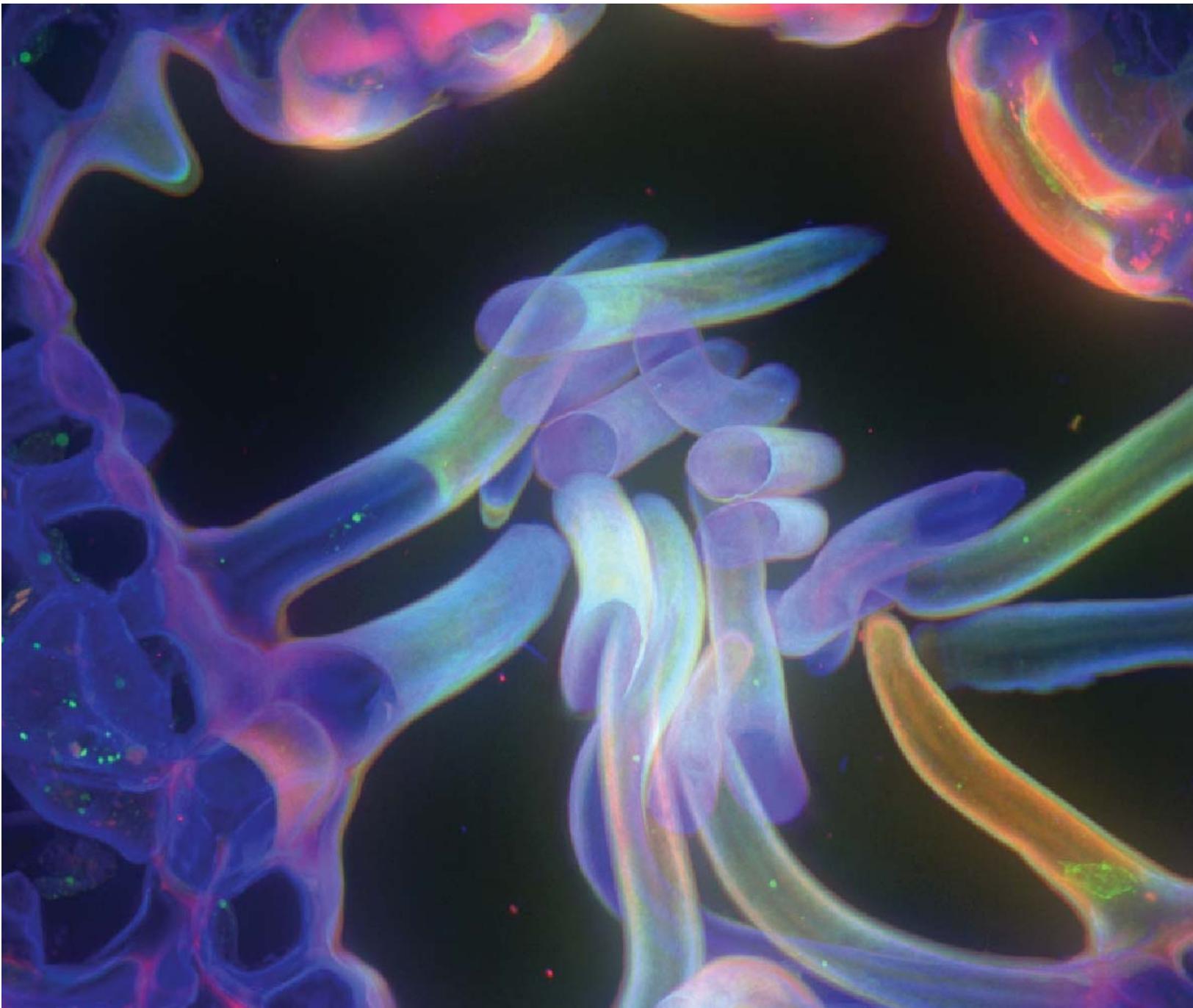
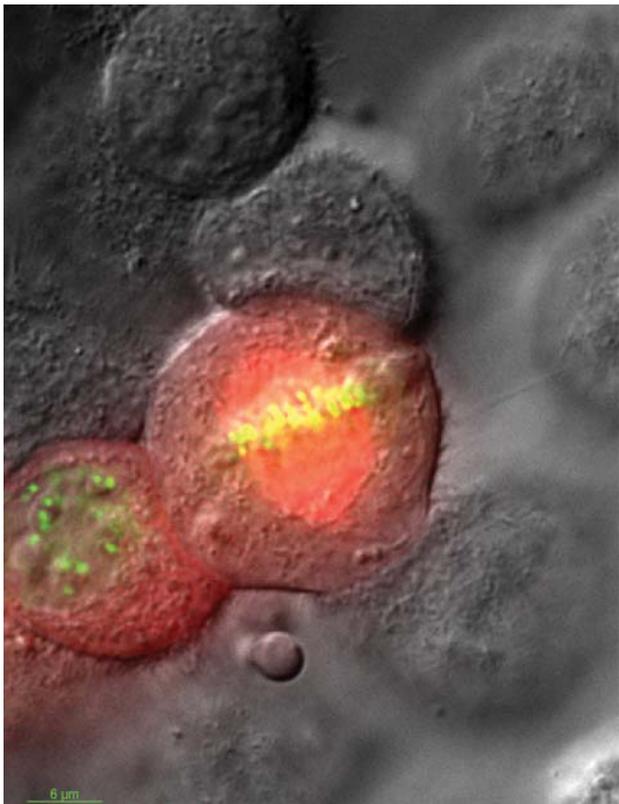


AppliedPrecision®

DeltaVision® Imaging Systems

widefield restoration microscopy





HeLa cell stained with mCherry-tubulin and GFP-cb (a gene on the chromosome) - Image courtesy of Jennifer DeLuca, Colorado State University.

restoration

Contrast and resolution in microscopes are limited by the amount of information that the objective lens can collect. This loss of information results in images that do not fully portray the location and concentration of signals in the actual sample. Restoration Deconvolution is a mathematical treatment that restores the contrast and resolution lost in the microscope to generate an image that more closely represents the original sample.

optimization

Applied Precision has pioneered the application of deconvolution to live cell imaging and, as a result, many companies sell deconvolution software for virtually any microscope. However, it is not enough to merely apply a deconvolution method to images from the microscope. The microscope system must be designed as a whole in order to obtain the highest quality images and data before applying deconvolution. DeltaVision systems are designed from the ground up for restorative deconvolution and live cell imaging. The results are obvious. Superior raw data and quantitative results.

quantitation

Other systems may deblur the image or provide simple smoothing operations to sharpen the image contrast. DeltaVision's sophisticated constrained iterative algorithm enhances the image contrast while actually increasing the numerical accuracy of the image. No data is lost or thrown away. This algorithm has been quantitatively verified to accurately represent the original 3D object (Swedlow et al, PNAS 2002). A truly restorative algorithm, it reassigns the out-of-focus light to its calculated point of origin rather than simply subtracting it from the image as with non-quantitative deblurring methods.



