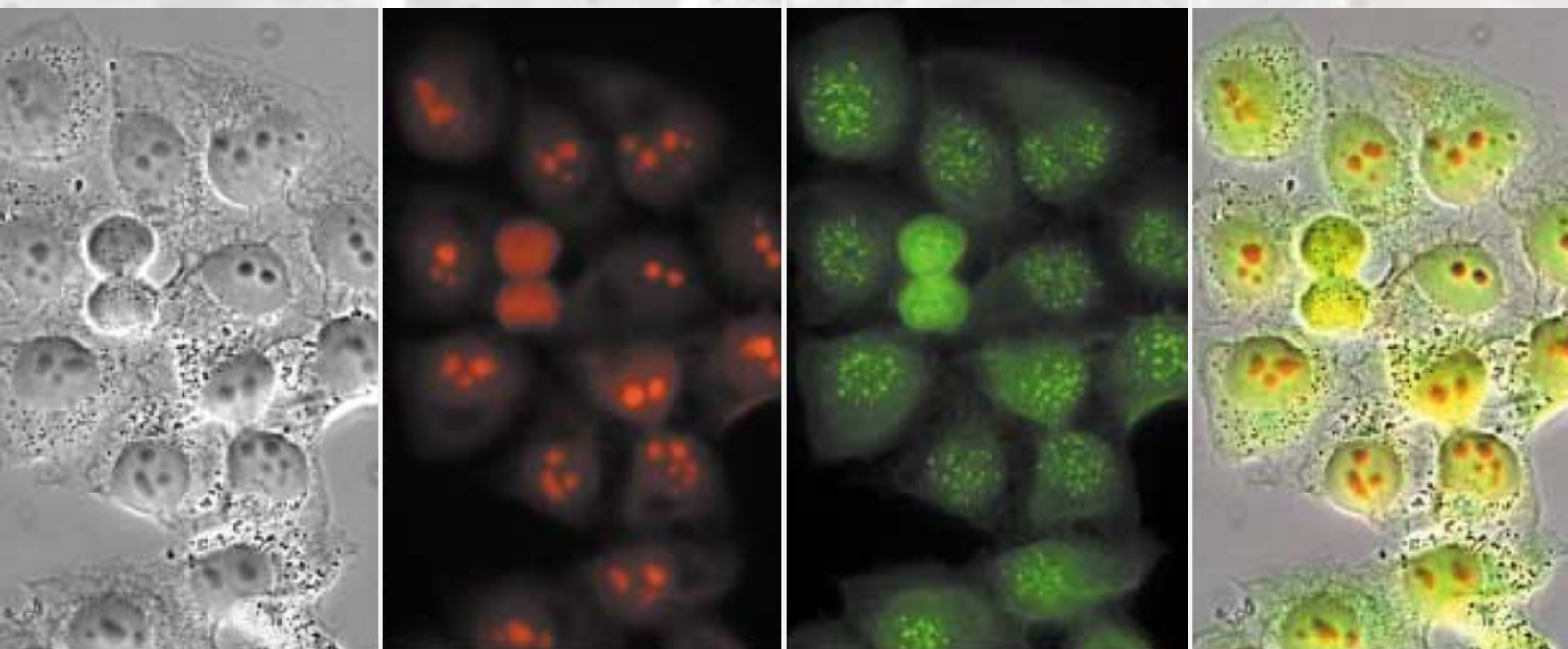


Integrated System for
Biological Imaging



MetaMorph[®] Imaging System



CHO cells shown (l-r) in Phase Contrast, Rhodamine, RFP and GFP Channels and Overlaid

Image Acquisition, Processing and Analysis



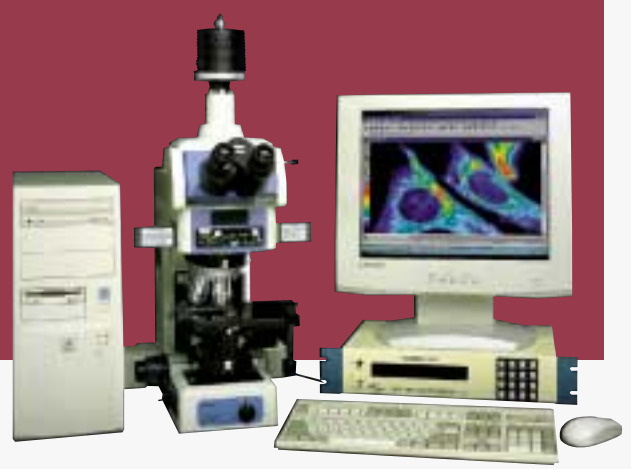
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UNIVERSAL IMAGING CORPORATION

Universal Imaging Corporation was established in 1983 with the goal of developing powerful yet affordable imaging systems for use in basic biological research. Today, MetaMorph continues to demonstrate our commitment to that goal.



MetaMorph® Imaging System

The MetaMorph imaging system is a powerful and affordable integration of software and hardware from Universal Imaging Corporation that enables scientific researchers to automatically capture and analyze images from digital and video microscopy.

Automated image processing and analysis

The MetaMorph system is especially valuable in biological studies that involve live cell imaging. Developed in conjunction with leading bioscience researchers, the system offers an array of tools.

The system offers an array of tools for image acquisition, processing and analysis. It enables operations such as time-lapse, multi-dimensional acquisition and 3-D reconstruction, and makes measurements such as morphometry, quantitation, colocalization, and brightness.

Sophisticated automation features such as image stacks, a macro function and morphometric classification enable unattended operation. Drivers are available to control automated microscopes, filter wheels and shutters, monochromators, focus motors and Piezo electric focus devices, motorized stages, robotic peripherals, and most digital or serial input/output devices. The system can employ monochrome and color cooled CCD digital and video cameras, as well as TWAIN compliant devices.

Sales, service and support in a complete package

Universal Imaging's resellers and representatives are trained to evaluate a researcher's imaging needs, and to configure a fully integrated MetaMorph system to meet those needs.

As part of this evaluation process, an on-site demonstration is often conducted using the customer's samples and fully functioning MetaMorph imaging software controlling such devices as digital cameras, filter wheels, illumination sources, focus motors, and stages.

Our resellers and representatives have the experience to help customers select components best suited for their research and budget. On-site installation and application-specific training are provided with each sale, and phone support staffed by Ph.D.s and seasoned imaging specialists is included.

Universal Imaging...responding to the research community's needs

The technologists at Universal Imaging Corporation maintain close, long-term relationships with leading scientists around the globe to stay uniquely responsive to the imaging needs of the research community. To ensure that the most advanced tools and technology are incorporated into its products, the company invests heavily in bio-imaging R&D.

Since its inception in 1983, Universal Imaging has provided personnel and equipment to the many microscopy courses conducted each year at the Marine Biological Laboratory in Woods Hole, Massachusetts. With its staff of application specialists, the company also supports courses at New York's Cold Spring Harbor Laboratory and numerous other microscopy and imaging courses, seminars, and workshops worldwide.

