Researchers demonstrate hyperspectral imaging for multiphoton microscopy

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Traditionally, the study of diseases at the molecular level has required scientists to extract cells and tissues from animal models and then look for clues in the samples that can determine the mechanisms underlying the disease and driving its progression. According to Chris B. Schaffer, associate professor of biomedical engineering at Cornell University, New York, USA, that is "like a person guessing who is winning a battle based on a single photograph from the warzone."

A better way, Schaffer said, is a method known as multiphoton imaging, by which individual cells can be tracked in living tissue using fluorescent labels and their three-dimensional structures visualized in detail while functioning in a natural environment. However, current multiphoton imaging systems can "become confused" when two or more fluorophores (the fluorescent labels) are used together to examine the behavior and interactions of different cell types. Called hyperspectral multiphoton microscopy, a new innovation by Schaffer's lab significantly extends the current capability of this imaging technology by allowing users to see and distinguish more colors of fluorophores at the same time.

This new solution to the problem will be presented by the research team at the Frontiers in Optics (FiO) / Laser Science (LS) conference in Rochester, New York, USA on 17-21 October 2016.

To use traditional multiphoton microscopy, cells of interest are tagged with a fluorescent dye or genetically encoded fluorescent markers such as green fluorescent protein (GFP) and then hit with a high-power, short-

pulse infrared wavelength laser. These fluorophores are excited—and define the cells they tag—only at the laser focus, where the laser energy is concentrated to create a high intensity.

"By scanning the laser focus throughout the sample, a 3-D reconstruction of all labeled cells and tissues is generated," Schaffer said. "These images can be collected rapidly, giving researchers the ability to track single cells in a living system, which is more relevant to understanding human disease than tracking cells in a Petri dish."

The problem with many fluorophores, Bares said, is that they aren't one distinct color.

"A red fluorophore, for example, will emit light across a range of wavelengths, say from 600 to 650 nanometers, so that there's overlap with a red-orange fluorophore emitting light from 580 to 620 nanometers," she explained. "The photodetector used to locate and visualize the target cells has no way to tell which one is the source for a 600-nanometer signal, and that's bad, because the red and red-orange fluorophores may mark cells with very different biological functions."

Bares said that in the Cornell hyperspectral technique, the entire visible light spectrum is used to better characterize a sample.

"It's similar to a simple multicolor imaging system, the standard red-green-blue, or RGB, signal used in television where an image has three color channels and each pixel gets a value in each of the three channels," she said. "In hyperspectral imaging, we collect the same image over 48 color channels, yielding multiple values for each pixel—and that provides a wealth of data for distinguishing very precisely between fluorophores, and in turn, cells."

To enhance its technique even further, the Cornell team used different

excitation laser colors. "For example, we eliminate the red versus red-orange overlap problem completely if we collect images first using a laser that only excites red fluorophores and then repeat the process with another laser that only stimulates red-orange labels," Bares said.

Schaffer, Bares and their colleagues have demonstrated the capabilities of hyperspectral imaging with a 48-channel multiphoton microscope in a number of samples, including identifying 10 different colors of fluorescent beads in agarose, tracking proteins of interest in living cell cultures by fusing them with fluorescent proteins, and distinguishing between five cell types in the dense cortical tissues of a live mouse brain. With more refinement—such as improving the image analysis algorithms, increasing imaging speed and providing real-time image processing—the researchers believe that their technique has a very promising future.

"Having access to a microscope that allows researchers to see and distinguish all of the cells in a tissue volume, as well as determine which interactions between these cells cause disease symptoms, could speed the development and deployment of new therapies," Schaffer said.

More information: "Hyperspectral Imaging in Live Mouse Cortex Using a 48-Channel Multiphoton Microscope," by A.J. Bares, M.A. Pender, M.A. Mejooli, S. Tilley, K.E. Chen, J. Dong, P.C. Doerschuk and C.B. Schaffer, will take place from 15:45-16:00, Thursday, 20 October 2016, in the Grand Ballroom D, Radisson Hotel Rochester Riverside, Rochester, New York, USA.

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