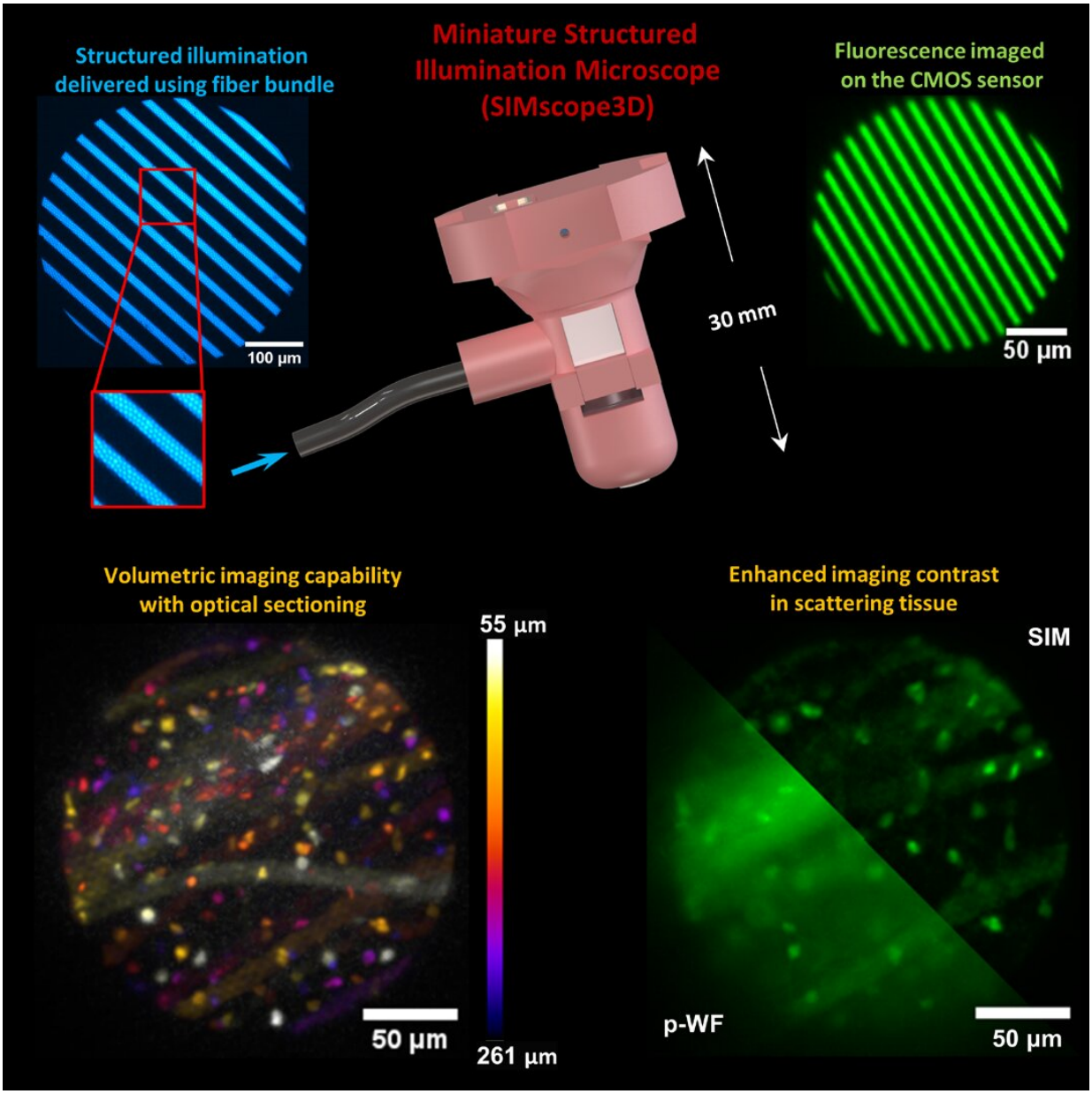
# Head-mounted microscope reaches deeper into mouse brains

March 29 2022



Researchers developed a head-mounted microscope that uses structured illumination to remove out-of-focus light with optical sectioning. This enables deep imaging while also enhancing image contrast in scattering tissue. Credit: Omkar D. Supekar, University of Colorado Boulder; Emily Gibson, University of Colorado Anschutz Medical Campus

Researchers have developed a miniature microscope that is designed for high-resolution 3D images inside the brains of living mice. By imaging deeper into the brain than previously possible with miniature widefield microscopes, the new lightweight microscope could help scientists better understand how brain cells and circuits operate.

