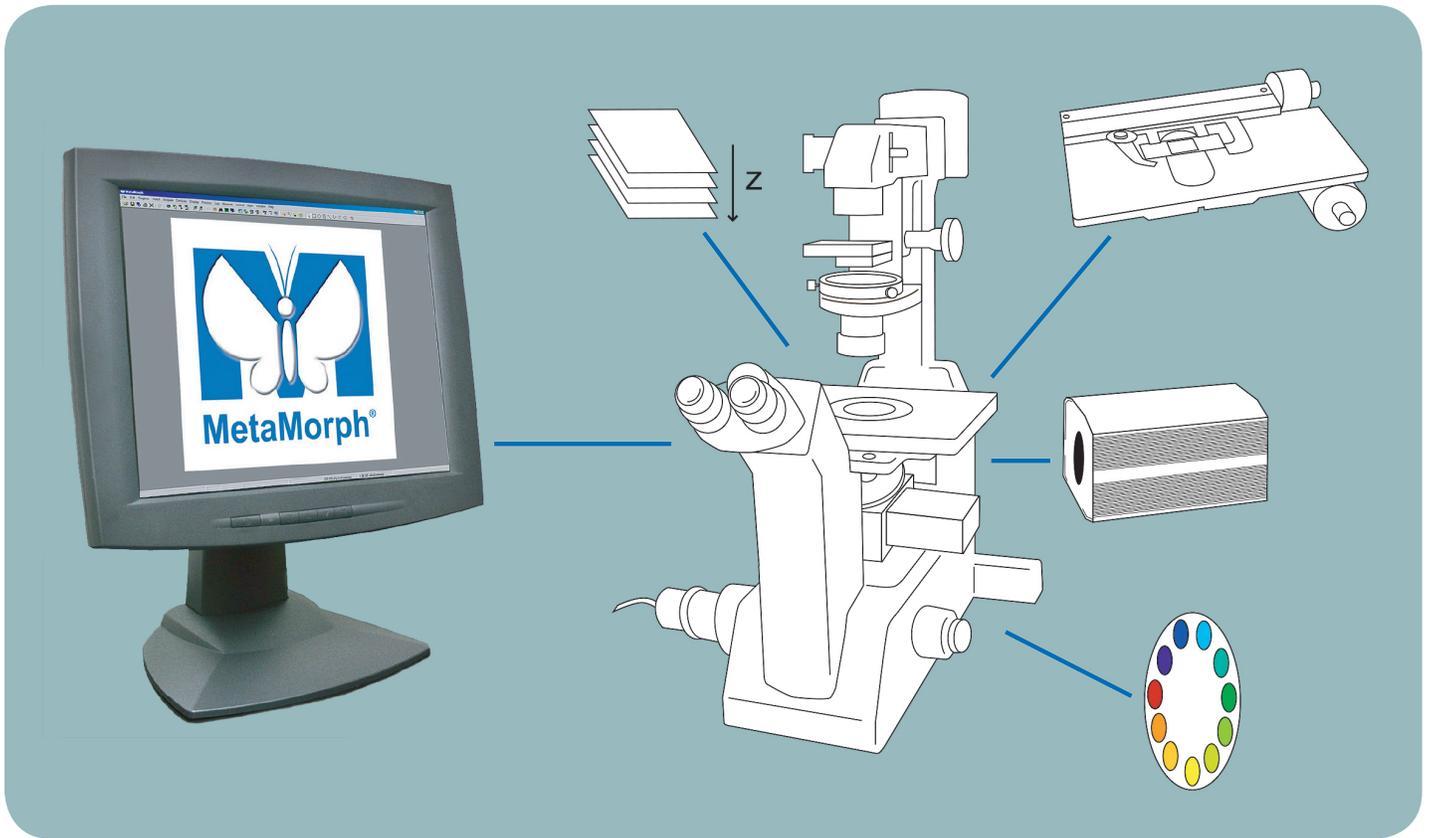




MetaMorph[®] Microscopy Automation and Image Analysis Software

An integrated system for bioimaging

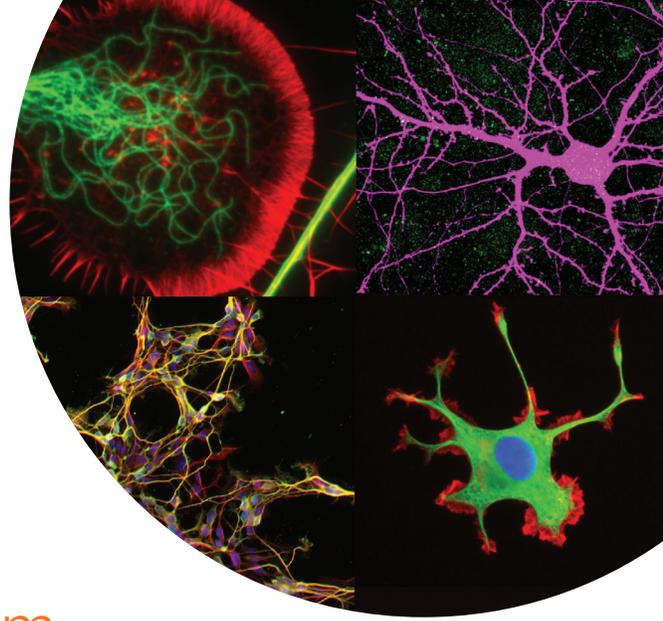


Bioimaging techniques contribute to a growing number of scientific breakthroughs.

The MetaMorph® Microscopy Automation and Image Analysis Software from Molecular Devices plays a large role in this revolution. With its image acquisition, processing and analysis capabilities, and complete set of tools for automation, MetaMorph software opens the door for new insights into cellular function.

MetaMorph software'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, MetaMorph software combines the speed, flexibility, and unmatched customer support required to get better results, faster.



An integrated imaging system for maximized control

Device automation for easy acquisition

MetaMorph software provides high-end control for devices including microscopes, filter wheels, shutters, cooled CCD cameras, video cameras, monochromators, focus motors and Piezo electric focus devices, motorized stages, digital and serial input/output, and robotic devices.

A complement to your confocal system

MetaMorph software is a great addition to your core facility's confocal station. With the MetaMorph Offline package, you can measure, analyze, and display multi-dimensional data acquired from a confocal system.

A variety of applications to suit your needs

Developed in conjunction with leading bioscience researchers, MetaMorph software offers tools for imaging applications such as:

