Universität Zürich[UZH]

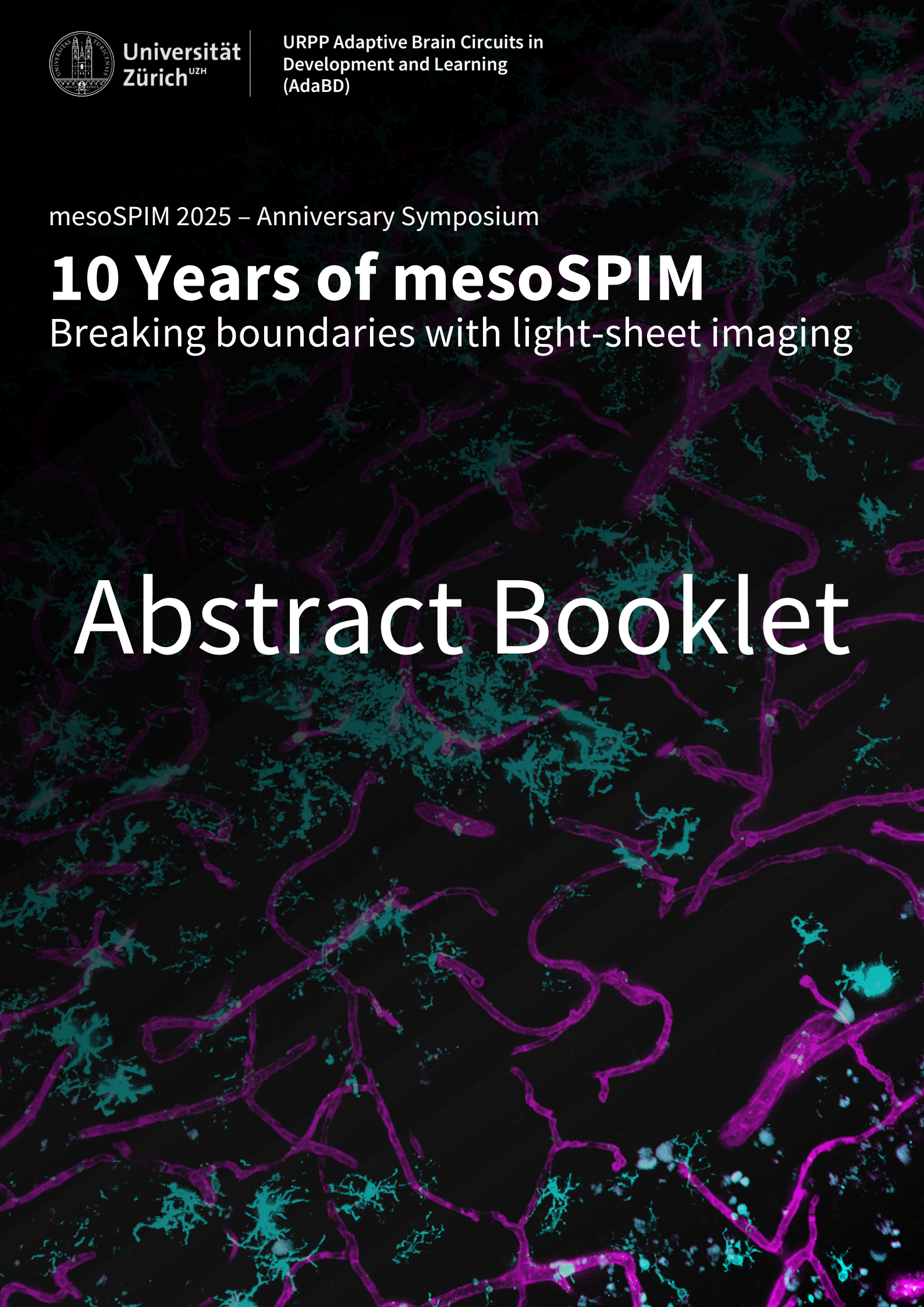
URPP Adaptive Brain Circuits in Development and Learning (AdaBD)

mesoSPIM 2025 – Anniversary Symposium

10 Years of mesoSPIM

Breaking boundaries with light-sheet imaging

Abstract Booklet



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Venue

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Program

Monday, October 13

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9:00 - 9:10 Fritjof Helmchen (University of Zurich, Switzerland)
"Welcome note".

9:10 - 9:40 Fabian Voigt (Harvard University, MA, USA)
"Expanding the bag of optical tricks for neuroscience".

9:40 - 10:00 Nikita Vladimirov (University of Zurich, Switzerland)
"The 10 years of mesoSPIM: lessons, updates and outlook".

10:00 - 10:40 (keynote, online) Simon C. Watkins (University of Pittsburgh, PA, USA)
"Pushing the mesoSPIM to submicron resolution with chemistry and computing".

10:40 - 11:00 Coffee break

11:00 - 12:20 Session 2. Tissue clearing (chair: Esther Stoeckli)

11:00 - 11:30 Anna Maria Reuss (University Hospital Zurich, Switzerland)
"aDISCO - A Broadly Applicable Method for 3D Microscopy of Archival Paraffin-Embedded Human Tissues".

11:30 - 12:00 (online) Hei Ming Lai (The Chinese University of Hong Kong)
"Customizing mesoSPIM-based workflows for clinical 3D tissue diagnostics".

12:00 - 12:20 Christophe Lamy (University of Geneva, Switzerland)
"Human anatomy at the age of optical dissection".

12:20 – 12:30 Martina Schättin Award

12:30 - 13:00 Lunch

13:00 – 13:50. Poster session, industry demos.

13:50 - 15:30. Session 3. Industrial solutions to imaging (chair: Fabian Voigt)

13:50 - 14:10 Ando Zehrer (Teledyne Photometrics, Germany)
"The Iris15: Teledyne Photometrics contribution to the MesoSPIM initiative".

14:10 - 14:30 Jon Daniels (Applied Scientific Instrumentation, USA)
"Modular Microscope and Automation Solutions".

14:30 - 14:50 Ralf Kruse (Mitutoyo Europe)
"TAGLENS – Ultrafast Focus Shift by Sound".

14:50 - 15:10 Otmane Buchareb (INSCOPER, France)
"Imaging software solution for automated and smart microscopy".

15:10 - 15:30 Nicolaas van der Voort (SVI Huygens, Netherlands)
"Powerful mesoSPIM image processing in the Huygens Software: a journey from Deconvolution, Fusion & Stitching to image visualization".

15:30-16:00 Coffee break

16:00-18:00 Session 4. Microscopy methods (chair: Nikita Vladimirov)

16:00 - 16:30 Kevin Dean (University of Texas Southwestern, TX, USA)
"Navigate, MCT-ASLM, and Altair-ASLM: Intelligent, Multiscale, Easy-to-Assemble Light-Sheet Platforms".

16:30 - 17:00 Prayag Murawala (MDI Biological Laboratory, ME, USA)
"Enhanced mesoSPIM for high-resolution mapping of brainstem locomotor circuits".

17:00 - 17:20 Jenna Hanmer (University of Nottingham, UK)
"Whole-brain imaging in rodents using MRI and light-sheet microscopy: A cross-scale, multi-modal approach".

17:20 - 18:00 (keynote, online) Adam Glaser (Allen Institute, WA, USA)
"New technologies for mapping centimeter scale tissues with nanoscale resolution".

18:00-19:30 Apero and get-together.

Tuesday, October 14

8:30 Welcome coffee

9:00 - 10:30 Session 5, Big data analysis and AI (chair: Joana Delgado Martins)

9:00 - 9:30 Stephan Preibisch (HHMI Janelia Research Center, VA, USA)
"BigStitcher-Spark: Software for peta-scale alignment of light sheet microscopy acquisitions".

9:30 - 10:00 Alan Watson (University of Pittsburgh, PA, USA)
"Automating MesoSPIM Data Postprocessing".

10:00 - 10:20 Dimokratis Karamanlis (University of Geneva, Switzerland)
"LightSuite: A high-throughput pipeline for localizing experience in the cleared mouse brain".

10:20 - 11:00 Coffee break

11:00 - 12:30, Session 6. Data analysis solutions (chair: Csaba Földy)

11:00 - 11:20 Michael Mahlert (Oxford Instruments Imaris)
"Interactive 3D and 4D image visualization and high throughput analysis with Imaris".

11:20 - 11:40 Michael Morehead (SyGlass)
"syGlass: Visualization, Annotation, and Communication of Very Large Image Volumes in Virtual Reality".

11:40 - 12:00 Tamara Manuelian (ZEISS Research Microscopy Solutions)
"ZEISS Beyond Imaging: How to manage, explore & analyze your imagery with ZEISS Software Solutions".

12:00 - 12:20 Joel Lüthi (University of Zurich, Switzerland)
"Fractal: An open-source framework for reproducible bioimage analysis at scale using OME-Zarrs".

12:20 – 13:00 Lunch

13:00 – 13:50 Poster session, industry demos

13:50 - 15:20 Session 7. Applications (chair: Fritjof Helmchen)

13:50 - 14:20 Esther Stoeckli (University of Zurich, Switzerland)
"mesoSPIM facilitates the analysis of neural circuit formation".

14:20 - 14:50 Laura Baudis (University of Zurich, Switzerland)
"Probing dark matter and neutrinos with light sheet microscopy".

14:50 - 15:20 Christelle Langevin (INRAE, University of Paris-Saclay France)
"Advancing animal health with tissue clearing and deep tissue imaging".

15:20-15:50 Coffee break

15:50 – 17:40 Session 8. Applications (chair: Martin Müller)

15:50 - 16:20 Laura Batti (Wyss Geneva, Switzerland)
"Light-Sheet Microscopy: empowering advancement in neuroscience and medicine."

16:20 - 16:40 Akanksha Jain (ETH Zürich, Switzerland)
"Unveiling the choreography of human brain development: Longterm lightsheet imaging unveils morphodynamics in human brain organoids".

16:40 - 17:00 Melvin Alappat (University of Zürich, Switzerland)
"Clearly sweet and painful: Investigating the effect of western diet on myelin homeostasis".

17:00 - 17:30 Thomas Naert (Ghent University, Belgium)
"Pythia: AI-predictable CRISPR-Cas9 editing meets mesoSPIM for visualizing precise genomic integrations and edits".

17:30 - 17:40 Nikita Vladimirov
"Concluding remarks".

17:40-19:00 Apero and farewell.

Abstracts of oral presentations

Session 1 - Developers

Expanding the bag of optical tricks for (neuro)science

Fabian Voigt

Harvard University

Starting with a retrospective on the development of the mesoSPIM platform, I will present an overview of various projects aimed at providing the bioimaging community with better instrumentation - including more recent work involving metasurfaces.

