Molecular Devices

The MetaMorph® System

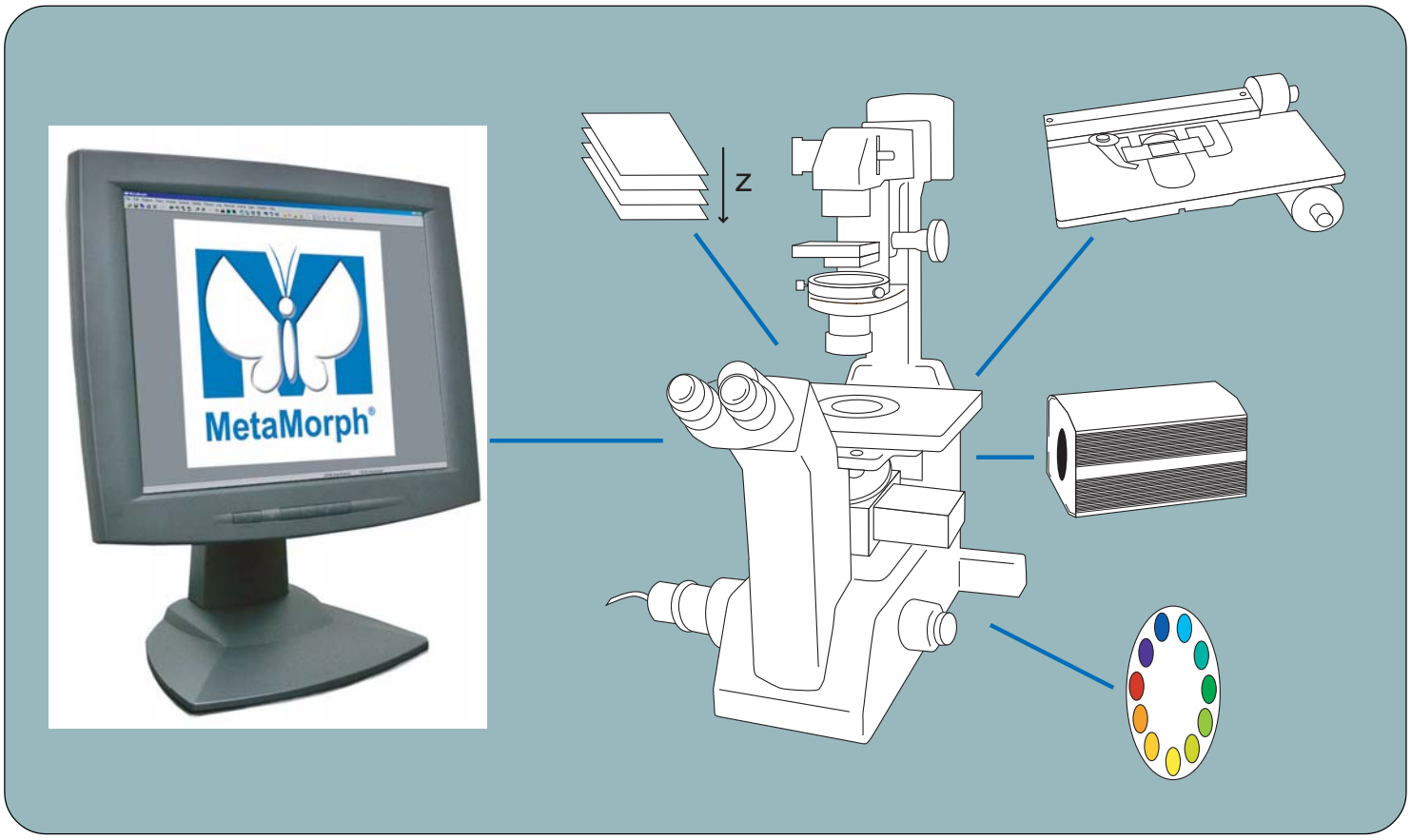
INTEGRATED SYSTEM FOR BIOIMAGING

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

MetaMorph'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 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 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 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 offers tools for imaging applications such as:

