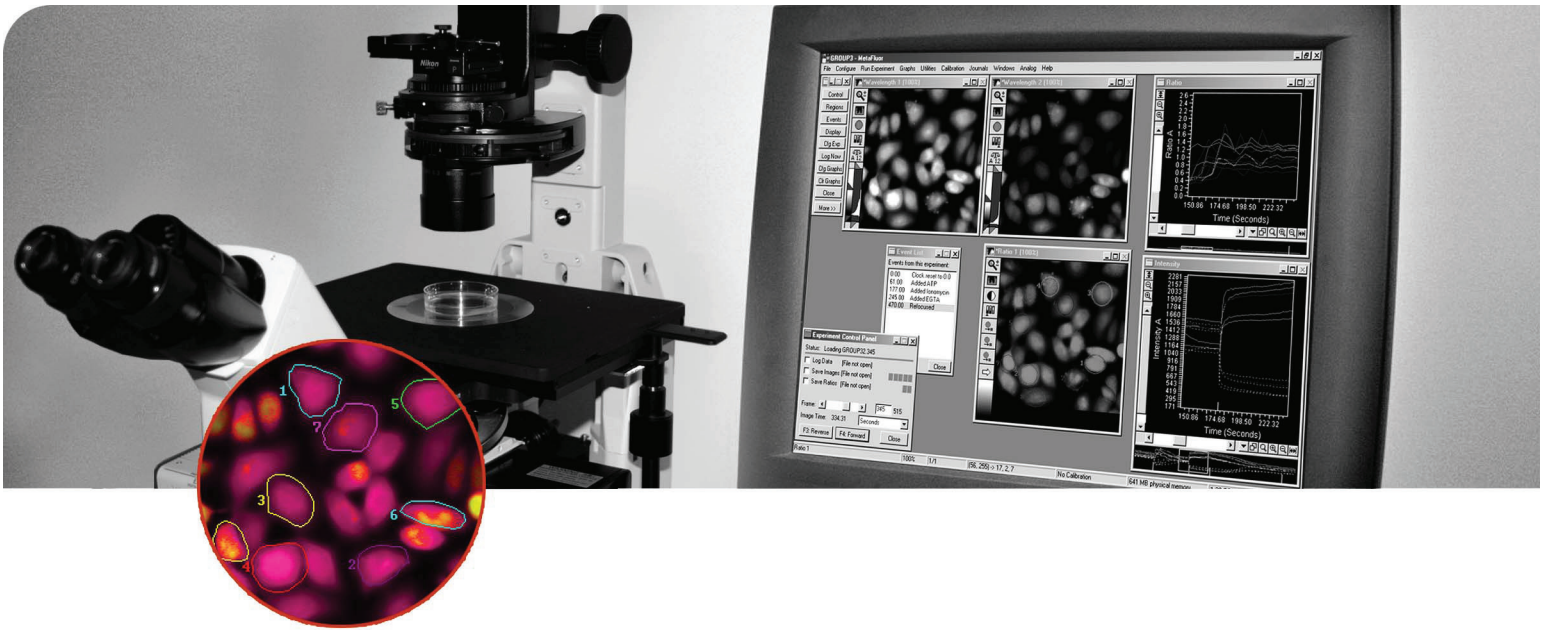
Molecular Devices

The MetaFluor® System

FLUORESCENCE RATIO IMAGING



- → RATIO IMAGING
- → CALCIUM IMAGING
- → FRET
- → pH MEASUREMENTS
- → ION CONCENTRATION
- → INTENSITY-OVER-TIME

Fluorescence ratio imaging is the monitoring of live cells in which a fluorescent indicator of intracellular ions is introduced. Indicator dyes have been designed to shift their fluorescence excitation or emission spectrum when binding with specific ions. Images are obtained at two different wavelengths, typically matching the absorption bands at the high and low binding conditions.

By ratioing the intensities in the images, it is possible to construct a map showing the local ion concentrations throughout the field of view. Since the monitoring process is nondestructive, image acquisition can be repeated frequently to trace and monitor the time course of cellular responses.

The MetaFluor® Imaging System is designed for dual-wavelength intracellular ion measurements.

The system provides simultaneous display of the raw data, ratio image, graphs of intensities, ratios and ion concentrations, and a non-ratiometric image such as a brightfield or phase-contrast image. Two different ratiometric indicators can be imaged and measured simultaneously.