The 10 years of mesoSPIM: lessons, updates and outlook

Nikita Vladimirov[1,2], Fabian Voigt[3], Fritjof Helmchen[1,4]

1 URPP AdaBD, Brain Research Institute (HIFO), University of Zurich, Zurich, Switzerland
2 Center for Microscopy and Data Analysis (ZMB), University of Zurich, Zurich, Switzerland
3 Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA, USA
4 Neuroscience Center Zurich (ZNZ), University of Zurich, Zurich, Switzerland

The mesoSPIM is an open-source light-sheet microscopy project for imaging cleared tissues. Our goal is to develop and share knowledge on how to build and use your own facility-grade light-sheet microscope - compatible with all tissue clearing techniques, at affordable cost, with open-source hardware and software, thus making light-sheet imaging more accessible for cutting-edge biological and medical research. Currently, there are over 37 mesoSPIM systems in operation around the world, used for a variety of research projects in neuroscience, pathology, anatomy, developmental biology, and physics. There are more than 100 publications that used mesoSPIM microscopes, and the project's scientific impact is rapidly growing with new systems being actively built and used around the world.
I will share the lessons I learned from developing and disseminating this open-source project and discuss factors that contribute to its longevity and success, current challenges, and future directions.

Pushing the mesoSPIM to submicron resolution with chemistry and computing

Simon Watkins, Alan Watson, Reese Smith, Iana Vasylieva

University of Pittsburgh, Pittsburgh PA

The CBI which I founded and direct is a very large (multifaculty) research center directed to implementing computational microscopy to solve complex problems in biomedical research. The mesospim platform has become an integral part of our technology platform and its use continues to expand. We have built 3 mesoSPIM systems at this point. I will discuss why we started down the road of building and using this particular platform, the customizations we have made to the scope design using high NA macro optics and our Cuvette design which removes any possibility of astigmatism due to misalignment. I will also discuss our attempts to push the final image resolution using the mesospim platform into the submicron range using tissue expansion and image processing/deconvolution approaches .

Session 2 - Tissue Clearing

aDISCO - A Broadly Applicable Method for 3D Microscopy of Archival Paraffin-Embedded Human Tissues

Anna Maria Reuss [1,2]

1 Institute of Neuropathology, University Hospital Zurich, Zurich, Switzerland
2 Brain Research Institute, University of Zurich, Zurich, Switzerland

During human surgeries and autopsies, specimens are regularly sampled and stored as formalin-fixed paraffin-embedded (FFPE) tissue blocks, giving rise to enormous archives of healthy and diseased human tissues. In my talk, I present 'archival DISCO' (aDISCO), a versatile and robust clearing method for whole-mount archival FFPE human tissue blocks, stored for at least 15 years. aDISCO enables complete clearing and consistent antibody staining of the entire tissue blocks from various organs and is compatible with a wide range of commonly used antibodies.

Customizing mesoSPIM-based workflows for clinical 3D tissue diagnostics

Hei Ming Lai

The Chinese University of Hong Kong

Clinical 3D tissue analysis based on deep immunostaining, tissue clearing, and light-sheet microscopy can constitute an ideal workflow for non-consumptive extraction of complete information within clinical specimens with pragmatic turnaround time and logistics. We report our progress in developing such a pipeline, involving reproducible multiplexed immunostaining for reliable automated single-cell segmentation to quantify actionable biomolecular expression in formalin-fixed and FFPE specimens. This also consists of customizing mesoSPIM as the hardware backbone for clinical diagnostics, which we invite the community to comment on and collaborate further.

Human anatomy in the age of optical dissection

Christophe Lamy

University of Geneva

The combination of large-scale tissue clearing methods and mesoscopic imaging tools enables the optical dissection of human organs. This approach merges the historical gap between histology, that resolves the cellular and subcellular structure of tissues, and macroscopic anatomy, that describes organs in their entirety and in their anatomical relationships. It leads to new descriptions of human anatomy. It helps to characterize more accurately extended structures, scarce components and non-homogeneous tissues. It enables multimodal and multiscale studies linking molecular and cellular features to medical imaging. We optimized tissue processing, imaging and analysis pipelines to study human cadaveric specimens. We demonstrated their application to all organ systems, from brain, to viscera though to the locomotor system. Further, we showed their suitability to study pathological tissues. Optical dissection opens new avenues in the understanding of normal and pathological anatomy.

Session 3 - Industrial solutions to imaging

The Iris15: Teledyne Photometrics contribution to the MesoSPIM initiative

Ando Zehrer[1], Rachit Mohindra[2]

1 Teledyne Photometrics, Germany
2 Teledyne Photometrics, USA

Teledyne produces a wide range of sensors and cameras which find relevance in various industries, whether commercial or research. Our Teledyne Scientific Imaging branch and in particular Photometrics has a portfolio specialized in scientific cameras for microscopy, astronomy and spectroscopy. While many of our cameras can be used for light-sheet microscopy, the emphasis will be put on the Iris15, an established solution for producing high quality datasets on the mesoSPIM system, and our revolutionary KINETIX. Several imaging modalities provided by both, IRIS15 and KINETIX, are relevant to synchronize the exposure progression with the illumination (i.e. our programmable scan mode). The efficacity of our solution can be shown with different projects where the mesoSPIM system recorded high-quality data using the Iris15 and the KINETIX. In addition to the image quality, Teledyne Photometrics can also accelerate and enhance the user's research through our high-end technical support, our resources and open-SDK which supports a plethora of platforms.

Modular Microscope and Automation Solutions

Jon Daniels

ASI / Applied Scientific Instrumentation

Applied Scientific Instrumentation (ASI) has been advancing the state of the art in optical microscopy in close collaboration with leading scientists around the world for 35 years.
I will give an overview of ASI's motorized stages, modular microscope components, and objective lenses for cleared tissue imaging. I will also share a few case studies about collaborations with researchers that have led to new scientific capability.
ASI hardware allows researchers to quickly create customized automated microscopes. These microscopes are modifiable, affordable, and easily replicated which allows for rapid dissemination of advances.

TAGLENS – Ultrafast Focus Shift by Sound

Ralf Kruse

Mitutoyo Europe

Light sheet microscopes consist of various optical components. A core component is a laser with defined focal region. A rather new optical sensor can shift the laser focus with ultra-high speed to reduce data capturing time. In this talk I will explain the technical principle of this new optical device. TAGLENS is a special designed optics filled with a liquid, surrounded by piezo elements that produce a dynamic gradient index in the center of the TAGLENS body. Thus, a light beam, such as a laser beam, will change its propagation behavior according to the dynamical optical property of the fluid. The controlled electrical signal of the piezo elements with a frequency of 70 kHz results in an ultra-fast focus shift of the same speed. Integrating a TAGLENS with its fast axial scanning in the optical setup of the laser beam allows a uniform illumination with high NA (high resolution) and large FOV (field of view).
This new technology is already implanted in commercial light sheet microscopes for optimizing the uniformity of illumination, resulting in a high signal-to noise ratio and reduced measuring time.

Imaging software solution for automated and smart microscopy

Otmane Bouchareb

INSCOPER

Today's imaging solutions must coordinate an expanding array of cameras, stages, lasers, and motorized optical components, each typically driven by its own dedicated software. INSCOPER brings the entire system together into a single, universal solution that synchronizes every device down to the millisecond, streams data to disk without bottlenecks, and delivers fully datasets ready for analysis.
In this talk we will:
1. Showcase INSCOPER's imaging advantages
   - Multimodal integration: from simple time-lapse to complex multi-day acquisitions.
   - Universal compatibility: works with any camera-based microscope and application.
   - Intuitive workflow: build complete protocols in minutes with a clean and user-friendly interface.
   - Higher performance: ensures reproducibility, delivers sustained throughput, and is maintenance-friendly even on aging Windows systems.
2. Highlight features tailored for mesoSPIM
   - Automatic system calibration.
   - Precise synchronization between light-sheet and sample for flawless 3-D imaging.
   - Coordinated control of scanners, lasers, and camera for light-sheet mode.
   - Optimized data streaming to keep up with high-volume acquisitions.

Whether you run a commercial microscope, build a custom system, or retrofit legacy systems, INSCOPER handles every step from hardware setup to image acquisition, so you spend less time wrestling with the equipment and more time answering biological questions.

Powerful mesoSPIM image processing in the Huygens Software: a journey from Deconvolution, Fusion & Stitching to image visualization.

Nicolaas van der Voort

SVI Huygens, Netherlands

Huygens deconvolution has a proven track record in deconvolving complex datasets, performing well over a range of complex 3D datasets. For Gaussian-type lightsheets, the Huygens PSF is position dependent and accounts for the deteriorating z-resolution away from the lightsheet center. Thereby, it can increase the useful range of the lightsheet far beyond the rayleigh range. For axially-swept lightsheet types, Huygens has a separate PSF model, also ensuring high-quality deconvolution for those types.
Commonly, two oppositely placed cameras are used to counteract shadowing artifacts, in turn creating the need for multi-view fusion. A straightforward approach is to overlay the two images directly. However, this requires careful (re)alignment of the two cameras to avoid object doubling, increasing the operation skill bar. The Huygens software comes with a Fusion option that automatically optimizes the position of each view, thereby decreasing the skill bar to operate the microscope. It can flexibly handle many views, for example a 2, 4 or 8 view configuration.
After Fusion, image stitching is ubiquitously needed. Drift during acquisition of large z-stacks may create the need for stabilization prior to stitching. For the stitching process itself, the tile position needs to be optimized to avoid object doubling and vignetting correction may be necessary. The Huygens Stitcher option is built for large data and can handle the aforementioned issues, while providing fast preview feedback.
Clearly, processing speed is key. The best processing speeds can be achieved by a combination of fast algorithms and efficient use of computational resources. Huygens is very nimble in dividing up the whole dataset into relevantly sized chunks that fit on the GPU, thereby enabling use of the full processing power available on the system. Further, the need for images to fit in the computer RAM is a hurdle for many users. Huygens alleviates this by using memory-mapped files allowing the processing of images larger than the RAM.
In conclusion, Huygens is a mature image analysis software package that can deal with a near-complete set of image analysis challenges associated with SPIM. It has a proven track-record of supporting leading researchers around the world. By providing fast feedback on restoration choices, it puts users in the driver's seat, lowering the operation skill-bar and enabling researchers to focus on what really matters to them.

Session 4 - Microscopy Methods

Navigate, MCT-ASLM, and Altair-ASLM: Intelligent, Multiscale, Easy-to-Assemble Light-Sheet Platforms

Kevin M. Dean

University of Texas Southwestern Medical Center, Dallas, TX, USA.

Axially Swept Light-Sheet Microscopy (ASLM) synchronizes aberration-free remote focusing with the rolling shutter of a scientific CMOS camera to achieve isotropic, high-resolution, and large-field-of-view imaging. Building upon this foundation and integrating open-source design elements from the mesoSPIM Initiative, we developed Multiscale Cleared-Tissue ASLM (MCT-ASLM), an imaging platform that supports both organelle- and tissue-scale imaging in a unified format. To enable adaptive, event-driven imaging workflows, we created Navigate, an open-source software suite that dynamically tunes microscope operation in real time using image-based feedback. Navigate is highly modular and compatible with varied hardware, facilitating self-driving acquisition workflows on diverse imaging systems. To improve accessibility, we also developed Altair-ASLM, a plug-and-play light-sheet microscope platform featuring a precision dowel-pin baseplate for rapid, reproducible optical alignment. All designs, parts lists, and software are publicly hosted on GitHub, supporting widespread adoption and community-driven innovation. Together, Navigate, MCT-ASLM, and Altair-ASLM provide an integrated toolkit for intelligent, scalable, and high-performance light-sheet imaging.

