

# the MetaMorph<sup>®</sup> system

INTEGRATED SYSTEM FOR BIOIMAGING



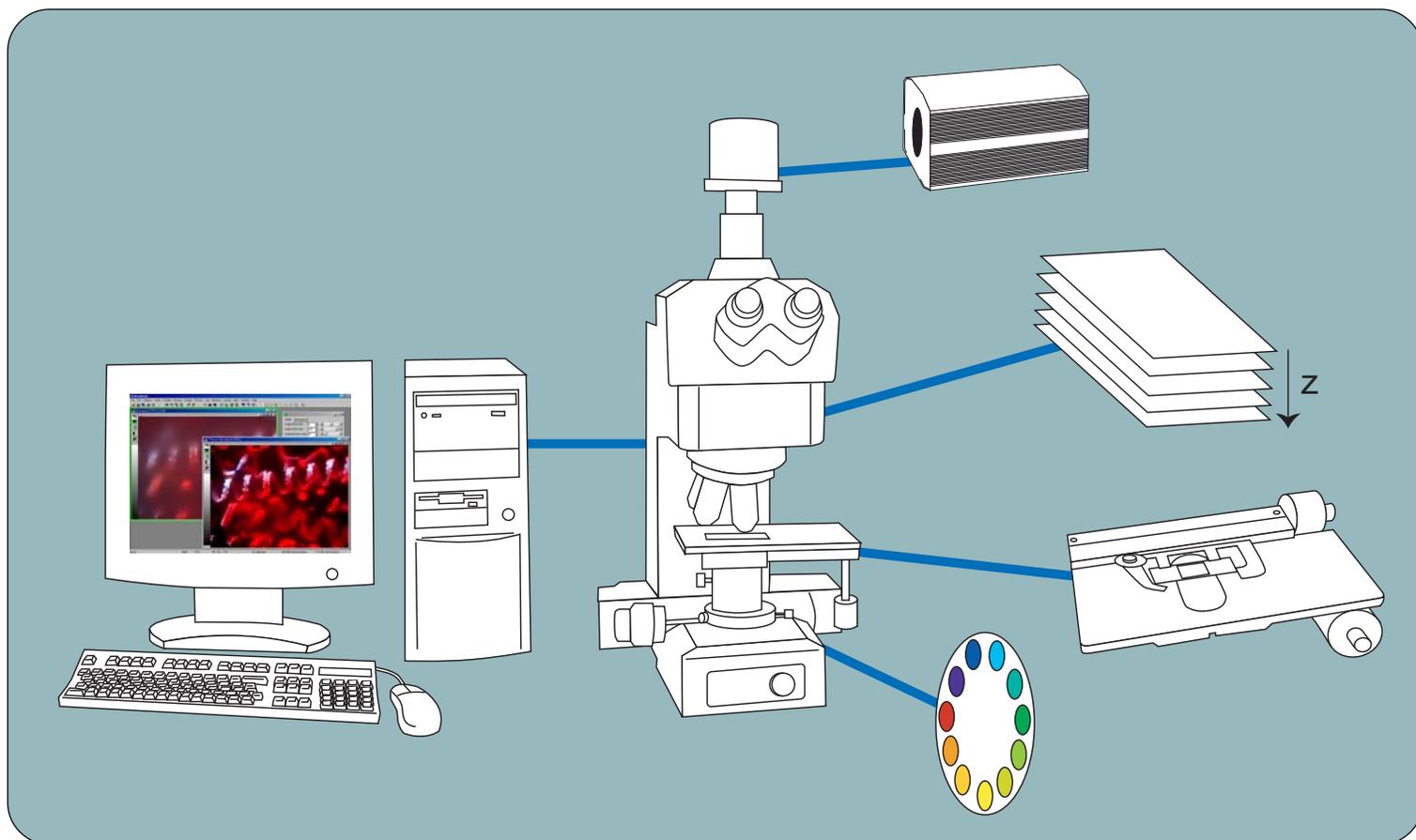
Bioimaging techniques contribute to a growing number of scientific breakthroughs.

