X-Cite®
Fluorescence Illumination • In Control

Fura-2 Imaging with the X-Cite NOVEM™

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FURA-2 IMAGING

Intracellular calcium signals drive many cellular processes including muscle contraction, cell proliferation, synaptic transmission, and gene transcription. Detecting changes in intracellular calcium is of interest to researchers because it provides insight to these dynamic processes in living cells.

Techniques for detecting intracellular calcium signals using Fura-2 imaging are well established. The excitation maxima of Fura-2 shifts from 380nm to 340nm upon binding of calcium. This shift can be used to quantify intracellular calcium fluxes.

Xenon lamps have been the gold standard for Fura-2 imaging because they provide a uniform intensity of light over a wide range of wavelengths. These arc lamps require frequent replacement, making them a nuisance, and expensive to maintain. In addition, since arc lamps are broad spectrum, they must be used in combination with filter wheels to isolate and constantly flip between the 340nm and 380nm excitation wavelengths, putting a mechanical limit on imaging speed. Hence, there has been a general move toward dyes like Fluo-4, which are single excitation. Single wavelength dyes can detect changes in calcium but cannot measure the calcium concentrations in the cytoplasm.

Fura-2 imaging requires alternately exciting the fluorophore at 340nm and 380nm. Image pairs are recorded and the signal excited by 340nm is divided by the signal excited by 380nm to produce a ratiometric image. A change in the ratio over time corresponds to a change in intracellular calcium. With the development of LEDs at lower wavelengths, it is now possible to use solid-state technology for this application. In an LED system, each wavelength can be controlled separately, allowing the user to simply adjust the 340nm vs. 380nm LEDs to capture the images at the two wavelengths at similar excitation intensities.

This alleviates the need for using cumbersome Xenon lamp technology for this application, which requires the user to align and replace bulbs every few hundred hours. LED technology can be used reliably for tens of thousands of hours without replacement.

Having a powerful, fast switching light source providing filtered LED excitation negates the need for reliance on a filter wheel thereby speeding up data acquisition.

Here we demonstrate how the X-Cite NOVEM allows researchers to exploit the benefits of Fura-2 imaging (quantitation and ability to correct for bleaching) without the need for arc lamps and providing the speed and economy of use of single wavelength probes.

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CASE STUDY 1

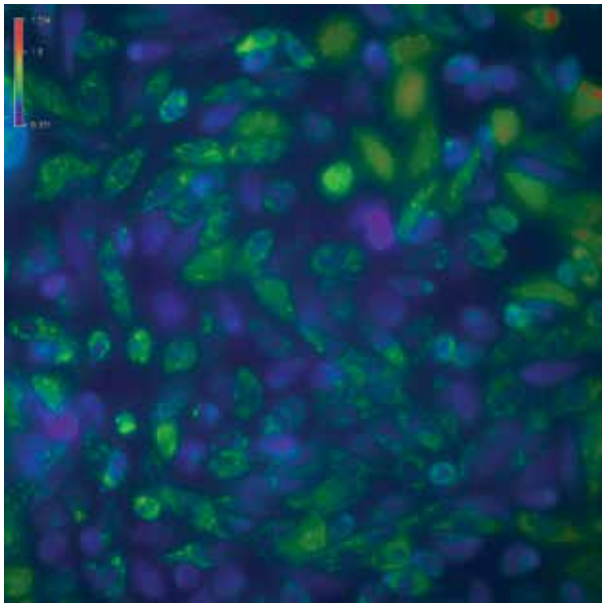
Fura-2 calcium imaging of HeLa cells using Ionomycin.

Callen T. Wallace, Senior Research Specialist Center for Biologic Imaging, University of Pittsburgh.

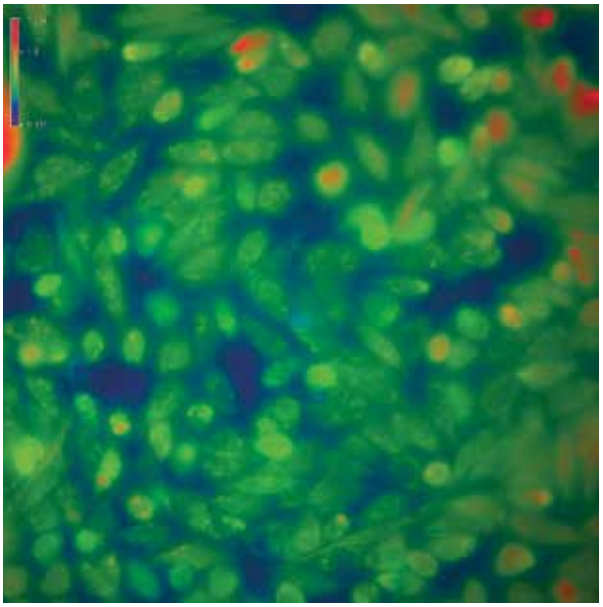
Images were acquired on a Nikon Ti Inverted Microscope with a 40x 1.30 N.A. objective and a Photometrics Prime BSI sCMOS camera using the X-Cite NOVEM™ light source. HeLa cells were loaded with Fura-2 AM at 5µM concentration for 30 minutes before imaging. Time- lapse imaging was performed, triggering between 340nm and 385nm excitation at 100ms intervals with a continuous acquisition to capture baseline fluorescence in each channel and the ratiometric change due to agonist. To induce calcium flux, 1mM of Ionomycin was added to 2mL of media in real-time during imaging.