Enhanced mesoSPIM for high-resolution mapping and its many applications

Prayag Murawala

MDI Biological Laboratory, Bar Harbor, ME - 04609, USA

I will describe 1) how my lab which is focused on tissue regeneration and morphogenesis got into mesoSPIM, 2) how mesoSPIM variant at MDIBL is different from the standard set up, 3) the innovative visiting scientist program (VSP) at MDIBL which provides access of mesoSPIM to external users and 4) how we are leveraging it to answer many different questions in biology.

Whole-brain imaging in rodents using MRI and light-sheet microscopy: A cross-scale, multi-modal approach

Jenna Hanmer[1], JP Manzano-Patron[1], M Craig[1], M Prior[1], C Lai[2], E Radfar[3], GJ Hickman[4], E de Guzman[5], C Tisca[5], M Tachrount[5], Q Shen[5], C Ligneul[5,6], AFD Howard[5,7], RC Trueman[8], K Chisholm[8], S Jbabdi[5], KL Miller[5], PS Morgan[1], TD Farr[9], J Lerch[5], SN Sotiropoulos[1,5]

1 Sir Peter Mansfield Imaging Centre, School of Medicine, University of Nottingham, UK

2 MRC Weatherall Institute of Molecular Medicine, University of Oxford, UK

3 Centre of Membrane Proteins and Receptors (COMPARE) Advanced Imaging Facility, University of Birmingham, UK

4 School of Science and Technology, Nottingham Trent University, UK

5 Oxford University Centre for Integrative Neuroimaging, University of Oxford, UK

6 Commissariat à l'énergie atomique et aux énergies alternatives (CEA), Paris, France

7 Imperial College London, Faculty of Engineering, UK

8 School of Life Sciences, University of Nottingham, UK

9 Centre for Cardiovascular Science, University of Edinburgh, UK

Diffusion Magnetic Resonance Imaging (dMRI) enables non-invasive investigation of brain connectivity and microstructure, but its measurements are indirect, noisy, and limited in resolution. In contrast, optical imaging of post-mortem tissue offers a cellular-scale ground truth for validating dMRI contrasts. However, aligning traditional 2D histology with 3D dMRI is challenging. Fortunately, recent advances in tissue clearing and light-sheet microscopy now enable 3D optical imaging of intact rodent brains, facilitating direct comparisons with dMRI. Here, we leverage these emerging technologies to combine high-resolution ex-vivo dMRI with whole-mount immunolabelling using standard antibodies. This approach extends capabilities beyond transgenic fluorescent reporter models, enabling application to wild-type mice, rats, and potentially human tissue. Adult rodents were perfused, with or without gadolinium contrast agent, and multi-shell dMRI (100–200µm isotropic) was acquired across multiple 7T Bruker scanners, forming the basis of a robust, generalisable processing pipeline. Following MRI, brains were cleared, immunolabelled (for neurons, astrocytes, microglia, neurofilaments, and myelin), and imaged volumetrically at sub-5µm resolution with UltraMicroscope II or BLAZE light-sheet systems. Image stacks were stitched, converted to NIfTI format, and registered to dMRI volumes. Our results demonstrate that clearing and immunostaining are compatible with gadolinium contrast, enabling shorter dMRI scans with improved signal-to-noise. Importantly, we achieved robust myelin staining, addressing a known challenge in cleared tissue. Whole-brain 3D microscopy revealed microstructural features that aligned directly with dMRI, avoiding the complex methods required to map histology onto dMRI. From these datasets, fibre orientation, neuronal density, and glial metrics can be extracted and compared with dMRI-derived measures. Together, this pipeline integrates dMRI, tissue clearing, immunolabelling, and light-sheet microscopy in the same sample, providing a scalable framework to link macroscopic dMRI contrasts to the underlying cellular architecture, and advancing dMRI techniques in both basic and translational neuroscience.

New technologies for mapping centimeter scale tissues with nanoscale resolution

Adam Glaser

Allen Institute

A mechanistic understanding of biological systems requires probing biological structure at the level of entire organs, the constituent ‘cell-types’, subcellular organelles, and the underlying molecular assemblies. In many cases it is essential to probe systems on all of these scales at the same time in a multiplexed manner, producing unprecedented challenges for imaging and huge data rates and volumes. Here I will present our recent efforts in this area, and the development of multiple new imaging technologies capable of mapping centimeter scale tissues with nanoscale resolution.

Session 5 - Big Data Analysis and AI

BigStitcher-Spark: Software for peta-scale alignment of light sheet microscopy acquisitions

Tobias Pietzsch[1], Sharmishtaa Seshamani[2], John Bogovic[3], Mark Kittisopikul[3], Diyi Chen[3], Benquan Wang[3], Stephan Saalfeld[3], Mojtaba Tavakoli[3], Philipp Keller[3], **Stephan Preibisch**[3]

1 Independent Scientist
2 Allen Institute
3 HHMI Janelia

Lightsheet microscopy is an emerging technology that enables imaging of large, fixed samples such as adult mouse brains with single-cell resolution. Excitingly, it even holds the potential for performing connectomics studies using light microscopy (LICONN) when combined with expansion microscopy.

Previously, we developed the BigStitcher software that efficiently handles and interactively reconstructs large lightsheet acquisitions up to the terabyte range. While being convenient and user-oriented, it does not scale to data produced by modern, peta-scale lightsheet systems such as the ExaSPIM in terms of compute time and functionality.

To address these bottlenecks, we developed BigStitcher-Spark, a command-line tool. It is able to execute compute-heavy steps of the alignment and fusion pipeline distributed on clusters and the cloud, while the user can conveniently switch between both, the user-oriented BigStitcher, and the distributed command-line tool BigStitcher-Spark. We further improved fusion performance significantly, and added functionality such as 3D intensity correction, OME-ZARR support, efficient interactive rendering of thousands of image tiles, support for deformation fields, or splitting of datasets for improved alignment quality.

We will highlight BigStitcher(-Spark) on datasets acquired by the ExaSPIM (Allen Institute), and a new lightsheet system developed by the Keller lab at Janelia applied to expanded samples for connectomics studies by the Tavakoli lab.

BigStitcher and BigStitcher-Spark constitute a powerful solution for user-and developer friendly lightsheet microscopy reconstruction that scale from small, tiled confocal datasets to peta-scale lightsheet datasets consisting of thousands of tiles.

Automating mesoSPIM Data Postprocessing

Alan Watson, Simon Watkins, Reese Smith, Iana Vasylieva and Kelin He

The Center for Biologic Imaging and the Department of Cell Biology; University of Pittsburgh

As mesoSPIM achieves a decade of innovation in open-source light-sheet imaging, handling the vast volumes of data it generates remains a formidable challenge. Here we introduce an end-to-end framework which was designed to automate data post processing for multiple mesoSPIM systems. Our pipelines use high-performance computing systems to manage data collection, deconvolution, stitching and visualization. Ultimately, these tools reduce manual interventions and enable fast, reproducible and centralized data handling.

LightSuite: A high-throughput pipeline for localizing experience in the cleared mouse brain

Dimokratis Karamanlis , Yuqiao Xie, Anas Masood, Sami El-Boustani

Department of Basic Neurosciences, University of Geneva, Switzerland

Light-sheet microscopy enables brain-wide surveys of neuronal activity by profiling immediate-early gene expression. Interpreting these large-scale 3D datasets requires scalable, automated methods for fluorescent cell detection and registration to standard atlases that minimize manual curation. Here, we introduce LightSuite, a pipeline for cell counting and brain registration that efficiently combines automated processing with user input to quantify neural activity across the entire adult mouse brain. LightSuite leverages GPU-based batch processing to rapidly analyze brain volumes at cellular resolution. Its cell detection algorithm is robust to non-uniform illumination, a common artifact in cleared tissue. We achieve highly accurate atlas registration by first modeling brain structures as point clouds and then refining the alignment with manually selected anatomical landmarks. This supervised approach makes our registration robust to tissue damage, such as missing olfactory bulbs. We validate LightSuite by demonstrating its ability to resolve activity differences across cortical layers. Applying our method to cFos-labeled brains, we uncovered distinct patterns of prefrontal cortex activity in mice performing a decision-making task alone versus in pairs. Furthermore, we have extended LightSuite's application to the analysis of brain sices from conventional wide-field microscopy. As a high-throughput tool, LightSuite paves the way for rapidly localizing behaviorally relevant neural activity in large-scale datasets.

Session 6 - Data Analysis Solutions

Interactive 3D and 4D image visualization and high throughput analysis with Imaris

Michael Mahlert

Oxford Instruments Imaris

Visualization and interactive analysis of very large multidimensional images is the key step for extracting scientific data. Imaris provides a powerful interactive and easy to learn interface for visualization, analysis and date extraction.
Imaris renders very large 3D and 4D datasets in real time and provides powerful slicing, clipping and animation tools.
On top of the visualisation, Imaris offers a complete toolbox for quantitative analysis of image data. It provides very flexible machine learning tools for segmentation of objects and extracting morphological measurements and spatial relationships.
The talk will provide a live demonstration of Visualisation and of AI segmentation and classification and also give a preview on our future Imaris high throughput image analysis pipeline approach.

syGlass: Visualization, Annotation, and Communication of Very Large Image Volumes in Virtual Reality

Michael Morehead

syGlass, Inc

Interacting with 3D volumetric datasets on traditional 2D monitors is the de facto standard but is generally nonoptimal. Stereoscopic immersive virtual and augmented reality can provide a 3D environment in which users can interact with volume data in 3D, providing improvements in spatial understanding, intuitive human-centric controls, and faster 3D operations.
We present our work in bringing high-end volume rendering to virtual reality. We couple hardware- and data source- agnostic direct volume rendering with multi-resolution volume octree technology to support very large volumes (>20TB). On top of this custom engine, we've built online multi-user collaboration systems, virtual reality publishing tools, cloud rendering, and a suite of analytical tools for the measurement or annotation of 3D structures.
Our platform now integrates CellposeSAM for automated cellular segmentation, enabling precise identification and delineation of cellular structures within volumetric datasets. This advancement facilitates comprehensive downstream analysis through integrated morphometric analysis and object classification workflows, allowing researchers to quantitatively characterize cellular phenotypes and automatically categorize structural features at scale. These capabilities transform our VR environment into a complete analytical pipeline for cellular and structural biology applications.

ZEISS Beyond Imaging - How to manage, explore & analyze your imagery with ZEISS Software Solutions

Tamara Manuelian

ZEISS Research Microscopy Solutions, Carl Zeiss Microscopy GmbH

ZEISS Microscopy, known to researchers and microscopists for more than a century to deliver high-precision innovative optical systems, is ready to support and streamline digitization of imaging workflows in research & industry with a comprehensive, file- and vendor-agnostic software suite. With this we are providing novel technology & toolsets and this we do in a platform-of-choice approach. Our portfolio is applicable at any required scale, to our customers who are aiming to manage, explore and quantify their image data in an adaptive yet automatable manner. We will deliver an overview on how in particular the ZEISS arivis ecosystem adapts to meet user requirements in handling and analysing multi-dimensional imagery. We will demonstrate sample applications including experimental data as of selected MesoSpim and other “large data” projects.
ZEISS arivis Advanced Image Analysis
ZEISS arivis platform supports multiple file formats, optimizing computing resources and storage to easily handle large, multi-dimensional datasets. The software suite makes it easy to train AI models and integrate them into your analysis protocols. Then to automate and scale up your image analysis. Overcome the image analysis bottleneck and gain fast, reliable, and reproducible results.

