TechNote #2601

Controlling Biological Processes with the Luxendo Photomanipulation Module

Light-sheet fluorescence microscopy, also known as selective plane illumination microscopy (SPIM), is a powerful imaging technique that allows the visualization of biological specimens with exceptional precision and minimal photodamage. By illuminating and imaging a thin plane of a sample and using the latest camera-based detection, light-sheet microscopy allows rapid three-dimensional imaging of living organisms and tissues over extended periods, hours to days, in their native environments. This non-invasive imaging approach has widespread applications and has facilitated groundbreaking discoveries and invaluable insights into the fundamental mechanisms governing life at the cellular and subcellular levels. Futhermore, Bruker's Luxendo light-sheet microscopes utilize industry-leading SPIM technology that has become widely known for studies in fields ranging from developmental biology and neurobiology to drug discovery and plant biology.

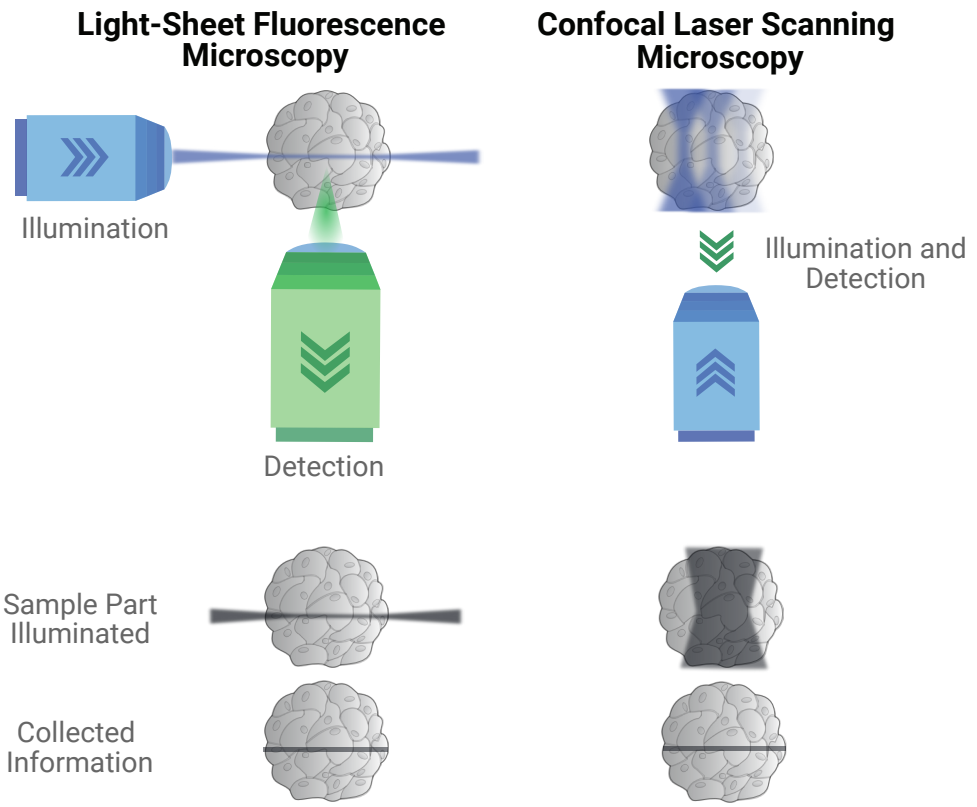


FIGURE 1.
Light-sheet microscopy illuminates only a thin sample section and data is collected simultaneously by an uncoupled detection objective.

Bruker's Luxendo SPIM Technology

Luxendo SPIMs enable precise and laser-based manipulations at specific regions of interest when equipped with the innovative photomanipulation module (PM). This setup is particularly well-suited for spatially resolved biophysics and optogenetics of cells and tissues. Furthermore, the PM functionality is fully integrated into the experimental workflow provided by the LuxControl microscope software and is intuitive to use. This groundbreaking addition allows researchers to investigate intricate biophysical processes, activate fluorophores and proteins, and unlock new avenues of scientific discovery.

What is Photomanipulation?

Photomanipulation refers to a powerful experimental technique used in various scientific disciplines, particularly in the fields of biology, cell biology, and neuroscience. It involves precise and controlled manipulation of biological samples, including cells, tissues, or organisms, using lasers. Photomanipulation uses light beams with high spatial and temporal accuracy to allow researchers to alter specific regions within their sample.

This technique has become instrumental in studying cellular processes, probing the functionality of specific proteins and organelles, and understanding the complex interactions within living systems. As a result, photomanipulation has proven to be an indispensable tool in advancing our knowledge of biological mechanisms and has paved the way for groundbreaking discoveries in modern scientific research.

