

3 – 6 June 2025

EMBL Advanced Training Centre and Imaging Centre

## INDUSTRY AND COMMUNITY ROOM ABSTRACTS

## **WORKSHOP 4**

THURSDAY | 5 JUNE | 14:30 - 15:30

## High-Throughput STORM Imaging with Abbelight's SAFe System Featuring ASTER Illumination

### Presenter(s):

Yashar Rouzbahani - Abbeliaht, France

Dassine Zouaoui - Abbelight, France

Unlocking More Data through Expanded Fields of View in SMLM Single Molecule Localization Microscopy (SMLM), including techniques like STORM, has transformed the way we explore biological systems, offering 10 to 20 times increase in spatial resolution of conventional light microscopy. This enables the visualization of molecular structures and spatial organization at the nanoscale, far beyond the diffraction barrier. Despite these advantages, many SMLM workflows remain limited by narrow fields of view and time-intensive acquisitions, constraints that reduce throughput and hinder large scale analysis.

In this workshop, we present Abbelight's high throughput STORM imaging workflow based on the SAFe system, which features ASTER illumination, a proprietary flat field excitation technology embedded in the illumination module of the SAFe platform. This integrated approach allows users to perform SMLM across ultra-wide fields of view while maintaining uniform excitation and precise single molecule localization throughout the entire imaging area. Unlike conventional Gaussian beam profiles that introduce intensity variation across the field, ASTER delivers homogeneous excitation, ensuring consistent fluorophore activation from center to edge. This enables high resolution STORM imaging at fields of view up to 150  $\mu$ m × 150  $\mu$ m using a 100X objective and 230  $\mu$ m × 230  $\mu$ m using a 60X objective, a significant expansion over standard size of 50  $\mu$ m. The result: more cells per frame, higher statistical power, and data rich acquisitions that open the door to population level insights and rare event detection.

#### We will cover:

- $\bullet$  Scaling the SMLM field of view: from 50  $\mu m$  to 230  $\mu m$
- How larger FOVs increase data volume, cell counts, and biological outcomes

#### Schedule

10 min Introduction to STORM imaging

10 min Introduction to Abbelight Safe MN 360 and ASTER technology

25 min STOTM multi-color imaging with different FOV size

15 min Summary of the workshop & Questions

## Abberior STED-MINFLUX Workshop: Biological Imaging Across Scales

### Presenter(s):

Bastian Klußmann-Fricke - Abberior Instruments GmbH, Göttingen

Clara-Marie Gürth - Abberior Instruments GmbH, Göttingen

Ulf Matti - Abberior Instruments GmbH, Göttingen

Biological samples span a vast range of sizes, from millimetre-scale organisms and tissues to nanometre-sized single molecules. Investigating these diverse samples necessitates a suite of optical microscopy functionalities. This includes large fields of view and high working distances for macroscopic specimens, optical compensation for thick tissues, and high resolution for subcellular and in vitro studies. Traditionally, these requirements demanded multiple specialized microscopes. The MIRAVA POLYSCOPE overcomes this limitation by integrating confocal, MATRIX, STED, and MINFLUX modalities into a single versatile platform.

Investigating the nuclear pore complex (NPC) at various levels of detail perfectly illustrates the need for a wide range of microscopy modalities. Confocal microscopy can reveal its cellular-level organization, while super-resolution techniques like STED and MINFLUX unveil its detailed macromolecular arrangement. To highlight the MIRAVA POLYSCOPE's comprehensive features, we will focus on the NPC as a prime example.

This workshop offers participants a unique opportunity to learn how to investigate a single biological structure across several orders of magnitude, spanning from diffraction-limited overviews to true molecular insights. Through hands-on demonstrations on the abberior MIRAVA POLYSCOPE, we will showcase how confocal, STED, and MINFLUX imaging can be strategically employed to answer different questions about the NPC at corresponding scales and resolutions.

## Abberior MINFLUX Workshop: 3D and 2 Colour MINFLUX Nanoscopy

### Presenter(s):

Ulf Matti - Abberior Instruments GmbH, Göttingen

Clara-Marie Gürth - Abberior Instruments GmbH, Göttingen

Bastian Klußmann-Fricke - Abberior Instruments GmbH, Göttingen

Achieving molecular-scale imaging of protein complexes has long been a goal in the life sciences. MINFLUX nanoscopy addresses this challenge by precisely localizing single fluorophores through sequential fluorescence readout at defined positions surrounding the molecule. The use of a donut-shaped excitation beam enables localization with exceptional precision while minimizing photon requirements and thereby surpasses limits of other super-resolution techniques. Previously, such high-performance microscopy remained largely accessible only to specialized optics experts.