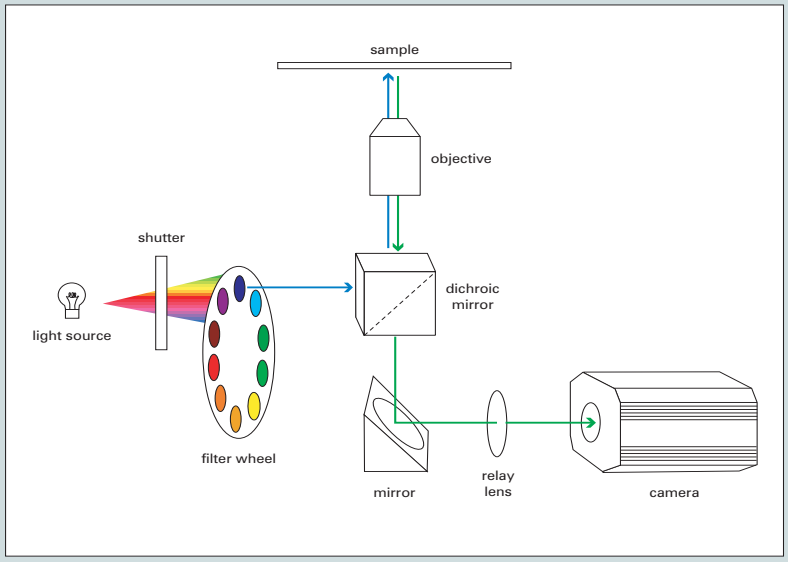
CUSTOM CONFIGURATION

Toolbars, menus, wizards and dialog boxes help move you through the image processing steps quickly. Features such as multiple image windows, flexible device control, synchronization and timing, and journals allow for automated image acquisition and analysis unlike any other system.

With the MetaFluor System, you customize the set-up once, then let the experiment run by itself. You are able to collect a large amount of data online and process it with either MetaFluor or an analysis-only copy of the software.

device control and system automation

typical system configuration for fluorescence ratio imaging



DEVICE CONTROL

MetaFluor works with microscopes equipped with epi-fluorescence illumination.

The system includes device drivers for numerous commercially-available filter wheels, shutters, monochromators and high speed filter changers for illumination control.

Camera drivers are optional. The MetaFluor system's camera drivers support acquisition from a wide variety of digital cameras.

MetaFluor enables sub-region, binning and analog-to-digital (A/D) selection if the camera allows it. Gain and exposure time can be set per wavelength for acquisition.

Streaming can be used as an acquisition option. With the appropriate devices, streaming allows you to acquire a predefined number of images at the maximum frame rate of the camera (patented).

JOURNALING AND TASK AUTOMATION

Journals are sophisticated and customizable macros that execute many tasks without requiring you to know any programming language.

The software's Journal Editor allows you to create functions to simplify system operations, automate acquisition and device control, and sequence events.

User-definable taskbars make it easy to achieve “one-button” control of your system.

fluorescence ratio imaging applications

Spatio-temporal activation of caspase revealed by indicator that is insensitive to environmental effects

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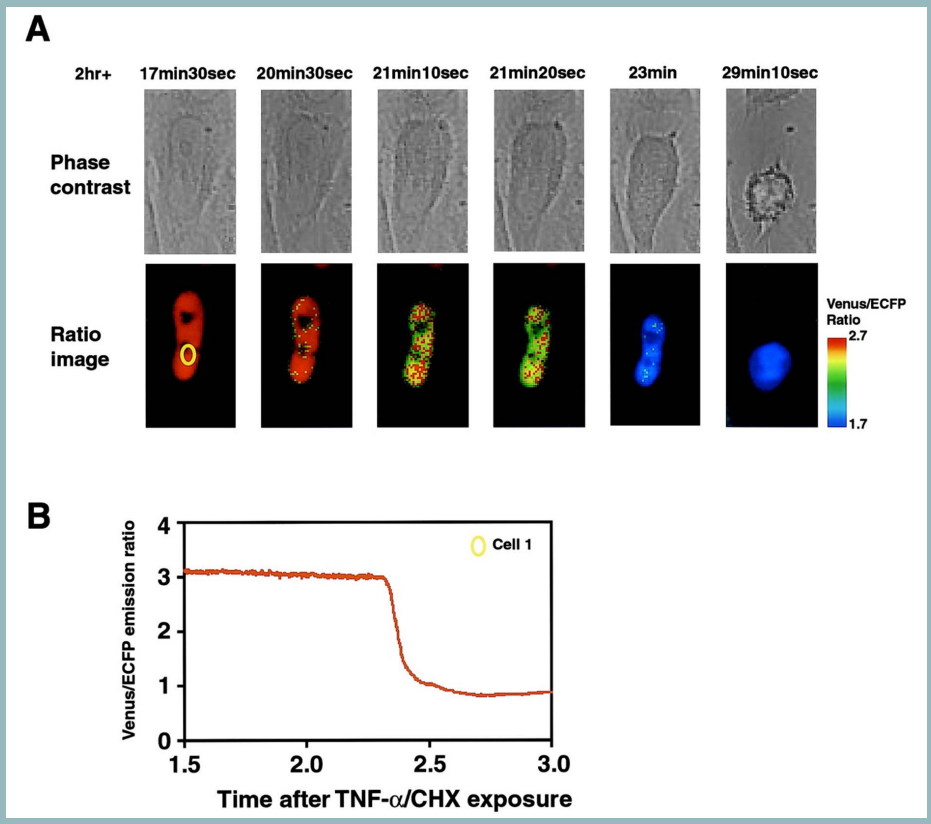
[3] Laboratories for Cell Biology and Neuroscience, Graduate School of Medicine, Osaka University, Suita, Osaka 565-0871, Japan
[4] Structure and Function of Biomolecules, Precursory Research for Embryonic Science and Technology (PRESTO), Japan Science and Technology Corporation (JST), Nittochi 535, Akinono-cho, Nakagyo-ku, Kyoto 604-0847, Japan