Fractal: An open-source framework for reproducible bioimage analysis at scale using OME-Zarrs

Joel Lüthi

BioVisionCenter, Department of Molecular Life Sciences, University of Zurich

Analyzing large amounts of microscopy images in a FAIR manner is an ongoing challenge, made harder by the large diversity of image file formats and processing approaches. Recent work on an OME-Zarr, a community-driven next-generation file format, offers the chance to create more shareable bioimage analysis workflows. Building on OME-Zarr and tackling issues related to the scalability & accessibility of bioimage analysis pipelines, the BioVisionCenter is developing Fractal, an open-source framework for processing images in the OME-Zarr format. The Fractal framework consists of a server backend & web-frontend that handle modular image processing tasks. It facilitates the design and execution of reproducible workflows to convert images into OME-Zarrs and apply advanced processing operations to them at scale, without the need for expertise in programming or large image file handling. Fractal comes with pre-built tasks to perform instance segmentation with state-of-the-art machine learning tools, to apply registration and stitching in 2D and 3D, and to extract high-dimensional measurements from multiplexed, 2D and 3D image data at the TB scale. By relying on OME-Zarr-compatible viewers like napari, MoBIE and ViZarr, Fractal enables researchers to interactively visualize terabytes of image data stored on their institution’s remote server, as well as the results of their image processing workflows**.**

Session 7 - Applications

mesoSPIM facilitates the analysis of neural circuit formation

Esther Stoeckli

University of Zurich, Dept. Molecular Life Sciences

Neural circuit formation is a multi-step process, including cell migration, axon growth and guidance, as well as synapse formation. We favor the chicken embryo as a model organism due to its accessibility during neural development that allows for precise temporal control of loss- and gain-of-function approaches. The comparative analysis of neural circuits in the peripheral or central nervous system can be time consuming and tricky when done in sections of control-treated and experimental animals, as the angle or the precise location of the section can confound the readout. Using mesoSPIM light-sheet imaging of whole-mount embryos allows for an unbiased initial assessment of neural circuit differences between control and experimental embryos. We have developed protocols to study neural circuit formation in the peripheral nervous system and in the cerebellum.

Looking for dark matter with colour centres

Laura Baudis

Physics Institute, University of Zurich

The fundamental nature of dark or invisible matter remains one of the great mysteries of our time. A leading hypothesis is that dark matter is made of new elementary particles, with proposed masses and interaction cross sections spanning an enormous range. Among these, particles with masses in the MeV-TeV range could be directly observed via scatters with atomic nuclei in transparent crystals. The resulting low-energy nuclear recoils lead to the formation of color centres, which can be observed via fluorescence spectroscopy. After a brief introduction to the principles of dark matter detection, I will present the direct detection scheme based on light-sheet fluorescence microscopy to image nuclear recoil tracks. I will show first results from cubic-centimeter sized crystals and discuss plans to scale up the technology.

Advancing animal health with tissue clearing and deep tissue imaging

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Recent imaging technologies advances have rendered possible the examination of whole-organ and organism in three-dimensions (3D). These cutting-edge methods have revolutionized several fields of biology like neurosciences, development and infectiology by allowing deep visualization of complex tissues at cellular resolution. Hence, 3D molecular phenotyping of complex tissues promote significant progress in animal health diagnostic. In parallel with instrumental developments in imaging, the emergence of tissue clearing techniques is a significant step-forward allowing optical inspection of over cm biological organism. Our laboratory's phenotyping platform has pioneered optimized techniques for tissue clearing and whole-mount immunostaining in a broad spectrum of livestock and aquatic models, including zebrafish, carp, and trout. We refined our protocols a step-further to a so-called "3D histology" pipeline for applications in development biology, comparative anatomy and infectiology. Faced with the technical constraints associated with imaging such large samples, we assembled the first mesoSPIM in France in late 2024 together with physicists specialising in optics at ENS Paris-Saclay.
Ongoing developments aim to develop novel tools for the detection and recognition of pathological patterns guided by AI leading to computer aided diagnosis of fish pathology.
Positioned as an open and collaborative platform at the forefront of animal health research, we welcome all forms of collaboration and are eager to engage with partners in building interdisciplinary projects that drive innovation in 3D histology.

Session 8 - Applications

Nine Years of mesoSPIM at the Wyss Center: Building and Evolving a Translational Imaging Platform for Neuroscience

Laura Batti

Wyss Center for Bio and Neuroengineering in Geneva

Over the past nine years, the Wyss Center has developed a robust and evolving lightsheet imaging platform to support neuroscience and translational research. At the heart of this platform is our mesoSPIM, complemented by COLM, Clearscope and more recently, LifeCanvas technologies. From the very beginning, our vision was to build a multi-user, flexible infrastructure capable of meeting the needs of diverse neuroscience projects, from brain mapping to biomarker discovery in neurodegenerative disease.
I will give an overview of how and why this platform came to life, reflecting on the historical milestones that shaped its development. Today, the platform spans the full pipeline from tissue clearing—using protocols adapted for high-throughput, reproducibility, and flexibility—to high-resolution lightsheet imaging and data processing. I will present the specific customizations and upgrades implemented on our mesoSPIM over the years, emphasizing how they have helped us meet growing user demands and experimental complexity.
Notably, mesoSPIM remains the one of the most heavily used microscopes in our facility. I will share usage statistics that illustrate its central role and widespread adoption across academic and translational projects. Several case studies will demonstrate how mesoSPIM-based workflows have enabled scientific discoveries and contributed to translational pipelines, serving as a powerful tool for guiding targeted therapeutic interventions.
Finally, I will reflect on the future of high-throughput lightsheet imaging, advocating for a strategic shift toward experiment batching and standardized workflows—key steps to scale access, ensure reproducibility, and accelerate discovery in brain research.

Unveiling the choreography of human brain development: Longterm lightsheet imaging unveils morphodynamics in human brain organoids

Akanksha Jain[1], Gilles Gut[1], Fátima Sanchis-Calleja[1], Reto Tschannen[1], Zhisong He[1], Nicolas Luginbühl[1], Fides Zenk[1], Antonius Chrisnandy[3], Simon Streib[1], Christoph Harmel[2], Ryoko Okamoto[1], Malgorzata Santel[1], Makiko Seimiya[1], René Holtackers[1], Juliane K. Rohland[1], Sophie Martina Johanna Jansen[1], Matthias P. Lutolf[3], J. Gray Camp[2] & Barbara Treutlein[1]

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Brain organoids enable mechanistic study of human brain development and provide opportunities to explore self-organization in unconstrained developmental systems. We have established long-term light sheet microscopy on unguided multi-mosaic neural organoids (MMOs) generated from fluorescently labeled human induced pluripotent stem cells (iPSCs), which enables tracking of tissue morphology, cell behaviors, and subcellular features over weeks of organoid development. We demultiplex multi-mosaic neural organoids using morphometrics to provide quantitative measurements of tissue and cellular dynamics, using Actin, Tubulin, plasma membrane, nuclei, and Lamin labels, and show that the organoids exhibit tissue state transitions through neural induction, lumenization, and regionalization. We find that despite morphological heterogeneity, different organoids exhibit lumen formation and expansion at a consistent time, coinciding with early neurectoderm switching to late neurectoderm fate. This morphological tissue transition coincides with a switch in underlying gene regulatory networks (GRNs) involving extracellular matrix (ECM) pathway regulators. Presence of a basement membrane rich external ECM promotes cell polarization, cell alignment to form a neuroepithelium, lumen expansion and leads to formation of telencephalic progenitors. However, in absence of external ECM, the tissue transition switch is perturbed forming a heterogenous neuroepithelium with mixed cellular alignment and polarity. This promotes formation of increased neural crest cells and non-telencephalic progenitors. Finally, we show ECM induced patterning guidance is linked to modulations of the WNT and HIPPO signaling pathway, including spatially restricted induction of WLS and YAP1. Altogether, our work provides a new inroad into studying human brain morphodynamics, and supports a view that mechanosensing dynamics play a central role in constraining brain regionalization.

Clearly sweet and painful: Characterizing western diet-induced pain-related myelin adaptations throughout the mouse central nervous system.

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Chronic pain affects ~20% of the adult population, yet its treatment remains highly challenging due to variable drug efficacy and side effects. Emerging evidence suggests that lifestyle factors, particularly diet, influence pain outcomes. A Western diet (WD), high in saturated fat and sugar, has been linked to both chronic pain and demyelinating diseases, largely through systemic inflammation and oxidative stress. Since oligodendrocytes (OLs), the myelin-producing cells of the central nervous system (CNS), are particularly vulnerable to metabolic imbalance and oxidative stress, and their depletion alone induces pain-like states in mice, they may represent a key factor in WD-induced pain.

To investigate the interplay of WD, CNS myelination, and pain, we use transgenic mouse models of both sexes combined with behavioral nociceptive testing. A triple transgenic reporter line (PDGFRa-creERT; Ai14; CNP-mEGFP) is employed to fluorescently label OLs and myelin with EGFP, and oligodendrocyte precursor cells with tdTomato. During the 12 weeks of WD supplementation, the nociceptive response of the mice to a mechanical stimulus is monitored to identify those that develop a pain-like phenotype. At the end of the experiment, the whole CNS is dissected, cleared, and imaged with the mesoSPIM light-sheet microscope to identify areas prone to myelin deficits. So far, our efforts have focused on optimizing tissue processing, imaging, and analysis. Our preliminary results demonstrate that SHIELD transformation is the optimal clearing method in our hands, preserving fluorescence and tissue integrity in whole-CNS preparations. Upon image acquisition with mesoSPIM V6, data are stitched, downsampled, and registered to the Allen Brain Atlas at a 25 µm isotropic resolution using the Brainreg tool provided by the BrainGlobe initiative.

This approach provides the technical basis for quantitative mapping of WD-induced pain-related changes in CNS myelination through light-sheet imaging of OL and myelin dynamics.

Pythia: AI-predictable CRISPR-Cas9 editing meets mesoSPIM for visualizing precise genomic integrations and edits

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Achieving precise DNA editing with CRISPR remains challenging because repair outcomes are often unpredictable. We developed AI-designed repair templates that use repeat homology arms to promote accurate cassette integration and minimize unwanted deletions. Implemented in our design tool Pythia, these strategies enable precise edits in human cells, germline-transmissible integrations in Xenopus, and protein tagging in neurons of the mouse brain. To place these edits in the context of whole organisms, we pair these strategies with mesoSPIM light sheet microscopy. This approach allows us to visualize genomic integrations and transgene expression across complex tissues and during early embryonic development. The combination of AI-guided CRISPR design with large-scale imaging provides a powerful framework for both experimental biology and therapeutic genome engineering.