- Multi-dimensional imaging
- 3D deconvolution
- 3D reconstruction
- Colocalization and brightness measurements
- Particle tracking and motion analysis
- Fluorescence, FRET, FRAP, and FISH
- Morphometry
- Multi wavelengths cell segmentation
- Neurite outgrowth
- Angiogenesis tube formation
- Time lapse
- Z-series
- And more

Custom configured for you

MetaMorph software is available in multiple custom configurations:

- **MetaMorph Basic Acquisition for Microscope** (Part no. MMACQMIC): Includes Camera Acquisition Option and Complete Microscope Control.
- **MetaMorph Advanced Acquisition** (Part no. MMADVACQ): Includes all Acquisition Options and Device Drivers.
- **MetaMorph Premier Acquisition** (Part no. MMPREACQ): Includes all Acquisition and Control Options and Device Drivers. Includes 5 Optional Application Modules.
- **MetaMorph Premier Plus Acquisition** (Part no. MMPREPLU): Includes all Acquisition and Control Options and Device Drivers. Includes all available Optional Application Modules except AutoQuant and Super Resolution Modules.
- **MetaMorph Offline Packages:**
The offline packages have any or all of the processing and analysis capabilities depending on the package without hardware control and is perfect for multi-user facilities. Available for Basic and Premier.
- **MetaMorph Network Premier Package:**
This offline package is available for three seats or more.

A powerful multi-dimensional imaging tool

MetaMorph software is optimized for multi dimensional experiments.