Figure 5. Nuclear activation of caspase-3 precedes apoptotic nuclear changes. (A) Ratio images and phase contrast images of NLS-SCAT–expressing cells. HeLa cells were transfected with 0.5 μg pcDNA-SCAT3. Imaging analysis was started 18 h after transfection. (B) Venus/ECFP emission ratio changes of individual cells examined in A.



MetaFluor is an ideal tool for:

- → Ratio imaging
- → Calcium imaging
- → FRET
- → pH measurements
- → Ion concentration
- → Intensity-over-time

MetaFluor provides the flexibility to measure Fluorescence Resonance Energy Transfer (FRET). FRET involves the non-radiative transfer of energy from a fluorophore in an excited state to a nearby acceptor fluorophore. FRET will occur when fluorophores are within angstroms of one another. This technique is used to infer protein-protein interaction and colocalization.

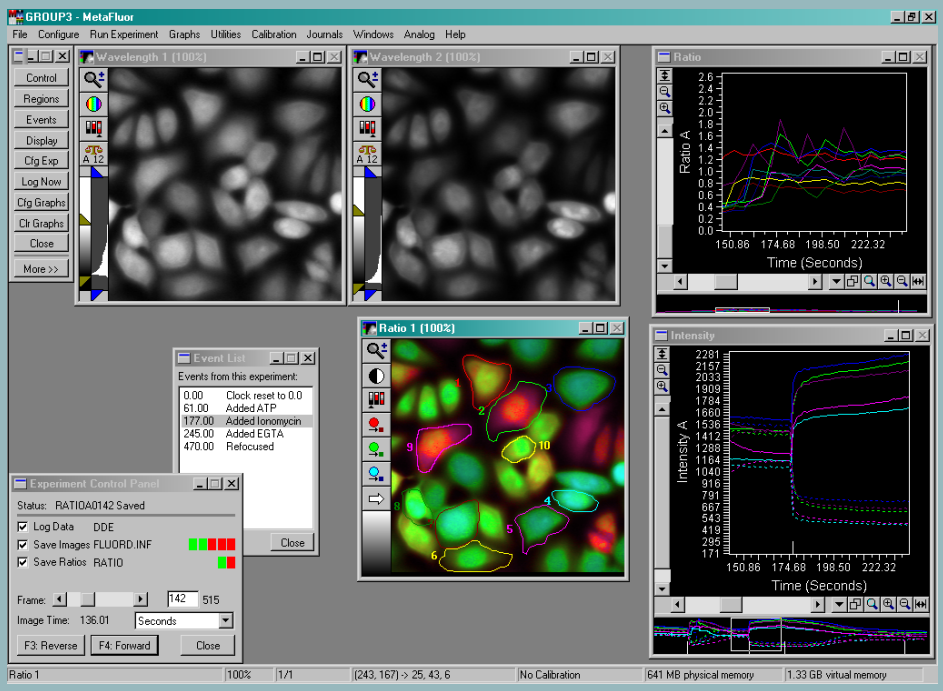
SIMULTANEOUS EMISSION-SPLITTING

MetaFluor supports multi-wavelength emission-splitter acquisition. The Dual-View™ device option separates the fluorescent image into a set of two or four spectrally-discrete images and acquires them on a single CCD chip with a single exposure without overlap. Using the TwinCam option, the Dual-Cam™ multi-wavelength emission splitter device is used to project one wavelength to one camera and a different wavelength to a second camera, allowing simultaneous acquisition from two cameras. This allows the measurement of emission-shifted probes (Indo-1, SNARF, JC-1) or FRET-based sensors (CFP, YFP) at very high speeds, without any moving parts.