Here, we present the MINFLUX microscope built upon a conventional fluorescence microscope stand, providing this powerful technique for a broader range of users. This system seamlessly integrates ultra-high localization precision with standard experimental workflows, empowering non-experts to readily apply MINFLUX. We demonstrate its capabilities by visualizing intricate structures, such as the nuclear pore complex (NPC), at the nanoscale in three dimensions. Furthermore, through the labelling of different components within this complex, we achieve nanoscale resolution in two colours.

This workshop will provide participants with practical insights into the 3D analysis of biological structures using MINFLUX. We will showcase how two-colour MINFLUX experiments enable the detailed investigation of spatial relationships between different proteins at the nanoscopic level.

## LCS SPIM – Light Sheet Microscopy for Large Cleared Samples in high resolution

### Presenter(s):

Dr. Jürgen Mayer, Bruker Fluorescence Microscopy, Germany

The LCS SPIM is an inverted light sheet setup that was designed to image large, cleared samples in an easy way. The cuvette based sample mounting allows researchers to mount their samples under a fume hood in a safe way. The cuvettes can be sealed with a lid before installation on the microscope, such that there is no exposure to toxic fumes. The depth-dependent focussing assures best possible image quality throughout the entire depth of the sample.

Traditionally, the LCS is equipped with a 4x detection objective, emphasizing the acquisition of centimeter sized samples. Here, we present a new implementation that allows to image with higher magnification and higher resolution while keeping the easy and safe sample mounting of the inverted design of the LCS SPIM.

### **Carl Zeiss Microscopy Deutschland GmbH**

## Unlocking the Future of Imaging with ZEISS Lattice SIM 3

### Presenter(s):

Dr. Christine Strasser, Carl Zeiss AG, Switzerland

Cellular processes are complex yet delicate. Therefore, we require an imaging solution that is gentle but allows visualization of finer details. The ZEISS Lattice SIM 3 represents a significant advancement in imaging such multicellular samples, designed to address the evolving needs of researchers studying developing organisms, organoids, 3D cell cultures, and intricate tissue sections. This system is optimized for objectives ranging from 10× to 40×, ensuring the capture of finer details critical to biological research.

Key features include the integration of SIM Apotome technology, which facilitates rapid optical sectioning through Leap Mode, delivering high-quality images with a remarkable speed of 85 fr/s. The Lattice SIM 3 offers a large field of view while allowing access to smaller regions of interest, enabling near-isotropic resolution in large volumes and gentle super-resolution imaging.

Additionally, Burst Mode enhances time-lapse imaging, allowing researchers to monitor dynamic changes over time with clarity. The innovative Lattice SIM imaging and SIM<sup>2</sup> image reconstruction techniques enable resolution of structures down to an impressive 140 nm.

The seamless integration of arivis software further enhances the imaging system, providing advanced image analysis tools for sophisticated particle and cell tracking. This combination empowers researchers to transform their data into actionable insights, yielding faster and more accurate results while streamlining workflows. The ZEISS Lattice SIM 3 maintains compatibility with standard dyes and fluorescent proteins, offering flexibility for simultaneous two-colour imaging with clean channel separation.

In summary, the ZEISS Lattice SIM 3 not only elevates research capabilities but also unlocks new dimensions in imaging, paving the way for future discoveries in the field.

## Ultra-Large-Field-of-View Imaging Using Innovative Photonic Chip Technology

### Presenter(s):

Merete Storflor - Chip Nanolmaging, Norway

Luis Villegas - Chip NanoImaging, Norway

Jon Kristian Hagene - Chip Nanolmaging, Norway

Explore the full complexity of biological processes with the largest uniform field of view available. Chip Nanolmaging offers multi-channel TIRF imaging at the millimeter scale, delivering exceptional signal-to-noise performance through high refractive index contrast. Our multimode waveguide technology is seamlessly integrated into the sample carrier, providing tight light confinement, high intensity, and a flat illumination profile - ideal for super-resolution imaging. Our bio-compatible carriers are well-suited for both live and fixed cell imaging as well as for use with tissue sections. Further, our landmarked carriers enable robust correlative microscopy workflows. The system also integrates EPI fluorescence with advanced post-processing tools for 2D/ 3D deconvolution, along with GPU-accelerated reconstruction software for single-molecule localization microscopy (SMLM) data. Its versatility makes it ideally suited for studying membrane dynamics and drug interactions with precision and ease.

