



# Application Note #2014

## Light-Sheet Imaging Reveals the Secrets of Neuroscience

The brain is highly complex and the most energy-demanding organ. It is composed of various cell types, including neurons that transmit information, immune cells that protect brain functions, astrocytes that regulate the chemical environment, and glia that provide structural and functional support. Additionally, non-cellular components, such as the cerebrospinal fluid (CSF) and (g)lymphatic system, are integral for maintaining brain health, homeostasis, and overall functionality. Due to its critical function, the brain, its health, and its development are major research areas.<sup>1</sup> This application note discusses how light-sheet microscopy overcomes challenges in brain imaging to look at brain tissues and components, from the eye and organoids, to the spinal cord and peripheral nervous system.

### Challenges in Brain Imaging

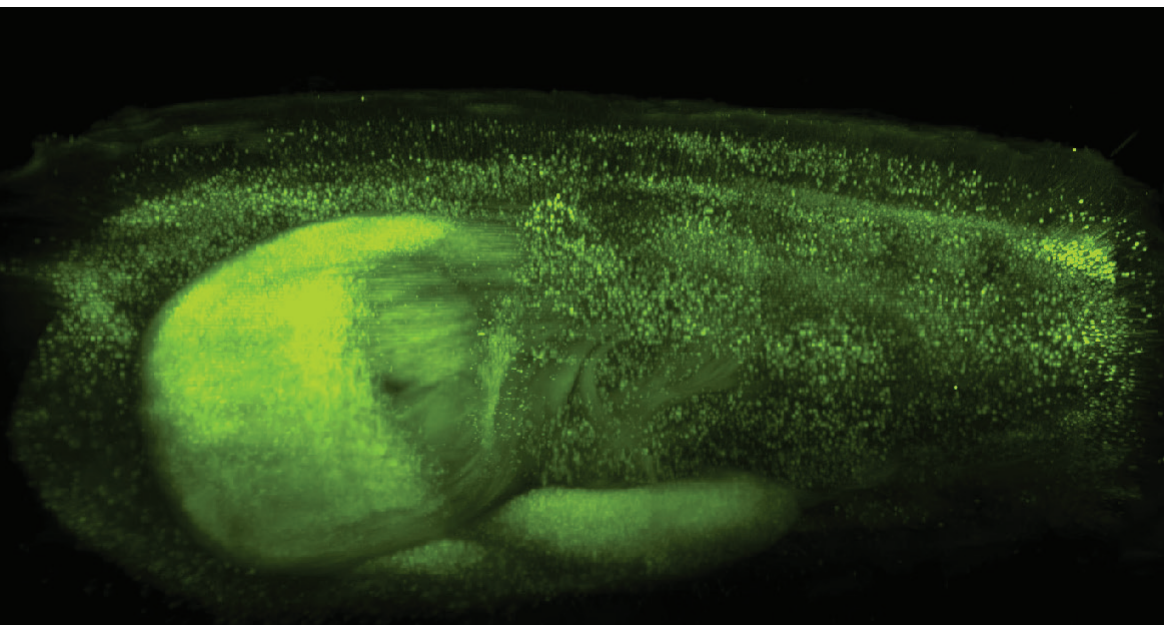
The brain is notoriously challenging to study. It is composed of countless small cells spread throughout a considerably large organ, often necessitating a trade-off between imaging the entire brain and achieving high-resolution images. For imaging in humans and other vertebrates, imaging at non-embryonic stages is challenging due to the skull encasing the brain with hard, opaque bone.

Advances in imaging correspond with changes in technology. In medicine, one of the gold standards for brain imaging in humans is functional magnetic resonance imaging (fMRI).<sup>2</sup> The medical imaging landscape is rapidly changing because deep learning and artificial intelligence have a high impact on new developments for data acquisition and analysis.<sup>3</sup> In contrast, for

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COVER IMAGE.