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:

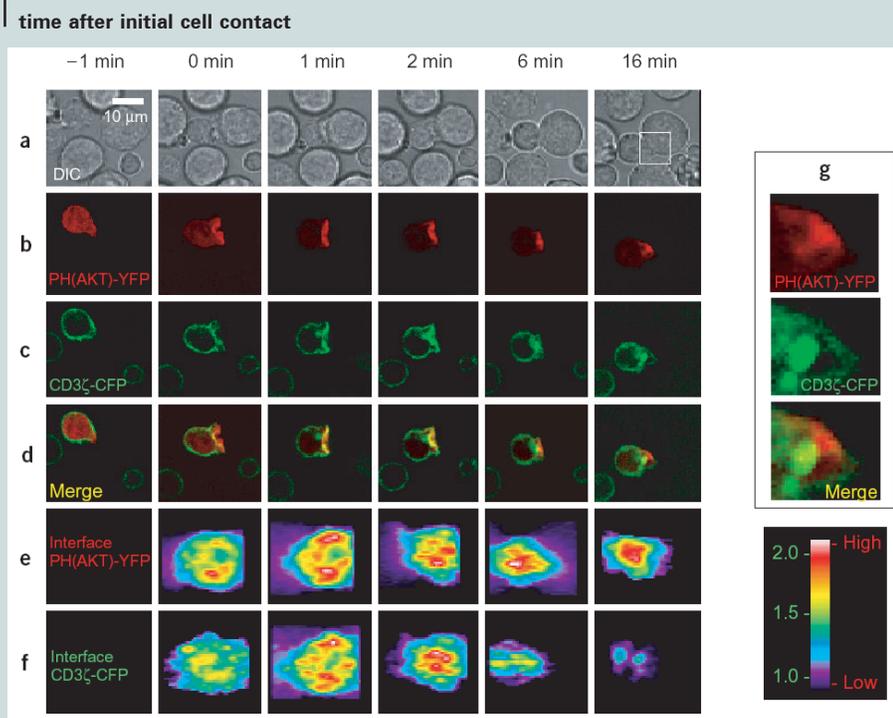
- Multi-dimensional imaging
- 3D deconvolution
- 3D reconstruction
- Colocalization and brightness measurements
- Particle tracking and motion analysis
- Fluorescence, FRET, FRAP, and FISH
- Morphometry
- Cell counting
- Time lapse
- Z-series, and more

### CUSTOM CONFIGURED FOR YOU

MetaMorph is available in three custom configurations:

- **MetaMorph Premier** has all the device drivers (camera drivers optional) and most 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.
- **MetaMorph Offline** has any or all the analysis capabilities of either Basic or Premier without external devices control, perfect for multi-user facilities with multiple MetaMorph systems.

## a powerful multi-dimensional imaging tool



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

Johannes B. Huppa<sup>1,2</sup>, Michael Gleimer<sup>1</sup>, Cenk Sumen<sup>1,3</sup>, and Mark M. Davis<sup>1,2</sup>

<sup>1</sup>Stanford University School of Medicine, Department of Microbiology and Immunology

<sup>2</sup>Howard Hughes Medical Institute, Stanford, CA 94305

<sup>3</sup>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  $\alpha\beta$  TCR transgenic mice and infected with two batches of retroviruses expressing PH(AKT)-YFP and CD3 $\zeta$ -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  $\mu$ 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 $\zeta$ -CFP (c) and their corresponding overlays (d). (e,f) Three-dimensional interface reconstructions of PH(AKT)-YFP (e) and CD3 $\zeta$ -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 $\zeta$ -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 $\zeta$ -CFP).

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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)

Keep lengthy time-dependent events in focus with MetaMorph's customizable auto-focus capabilities.

