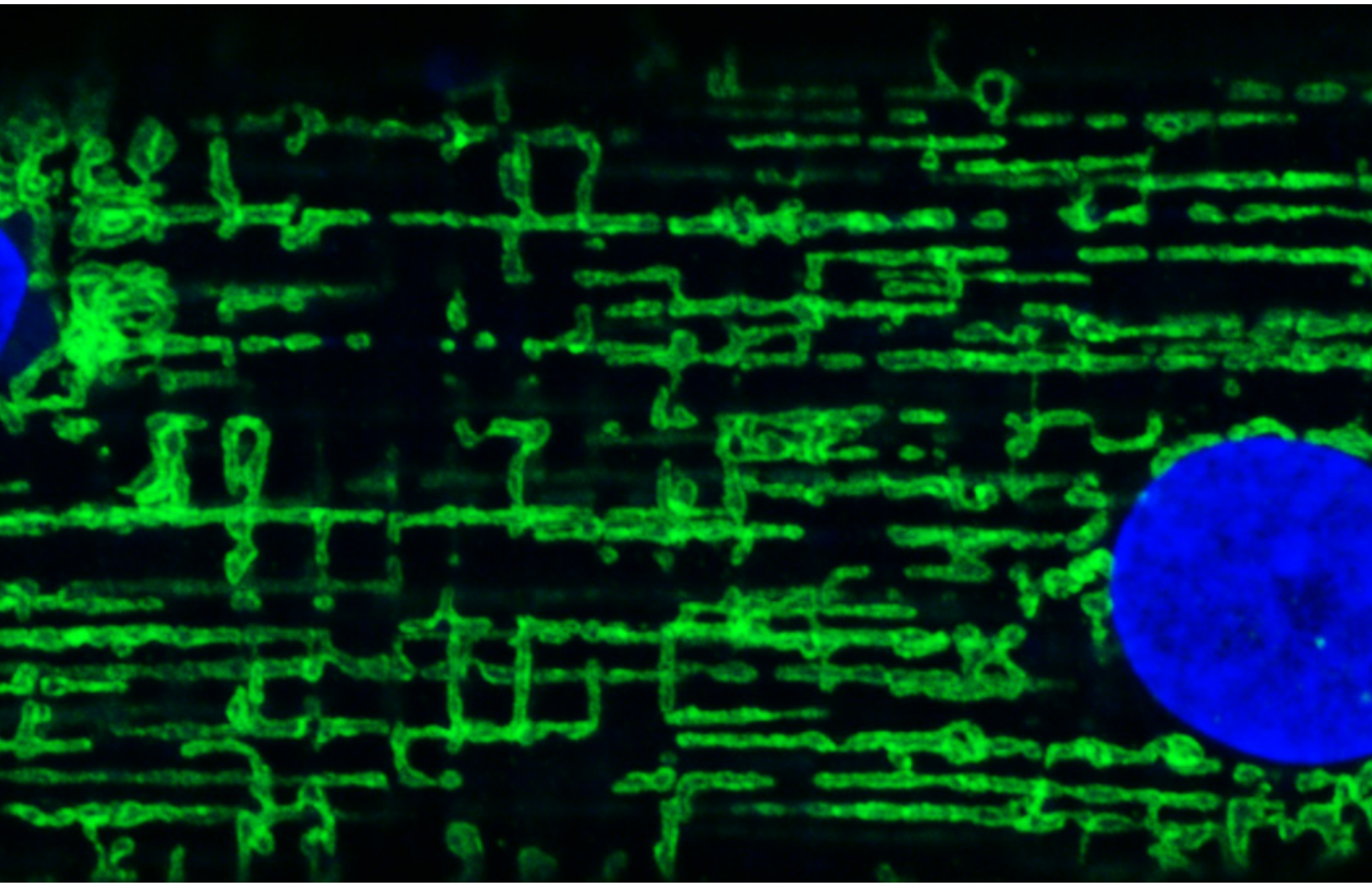
INVITED SPEAKERS

Holistic Imaging of Microsized Axons: From Morphology to Topological Organization in the Brain Network