DELTAVISION



DeltaVision Core

Bring the power of an imaging facility into your lab. DeltaVision Core is powerful enough to acquire, analyze and archive your most complex image data. DeltaVision systems are engineered and built specifically to provide uncompromised imaging and visualization of live-cell biology.

DeltaVision Core is a fully integrated, optimized microscopy system designed to increase the ability to look at more probes and samples over longer periods of time than any competitive imaging system.

Every DeltaVision is fully assembled and tested at our facility prior to shipment. Once you receive your system, it can be reassembled in your lab, allowing you to collect data that very same day!

personalDV

Whether you're starting your own lab or expanding an existing one, the personalDV is the perfect choice! Start imaging faster with a fully integrated system that delivers high-performance system imaging at a component price.

Small and dim samples? No problem. personalDV minimizes photodamage and enables a very high signal-to-noise ratio. Combined with cell tracking and autofocus, live-cell imaging just got easier.

The personalDV was designed with space in mind and can easily fit on a lab bench. The Vibration Isolation Platform dampens local movement from the surrounding lab environment. Add an Opaque Environmental Chamber to the personalDV and you no longer need a dedicated room for microscopy.

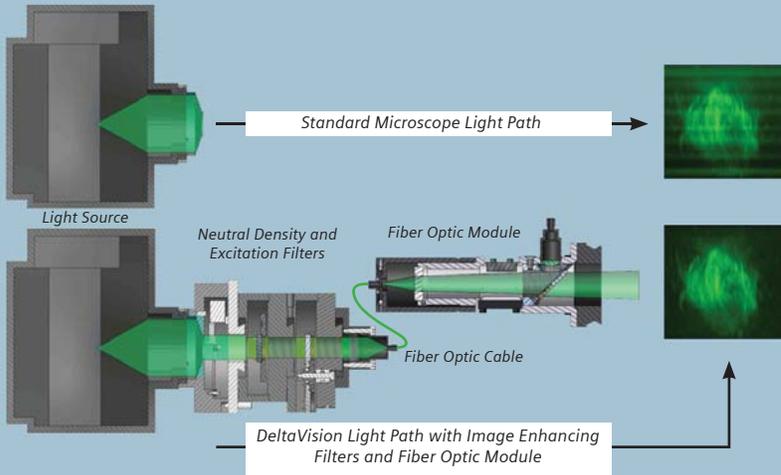
Factors for Excellent Image Quality

hardware

- API certified objectives
- Quality control of the camera
- Proprietary illumination of the sample for quantification, and light intensity correction for proper deconvolution
- Efficient photon collection from the sample
- Nanomotion stage control

software

- Truly restorative algorithm that reassigns the out-of-focus light to its expected point of origin, as opposed to simply subtracting it from the image as with deblurring methods
- Photon conservation - information is not lost, it is reassigned to the origin
- Quantitative data- reliable analysis made possible with accurate raw data



uniform illumination

Wide field illumination systems utilize white light sources to provide maximum flexibility in wavelength selection. However, arc lamps can suffer from variations in intensity over the course of an experiment. Additionally, the absolute position of the arc changes in 3-dimensional space. These variations in position and intensity lead directly to errors in quantification of acquired 2D images and 3D volumes.

Only DeltaVision systems have implemented proprietary hardware and software to remove the spatial and temporal variations in light intensity. Integrated components are critical to the success of creating uniformly illuminated 2-dimensional and 3-dimensional images: the FOM (Fiber Optic Module) with PhotoSensor. Another DeltaVision exclusive!

superior illumination

Xenon lamps generate a more stable fluorescent illumination spectrum, a striking contrast to the peaks and valleys of a mercury arc lamp. This allows greater flexibility in choosing fluorescent markers. Xenon lamps are not subject to the flicker that occurs in mercury arc lamps. The result is uniform sample illumination.

Halogen bulbs can appear yellow and lose intensity near the edges of the field of view. LED transillumination creates a clean, consistent, white background for phase contrast and DIC applications. Superior illumination technology for cutting edge science.

NanoMotion III

Applied Precision's Nanomotion III Precision Control with our patented Flexure Stage is an essential component of the DeltaVision Imaging Systems. No other stage in the world has the same accuracy and repeatability over its full range of motion. Precise stage movement is critical in sampling and resampling points in a time-lapse experiment.

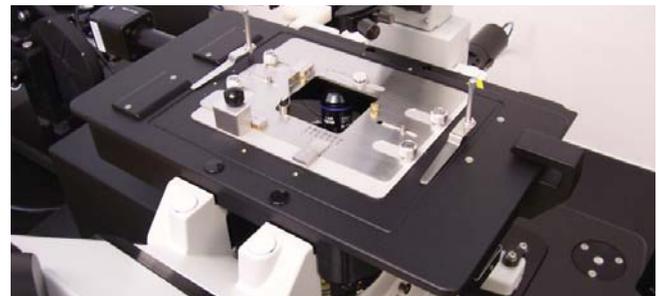
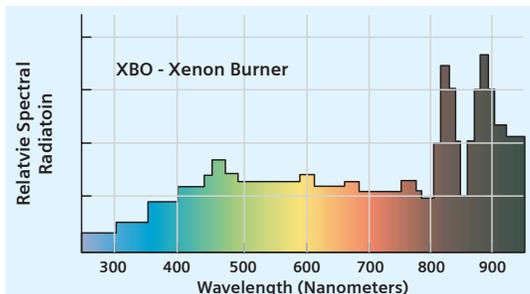
Z axis

Absolute accuracy	< 0.6 μm per 13 μm Z scan
	< 2.0 μm per 100 μm Z scan
Out-of-axis motion	< 0.6 μm per 13 μm Z scan
	(< 0.3 μm typical)
Step resolution	5 nm (theoretical)
Unidirectional repeatability	< 0.2 μm (< 0.1 μm typical)
Maximum travel	1 mm
Typical speed	1000 $\mu\text{m}/\text{sec}$

XY axes

Absolute accuracy	< 10.0 μm per 25 mm of travel
Unidirectional repeatability	< 0.4 μm per axis
	(< 0.2 μm typical)
Step resolution	20 nm (theoretical)
Maximum travel	25 mm (X) x 25 mm (Y) or
	25 mm (X) x 50 mm (Y)
Typical speed	2000 $\mu\text{m}/\text{sec}$

Xenon Arc Lamp Emission Spectrum



what is deconvolution?