The company's other products

MetaMorph® Offline is a low-cost image processing system designed for multi-user facilities. The Offline system has all the analytical and processing capabilities of the complete MetaMorph system, but without the advanced image acquisition or hardware control. To meet the needs of multiple users, the full MetaMorph system is installed to acquire images, and then several Offline systems can be added for post-acquisition processing.

MetaFluor® is specifically designed for single or dual wavelength intracellular ion (FURA2, BCECF, INDO, etc.) measurements.

My group has been very happy with the MetaMorph system for a variety of imaging applications. Until recently we used home-written software, but we decided we needed a program with professional support. MetaMorph worked right out of the box for a number of different microscope configurations. The software has been very stable, and new users get up to speed quickly. I am happy with the speed with which drivers are produced for new hardware products, and the compatibility of MetaMorph's image file architecture with other imaging programs. We are using a modified version of the MetaMorph system for high throughput imaging applications (collection and analysis of up to 5,000 images per day). Overall the system has worked very well and performed stably in this challenging application.

Dr. Timothy J. Mitchison
Harvard Medical School

Acquire Over Time, Z-Distance and Wavelength

Scientists, striving for excellence, know the importance of building a strong foundation of research fundamentals. The MetaMorph imaging system delivers excellence with a reliable foundation of tools for image acquisition.

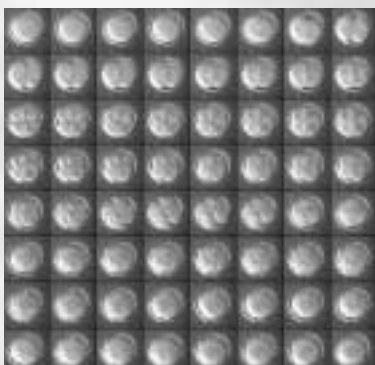
The system can acquire images over a defined period of time, a range of Z-axis focal distances (Z-distance), a range of excitation wavelengths or any combination of these three parameters. Using one or all of these acquisition methods, users can make measurements of region intensity levels over a series of images.

In the *Acquire Timelapse* dialog box, the system asks for the number of frames to acquire as well as the desired amount of time between them and the type of image storage required. MetaMorph also can be configured to ask for an acquisition subregion, the type of autoscaling required and other preferences.

During acquisition of images over a Z-distance, the MetaMorph system provides support for Z-axis focus motors. When acquiring a range of excitation wavelengths (also known as a spectral scan), MetaMorph supports monochromators and other devices that utilize continuous wavelength control. The system prompts the user to select starting and ending wavelength as well as wavelength increment and storage options.

Video Acquisition

With the *Acquire from Flashbus* command, the MetaMorph system can acquire images, background reference images, and shading reference images from a video camera. Easy-to-use interfaces enable integration and averaging of both the “live” display after background subtraction and of acquired frames.



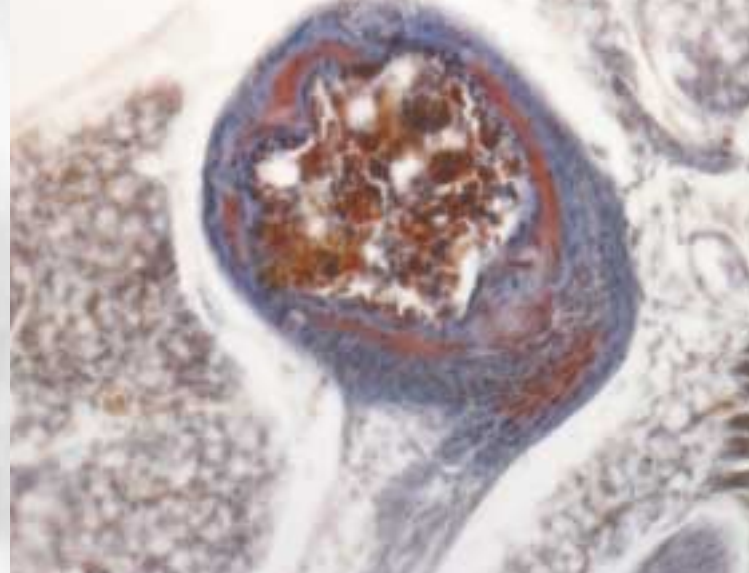
Early Ascidian development shown using Differential Interface Contrast Timelapse Microscopy

can be acquired at 30 fps. Digital camera images can be acquired at rates exceeding 100 fps (camera and acquisition region limited).

Combining its most powerful acquisition modes and available hardware, the MetaMorph system can acquire stacks of Z-distance images and stream them directly into RAM at fast rates. Coupled with the Piezo electric focusing device, this Z-streaming application dramatically reduces experimentation time.

Stream Acquisition

In *Stream Acquisition* mode, the MetaMorph system transmits image data at top speed, directly from the digital camera into the computer's RAM. Once all of the requested frames in this bit-stream have been acquired, MetaMorph performs data logging tasks and builds an image stack for easier processing and analysis. Video images



Acquired using the Photometrics CoolSnap™ digital camera

Cooled CCD Cameras

With their high resolution and broad dynamic range, cooled CCD cameras deliver the best results for most light microscopy applications. The MetaMorph system supports a wide variety of scientific grade monochrome and color cooled CCD cameras, and has an intuitive set of control panels that simplify operations for users. Supported cameras include models with frame transfer or back-thinned chips, which are high sensitivity, low noise devices.



Using MetaMorph's *Acquire* command, operators can set exposure time, region for the acquisition, binning type used, gain, camera speed, bit depth, shutter state, if an external shutter is used, and other parameters specific to camera type and model.

To improve visualization, operators can set a variety of parameters including background subtraction, shading correction, and scaling.

Video Cameras

The MetaMorph system acquires and displays images from a video camera connected to the Flashbus video acquisition board.

Color or monochrome VCRs, NTSC or PAL cameras, intensifiers and other devices can be used for video input.

The Flashbus frame grabber board supports the use of RS-170, CCIR, RGB, Composite, and S-Video input signals.

TWAIN Compliant Devices

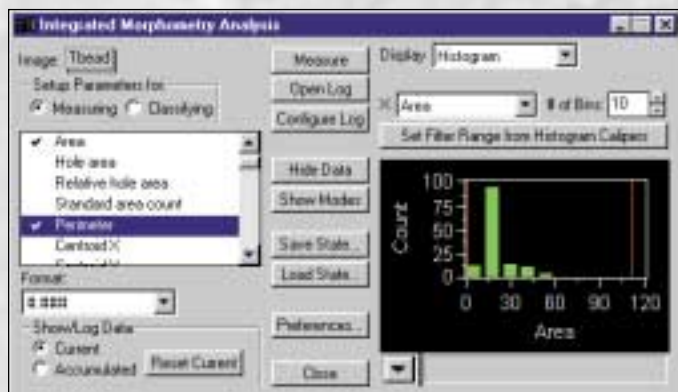
The MetaMorph system supports a large number of TWAIN compliant devices including cameras, scanners, and other hardware.