Tim Dyrby
Professor
Danish Research Centre for Magnetic Resonance (DRCMR)
Copenhagen University Hospital - Amager and Hvidovre

The communication between different functional brain regions is transmitted via bundles of microsized axons. It is believed that axon diameter modulates the speed of neuronal signal transmission along the axon, thereby influencing the timing of the brain network. Mathematical models have been established to predict the structure-function relationship of axons by assuming a cylindrical shape. However, the question remains: what does an axon look like in 3D, and how is it organized when forming larger pathways connecting functional regions? Here, we combine Magnetic Resonance Imaging (MRI) and X-ray Nano Holotomography (XNH) imaging of the same brain sample to quantify 3D axon morphology and its topological organization across anatomical length scales ranging from millimeters to nanometers.

SHORT TALKS ABSTRACTS



SHORT TALKS

3D ultrasound to improve surgical precision during oral cancer treatment

by Fatemeh Makouei, Rigshospitalet

The primary treatment for most cancer types is surgery, intending to remove all cancerous tissue with a free margin of healthy tissue around it. However, accurately identifying the tumor's edge during surgery can be challenging, and inadequate surgical margins will potentially leave cancer cells in the body.

Approximately 50% of oral cancer surgeries result in unclear margins, requiring additional interventions to ensure complete tumor removal. This will decrease the chance of survival and necessitate additional surgery and/or radiation therapy, which significantly will impact the patient's quality of life negative.

To address this issue, we have developed a new 3D ultrasound scanning technique that can be used in the operating room to analyze the margins of removed cancer tissue. This provides surgeons with real-time information on the success of the surgery, allowing them to determine if more tissue needs to be removed.

Seeing is believing: abundance and function of G-quadruplex DNA in Staphylococcal biofilms

by Gabriel Antonio Salvador Minero (antonio.minero@inano.au.dk), Line Mørkholdt Lund, Andreas Møllebjerg, Mikkel Illemann Johansen, Nis Pedersen Jørgensen, Daniel Erik Otzen, Victoria Birkedal, Rikke Louise Meyer. Interdisciplinary Nanoscience Center (iNANO), Aarhus University, Gustav Wieds Vej 14, 8000 Aarhus, Denmark

Extracellular DNA (eDNA) is a major matrix component of many biofilms. Until recently, eDNA was assumed as a canonical right-handed B-form. However, eDNA appears to become resistant to nuclease digestion as the biofilm matures. This can be due to formation of non-canonical structures such as Z-DNA, G-quadruplex (GQ), and DNA: RNA hybrids. First, we applied immunostaining to visualize Z-DNA, GQ-DNA as well as B-DNA in staphylococcal biofilms grown under adverse conditions including nuclease treatment. Then we developed a faster and cheaper staining method of GQ-DNA using SYTO/TOTO (live/dead) dye combination. While TOTO has superior affinity to B-DNA, both SYTO and TOTO bind GQ-DNA resulting in FRET. Finally, we demonstrate novel function of GQ-DNA such as peroxidase activity of GQ-DNA complexed with hemin.

The eDNA structures were studied in vitro in S. epidermidis (WT, ΔicaADBC, ΔatlE) biofilms grown in TSB with 200 mM NaCl and 5 µM hemin, and in vivo in a murine implant-associated osteomyelitis model with S. aureus. The biofilm macroscale structure was imaged by optical coherence tomography. B-DNA, Z-DNA and GQ structures were visualized by confocal laser-scanning microscopy, using immunolabeling (antibodies Ab35I9, Z22 and BG4, respectively) and using DNA-binding stains SYTO™60 and TOTO™-1. Fluorescent tyramide and H_2O_2 were used for detection of the peroxidase activity from the GQ/hemin DNAzyme.

We believe that an important role of non-canonical DNA in biofilm has been overlooked until now. Macroscale web-like matrix structures appeared in biofilms formed in TSB with hemin, in which both GQ- and Z-DNA were detected. GQ-DNA were both associated with the cell surface and in spiderweb-like strings connecting micro-colonies, and GQs were abundant in both in vitro and in vivo biofilms. Staining biofilms with fluorescent tyramide and H_2O_2 resulted in detection of heme/GQ DNAzyme activity in biofilms, and future studies will reveal how bacteria benefit from this activity. Staining biofilms with SYTO™60 and TOTO™-1 resulted in GQ-specific FRET in the extracellular matrix.

