Leica MM AF powered by MetaMorph®

Integrated System for Bioimaging

Living up to Life

Leica
MICROSYSTEMS

Integrated System for Bioimaging -

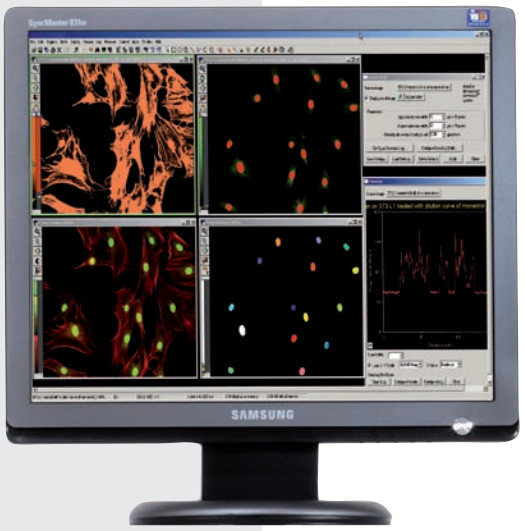
A variety of applications to suit your needs

Developed in conjunction with leading bioscience researchers, Leica MM AF offers tools for imaging applications such as:

- Multi-dimensional imaging
- 3D deconvolution
- 3D reconstruction
- Colocalization
- Brightness measurements
- Particle tracking and motion analysis
- Fluorescence, FRET and FISH
- Morphometry

Bioimaging techniques contribute to a growing number of scientific breakthroughs. The Leica MM AF Imaging System powered by MetaMorph, plays a large role in this revolution. With its image acquisition, processing and analysis capabilities, and complete set of tools for automation, Leica MM AF opens the door for new insights into cellular function.

Leica MM AF's flexibility and versatility make it a powerful system for performing operations such as time lapse, multi-dimensional acquisition and 3D reconstruction, and for making measurements such as morphometry, colocalization and brightness. In biological experiments using live cell imaging, Leica MM AF combines the speed, flexibility and unmatched customer support required to get better results, faster.



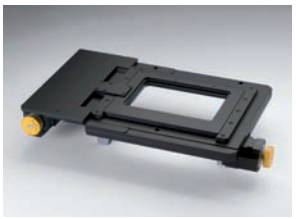
Leica DMI6000 B

– the New Leica MM AF

Device automation for easy acquisition
The Leica MM AF provides high-end control for devices like filter wheels, shutters, cooled CCD cameras, including the Leica DFC cameras, SuperZ Galvo focus and Piezo electric focus devices, motorized stages, digital and serial input/output and of course the automated Leica Microsystems research microscopes.

An integrated Leica system solution
Leica MM AF is an integrated system solution based on the Leica automated research microscopes. All systems are integrated, tested and installed by Leica specialists. Whether you have questions or requests on software, hardware, accessories or applications, Leica Microsystems is your partner.


SuperZ Galvo


Scanning stage


Leica DFC360 FX

Custom configured for you

Leica MM AF is available in two custom configurations:

- **Leica MM AF Acquisition and Analysis**
  Complete software package for control and integration of all automated Leica research microscopes, peripherals and Leica cameras. Multi-dimensional image acquisition, image processing and analysis. Drivers for other cameras suppliers optional.

- **Leica MM AF Offline**
  Complete software package for image measurement, processing and analysis. Includes all the analysis capabilities of Leica MM AF Acquisition and Analysis without devices control. Perfect for multi-user facilities.