RATIO IMAGING

Once acquired, the wavelengths are grouped into two pairs of ratiometric wavelengths, and one isosbestic or transmitted-light image. With this arrangement, it is possible to monitor two indicators simultaneously, such as BCECF and Fura-2 for pH and calcium respectively, while also obtaining a brightfield image of cellular morphology.

powerful real time processing acquisition



A simultaneous display of multiple wavelengths images and various customizable graphs provide an easy point-and-click interface. When playing back an experiment, clicking on the graph will rewind or fast-forward the experiment to show the images that correspond to that location on the graph.

CHO cells loaded with Fura-2. Experiment from the Optical Microscopy and Imaging in the Biomedical Sciences short course at the Marine Biological Laboratory, Woods Hole, MA. Courtesy of Lynda Pierini, PhD, Cornell Medical Center, Ken Dunn, PhD, Indiana University-Purdue University, and Professor Colin Izzard, SUNY University of Albany.

When acquiring from video sources, MetaFluor can average up to 256 images per time point, significantly reducing random image noise. Background subtraction is also used to improve accuracy by correcting for stray light, camera noise and auto-fluorescence.

REAL TIME PROCESSING

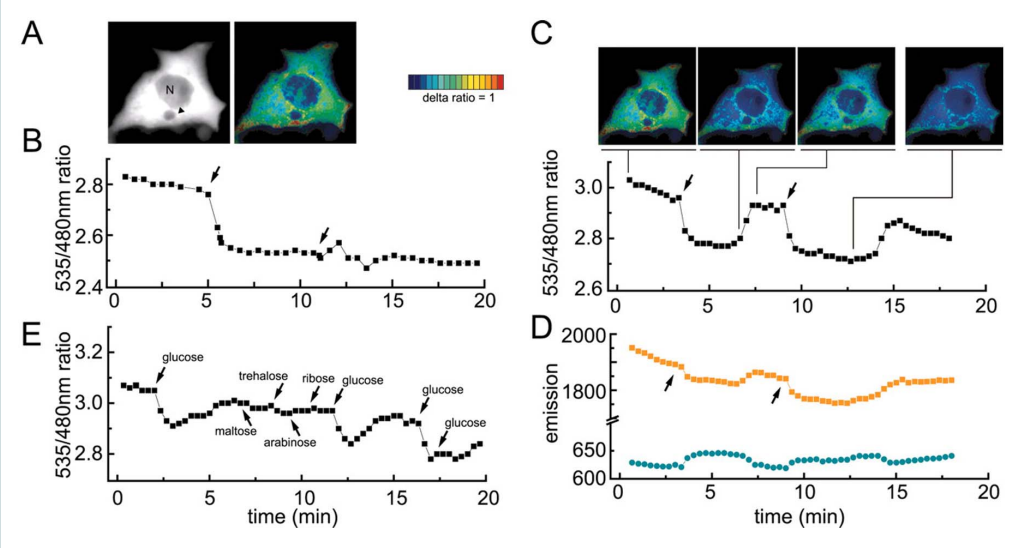
MetaFluor will perform frame integration or averaging and background subtraction on your image as your experiment progresses. Ratio shifts or ion fluxes are observed immediately, providing instant feedback on your experiment.

CALIBRATION

A direct display of intracellular ion concentrations is obtained by using the various calibration options offered; the Grynkiewicz equation (Grynkiewicz *et al.*,1985) and titration equation for both *in situ* and *in vitro* experiments. These calibrations can then be stored for future use.