Movie and figure legend: Images were acquired using triggered acquisition with the light source at 100ms exposures for each channel. The ratiometric shift occurs instantaneously on the addition of Ionomycin. Left: Pre-stimulation, Right: Post stimulation

Pre Stimulation



Post Stimulation



To view the video depicting the ratiometric switch on addition on Ionomycin, ***CLICK HERE.***

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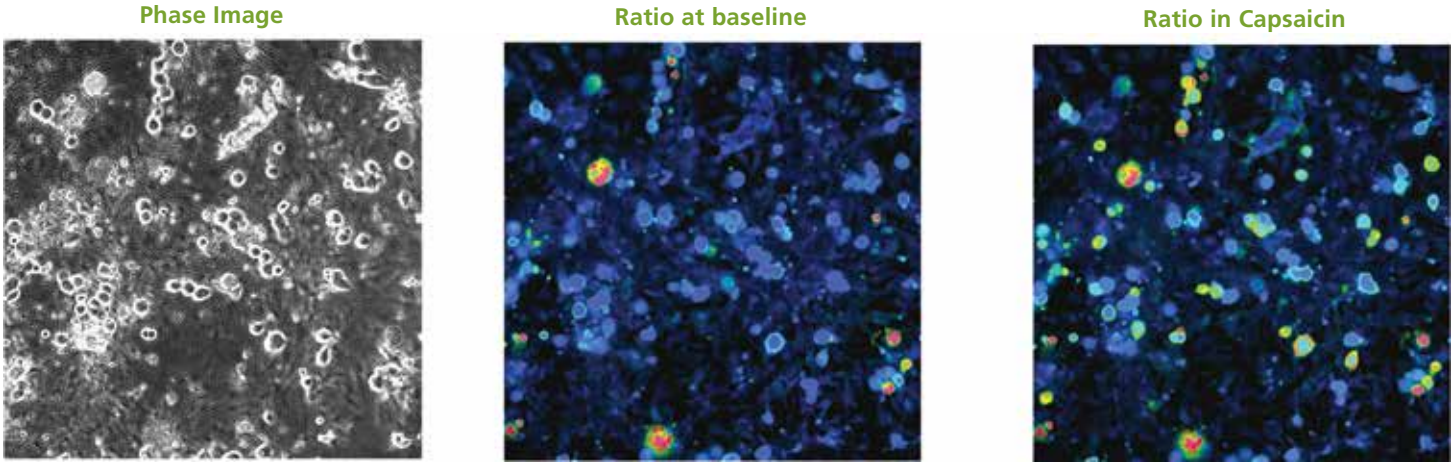
CASE STUDY 2

Capsaicin elicits calcium responses in rat DRG sensory neurons.

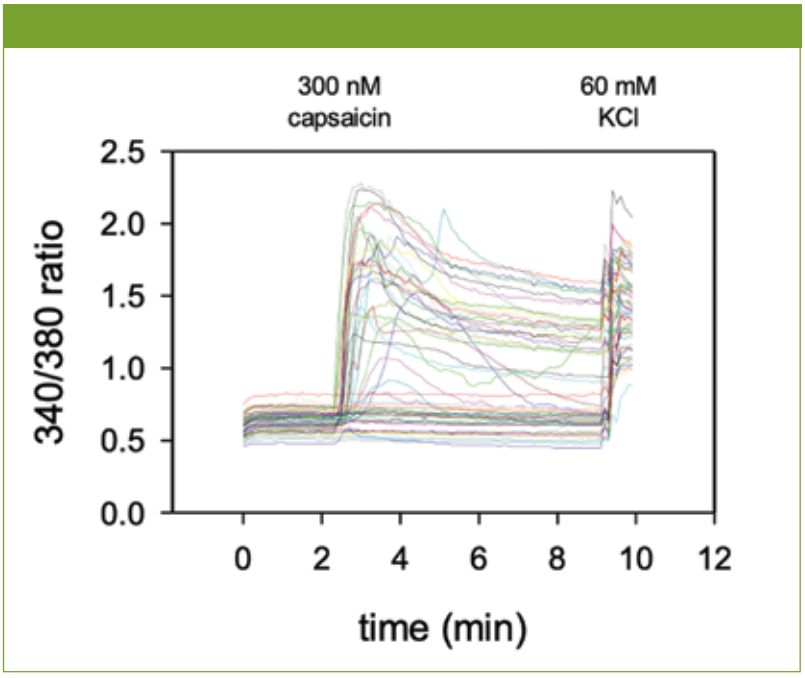
Neuroservice USA*. Dong Liu, Matt Reissman, Robert Petroski

Sensory neuron cultures were prepared from adult rat dorsal root ganglia and plated on a monolayer of astrocytes growing on PDL-coated glass imaging dishes (MatTek). Calcium imaging was performed on Fura-2 AM loaded cells at nine days in vitro.

