Imaging is NOT just a black and white issue.

Application Notes: Ratiometric Calcium Imaging

INDO-1 IMAGING

Indo-1 is a UV-excitable ratiometric indicator that is generally used to study the role of calcium in cellular regulation. Ratiometric indicators are very popular in the microscopy community because they have significant advantages over single emission probes where changes in ion concentration cause changes in emission intensity only at a single wavelength. Measurements of ion concentrations, such as calcium, using fluorescence microscopy are sensitive to the effects of uneven dye loading, photobleaching, leakage of dye, and unequal cell thickness. By using ratioing techniques, the measurements will be considerably less sensitive to these effects.

With the advent of the ***Dual-View™*** system, the emission ratioing approach has been significantly simplified with the capability to simultaneously acquire two emission images on a single CCD. This simplification has led to a renewed interest in emission ratioing techniques especially since they do away with rotating filter wheels and sequential imaging and all of their associated problems. Since Indo-1 dye exhibits a shift in emission wavelength with changes in ion concentration, it is a perfect candidate for ratio imaging and all the advantages that this technique provides. Despite issues with photobleaching, Indo-1 is regaining its popularity for accurate measurement of intracellular calcium concentrations in applications beyond flow cytometry.

SIMULTANEOUS CALCIUM AND PH?

While Indo-1 is effective at targeting calcium ions, it will also be effective at targeting other ions with similar properties such as hydrogen ions.[1] As a result, the pH of the environment will have an impact on the measurement and needs to be monitored at the same time. Previous research monitored two wavelengths for calcium and two wavelengths for SNARF using a four camera video microscope. Figure 1 shows an image of rat pituitary intermediate lobe melanotropes obtained with this system.[2]

Optical Insights' ***Quad-View™*** simplifies the instrumentation considerably providing the perfect solution for this application. By acquiring four separate spectral images simultaneously on a single CCD, the ***Quad-View™*** can monitor the two wavelengths of Indo-1 for calcium and two wavelengths for a pH indicator such as SNARF-1.

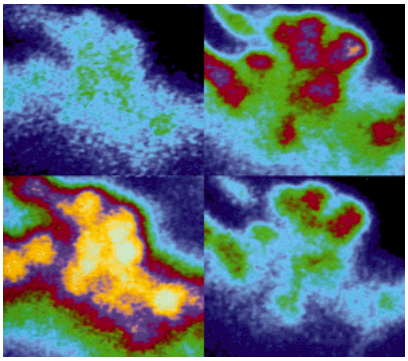

Figure 1. Rat pituitary intermediate lobe melanotropes labeled with indo-1 AM and carboxy SNARF-1, AM, acetate indicators. Pseudocolored fluorescence from indo-1 is shown at 405 and 475 nm (left panels). Pseudocolored fluorescence from carboxy SNARF-1 is shown at 575 and 640 nm (right panels).

FURA-2 / DIC IMAGING

Until recently, Fura-2, which is an excitation ratio indicator, has been the indicator of choice for calcium concentration measurements because excitation ratioing has been more practical than emission ratioing in the past. If Fura-2 is still your dye of choice, the ***Dual-View™*** system comes in handy when you want to monitor cell morphology at the same time you are monitoring calcium concentration. When configured for simultaneous fluorescence/DIC imaging, the Dual-View™ will allow you to visualize the location and concentration of the calcium in the fluorescence channel, and visualize changes in cell morphology in the DIC channel. The fluorescence image can be overlaid on the DIC image and your whole sequence of images can be played in time to provide a multi-dimensional view of the cellular environment!

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[1] Stephen J. Morris, "Simultaneous Multiple Detection of Fluorescent Molecules," *Optical Microscopy*, eds. Brian Herman, John LeMasters (New York: Academic Press, ©1993) pg. 184.

[2] Stephen J. Morris, "Simultaneous Multiple Detection of Fluorescent Molecules," *Optical Microscopy*, eds. Brian Herman, John LeMasters (New York: Academic Press, ©1993) pg. 188.

