FLUORESCENCE MICROSCOPY

Imaging Organoids with Light-Sheet Fluorescence Microscopy

App Brief 2000

Organoids: a trending research tool in life sciences

The use of organoids as biological samples has become increasingly popular across a wide range of life science disciplines. In fact, organoids were named the Nature Method of the Year in 2017 for their potential as tools for studying human biology and disease.[1] Organoids are defined as three-dimensional stem cell structures that comprise ex vivo miniature organs,[2] and can be cultured to represent a variety of tissue types (e.g., the brain organoid in Figure 1). Studying tissue and organ biology is challenging, particularly in mammals, given sample accessibility and ethical concerns.[3] The use of organoids alleviates these challenges, supporting researchers in exploring questions that previously could not have been investigated without them. Recently, organoids have been used to answer questions in the fields of developmental biology, cancer biology, neuroscience, drug discovery, regenerative medicine, disease modeling, and more.

One common way that organoids are studied is imaging with fluorescence microscopy. Traditionally, researchers have used several approaches for imaging organoids, including confocal and multiphoton microscopy. More recently, researchers have used light-sheet fluorescence microscopy (LSFM) to image organoids. LSFM enables researchers to gently image organoids, and even their cellular structures, in three dimensions.

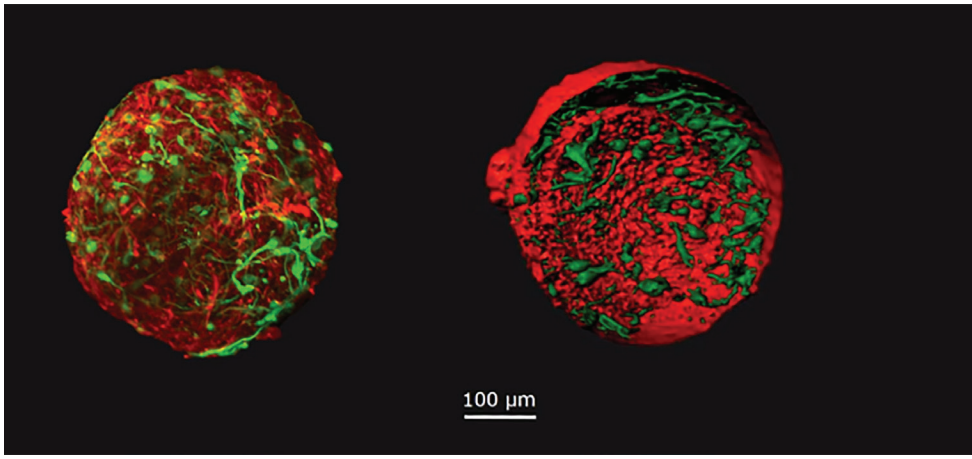

Figure 1.
Fixed Astrocyte Spheroid: Spheroid stained with anti-GFAP (Alexa 488) to label astrocytes and anti-Neurofilament200 (Alexa555) to label neurons. Imaged on the InVi SPIM.
Image courtesy of Markus Bruell and AG Leist at University of Konstanz, Konstanz Germany.

Principles and advantages of light-sheet microscopy for organoid imaging

LSFM has emerged as a key tool for studying biological systems– from subcellular processes to entire organisms. It is well-suited for organoid imaging due to its ability to gently collect 3D images of large samples over extended periods of time. This is important as organoids are fragile and research questions using them, particularly in tissue development, often require time-lapse studies. The large field of view and high resolution of LSFM allows researchers to view whole organoids, ranging from microns to millimeters, as well as the cellular structures located within them.

Unlike a confocal microscope, which uses a single objective for illumination and detection, a light-sheet microscope utilizes two objectives placed orthogonally to each other– one for illumination and one for detection (Figure 2). This geometrical arrangement of objectives in a LSFM allows for the illumination of a single thin section of the sample by one objective that corresponds with the focal plane of the detection objective. This illumination scheme results in only the focal plane of the corresponding detection objective to be illuminated, while the rest of the sample is unexposed to the light, resulting in limited phototoxicity and low photobleaching. Scanning the light-sheet through the sample allows researchers to acquire complete three-dimensional (3D) volume data with high resolution. Additionally, LSFM has an advantage of using camera-based acquisition resulting in fast acquisition speeds.

When imaging organoids, it is necessary to strike a balance between acquisition time, sample size and field-of-view requirements, and the duration of the experiment. Light-sheet microscopy allows researchers to increase acquisition speed, decrease phototoxicity, and obtain high-resolution, low-noise images of organoid samples, effectively opening new frontiers in organoid research.