G. Grynkiewicz, M. Poenie, R.Y. Tsien (1985) A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. The Journal of Biological Chemistry. 260(6):3440-3450.

image analysis and processing



***In Vivo* Imaging of the Dynamics of Glucose Uptake in the Cytosol of COS-7 Cells by Fluorescent Nanosensors**
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Figure 3. *In vivo* characterization of FLIPglu-600µ. *A*, averaged YFP-CFP emission image shows FLIPglu-600µ in cytosol and exclusion from nuclei (*N*) and lysosomes (triangles). Emission intensities were higher in thicker layers of cytosol adjacent to the nucleus. Ratio images are pseudocolored to demonstrate glucose-dependent ratio changes. *Red* indicates high ratio, and *blue* indicates low ratio. Integration of the ratio over the entire cells was used to quantify the ratio change. *Arrows* indicate the addition of 10 mM sugar. Each graph shows ratio changes for a single cell. *B*, direct addition of 10 mM glucose led to a decrease in ratio. Because of continuous external glucose supply, the ratio remained constant. Increasing the external concentration to a total of 20 mM did not cause further changes. *C*, following addition and detection of glucose, external glucose was removed by perfusion with glucose-free solution. The subsequent increase in ratio indicates reversible glucose detection. *D*, according to the FRET theory, the decrease in YFP emission is accompanied by an increase in CFP emission. Probably, because of photobleaching, YFP emission decreased. *E*, the addition of glucose but not of 10 mM of other sugars led to ratio changes.

Regions of interest can be generated automatically or manually placed on your image to monitor intensity, ratio value or ion concentration. Measurements are then made simultaneously on all the regions of interest and update continuously on a scrolling graph, allowing you to follow dynamic changes as they occur in your living samples.

INTERACTIVE GRAPHS

A display of multiple graphs gives flexibility in the presentation of your experiment's data. MetaFluor enables you to click on graph traces to display a readout of the time and data value for the region nearest to the click.

The Event Mark function is useful to record when drugs or solutions were added, experimental conditions changed, triggers were received or sent or other events occurred. You have the option to associate a timer and an alarm bell to each event. Additionally, for perfused samples, ambient conditions can be logged and tracked.

Each image has an annotation that is saved within the TIFF file format. The annotation will record wavelength-dependent settings. Additional information can be stored in a protocol file.

EXPORT FOR DATA ANALYSIS

If needed, MetaFluor can log and export all measurements to either a text file or to a spreadsheet program such as Microsoft® Excel®.

COMPATIBLE WITH METAMORPH

Because MetaFluor saves images in TIFF file format, you can import them into MetaMorph for further processing and analysis.

Technical Summary

COMPUTER REQUIREMENTS
- Intel® Pentium-4 processor or later
- Microsoft® Windows® XP
- CD-ROM drive
- 512MB or more system memory (RAM) (more memory may be required for processing large image data sets)
- 200MB free hard disk space for program only (image storage requires more space)
- 24-bit graphics display

ACQUISITION
- Up to five wavelengths per cycle
- Real time background subtraction (independent background for each wavelength)
- Real time shading correction (independent shading reference for each wavelength)
- Time lapse

AUTOMATION
- Control for multiple shutters, filter wheels, monochromators and other wavelength-changing devices
- Device triggers for pumps, valves, strobes or flash lamps using TTL outputs
- Customizable journals and taskbars

DIGITAL CAMERA ACQUISITION FEATURES
(depends on imaging hardware used)
- Exposure time, gain, A/D transfer speed, bits-per-pixel for each wavelength
- On-chip gain multiplication
- Binning and sub-region selection
- Control of integrated camera shutter
- Support for frame transfer, interline, full frame, back illuminated sensors
- Streaming of data for high speed applications

VIDEO-BASED ACQUISITION FEATURES
(depends on imaging hardware used)
- RS-170 or CCIR video inputs
- Frame averaging, on-chip camera integration, summation into a 16-bit buffer
- Analog gain and black level offsets for each wavelength
- Adjustable intensifier gain for each wavelength
- Compensation for camera lag

CALIBRATIONS
- Grynkiewicz equation
- Titration calibrations with choice of curve fits
- Calibration maps to directly display pH, calcium or other ion concentrations

ANALYSIS
- Ratio of up to two indicators per cycle
- Automatic generation of multiple regions of interest
- Fluorescence Resonance Energy Transfer
- Multiple graphs display
- Event Marks and image annotation
- Tracking of experiment conditions
- IMD, pseudocolor or monochrome display
- QuickTime® or AVI formats for movies
- Data logging to text file or spreadsheet such as Microsoft® Excel®
- Compatible with MetaMorph®

CUSTOM CONFIGURATION
- Multi-users environment available
- Settings storage for each type of experiment

SUPPORT
- Technical support via phone, e-mail or online at support.universal-imaging.com
- Electronic documentation

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