A Powerful Multi-dimensional Imaging Tool

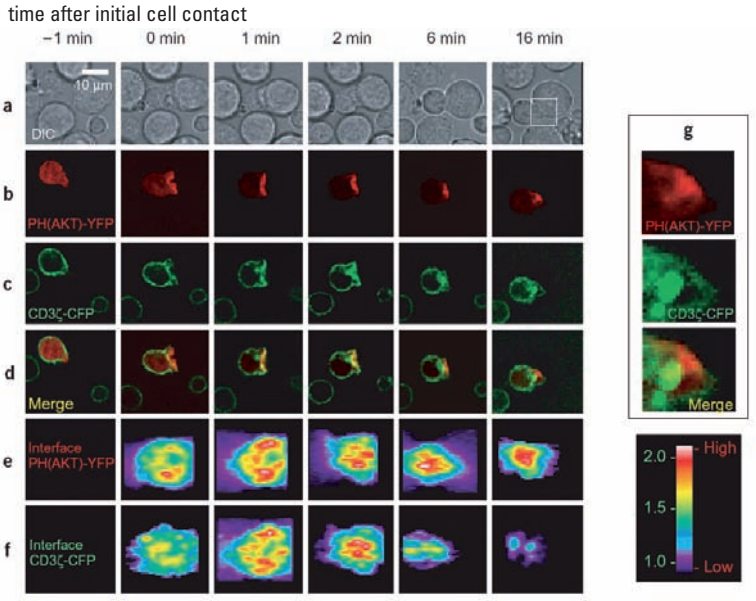
Leica MM AF is optimized for multi dimensional experiments. In addition to X and Y dimensions, you can acquire and display:

- Z-axis or multiple focus series (Z dimension)
- Multiple fluorochromes (Wavelength dimension)
- Time lapse (Time dimension)
- Multiple stage positions (Stage dimension)

For any multi-dimensional experiment, you can:

- Align images within a stack
- Create a montage
- Create and play a movie exportable as QuickTime® or AVI
- Render a 3D reconstruction
- Create Z-series projections
- Color-combine images
- Measure through all planes automatically
- Enhance any or all images
- Deconvolve the images with nearest and no neighbour. 3D deconvolution optional
- Equalize light
- Create topographic surface maps
- Perform arithmetic operations
- View orthogonal planes
- Stitch a stack of images (optional)
- Visualize the experiment in 3 dimensions and obtain 3D measurements

A simple interface guides you through each dimension and settings can be modified after acquisition is initiated. The Leica microscope peripheral controls are integrated in the Leica MM AF toolbar, displaying current illumination, magnification and XYZ location settings. Leica MM AF's customizable auto-focus capabilities keep lengthy timedependent events in focus.



Continuous T cell receptor signaling required for synapse maintenance and full effector potential
Johannes B. Huppa[1,2], Michael Gleimer[1], Cenk Sumen[1,3] and Mark M. Davis [1,2]
[1]Stanford University School of Medicine, Department of Microbiology and Immunology
[2]Howard Hughes Medical Institute, Stanford, CA 94305
[3]Center for Blood Research, Harvard Medical School, Boston, MA 02115

Figure 1: Antigen-induced PI3K activity colocalized with TCR-CD3 complexes within the nascent immunological synapse and remained mainly synapse associated at later stages despite substantial TCR internalization. T lymphocytes were isolated from 5c.c7 αβ TCR transgenic mice and infected with two batches of retroviruses expressing PH(AKT)-YFP and CD3ζ-CFP. Usually 15% of the T cells were positive for the expression of both constructs at the time of imaging (day 6). CH27 B cells had been pulsed with the MCC peptide (0.4 µ M) and were pooled with transduced T cells. (**a**) Differential interference contrast (DIC) acquisitions. (**b–d**) Epifluorescent midplane acquisitions of PH(AKT)-YFP (**b**), CD3ζ -CFP (**c**) and their corresponding overlays (**d**). (**e,f**) Three-dimensional interface reconstructions of PH(AKT)-YFP (e) and CD3ζ-CFP (**f**). (**g**) A ‚close-up' view of the area of contact at the 16-min time point (white rectangle, far right panel of a) of PH(AKT)-YFP (red) and CD3ζ-CFP (green) and their corresponding overlay.

To improve image quality, out-of-focus light was removed from fluorescent image stacks using a blind deconvolution algorithm. The white bar (far left panel of **a**) indicates object size; the "false-color look-up table" (bottom right) indicates intensity values for interface reconstructions (high-low representation for PH(AKT)-YFP and fold increase (left margin) over average surface intensity for CD3ζ-CFP).