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

## increase image information with deconvolution and segmentation tools

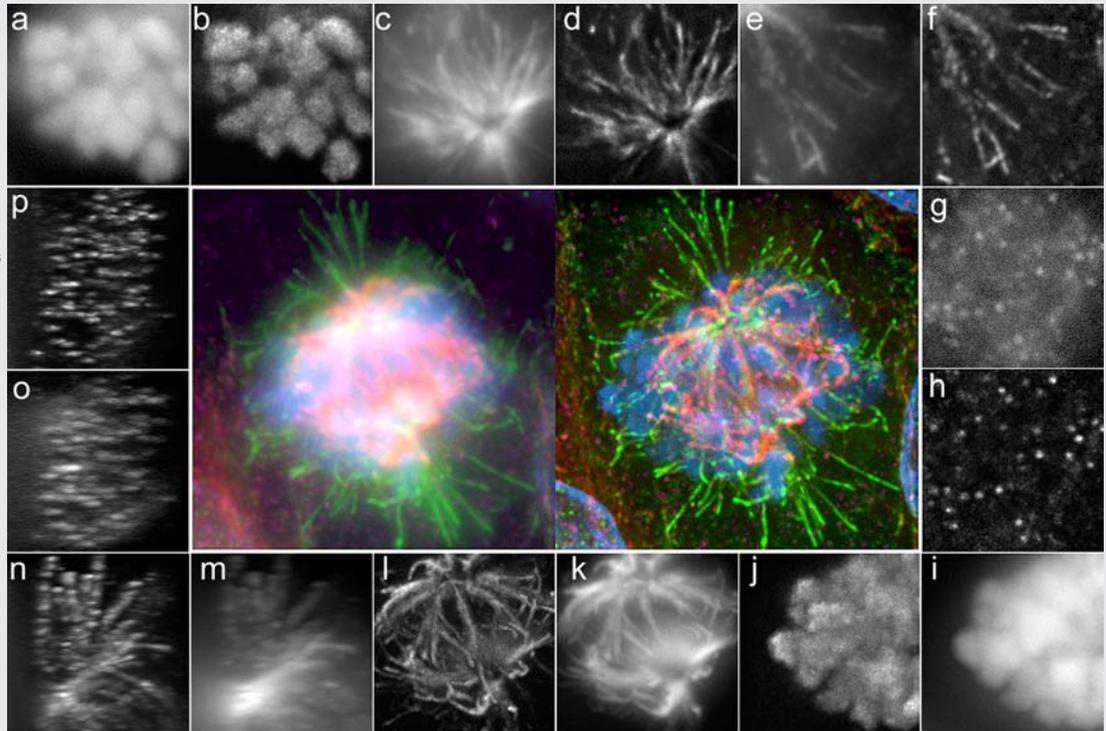
### 3D deconvolution of mitotic HeLa cell rendered with PSF-based deconvolution module

Fabrice Cordelières and Jean-Baptiste Sibarita  
Institut Curie, Research Division, France

The mitotic spindle participates in the correct distribution of the chromosomes (blue and centromeric regions in magenta) between the two daughter cells. It is composed of tubulin (red) from which astral microtubules expand toward the cell cortex (as shown in green, detection of the dynein/dynactin motor complex).

Center color images: 3D reconstruction before (**left**) and after (**right**) deconvolution.

Surrounding images: Chromosomes before (**a, i**) and after (**b, j**) deconvolution, tubulin before (**c, k**) and after (**d, l**) deconvolution, astral microtubules before (**e, m**) and after (**f, n**) deconvolution, and centromeres before (**g, o**) and after (**h, p**) deconvolution.



Images courtesy of Fabrice Cordelières and Jean-Baptiste Sibarita, Institut Curie, Research Division, France.

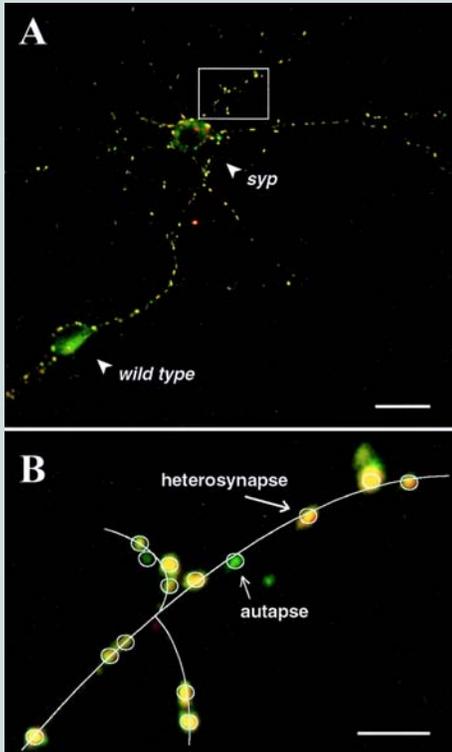
Out-of-focus intensities are present in all acquired images. These intensities can be accounted for by observing the behavior of light originating in a point source and passing through the microscope optics.