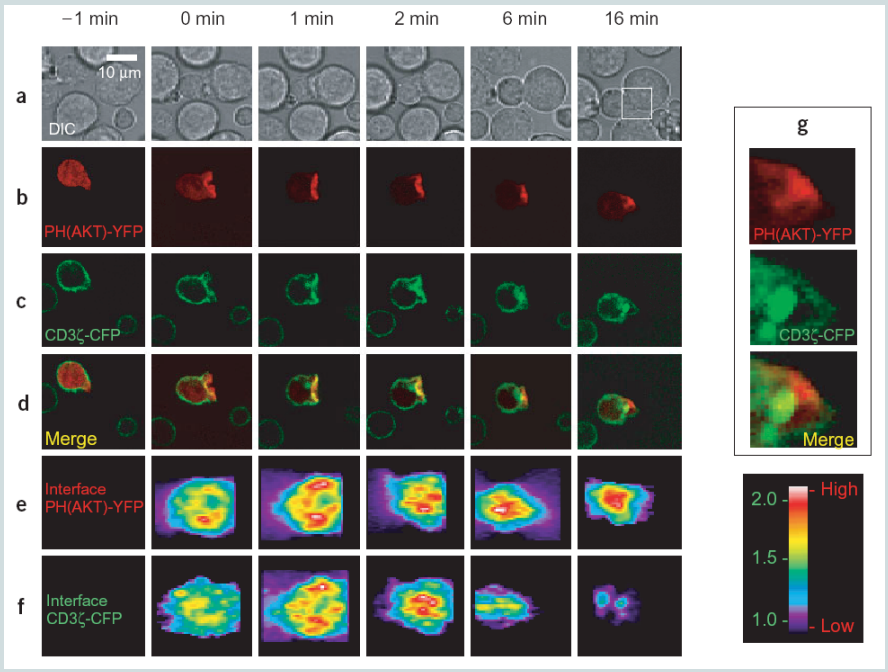
- → Multi-dimensional imaging
- → 3D deconvolution
- → 3D reconstruction
- → Colocalization and brightness measurements
- → Particle tracking and motion analysis
- → Fluorescence, FRET, FRAP, and FISH
- → Morphometry
- → Multi wavelengths cell segmentation
- → Neurite outgrowth
- → Angiogenesis tube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

CUSTOM CONFIGURED FOR YOU

MetaMorph is available in three custom configurations:

- → **MetaMorph Premier** has all the device drivers (camera drivers optional) and many of the advanced processing capabilities built in for maximum flexibility.
- → **MetaMorph Basic** has a robust set of image acquisition, processing and analysis tools built in, for a more cost-effective solution.
- → **MetaMorph Offline** has any or all the analysis capabilities of either Basic or Premier without external devices control, perfect for multi-user facilities.

a powerful multi-dimensional imaging tool



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]
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[2] Howard Hughes Medical Institute, Stanford, CA 94305
[3] Center for Blood Research, Harvard Medical School, Boston, MA 02115

Figure 2: 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).

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

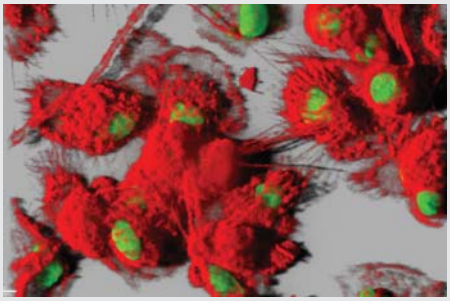
- → Z-axis or multiple focus series (Z dimension)
- → Multiple fluorochromes (Wavelength dimension)
- → Time lapse (Time dimension)
- → Multiple stage positions (Stage dimension)

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 MetaMorph toolbar, displaying current illumination, magnification and XYZ location settings. MetaMorph'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 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
- → Equalize light
- → Create topographic surface maps
- → Perform arithmetic operations
- → View orthogonal planes
- → Stitch a stack of images
- → Visualize the experiment in 3 dimensions and obtain 3D measurements

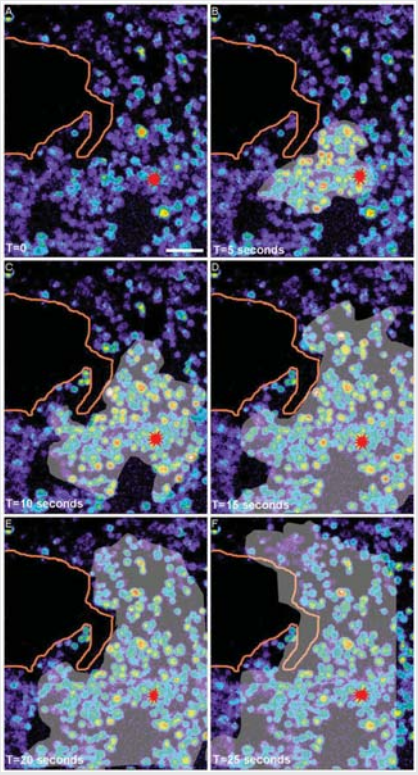
observe changes over time



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 controlling the PerkinElmer UltraVIEW™.

Dendritic cells loaded with FURA 2 AM
Simon C. Watkins Ph.D.
University of Pittsburgh School of Medicine, Pittsburgh, PA 15213

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. 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 nanotubules. Results show that the flux did not cross the gap where the scrape occurred.