When light strikes an object, the light scatters in all directions: x, y and z. Every sample is comprised of points, all scattering light. Light from one plane of origin may appear in a different plane. The net effect of the scattered light is that samples look slightly blurred under a microscope. The measurement of property of light scattering is known as the Point Spread Function, or PSF.

Microscopes are limited by the amount of information that the objective lens can collect. This loss of information results in images that do not fully express the location and concentration of signals in the actual sample. Deconvolution is a mathematical treatment that uses the PSF to restore the contrast and resolution and generate an image that more closely represents the original sample.

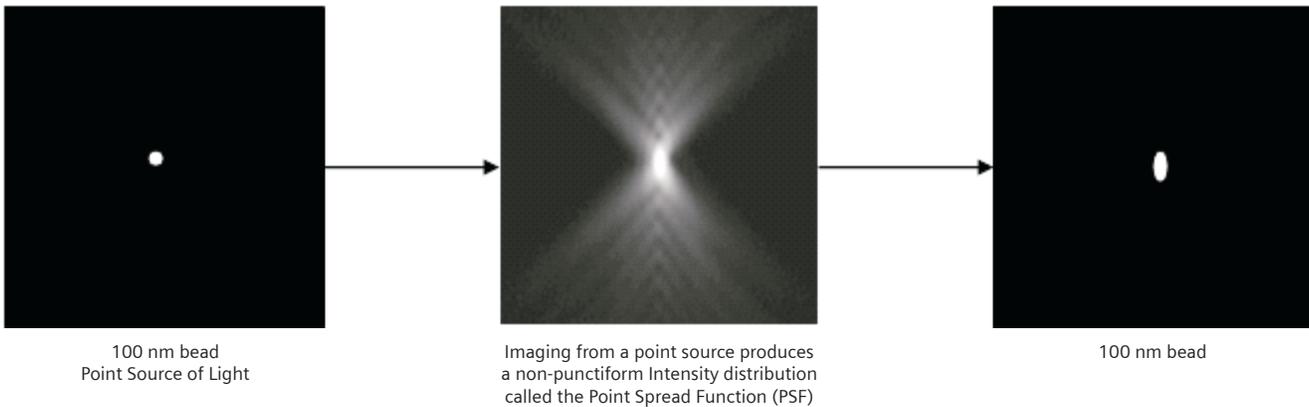
restorative deconvolution

Contrast and resolution in microscopes are limited by the amount of information that the objective lens can collect. This loss of information results in images that do not fully express the location and concentration of signals in the actual sample. Deconvolution is a mathematical treatment that restores the contrast and resolution lost in the microscope to generate an image that more closely represents the original sample.

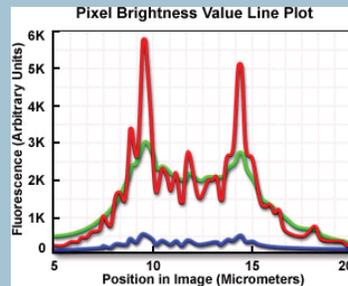
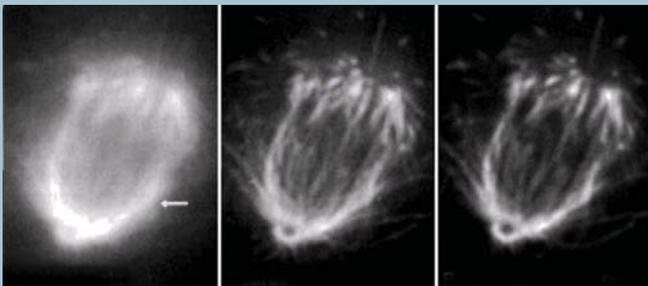
Applied Precision has pioneered the application of deconvolution to live cell imaging and, as a result, many companies sell deconvolution software for virtually any microscope. However, it is not enough to merely apply a deconvolution method to images from the microscope. The deconvolution system must be designed as a whole in order to obtain the highest quality images and data. The DeltaVision High Resolution Imaging Systems are designed from the ground up for deconvolution and the results are obvious.

point spread function (PSF)

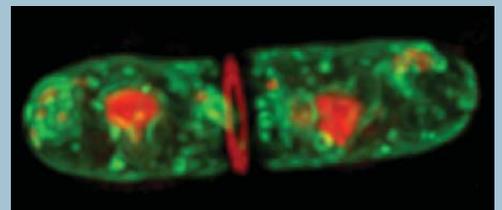
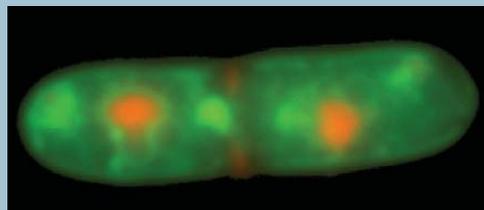
Appearance of a 100 nm (0.1 μ m) Fluorescent Bead in a Light Microscope



Nearest Neighbor vs Restoration Deconvolution

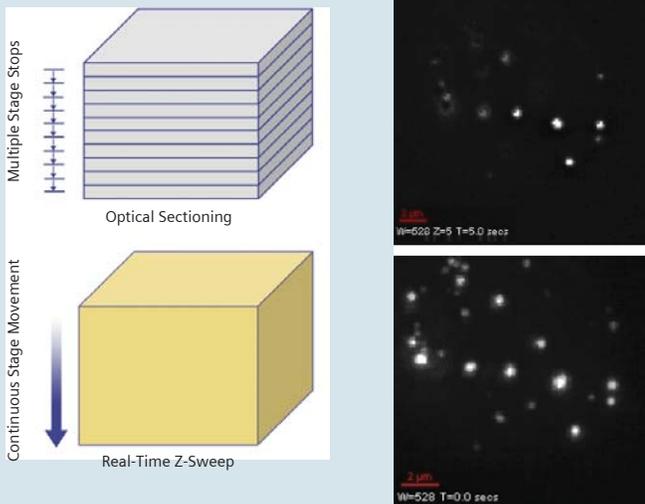


Nearest Neighbor, or Deblurring, is a subtractive process used in image analysis. Data is thrown away for ease of computation and the resulting images that cannot be used for quantitative analysis. Restorative Deconvolution reassigns the light to the original, ensuring the validity of data. (Images Courtesy of BioTechniques 31(2001): 1076-1097)



OAI

OAI (optical axis integration), a DeltaVision exclusive, is a rapid approach for acquiring and displaying 3D Z projections. Instead of creating projections from multiple optical sections, it collects and integrates image data throughout a single continuous stage sweep. OAI has significant advantages for applications such as Leading Edge Motion Analysis, Fast Organelle Dynamics, Microtubule Dynamics, and Fluorescence in situ Hybridization (FISH). It is especially valuable for studies of objects that are moving in 3D space (e.g., kinetichores in a cell nucleus or other rapidly moving structures).