The Photomanipulation Module (PM) can be used for experiments such as:

- Photoablation
- Cauterization
- Photobleaching
- Fluorescence Recovery After Photobleaching (FRAP)
- Uncaging
- Optogenetics
- Photoactivation
- Photoconversion

Technical Specifications

The PM is an add-on module that supports integration of various lasers including visible CW (such as for FRAP) or pulsed IR (such as for cauterization) with the detection objectives of Luxendo SPIMs. The PM laser is coupled into the detection objective lens to create a diffraction-limited illumination spot. The PM laser spot size is mostly determined by the numerical aperture (NA) of the detection objective lens.

By controlling a 3D beam scanner from the acquisition software, researchers gain the flexibility to freely position the illumination spot in three-dimensional space within the sample during imaging. In this way, the PM supports the creation of complex illumination regions, such as points, circles, squares, straight, and freeform lines. These regions can either be pre-defined as part of the experimental workflow or interactively determined during 3D imaging experiments. This sophisticated functionality offers full flexibility for advanced photomanipulation experiments, enabling precise and targeted investigations of specific regions of interest within biological samples.

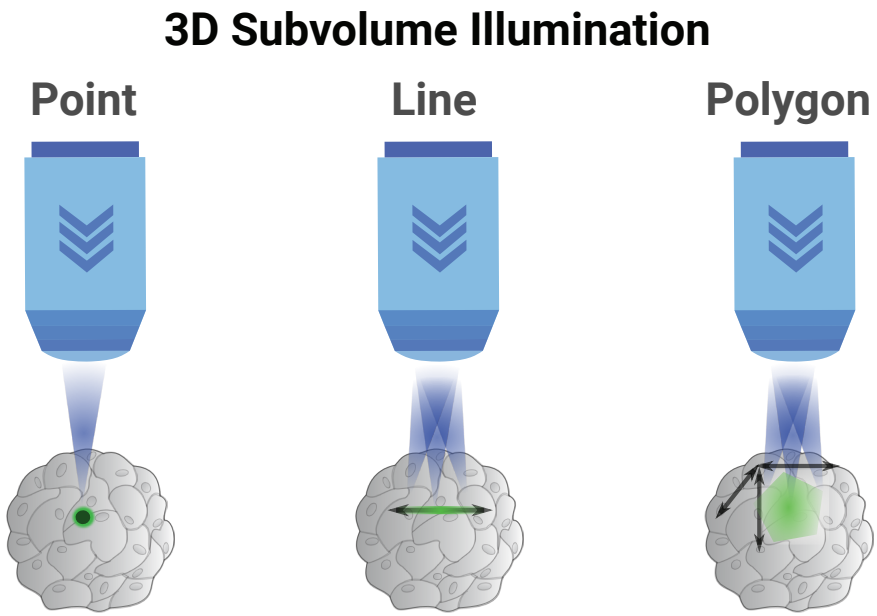


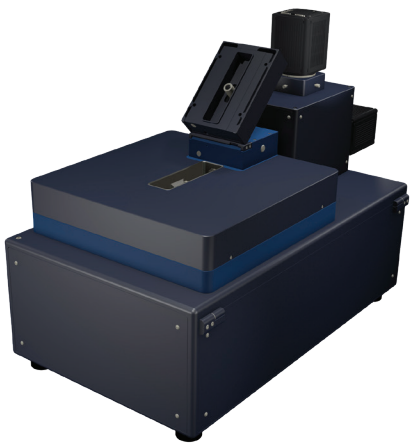
FIGURE 2.
The PM laser can be shaped based on a point, line, or polygon. Importantly, it can be freely positioned in 3D in the sample.

Luxendo's PM-Compatible Light-Sheet Systems

Thanks to careful design and engineering, the add-on PM module is compatible with three of Bruker's Luxendo SPIMs including:

- **TruLive3D Imager**: Dual-sided illumination for highly multiplex 3D cell culture live imaging
- **MuVi SPIM**: Multiview design for versatile imaging of live and cleared samples
- **InVi SPIM Lattice Pro**: Ideal for high-resolution, fast imaging of single cells to 3D cell cultures or organisms

Without PM



With PM

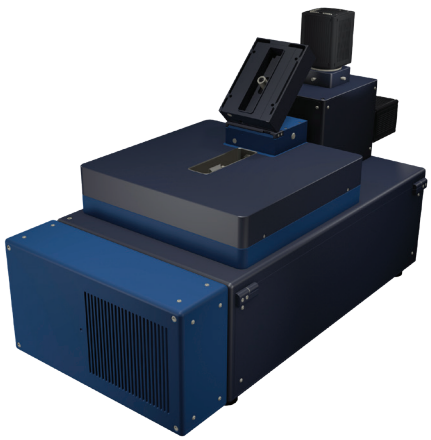


FIGURE 3.
TruLive3D Imager SPIM without (left) and with (right) PM module.