Abstracts of posters

Block 1: mesoSPIM Makers

(1) Enhancing mainframe mesoSPIM with Near-Infrared (NIR) Imaging for Multiplexed Large-Scale Tissue Imaging

Yoseline Cabara, Clémence Hurni, Ivana Gantar, Domitille Rajot, Laura Batti, Stéphane Pagès

Wyss Center for Bio and Neuroengineering - Geneva

The mesoSPIM versatile and modular design enables scalable multiplexing, theoretically allowing unlimited spectral channels constrained only by optical component specifications (ETLs, camera sensitivities). We integrated a 785 nm NIR channel into our existing four-channel mesoSPIM (405, 488, 561, 647 nm), creating a five-channel system with minimal spectral crosstalk. NIR imaging significantly enhances performance for centimeter-sized samples through reduced autofluorescence and increased tissue penetration, which could potentially improve signal-to-noise ratios.
The large spectral range (405-785 nm) presents challenges for fiber-based combination, the broadband nature results in wavelength-dependent losses and poor coupling efficiencies in optical fibers. We implemented a simple approach consisting of free-space laser combination. This is stable but requires occasional alignment adjustments to maintain wavelength co-registration in the same illumination plane. Integration was validated with multiplexed glioblastoma cryosections demonstrating robust alignment, efficient NIR excitation and photostability. Complete integration can be achieved within one week.

(2) A Lower Cost Solution for Up to 6 Laser Lines: The UCL Benchtop-MesoSPIM

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In order to visualise up to 5 fluorophores (CFP, GFP, YFP, TdTomato and AlexaFluor647) in our confetti mouse models, the original 4-colour Benchtop MesoSPIM design was modified with updated electronics to deliver a lower cost design for up to 6 laser lines. Hardware components are now controlled via a single signal generation card with compoents physically connected through a low-cost screw terminal block. Further modifications include an 8-slot filter wheel and re-sized 3-D printed components in the detection path to accommodate the new filter wheel. Specifications are available through the UCL-Benchtop-London folder in the mesospim/benchtop-hardware Github respository.
The team at the UCL Cancer Institute is now using this system to visualise glioblastoma in mouse brains, using BrainGlobe for analysis and visualisation.

(3) Mechanical de-skewing for imaging of laterally extended cardiac tissues with a mesoSPIM light-sheet microscope

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Light-sheet microscopy ideally provides cellular resolution over centimetre fields of view and is hence a fitting tool for observing structural remodelling post myocardial infarction (MI). Commonly, light-sheet microscopes employ inner cuvettes to embed large, cubic cm samples in refractive index matched media. Our research, however, necessitates imaging vibratome-sliced ventricular samples with thickness of a few hundred microns to expediate the tissue clearing process. The slices are cleared and mounted in refractive index matched glass slides and held in 3D printed frames at 45 degrees between illumination and detection axes. Imaging these samples with a conventional axial scan results in a “shearing” of the image where the illuminated region of interest moves laterally in the field of view. This results in large data sets and can cause diminished image quality due to parts of the sample being illuminated by parts of the beam thicker than the waist.

We present a protocol for mounting and imaging tissue slices for the mesoSPIM [1] light-sheet microscope, implementing a mechanical method of deskewing to provide 3D structural information with a 78% decrease in computational time from conventional pre-processing algorithms. Additionally, we discuss the improvement in axial resolution when imaging with this new technique. We present a full characterization of the optical arrangement and demonstrate the feasibility of this approach on the example of fluorescent beads embedded in agarose and ventricular slices of rabbit heart – expanding the use of the mesoSPIM to thin tissue sections.

[1] Voigt, F.F., Kirschenbaum, D., Platonova, E. et al. The mesoSPIM initiative: open-source light-sheet microscopes for imaging cleared tissue. Nat Methods 16, 1105–1108 (2019). https://doi.org/10.1038/s41592-019-0554-0

[2] Dean, K.M., Chakraborty, T., Daetwyler, S. et al. Isotropic imaging across spatial scales with axially swept light-sheet microscopy. Nat Protoc 17, 2025–2053 (2022). https://doi.org/10.1038/s41596-022-00706-6

(4) The Microscopy Innovation Platform (MIP): an advanced microscopy platform for imaging, tissue clearing and expansion

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With the demands from life science research pushing to image larger volumes at a better resolution, the mesoSPIM microscope has been a valuable instrument thanks to the ability to fit and image samples of considerable size in a relatively short time down to cellular resolution. As a development and service-based platform, our group has been supporting research from a wide array of fields both inside the University of Zurich and worldwide by sharing knowledge, collaborating, treating and imaging samples.
Funded by the University Research Priority Program (URPP) in Adaptive Brain Circuits in Development and Learning (AdaBD), a multidisciplinary network connecting research labs spanning from cells to humans, we offer an open-source, customizable, easy-to-use and build microscopes, compatible with all tissue clearing techniques, at a fraction of the cost of an equivalent commercial system. We are further developing and providing user access to the multi-photon Schmidt microscope for all-immersion, high-resolution imaging of fixed or live samples. With these two types of microscopes, we support users in imaging, sample processing (clearing, staining and tissue expansion), and consult them on computational processing of large imaging datasets (stitching, fusion and visualization).
Outside of the lab, MIP is strongly involved in outreach projects. We take part in multiple courses hosted by the University of Zurich, participate in science communication events, and collaborate with different organizations to introduce the younger generations to the world of microscopy.
Here, we give an overview of undergoing projects, new developments of the platform, exciting case studies and initiatives stemming from our team.

(5) Reuse-SPIM: Integrating old lasers into a beam combiner: the mesoSPIM project at MPI-BI

Polisseni Claudio, Paynter Danielle

Max Planck Institute for Biological Intelligence, Germany

At the Max Planck Institute for Biological Intelligence in Munich, we are developing a benchtop mesoSPIM microscope for imaging diverse samples, ranging from zebrafish larvae to entire pigeon brains. This poster highlights our system, with a particular focus on a custom-built laser combiner.
We repurposed individual lasers from a discontinued spinning-disk microscope and, in collaboration with the original manufacturers, adapted these OEM components to ensure safety and optimal performance. In addition to the standard excitation lines (405, 488, 561, and 640 nm), we incorporated legacy 442 nm and 514 nm laser sources that had previously remained unused. Furthermore, we investigated optimal excitation strategies for near-infrared dyes such as Cy7 and Alexa Fluor 750. Since no 750 nm laser diode is readily available, we optimized the selection and operating temperature of a 730 nm diode to induce a red-shift of more than 8 nm.
All seven lasers from different manufacturers were integrated into a custom beam combiner. Motorized waveplates and shutters enable precise delivery of adjustable power to different arms of the light sheet, allowing either simultaneous or sequential illumination. We are developing dedicated software to control this combiner, which will be shared with the community along with a step-by-step implementation guide.
In summary, we have constructed a seven-color combiner spanning wavelengths from 405 nm to 730 nm. By repurposing lasers from obsolete systems, our approach promotes sustainability and cost-effectiveness while enhancing the flexibility of mesoSPIM instrumentation.

(6) Access to large range of imaging scales via dual detection path mesoSPIM

Sharon King, Rebecca Peterson, Daniel Stabley

St. Jude Children's Research Hospital

Tissue clearing and expansion combined with axially scanned light-sheet microscopy enable micron resolution studies of continuous, unperturbed neuron connectivity at depth within adult mouse brain and spinal cord. This approach has key advantages for studying the unique scale and architecture of such systems, chiefly that it does not disturb the integrity of the tissue and allows for multiple image acquisitions of the same area. Furthermore, mapping and probing neuronal connectivity in samples while screening for specific biological phenotypes over a large field of view benefits greatly from imaging intact tissue. This is the case, for example, when verifying that tumor models recapitulate the appropriate tumor characteristics and similarly when mapping interneuron subpopulation connectivity to the spinal cord.
As one of the first open-source ASLM designs, mesoSPIM enables imaging of the whole adult mouse brain within a short time-frame and also with uniform optical sectioning over a larger field of view for cell-body level resolution with isotropic sampling. Its original design indicated two separate optical configurations with detection paths employing either an objective or a telecentric imaging lens. The two configurations present access to a large range of resolution but converting between them is laborious and not compatible with the demands of imaging in a core facility. In order to take advantage of the entire range of resolution without the need for continuous system reconfiguration, we chose to build both of these detection path types with a shared excitation path. This allows, for example, whole adult mouse brain images to be captured in a single FOV volumetric stack at cell-body level resolution and higher resolution imaging of a portion of the same FOV without moving the sample or any instrumentation. We share the details of this dual mesoSPIM configuration and show its advantages for large field of view and subcellular resolution imaging in samples representing a variety of neuroscience applications.

(7) Innovative sample holders for light-sheet microscopy

Fabian Eggimann**, Lukas Lüchinger**

Additive Manufacturing Facility, University of Zurich, Switzerland

Precise positioning of samples is crucial for imaging large, cleared tissues in mesoSPIM. Our development, with the help of mesoSPIM users at the Additive Manufacturing Facility (AMF), focuses on the production of customizable 3D-printed holders (from solvent-resistant plastic like PA12) that improve the flexibility and efficiency of microscopy.
We have developed different types of holders:
Clamps: For immersion in imaging media (iDISCO, ECi, MASH, CUBIC), with kinematic bases (e.g. Thorlabs KB25/M) for stable fixation. Clamps provide flexible rotation and reduce refractive index mismatches during immersion use.
Immersion cuvettes: Allow separation of imaging media (iDISCO, ECi, MASH, CUBIC, CLARITY). Immersion cuvettes facilitate parallel processing of multiple samples with minimal media loss.
These 3D-printed holders offer cost-effective, customizable solutions for the mesoSPIM community. They promote sample preparation efficiency and open up new possibilities for research in light-sheet microscopy.

Block 2: Applications

(8) Towards 3D Mesoscale Atlases of Human Brain Structures

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The human brain's complexity arises not only from long-range connectivity but also from the complex organisation of local neuronal populations. Inhibitory interneurons play a critical role in shaping higher-order functions, yet we know little about their organization in subcortical structures, particularly the thalamus. To address this, we combined structural and diffusion MRI with advanced histological approaches. We used high-field MRI and diffusion imaging to provide an anatomical framework and connectivity context with the goal to reconstruct major axonal bundles within the tissue block. We then applied optical clearing and fluorescent labelling to centimetre-scale blocks of post-mortem human tissue. We stained samples for neurons (NeuroTrace), neurofilament heavy chain (NF-H), and inhibitory interneuron subtypes (GAD67, PV, Prox1…), and we imaged them with mesoSPIM light-sheet microscopy. This workflow allowed us to visualize neuronal populations and interneuronal networks in 3D at high resolution across mesoscale volumes. Our preliminary data demonstrate that mesoSPIM imaging of cleared human tissue can map brain architecture at previously inaccessible scales and generate a 3D mesoscale atlas, establishing a methodological framework for systematically characterizing inhibitory circuitry in large human tissue volumes.

