Scope of the symposium

Bruker is dedicated to providing a complete range of imaging solutions for the advancement of neuroscience research. Our fluorescence microscopy solutions are uniquely optimized to address specific challenges when studying the brain.

Our multiphoton systems provide superior imaging depth, speed, and resolution—enabling the in vivo study of mechanisms in deep portions of the brain on a timeline that is reflective of the underlying biology. Our light-sheet microscopes from Luxendo enable long-term imaging of organoids and other live tissues. Our super-resolution microscopes utilize a quantitative single-molecule localization technique which supports the direct investigation of the molecular distribution of specifically labeled proteins at a resolution beyond the optical diffraction limit. Our recent addition, Canopy Biosciences, adds multi-omics and high-content cytometry technologies enabling single cell, tissue and suspended cell-based discovery and validation in immunology and targeted proteomics, as well as a suite of complementary multi-omics services. While we offer a variety of imaging solutions, they all have one commonality—the ability to take your neuroscience research to the next level.

This virtual workshop will include several brief presentations and demonstrations, address common questions posed by neuroscientists, and provide insight on a variety of innovative solutions for advancing neuroscience research.

Key topics covered will include:

- Executing holographic optogenetics experiments with a multiphoton microscope
- Imaging large cleared biological samples with light-sheet microscopy
- Utilizing single-molecule localization techniques to advance neuroscience research
- Performing precise spatial multiplexing in brain tissue

Featured systems:

- Ultima 2Pplus Multiphoton Microscope
- LCS SPIM Light-sheet Microscope
- Vutara VXL Super-Resolution Microscope and Plexflo Microfluidics Platform
- ZellScannerONE ChipCytometry Instrument

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Program – Wednesday, October 13th, 2021

1:00 PM – 3:00 PM EDT

1:00 **Welcome Address**

Philip Golding, Fluorescence Microscopy Sales Manager Sales Manager, Bruker

1:05 – 1:30 **Beginner's Guide to Performing Optogenetics Experiments on the Ultima 2Pplus, the All-optical Multiphoton Workstation**

Ewa Zarnowska, Ph.D., Sales Applications Scientist, Bruker

1:30 – 1:55 **Fast Imaging of Large Cleared Biological Samples with Light-sheet Microscopy**

Jürgen Mayer, Ph.D., Senior Application Specialist and Product Manager, Luxendo

1:55 – 2:20 **Super-resolution Microscopy in Neuroscience**

Winfried Wiegraebe, Ph.D., Product Manager for Super-Resolution Microscopy, Bruker

2:20 – 2:45 **Precise Spatial Multiplexing for Neuronal Cell Profiling in Mouse Brain Fresh Frozen Tissue with ChipCytometry**

Adam Northcutt, Ph.D., Senior Scientist, Canopy Biosciences

2:45 – 2:55 **Final Question and Answer**

2:55 – 3:00 **Closing**

Philip Golding, Fluorescence Microscopy Sales Manager Sales Manager, Bruker

Imaging Application in Neuroscience:
Assessing Neuronal Structure and Function

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Talk abstracts

Beginner's guide to perform optogenetics experiments on the Ultima 2Pplus, the all-optical multiphoton workstation.

Ewa Zarnowska, Ph.D., Sales Applications Scientist, Bruker

Optogenetics is an ingenious research technique that can help to solve mystery of the brain's works. In the process, the neuronal cells are virally transduced to be receptive to the light which is then used for manipulation of brain's cells such as to activate them or to inhibit them.

Brain tissue is highly photon scattering where networks of neuronal cells are intricate and span across 3-dimensions. Therefore, observation and optical manipulation of the brain's cells requires advanced technical developments that provide adequate time- and spatial-resolutions.

Bruker's answer to the needs of modern neuroscience was to design the state-of-the-art all-optical multiphoton workstation, the Ultima 2Pplus, which has larger than others field of view and is equipped with Neuralight 3D spatial light modulator (SLM), remote focusing and software developments to facilitate reading via imaging and writing via holographic optogenetics the activity in specific ensembles of neurons during behavior.

During this presentation I will highlight Bruker's unique solutions within the Ultima 2Pplus. Also, I will talk about the Prairie View software developments that allow targeting with the light multiple cells simultaneously. I will demonstrate the use of the software for the optogenetics experiments synchronized with imaging.

After attending this presentation, you will be familiar with functionality of the Ultima 2Pplus, and you will be able to independently run the optogenetics experiment on Bruker's multiphoton workstations.

Ewa is a neuroscientist specialized in advanced microscopy and electrophysiological techniques. She received her Ph.D. degree in Biophysics from the Medical University of Wroclaw, Poland. She was a tenured Assistant Scientist in the Department of Anesthesiology at the University of Wisconsin in Madison.