Nerve-tumor interaction. *Thy1-YFP-16* mouse line; 14 days after implanting breast cancer cells. Sample courtesy of Chenchen Pan.



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FIGURE 1.

Neurons of a transgenic mouse expressing fluorescent protein YFP. The brain was cleared with Clarity. Image courtesy of Dr. Zhang Dan, Tsinghua University, China.

biomedical and preclinical models, changes in sample preparation techniques—such as brain expansion—often come into play. By physically expanding the brain tissue, the resolution issue can be overcome by increasing the sample rather than decreasing the resolution.

Another challenge in brain imaging is that in most organisms, the brain itself is not transparent but opaque, reducing light penetration and thus impacting imaging beyond the surface. To overcome this hurdle, tissue clearing can be used. Tissue clearing renders tissues transparent, enabling light penetration and imaging within the tissue. This allows the visualization of intricate neural networks and other brain structures.<sup>4</sup>

## Neurobiology Versus Neurodevelopment

When it comes to brain research, two major areas of interest are brain development and neurobiology. Brain development studies the processes of brain formation and its maturation throughout life. It provides crucial insights into how abnormalities during development can lead to brain disorders.

Conversely, neurobiology is concerned with brain function, including memory, decision-making, sensory perception, and more. This includes the understanding of neurotransmitters, neuroplasticity, and neurochemistry. Understanding neurobiology helps diagnose and treat various neurological conditions.

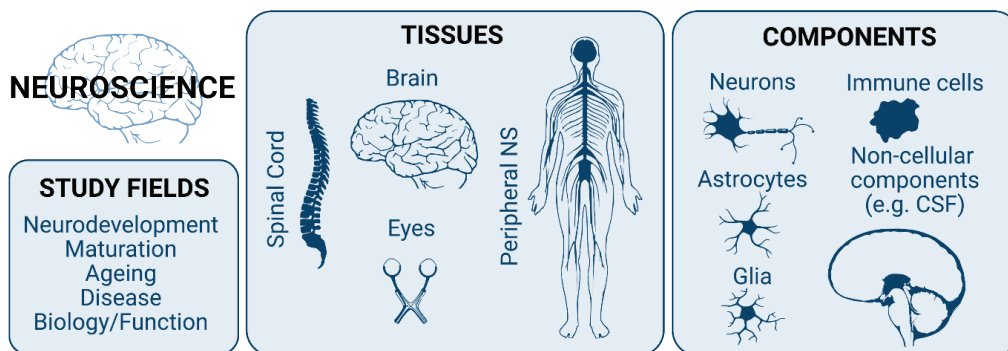


FIGURE 2.

The term *neuroscience* encompasses different study fields, tissues, and nervous system components.

## Light-Sheet Fluorescence Microscopy in Brain Imaging

Light-sheet fluorescence microscopy (LSFM) is a highly effective tool for brain imaging, offering practical advantages for neuroscientific research. Its notable features include penetration depth, reduced phototoxicity, and the ability to image fast processes.<sup>5</sup> LSFM's versatility is evident in its applications for live imaging dynamic processes, such as calcium dynamics or long-term developmental changes in the brain. It is also ideally suited for imaging cleared and expanded tissues, offering cellular-level resolution of whole brains.<sup>6</sup>

### Application Example—Hindbrain Morphogenesis

In one study, the role of Notch-3 in the neurogenic fate of hindbrain boundaries was described.<sup>7</sup> The group used intricate time-lapse imaging and data analysis to show specific boundary cells during hindbrain morphogenesis depending on Notch-3 signaling. Bruker's Multi-View Selective-Plane Illumination Microscope (MuVi SPIM) was employed for cell lineage analysis.