"With further development, our microscope will be able to image neural activity over time while an animal is in a naturalistic environment or performing different tasks," said lead author Omkar Supekar from the University of Colorado Boulder. "We show that it can be used to study cells that play an important role in neurological disorders such as multiple sclerosis."

In the journal *Biomedical Optics Express*, the researchers describe their new SIMscope3D, which images fluorescence emitted from tissue or fluorescent tags after the sample is exposed to certain wavelengths of light. The new device is the first miniature microscope to use structured illumination to remove out-of-focus and scattered light, which allowed imaging as deep as 260 microns on fixed brain tissue with an LED light source.

"Developing new treatments for neurological disorders requires understanding the brain at the cellular and circuit-level," said research team lead Emily Gibson from the University of Colorado Anschutz Medical Campus. "New optical imaging tools—particularly those that

can image deep into brain tissue like the microscope our team developed—are important for achieving this goal."

## Seeing deeper

Head mounted microscopes are used to image the brains of small rodents through transparent windows implanted into their skulls. Researchers have previously developed head-mounted widefield fluorescence microscopes, but light scattered by tissue prevents imaging deep into the brain. Miniature two-photon microscopes can overcome this drawback by eliminating out-of-focus light in each focal plane—a process known as optical sectioning—but typically require expensive pulsed lasers and complex mechanical scanning components.

To design the new microscope, Andrew Sias, Sean Hansen, Gabriel Martinez and Emily Gibson from the Department of Bioengineering at the University of Colorado Anschutz Medical Campus; Douglas Shepherd from the Department of Physics at Arizona State University; Omkar Supekar and Juliet Gopinath from the Department of Electrical, Computer and Energy Engineering, and Victor Bright from the Department of Mechanical Engineering at the University of Colorado Boulder collaborated closely with neuroscientists Graham Peet, Diego Restrepo and Ethan Hughes from the Department of Cell and Developmental Biology and Xiaoyu Peng and Cristin Welle from the Department of Physiology and Biophysics at the University of Colorado Anschutz Medical Campus to optimize it for studying the brain.

Volumetric imaging is accomplished by using an imaging fiber to deliver spatially patterned light to the miniature microscope objective. This process also removes out-of-focus light, enabling optical sectioning similar to that accomplished with two-photon approaches but without the complex components or expensive laser.

The microscope includes a compact tunable electrowetting lens that allows 3D visualization of brain structures by changing the microscope's focal depth without requiring any moving parts. The researchers also integrated a CMOS camera directly into the microscope. This enables imaging with high lateral resolution while avoiding artifacts that might be induced if the images traveled through the fiber bundle. Using an LED light source, the new microscope can produce sharp contrast even when imaging deeply into highly scattering tissue.

## Capturing glial cells

The researchers demonstrated their new system by imaging oligodendrocytes and microglia labeled with a fluorescent protein in mice that were awake but placed in a device that kept their head stationary. In people with multiple sclerosis, oligodendrocytes—which form an insulating layer around axons—are destroyed. This causes the connections in the brain to slow down, leading to impairment of vision, motor skills and other problems.

"We used our miniature microscope to record a time series of glial cell dynamics in awake mice at depths up to 120 microns in the brain," said Supekar. "Scientists don't fully understand exactly how these cells work or their repair processes. Our microscope opens the possibility of long-term studies examining how these cells migrate and are repaired."

The researchers are now working to improve the microscope's acquisition speed and weight. With minor upgrades, the microscope will be able to image faster dynamics, such as neuronal electrical activity, while the mouse performs different tasks. The researchers say that because the microscope does not require expensive components it could be easily developed into a commercial system for use in neuroscience labs.

**More information:** Omkar D. Supekar et al, Miniature structured illumination microscope for in vivo 3D imaging of brain structures with optical sectioning, *Biomedical Optics Express* (2022). DOI: 10.1364/BOE.449533