Join our workshop and experience how advanced photonic chip-based TIRF increases the power of imaging techniques like SMLM - by allowing for super-resolution imaging across ultra-large fields of view. For those interested, we can also demonstrate on-chip histology. We will also showcase our user-friendly reconstruction software, designed to efficiently reconstruct large datasets, ensuring a seamless and high-performance imaging workflow.

### **Community room workshop**

## Hands-on: REMBI-compliant annotations in OMERO

#### Presenter(s):

Tom Boissonnet - Heinrich Heine Universität - Germany

Christian Schmidt - DKFZ German Cancer Research Center - Germany

The FAIR (Findable, Accessible, Interoperable, Reusable) guiding principles for data management and datastewardship (Wilkinson et al., 2016) provide a framework to increase the value of scientific data. However, theseguidelines are not "a standard", nor do they dictate discrete technical solutions. Research communities must establish a consensus on what FAIR means in their field and how FAIR data can be achieved. A key focus of the FAIR principles is onmetadata, the important accessory information around the measurement data required to understand the data from abioimaging experiment (Kunis & Dohle, 2022). Moreover, achieving machine-readability is an important aspect for FAIR data. Therefore, metadata should adhere to consented standards with respect to which information is stored, how it is implemented in a data model, and how the description becomes well-structured and unambiguous by means of controlled vocabularies and ontologies. The Recommended Metadata for Biological Images (REMBI) is a set of community-established metadata items, structured into modules that help data producers annotate their data with a minimum set of metadata (Sarkans et al., 2021). In this workshop, we show how to get started with metadata enrichment from the ground up and how to use REMBI-compliant annotation within the data management system OMERO. We demonstrate the integration of REMBI items within OMERO's "Project" and "Dataset" structure using Key-Value Pair annotations with ISA-aligned linkage to ontologyterms.

Kunis S & Dohle J (2022). Structuring of Data and Metadata in Bioimaging: Concepts and technical Solutions in the Context of Linked Data. Zenodo. https://doi.org/10.5281/zenodo.7018750

Sarkans, U., Chiu, W., Collinson, L. et al. REMBI: Recommended Metadata for Biological Images—enabling reuse of microscopy data in biology. Nat Methods 18, 1418–1422 (2021). https://doi.org/10.1038/s41592-021-01166-8

Wilkinson, M., Dumontier, M., Aalbersberg, I. et al. The FAIR Guiding Principles for scientific datamanagement and stewardship. Sci Data 3, 160018 (2016). https://doi.org/10.1038/sdata.2016.18

### **Community room workshop**

## PixelQuality: best practices for publishing images (QUAREP-LiMi)

### Presenter(s):

Sergiy Avilov - Max Planck Institute of Immunobiology and Epigenetics - Germany

Christopher Schmied - Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP) - Germany

Rapid technological advancements are steadily increasing the complexity of documenting and explaining scientific methods and their results. Imaging using microscopy has seen dramatic changes over the last decades with novel microscopy systems allowing imaging at higher spatial and temporal resolution. Furthermore, image and data analysis methods have progressed particularly in the application of machine learning and artificial intelligence. Scientists are now expected to be experts in many disparate fields to produce high-quality results in their publications and provide detailed accounts of a wide range of technologies. Unified standards for publishing and documenting image processing are currently lacking. This shortfall, combined with insufficient training on documenting image acquisition and processing, often leads to poor quality of published images and incomplete reporting.

To tackle this gap, the working group 12 of Quality Assessment and Reproducibility for Instruments & Images in Light Microscopy (QUAREP-LiMi) has developed comprehensive checklists for authors, reviewers, and editors for publishing images and image analysis [1, 2]. These guidelines aim to enhance the quality and reproducibility of scientific images. The adoption of these checklists by several scientific journals, including a trial adoption in 3 major Springer-Nature journals planned for 2025, highlights the growing recognition of the need for rigorous image and image analysis quality standards.

This workshop will introduce the checklists and training material [3] and aims to show by example how to use this material to improve the publication of images and image analysis.

