Photometrics Customer Profile

Ratiometric Imaging of Fluorescence and Absorption Signals

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>
> **– Daryl W. Hochman,** Ph.D., Assistant Professor in the Departments of Surgery (Division of Surgical Sciences) and Pharmacology and Cancer Biology at Duke University

BACKGROUND

Epilepsy that is untreatable by medication can be relieved by surgically removing excitable brain tissue. However, seizures originating in the cerebral cortex cannot be localized using non-invasive imaging techniques such as fMRI or PET. To localize the site of seizure onset, electrodes are surgically placed atop the cortex to monitor and triangulate epileptic seizure activity. The procedure poses significant discomfort and infection risk to the patient, as the boreholes through which electrodes are placed remain open for the seven-day monitoring period.

Daryl Hochman, Ph.D., and his colleagues at Duke University are developing a novel, nanoparticle-based imaging technique that has potential to quickly localize excitable cortical tissue and eliminate the electrode-based procedure.

The nanoparticles, developed in the lab of Cassandra L. Fraser, Ph.D. at the University of Virginia, exhibit oxygen-independent fluorescence at 450 nm and oxygen-dependent phosphorescence at 550 nm under UV illumination. Dual wavelength imaging and ratiometric calculations can reveal dynamic oxygen concentrations in the pial arterioles across the cortical surface and provide an indication of local neural activity.

CHALLENGE

"Typically, cameras I've looked at that are good for fluorescence are not that good for absorption. They don't have the needed combination of dynamic range and sensitivity," explained Hochman. The project required a wide dynamic range to detect small oxygen-dependent changes in light absorption against the strong intrinsic signal at 550 nm, as well as exquisite sensitivity to capture dim fluorescent signal at 450 nm.

"We also needed a camera that had the potential to do ultrafast imaging to capture bursts of neural activity," Hochman said. "We didn't know the time course of the changes. Will they be the duration of an action potential, or much slower because of the diffusion of oxygen?"

Finally, to definitively quantify the nanoparticles' response to specific stimuli as well as compensate for fluctuations in dye loading and interference from intrinsic optical signal, Hochman had to economically image two wavelengths simultaneously. "I needed to acquire both oxygen-sensitive and oxygen-insensitive fluorescence signals at the same time. You can't just use filter wheels. The alternative, two cameras and two computers, was double the cost. I'd prefer to use my resources another way."

OVERVIEW

Using ratiometric imaging of fluorescence and absorption signals, Daryl Hochman, Ph.D. is developing a novel method to measure cortical activity based on changes in oxygen concentration.

FACILITY

Duke University

IMAGING EQUIPMENT

Photometrics Evolve™ camera for both absorption and low-light fluorescence

Photometrics DV2™ emission splitter for simultaneous imaging of two wavelengths, polarizations, or amplitudes

KEY FEATURES

- *Wide dynamic range and high sensitivity for both absorption and low-light fluorescence imaging in one camera (dual amplifiers and 16-bit digitization)*
- *Clear, ultrafast image capture (highest quantum efficiency and lowest read noise of any EMCCD camera. 10MHz readout speed)*

SOLUTION

Hochman placed the Evolve EMCCD camera directly into his surgical macroscope and began imaging his primate models. The camera's dual amplifiers met his demands for high sensitivity and wide dynamic range.

"The Evolve can be used in traditional CCD mode, where it has dynamic range, and EM mode, where it is still very sensitive for fluorescence. Without the Evolve, we would need two expensive cameras that we would have to swap on and off the microscope. It wouldn't be a feasible thing to do," Hochman explained.

"We are really taking advantage of the camera's high sensitivity," he continued. The Evolve's deep-cooled engineering, providing the highest quantum efficiency (>90% peak QE) and the lowest read noise of any EMCCD camera, enabled Hochman to capture clear, usable images at ultrafast frame rates and thus resolve the nanoparticles' behavior over the time course of bursts of neural activity.

As for economically capturing two wavelengths simultaneously, Hochman placed Photometrics' DV2 two channel simultaneous imaging system between the Evolve and the macroscope. "I put the DV2 on and the optics project two wavelengths of the same field of view, one projected on each half of the camera. A quick run of 1,000 two-channel images then acquires quantitative changes in brain oxygenation."

Hochman also relies on Photometrics' expert technical support as another essential feature for experimental success. "We neuroscientists aren't necessarily optics or CCD imaging experts. I know I can rely upon the help from Photometrics to solve anything that comes up. Time is everything when doing these experiments. You don't want to be delayed by three weeks because you can't figure out how to get something to align or don't have the right filter or lens."

RESULTS

"We are able to use the Evolve camera for all our imaging requirements. We can flip to one wavelength, remove the emission filters and measure the light absorption. We can flip to another wavelength and image the fluorescence with high sensitivity. In that way, the camera is uniquely suited for performing both types of imaging at the same time," Hochman said.

The DV2 also enabled Hochman to economically quantify changes. "Without the DV2, we would have to do multiple runs under each wavelength and average them. We'd have to hope that the behavior of the neurons was similar. It's a matter of accuracy. I have a reliable ratio with those images, which have the absolute same field of view and extent of time," he continued. "There's also the time involved. It's a primate study, so it's expensive. The DV2 allows us get twice as much data in the same time span."

Hochman's lab has spent several weeks labeling primate brains with nanoparticles and measuring responses to electrical stimulation for epileptic activity. Hochman's team is on course for developing the nanoparticle model for measuring neocortical activity: "We've obtained clear results. Fluorescence increases as oxygen concentration decreases. The converse is also true."

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LOOKING FORWARD

With a better understanding of how the nanoparticles reflect cortical activity, Hochman plans to develop the bio-degradable, nontoxic nanoparticles for use as a clinical diagnostic capable of quickly localizing inter-epileptic activity.

Hochman considers the Evolve's Quant-View™ mode, which reports imaging data in standardized, reproducible units, key to that process: "We're making quantitative measurements of blood oxygenation, based on ratios of two signals. Expressing those in physically meaningful units raises questions that wouldn't be obvious otherwise. With the Evolve, we've gained the ability to collect quantitative data to better analyze accuracy as we consider clinical applications. In the end, it might not be essential in the clinic. However, I think it will be essential in developing the application."

COLLABORATING RESEARCHERS

Hochman collaborates with several researchers. **Cassandra L. Fraser, Ph.D.**, Professor of Chemistry at the University of Virginia and graduate student **Guoqing Zhang** developed the oxygen-sensing nanoparticles. **Michael M. Haglund, M.D., Ph.D.**, Professor of Neurosurgery and Neurobiology at Duke University surgically prepares the primate models for imaging. **Greg Palmer, Ph.D.**, Assistant Professor in Radiation Oncology and Cancer Biology at Duke University pioneered the use of these oxygen-sensitive nanoparticles for studying rodent tumor models.

www.photometrics.com
1 520.889.9933 sales@photometrics.com
3440 E. Britannia Dr. | Tucson, AZ 85706