The MetaMorph tools available for image acquisition provide the necessary groundwork for the system's analysis and measurement capabilities.

General Image Analysis

Once a set of interesting images has been acquired, the researcher can use the MetaMorph system's functions for analysis and measurement.

Versatile region and line creation tools are the basis for all manual measurements in the MetaMorph Imaging System. The user defines regions and the system calculates distances along straight or curved paths, angles between lines, and areas of regions. Densitometric and intensity measurements can be made on or within regions.



Integrated Morphometry and Analysis (IMA) is one of the powerful tools the MetaMorph system provides for image measurement and analysis.

Morphometric Analysis

The Integrated Morphometry Analysis (IMA) function uses an advanced point-and-click interface to make classification of objects easy. Employing binary image operations such as thresholding, erosion and dilation, the user can prepare an image for subsequent morphometric analysis. Objects can be measured within the image and separated into user-definable classes with any combination of morphometric parameters.

Region Measurements

The Region Measurements function simplifies manual region measurements by combining multiple data parameters like area, length, and angle into one easy-to-use interface. The user can view the data in a configurable display similar to a spreadsheet, making data logging simple and clear. Users can also choose to label regions on the image with numbers, data, or other text that they define. Whether dealing with single images or multi-image stacks, dozens of region measurements can be analyzed at the same time.

Data Storage and Exporting

All morphometric and densitometric measurements may be exported to an ASCII text file, Microsoft Excel, or other spreadsheet application using dynamic data exchange (DDE).

Image Analysis Features:

- Curved path distance measurement
- Densitometric calibration to standards
- Feature analysis of width, height, Z-position and other morphometric parameters
- Histogram computation and display
- Brightness measurements within user-definable regions on “live” or stored images
- Line intensity scans or Kymographs
- Object size distribution analysis
- 3-D distance measurements with XYZ calibration
- Centroid computation
- Object classification or sorting using morphometric parameters
- Position, distance and angle measurement
- Shape factor analysis
- Statistical reporting and data logging

Time Dependence

The MetaMorph system simplifies measurements involving intensity changes over time. Such measurements are important to studies of protein motility or stability, FRAP (fluorescence recovery after photobleaching), protein-protein interactions, FRET



Basic parameters of the image here above are compiled by the Region Measurements table.

Region Label	Area	Height	Threshold Area
Area = 111	111	10	111
Area = 62	62	7	62
Area = 150	150	21	150
Area = 124	124	9	124
Area = 47	47	7	47
Area = 29	29	3	29
Area = 50	50	5	50
Area = 79	79	9	79
Area = 134	134	18	134
Area = 132	132	12	132
Area = 152	152	17	152
Area = 126	126	18	126
Area = 135	135	15	135

(fluorescence resonance energy transfer), gene translation (luciferase assays), calcium transients (aequorin or Fluo-3) and other types of studies.

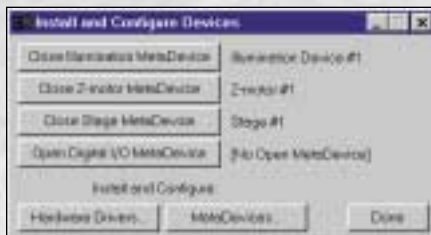
With the MetaMorph system's image stacks, it is easy to measure colocalization, morphometry or intensity over time. Motion analysis is achieved with automated tracking features.

Powerful Tools For Device Automation

The MetaMorph system provides many powerful automation tools, which can simplify and increase the speed and reliability of experiments. Dedicated tools collect and combine multiple wavelengths, multiple positions, and time-lapsed images for optimum flexibility.

Device Control

The MetaMorph system provides standardized interfaces for illumination, stage and Z-motor devices, as well as providing digital I/O to communicate with unique devices.



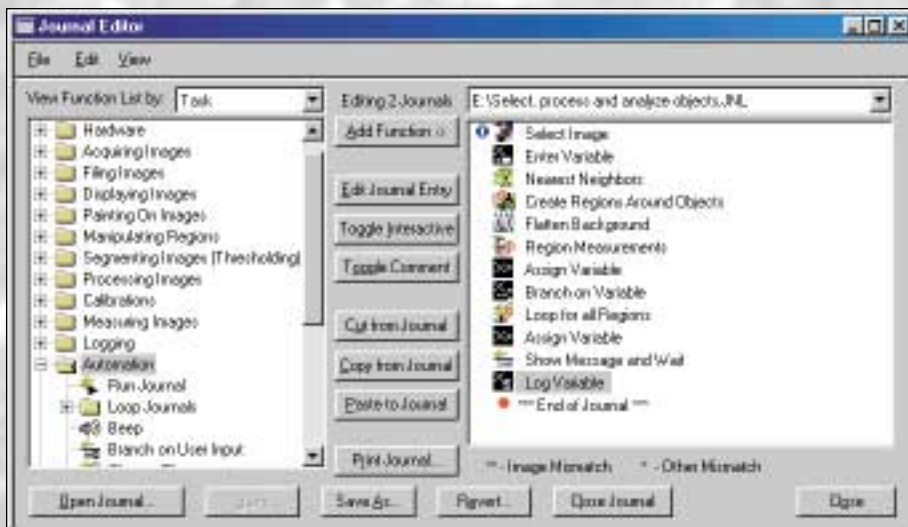
Journaling and device controls can:

- Control the illumination wavelength by employing filter wheels, shutters, liquid crystal tunable filters, monochromators or other wavelength selectors.
- Synchronize image acquisition and analysis with the opening and closing of shutters.
- Scan slides or multi-well plates with a motorized stage.
- Acquire images of a through-focus Z-series for subsequent deblurring, deconvolution, and 3-D reconstruction.
- Control custom devices that accept commands through a serial or parallel port connection.
- Autofocus at multiple stage positions or at specific intervals in a time-lapse environment.