Application Examples

Cytokine Dynamics in a Tail Wound Assay

The add-on PM enables the study of immune system responses in a zebrafish tail wound assay. By using laser-based PM, researchers introduce wound sites with high-precision to study immune system responses in tissue repair and regeneration. This approach provides valuable information about immune system dynamics and offers potential applications in developing wound healing therapies for both zebrafish and higher vertebrates, including humans.

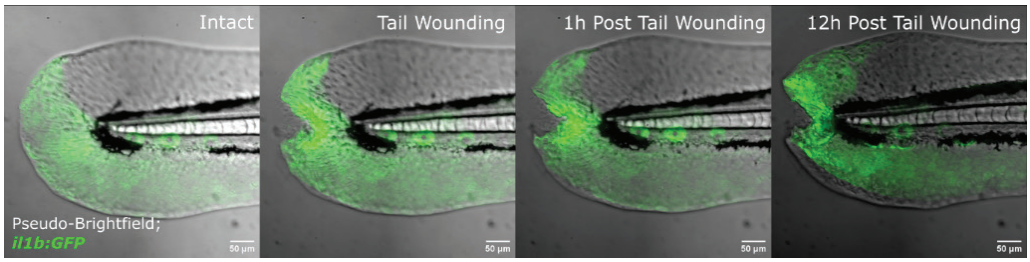


FIGURE 4.
Timelapse of cytokine dynamics in a tail wound assay. Pseudo-Brightfield and il1b:GFP (green) merged. Captured every 5 min for 12 hours. Sample courtesy of Dr. Elizabeth Jerison, Stanford University.

Tissue Morphogenesis in Drosophila

In developmental biology, photomanipulation provides invaluable insights into the role of biophysical forces in tissue morphogenesis. The Bruker webinar "Studying Morphogenetic Waves with Photo Manipulation Coupled to Multi-View Light-Sheet Microscopy" showcases groundbreaking research that goes beyond traditional 2D embryo imaging approaches. Guest speaker Dr. Matteo Rauzi from the University Côte d'Azur discusses his lab's work using a novel computational model based on light-sheet imaging, photomanipulation, and multidimensional image analysis. The webinar highlights how photomanipulation creates new avenues to explore crucial processes like Drosophila embryo gastrulation, neurulation, and the shaping of the animal body.[1]

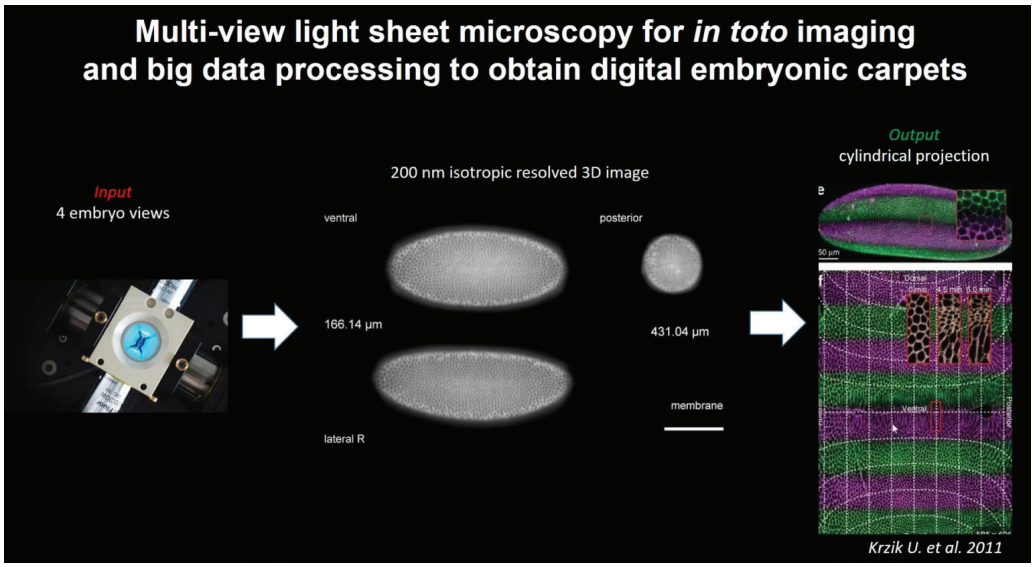


FIGURE 5.
Dr. Rauzi's research showing multi-view light-sheet microscopy for in toto imaging and big data processing to obtain digital embryonic carpets[1].

