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 MetaFlouor, 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**
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.
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.

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.
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.

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, strobos or flash lamps using TTL outputs
- Customizable journals and taskbars

### Digital Camera Acquisition Features
(depend 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
(depend 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