This 2D image of endosomes in a HeLa cell (left) and an instant 3D Z projection (bottom) of the same area were acquired under similar conditions. The additional data in the instant 3D Z projection includes objects that moved out of the depth of field of the 2D image during the data acquisition process.

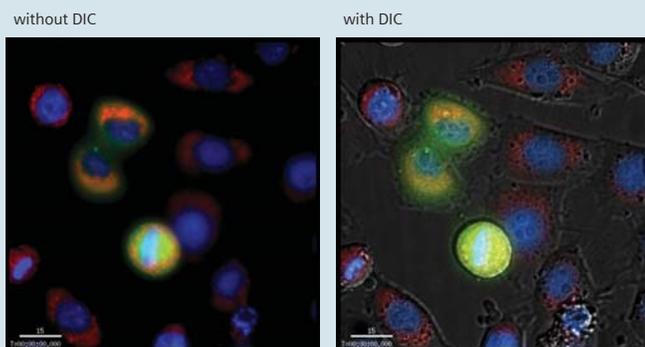
autofocus

The DeltaVision High Resolution Imaging System's unique contrast-based AutoFocus feature requires minimal light exposure, helping maintain live-cell viability while optimizing focus during experiments. This feature can be turned on or off as needed and is easily combined with point-visiting, time-lapse, and integrated cell tracking experiments.

Autofocus is especially useful for long time-lapse experiments. It allows users to acquire focused images without closely monitoring experiments.

DIC

Differential Interference Contrast (DIC) Light Microscopy produces high contrast and finely detailed images. Combined with fluorescence microscopy it creates a powerful tool to view your image in the context of the whole cell.



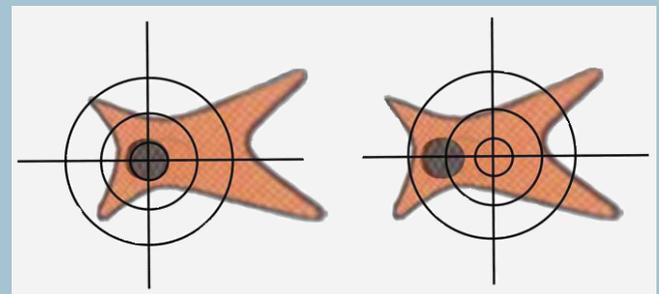
Hela Cells, Hoechst, GFP, Mitotracker red, DIC - Images courtesy of Pearl Lee, BMS, S. Korea

cell tracking

The integrated Cell Tracking feature of the DeltaVision High Resolution Imaging Systems automatically moves the stage to follow cells as they move during a time-lapse experiment. The user specifies an ROI around an object of interest. The software then determines the center of the cell and establishes a recognizable pattern within the ROI. In subsequent images, the software recognizes this pattern and recalculates the center of the cell. The position of the new center is compared with the position of the previous center and, if the cell has moved beyond a specified threshold, the system automatically moves the stage and re-centers the cell.

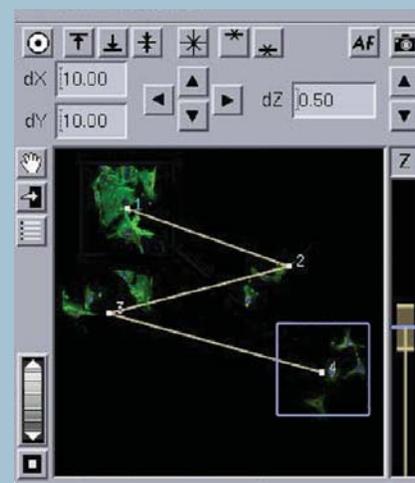
DeltaVision allows you to adjust the following cell tracking parameters:

- Tracking Method
- Threshold
- Reference Channel
- ROI Percent



point-visiting

For live cell imaging, Applied Precision's exclusive Nanomover® technology enables an important addition to live cell imaging: Point Visiting. The DeltaVision system's highly accurate and repeatable 3D motorized stage allows a user to repeatedly visit a series of points on a coverslip or chambered slide. The point-to-point accuracy, precision, and total travel of the DeltaVision stage are unmatched in the industry. Easy-to-use softWoRx® software allows programming of virtually an unlimited number of points on a single slide. Each of these points can be visited in succession and the fast, accurate, Nanomover motors ensure that minimal time is lost moving between different imaging sites while maintaining industry-leading accuracy and precision.

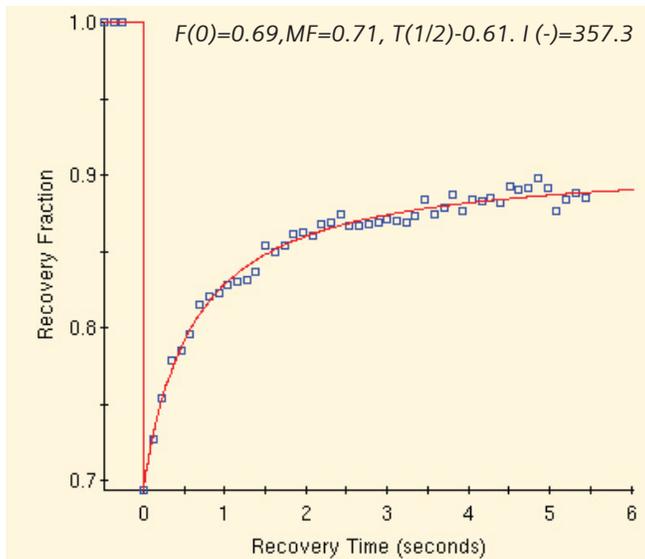
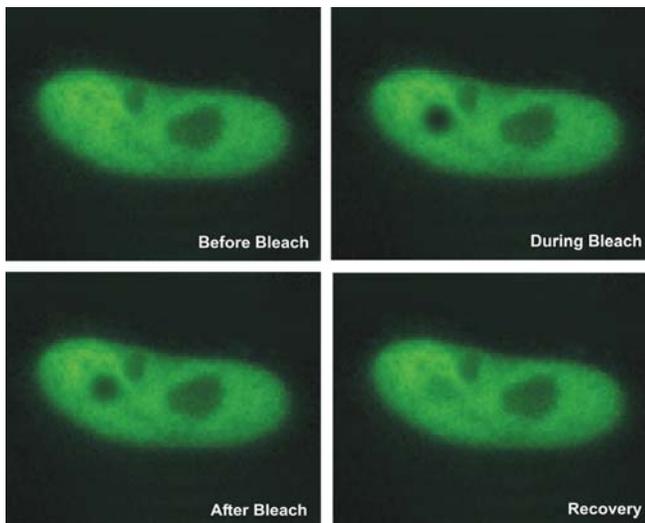