Fast imaging of large cleared biological samples with light-sheet microscopy: Luxendo's LCS-SPIM

Jürgen Mayer, Ph.D., Senior Application Specialist and Product Manager, Luxendo

Imaging volumetric biological tissue with cellular or sub-cellular resolution has become a key factor in neuroscientific research. Light-sheet microscopy is the state-of-the-art methodology to achieve this, enabling non-destructive data acquisition of intact organs such as an entire mouse brain.

A prerequisite for optical imaging in large (i.e. mesoscopic) samples is tissue clearing. There are numerous different clearing techniques, but all of them have in common that they render biological samples optically transparent. The combination of cleared tissues with light-sheet microscopy is an ideal synergy that allows addressing new questions in neurobiology.

Minimizing sample mounting time, and a fast acquisition followed by a robust processing pipeline is important, especially as the desire to image ever larger samples becomes more and more common.

In this session, we will explain the principle of light-sheet microscopy, highlight a few applications, and have a detailed look in the cleared sample light-sheet segment of Luxendo/Bruker's light-sheet portfolio, specifically on the latest development in this segment: the LCS-SPIM (Large Cleared Sample – Selective Plane Illumination Microscope).

Dr. Jürgen Mayer is a Senior Application Specialist and the Product Manager for cleared system microscopes at Luxendo, the light-sheet microscopy branch of Bruker's FM division. Before joining Luxendo, during his pre- and post-doctoral research, he developed and implemented a method to compensate attenuation artefacts in light-sheet microscopy via multimodal imaging combining light-sheet with optical projection tomography.

Super-Resolution Microscopy in Neuroscience

Winfried Wiegraebe, Ph.D., Product Manager for Super-Resolution Microscopy, Bruker

Single-Molecule Localization Microscopy (SMLM) was essential in tackling neuroscience questions about the organization of synapsis and the internal structure of neurons. With an optical resolution of 20 nm, SMLM is uniquely qualified to address biological mysteries that require specific labeling as used in fluorescence microscopy but higher resolution than can be achieved with diffraction-limited microscopy.

In addition to applications, we will discuss the basics of super-resolution microscopy and different implementations like dSTORM, PALM, and DNA-PAINT. We will discuss how the unique features of the Bruker Vutara VXL – its workflow-oriented software, biplane detection for improved z-resolution, and fluidics for multiplexed applications – will help you focus on neuroscience and getting superior results.

Winfried Wiegraebe is the product manager for super-resolution microscopy at Bruker. He has close to 30 years of experience in advanced microscopy in biology - including AFM, FCS, confocal microscopy, and super-resolution microscopy. Before joining Bruker, Winfried managed the Stowers Institute for Medical Research microscopy infrastructure in Kansas City, MO, US, and built the imaging pipeline at the Allen Institute for Cell Science in Seattle, WA, US. He studied Physics at the Technical University in Munich, Germany, and did his Ph.D. studies at the Max-Planck Institute in Martinsried, Germany.

Precise Spatial Multiplexing for Neuronal Cell Profiling in Mouse Brain Fresh Frozen Tissue with ChipCytometry

Adam Northcutt, Ph.D., Senior Scientist, Canopy Biosciences

Understanding the spatial distribution of key neuronal cell populations is critical in advancing our understanding of disease to inform the development of novel therapeutics. Here we present the analysis of fresh frozen (FF) tissue samples from mouse brain using a novel precise spatial multiplexing technology called ChipCytometry, which combines iterative rounds of targeted fluorescent staining with high dynamic range imaging to facilitate quantitative phenotyping with single-cell resolution. Standard FCS files are generated from multichannel OME-TIFF images, enabling identification of cellular phenotypes via flow cytometry-like hierarchical gating. In this study, a 13-plex assay was used to identify and quantify relevant cellular phenotypes and subtypes for neurobiology and neuro-oncology applications. The results show precise expression levels for each marker in the assay in each individual cell in the sample, while maintaining spatial information about each cell. Spatial analysis of the samples reveals neuronal cell heterogeneity including clusters of microglia and cholinergic neurons in a region rich with glutamatergic neuronal cells, demonstrating the utility of the ChipCytometry platform for the in-depth cellular profiling of mouse FF tissue samples.

Adam Northcutt is a Senior Scientist at Canopy Biosciences focused on application development for the ChipCytometry platform. Adam holds a PhD in Molecular Biology from the University of Missouri with an emphasis in neuroscience and bioinformatics.

Please don't hesitate to contact us at productinfo@bruker.com if you have any questions.