



Leica MM Fluor powered by MetaMorph®

Fluorescence Ratio Imaging

Living up to Life

Leica
MICROSYSTEMS

Fluorescence Ratio Imaging

The Leica MM Fluor is an ideal tool for:

- 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 non-destructive, image acquisition can be repeated frequently to trace and monitor the time course of cellular responses.

The Leica MM Fluor 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 Leica MM Fluor, 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 the Leica MM Fluor or an analysis only copy of the software.



Leica EL6000

Leica EL6000 with its long-life (+2000 h) metal halide lamp. Its integrated high speed shutter with its 6 ms switching time makes this light source perfectly suited for live cell applications by minimizing the light exposure to the cells whenever possible.

Device Control and System Automation

Device control

The Leica MM Fluor works with automated Leica research microscopes equipped with epi-fluorescence illumination. For device control, the Leica MM AF Acquisition and Analysis package is required including drivers for external filter wheels, the Leica EL6000 high speed shutter and the Leica DFC Cameras. Drivers for third party cameras are optional.

Leica MM Fluor 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.

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.



"MetaMorph® is a Registered Trademark of MDS Analytical Technologies"

Fluorescence Ratio Imaging Applications

The Leica MM Fluor 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.

A variety of filter sets are available to fit specific applications; from standard multi-color fluorescence to Calcium-ratioing to various FRET pairs and many others.

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.

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

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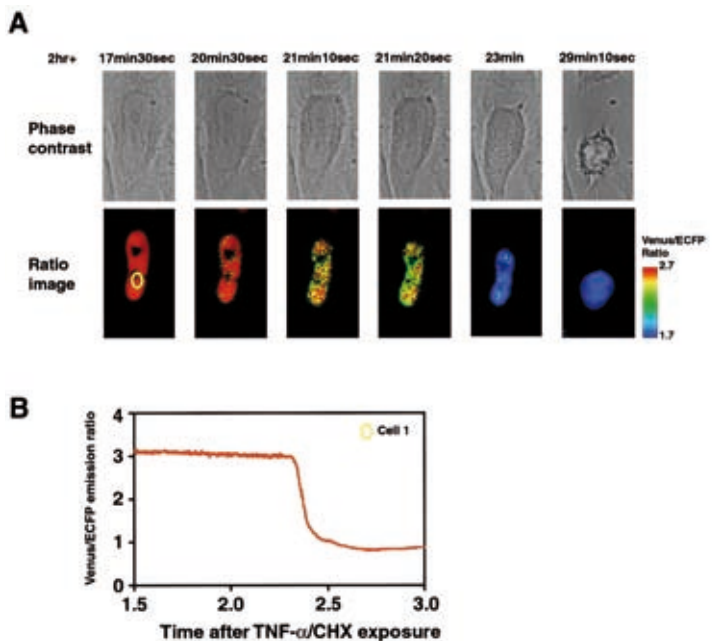
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Figure 1: Nuclear activation of caspase-3 precedes apoptotic nuclear changes. (A) Ratio images and phase contrast images of NLS-SCAT-expressing cells. HeLa cells were transfected with 0.5 µg pcDNA-SCAT3. Imaging analysis was started 18 h after transfection. (B) Venus/ECFP emission ratio changes of individual cells examined in A.

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Powerful Real Time Processing Acquisition

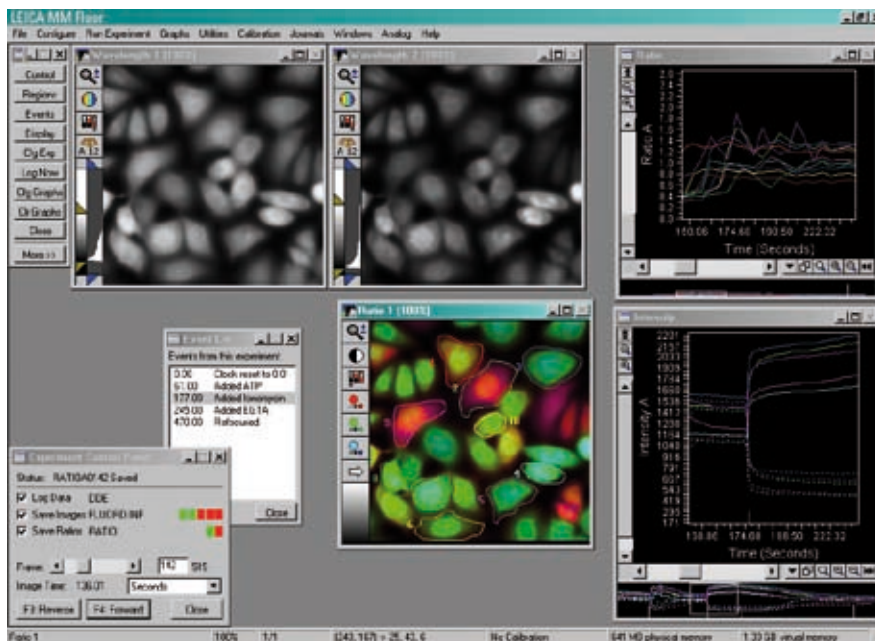
Background subtraction is used to improve accuracy by correcting for stray light, camera noise and auto-fluorescence.