Some of the results from this work are published in Nucleic Acids Research (2024) (DOI: 10.1093/nar/gkae034).

SHORT TALKS

Drug Distribution Analysis: A Comprehensive Approach Using Whole Mouse Organ Clearing and Lightsheet Microscopy

by Sara Elgaard Jager, Kathrine Jensen, Laura R. Knudsen, Grace A. Houser, Tomek Topilko, Kenneth Lindegaard Madsen.Department of Neurosciences, University of Copenhagen

In the Madsen lab we have implemented tissue clearing and lightsheet microscopy to visualize the distribution of a fluorescently conjugated drug within mouse organs. By integrating tissue clearing techniques with lightsheet microscopy, we devised a protocol amalgamating features from iDisco and PEGASOS clearing protocols, ensuring optimal tissue translucency while preserving drug fluorescence. Our investigation spans various anatomical regions including the leg, brown fat, white fat, brain, and spinal column, aiming to discern the drug's site-specific effects.

Additionally, employing whole-brain cfos staining enabled elucidation of the drug's impact on brain activity. We established a comprehensive pipeline encompassing automated stitching, alignment to the Gubra brain atlas, segmentation, and statistical analysis to achieve unbiased quantification of cfos-positive cells.The implementation of whole organ tissue clearing and lightsheet microscopy has markedly advanced our understanding of the experimental drug molecule, propelling our project forward.

Non-invasive label-free assessment of cellular growth and lipid storage in a free-living dinoflagellate Symbiodinium sp.

Walter Dellisanti, Swathi Murthy, Michael Kühl. Marine Biology Section, University of Copenhagen, Helsingør

Symbiodiniaceae dinoflagellates play a pivotal role in crucial processes such as photosynthesis, nutrient cycling, and calcium carbonate production in coral reef ecosystems. Despite iron is recognized in the physiological functions of Symbiodiniaceae, its precise impact on cellular development and metabolic health remains elusive. Here, we investigated the influence of varying iron concentrations, ranging from 0 to 100 nM Fe(III), on Symbiodinium sp. ITS2 type A1 cultures, with a particular focus on non-invasive label-free holotomography analysis. We show that moderate levels of dissolved iron (50 nM) increase growth rates and the development of cellular components, including lipids and proteins. We observed distinct growth patterns, alterations in pigment concentrations, and shifts in cellular morphology contingent upon iron availability. Non-destructive, label-free holotomographic imaging revealed higher intracellular lipid accumulation (+57%) in response to 50 nM Fe(III) enrichment. Our findings contribute to a deeper understanding of the relationship between iron availability and Symbiodinium sp. growth and cellular development, with implications for coral health and reef resilience in the face of environmental stressors.

Deciphering functional tumor-immune crosstalk through highly multiplexed imaging and deep visual proteomics.

Xiang Zheng, Andreas Mund, and Matthias Mann. University of Copenhagen, Copenhagen, Denmark

Introduction: To address the challenge of capturing diverse cellular types while maintaining spatial context, we adopted multiplex staining techniques, enhancing our comprehension of tumor-stroma interactions. We introduced deep visual proteomics (DVP), a spatial technology that integrates imaging, cellular phenotyping, single-cell laser microdissection, and mass spectrometry (MS). We further introduced a robust dimethyl labeling-based multiplex-DIA workflow to enhance throughput and proteome depth. Our study integrates multiplex staining with multiplex-DIA DVP for spatial proteome profiling, leveraging MS capabilities while ensuring clinical feasibility. This approach demands only a single FFPE section for spatial proteome analysis at the resolution of single cell types, unraveling the complexities of the cancer ecosystem with direct clinical implications.

Material and method: We conducted multiplex immunofluorescent staining on human colorectal, skin, head and neck cancers, followed by cellular phenotyping and automated single-cell laser microdissection. Subsequently, we loaded dimethyl-labeled 3-plex or label-free tryptic peptides onto Evotips and performed LC-MS analysis using the Evosep One coupled to timsTOF SCP or latest generation of OrbitrapAstral MS.