LASER APPLICATIONS

FRAP/FLIP

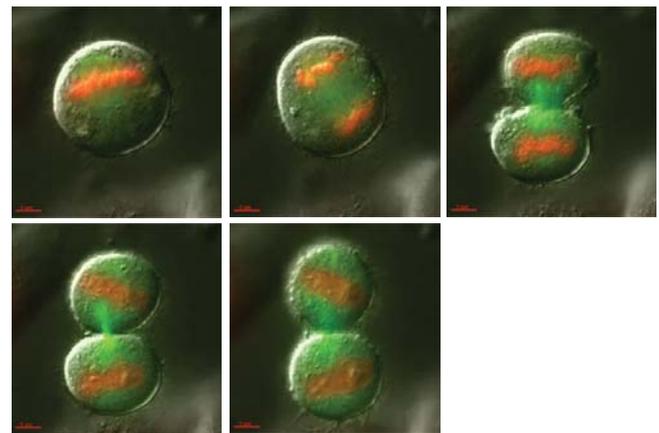
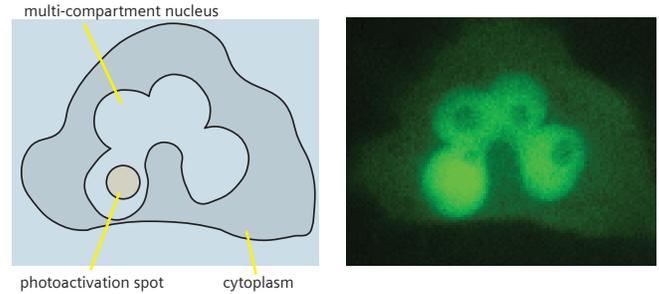
The Fluorescence Recovery After Photobleaching (FRAP) experiment method consists of photobleaching a point (or points) of interest and then measuring the recovery of fluorescence in the bleached area. The observation of recovery indicates that the fluorescent molecules are mobile. The rate of recovery determines the speed at which the molecules are moving.

When you use the DeltaVision QLM Laser Module to perform FRAP, the point of interest is photobleached with a laser pulse and time-lapse images are captured to monitor the bleached area. DeltaVision's Fast FRAP feature minimizes the time between the laser event and camera acquisition, resulting in accurate, real-time FRAP results. The recovery of fluorescence in the bleached area is measured as a function of time. From these measurements, you can discern information about the environment of the fluorescent molecules and their affinities.



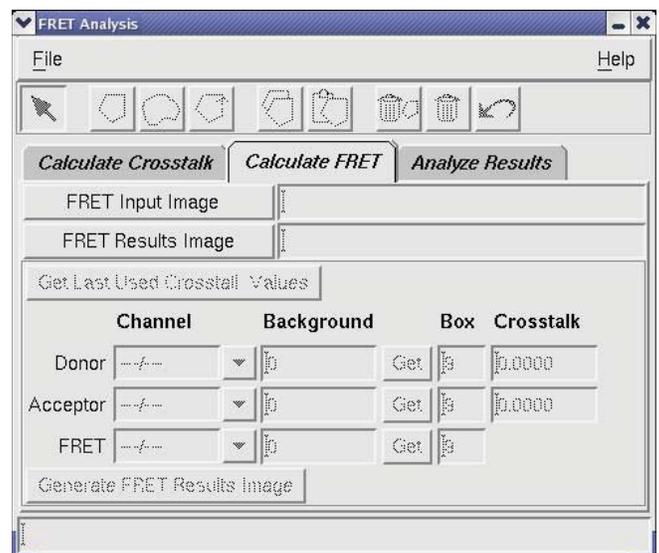
PA-GFP

Photoactivatable GFP (PA-GFP) is a powerful reagent to observe a specific group of proteins fused to GFP. PA-GFP does not emit GFP-spectrum photons until excited. PA-GFP can be activated by either the arc-lamp or laser, enabling the scientist to observe movement and measure kinetics of the sample.



FRET

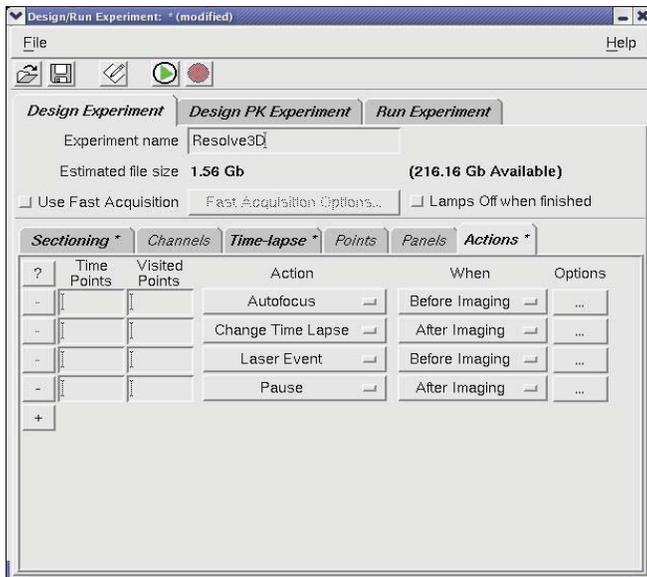
Fluorescence Resonance Energy Transfer (FRET) has gained importance as a technique to measure the distance between two fluorophors. softWoRx has the tools to collect and analyze FRET data- no additional software required.



softworx linux 3.7.0

Acquire

The Experiment Designer supports multi-wavelength, multi-point, time-lapse imaging in Z. The Actions feature makes designing complex experiments easy to maximize time, reagents and productivity. Picture an eight well chambered slide with a different experimental condition in each well. Users can select multiple points to visit not only within one well, but in all eight wells. Programmable autofocus at user defined time points keeps live cells in the field of view while preserving cell viability.