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

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

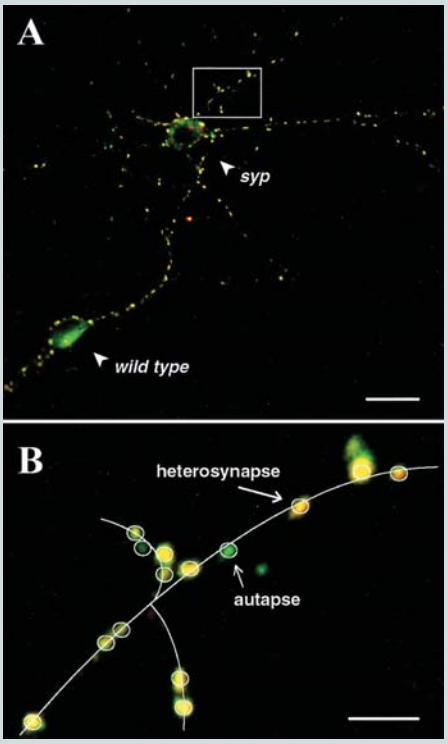
Another feature for time lapse is the Live Replay option. With appropriate devices, and when viewing live images, you can press a key when an interesting event occurs and capture a stack containing some past history of the event as well as some data after the event happened.

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.

plot colocalization and brightness measurements for visual representations



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.

While good experiment data can be obtained by analyzing a single fluorescent probe, you often get better results by examining more complex interactions. MetaMorph'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 MetaMorph excels at providing this type of information.

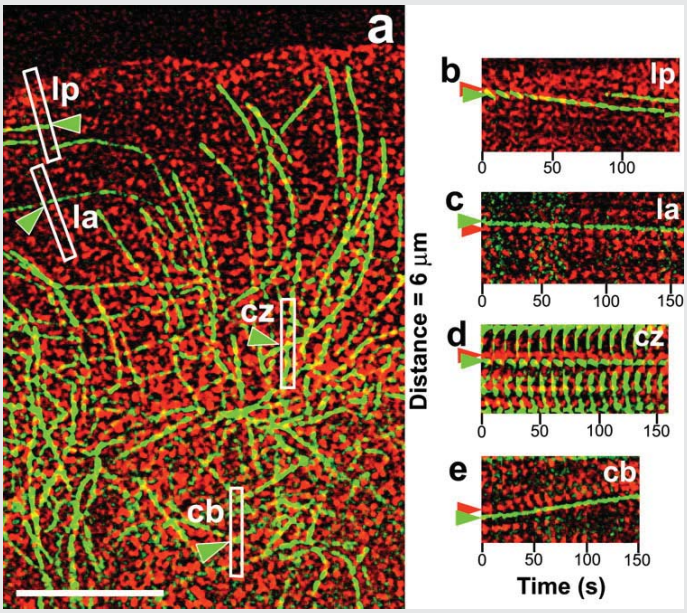
With MetaMorph, you can log intensity data from selected regions in an image stack or live video image over time and choose which parameters to capture.

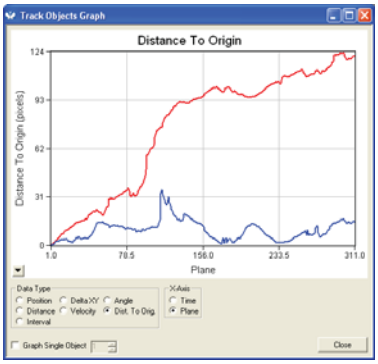
algorithms for particle tracking and motion analysis

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 2. 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 X-rhodamine 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.





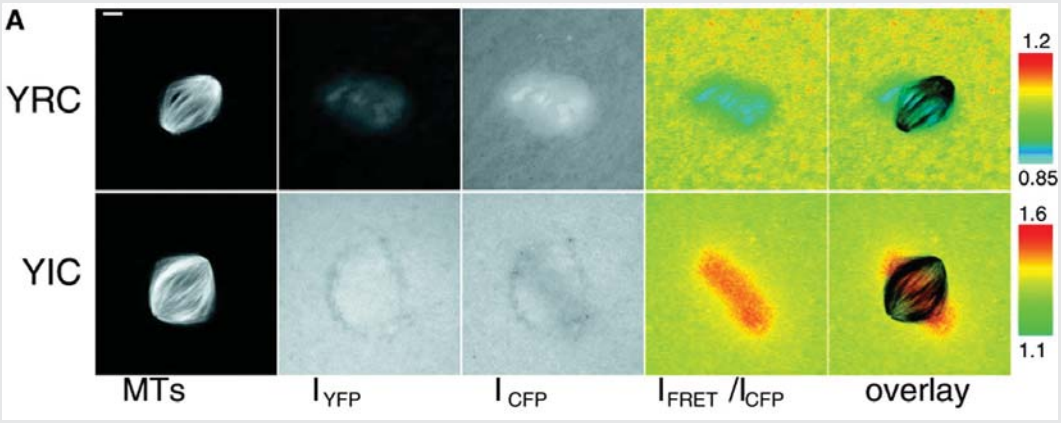
Sample display of captured data as a graph.