Results and discussion: Pre- and post-22-plex staining proteome analysis confirmed minimal protein loss, validating microdissection compatibility and notable protein recovery. Key findings include: 1) profiling of 21 cellular (sub)types and identification of approximately 5000 protein groups from a cellular volume of 111,000 µm3, equivalent to only 55 single cells; 2) enhanced adaptability of tumor-infiltrating cytotoxic T cells with to hypoxic environments; 3) significant heterogeneity of tumor cells in relation to their proximity to cytotoxic T cells in tonsil cancer; 4) formation of an immunosuppressive barrier by macrophages hindering T cell infiltration; 5) distinctive spatial proteome signatures of T cell subsets in the colorectal tumor microenvironment of the lamina propria and tumor epithelium region; 6) identification of IL4 inhibition to address chemoresistance and promising targeted therapeutic candidates, including CDK and proteasome inhibitors, for composite lymphoma.

Conclusion: Our workflow showcases remarkable versatility across diverse cancer types and MS platforms, characterizing the spatial proteome of the tumor microenvironment and advancing personalized oncology.

Label free Raman based microscopy for studies in skin physiology and pharmacology

J. Brewer, I. Iachinaa, MA. Lomholta, K. Nielsen, M. Ebbesena. University of Southern Denmark

The utilization of Coherent anti-Stokes Raman Scattering (CARS) microscopy in biomedical research marks an advancement in our capability to examine complex biological systems with unparalleled resolution and specificity. This work emphasizes the application of CARS microscopy to investigate diverse aspects of skin physiology and pharmacology.

The application of CARS microscopy facilitated the direct observation of time-dependent, spatially resolved diffusion of water (D2O) within human skin tissue, revealing significant variations in diffusion coefficients across different layers of the Stratum Corneum (SC). This research demonstrates CARS microscopy's capacity to quantitatively assess diffusion coefficients at various tissue depths and locations.

Moreover, the investigation of dissolvable microneedles for transdermal drug delivery highlights CARS microscopy's distinctive ability to visualize the penetration of microneedles and track their morphology within the skin. By providing detailed images of microneedle decomposition and drug dispersion, CARS microscopy delivers insights into the mechanisms of drug release and absorption.

Overall, these studies illustrate the strength of CARS microscopy in providing detailed, molecule-specific insights into complex molecular interactions within tissues.

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SHORT TALKS

Ultrastructural alterations in the Slitrk5-/- Mouse Model using SBF-SEM and vEM.

Ole Borup Svendsen, Aarhus University

Using Serial Block-Face Scanning Electron Microscopy (SBF-SEM) to study the striatal ultrastructure of an Obsessive-Compulsive Disorder (OCD) mouse model, Slitrk5 KO, we identified Wallerian-like pathologies in a population of myelinated axons. Slitrk5, a protein known to play a role in neurite outgrowth, dendritic morphology, and neural survival. Knocking out this protein in mice leads to OCD-like phenotypes, potentially due to a deficient corticostriatal circuit.

In our study, we segmented and rendered healthy myelinated axons, nodes of Ranvier, glial cells, and pathological profiles. While no structural alterations were observed in healthy myelinated axons, Wallerian-like pathologies such as condensed axoplasm and myelin whorls appeared in the knockout mouse model. These structures lacked typical axoplasmic fine structure and were accompanied by either compacted electron-dense axoplasm, empty myelinated axonal profiles, multilamellar myelin structures, or swollen axons with heavy organelle accumulations. This type of pathology has not been previously linked to OCD, though it is seen in studies of traumatic brain injuries, multiple sclerosis, and Wallerian degeneration. 3D reconstructions showed myelin whorls as periodically disorganized remnants of myelin, while condensed axoplasm appeared as long pathological structures with preserved myelination. The morphology of condensed axoplasm varied significantly with some structures being elongated and/or encapsulated healthy axons.Our study using SBF-SEM and volume electron microscopy (vEM) provides new insights into OCD pathogenesis and axonal degeneration processes.