This behavior is described by the Point Spread Function (PSF). It can be used to quantitatively compensate for the blurring of images due to out-of-focus information. This process is called deconvolution.

MetaMorph's deconvolution module helps you improve images by reassigning out-of-focus intensities back to the spatial locations they originated in.

The results are images with sharper definition and lower background, better contrast, and improved signal-to-noise ratio. You can then use a number of image processing, segmentation and enhancement features to facilitate measurements.

## plot colocalization and brightness measurements for visual representations



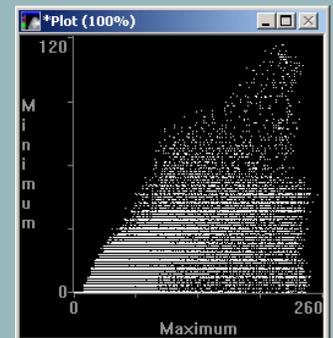
### Synaptophysin regulates activity-dependent synapse formation in cultured hippocampal neurons

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 heterotypic 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  $\mu\text{m}$  (A) and 5  $\mu\text{m}$  (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.

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.



Sample correlation plot shows coefficient of 0.6653.

While good experimental 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.

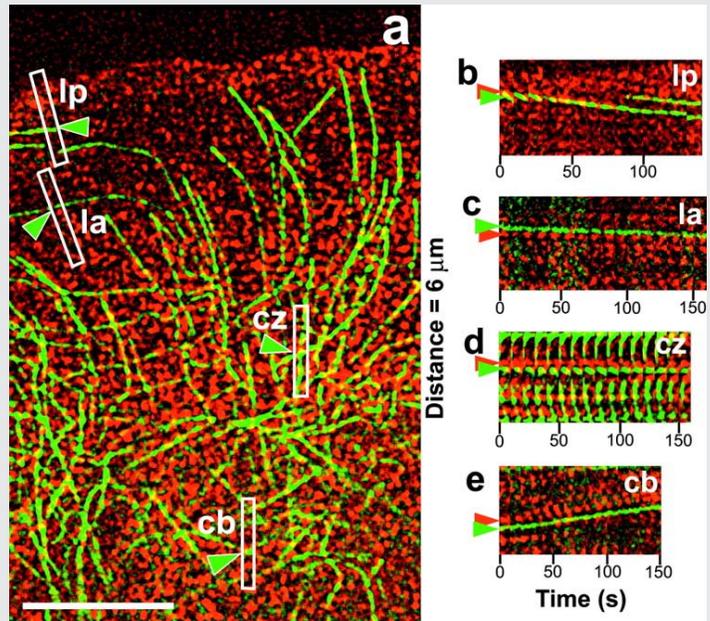
With MetaMorph, you can log intensity data from selected regions in an image stack or live video image over time and choose which parameters to capture.

## algorithms for particle tracking and motion analysis

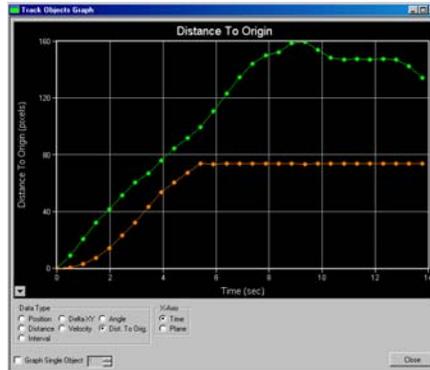
### Dual-wavelength fluorescent speckle microscopy reveals coupling of microtubule and actin movements in migrating cells

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  $\mu\text{m}$ .