- [1] Schmied et al. Community-developed checklists for publishing images and image analyses. Nat Methods (2024) https://doi.org/10.1038/s41592-023-01987-9
- [2] https://award.einsteinfoundation.de/award-winners-finalists/recipients-2024/pixelquality
- [3] https://quarep-limi.github.io/WG12\_checklists\_for\_image\_publishing/intro.html

## Unlocking 3D Complexity: Integrated Confocal and Super-Resolution Microscopy for Organoid and Model Organism Analysis

#### Presenter(s):

Greg Perry - Business Development Manager EMEA, CrestOptics Spa

Francesco Bacchi - Business Development Manager EMEA, CrestOptics Spa

The study of complex biological systems, such as organoids and model organisms, demands imaging techniques that can capture both large-scale architecture and subcellular details in three dimensions. This workshop presents a powerful approach combining CrestOptics' X-Light V3 spinning disk confocal microscopy for rapid volumetric imaging with DeepSIM super-resolution microscopy for nanoscale resolution. The X-Light V3 enables efficient acquisition of large 3D datasets, while DeepSIM provides the necessary super-resolution to resolve intricate structures within these models. We will demonstrate the capabilities of this integrated workflow for high-content analysis of organoids and whole-organism imaging, highlighting its potential to accelerate discoveries in developmental biology, disease modeling, and drug discovery.

## Unlock the Dynamics of Life: Experience High-Speed, Super-Resolution Live-Cell Imaging with the MI-SIM

#### Presenter(s):

Christiaan Stuut - CSR Biotech, Heidelberg, Germany

Jinging Wang - CSR Biotech, Guangzhou, China

Shutang Zeng - CSR Biotech, Guangzhou, China

Ready to capture the dynamic processes of life at unprecedented speeds and resolution? The MI-SIM system is your gateway to dynamic live-cell microscopy, achieving imaging speeds of over 500 frames per second while resolving details down to 60 nm with Sparse deconvolution. Imagine visualizing the fastest cellular processes with stunning clarity and minimal artifacts. Whether you're exploring single-molecule dynamics at the cell surface using TIRF-SIM or delving deep into tissue samples with 3D-SIM, the MI-SIM provides the versatility and performance you need to push the boundaries of your research.

In this workshop, you'll discover the fundamental principles behind Structured Illumination Microscopy (SIM), the powerful super-resolution technique that empowers the MI-SIM. We'll explain how structured illumination patterns and advanced algorithms work together to overcome the diffraction limit of light, revealing cellular structures with remarkable detail in both 2D and 3D.

Gain hands-on insights into the MI-SIM experiment workflow. We'll guide you through efficient image acquisition, the crucial reconstruction process that generates super-resolution images, and the power of our advanced Sparse deconvolution algorithms to further enhance resolution and clarity.

Join us to experience the transformative capabilities of the MI-SIM for your live-cell imaging studies. From capturing rapid molecular interactions to resolving intricate organelle dynamics within complex tissues, see how the MI-SIM can revolutionize your understanding of cellular life.

### **EVIDENT Europe GmbH**

## Pushing the limit of spinning disk confocal with the IXplore IX85

Presenter(s):

Wojciech Brutkowski, Evident Europe

Advancements in spinning disk confocal microscopy are unlocking new frontiers in live cell imaging and high-speed volumetric acquisition. Explore at ELMI 2025, how IXplore™ IX85 with an industry-leading 26.5 FOV across two integrated imaging ports, redefines the potential of confocal research by integrating cutting-edge innovations designed to enhance usability, stability and resolution.

Experience seamless and efficient workflows supported by AI-based macro-to-micro imaging, auto-correction collar and intelligent shading correction. Learn, how our revolutionary LUPLAPO25XS silicone gel objective enhances organoid imaging and multi-well plate screening, providing deeper insights, more stable time-lapse imaging and precise data with ease.

Paired with spinning disk technology, the IXplore IX85 offers a powerful solution for advanced imaging. Researchers can now achieve higher resolution, improved signal-to-noise ratios, and enhanced reproducibility with minimal manual intervention. With a system built for adaptability and precision, the IX85 ensures that confocal imaging workflows are faster, more reliable, and capable of delivering unprecedented insights into complex biological systems.

### Leica Microsystems

## Advancing 3D Spatial Omics with High Multiplex Imaging

#### Presenter(s):

Luis Alvarez - Leica Microsystems, Germany

Irmtraud Steinmetz - Leica Microsystems, Germany

Julia Roberti - Leica Microsystems, Germany

The complexity of biological processes demands innovative methods for comprehensive study. Recent developments in fluorophore technology have enabled imaging techniques to detect an increasing number of signals within a single sample. This progress has paved the way for advanced multiplexing and imaging "omics" approaches, revealing crucial insights into tissue organization, cancer progression, tumor-immune interactions, therapy prognosis, and infectious diseases.

