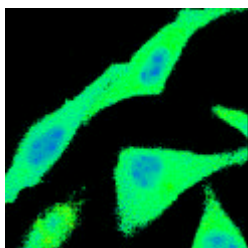
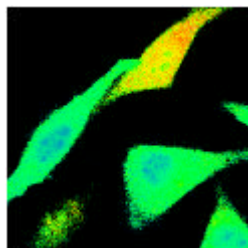




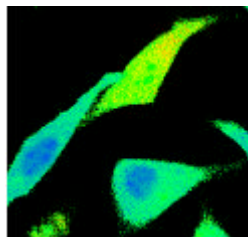
Response of CHO cells to 5-hydroxytryptamine



t = 0s (basal level)



t = 10s



t = 15s

Ratio images of calcium changes in CHO cells labelled with Fura-2.

Professor Steve Hill and Dr Steve Briddon work within the Institute of Cell Signalling at the University of Nottingham. This Institute is a multidisciplinary center with complimentary skills in cell biology, molecular pharmacology and cell signaling. The research performed there uses the advances being made in molecular genetics and cell biology to study the regulation of cell signaling pathways in healthy and diseased human tissues.

Professor Hill and Dr Briddon have an interest in 5-hydroxytryptamine (5-HT), which acts as a neurotransmitter in the central and peripheral nervous systems in addition to being an endocrine/autocrine messenger in a variety of tissues. It acts upon 5-HT receptors which signal intracellularly through G-proteins.

The images shown here were captured and ratioed using Openlab. The cells used were Chinese Hamster Ovary (CHO) cells and the experiment was carried out to investigate the interaction between the 5-HT_{1A} receptor and a transfected insulin receptor. The 5-HT_{1A} receptor on CHO cells is expressed at a very low level and this signaling pathway cannot be detected by conventional population calcium studies or inositol phosphate determination.

The cells were loaded with Fura-2 and stimulated by the addition of 1 μ M 5-HT. Ratio pairs of images at 340nm/380nm were captured at intervals using the Openlab cell imaging system. The changing intracellular calcium level can be seen in the middle and lower images, at 10s and 15s after stimulation. If required, this information could be extracted and shown graphically using the Openlab Graphing module. By calibration, ratio units can be converted to μ M Ca²⁺.

Experimental protocols for ratio imaging experiments are created in Openlab using the Automator. This is an icon based programming language of almost unlimited power and versatility, but which is also easy to understand. In particular, the Automator allows the user to include Variables so that, for example, the speed of image capture does not occur at fixed intervals but can be varied during the experiment so that no valuable data is lost should events occur more rapidly than expected.



Module configuration

Module Families

Camera

Snapper Video

Hardware

Filters & Shutters

File Filter

Quick Time file filter
TIFF file filter
PICT file filter

Application

Ratio

Automation

Automator Pro including
Graphing

Critical Points

- High speed dual filter wheel allows for rapid change of excitation wavelength
- The CoolView camera allows for high speed image Capture
- The raw A and B images can be ratioed and viewed during the experiment
- Changes in the Ratio values can be plotted as a function of time during the experiment
- All data sets can be exported in a format suitable for statistical analysis in other programs
- The ratioed images can be calibrated by converting ratio values to ion concentrations