In addition to X and Y dimensions, you can acquire and display:

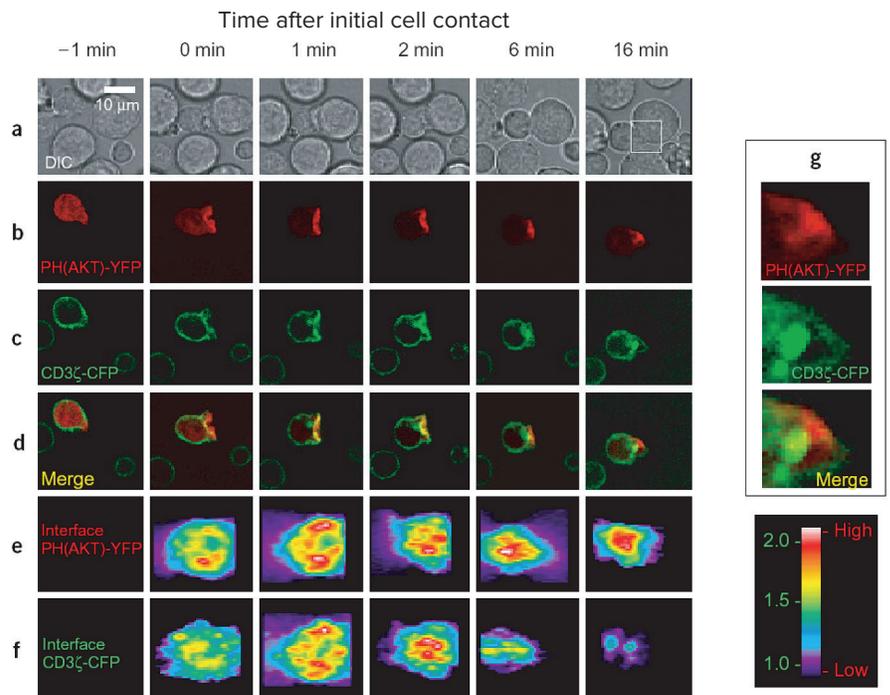
- Z-axis or multiple focus series (Z dimension)
- Multiple fluorochromes (Wavelength dimension)
- Time lapse (Time dimension)
- Multiple stage positions (Stage dimension)

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

For any multi-dimensional experiment, you can:

- Align images within a stack
- Create a montage
- Create and play a movie, export as 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
- Equalize light
- Create topographic surface maps
- Perform arithmetic operations
- View orthogonal planes
- Stitch a stack of images
- Visualize the experiment in 3 dimensions
- Obtain 3D measurements

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 $\alpha\beta$ TCR transgenic mice and infected with two batches of retroviruses expressing PH(AKT)-YFP and CD3 ζ -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), CD3 ζ -CFP (c) and their corresponding overlays (d). **(e,f)** Three-dimensional interface reconstructions of PH(AKT)-YFP (e) and CD3 ζ -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 CD3 ζ -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 CD3 ζ -CFP).



Continuous T cell receptor signaling required for synapse maintenance and full effector potential

Johannes B. Huppa^{1,2}, Michael Gleimer¹, Cenk Sumen^{1,3} and Mark M. Davis^{1,2}

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Observe changes over time

Intensity over time measurements are important in studies such as protein motility, FRAP, FRET, and protein-protein interactions.

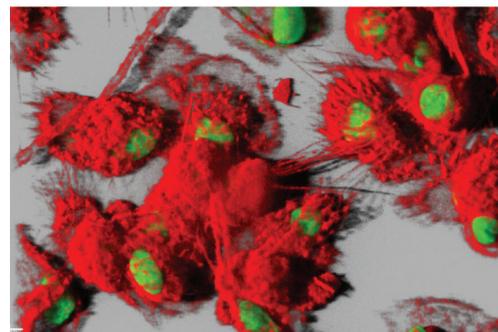
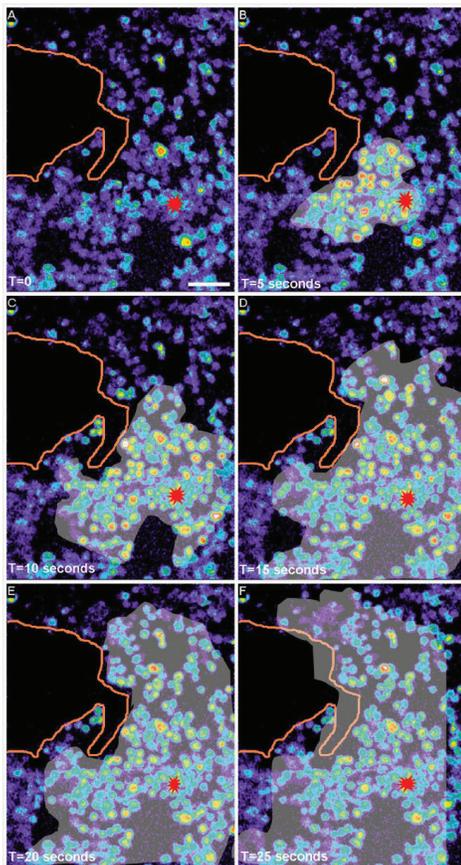
MetaMorph software facilitates time lapse acquisition by offering streaming as an acquisition option. With the appropriate devices, our patented streaming allows you to acquire at the maximum rate of the camera.