The STELLARIS confocal platform is ideally equipped for 3D high multiplexing applications, featuring a tunable white light laser excitation (WLL, range 440 nm - 790 nm) and up to five highly sensitive spectral Power HyD detectors, providing detection flexibility from 410 nm to the NIR range. This combination supports a wide array of fluorophores, optimizing various configurations.

In this workshop, we will explore the experimental design and considerations for conducting high multiplexing imaging experiments with 15+ fluorophores on a single sample, with the new functionality SpectraPlex for the STELLARIS platform. Topics will include selecting appropriate fluorophore panels, preparing samples, and addressing imaging challenges. We will also demonstrate how high multiplexing targets can be utilized for 3D omics together with image analysis to derive meaningful insights from complex spatial biology experiments.

#### References

3D multiplexing imaging in cancer immunology. Leo Kunz, Dario Speziale, M. Julia Roberti, Susanne Holzmeister, Frank Hecht, Luis A. J. Alvarez, Irmtraud Steinmetz. Nat. Methods (2024). https://www.nature.com/articles/d42473-024-00260-7

SpectraPlex: A powerful toolbox for advanced 3D high-multiplex imaging. Roberti, M. J., Hecht F., Gai E., Straka T., Holzmeister S., Steinmetz I., Wong H., Alvarez L. Nat. Methods (2024). https://www.nature.com/articles/d42473-024-00262-5

## Fast, Label-Free, Live-Cell Analysis with Digital Holographic Microscopy

Presenter(s):

Benjamin Rappaz

Head of Life Sciences

Lyncée Tec, Lausannne, Switzerland

Digital Holographic Microscopes (DHM®) by Lyncée Tec offers a fast, label-free, noninvasive imaging approach based on quantitative phase measurements. This technique enables real-time monitoring of living cells, providing metrics such as cell morphology, dry mass, and intracellular dynamics without the need for exogenous labels or dyes. Thus, allowing to quantify cell health status and ongoing bioprocesses without perturbing your cells.

In this workshop, we will present the principles and key applications of DHM® in life sciences:

Quantitative analysis of morphology, proliferation, and cytotoxic responses

Applications for high-content screening and long-term live-cell monitoring

4D tracking of live cells

The system offers multi-objective support (2.5x to 100x), a large field of view (up to 1.3 mm at 5x), and fast acquisition speeds (up to 194 fps), allowing robust and scalable live-cell imaging. DHM® can also be correlated with fluorescence for simultaneous recording and correlative studies.

Case studies and live demonstrations will illustrate how DHM® enables reproducible, high-throughput analysis of cellular processes, with minimal perturbation to the biological system. The session is intended for researchers seeking robust, quantitative, and label-free imaging methods for live-cell studies.

More info:

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## Integrated 3D Imaging Workflows: From Sample Prep to Stunning Results with the UltraMicroscope Platform

#### Presenter(s):

Luigi Prisco, Miltenyi Biotec B.V. & Co. KG
Zahra Labbaf, Miltenyi Biotec B.V. & Co. KG
Rafael Kurtz, Miltenyi Biotec B.V. & Co. KG

L Prisco1, Z Labbaf1, R Kurtz1, C Ahlert1

1 Miltenyi Biotec B.V. & Co. KG.

Visualizing the three-dimensional architecture of complex and large biological specimens has traditionally been a time-intensive task, often taking hours to days. Miltenyi Biotec simplifies this process with a complete, end-to-end workflow encompassing sample preparation, imaging, and data processing – all centered around the UltraMicroscope Platform.

Featuring both the UltraMicroscope Blaze™ and the UltraMicroscope Choros™, our platform delivers fast, high-resolution light sheet imaging for a wide range of sample sizes. In this workshop, we invite you to join us for a live demonstration that walks through the entire 3D imaging process, showcasing our latest innovations: LightSpeed Mode, which accelerates imaging performance; MACS UltraMounts, enabling high-throughput 3D imaging; and the MACS iQ View – 3D Large Volume software, which simplifies image processing.

Discover how our cutting-edge technology delivers scalable, efficient 3D imaging to accelerate your research.

A whole mouse brain cleared with the MACS Deep Clearing Kit and stained with antibodies for tyrosine hydroxylase (Vio G570, magenta), NeuN (Vio R667, purple), and neurofilament (Vio 780 Yellow) and imaged on the UltraMicroscope Blaze™ light sheet system.