Process

The Task Builder feature streamlines post-acquisition processing chains (such as deconvolution, volume rendering and data export) to entire sets of data, not just one file at a time. Data can be exported as tiffs for quantitative analysis or as movie files for convenient viewing of time-lapse experiments. System level Queue Management ensures seamless data acquisition by the DeltaVision Microscopy System while data processing runs the background.

Analyze

softWoRx 3.7.0 has analysis tools available for all levels of complexity. From simple data inspection to 3D modeling, FRET ratio imaging to kinetic analysis, softWoRx 3.7.0 enables scientists to visualize and quantitate data.

Organize

The Data Management Solution (DMS) was created to manage active and archived data based on the OMERO platform.

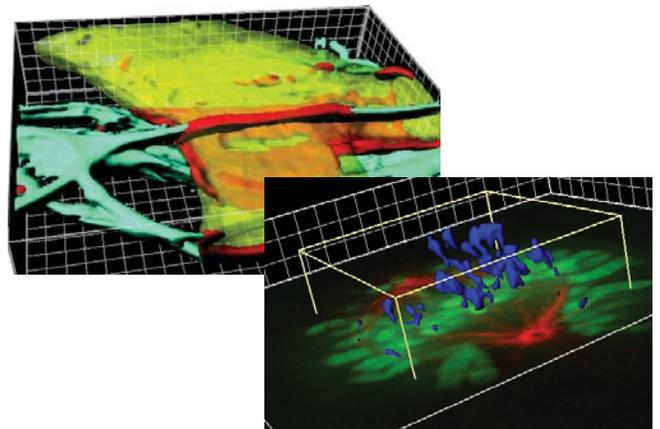
softworx suite

softWoRx Suite provides sophisticated multi-dimensional data visualization, analysis, image restoration, image correction and image viewing management, all within a Windows®-based, easy-to-use, streamlined browser interface. From the browser window, users can access files across networks through FTP or secure FTP connections. The browser displays interactive image previews and file metadata. Files in the browser window can be sent to off-the-shelf tools such as Photoshop® or to the three other softWoRx Suite components:

- Image Viewer
- 3D Image Restoration
- 3D Visualization and Analysis

Images can be processed on a Windows desktop with the industry acclaimed Constrained Iterative 3D Image Restoration and Image Correction tool, a quantitatively validated deconvolution solution generating the most accurate measure of sample fluorescence available.

The softWoRx Suite provides the most advanced visualization and analysis package available on a Windows based platform.



multiplexed wavelengths

The MPX2 is a solid-state illumination unit that enables rapid image acquisition of single channel images without degradation of image quality. This technology enables the DeltaVision systems to Sequential image capture images which is the only way to acquire uncompromised high quality images.

Colocalization of rapid cellular processes is a challenging imaging technique. Simultaneous imaging techniques sound easy but in practice introduce crosstalk and confound data. For more technical details on sequential imaging and the drawbacks of simultaneous imaging, talk to an Applied Precision representative.

Laser Module

The DeltaVision Laser Module (QLM2) is an optional component for the DeltaVision CORE system that adds a laser beam into the back aperture of the microscope objective to provide a focused illumination spot in the center of the optical field.

The QLM2 is designed for photokinetic experiments that involve the interactions of light with biomolecules and fluorophores. A photokinetic event is an event within an experiment in which the sample is illuminated with a laser. It can be a simple event such as photobleaching a single diffraction-limited location in a cell. More complex experiments entail repeatedly activating a pattern of points using one laser and then switching to a different laser to photobleach a smaller region within that pattern. Whether this event causes photobleaching, photoactivation, or some other phenomenon is largely dependent on the molecules that are present and the parameters of the photokinetic event (e.g., laser wavelength, laser power, and spot size).

Sample applications include:

- Fast FRAP
- FLIP
- Laser FRET
- Photoactivation (PA-GFP)
- Photoconversion

Configuration	Laser	Sample Applications
Base System	488nm	Fast FRAP (GFP, YFP, Alexa488), FLIP, Dronpa
Option 1	405 nm	Photoactivation (PA-GFP), Laser FRET (CFP), Dronpa
Option 2	514 nm	Laser Acceptor Depletion FRET (YFP)
Option 3	561 nm	Fast FRAP, FLIP (mCherry, mApple)

TIRF

Total Internal Reflection Fluorescence Microscopy is a microscopy technique that limits fluorescence imaging to a thin area at the surface of a substrate. Typically, the TIRF sample is only illuminated 100-200nm into the specimen, resulting in an enhanced signal-to-background ratio and higher imaging contrast. This technique utilizes a simple yet elegant law of physics to improve biological imaging. When light passes from a medium of high refractive index to a medium of low refractive index and the angle of incidence is greater than or equal to the critical angle, the light will reflect off of the interface and not actually enter the second medium.



conventional



TIRF

EMCCD

The Cascade II EMCCD camera is optional on DeltaVision systems and is ideal for low light, live cell experiments with low background. The EMCCD utilizes a back-thinned chip with 90-95% quantum efficiency. PCI communication with the instrument controller enables the fastest imaging conditions. Talk to an Applied Precision representative to determine if the EMCCD camera is the right choice for your application.



microtiter stage

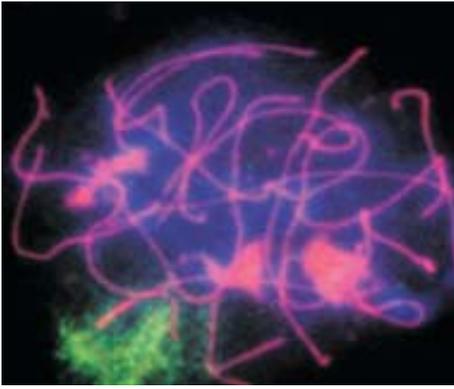
Increase the flexibility of the DeltaVision with the microtiter stage, designed to accommodate 96-, 384- and 1536 well microtiter plates. User-friendly software to collect and review multiple fields of view per well enables preset imaging patterns to tackle large screening assays. Ideal for high-throughput assays such as signaling molecules, drug efficacy or antibody titrations.