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 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.



Single time point of a time lapse series. Dendritic cells were surface labeled with an antibody to MHC class one and DRAC 5 to stain the nucleus. A data stack was collected using MetaMorph software.

Dendritic cells were loaded with FURA 2 AM and a scrape was made across the field of view using a fine micropipette tip. Following the scrape, the cells were left to stabilize for 10 minutes and then imaged using a Nikon 2000e2 microscope and MetaMorph software. The cells were poked with a micro injection tip. A calcium flux is seen to radiate out from the original cell across the dish and the flux is carried down the tunneling nanotubes. Results show that the flux did not cross the gap where the scrape occurred.

Dendritic cells loaded with FURA 2 AM

Simon C. Watkins Ph.D., University of Pittsburgh School of Medicine, Pittsburgh, PA 15213

Images courtesy of Simon C. Watkins Ph.D., Department of Cell Biology and Physiology and Department of Immunology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15213.

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.

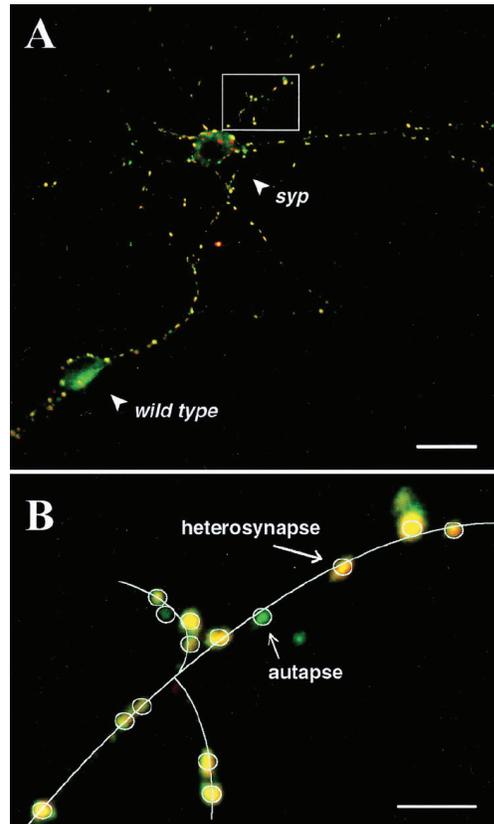
Colocalization tools provide a higher level of detail, with quantitative data regarding regions of overlap between two fluorescent probes.

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 MetaMorph software excels at providing this type of information.

With MetaMorph software, you can log intensity data from selected regions in an image stack or live video image over time and choose which parameters to capture.



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 μ m (A) and 5 μ m (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.

