Shedding Light On Deep Tissue: Multiphoton Microscopy

Multiphoton microscopy and other approaches for more precise imaging of tissue samples—such as lightsheet imaging and tissue-clearing methods—are growing in popularity as they turn more mainstream. As more scientists use these deep-imaging methods, new applications are continually arising, such as watching some of the cellular processes that control brain development, testing bones for lead levels, and studying a wide range of disease processes. **By Mike May**

Throughout the history of biological imaging, scientists and engineers have worked together to see more. Sometimes their advances enabled us to see things with higher resolution; other times they helped us to see deeper into a sample. Yet biologists want to do both things at once, which has led to new methods such as multiphoton microscopy.

In confocal microscopy, a single photon excites a fluorescent label in a sample. In two-photon microscopy—the most common form of multiphoton microscopy—two photons are absorbed by the label at virtually the same instant. Multiphoton microscopy also uses longer-wavelength photons, which are lower energy and penetrate more deeply, creating less tissue damage while imaging farther into a sample.

The ability to resolve structures deeper in biological samples keeps evolving with improvements in technology and modified or new methods—while still requiring considerable expertise in preparation, operation, and analysis. In many ways, these two key elements of scientific progress—technology and techniques—are moving in tandem to help imaging reveal more of the biological world.

Putting the tech together

To expand the use of multiphoton imaging, scientists need more and better commercial platforms to consider. One of them is the A1R HD multiphoton confocal microscope from **Nikon** in Melville, New York. A key feature of this microscope is speed—capturing data from the entire field-of-view at 30 frames per second. "With the A1R HD, scientists can capture great quality images at a high rate," says Adam White, a biosystems product manager for Nikon.

The LSM 880 with Airyscan from **ZEISS,** headquartered in Oberkochen, Germany, also provides multiphoton imaging. "Adding the Airyscan detector improves the signal-to-noise ratio by four to eight times," says Joseph Huff, solution manager and application development engineer at ZEISS. "Using this setup on mouse brain, we can acquire data at depths up to 500 microns."

However, there are drawbacks that come with deeper focus. "As you go deeper in tissue, the amount of spherical aberration increases," says Carlo Alonzo, product manager at **Olympus** in Waltham, Massachusetts. "This degrades the focus and results not just in poorer resolution, but also dimmer images because of less efficient multiphoton excitation."

For that reason, Olympus developed TruResolution Objectives. "A motorized optical correction collar dynamically ad- cont.>

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