## Introducing the New Scanning Solution from Nikon — Slide Scanning Made Easy Without Compromising on Image Quality

Presenter(s):

Orsolya Szilagyi

Philipp Strunz

The NIS-Elements Slide Scanning Module is a powerful software tool that was developed specifically for our reliable Ni-E upright microscope. This state-of-the-art system features:

Scanning of up to 8 slides at a time

Full automation with premium-grade optics

High resolution, fast imaging capabilities

Intuitive, user-friendly interface

You don't need to spend hours getting familiar with a complicated system to start using our scanning solution. Whether you're a seasoned microscopist or new to the field, our step-by-step workflow and preoptimized settings ensure a seamless operation, making this an ideal tool for any imaging facility. Blurry images can be a thing of the past thanks to our high-quality optics. Furthermore, our AI-powered tools ensure that your samples are always perfectly in focus and all regions of interest are automatically detected. No need to spend countless hours in front of a scanner as our high-speed stage and precise tiling technology enable whole tissue section scanning in under a minute. You can easily navigate and visualize your high-quality scans in the convenient gallery view and use them for further analysis within NIS-Elements if required.

Experience the future of slide scanning firsthand by registering for our upcoming workshop. Discover how this innovative solution can transform your research capabilities and enhance your laboratory efficiency.

Reserve your spot today and take the first step toward elevating your microscopy experience with Nikon.

NIS-Elements Slide Scanning Module webpage:

https://www.microscope.healthcare.nikon.com/en\_EU/products/slide-scanning/nis-elements-slide-scanning-module

# Are you ready to begin your dSTORM journey? An introduction to the Nanoimager and the ONI Training Kit: dSTORM

#### Presenter(s):

Dr. rer. nat. Nensi Alivodej - Field Application Scientist, EU - nalivodej@oni.bio

Pip Timmins - Sales Executive - phillipa@oni.bio

Are you ready to begin your dSTORM journey? An introduction to the Nanoimager and the ONI Training Kit: dSTORM

The Nanoimager is a compact and state-of-the-art microscope, offering quantitative analysis for localization-based imaging (dSTORM and PALM), single-particle tracking and single-molecule FRET.

The Nanoimager is designed to operate on a standard lab bench and has a footprint smaller than a piece of A4 paper, making it more accessible to researchers.

The ONI Training Kit<sup>™</sup> for dSTORM is designed to provide a simple workflow for new and existing users to learn the fundamentals of single-molecule localization microscopy. Purchase of the training kit gives a free pass to one of our certified 'Live from the Lab' On-line training courses.

This workshop is intended for people who are new to dSTORM imaging or who want to brush up on their knowledge.

Workshop attendees will receive a 10% discount voucher to be redeemed against the purchase of a Training Kit

### **Oxford Instruments**

## When Seeing Fails and Objective (Lens) Truths Prevail: Linking Microscope Quality Control Metrics to More Precise Biological Readouts

#### Presenter(s):

Geraint Wilde - Oxford Instruments

How much do optical component variations in the microscope affect common biological imaging assays? What are the impacts of these detectable variations on the repeatability and/or reproducibility of microscopy experimental results, and under what conditions do they really matter when observing a biological phenomenon?

The efforts of the QUAREP-LiMi consortium have established a rigorous and standardized approach to microscope quality control and have clearly highlighted large differences in optical performance between not only different types of microscopy technology, but also between identical microscope models using the same optics.

Building on this drive towards light microscopy standardization, we designed a set of experiments where different objective lenses are used to image the exact same field of view on the same microscope platform and thereby demonstrate the direct correlation between a measured quality control metric using a calibration sample and an image measurement on a real biological sample that depends on the metric's magnitude. We show how subtle yet quantifiable differences in a biological sample's sub-cellular component volumes, cross-channel colocalization, and cross-field of view intensities - which are difficult to ascertain by eye from the images - are directly relatable to measurable differences in point spread function (PSF), chromatic aberration, and field illumination uniformity obtained with each objective lens.

Our study illustrates how ascertaining a clear understanding of the microscope quality and performance metrics facilitates a better comparison and reproducibility of inter-microscope experiments with increased confidence. Seeing is not always believing.