FLUO-3 / FURA RED IMAGING

Indo-1 and Fura-2 are both fluorescent dyes that require UV excitation. As such, the experimental setup is more involved because of the need for UV optics and objectives. As an alternative, a combination of visible dyes can be used to monitor free versus bound intracellular calcium levels. However, these dyes exhibit changes in intensity instead of shifts in the emission wavelength with changes in intracellular calcium levels. Since the application of a ratio imaging is not possible to compensate for the effects described previously, calibration of the system is dependent on the measurements of maximal and minimal fluorescence after respective additions of a calcium ionophore.[3]

[3] LeMasters, J.J., Qian, T., Trollinger, D.R., Muller-Borer, B.J., Elmore, S.P., Cascio, W.E.., "Laser Scanning Confocal Microscopy Applied to Living Cells and Tissues," *Methods In Cellular Imaging*, ed. Periasamy, A., Oxford University Press, NY, Chapter 5, pg.78, © 2001

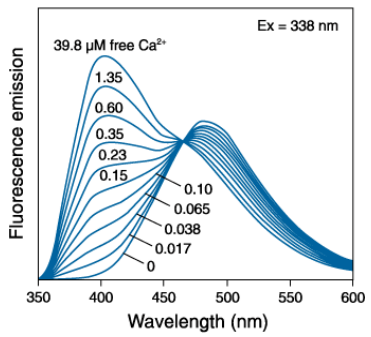
DUAL-VIEW™ CONFIGURATIONS FOR CALCIUM IMAGING

Indo-1 Imaging

Filter Sets	Part Number	
	Optical Insights	Chroma Tech.
Microscope Exciter Filter Set	OI-12-EX	OI-12-EX
Dual –View™ Emission Filter Set	OI-12-EM	OI-12-EM

Comments:
- This filter set is designed to excite Indo-1 at 365 nm and monitor the emission at 405 nm (saturated calcium) and 485 nm (free calcium). The emission filter set uses a 440 nm dichroic to split the two emission images.

Indo-1 Emission Spectra (Molecular Probes)

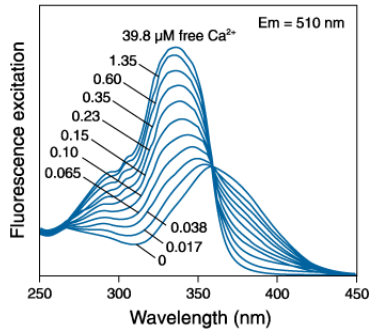


Fura-2/DIC Imaging

Filter Sets	Part Number	
	Optical Insights	Chroma Tech.
Microscope Exciter Filter Set	MS-455LD	MS-455LD
Dual –View™ Emission Filter Set	OI-19-EM	OI-19-EM

Comments:
- The excitation system will need a monochromator or filter wheel to allow for dual excitation of the Fura-2 at 340 nm & 380 nm. The emission filter set uses a 565 nm dichroic to split the fluorescence at 510 nm and visible DIC (brightfield channel) into separate images.

Fura-2 Excitation Spectra (Molecular Probes)

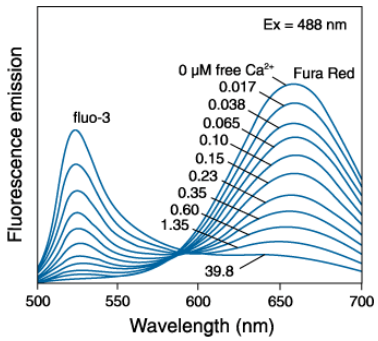


Fluo-3/Fura Red Imaging

Filter Sets	Part Number	
	Optical Insights	Chroma Tech.
Microscope Exciter Filter Set	OI-10-EX	OI-10-EX
Dual –View™ Emission Filter Set	OI-10-EM	OI-10-EM

Comments:
- This filter set is designed to excite Fluo-3 & Fura Red, which are visible dyes used for ratiometric imaging of calcium. The emission filter set records images at 535 nm (sat. Ca2+) & 660 nm (free Ca2+). The emission filter set uses a 565 nm dichroic to split the two images.

Fluo3/Fura Red Emission Spectra (Molecular Probes)



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