Observe Changes Over Time

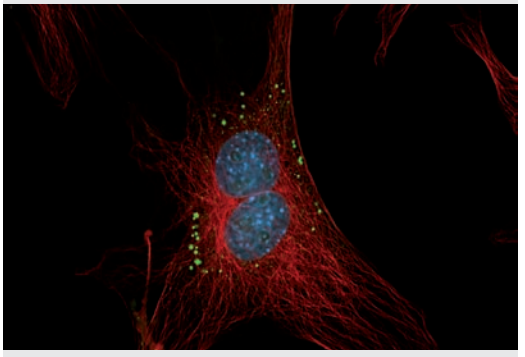
Intensity over time measurements are important in studies such as protein motility, FRET and protein-protein interactions. Leica MM AF facilitates time lapse acquisition by offering streaming as an acquisition option. With the appropriate devices, streaming allows you to acquire at the maximum rate of the camera (patented).

Meeting advanced requirements
Another feature for time lapse is the Live Replay option. With appropriate devices, and when viewing live images, you can press a key when an interesting event occurs and capture a stack containing some past history of the event as well as some data after the event happened.

Customization through journaling
Journals are sophisticated, customizable and powerful macros that record and perform a series of tasks without the need for a 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 and custom menus make it easy to achieve one-button control of your system.

Leica EL6000 external light source with high-speed shutter
As light source the Leica EL6000 with its long-life (+2000 h) metal halide lamp is used. 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.

Leica EL6000 external light source with high-speed shutter for fluorescence excitation.



FluoCells prepared slide #2 (F-14781) also contains BPAE cells, but this time stained with red-fluorescent Texas Red®-X phalloidin for labeling F-actin, mouse monoclonal anti-α-tubulin, deconvolved

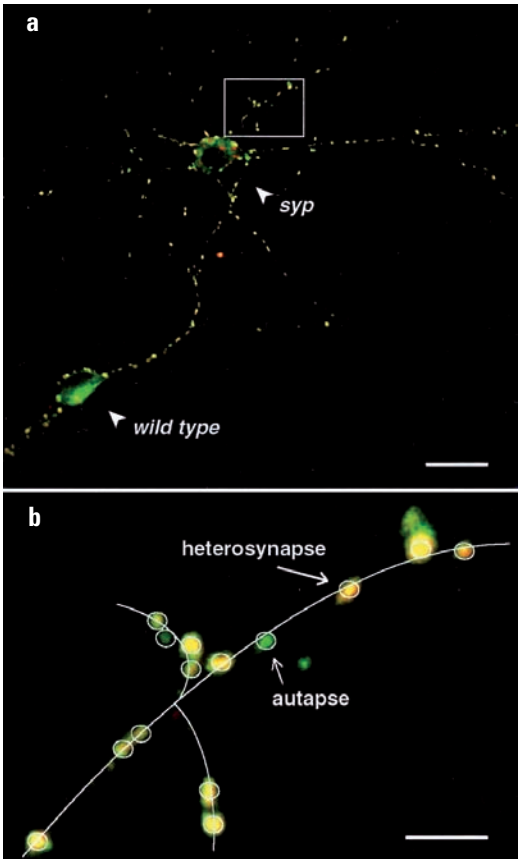
Plot Colocalization and Brightness Measurements for Visual Representations

While good experiment data can be obtained by analyzing a single fluorescent probe, you often get better results by examining more complex interactions. Leica MM AF's colocalization tools provide a higher level of detail, with quantitative data regarding regions of overlap between two fluorescent probes.

These tools enable you to graphically represent the intensities of each probe on a pixel-by-pixel basis and calculate a correlation coefficient to give a measure of both positive and negative colocalization. Your data can then be exported to a spreadsheet or text file.

Measure brightness over time

Many fluorescence experiments depend on measuring brightness parameters and Leica MM AF excels at providing this type of information. With Leica MM AF, you can log intensity data from selected regions in an image stack or live video image over time and choose which parameters to capture.



Synaptophysin regulates activity-dependent synapse formation in cultured hippocampal neurons
Leila Tarsa and Yukiko Goda Division of Biology, University of California at San Diego, La Jolla, CA 92093-0366

Figure 2: Counting synapses along the syp-mutant dendrite based on overlaid images of syp and syt immunofluorescence. For the 12-day-old heterogenotypic cell pair shown (A), determination of autapses and heterosynapses along a mutant dendrite is illustrated for the boxed area (B). Autapses are devoid of syp fluorescence and display syt immunofluorescence (green), whereas heterosynapses are positive for both syp and syt immunofluorescence (yellow). Lines were drawn along the dendrites to determine their lengths. [Bar = 20 µm (A) and 5 µm (B)]. Note that several fluorescence puncta that appear after immunolabeling for syp in the rhodamine channel (red) do not contain syt. They represent less than 3% of total syp- or syt-positive fluorescence puncta (unpublished data) and have been excluded from analysis.