Dr. Rauzi's innovative approach has allowed for discoveries about epithelial furrowing, challenging previous ideas about its role in embryo development and revealing the molecular signals and mechanical forces responsible for this process.[1,2]

Microglia Response to Axonal Damage

IR laser ablation with a Luxendo MuVi SPIM can selectively dissect the axon of a neuron in the zebrafish brain and track the movement of microglia to the damaged axon after ablation.[3]

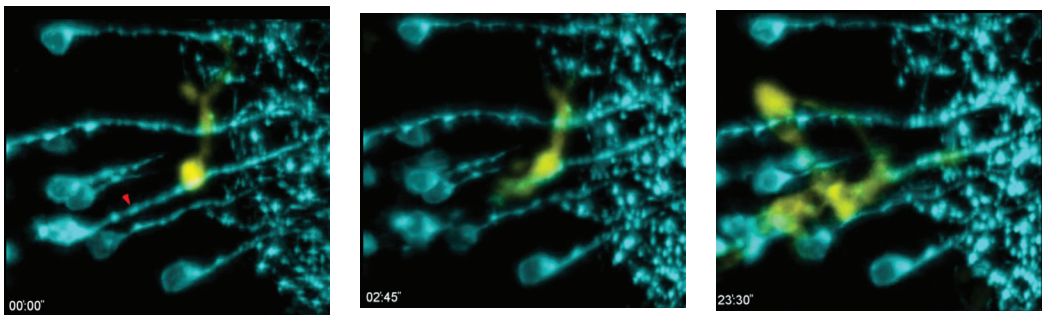


FIGURE 6.
After ablation, it took approximately thirty minutes for four microglia to reach the damaged axon. Image used with permission under Creative Commons Attribution (CC-BY 4.0 DEED).[3]

Mouse Peri-Implantation

To gain insights into mouse peri-implantation, a pulsed infrared (IR) laser at 1040 nm, 200 fs pulse length and 1.5 W output power (Spectra-Physics, HighQ-2) is coupled into the detection objective. Results from this experimental set up show that the polar trophectoderm (pTE) cells shortened in one direction (apically) and elongated in another (along their apico-basal axis), which provides valuable insights into the development of these cells.[4] This study demonstrates the utility of advanced imaging and laser manipulation techniques in understanding embryonic development.

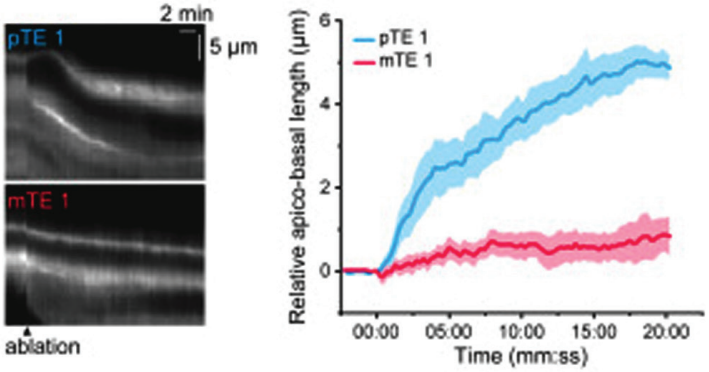


FIGURE 7.
Kymographs of GFP-Myh9 signal along with blue and red lines in (F), and measurement of the apico-basal length of pTE cells upon laser ablation. Used with permission under Creative Commons Attribution (CC BY 4.0).[4]

Groundbreaking Investigations with the PM Module

This technical note discussed the capabilities of Bruker's Luxendo light-sheet microscopes when equipped with the photomanipulation module. These innovative technologies enable precise and laser-based manipulations of specific regions of interest and are well-suited for non-invasive imaging of living organisms and tissues in their native environments. The PM module is an add-on that is compatible with three of Bruker's Luxendo SPIMs and has enabled groundbreaking investigations in developmental biology, neurobiology, and more.

References

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2. Popkova, A., Pagnotta, S., Rauzi, M. "A mechanical wave travels along a genetic guide to drive the formation of an epithelial furrow." *bioRxiv*, 12.08.518365, (2022). https://doi.org/10.1101/2022.12.08.518365. (preprint).

3. Medeiros, G., Kromm, D., Balazs, B., et al. "Cell and tissue manipulation with ultrashort infrared laser pulses in light-sheet microscopy." *Sci Rep*, 10, 1942, (2020). https://doi.org/10.1038/s41598-019-54349-x.

4. Ichikawa, T., Zhang, H.T., Panavaite, L. An ex vivo system to study cellular dynamics underlying mouse peri-implantation development." *Developmental Cell*, 57(3), 373-386, (2022). https://doi.org/10.1016/j.devcel.2021.12.023.

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