Working with a new instrument (rev. 2010)

Thanks to grants from the National Center for Research Resources (NIH) and Rush University’s Hasterlik Philanthropic Fund, our department acquired a novel instrument. This is a two-photon LSM 510-Meta/LIVE combination (or LSM 5-DUO), built by Carl Zeiss Microimaging.

Confocal systems include a microscope, a group of lasers, and a scanner, which guides the excitation light into the microscope and the emitted light into one or more photosensors. In the DUO two scanners, each with a set of lasers, converge into one microscope. The main purpose of the 2nd scanner (operator) is to provide additional irradiation of the sample, while the first scanner independently images fluorescence. The operator permits a number of operations on the sample, all related to *photoconversion*. In our case we will use it to photorelease Ca^{2+}, protons, or IP_3, at precisely defined locations. A second advantage is the possibility of using 2-photon irradiation (from a so-called DeepSee infrared laser, built by SpectraPhysics) to provide highly penetrating energy that will produce photoconversion in microscopic areas without unduly irradiating the rest of the sample. Finally, the first scanner is a “LIVE”, a confocal imager that, using a different principle, acquires images at a much greater rate.

We are now applying these features for the purposes of monitoring (i) calcium inside cytosol, mitochondria and storage organelles of skeletal and cardiac muscle and brain vascular endothelia, (ii) proton concentration and evolution in phagosomes and submembrane domains, (iii) membrane curvature and lipid rafts on living cells and cell fractions by FRET between raft-preferred or raft-excluded molecules, (iv) unidirectional flux through individual channels in bilayers and (v) cAMP in endothelial cells. This monitoring is simultaneous with operator-induced photorelease as described above. An update on current work can be found on the next page. This instrument is available to other labs at Rush University, as well as qualified extramural users, on a fee-for-service basis. Inquire with Dr. John Tang; jtang@rush.edu.

Current work with photorelease of calcium and simultaneous monitoring of cellular responses is best summarized in abstracts of work to be presented at the annual meeting of the Biophysical Society, in San Francisco (February of 2010).
Flux in artificial Ca sparks generated by 2-photon release from a novel cage confocally imaged at microsecond resolution.

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Control of calcium signaling in striated muscle relies on concurrent actions of calcium ions to promote and inhibit release channel opening. To understand these actions we developed artificial Ca sparks generated by 2-photon (2P) release from NDBF-EGTA (Momotake, Nature Methods 2006) as quantifiable local stimuli. A “Dual Scanner” (Zeiss) delivers IR laser flashes through a LSM 510 scanner, while rapidly imaging fluorescence of a [Ca$^{2+}$] monitor via a slit scanner (5-LIVE; ca 100 μs/line). Ca sparks of 0.1 to 10 μM (A, B) are elicited in a droplet after microseconds of 2P irradiation at 720 nm and imaged with the low affinity dye fluo 4FF. Reaction-diffusion analysis (Ríos, JGP 1999) yields the flux of Ca photorelease (C). This flux, which initially reaches several hundred mM/s, decays with τ of 2-3 ms. The technique is used to measure physico-chemical properties of calcium ligands, including bio-sensors. Applied inside muscle fibers (Figueroa, this meeting) it serves to quantitatively characterize calcium control in cells.

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Local calcium stimuli (artificial sparks) generated by 2-photon breakdown of the cage NDBF-EGTA were applied to evoke Ca release from the SR in single skeletal or cardiac muscle cells undergoing fast Ca imaging with the low affinity dye fluo 4FF. The figure shows selected sequential images of the Ca transient generated by a frog skeletal muscle fiber with permeabilized plasmalemma, in response to a spark (elicited outside the fiber to avoid photodamage). Two types of responses were observed: (i) an all-or-none wave –shown– that propagates over the entire cell and (ii) graded responses, which fail to propagate. Release analysis (Rios, JGP 1999; Figueroa, this meeting) separates SR release from simple diffusion of photo-released Ca into cells. The technique yields a sensitive measure of threshold \([\text{Ca}^{2+}]\) for release activation, which in the example (0.3 mM \([\text{Mg}^{2+}]_{\text{cyt}}\)) was 1 \(\mu\)M, and can monitor inactivation by combining multiple stimuli. Modeling of these responses aims at describing quantitatively the properties of activation, as well as the roles of inactivation and depletion in the control of Ca release. Other details and acknowledgments are presented elsewhere (Figueroa, this meeting.)