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 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.

the speed and precision needed for fluorescence



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 3. 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 I_{YFP}, I_{CFP}, and FRET ratio (I_{FRET}/I_{CFP}) 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 I_{CFP} in regions where FRET occurs.

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 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 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. MetaMorph is ideal for the analysis of live cell laser illumination experiments.

AN IDEAL TOOL FOR FRET

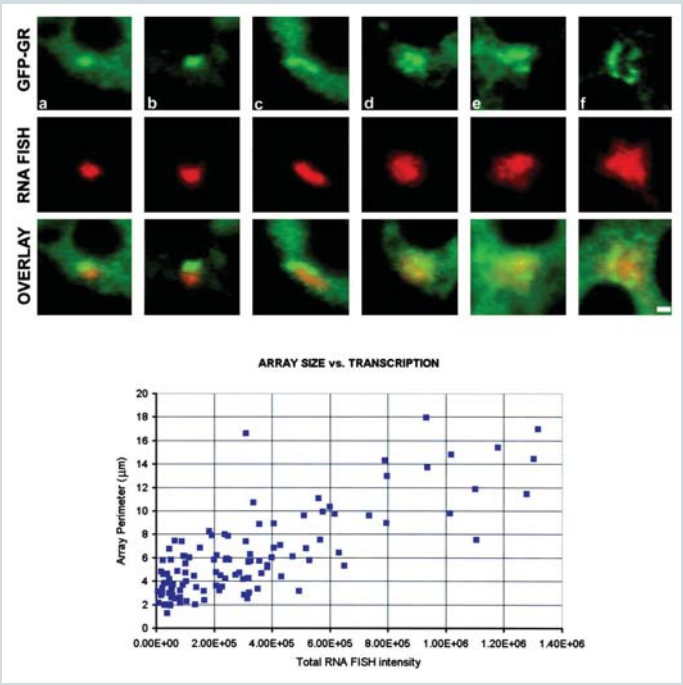
Several key features make the MetaMorph system a powerful platform for FRET imaging. First, FRET takes place at extremely low light levels and dark current noise must be minimized. MetaMorph supports highly-sensitive, cooled CCD cameras with high quantum efficiency (less noise) and fast readout rates.

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

Third, speed is key to FRET experiments and MetaMorph 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.

count, classify and measure multiple cell parameters



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 7. 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.

MODULES FOR SEGMENTATION

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

technical summary

MINIMUM COMPUTER REQUIREMENTS

- Computer with Intel® Pentium 4 processor
- Microsoft® Windows® 2000 or 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

MICROSCOPE CONTROL OPTIONS

- Popular automated microscope models from major manufacturers
- 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 parallel)

ACQUISITION OPTIONS

- Digital CCD cameras, both monochrome and color, including: cooled, full frame, frame transfer, interline, back thinned, intensified and on-chip multiplication gain from major manufacturers
- Video cameras, both monochrome and color, including: RS-170, CCIR, on-chip integration, intensified, CCD and tube from major manufacturers
- Simultaneous acquisition from two cameras or control of an image splitting device for projection of two or four emission wavelengths onto a single camera (appropriate hardware required)
- Wavelength streaming and/or Z-axis streaming (patent pending)

For complete details of compatible microscopes, cameras and other supported devices, consult our website at support.universal-imaging.com.

STANDARD FEATURES

(with MetaMorph Premier and Basic systems)

- 8-, 16-, 24-, 48-bit image and stack display and processing, including: morphology operators, arithmetic operations, Fast Fourier Transform processing, shading correction and background subtraction
- 3D reconstruction
- 2D deconvolution
- Cell counting
- 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

OPTIONAL FEATURES FOR BASIC SYSTEMS

(standard with MetaMorph Premier systems)

- Multi-dimensional imaging
- Overlay multi-fluorescent images
- Image stitching
- Motion analysis and particle tracking
- Colocalization and correlation measurements
- Angiogenesis, Neurite Outgrowth, Count Nuclei/Cell Scoring, Cell Cycle and Multi Wavelength Cell Scoring Application Modules
- Live Replay
- Automated Stage Scanning

ADDITIONAL MODULES

(not standard)

- 3D deconvolution
- 4D visualization and 3D measurements
- Cell Health, Granularity, Live/Dead, Mitotic Index, Monopole Detection Application Modules
- Network licenses

SUPPORT TOOLS

- Support site: support.universal-imaging.com
- Electronic documentation
- Interactive Tutorial CD

For the latest features and options, visit our website at www.moleculardevices.com.

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