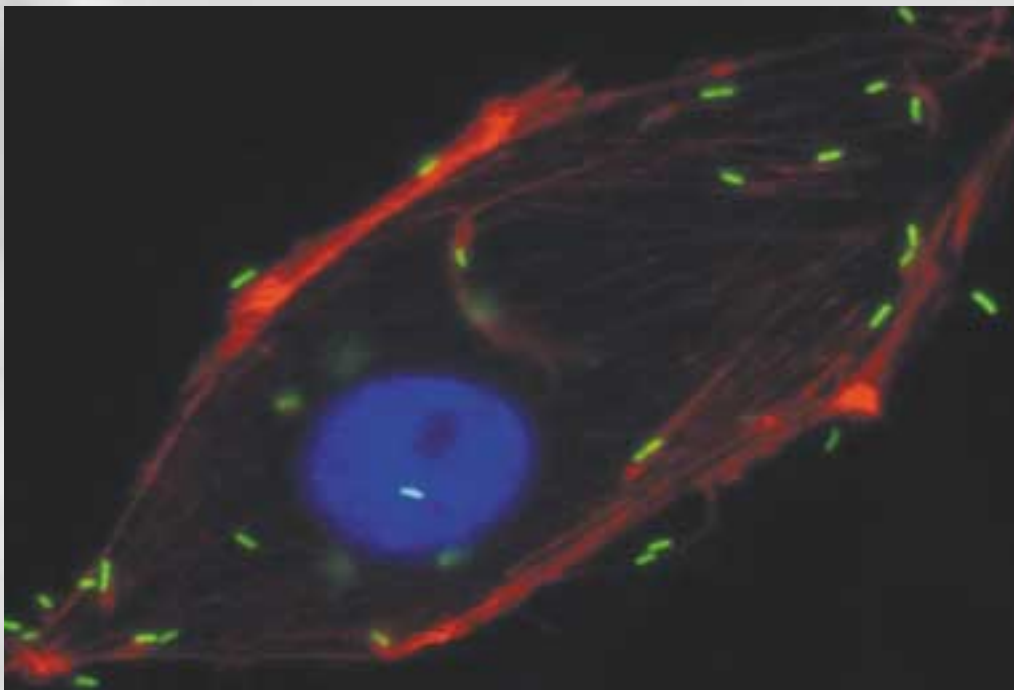
Automated microscope controls can:

- Rotate objectives and change cube settings, open and close the shutter, switch the illumination lamp on or off, and change the lower and upper prism positions.
- Control the X, Y, and Z axis positioning of a stage, move the stage and store the positions for future use.
- Control automated stages to image multiple locations in a sample or multiple samples including dishes and multi-well plates.
- Use focus motors to collect Z-series or through-focal image sets.
- Acquire images using eight different sets of settings for wavelength, intensity, and exposure duration.
- Acquire a spectral scan in a range of excitation or emission wavelengths.
- Capture a timelapse series of images at a specified intervals and durations.



More advanced automated experiments can require the acquisition of images from more than a single plane. This need is met by the multi-dimensional imaging functions described next.

The MetaMorph system's unique journaling capability speeds an experiment by automating repetitive tasks. Similar to a macro, a journal is a user-specified sequence of commands recorded using a 'learn mode' as they are performed.



PUK2 cell infected with *Listeria* and three different acquisitions. Green bacteria, blue nuclei and red F-actin are visible in the host cell.

(Multi-dimensional images on pages 6-7 courtesy of Darren E. Higgins, Ph.D., Assistant Professor, Department of Microbiology and Molecular Genetics, Harvard Medical School)

Multi-Dimensional Imaging

Live cell studies often involve the collection of images in multiple dimensions and wavelengths, over time, and from various stage positions. Managing the many devices that provide this degree of flexibility is the strength of the MetaMorph system's Multi-Dimensional imaging function.

Two modules support the Multi-Dimensional capability: The Multi-Dimensional Acquisition menu and the Review Multi-Dimensional Data menu. These new tools enable the user to simply and quickly acquire any combination of image dimensions, and to review all those images without having to create a journal.

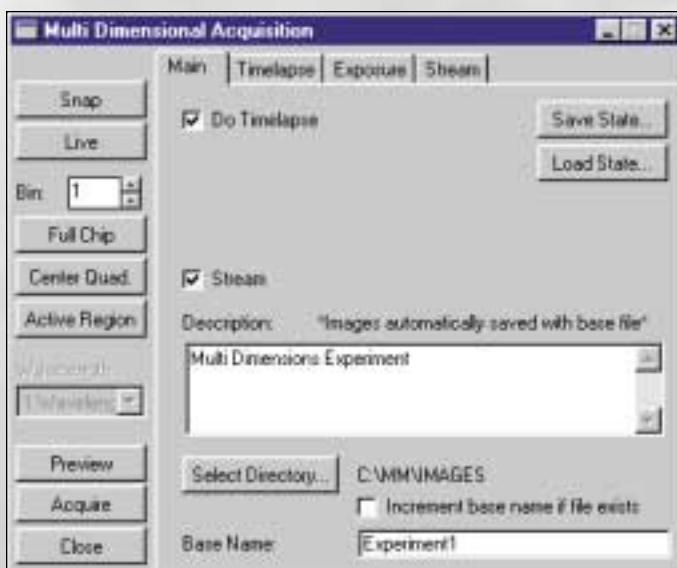
As an example, an experiment could require DAPI and FITC wavelength images, acquired through ten different focus "Z" planes, taken every two minutes for an hour, for a total of 600 images (2 wavelengths x 10 planes x 30 time points). The Multi-Dimensional Acquisition menu can set this up without the user having to program a journal or macro. Then, the Review Multi-Dimensional Data dialog box allows the user to easily view and process all or parts of the image data.

Multi-Dimensional Acquisition

The MetaMorph system's capability for Multi-Dimensional Imaging can automatically acquire and display digital microscopy images in all of the following dimensions:

- X and Y dimensions of the image itself
- 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, users can select more than 200 different combinations of features. The user can produce movies, montages, color-combine images, measure images, and perform 3-D reconstruction on all or any part of the saved images.



Multi-Dimensional Imaging and other MetaMorph system tools can:

- Align images within the stack
- Create and play a movie
- Render a 3-D construction
- Create a montage
- Save the stack to disk
- Measure through all planes automatically
- Enhance any or all of the images
- Deblur the images
- Create topographic surface maps
- Perform arithmetic operations
- View orthogonal planes
- Select a plane or planes
- Equalize light