High-throughput classification of S. cerevisiae tetrads using deep learning.

Balint Szücs, University of Copenhagen

Meiotic crossovers play a vital role in proper chromosome segregation and evolution of most sexually reproducing organisms. Meiotic recombination can be visually observed in Saccharomyces cerevisiae tetrads using linked spore-autonomous fluorescent markers, placed at defined intervals within the genome, which allows for analysis of meiotic segregation without the need for tetrad dissection. In order to automate the analysis, we developed a deep learning-based image recognition and classification pipeline for high-throughput tetrad detection and meiotic crossover classification. As a proof of concept, we analyzed a large image dataset from wild-type and selected gene knock-out mutants to quantify crossover frequency, interference, chromosome missegregation and gene conversion events. The deep learning-based method has the potential to accelerate the discovery of new genes involved in meiotic recombination in S. cerevisiae such as the underlying factors controlling crossover frequency and interference.

Combining Light Sheet Microscopy and Large Data Analysis to Uncover Intricacies of Tumor Immunotherapy.

Serhii Kostrikov1, Hólmfridur Rósa Halldórsdóttir1, Sven Weller1, Anders Elias Hansen1, Thomas Lars Andresen1
Department of Health Technology, Technical University of Denmark

Various bioimaging modalities have been rapidly progressing towards providing more data. Large spatial coverage modalities like MRI and CT have been improving resolution, while high-resolution techniques like fluorescent microscopy have been expanding their spatial coverage. All these provide a fascinating opportunity to study heterogeneous biological structures like tumors in high detail. At the same time techniques for comprehensive data extraction and high-throughput analysis of massive amounts of data from such images, as well as incorporating these in large-scale studies with meaningful number of samples still presents a challenge.

SHORT TALKS

In the proposed presentation, we showcase a detailed large-scale study of T-cell based tumor immunotherapy. From a biomedical standpoint, our study dives deep into such key aspects of T-cell immunotherapy as tumor targeting, cell proliferation and migration on one hand, and features of tumor vasculature and its response to such therapeutic intervention on the other. From a methodological standpoint, our study presents techniques for pre-labelling of the structures of interest without time-consuming immunolabelling of large tissue samples; development and implementation of image analysis workflows for extracting cell distribution data in 3D space and details of 3D vessel architecture, together with feature engineering techniques such as clustering analysis and advanced 3D network analysis; automating statistical analysis and data visualization for such large multi-faceted datasets.

The case-study demonstrates how the implementation of advanced imaging and image analysis techniques helps us characterize intricacies of tumor-specific versus tumor non-specific T-cell therapies and uncover vascular remodeling observed as a differential response to cell specificity, which would be impossible to detect without these methods. Ultimately, it exemplifies how we can integrate these techniques in routine large-scale studies in a manner, which provides reasonable throughput and helps to translate large imaging data into valuable biomedical insights.

3D Image Analysis with qim3d: An Open-Source Python Library

Felipe Delestro, Technical University of Denmark

The field of image analysis demands robust and versatile tools to extract meaningful insights from complex 3D datasets. We introduce qim3d, an open-source Python library designed to streamline and empower image analysis for volumetric data. It offers a comprehensive suite of functionalities ranging from fundamental processing tools to intuitive graphical user interfaces, catering to the diverse needs of researchers and image analysts. Getting started is as easy as pip install qim3d.

At its core, qim3d provides essential functionalities for handling data, processing, and visualization. Leveraging state-of-the-art algorithms, the library facilitates tasks such as filtering, segmentation, and feature extraction, enabling users to efficiently preprocess raw data and extract relevant information. With a modular design, qim3d ensures flexibility and extensibility, allowing researchers to seamlessly integrate custom processing pipelines tailored to their specific research objectives.

One of the distinctive features of qim3d is its rich graphical user interfaces (GUIs), which empower users with intuitive tools for interactive analysis. For example, the library includes tools such as the Data Explorer for quick data insights. These GUIs not only enhance usability but also facilitate collaboration and knowledge sharing among researchers with varying levels of expertise.