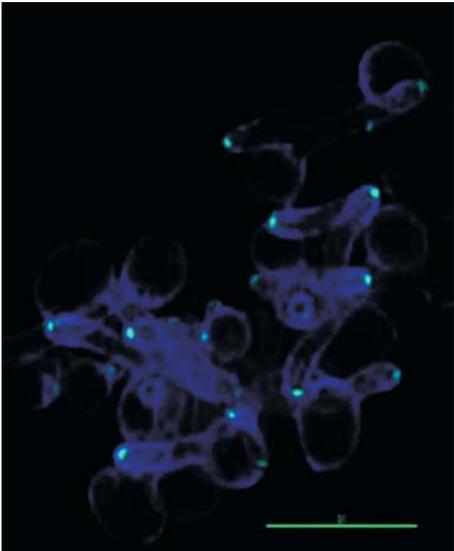
environmental control

- Precise temperature control
- Quick and easy installation
- Integrated CO₂ control & accessories
- Safe operation inside chamber - no moving mechanical parts or high voltage electrical components
- Ample working space inside the chamber
- Controllable temperature and CO₂
- External, low-vibration & low-noise design heater



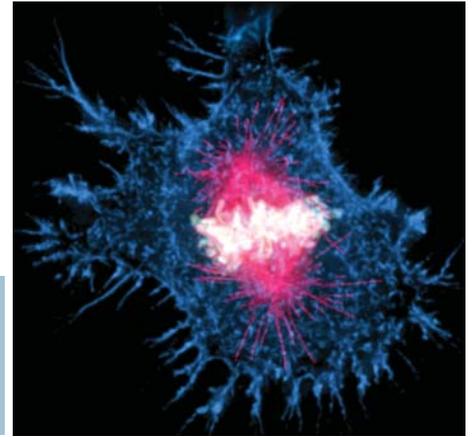


Identification of the X chromosome in female meiotic cells - This image shows 3 female germ cell nuclei (DAPI) undergoing meiotic pairing and recombination, which is mediated by the synaptonemal complex (sc red). The behaviour of the X chromosome can be traced by labelling with an antibody to XLR (green), a protein that preferentially associates with sex chromosomes. Image courtesy of James Turner, The National Institute for Medical Research, London, England.

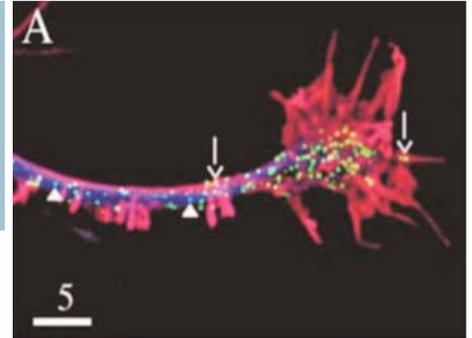


A *Xenopus* kidney tissue culture cell (XLK2) stained with anti-tubulin antibody to show microtubules. Rhodamine-Phalloidin to stain actin and DAPI to stain DNA. Image is a maximum intensity projection of a 3D deconvoluted data set. Image courtesy of Paul Andrews, Wellcome Trust Biocenter, University of Dundee, Scotland, U.K.

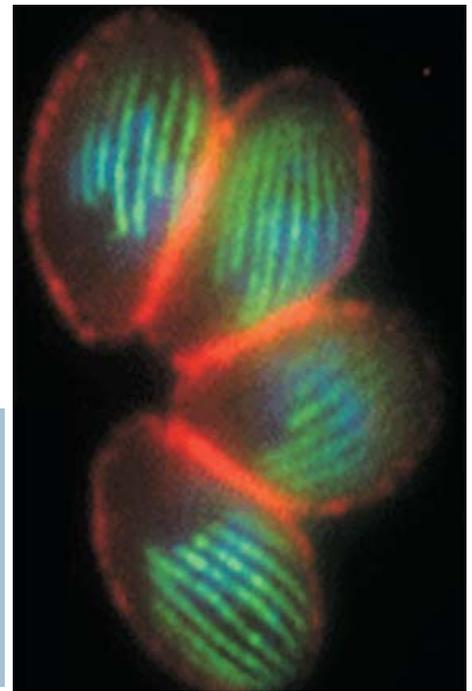
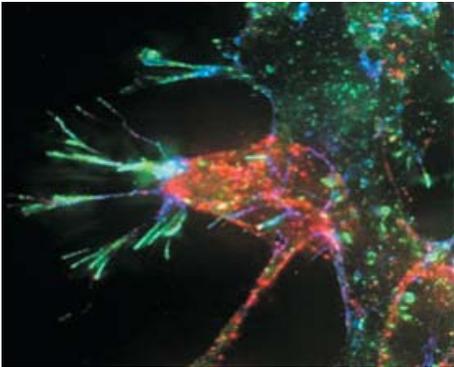
Fluorescence image of a growth cone and process of a neuron expressing tPA-EGFP (green) that was stained with anti-tubulin antibodies (blue) and Texas Red phalloidin (red). The arrowheads show DCGs that colocalize with microtubules, whereas the arrows show DCGs that colocalize with actin filaments. *The Journal of Neuroscience*, March 23, 2005, 25(12):3095-3106, 3095. Image courtesy of Dr. Beth A. Scalettar, Department of Physics, Lewis and Clark College, Portland, OR.



This picture shows the localisation Myosin light chain (Mlc1) in developing hyphae. This protein has been visualized by fusion to YFP. It localises to the Spitzenkörper forming a discrete yellow spot at the tips. The cell walls have been stained blue with Calcofluor. *Candida albicans* causes yeast infections (candidal vaginitis and vulvovaginitis (in women) and balanitis (in men)).



Fixed HEK293 cell with red labelled potassium channel, blue labelled actin filaments and green labelled cortactin in a projection near the coverslip. Image courtesy of Tony Morielli, University of Vermont.



Spindle microtubules form an intra-nuclear spindle to coordinate chromosome segregation during mitosis. The spindle microtubules originate in a dense plaque structure that is embedded in the nuclear membrane adjacent to cytoplasmic centrioles. The characteristic crescent shape and apical polarity of *Toxoplasma* is maintained by an interaction between the pellicle and the underlying twenty-two subpellicular microtubules. These microtubules are nucleated by the apical polar ring. Image courtesy of Naomi Morrisette, University of California, Irvine.