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

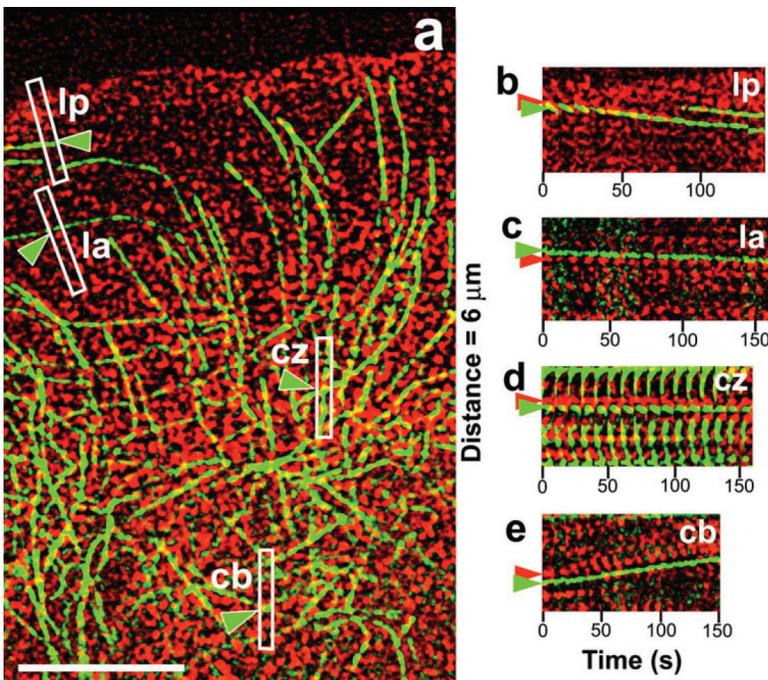
Leila Tarsa and Yukiko Goda (2002) Synaptophysin regulates activity-dependent synapse formation in cultured hippocampal neurons. *PNAS*. 99(2):1012-1016. © 2003 National Academy of Sciences, U.S.A.

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.

MetaMorph software 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.

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 μ m.

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

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The speed and precision needed for fluorescence

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

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

Live cell studies, such as Fluorescence Recovery After Photobleaching (FRAP) and Fluorescence Loss In Photobleaching (FLIP), 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.

MetaMorph software supports rapid shuttering for illumination control to minimize photobleaching before exposure to the laser light and while monitoring recovery. Maximal temporal resolution can be achieved with cameras that support streaming subsequent to laser illumination. The software is ideal for the analysis of live cell laser illumination experiments.

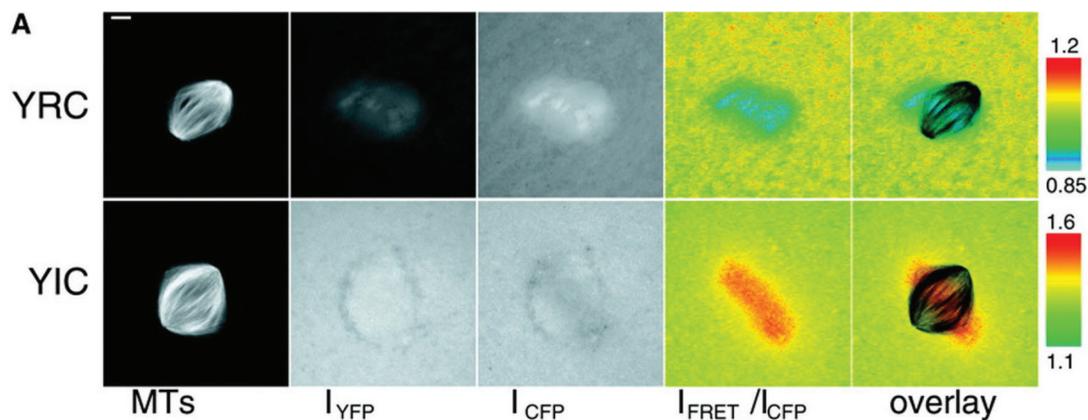
An ideal tool for FRET

Several key features make MetaMorph software a powerful platform for FRET imaging. First, FRET takes place at extremely low light levels and dark current noise must be minimized. The software supports highly-sensitive, cooled CCD cameras with high quantum efficiency (less noise) and fast readout rates.

Second, FRET images are taken at different wavelengths. The software makes it easy to handle automated wavelength devices and automatically aligns multiple images.

Third, speed is key to FRET experiments and the software meets this challenge with its support for multi-wavelength streaming using appropriate devices.

Finally, a FRET-specific dialog box automates the complex arithmetic needed to account for and correct fluorescent background and bleedthrough in your images.



A gradient of Ran-GTP surrounding chromosomes visualized in egg extracts and abolished by the addition of Ran mutants. Scale bars, 10 μ m.

(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.