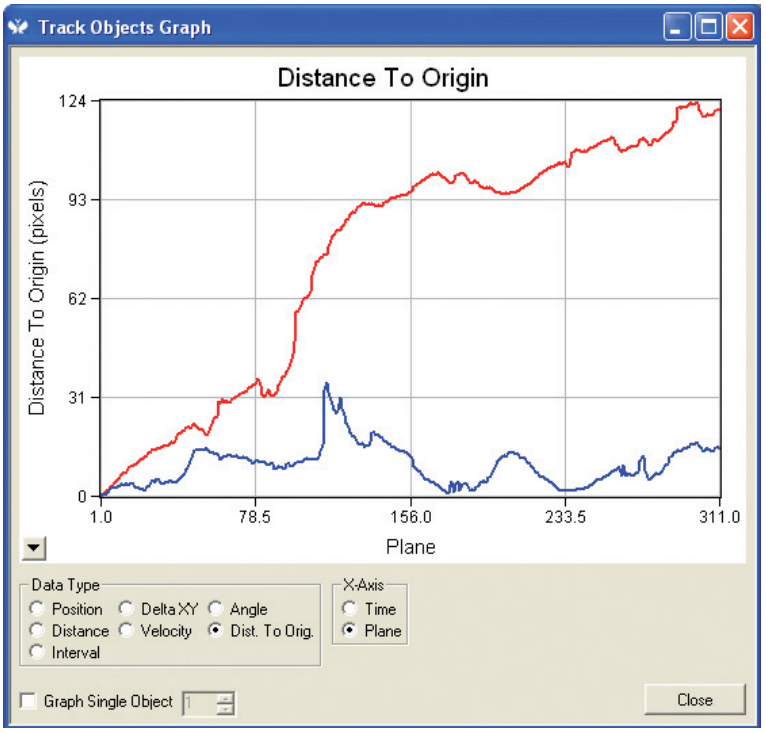
Leila Tarsa and Yukiko Goda (2002) Synaptophysin regulates activity-dependent synapse formation in cultured hippocampal neurons. PNAS. 99(2):1012-1016.

Algorithms for Particle Tracking and Motion Analysis

Follow the movement of tagged particles over time such as fluorescently-labeled cell surface molecules, microtubules, nucleic acids, lipids and other objects with sub-pixel resolution.

Leica MM AF facilitates your analysis with features for spatial calibration, point-to-point measurements, automated time stamping of images and tracking of objects.

Measure X and Y coordinates, velocity, mean displacement, mean vector length and more, then plot your measurements onto printable and custom-configurable graphs for easy visualization.



Sample display of captured data as a graph.

Dual-wavelength fluorescent speckle microscopy reveals coupling of microtubule and actin movements in migrating cells

Wendy C. Salmon, Michael C. Adams, and Clare M. Waterman-Storer, Department of Cell Biology and Institute for Childhood and Neglected Diseases, The Scripps Research Institute, La Jolla, CA 92037

Figure 3: MTs parallel to the leading edge are coupled to the movement of f-actin. (**a**) Image from Video 3 (available at http://www.jcb.org/cgi/content/full/ jcb.200203022/DC1) of Cy2 MTs (green) and Xrhodamine f-actin (red). Boxes highlight the regions in the lamellipodium (**lp**), lamellum (**la**), convergence zone (**cz**), and cell body (**cb**) that were used to construct the kymographs in (**b-e**). The long axis of the boxes was tilted to match the trajectory of speckles as determined by watching Video 3. Green arrowheads highlight the parallel MTs being analyzed. (**b-e**) Dual wavelength kymographs of the regions highlighted in panel a. Green and red arrowheads highlight speckles in parallel MTs and the actin meshwork, respectively. Bar, 10 µm.