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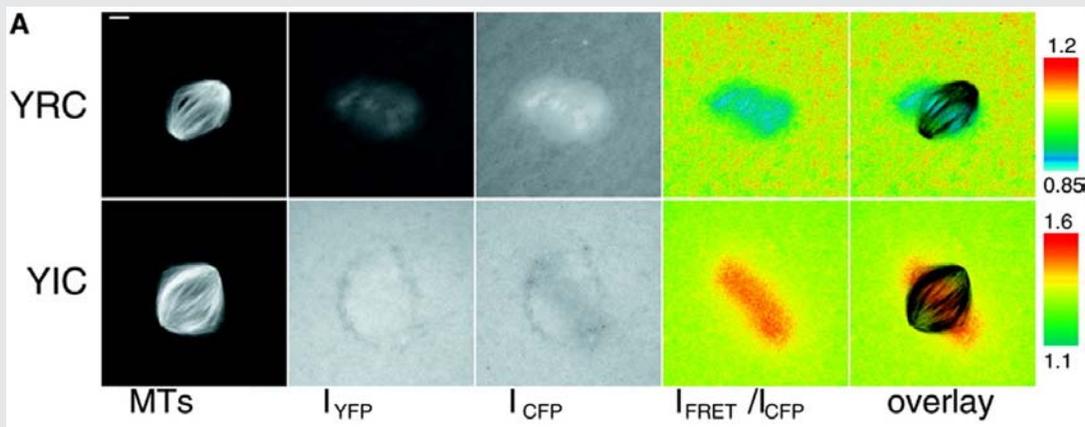
Sample display of captured data as a graph.

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  $\mu\text{m}$ . (A) Fluorescence images of mitotic spindles showing microtubules (MTs) and  $I_{\text{YFP}}/I_{\text{CFP}}$  and FRET ratio ( $I_{\text{FRET}}/I_{\text{CFP}}$ ) 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  $I_{\text{CFP}}$  in regions where FRET occurs.

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### FLUORESCENCE, FRAP, FISH

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

### AN IDEAL TOOL FOR FRET EXPERIMENTS

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.

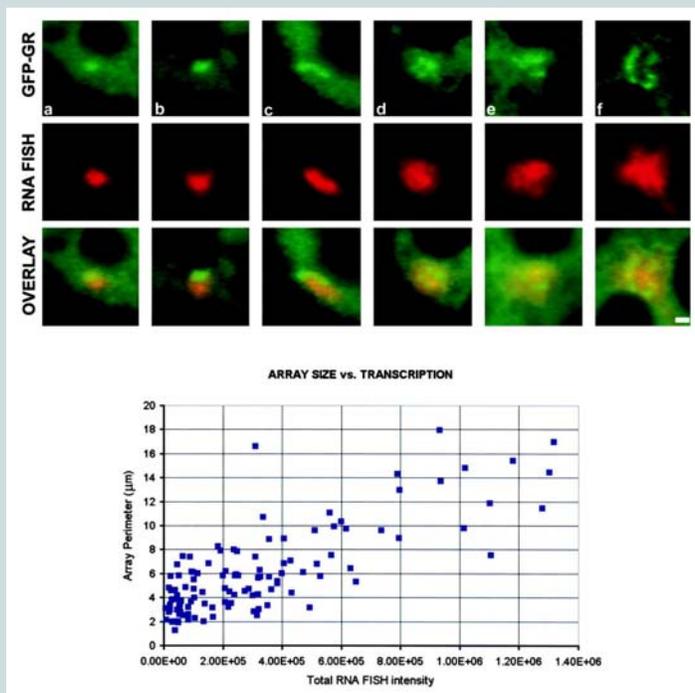
## count, classify and measure multiple cell parameters

### 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  $\mu\text{m}$ .



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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

### COMPUTER REQUIREMENTS

- Intel® Pentium-3 or -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

- Most popular automated microscope models from 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 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 microscope control, acquisition, and other supported devices options, consult our website at

[support.universal-imaging.com/hardware.cfm](http://support.universal-imaging.com/hardware.cfm)

### STANDARD FEATURES

- (with MetaMorph Premier and Basic systems)
- 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

### 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

### ADDITIONAL MODULES

(not standard)

- 3D deconvolution
- Advanced 3D visualization

### SUPPORT

- Technical support via phone, e-mail or online at [support.universal-imaging.com](http://support.universal-imaging.com)
- Electronic documentation



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