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

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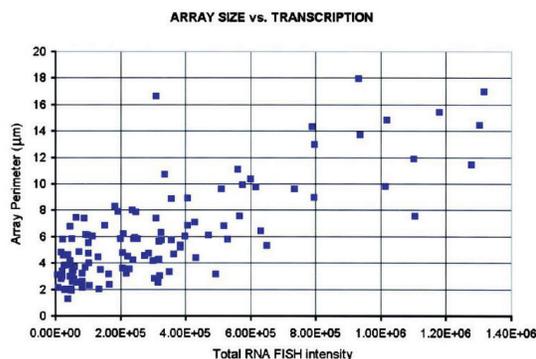
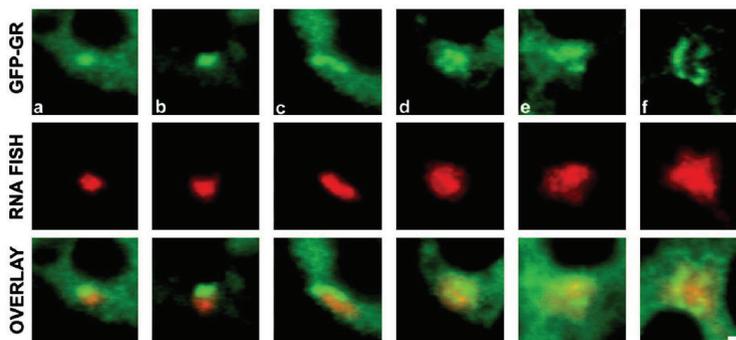
Count, classify, and measure multiple cell parameters

Modules for segmentation

Canned, application-specific analysis modules are available for MetaMorph software: 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.

MetaMorph software'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

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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

Minimum computer requirements

- Microsoft® Windows® 7 or 10
- 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

Microscope control options

- Most popular automated microscope models from major manufacturers Leica, Nikon, Olympus, and Zeiss
- Digital auto-focus
- XY stage device control for popular models from major manufacturers
- Z-axis device control for popular models from major manufacturers
- Piezo-actuated Z and XY device control
- Monochromator control for illumination
- Filter wheel and shutter control
- UniBlitz® shutters
- AOTF for laser control
- Liquid Crystal tunable filters
- Custom I/O (RS-232 serial and TTL)
- Custom drivers

Acquisition options

- Digital CCD and CMOS cameras, both monochrome and color, including: cooled, full frame, frame transfer, interline, back thinned, intensified and on-chip multiplication gain from major manufacturers
- Simultaneous acquisition from two cameras or control of an image splitting device for projection of two or four emission wave lengths onto a single camera (appropriate hardware required)
- Wavelength streaming and/or Z-axis streaming (patent pending)

For complete details of microscope control, acquisition, and other supported devices, consult our website at

<http://support.metamorph.com>.

Support

- Technical support via phone, e-mail, or online at <http://support.metamorph.com>
- Electronic documentation

For complete details of features and options, consult our website at www.metamorph.com

Basic Configuration Standard Features

- 8-, 16-, 24-, 48-bit image and stack display and processing, including: over 15 morphology operators, arithmetic operations, Fast Fourier Transform (FFT) processing, shading correction, and background subtraction
- 3D reconstruction
- FRET
- Auto expose from digital cameras
- Time lapse acquisition
- Spectral scan acquisition
- Z-series acquisition (with Z-motor driver)
- 2D deconvolution
- Morphometry and distance measurements
- Data logging and exporting
- Automation through journals and taskbars
- Customizable toolbars and windows
- Multi-dimensional acquisition
- Overlay multi-fluorescent images
- Colocalization and correlation measurements
- Premier configuration standard features and optional modules can also be purchased individually for Basic configuration

Premier Configuration Standard Features

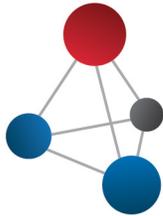
- Includes all Basic Configuration Standard Features
- Scan slide to scan and stitch together multiple fields of view
- Stitching of individual images
- 4D viewer – 3D measurements
- Motion analysis and particle tracking
- Includes a selection of 5 optional modules

Optional modules

- Custom module analysis
- Screening acquisition
- Screening playback
- Multi-dimensional motional analysis
- Angiogenesis
- Cell cycle
- Cell health
- Cell scoring
- Count nuclei
- Granularity
- Live/dead
- Micronuclei
- Mitotic index
- Monopole detection
- Multi Wavelength Cell Scoring (MWCS)
- Neurite outgrowth
- Transfluor
- Translocation

Additional non-standard modules

- 3D deconvolution
- Super resolution



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