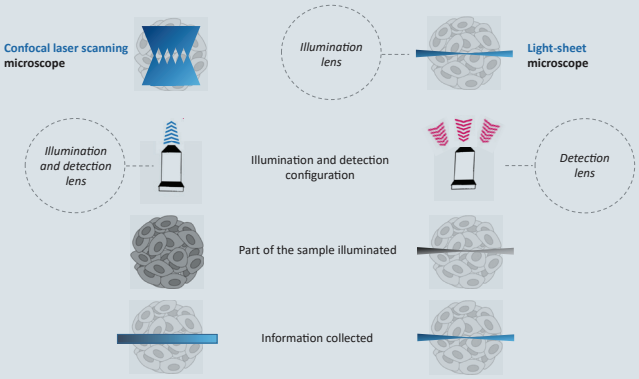

Figure 2.
Comparison of confocal laser-scanning and light-sheet fluorescence microscopy.

Case study: organoids in cancer biology research

LSFM has been used for imaging organoids to answer questions across several life science disciplines. Presented here is an example of using LFSM in cancer biology, particularly to study tumorigenesis in mammary organoids.[4] How tumors develop from a few malignant cells within an intact epithelium is a critical question that was previously unanswered. The researchers developed a method to switch on oncogenes in single cells within an otherwise normal epithelial cell layer, and combined this with LSFM to study single-cell behavior within the mammary organoid over time (Figure 3). With this methodology combined with LSFM, they found that small local clusters of transformed cells formed tumors, while isolated transformed cells did not. This novel finding, uniquely supported by the capabilities of LSFM, has advanced knowledge in the field of cancer biology.

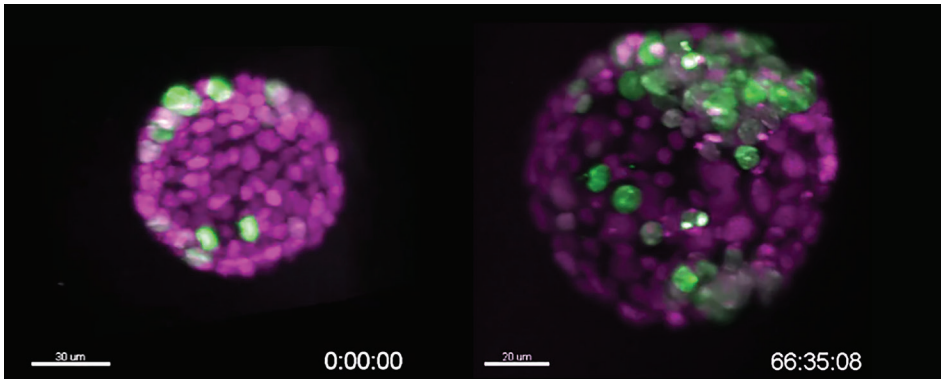

Figure 3.
Light-sheet imaging of stochastic tumorigenesis in mammary acini. Panels are 3D images of selected timepoints during live-cell, time-lapse microscopy of induced T acini transduced with a reporter virus. Left panel shows the organoid at T0 and right panel shows the proliferative phenotypes seen with stoachastic transformation after about 66 hours. Imaged with InVi SPIM.

Bruker solutions and additional LSFM information

Bruker offers a range of advanced solutions for light-sheet microscopy research. In particular, Bruker's **TruLive3D** and **MuVi SPIM** technologies are specifically optimized for organoid research experiments.

To learn more about Bruker's light-sheet microscopes and their capabilities, visit www.bruker.com/Luxendo.

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References

1. 2018. Method of the Year 2017: Organoids. *Nature Methods* 15(1). https://doi.org/10.1038/nmeth.4575
2. Rios, A.C. and Clevers, H., 2018. Imaging organoids: a bright future ahead. *Nature Methods*, 15(1), pp.24-26.
3. Rossi, G., Manfrin, A. and Lutolf, M.P., 2018. Progress and potential in organoid research. *Nature Reviews Genetics*, 19(11), pp.671-687.
4. Alladin, A., Chaible, L., Del Valle, L.G., Sabine, R., Loeschinger, M., Wachsmuth, M., Hériché, J.K., Tischer, C. and Jechlinger, M., 2020. Tracking cells in epithelial acini by light sheet microscopy reveals proximity effects in breast cancer initiation. *Elife*, 9, p.e54066.

TruLive3D Imager

Deep and Crisp Imaging

Dual-sided illumination and single-lens detection from below for fast acquistition speed, high-resolution imaging, and minimal shadowing effects.

Native and Natural

Close-to-natural environmental conditions for live-cell, long-term imaging of sensitive samples with reduced phototoxic effects.

Multiplexing

A large sample holder enables analyzing up to 100s of samples in one experiment with up to six different experimental conditions.

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