## Ultra-thin Light Sheet Microscopy Providing Multi-user Experience Combined with Automated Multi-scale Imaging Capabilities

Presenter(s):

Igor Lyuboshenko - PHASEVIEW

Alpha3 Facility Edition is an automated light sheet microscope designed for imaging facilities and research laboratories. It integrates key technologies to deliver ultra-thin light sheet thickness and a multi-user experience, from rapid sample screening to high-resolution image acquisition of fixed or live samples and whole cleared specimens. The system offers a seamless multi-scale imaging experience for diverse applications, enabling multiple users to observe the same sample at various scales without compromising imaging resolution.

As a fully automated system, the Alpha3 Facility Edition configures itself when changing magnification, allowing users to focus on capturing images. It recovers all settings and calibration parameters, providing a personalized multi-user and multi-application experience. Intelligent illuminators coupled to a wide-field detection microscope ensure maximum homogeneous light coverage for both small and large samples. Illumination from both sides simultaneously covers the maximum area of the sample. Observation is possible through oculars and with a camera.

The system features an extended working distance and objectives with high numerical aperture, providing a very thin optical section with optimized sharpness. It allows varying the orientation of the sample sectioning plane within the specimen, maintaining homogeneous image sharpness over the entire field of view. It automatically configures lenses for illumination and detection, optimizing image quality and ensuring excellent colocalization of images acquired with multi-wavelength light beams, thanks to the dynamic focusing module. Alpha3 Facility Edition offers advanced capabilities in light sheet microscopy, providing a versatile and automated solution for multi-user environments and diverse imaging applications. You are welcome to join us to explore these innovative features and discuss potential collaboration opportunities to further advance your research.

## Pushing Boundaries in FLIM to Enhance Efficiency, Quality and Reproducibility

#### Presenter(s):

Isabel Gross - Picoquant GmbH, Germany

Matthias Patting - Picoquant GmbH, Germany

Quantitative time-resolved fluorescence techniques like Fluorescence Lifetime Imaging (FLIM) have become more attractive recently to study mechanisms driven by phase separation or to sense the cellular environment, for example.

PicoQuant's innovative confocal microscope Luminosa combines state-of-the-art hardware with cutting edge software to deliver high quality data while simplifying daily operation. The software includes several features which improve the ease of use and reproducibility of experiments, including context-based workflows, sample-free auto-alignment and excitation laser power calibration. Still, if required for new method development every optomechanical component can be fully accessible.

We will show how FLIM is streamlined with Luminosa.. In combination with GPU-accelerated algorithms, this enables high-speed automated analysis of FLIM images. The InstaFLIM analysis workflow suggests the best fitting model based on statistical arguments, requiring minimal user interaction. The additional NovaFLIM software package enables more extensive and advanced image analysis.

Many recent initiatives have focused their efforts on improving the aspects of Quality Assessment (QA), Quality Control (QC), and reproducibility in time-resolved fluorescence microscopy. In another push, an increasing number of funding and research institutions commit to FAIR principles as well as promoting open-science initiatives.

The design of Luminosa's software makes all data easily accessible. It works with the open, well documented PTU data format, enabling custom analysis. Moreover, it includes various data export options.

### Rapp OptoElectronic GmbH

## Automating Photomanipulation Experiments with RAPP OptoElectronic Devices Using Adaptive Feedback Microscopy

### Presenter(s):

Dr. Aliaksandr Halavatyi - EMBL Heidelberg, Germany

Dr. Manuel Gunkel - EMBL Heidelberg, Germany

Dr. Anja Zimmermann - RAPP OptoElectronic GmbH, Germany

Photomanipulation is an important technique in microscopy: it is essential for studying molecular kinetics and interactions, modulating live sample properties via optogenetics, laser ablation and more. For these types of experiments, specialized photomanipulation devices are required to meet specific needs, such as photomanipulation during imaging, complex illumination patterns or specific light sources. RAPP OptoElectronic (RAPP) devices are designed to fulfill these requirements and can be integrated with various microscope types.

Automation is vital to enhance throughput and ensure unbiased selection of photomanipulated regions. Such workflows are implemented with Adaptive Feedback Microscopy technology, where each image is analyzed in real-time by a preconfigured routine to identify target areas. Once analysis is complete, the appropriate photomanipulation action is initiated.

In this workshop, we will showcase the Adaptive Feedback Microscopy pipeline using a RAPP UGA-42 FireFly device on an Evident FluoView 3000 confocal microscope. We will demonstrate fully automated photoactivation of a specific cell subpopulation identified through cellular phenotype profiling. This workflow builds on the AutoMicTools toolbox for Fiji, developed at the Advanced Light Microscopy Facility (ALMF) at EMBL Heidelberg.