Images were acquired with an Olympus IX73 inverted microscope with the X-Cite NOVEM and Hamamatsu ORCA Fusion BT camera. MetaFluor imaging software was used for data acquisition and analysis. Adult rat DRG neuron cultured cells were loaded with 3 uM Fura-2 AM in 0.025% Pluronic F-127. Capsaicin was used to evoke calcium signals by activating native TrpV1 receptors.



Phase-contrast (left) and ratiometric images of Fura-2 loaded DRG sensory neurons. 300 nM capsaicin (TRPV1 agonist) elicited calcium responses in ~50% of sensory neurons.



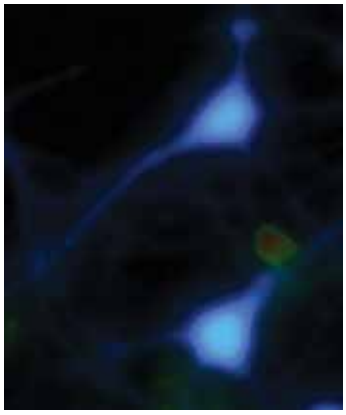
Time course of calcium responses in individual Fura-2AM loaded DRG sensory neurons indicated by colored lines. Following a 2 min baseline period, perfusion for 1 min with 300 nM capsaicin elicited calcium responses in ~50% of neurons. All cells responded to depolarization by 60 mM KCl indicating healthy, electrically excitable neurons.

CASE STUDY 3

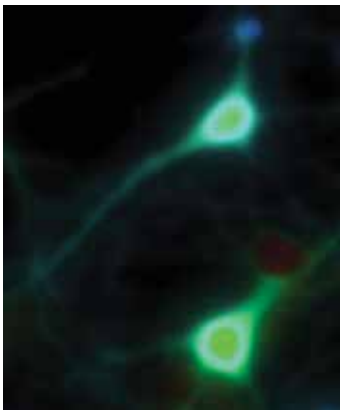
Glutamate elicits calcium fluxes in rat pyramidal neurons.

Neuroservice USA. Dong Liu, Matt Reissman, Robert Petroski

Neuron cultures were prepared from E18 rat cortex and plated on a monolayer of astrocytes growing on PDL-coated glass imaging dishes (MatTek). Calcium imaging was performed in Fura-2 AM loaded cells at 9 days in vitro.



Baseline calcium

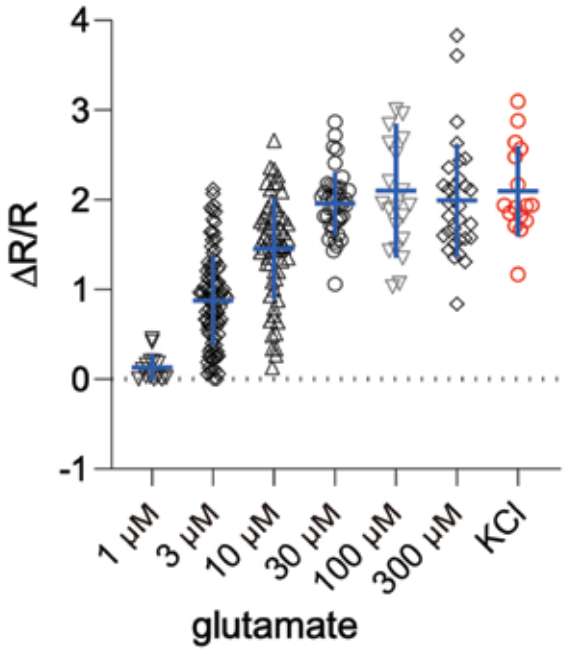


100µM Glutamate

Low resting baseline calcium level (blue) in rat cortical pyramidal neurons and the response to 100µM glutamate that triggers a rapid calcium flux (green).

*CASE STUDY 2 & 3 were completed in part by Neuroservices USA.

To view the video of the calcium response, please ***CLICK HERE.***



Calcium responses to glutamate in E18 pyramidal neurons were concentration dependent. Depolarization by 60 mM KCl also elicited a comparable calcium signal.

RESULTS AND DISCUSSION

These studies present an effective LED alternative to traditional Xenon technology used for ratiometric calcium imaging using Fura-2. Fura-2 is the gold standard for calcium ratiometric imaging but has been limited to expensive, outdated, and bulky Xenon technology.

The X-Cite NOVEM provides an LED option with higher power than Xenon lamps and fast switching between 340 and 380nm allowing for rapid acquisition of cellular calcium responses. Each NOVEM-Fura system also offers nine individual channels to allow imaging of Fura-2 along with most other popular fluorophores used in research and imaging.

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