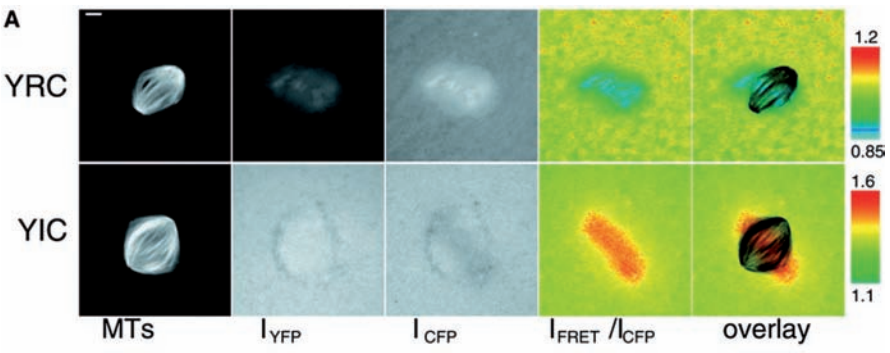
The Speed and Precision Needed for Fluorescence

Common applications of fluorescent-based methods are providing new insights into protein dynamics and the biological processes they regulate.

With a typical system configuration, Leica MM AF easily automates and simplifies the process of acquiring, color-combining and visualizing multiple fluorophores.

Live cell studies demand the rapid acquisition and low-light level imaging of highly sensitive, cooled CCD cameras with high quantum efficiency, low noise and fast readout rates.

FRET Applications with Leica MM AF
The Leica MM AF makes it easy to handle automated wavelength devices and automatically aligns multiple images. A FRET-specific dialog box automates the complex arithmetic needed to account for and correct fluorescent background and bleed through in your images.



Visualization of a Ran-GTP gradient in interphase and mitotic Xenopus egg extracts
Petr Kalab, Karsten Weis, and Rebecca Heald, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720-3200

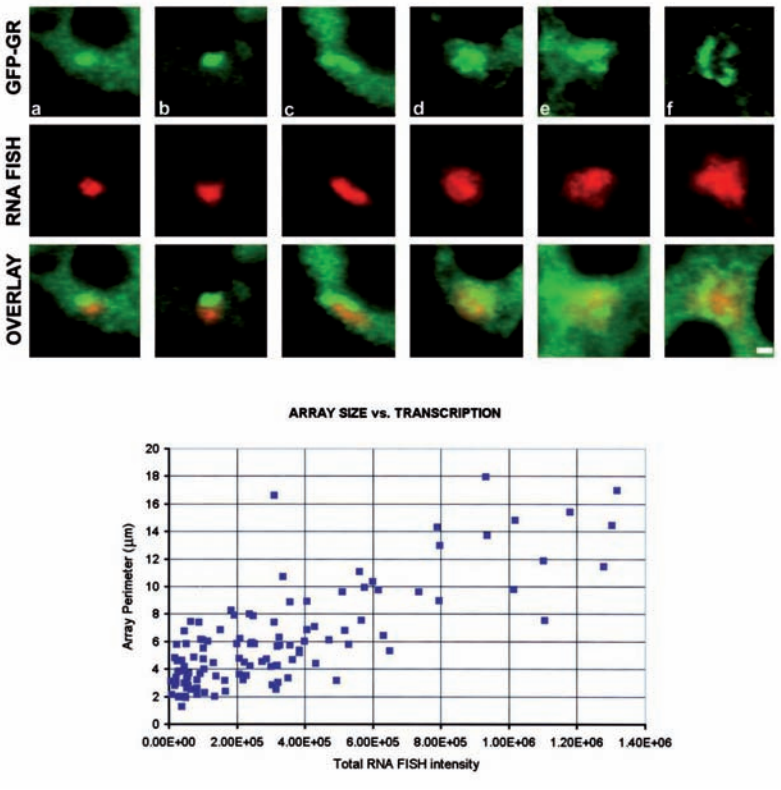
Figure 4: A gradient of Ran-GTP surrounding chromosomes visualized in egg extracts and abolished by the addition of Ran mutants. Scale bars, 10 µm. (A) Fluorescence images of mitotic spindles showing microtubules (MTs) and IYFP, ICFP, and FRET ratio (IFRET/ICFP) signals, and an MT-FRET ratio overlay showing a decrease in FRET surrounding chromosomes in the presence of YRC and an increase in the presence of YIC due to the presence of Ran-GTP. There is a decrease in ICFP in regions where FRET occurs.