(9) Quantitative analysis of brain wiring

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Axonal wiring in the adult brain has long been considered largely stable. However, emerging evidence indicates that adult neurons retain a notable capacity for axon elongation and circuit remodeling (Seng et al., 2022; Luo et al., 2021). Meanwhile, there is a pronounced loss of axons during aging (Marner et al., 2003). Together, these findings suggest that structural plasticity in adult neural circuits may be more extensive than previously thought.
Although major projection pathways have been anatomically and functionally mapped, little is known about how axonal connectivity may change over time. Despite recent advances in automating neuronal reconstructions, the difficulty of quantifying the absolute length and architecture of individual axons remains a major challenge, particularly for long-range projection neurons.
To address this, we use a combination of whole-brain tissue clearing, mesoSPIM light sheet microscopy, and advanced image analysis to trace axons from CA1 pyramidal neurons across the entire mouse brain. This approach allows us to characterize axonal arborization in detail during different ages and/or pathological conditions, providing new insights into the principles governing adult brain connectivity.

(10) Young adult-born dentate granule cells facilitate drift of CA3 spatial representations in the hippocampus

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Memory is fundamental to being human and memory-related information is encoded in sparse neural ensembles in the brain. Recent experiments reveal that neuronal representations of learned tasks continually change over extended timescales, a phenomenon termed representational drift, to adapt to the ever-changing environment. Hippocampus is a brain structure crucial for certain forms of memory such as spatial memory, the cognitive ability to remember the locations of objects and events. In the hippocampus subfield dentate gyrus (DG), throughout the lifespan, new granule cells (adult-born dentate granule cells, abDGCs) are continuously generated and incorporated into the local network, proving the brain a unique form of neural plasticity. Compared with mature abDGCs (> 8 weeks old), young abDGCs (4-7 weeks old) exhibit increased synaptic plasticity and intrinsic excitability. During their maturation, abDGCs send their major synaptic outputs to the hippocampal subfield CA3 and are reported to be important for many hippocampus-dependent brain functions, including learning, memory and emotional behaviors. However, it remains largely unknown how abDGCs affect the hippocampal, especially CA3 network activity and function. Here, while chemogenetically activating or inhibiting abDGCs, we use in vivo two-photon Ca2+ imaging to record CA3 neuronal activity in head-fixed mice performing a spatial learning task in virtual reality. After the formation of stable spatial memory, inhibiting (but not activating) young (but not mature) abDGCs leads to more rigid spatial representations, meaning less representational drift in CA3, without broadly modulating neural activity. Interestingly, more rigid spatial representations in CA3 are associated with behavioral impairments in suppressing the original spatial memory in a novel virtual environment. Taken together, our data suggests that young abDGC's activity facilitates drift of CA3 spatial representations, which is crucial for the brain to inhibit original memory to adapt to the ever-changing environment.

(11) Characterization of autism spectrum disorder-associated FoxP Genes in Peripheral Neural Circuit Formation using mesoSPIM

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FoxP genes have been linked to autism spectrum disorder (ASD), a neurodevelopmental disorder that is associated with aberrant connectivity of neural circuits. There is evidence that mutations in FoxP genes lead to impaired cognition, speech, and motor abilities. Our goal is to elucidate and characterize the molecular mechanisms by which FoxP genes orchestrate neural circuit formation. As a first step, we characterized the temporal and spatial expression pattern of the FoxP transcription factors. To study their impact on neural circuit assembly, we are using the chicken embryo, a powerful vertebrate model for developmental studies. We use in ovo RNAi to silence FoxP genes in temporally controlled and cell-type specific manner. Our studies demonstrate a role of FoxP transcription factors in the formation of neural circuits in the peripheral nervous system (PNS). For the analysis of the phenotypes, we are using whole-mount staining and tissue clearing protocols for light-sheet microscopy, mesoSPIM. We found aberrant cell migration and axon guidance in the absence of FoxP transcription factors. The aberrant formation of PNS circuits likely contribute to the sensory perception anomalies described in some ASD patients.

(12) A framework to determine active neurons and networks within the mouse brain

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The mouse brain's activity changes drastically over a day despite being generated from the same neurons and physical connectivity. To better understand this, we develop an experimental-computational pipeline to determine which neurons and networks are most active at different times of the day. We genetically mark active neurons of freely behaving mice at four times of the day with a c-Fos activity-dependent TRAP2 system. Neurons are imaged and digitized in 3D, and their molecular properties are inferred from the latest brain spatial transcriptomic dataset. We then develop a new computational method to analyze the network formed by the identified active neurons. Applying this pipeline, we observe region- and layer-specific activation of neurons in the cortex, especially activation of layer five neurons at the end of the dark (wake) period. We also observe a shift in the balance of excitatory (glutamatergic) neurons versus inhibitory (GABAergic) neurons across the whole brain, especially in the thalamus. Moreover, as the dark (wake) period progresses, the network formed by the active neurons becomes less modular, and the hubs switch from subcortical regions, such as the posterior hypothalamic nucleus, to cortical regions in the default mode network. Taken together, we present here a pipeline to understand which neurons and networks may be most activated in the mouse brain during an experimental protocol, and to explore how brain activity changes over the course of a day.

(13) A pipeline for gene perturbation and whole-embryo quantification of peripheral nerve development in the chicken

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The chicken embryo is a powerful model for studying peripheral nervous system (PNS) development, offering both accessibility and the ability to perform spatially and temporally precise genetic manipulations—while retaining key similarities to the human PNS. Here, we present an integrated method that combines targeted gene knockdown with high-resolution, whole-embryo imaging and AI-driven analysis to quantify peripheral nerve development. Genes of interest are downregulated in sensory and motor neuron progenitors before axon outgrowth using in ovo RNA interference. Embryos are then immunostained for neurofilament to label axons, optically cleared using an adapted whole-mount clearing protocol, and imaged with the mesoSPIM light-sheet microscope, enabling complete visualization of the PNS at embryonic day 6. To analyze the resulting large-scale datasets, we developed a custom image processing pipeline that uses a U-Net-based model for nerve segmentation and graph-based quantification of nerve morphology. This allows for automated, quantitative comparison of nerve trajectories, branching patterns, and structural features across experimental conditions. By combining gene perturbation, tissue processing, and scalable computational analysis, our method offers a robust framework for investigating the molecular mechanisms driving PNS development.

(14) Comprehensive analysis of Cajal-Retzius cell development reveals macroscopic organizational principles of the cortex

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Cajal-Retzius cells (CRc) constitute a transient, glutamatergic neuronal population implicated in the cortical formation and lamination during embryogenesis. Before their apoptosis, CRc are synaptically embedded in the early postnatal cortical circuit. Our previous work has revealed the significance of CRc timely death for the anatomical, functional and behavioral maturation of the somatosensory circuit.
In this work we mapped out the CRc apoptotic trajectories at mesoscale to unravel which cortical regions mature when during development (P0 to ≥P56). By deploying a comprehensive analysis pipeline including genetic fate-mapping, 3D-fluorescence light-sheet microscopy, semi-automatic registration and neuronal detection (using the DataDelv software), we found distinct apoptotic dynamics for different CRc populations across the cortex. Testing causally the importance of such dynamics at large-scale, we arrested the death of one CRc population (BaxcKO) and assessed long-range and cross-areal connectivity using 9.4T MRI scans and diffusion tractography analysis at P6 and P21.
Our anatomical analysis revealed that some CRc persist in other cortical areas beyond the adult hippocampus. In vitro electrophysiological recordings of persistent CRc revealed their continued immature functional characteristics. Ongoing immunohistochemical experiments in the adult mouse and human cortex will pave the way for investigating the persistence of CRc in disorders related to gyrification defects and developmental delay.

(15) Combining expansion microscopy and light sheet imaging to investigate synaptic pathology in Alzheimer's Disease Human brain tissue

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by the spread of pathological proteins throughout the brain in a stereotypical manner, following specific neuronal networks. Recent work suggests that pathological proteins may spread via vesicular viral-like trans-synaptic transmission. While this mechanism has been studied in animal models, its direct investigation has been hindered by limited access to high-quality samples, imaging constraints, and tissue preservation challenges. We develop new optimized methods to explore the mechanism in AD patients using postmortem brain samples from the Geneva Brain Bank (GBB). Coupling ExM with light-sheet fluorescence microscopy (LSFM) allows us to visualize large tissue volumes at subcellular resolution, offering access to the nanoscale 3D organization of synaptic proteins across disease-affected brain regions. This approach actively contributes to advancing the histological characterization of AD pathology in human tissues, promoting human-based investigations and opening new perspectives for translational research in AD.

(16) Spatial Mapping of Collagen Architecture in the Desmoid Tumor (Immune) Microenvironment Using Light-Sheet Microscopy

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Desmoid tumors (DTs) represent locally invasive soft tissue tumors, strictly driven by Wnt/β-catenin hyperactivation. Similar to other fibroproliferative disorders with persistent fibroblast activation (keloids, idiopathic pulmonary fibrosis), DTs are marked by excessive collagen secretion, contributing to extracellular matrix (ECM) stiffness. Our lab has established robust short-latency and high-penetrance desmoid tumor models in the aquatic tetrapod Xenopus tropicalis through CRISPR/Cas9-mediated modulation of the Wnt signaling pathway.
Despite the notion that DTs are 'simple' tumors that originate from a single oncogenic insult (APC or CTNNB1 mutation), the mechanisms by which tumor cells interact with their collagen-rich matrix and the surrounding tumor microenvironment (TME) remain poorly understood. To investigate the spatial relationship between collagen architecture and the cellular (immune) populations within the TME of DTs, we adapted collagen visualization withthe synthetic dye Fast Green FCF (Timin & Milinkovitch, iScience), coupled with DISCO-based tissue clearing and 3D light-sheet microscopy on the Benchtop mesoSPIM platform. To gain deep biological insights from our datasets, we are developing deep learning segmentation models for feature extraction and automated assessment of three-dimensional collagen architecture.

Leveraging this multidisciplinary approach, we aim to gain novel spatial and molecular insights into collagen biology, tumor-TME interactions and therapeutic vulnerabilities in DTs and related collagenous fibrotic lesions.

(17) Deep body peptidergic afferents lack Advillin expression: Implications for sensory neuron profiling

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A heterogenous population of peripheral neurons is housed in dorsal root ganglia (DRG). Those diverse somatosensory afferent neurons mediate key bodily sensations ranging from touch and proprioception to temperature and pain. Advillin (Avil), an actin-binding protein, is a commonly used marker of all sensory and sympathetic neurons [1]. However, recent findings question its universality, particularly in certain peptidergic subpopulations. We aimed to evaluate which afferents are not covered by Avil and thus could exhibit new interesting population of sensory neurons. Additionally, our aim is to investigate the spatial relationship between the nervous system and tumour tissue, and to compare neural pathways in healthy tissue and model of tissue affected by chronic pain.
We are currently establishing a mesoSPIM benchtop platform to enable high-resolution volumetric imaging, enhancing spatial accuracy and sustaining key findings. The system will be employed to visualize the spatial relationship between the nervous system and tumour tissue, as well as to characterize alterations in neural architecture between healthy tissue and model of tissue affected by chronic pain. To this end, we utilized three transgenic mouse lines—Avil-eGFP, Calca-eGFP, and ChRNA3-eGFP—targeting distinct sensory and sympathetic neuron populations. Targeted tissues were retrogradely labelled with Fast Blue, and lumbar and sacral DRGs were cryosectioned and immunolabelled for key sensory markers (CGRP, Avil, Tubulin-βIII, IB4). Confocal and digital light-sheet imaging followed PACT-based tissue clearing [2]. Data analysis was conducted in Fiji, Imaris, Python, and GraphPad Prism.
We demonstrate that Avil does not universally cover sensory neurons. Specifically, a subset of peptidergic afferents enriched in CGRP and innervating deep tissues exhibits low or undetectable levels of Avil. Data is supported by post hoc snRNAseq analysis.
We conclude that Avil is not a universal marker of sensory neurons but is biased towards superficial skin afferents. Planned studies using mesoSPIM are expected to provide more comprehensive spatial insight into this heterogeneity.

