

Dynamic Ratio Imaging of Intracellular Calcium

Cytosolic and organellar free calcium concentrations show dramatic spatial and temporal fluctuations associated with intracellular messaging; therefore, the ability to monitor intracellular Ca^{2+} flux has wide-ranging utility in the life sciences. Today, a significant number of *in vitro* and *in vivo* investigations involve the monitoring of calcium.

MAPPING THERMORESPONSIVE NEURONS WITH RATIOMETRIC FRET

Researchers at the Michael Welsh laboratory (Howard Hughes Medical Institute and the University of Iowa College of Medicine) employed a FRET-based optical approach using an engineered Ca^{2+} -sensitive fluorescent protein biosensor, yellow cameleon 2.1, to identify neurons responding to temperature changes.¹

The researchers utilized genetically encoded Ca^{2+} indicators, known as cameleons,² by sandwiching calmodulin, a peptide linker, and the calmodulin peptide-binding protein M13 between cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP). Binding of Ca^{2+} makes calmodulin wrap around the M13 domain, thereby closing the distance between the CFP donor and YFP acceptor (the FRET pair). Consequently, there is a large increase in measurable FRET signal from the biosensor.

When the stoichiometry of the donor and acceptor is fixed, as it is when they are fused in a single polypeptide chain, the ratio of acceptor fluorescence to donor fluorescence signal can be used as a convenient means to measure FRET efficiency (as well as changes in FRET efficiency) with superb temporal resolution. For CFP-YFP biosensors such as cameleons, numerator and denominator images can be taken using a single excitation wavelength (that of the donor). In this scenario, the ratio would be $(FYFP)/(FCFP)$, where FYFP is the fluorescent image from the YFP channel using the CFP excitation wavelength, and FCFP is the fluorescent image from the CFP channel using the CFP excitation wavelength.

In their study, Welsh *et al.* used transgenic *Drosophila* larvae that express cameleon in their neurons to identify thermosensitive cells (see **Figure 1**). For microscopy, the larvae were submerged in water and immobilized using a glass cover. An Photometrics® DV2™ was used to separate and project two wavelength channels simultaneously on a single CCD camera sensor. This strategy permitted the perfect spatial and temporal registration needed for unambiguous, dynamic, dual-channel FRET detection.

RESULTS

Neuronal activity was assayed in the peripheral neurons as the temperature was changed. The results of the study indicated that the terminal organ is a thermosensitive structure that responds to cool temperatures (see **Figure 2**). Its response profile was found to resemble the behavior documented for commonly observed mammalian cold receptors. Some body-wall neurons also showed a FRET response correlated to temperature shifts; however, they responded to warm temperatures (see **Figure 3**).

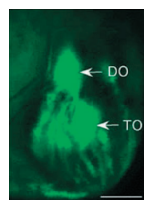


Figure 1. Cameleon fluorescence (YFP) in larval terminal (TO) and dorsal (DO) organs.¹

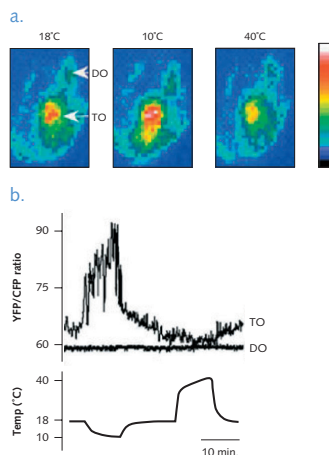


Figure 2. (a) Representative images of cameleon YFP/CFP ratios at the three temperatures indicated in terminal and dorsal organs; white represents a high fluorescence ratio, whereas purple is a low fluorescence ratio. (b) Effect (top) of temperature (bottom) on the YFP/CFP fluorescence intensity ratio from the terminal and dorsal organs.¹

ENABLING EQUIPMENT FOR DYNAMIC FRET IMAGING

One of the goals of modern microscopy is to correlate the spatial and temporal data-gathering ability of fluorescence microscopy to the functional activity of biochemical events. Toward this end, the advent of GFP-derived FRET biosensors has enabled researchers to study many dynamic cellular processes, including cellular transport, signaling, and development.