## Imaging Beyond the Brain—The Eye

Eye research is essential to understand and prevent vision loss. Still, eye research is also often used as a proxy to understand the brain, as the eye is a peripheral part of the brain but with much greater accessibility. The eyes share numerous similarities with the brain regarding cell types, functionality, and cell-to-cell interactions. This structural and functional resemblance makes the eye an invaluable model for fundamental biological processes. Thus, eye research is not only critical in itself but also serves as a bridge to broader neurology.<sup>8</sup>

### Application Example—Zebrafish Eye Laser Ablation

To understand macrophage response in the zebrafish eye, laser ablation can be used. Specifically, wounding of the eye is achieved using a laser, and a subsequent timelapse is acquired to understand macrophage recruitment and interaction with the wound. A transgenic line visualizing macrophages was used *Tg(mpeg::gfp)* with 488 nm excitation laser and collected with a 525/50 nm BP filter. Denoising and an unsharpen mask were applied to the maximum projection data. Each fish was a 150  $\mu\text{m}$  volume acquired every 2 min.

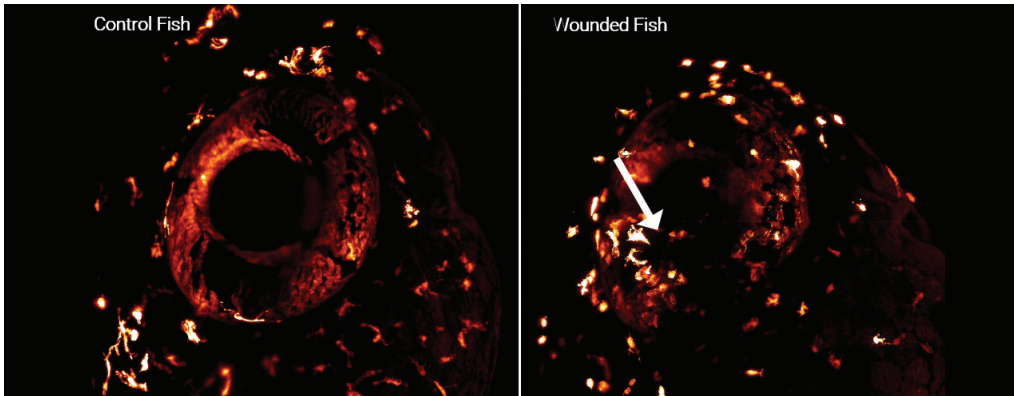


FIGURE 3.

Microglia and macrophage dynamics after wounding with photomanipulation. Courtesy of Dr Gordon Wang, Stanford University, USA.

### Application Example—Subcellular Mouse Retina Dynamics

A group investigated mouse retinas in 3D using a MuVi SPIM to study subcellular Golgi apparatuses and tuft morphology.<sup>9</sup>

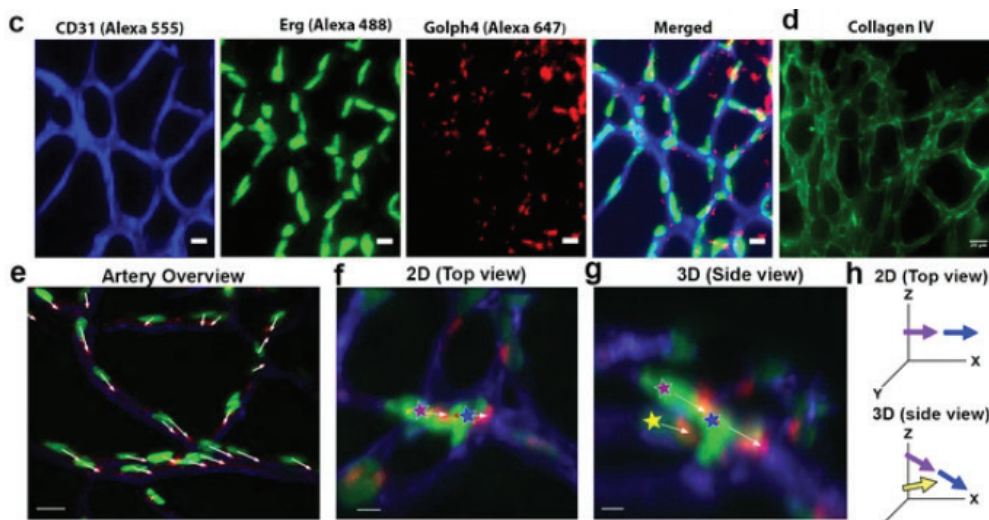


FIGURE 4.