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Bibliography

[1] Hasegawa, H. i inni, 2007. Analyzing Somatosensory Axon Projections with the Sensory Neuron-Specific Advillin Gene. The Journal of Neuroscience, 26 12.
[2] Treweek, J. B. i inni, 2015. Whole-body tissue stabilization and selective extractions via tissue-hydrogel hybrids for high-resolution intact circuit mapping and phenotyping. nature protocols, 22 10, pp. 1860-1896.

(18) Mesoscopic imaging of the human clitoris

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Lack of knowledge about the functional anatomy of the clitoris still persists and negatively impacts medical care for girls, women and gender diverse individuals with a clitoris. Precise knowledge of clitoral anatomy and histology is crucial in vulvar and vaginal surgeries for malignant and benign conditions, such as urinary incontinence or cysts, as well as in gender affirmation surgeries or reconstructive surgery after FGM/C. In addition, clitoral knowledge has been associated with endorsing less gendered sexual scripts, which shows a positive association with higher pleasure and orgasm experience.
To investigate the functional architecture of the clitoris, we developed a clearing protocol optimized for large human samples. Light-sheet microscopy was used to image those samples at a cellular resolution, to elucidate the vasculature and innervation of the organ.

(19) Multi-scale imaging of α-synuclein and microstructural changes in a mouse model of Parkinson's disease

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Parkinson's disease (PD) is characterized by the accumulation of α-synuclein (α-syn) aggregates, which are hypothesized to drive neurodegeneration in susceptible neurons, particularly within the dopaminergic nigrostriatal pathway. To explore the spatiotemporal relationship between α-syn inclusions and microstructural changes, we injected α-syn preformed fibrils (PFFs) into the substantia nigra pars compacta (SNc) of mice, using monomeric α-syn as a control. Brains were analyzed at 12- and 20-weeks post-injection using ex vivo T1-weighted (T1w) MRI, diffusion tensor imaging (DTI) at 9.4T, and light-sheet microscopy (LSM). PFF injections triggered dense α-syn pathology in SNc dopaminergic neurons, leading to neuronal loss and prion-like spread of aggregates to distant brain regions. High-resolution (<5 µm) whole-brain LSM mapping of α-syn pathology and dopaminergic degeneration, combined with T1w and DTI imaging, allowed detection of anatomical and microstructural connectivity changes within affected networks at 60–100 µm resolution. The PFFs-injected model displays abundant α-syn pathology and neurodegeneration, as seen in PD patients. The ex vivo MRI-LSM platform is an ideal tool for understanding the progression of the pathology at circuit and whole brain levels.

(20) From Molecules to Circuitry: Illuminating Cerebellar Circuit Assembly

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The cerebellum, traditionally linked to motor coordination, is now recognized for its role in higher cognitive functions. It forms complex connections with the cerebral cortex, pontine nuclei, inferior olive, thalamus, and spinal cord. Cerebellar abnormalities, such as disrupted Purkinje cell migration, reduced volume, and structural defects, are frequently reported in neurodevelopmental disorders, including autism spectrum disorder, intellectual disability, and attention deficit hyperactivity disorder. These findings highlight the importance of understanding cerebellar circuit development in the context of brain function and disease.

Although recent advances in tissue clearing and molecular labeling have improved deep-tissue visualization, many current methods remain time-consuming, technically complex, and dependent on specialized equipment. To overcome these limitations, we apply advanced clearing protocols combined with mesoSPIM-based light-sheet microscopy for rapid, high-resolution 3D imaging of the developing chicken cerebellum.

We have adapted our in ovo RNAi approach for functional gene analysis during neural circuit formation to the cerebellum of the chicken embryo. We used wholemount Calbindin staining to visualize zonal patterning of Purkinje cells allowing for the detection of cell migration deficits.

Block 3: Image Analysis Tools

(21) multiview-stitcher: An Interoperable Python Toolbox for Image Registration and Fusion

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Multi-view and multi-tile imaging offer great potential for enhancing field of view, resolution and penetration depth in microscopy. Here, we present multiview-stitcher, a versatile and modular Python package for 2D/3D image reconstruction that integrates existing registration and fusion algorithms into an extensible, standardized framework. Scalability to large datasets is achieved through chunk-aware processing with full support for affine spatial transformations. Multiview-stitcher provides interoperable interfaces via a Python API - usable as a library or within Jupyter notebooks - and a Napari plugin for interactive stitching of image layers. To demonstrate its usability and versatility in light-sheet imaging, we showcase reconstructions of multi-view and multi-tile acquisitions in several configurations.

(22) DataDelv software analysis of mesoSPIM Data

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Recent advances in data acquisition, as well as computing- and storage-technologies enable the creation of ever larger and more complex data sets in virtually all disciplines of sciences. The acquisition of microscopy data using mesoSPIM microscopes is one such example. However, organizing and maintaining such data sets and even more so rapidly exploring and interpreting them, creates new technical challenges that are often not adequately met by current software tools in academic settings. Here we present DataDelv, an open-source software tool for rapid data exploration and analysis in the life sciences and beyond. We introduce the key features of DataDelv and illustrate its uses on imaging data.

(23) Towards vascular connectomics in the human brain

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The vascular network of the human brain is highly complex and difficult to assess, encompassing multiple spatial scales, from large veins and arteries to small capillary branches. To evaluate the vascular connectivity in the human brain, it is essential to reconstruct and characterize the vascular network with high precision in 3D. We used aDISCO [1] - a recently developed tissue clearing method for whole-mount archival human tissue blocks - to process large human brain tissue blocks of up to 2 x 1.5 x 0.5 cm3 with immunolabeling for blood vessels. We used mesoSPIM [2] to acquire terabyte-sized 3D image stacks with a voxel size of 1-1.5 µm3.
To extract the vascular network from these large datasets, we developed a deep learning-based pipeline. First, raw image data is segmented using nnUNet [3], a state-of-the-art deep neural network. Then, the segmentation is converted into a graph representation through skeletonization [4], where graph nodes represent the vascular elements and graph edges represent the connections between these elements. To handle these large amounts of data, we rely on the flexible OME-Zarr format [5] and utilize high-performance computing resources to ensure scalability. Treating the graph as k-center problem and using massive post-processing, we can simplify that graph to capture both vascular connectivity and spatial anatomy.
We show that methods that are similarly used in the field of neuronal connectomics can be adapted to characterize the specific structure of vascular networks. We further demonstrate that our graph-based reconstruction of the vascular network enables detailed quantitative analysis and visualization of the vascular system, such as the vascular branching paths from arteries over arterioles, capillaries, and venules to veins. Together, our work lays the foundation for investigating the human "vascular connectome" - a comprehensive map of vascular connectivity in the brain.

[1] Reuss, A. M., etal (2025). aDISCO: A broadly applicable method for 3D microscopy of archival paraffin-embedded human tissues. bioRxiv. https://doi.org/10.1101/2025.05.21.655358
[2] Voigt, F. F., etal (2019). The mesoSPIM initiative: open-source light-sheet microscopes for imaging cleared tissue. Nature methods, 16(11), 1105–1108.
[3] Isensee, etal. nnU-Net: a self-configuring method for deep learning-based biomedical image segmentation. Nature methods, 18(2), 203-211.
[4] Kirst, C., et al. (2020). Mapping the fine-scale organization and plasticity of the brain vasculature. Cell, 180(4), 780–795. https://doi.org/10.1016/j.cell.2016.05.007
[5] Moore, J., et al. OME-Zarr: a cloud-optimized bioimaging file format with international community support. Histochem Cell Biol 160, 223–251 (2023).

(24) CellSeg3D, Self-supervised 3D cell segmentation for mesoSPIM & fluorescence microscopy

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Understanding the complex three-dimensional structure of cells is crucial across many disciplines in biology and especially in neuroscience. Here, we introduce a set of models including a 3D transformer (SwinUNetR) and a novel 3D self-supervised learning method (WNet3D) designed to address the inherent complexity of generating 3D ground truth data and quantifying nuclei in 3D volumes, namely cleared mouse whole-brain tissue imaged with mesoSPIM.
We developed a Python package called CellSeg3D that provides access to these models and a full workflow in Jupyter Notebooks and in a napari GUI plugin.
Recognizing the scarcity of high-quality 3D ground truth data, we created a fully human-annotated mesoSPIM dataset to advance evaluation and benchmarking in the field. To assess model performance, we benchmarked our approach across four diverse datasets: the newly developed mesoSPIM dataset, a 3D platynereis-ISH-Nuclei confocal dataset, a separate 3D Platynereis-Nuclei light-sheet dataset, and a challenging and densely packed Mouse-Skull-Nuclei confocal dataset. We demonstrate that our self-supervised model, WNet3D – trained without any ground truth labels – achieves performance on par with state-of-the-art supervised methods, paving the way for broader applications in label-scarce biological contexts.

(25) Large-Scale Data Storage, Processing, and Access for Data-heavy environments: The Bruker Acquifer HIVE

Clemens Schneider

Bruker Nano GmbH, Germany

Bruker's Acquifer HIVE is a powerful big data management system designed to handle large amounts of scientific data, including microscopy data. The HIVE is a centralized solution that serves the increasing need for data infrastructure with high-speed data transfer, secure storage, and parallel processing, allowing multiple users to analyze and process data simultaneously.
Ideal for research facilities and scientists working with large datasets, Acquifer HIVE supports all kinds of microscopes from light sheet systems to high content screening platforms. Its robust CPU and GPU capabilities ensure seamless integration with major imaging software, while its high-speed RAID architecture, firewall protection, and uninterruptible power supply provide reliable and secure storage.
This poster discusses integration possibilities and application examples of the Acquifer HIVE for big data management in microscopy environments.

Block 4: Tissue Clearing and Staining

(26) Detailed 3D architecture of adult human organs through new tissue clearing techniques

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Traditional histology relies on thin sections of small samples for studying human tissues, which limits our understanding of the complex structure and anatomical relationships of whole organs. Gross anatomy and medical imaging on the other hand lack the resolution and specificity to visualize histological structures. Tissue clearing methods are emerging as innovative solutions to transcend this constraint by revealing the spatial architecture of large tissues samples. However, making adult human organs transparent remains a challenge, given their size, density and endogenous fluorescence. We have developed and adapted novel techniques for clearing and labeling efficiently postmortem human organs, showing their compatibility with different organs and tissue types. Combined with light-sheet microscopy and advanced image-processing tools, we have reconstructed their cellular architecture, innervation and vascularization in three dimensions. Whole-organ tissue imaging enables a multimodal approach for the study of structure-function relationship in human anatomy.