Real Time Processing

The Leica MM Fluor 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.



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.

G. Grynkiewicz, M. Poenie, R.Y. Tsien (1985) A new generation of Ca²⁺ indicators with greatly improved fluorescence properties. *The Journal of Biological Chemistry*. 260(6):3440-3450.

Image Analysis and Processing

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. The Leica MM Fluor 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, the Leica MM Fluor can log and export all measurements to either a text file or to a spreadsheet program such as Microsoft® Excel®.

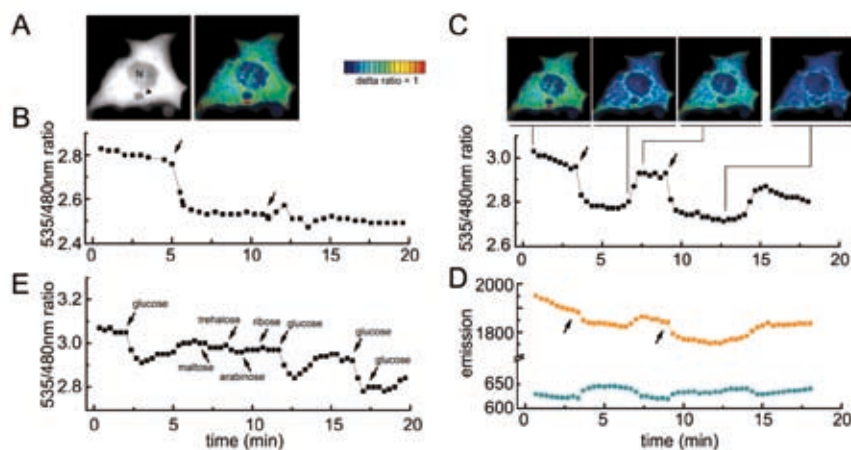
Compatible with Leica MM AF

Because the Leica MM Fluor saves images in TIFF file format, you can import them into Leica MM AF for further processing and analysis.

In Vivo Imaging of the Dynamics of Glucose Uptake in the Cytosol of COS-7 Cells by Fluorescent Nanosensors
Marcus Fehr, Sylvie Lalonde, Ida Lager, Michael W. Wolff, and Wolf B. Frommer, The Zentrum für Molekular Biologie der Pflanzen Tübingen, Plant Physiology, Auf der Morgenstelle 1, D-72076 Tübingen, Germany

Figure 2: *In vivo* characterization of FLIPglu-600 μ . **A**, averaged YFP-CFP emission image shows FLIPglu-600 μ 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 glucosedependent 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

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Technical Summary

Aquisition

- 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 Leica EL6000 high speed shutter
- Device triggers for pumps, valves, strobes or flash lamps using TTL outputs
- Customizable journals and taskbars

Digital camera aquisition features

(depends on system setup 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, back illuminated sensors
- Streaming of data for high speed applications

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 Leica MM AF

Custom configuration

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

“With the user, for the user”

Leica Microsystems

Leica Microsystems operates internationally in four divisions, where we rank with the market leaders.

• Life Science Division

The Leica Microsystems Life Science Division supports the imaging needs of the scientific community with advanced innovation and technical expertise for the visualization, measurement, and analysis of microstructures. Our strong focus on understanding scientific applications puts Leica Microsystems' customers at the leading edge of science.

• Industry Division

The Leica Microsystems Industry Division's focus is to support customers' pursuit of the highest quality end result. Leica Microsystems provide the best and most innovative imaging systems to see, measure, and analyze the microstructures in routine and research industrial applications, materials science, quality control, forensic science investigation, and educational applications.

• Biosystems Division

The Leica Microsystems Biosystems Division brings histopathology labs and researchers the highest-quality, most comprehensive product range. From patient to pathologist, the range includes the ideal product for each histology step and high-productivity workflow solutions for the entire lab. With complete histology systems featuring innovative automation and Novocastra™ reagents, Leica Microsystems creates better patient care through rapid turnaround, diagnostic confidence, and close customer collaboration.

• Surgical Division

The Leica Microsystems Surgical Division's focus is to partner with and support surgeons and their care of patients with the highest-quality, most innovative surgical microscope technology today and into the future.

The statement by Ernst Leitz in 1907, “with the user, for the user,” describes the fruitful collaboration with end users and driving force of innovation at Leica Microsystems. We have developed five brand values to live up to this tradition: Pioneering, High-end Quality, Team Spirit, Dedication to Science, and Continuous Improvement. For us, living up to these values means: **Living up to Life.**

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