In summary, qim3d emerges as a versatile tool for volumetric imaging, offering a blend of robust processing algorithms, intuitive graphical interfaces, and visualization capabilities. By democratizing access to sophisticated 3D image analysis tools, qim3d empowers researchers to unlock new insights and accelerate discoveries.

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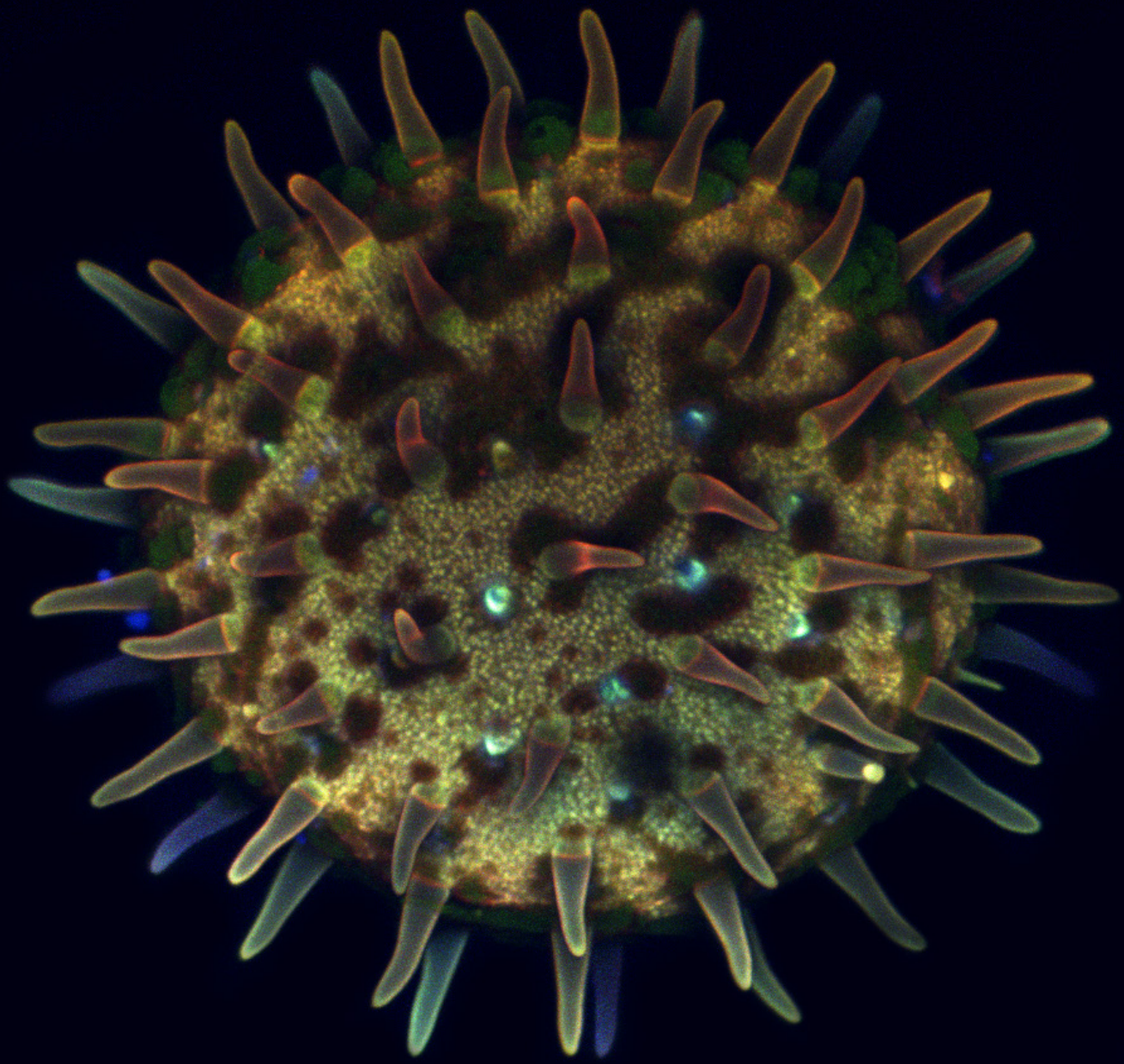

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