Light-sheet fluorescence microscopy was used to study subcellular components and cellular polarization. (c) Retina stained with antibodies for blood vessels using anti-CD31 (Alexa 555), vascular nuclei Anti-Erg (Alexa488), and Golgi apparatus using anti-Golgi (Golph4, Alexa 647). (d) Collagen IV stained retina. (e) Image showing aligned cell polarization. Figure 1—figure supplement 1 reproduced under CC BY 4.0 DEED license.<sup>9</sup>

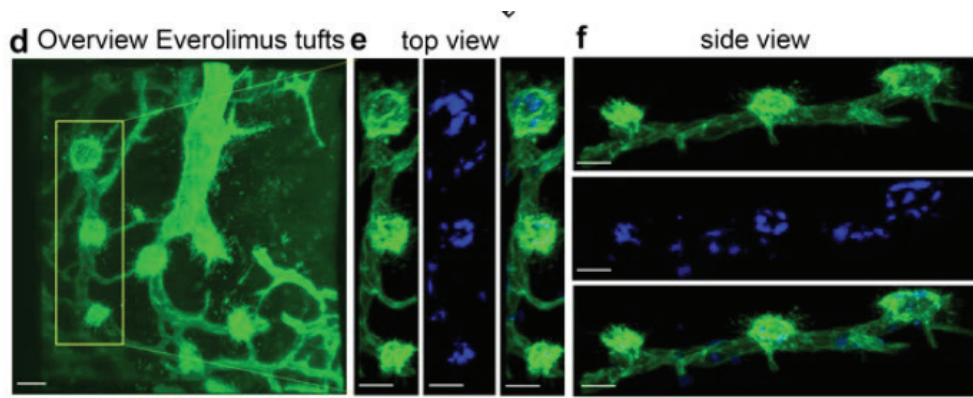


FIGURE 5.

Light-sheet fluorescence microscopy shows 3D tuft morphology. mTOR inhibition with Everolimus leads to highly active filopodia and cup morphology in vascular tufts. Figure 6—figure supplement 3 reproduced under CC BY 4.0 DEED license.<sup>9</sup>

### Imaging Beyond the Brain—The Spinal Cord

The spinal cord is central in transmitting signals between the brain and the rest of the body, influencing essential functions such as movement, sensation, and organ control. Advances in spinal cord research hold the promise of unlocking new treatments and therapies for spinal cord injuries, paralysis, and neurological disorders.<sup>10</sup>

### Imaging the Peripheral Nervous System

By studying the peripheral nervous system (PNS), scientists gain unprecedented access to the intricate details of peripheral nerves. This not only aids in mapping the structural intricacies of nerve fibers but also allows for the visualization of dynamic processes crucial for understanding neural function. Biomedical imaging is an essential tool for elucidating the connectivity between the PNS and the brain, enabling the tracing of neural pathways and investigating how information is transmitted and processed. Moreover, studying the PNS is pivotal in diagnosing and monitoring neurological disorders that may manifest in both peripheral nerves and the brain.<sup>11</sup> This integrated approach offers insights instrumental in advancing medical diagnostics, treatment strategies, and overall neurological research.