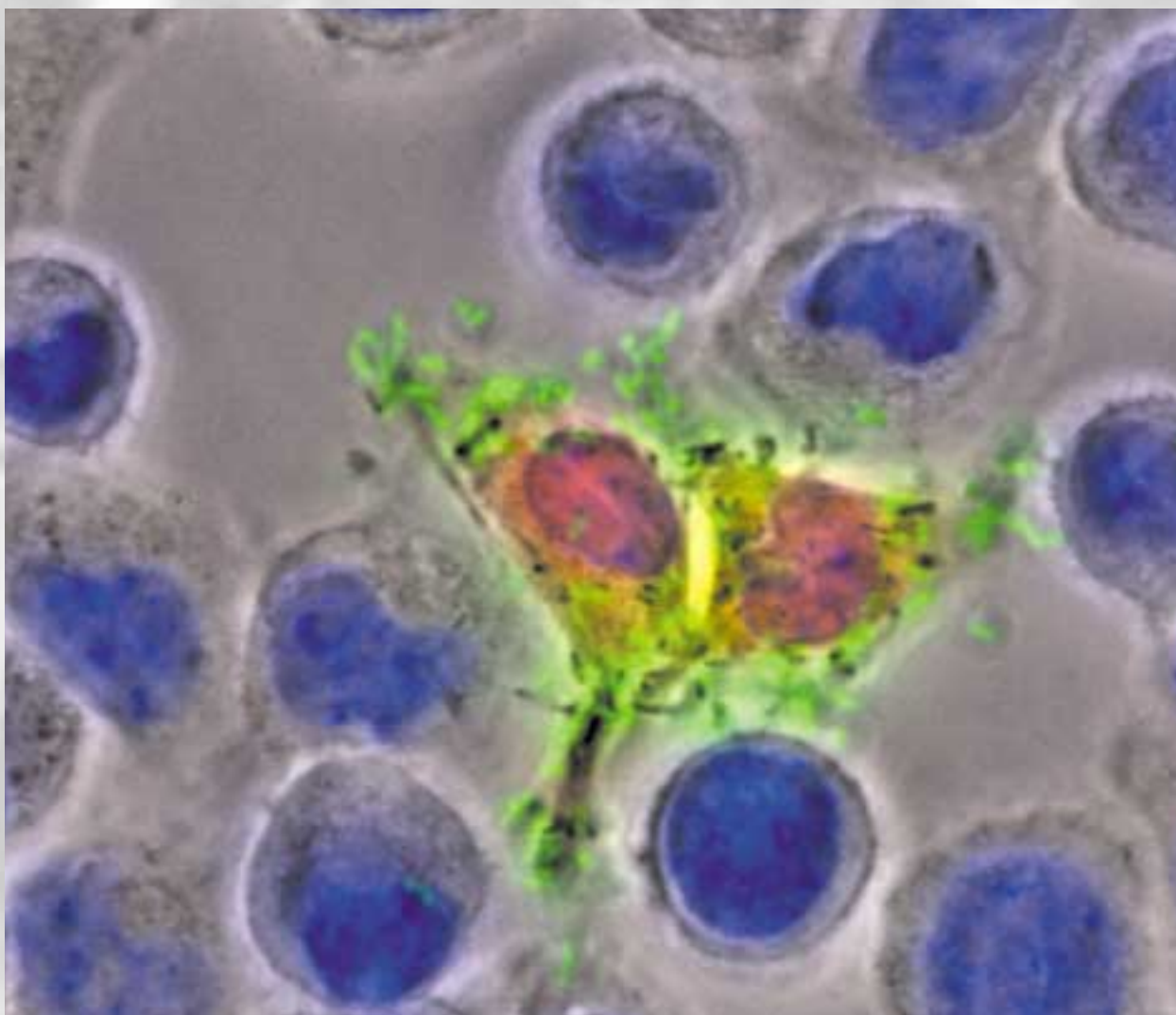
Review Multi-Dimensional Data

The Review Multi-Dimensional Data function enables the user to review and select data from a multi-dimensional (ND) data set.

For a selected data set, the system automatically describes the number of time points, stage positions, wavelengths and Z steps.

After selecting the data set to review, a grid opens on the Review Multi-Dimensional Data dialog box. The grid assigns time to the X-axis and Z position to the Y-axis.

Collecting, analyzing and presenting images from multiple dimensions gives the researcher strong analytical powers. Expanding this capability are the functions of image processing.



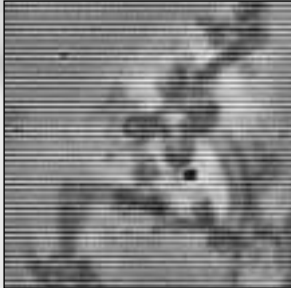
GFP-Listeria infected macrophages that have been stained with Cell Tracker Change. These cells are surrounded by L2 (fibroblast) cells used to examine the spread of bacteria from the macrophages. Blue nuclei in the L2 cells and red nuclei in the macrophages result from the cell tracker stain. Experiment was conducted live in temperature controlled environmental chamber.

Image Enhancement

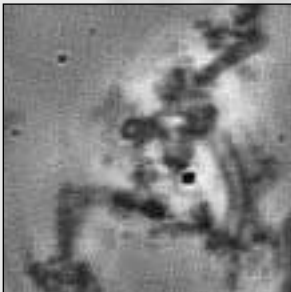
The MetaMorph system's processing functions bring out characteristics which may not be clear in the original image, making subsequent analysis and presentation more informative.

Processing and Enhancement Features:

- Processes stacks of images automatically to find the sum, minimum, maximum, average or best focus.
- Automatic contrast enhancement
- Image arithmetic and logic operators

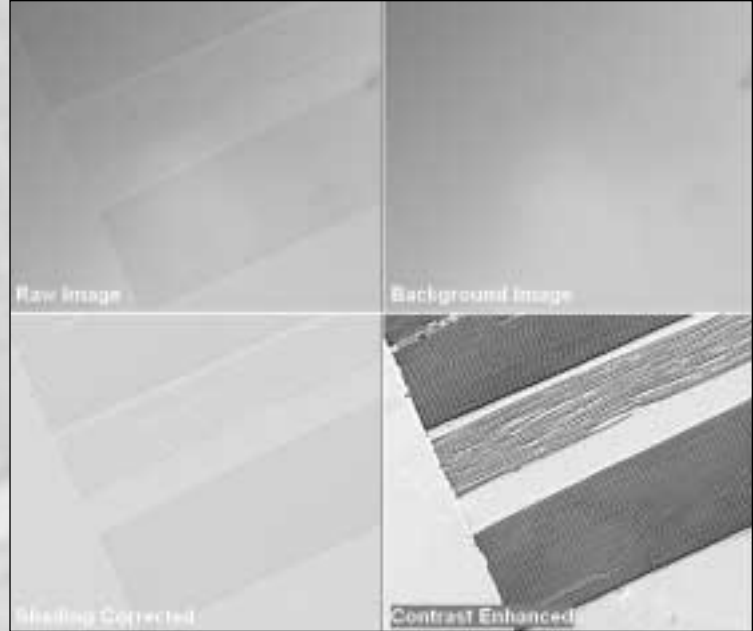


Original Image



Lines removed using FFT controls in MetaMorph

- 2-D Deconvolution (Nearest neighbor, No Neighbors)
- 3-D Reconstruction
- Image deblurring and haze removal
- Sharpening to enhance edges
- 8-bit distance map
- Edge detection
- Flatten background
- Low pass and median filtering
- Unsharp masking
- Intensity profiles
- Convolution using user-definable kernels
- Erosion
- Dilation
- Skeletonization
- Watershed segmentation