Count, Classify and Measure Multiple Cell Parameters

Modules for segmentation
Discrete, application-specific analysis modules are available for Leica MM AF: Angiogenesis, Cell Cycle, Cell Health, Count Nuclei/Cell Scoring, Granularity, Live/Dead, Mitotic Index, Monopole Detection, Multi Wavelength Cell Scoring and Neurite Outgrowth Application Modules. These modules provide users with a range of tools to automate processing and analysis of cellular images. No special microscopy or image analysis knowledge is required. Cellular segmentation and measurements are generated without the need for programming.

Leica MM AF's morphometry tools allow you to choose over 100 different parameters for morphometric measurement or classification of cells in monochrome or color images. Measure all the objects in your image or define filters which restrict the measurements to objects that meet specific criteria.

Set your preferences to increase the accuracy of the data gathered, such as the exclusion of cells that touch the edge of the image. Four interactive modes allow you to „point-and-click" as you work back and forth between the objects in the image window and data being displayed in a table, histogram or scatterplot. Your data can then be exported to a spreadsheet or text file for further analysis.



Large-scale chromatin decondensation and recondensation regulated by transcription from a natural promoter
Waltraud G. Müller, Dawn Walker, Gordon L. Hager, and James G. McNally, Laboratory of Receptor Biology and Gene Expression, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

Figure 5: The amount of transcript produced by the array is correlated with array size. Shown in the top row (**a–f**) are GFP-GR arrays from different cells fixed at 3 h of 100 nM dexamethasone. The corresponding RNA FISH signals are shown in the middle row and the overlay images in the bottom row. Note that progressive increase in array size (**a–f**) is accompanied by progressive increase in the RNA FISH signal. This correlation is confirmed by quantitative analysis of 113 cells as shown in the plot at the bottom of the figure. Each point in the plot represents an array, like those in panels a–f, whose total RNA FISH intensity has been measured and plotted as a function of the measured perimeter of the array. Bar, 1 µm.

Technical Summary

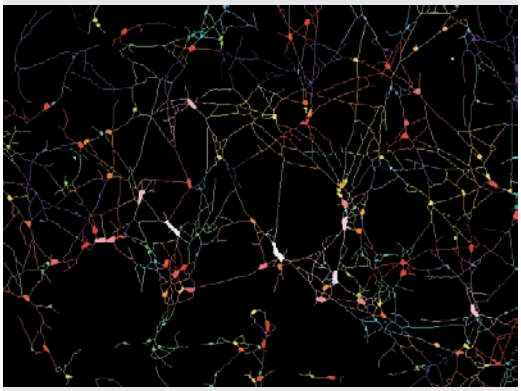
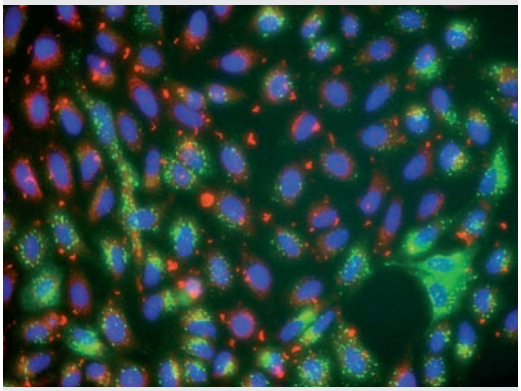
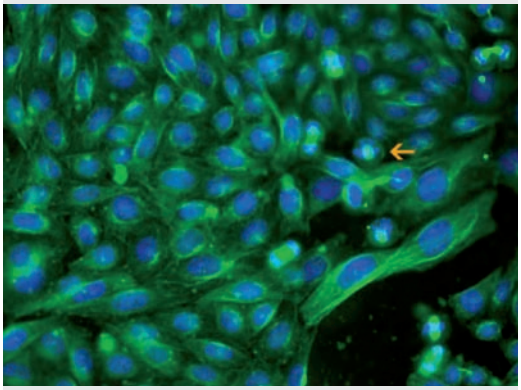


Image analysis
Each filament is assigned to a cell body. All the filaments and cell bodies are then measured.