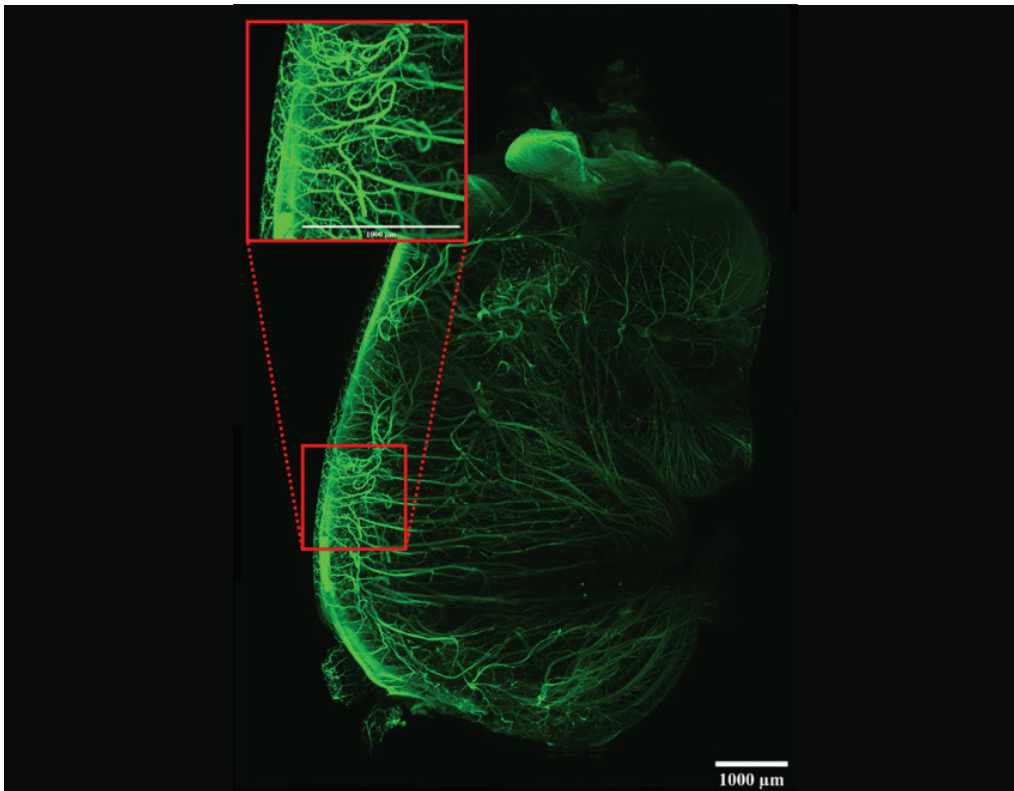


FIGURE 6.

Developing nerves in a whole mouse embryo. The sample was cleared with DBE. Tiled image (3 × 4) acquisition. Scalebars: 1mm. Imaged on the LCS SPIM. Sample courtesy of James Muller, MSKCC, New York, USA.

## Cell Culture and Organoids

3D cell cultures are fragile systems that require optimized experimental protocols and low phototoxicity during imaging to preserve samples. Light-sheet imaging is a unique solution for these challenges by enabling researchers to study 3D cell culture systems, including time-lapse imaging.

### Application Example—Astrocyte incorporation into neuronal organoids

A group studied how to establish human cell-based neural organoids and showed that the addition of astrocytes created a microphysiological system.<sup>12</sup> Organoids were imaged with the MuVi SPIM microscope.