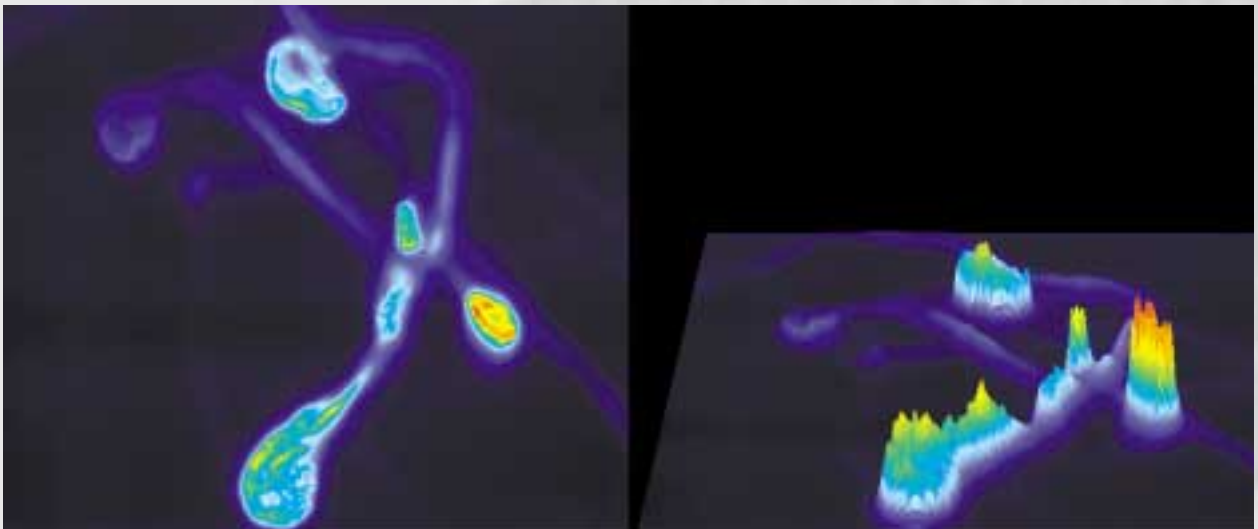


Differential Interference Contrast images of muscle tissue

- FFT (Fast Fourier Transform)
- Intensity profile
- User definable LUT (Look Up Table)
- Outline
- Single pixel removal

Examples of Enhancements:

- Extract object boundaries for feature analysis and enhancement using edge detection.
- Combine images using a variety of arithmetic and logical operators including addition, subtraction, division, logical AND, OR, and XOR.
- Sharpen images using a variety of enhancement filters.



Montage of GFP-labeled cells with intensity profile shown on the right.

Deconvolution

The MetaMorph system can improve experiment performance by mathematically reversing the image degrading effects common to optical microscopes.

In most microscopes, the lens aperture causes light from a point source to spread out or diffract, and light from out-of-focus planes can result in image contamination. This is known as convolution, and is caused by the optical system's Point Spread Function (PSF).

Two deconvolution commands in MetaMorph can be employed to offset these undesirable effects. The first command, called *Nearest Neighbors*, uses an estimation of the three-dimensional PSF to compute the contributions from adjacent out-of-focus planes, and combines this data with the original information to create a more visible resulting image. The researcher may enter experimental settings that help specify the PSF.

Secondly, the *No Neighbors* command uses an unsharp mask operator to blur an image plane,

thereby subtracting the undesired new input as well as the original out-of-focus information.

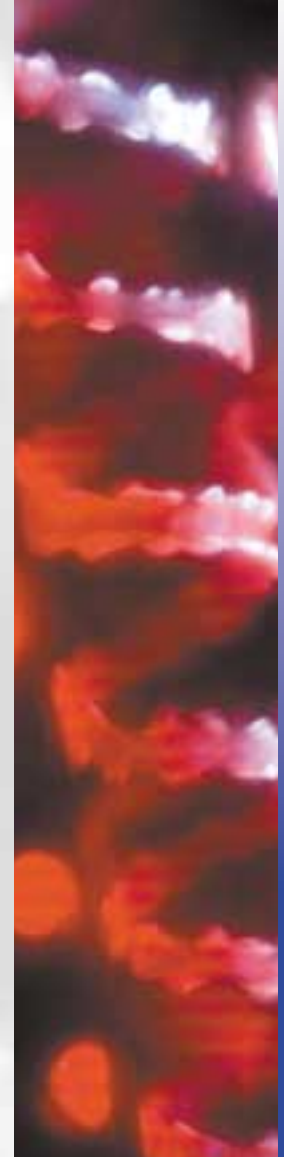
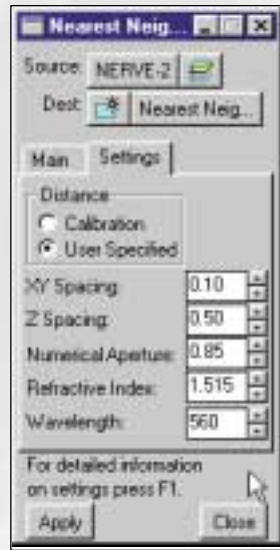
3-D Reconstruction

Experimental effects and outcomes are not always apparent when viewing images in two dimensions. The MetaMorph system helps to reveal more detail with its tools for *3-D Reconstruction*.

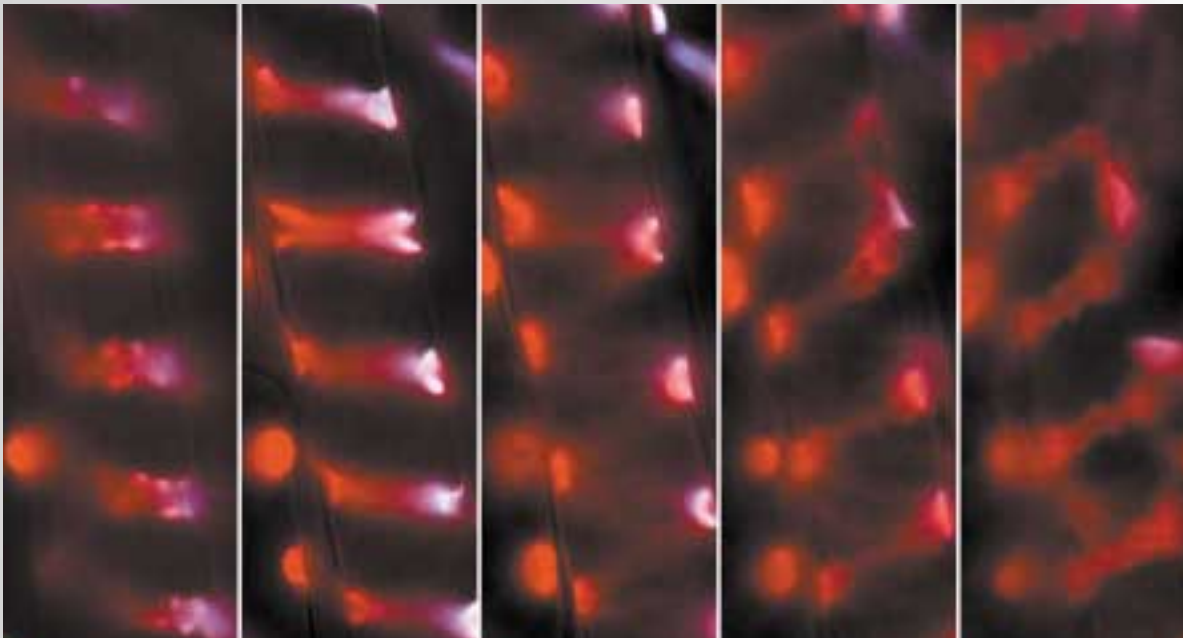
This command is used when the researcher wants to create a 3-D model consisting of rotated views from a stack of images. Using a stack of planes from a through-focus set of images, the user configures the *3-D Reconstruction* dialog box to set the angle, orientation, interpolation, Z-axis distance, and reconstruction type for the model. The resulting model

can be viewed with the MetaMorph system's *Movie* command.