(27) A multicolor labelling for cellular birth-date identification and light-sheet functional imaging of age-defined neuronal populations

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Understanding the development and function of neural circuits requires tools that can simultaneously track neuronal birthdate and monitor activity at cellular resolution. Current birthdate approaches are limited in-vivo, lack flexibility and temporal precision and their combination with optical functional imaging remains limited and challenging.
Recently, we developed CHLOK (Chromatic HaloTag Labeling for cell Ontogeny tracKing), a chemigenetic strategy that enables precise, multicolor birthdating of neurons in vivo. CHLOK makes use of HaloTag fused to neuronal promoters and a palette of Janelia Fluor dyes, applied sequentially to label neurons based on their birth date with high temporal resolution. This method allows simultaneous tagging of up to five neuronal cohorts and is compatible with a range of optical tools.
To validate CHLOK for functional imaging, we employed a custom-built light-sheet system. Using this setup, we performed fast fluorescent imaging and demonstrated CHLOK's compatibility with both WhaloCamp (a HaloTag-based calcium indicator) and Voltron2 (a chemigenetic voltage sensor), enabling multicolor calcium and voltage imaging in different birthdate-defined populations (Faini et al, CHLOK: a chemigenetic multicolor labeling system to visualize neuronal birthdate and circuit integration, under review). These recordings open the way for better tracking of the functional evolution of neuronal populations during brain development.

(28) Single channel segmentation of microglia, nuclei and vessels in cleared rodent brains with Tomato Lectin

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The Blood Brain Barrier regulates the exchanges between the brain and the rest of the body. A variety of cells orchestrate the exchange of signaling molecules between capillaries and the brain. In particular, recent research points to an important role of microglial cells in regulating the NeuroVascular Unit 1. Microglia can be found in contact with vessels and change shape in response to activation due to disease states. Examining their morphology using fluorescence microscopy can shed light on their function and relationship 2,3. However, varied morphologies and fine processes make 3D segmentation challenging. Automated segmentation methods minimize bias and can capture complex networks 4 but require high-quality staining of all capillaries and microglial cells.
Currently, the state-of-the-art pan-microglial staining is the anti-Iba1 antibody. It has been found that a considerable number of microglia do not express Iba1 at all 5, which could bias results toward specific phenotypes. Tomato Lectin (LEL) has also been used to stain microglia 5, while also targeting vessels broadly (i.e. arteries, veins and capillaries) 4. This is usually seen as a drawback. New deep learning methods could, however, disentangle the microglia and vessels from the single LEL fluorescence channel. Additionally, our newly optimised clearing protocol for immunostaining also stains nuclei with high specificity, creating an opportunity for multiple biological analyses with the imaging of a single channel.
We then plan to image cleared brain samples from multiple species (mouse, rat and human) in 3D over large areas with a cleared tissue dSPIM, then train neural networks to segment microglia, nuclei and vessels from the same LEL fluorescent channel. We will employ state of the art Unet convolutional models 7 and a new loss function (Skeleton Recall Loss 8), optimized for conserving the topology of thin structures. We will co-stain with anti-Iba1 antibodies to validate our microglia detections. The target of this method development is quantifying the overlap between the staining specificity of LEL and Iba1 for microglial morphology analysis.

1 Knopp, R. C et al. Cur Op in Neurobiology 77, 102648, 2022
2 Paolicelli, R. C. et al. Neuron 110, 3458–3483, 2022
3 Colombo, G. et al. Nat Neurosci 25, 1379–1393, 2022
4 Hildebrand, S. et al. Bioarxiv, 2024
5 Kenkhuis, B. et al. Neurobiology of Disease 167, 105684, 2022
6 Kettenmann, H. et al. Physiological Reviews 91, 461–553, 2011
7 Isensee, F. et al. Nat Methods 18, 203–211 2021
8 Kirchhoff, Y. et al. Arxiv, 2024

(29) Going Nuclear: An in-depth comparison of whole-brain bleaching, counterstaining, and analysis methods

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Autofluorescence is the enemy of every light-sheet imaging project. Reducing the autofluorescence while preserving the antigens is therefore critical. We systematically compared chemical, enzymatic and light-based bleaching methods for whole mouse brains, and test them for their compatibility with the INSIHGT staining (Yau et al., Nature Communications 2024) and solvent-based clearing protocols.

We find that photo bleaching during the preprocessing reduces the autofluorescence the 488nm channel such that it becomes useable as a third staining channel. We then evaluate several nuclear counterstains for their compatibility with whole-brain clearing.

We then analyze the whole-brain nuclear labeling with our DELiVR pipeline (Kaltenecker, Al-Maskari, Negwer et al., Nature Methods 2024) and post-process the cell location with the NuQLOUD clustering pipeline (Brambach et al., preprint at biorxiv 2024). This approach should be universal and enable detailed cytological maps in other organs as well.

(30) RatDISCO, a tissue clearing and immunolabelling protocol for large rat brains

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Understanding brain function requires 3D mapping of its neurochemical architecture. Manual sectioning, and stereology and cell quantification are the most used 2D anatomical methods. However, this poses challenges as traditional histology, imaging and analysis methods limit the amount of information we can obtain about the 3D structure of an organ. Advances in light-sheet microscopy allow us to take a global approach in studying development/disease. To observe our tissue in its entirety and use light-sheet microscopy, we must render our tissue transparent. The main steps of optical clearing are dehydration, delipidation and refractive-index matching. Our work utilised rat models of neurodevelopmental disorders to explore brain-wide changes in autism spectrum disorders (ASD). The large size of rat brain tissue can limit antibody penetration and analysis can be difficult due to the lack of a suitable atlas. As such, we developed RatDISCO - a whole-brain immunolabelling and clearing pipeline that detects proteins of interest throughout large rat brains.
To assess the depth of antibody penetration achieved using RatDISCO, we created a 3D rat brain atlas for our structure of interest, the amygdala, using ArivisVision4D. We then generated an analysis pipeline to quantify cell density of transcription-factor FOXP2 in the amygdala. To further test RatDISCO, we developed a pipeline to identify behaviourally activated neurons, using immediate-early gene cFos as a proxy for neuronal activation. We used our amygdala atlas to map and quantify the cell density of cFos+ neurons across wild-type and Fragile-X-knockout (Fmr1-/y) rats during a fear protocol.
Using a two-tailed, unpaired t-test, we compared the cell density of FOXP2 obtained in tissues cleared using RatDISCO to that obtained in tissues cleared using iDISCO+ (paired). Using a 2-way-ANOVA, we compared the cell density of cFos+ neurons in the amygdala of wild-type and Fmr1-/y rats across behavioural conditions.
RatDISCO offers substantial immunolabelling improvements compared to iDISCO+ in large rat brain tissue, achieving higher FOXP2 cell densities in the amygdala. RatDISCO allowed us to identify hypo-activation in the basal and basolateral amygdala in Fmr1-/y rats in response to fear. This correlates with observed amygdala-dependent processing alterations in individuals with Fragile-X-syndrome. RatDISCO is available at the light-sheet microscopy imaging facility at the University of Edinburgh (LSM3D https://discovery-brain-sciences.ed.ac.uk/light-sheet-microscopy-and-3d-analysis-facility-lsm3d).

(31) Clearing up incontinence: Developing optical tissue clearing techniques to unravel the 3D neuroanatomy of bladder control in the human spinal cord

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In our aging population, urinary incontinence is a prevalent and growing problem with a significant impact on patients' quality of life. Several nuclei in the sacral spinal cord (SSC), play a key role in regulating bladder muscles and, therefore, continence. However, little is known about their micro-anatomy or cell composition. This is largely due to the fact that these nuclei spread across several centimetres along the length of the SSC, making them challenging to image with traditional (2D) histology methods.

A better understanding of SSC neuroanatomy is crucial for advancing our knowledge of bladder control and improving treatment options for patients with urinary incontinence. Here we show an adjusted optical tissue clearing protocol that is potent enough to clear human spinal cord, with the aim to identify and characterize Onuf's nucleus in 3D.

(32) mesoSPIM Benchtop for Translational Eye Research

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We present ClearEye, a procedure enabling light sheet fluorescence microscopy (LSFM) of entire human eyes based on iDISCO+ tissue clearing and immunolabeling protocol. With a diameter of approximately 2.5 cm, the human eye presents a substantial imaging challenge; only mesoSPIM enabled complete volumetric acquisition. Leveraging the capabilities of the Advanced Lightsheet Imaging Center at the Wyss Center in Geneva, we achieved high-resolution three-dimensional (3D) lightsheet imaging. The approach overcomes limitations of traditional histology by allowing spatially accurate tracing of complex structures such as Schlemm's canal and anterior ciliary vessels.
This in toto imaging technique facilitates quantitative and customizable exploration of both anterior and posterior ocular structures, providing a valuable bridge between clinical and postmortem studies. Overall, ClearEye offers a powerful tool for comprehensive 3D histological assessment of human eyes in both research and clinical contexts.
Establishing our own Benchtop mesoSPIM imaging system will provide a crucial bridge between clinical and histological analysis by allowing direct comparisons between in vivo imaging and postmortem tissue from both anterior and posterior eye segments. This capability opens the door to customized, high-resolution exploration of complex ocular structures, supporting translational research in ophthalmology. In toto LSFM imaging will enhance mapping of focal pathologies and facilitate the study of diseases affecting multiple ocular compartments, such as age-related macular degeneration, uveitis, myopia, and ocular tumors. It will also enable the investigation of potential biomarkers for systemic and neurodegenerative diseases, using the eye as a window to the brain. Altogether, tissue clearing combined with large-volume 3D imaging presents a transformative tool for both clinical and basic vision science.

(33) Insights into whole tissue clearing and labelling protocols for various samples in a microcopy facility context

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Tissue clearing and immunolabelling have become essential components of imaging workflows for light-sheet fluorescence microscopy, especially when working with intact, complex biological samples. As new protocols continue to emerge, facility scientists and researchers must navigate a growing landscape of methods, each with different strengths, limitations, and practical considerations.
In this poster, we present an experimental overview of several clearing and labelling protocols, including iDISCO+, CLARITY, EZ Clear, and SmartBatch+, based on their use in our multi-user microscopy platform. Our focus is on sharing hands-on insights gathered from processing a range of samples, including mouse brains, spinal cords, muscles, human organoids, and other cm-scale tissues.
We aim to highlight practical observations around endogenous fluorescence preservation, antibody penetration across tissue types and sizes, preparation time, accessibility, and cost. Notably, EZ Clear stood out as a fast and accessible protocol, particularly effective in preserving endogenous signals. SmartBatch+ offers promising results for both endogenous fluorescence and deep labelling in large batches, although it requires more resources. iDISCO+ continues to be a reliable and sustainable option for whole organ labelling in routine lab settings.
We hope this poster will serve as a starting point for discussion within the tissue clearing community on how to navigate evolving technologies and match protocol choices to research needs and infrastructure.

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