The DV2 system enables simplified, automated capture of ratio images for biosensor imaging. The design of the beam path permits careful pre-alignment of the individual channels and incorporates a multiwavelength dichroic so that the images recorded at different wavelengths remain in perfect register throughout an experiment. Such precision is extremely difficult to accomplish using conventional automated filter wheels and dichroics, or manually positioned filter blocks.

Additionally, the lack of electromechanical instrumentation required for multiple-image capture with the DV2 reduces the complexity of software automation, minimizes the number of potential sources of vibration and electrical noise, and increases the overall dependability of the system.

It is important to note that when studying rapid biological processes, images of the CFP and YFP channels must be acquired simultaneously. If this is not done, the signal molecules may shift to a different pixel location and the donor and acceptor images will be misaligned in the resultant ratio images. In practice, this artifact is often misinterpreted as a loss of FRET signal.

The DV2 directly addresses this issue by projecting both images onto the imaging sensor at precisely the same moment. Thus, the system not only eliminates problems associated with poor temporal registration, it also removes electromechanical positioning of filter optics as the rate-determining factor with respect to the speed at which multichannel images can be recorded. In regard to the temporal resolution of data recording, the digital camera becomes the primary limiting factor; therefore, investigators can make use of the full potential of newer, low-light, high-speed imaging sensors such as the new Evolve EMCCD from Photometrics.

Citations

- Liu, L., O. Yermolaieva, W.A. Johnson, F.M. Abboud, and M.J. Welsh. Identification and function of thermosensory neurons in *Drosophila* larvae. *Nature Neuroscience* 6, 3, 267–73 (2003).
- Miyawaki, A., J. Llopis, R. Heim, J.M. McCaffery, J.A. Adams, M. Ikura, and R.Y. Tsien. Fluorescent indicators for Ca^{2+} based on green fluorescent proteins and calmodulin. *Nature* 388, 882–87 (1997).

RESOURCES

To learn more about Dr. Michael Welsh's research, visit <http://www.medicine.uiowa.edu/labs/welsh/MJWelsh.htm>.

To learn more about the Photometrics DV2, visit www.photomet.com/products/multichannel.

To learn more about high-performance CCD cameras, visit www.photomet.com and www.qimaging.com.

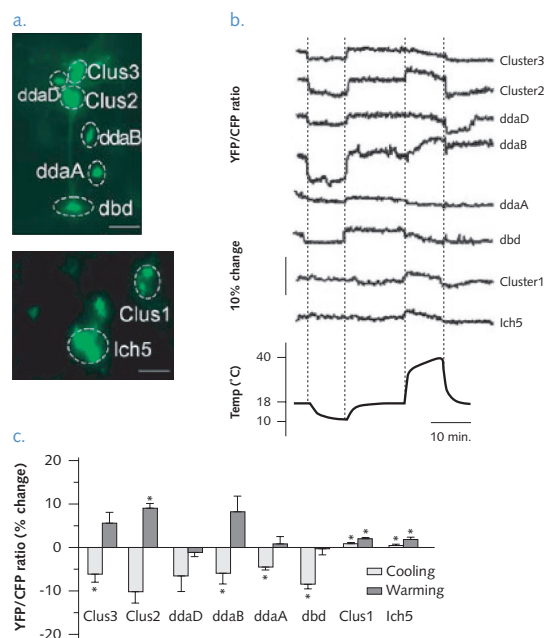


Figure 3. (a) Optical recording of cameleon fluorescence in the dorsal, lateral body wall segment identifying neurons. (b) Representative traces of YFP/CFP ratios during temperature changes (bottom) for specific neurons and clusters (top). (c) Changes in YFP/CFP ratio in response to heating and cooling for the indicated neurons and clusters.¹



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