With images now enhanced, sharpened and reconstructed, researchers can use the MetaMorph system's most advanced applications.



3D Reconstruction of a *Spyrogyra*



Individual Z-planes of a *Spyrogyra* taken with a Rhodamine filter set.

The advanced applications in the MetaMorph system give researchers the highest level of analytical power.

Particle Tracking

This command enables users to follow the movement of tagged particles, such as fluorescently labeled cell surface molecules, microtubules, nucleic acids, lipids, and other objects with sub-pixel resolution.

Researchers can measure X and Y coordinates, velocity, mean displacement, mean vector length, and more. These measurements can be displayed in a printable graph that the user configures.

Motion Analysis works very well when used with differential interference contrast microscopy (DIC) images because the tracking region can be made to encompass both the white and black spots produced by DIC. Researchers will thus have twice as many pixels to track, which leads to a proportional increase in the tracking position.

Measure Brightness

Many fluorescence experiments depend on measuring brightness parameters, and the MetaMorph system excels at providing this type of information.

Intensity data can be logged from selected regions in an image stack or live video image. Users can capture data such as average and integrated intensity, standard deviation of intensity, maximum and minimum gray scale areas, threshold area, and more.

Measure Colocalization

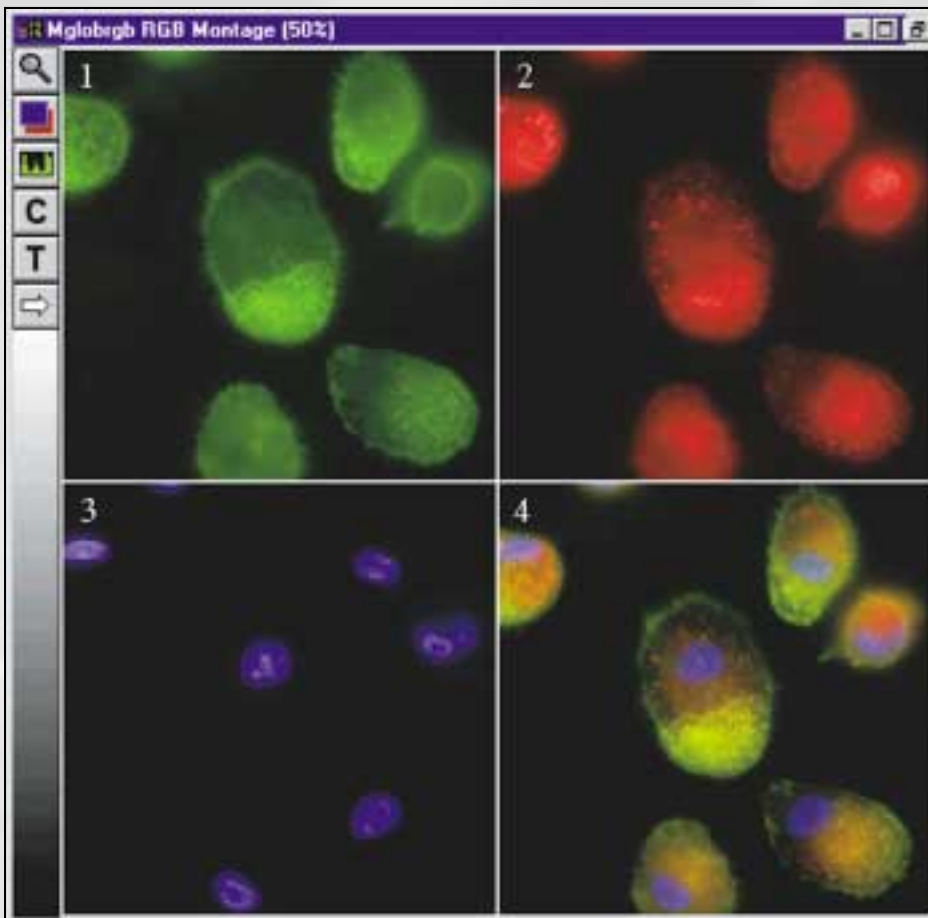
While good experimental data can be obtained by analyzing a single fluorescent probe, researchers often get better results by examining more complex interactions. The MetaMorph system's *Colocalization* function provides a higher level of detail, with quantitative data regarding regions of overlap between two fluorescent probes. Users can measure the area, average gray scale intensity, and integrated gray scale intensity in the region of overlap and save this data to a separate file.

Overlay Fluorescence

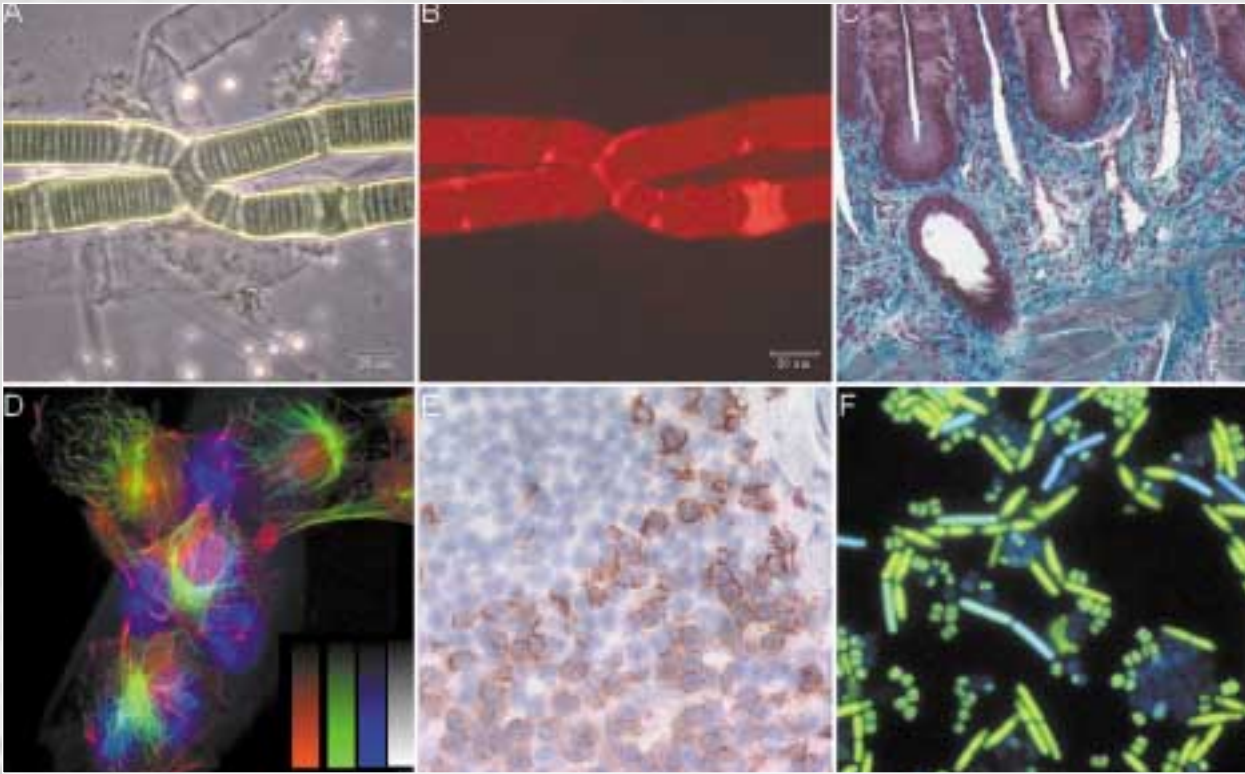
Greater levels of detail are also revealed by the *Overlay Fluorescence* function. This tool enables researchers to overlay up to six transparent fluorescence probe images over a background image. The information available in the background image is visible through the fluorescence image.