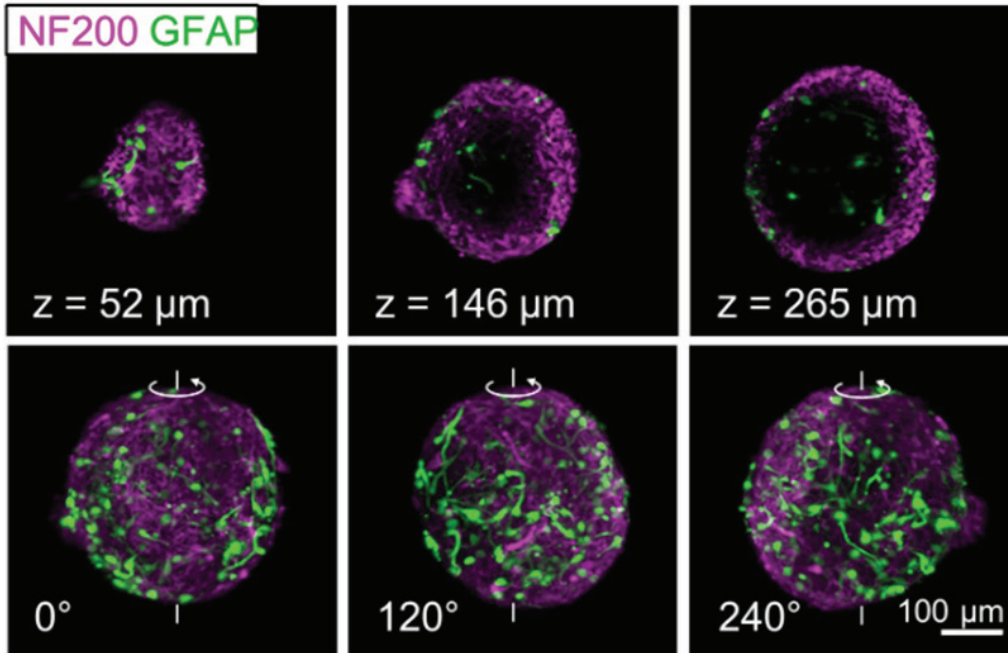


FIGURE 7.

Co-cultured organoids with neuronal cells (magenta) and astrocytes (green). The top row shows slices, and the bottom row shows 3D reconstructions and stills of rotations of organoids. Figure 4—altered from red-green to magenta-green to be colorblind-friendly. Adapted and reproduced under Creative Commons Attribution 4.0 International License (shared with author permission).<sup>12</sup>

## Conclusion

The brain's role as the body's central control hub makes understanding its health paramount. Despite the challenges posed by its complexity, innovative techniques and approaches are continually advancing our understanding of brain structure and function, paving the way for breakthroughs in brain development and neurobiology research. This knowledge is crucial for improving the diagnosis and treatment of neurological disorders, ultimately enhancing the overall well-being of individuals.

# The Best Light-Sheet Microscopes for Different Neuroscience Applications

Use Case	Scientific Requirements	Bruker Microscope
<b>Large Cleared Samples</b>	<ul style="list-style-type: none"><li>▪ Brain mapping or connectivity analysis</li><li>▪ Whole-body nerve assessment</li><li>▪ Whole organ studies</li></ul>	<b>LCS SPIM</b> Large, cleared sample light-sheet fluorescence microscope
<b>Fragile Samples</b>	<ul style="list-style-type: none"><li>▪ Organoid differentiation</li><li>▪ Organ-explant studies</li><li>▪ Fast imaging of developmental processes</li><li>▪ optional photomanipulation module</li></ul>	<b>TruLive 3D Imager</b> Dual-sided illumination light-sheet fluorescence microscope
<b>Live, Fixed, or Cleared Samples</b>	<ul style="list-style-type: none"><li>▪ Live, long-term or cleared sample imaging</li><li>▪ Multi-view imaging without rotation</li><li>▪ Optional photomanipulation module</li></ul>	<b>MuVi SPIM</b> Multiview imaging for live and cleared samples
<b>Specialized High-Resolution Imaging</b>	<ul style="list-style-type: none"><li>▪ High-resolution imaging of live or fixed samples</li><li>▪ Variable beam patterns</li><li>▪ Allows for specialized applications, such as SIM, FLIM, or FCS</li><li>▪ Optional photomanipulation module</li></ul>	<b>InVi SPIM Lattice Pro</b> Inverted view light-sheet fluorescence microscope with advanced illumination

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