The techniques presented can be adapted for various applications and microscopes equipped with RAPP devices. All RAPP devices are controlled via SysCon software, allowing a unified programming interface for photomanipulation. The modular architecture of AutoMicTools enables the integration of project-specific analysis routines and additional imaging, analysis and photomanipulation steps as needed.

See you in the workshop to discuss your potential applications and the minimal requirements for adapting the protocol to other microscope setups.

## Alignment-Free Two-Photon Microscopy with the Prelude® System

### Presenter(s):

Anja Huss - Thorlabs GmbH

Sergey Matveev - Thorlabs GmbH

Alexander Jelzow - TOPTICA Photonics AG

Multiphoton microscopy is a powerful method for probing neuronal activity and other complex biological events, yet it has long depended on intricate, specialist instruments that hinder accessibility. This workshop presents an alignment-free system that streamlines multiphoton imaging, expanding its reach to a wider community.

The Prelude® Functional Imaging Microscope by Thorlabs is a compact, fully integrated two-photon system designed to meet the demands of modern neuroscience and functional imaging. Developed in collaboration with researchers from Baylor College of Medicine and Columbia University, the Prelude addresses real-world experimental challenges by offering a maneuverable platform optimized for in vivo imaging of green fluorescent protein (GFP) and GCaMP6-labeled samples.

At its core, the Prelude features a 920 nm femtosecond pulsed laser coupled via fiber optics, eliminating complex alignment procedures and enhancing portability. Here, we use TOPTICA FemtoFiber Ultra 920 FD laser with high-power fiber delivery of femtosecond pulses. The system incorporates a silicon photomultiplier (SiPM) detector with greater than 38% photon detection efficiency at 500 nm, ensuring high-quality signal acquisition even in challenging specimens. Low profile and flexible XYZ translation and manual ±90° rotation provide versatile sample access, accommodating various experimental setups.

For fine Z-focus control, users can choose between a vibrationless liquid crystal remote focus system and a high-speed piezo objective scanner, facilitating rapid volumetric imaging without disturbing live specimens. The Prelude supports a range of long working distance objectives, including the TL10X-2P and TL15X-2P dry objectives, as well as the N16XLWD-PF water-dipping objective, catering to diverse imaging requirements.

This innovative microscope exemplifies Thorlabs' commitment to customer-inspired design, offering a flexible and user-friendly solution for advanced functional imaging applications.

### Tomocube Inc.

## Holotomography : the next imaging breakthrough is unlabeled

### Presenter(s):

Bruno Combettes - Tomocube Europe, Germany

Daniel Ghete - Tomocube Europe, Germany

"No stains. No labels. Just truth. See what you've been missing."

Label-free holotomography is redefining the way we explore live cells in 3D. By harnessing refractive index as intrinsic contrast, this cutting-edge technique enables high-resolution, quantitative imaging without the need for fluorescent dyes or stains. This workshop will introduce the fundamentals of holotomography, showcase applications in live-cell and tumoroid imaging, and provide insights into how it can complement your existing microscopy toolbox.

Whether you're managing an imaging facility or looking for new approaches in your research, this is your chance to discover the full potential of label-free imaging.

The showcased setup integrates holotomography with the CrestOptics spinning disk confocal module, giving you the best of both worlds — intrinsic contrast and molecular specificity, all in real time. Holotomography reveals structure and dynamics without labels. Spinning disk confocal adds molecular context with high-speed fluorescence imaging. Together, they offer a unique correlative approach — where cellular truth meets targeted insight.

그들은 함께 Cellular Truth가 목표 통찰력을 충족시키는 독특한 상관 접근법을 제공합니다.

## VisiScope Confocal based on Yokogawa CSU-W1 with Lumencor ZIVA 7 turn-key Multimode Laser illumination

### Presenter(s):

Dr. Andreas Babaryka - Sales and Application Support - Visitron Systems GmbH Puchheim Germany

Dr.Bernd Zobiak - Sales and Application Support - Visitron Systems GmbH Puchheim Germany

Typically spinning disk confocal are available for 3-dimensional imaging of live cells, tissues and microorganisms. However, the range of fluorescence excitation wavelengths provided by light sources installed on CSU systems is commonly covered by four or six lasers. Further, the cost of these single mode laser light sources often exceeds that of the CSU scanner itself. Lumencor's ZIVA Light Engine for Yokogawa increases the number of lasers from four to seven at a price significantly lower than that of 4 to 6 single mode lasers especially if NIR is required with the 748nm laser line.