Measure Grid

The *Measure Grid* function is very useful for gene chips and other images that have areas of interest at regular intervals. With this command, users can divide the image with a distinct grid, and run a macro that will measure parameters such as location, width, height, area, and various intensity values.



The yellow areas of the montage in panel 4 show colocalization of the green and red images in panels 1 and 2. Images courtesy of Philip L. Leopold, Barbara Ferris, and Ronald G. Crystal, Cornell University Medical College.



Montage and labeling of sample images taken with MetaMorph. Images labeled A and B courtesy of Microbial Diversity, Marine Biological Laboratory, Woods Hole, MA

As the final step in most research projects, scientists must convey their findings in a clear and compelling way. The MetaMorph systems flexible graphics.

Presentation & Publication

The MetaMorph system's flexible graphics and presentation functions enhance images for visual inspection and present the images in an appropriate format for publications or slide shows.

Presentation features:

- Draw
 - Text
 - Date and time labels
 - Calibration bars
 - Gray wedges
 - Arrows
- Change lookup table
- Convert monochrome images to color
- Convert color images to monochrome
- Produce intensity profile images
- Rotate or flip images
- Produce image montages
- Produce movies
- Copy images or regions of images to clipboard

Image overlay

- Superimpose multiple images for easier visualization.
- Help visualize the location of fluorescent markers within cells or subcellular structures.

Annotation

- Organize and label images
- Document the operations performed upon the images

Printing

- Send images and stacks of images to high resolution recorders, laser printers, slide makers and other Microsoft Windows compatible output devices for presentation and publication.

The MetaMorph System

By incorporating the latest advances in image acquisition, processing and analysis, experiment automation, and results presentation, the MetaMorph system demonstrates Universal Imaging Corporation's commitment to bioscience researchers.



Technical Summary

Computer Requirements

- Pentium-class processor
- Microsoft Windows 95/98/NT Workstation 4.0/2000* operating system
- CD-ROM drive
- 64MB or more system memory (RAM)
- 60MB free hard disk space (may vary due to cluster size) (for program only; image storage requires more space)
- 24-Bit graphics display

Standard Features *(with Basic and Complete systems)*

- Displays, loads and saves binary, 8-bit (monochrome and palette color) 16-bit, 24-bit and 48-bit (RGB) image types
- Color combine and color separate into 24 or 48-bit images
- Regions of interest include: rectangular, elliptical, line and free form
- Stack processing includes: stack arithmetic, 3-D reconstruction, best focus, montage, topographical surface, view orthogonal planes, create movie
- Auto Expose (standard with CCD cameras)
- Acquire Stream, Acquire Time-lapse
- Automated time-lapse and spectral scan acquisition
- Acquire Z series (standard with Z motor)
- Fast Fourier Transform (FFT) processing
- Image histogram
- Overlay/image colocalization measurements
- Shading correction and background subtraction
- Ratio images
- Arithmetic operations include: add, subtract, multiply, divide, logical AND, OR, XOR, minimum, maximum, average
- Morphology operators include: erode, dilate, skeletonize, outline, segment, remove single pixels, 8-bit distance map
- Filters include: sharpen, low pass, detect edges, unsharp mask, median filter, remove haze, custom kernels
- 2-D Deconvolution
- Integrated Morphometry Analysis includes measurements for total area, hole area, standard area count, perimeter, centroid, width, height, orientation, length, breadth, shape factor, average and total gray value, optical density, elliptical Fourier analysis and others
- Customizable toolbars and windows

* Universal Imaging Corporation's software is known to be Windows 2000 compatible. Not all hardware devices and drivers have been fully tested. Check with your sales representative for specific details.

- Data logging to text file or spreadsheet programs which support DDE (Dynamic Data Exchange)
- Experiment automation through journals and taskbars
- Technical support and applications advice available via phone or email
- Interactive training CD
- Electronic and online documentation

Optional Features for Basic System

(Standard with Complete system)

- 6-D, multi-dimensional image acquisition and analysis
- Wavelength streaming and/or Z axis streaming (patent pending)
- Illumination device control, including filter wheels, shutters, monochromators and liquid crystal tunable filters
- Auto Focus
- Motion Analysis, including automatic object tracking
- Image colocalization and correlation measurements
- Custom I/O (serial and parallel port)
- Stage device controls include: ASI, Nikon, Marzhauser, Prior, Ludl
- Z-axis device control includes: Nikon, Physik Piezoelectric Focus Device (PiFoc), Prior, Ludl, ASI
- Sutter DG-4 wavelength changers and Lambda 10-2 filter wheels
- Uniblitz shutters

Acquisition Options *(Partial List)*

- Monochrome and/or color video (RS-170, Composite CCIR, RGB) (NTSC or PAL)[requires imaging board]
- Roper Scientific (Princeton Instruments) digital cameras, including MicroMAX, PentaMAX and ST138
- Roper Scientific (Photometrics) PXL, SenSys, Quantix, CoolSnap
- Hamamatsu On-chip integrating CCD cameras including C4742-95, C4880 and the Orca series
- Diagnostic Instruments Spot cameras

Automated Microscope Control Options

- Leica
- Nikon
- Olympus
- Zeiss



UNIVERSAL IMAGING CORPORATION

One Ridgewood Place
402 Boot Road
Downingtown, PA 19335
610-873-5610 phone • 610-873-5499 fax
www.universal-imaging.com

Domestic US Sales

Tel: 610-344-9410
Email: sales@universal-imaging.com

International Dealers

For updated information:
www.universal-imaging.com/sales