Multiple wavelengths
Leica MM AF provides a flexible integrated solution for multi wavelength image acquisition and analysis. Up to seven wavelengths can be analyzed simultaneously by the module. U2OS rat β-arrestin 2-RrGFP cells treated with 1 µm isoproterenol. Blue: Hoechst 33342, red: Anti- Phospho-Histone H3 (Ser28), green: Transfluor® vesicles.



Multiple wavelength acquisition
CHO-K1 cells treated with monastrol and stained with mouse anti-beta tubulin primary antibody detected with a FITC conjugated goat antimouse secondary antibody. Nuclei are stained with Hoeschst 33342. Orange arrow shows monopole.

The Leica MM AF Acquisition and Analysis package includes controls for

- Leica Microsystems upright and inverted automated research microscopes
- Leica DFC cameras
- Motorized XY stages
- SuperZ galvo focus
- Leica EL6000 shutter control
- External filter wheels

Acquisition Options

- Third party cameras including cooled, frame transfer, interline, back thinned, intensified and on-chip multiplication gain from major manufacturers
- Wavelength streaming and/or Z-axis streaming
- Automated scan slide
- Live Replay

Additional Modules
(not standard)

- 3D deconvolution
- 4D visualization and 3D measurements
- Image stiching
- Automated motion analysis and particle tracking

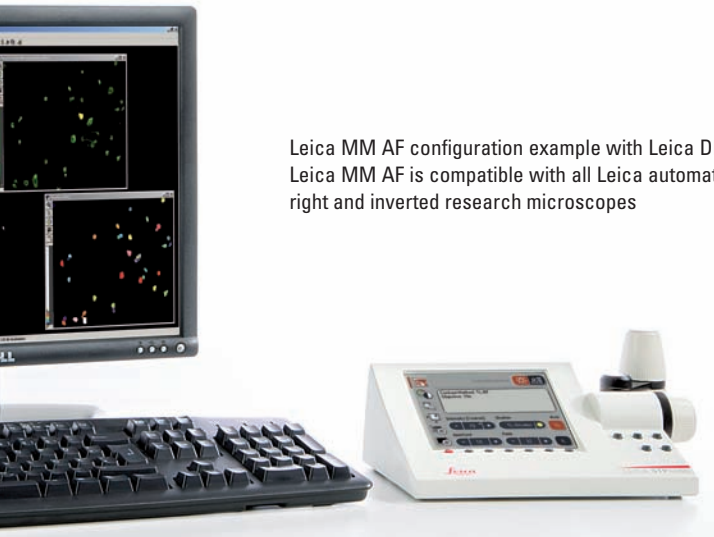
Standard Features

- Shading correction & background subtraction
- Morphometry and distance measurements
- Area/intensity measurements with graphing
- Basic filters & Morphology filters
- Arithmetic operations
- Create and play a movie exportable as QuickTime® or AVI
- Integrated Mophometry Analysis (IMA)
- Nearest neighbors and no neighbors
- 3D reconstruction
- 2D deconvolution
- FRET
- Time lapse and Z series
- Cell counting
- Kymograph for linear motility analysis
- Brightness measurements
- Colocalization
- Overlay multi-fluorescent images
- Data logging and exporting
- Digital autofocus

Also included is full journaling capability for individual customization of the application.

Application-Modules

- Leica MM Neurite Outgrowth
- Leica MM Angiogenesis
- Leica MM Count Nuclei and Cell Scoring
- Leica MM Multi Wavelength Cell Scoring
- Leica MM Cell Cycle
- Leica MM Cell Health
- Leica MM Granularity
- Leica MM Live Dead
- Leica MM Mitotic Index
- Leica MM Monopole Detection



Leica MM AF configuration example with Leica DM6000. Leica MM AF is compatible with all Leica automated upright and inverted research microscopes

“With the user, for the user”
Leica Microsystems

Leica Microsystems operates globally 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.

• Medical Division

The Leica Microsystems Medical 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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