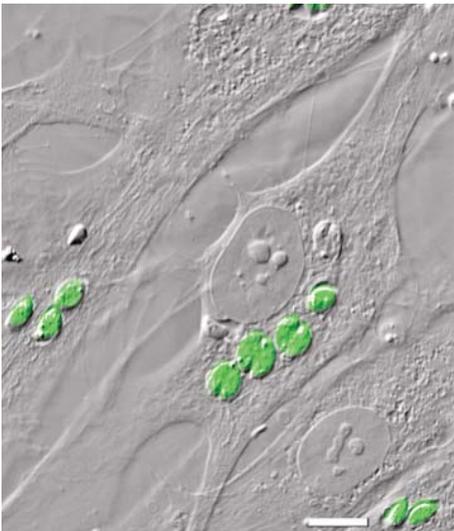
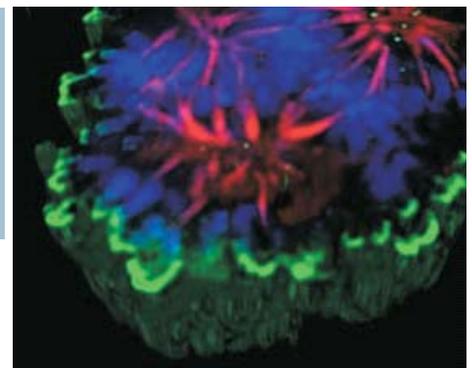
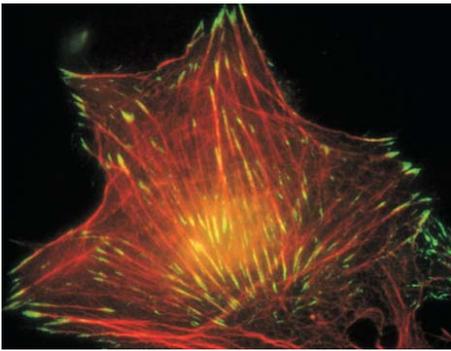


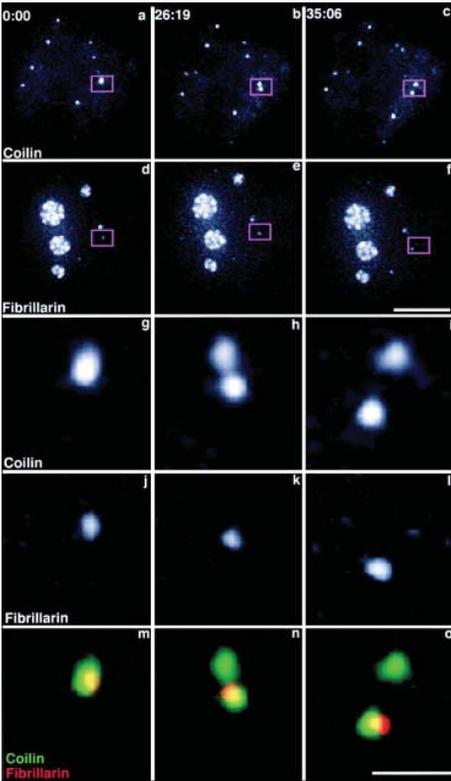
Image of an endomitotic megakaryocyte. The megakaryocyte is a bone marrow cell that is a precursor for blood platelets. It is called a megakaryocyte because it has many nuclei and is very large. The cell was stained for nuclei (blue), phosphorylated histone H3 (green) and alpha tubulin (red). Moeres UCSD Cancer Center. Image courtesy of Amy Geddis of the Division of Hematology/Oncology, Moeres UCSD Cancer Center, with assistance from Steve McMullen.



Living *Toxoplasma gondii* in Human Fibroblasts YFP-a-tubulin. Image courtesy of Ke Hu, David Roos, John Murray, University of Pennsylvania.

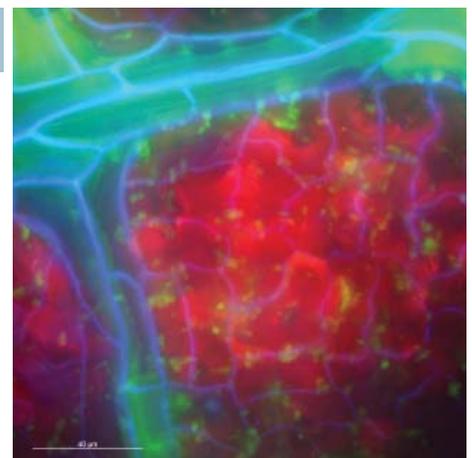
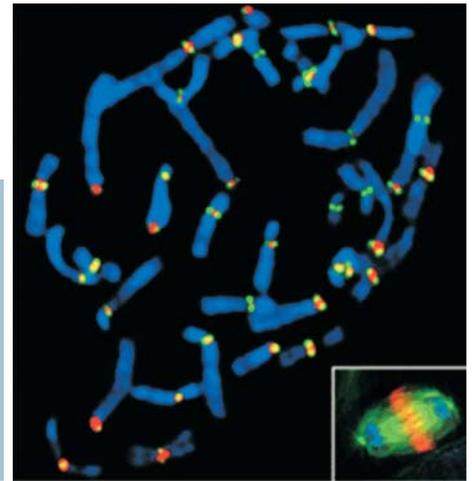


Porcine Aortic Endothelial Cells. Image courtesy of Ronen Zaidel-Bar, Benjamin Geiger. Wizeman Institute of Science.

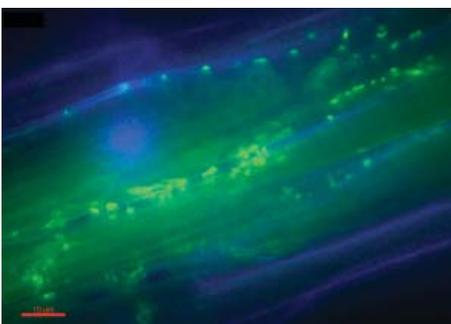


Localization of INCENP in the inner centromere of a metaphase-arrested cell and at the spindle midzone of an anaphase cell (lower right). The larger image shows spread chromosomes (stained blue with DAPI) from pig kidney epithelial cells (LLC PK) expressing chicken INCENP protein (stained red). The kinetochores are stained with human autoimmune serum (green). INCENP is concentrated in the inner centromere. At anaphase, INCENP transfers to the midzone of the spindle (inset in the lower right - INCENP = red; microtubules = green; DNA = blue). Image courtesy of Alastair Mackay, Bill Earnshaw, University of Edinburgh.

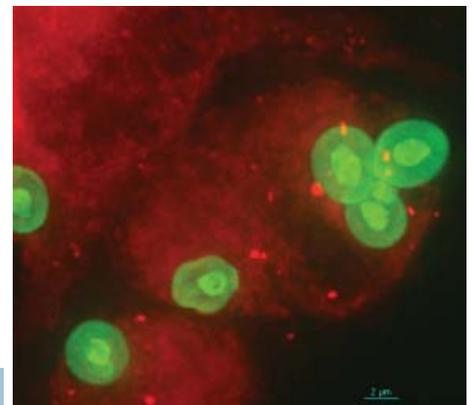
Imaging of 100 micron slices of autumn maple leaf. Image courtesy Kyla Teplitz and Kathryn Buchanan, Applied Precision, Inc.



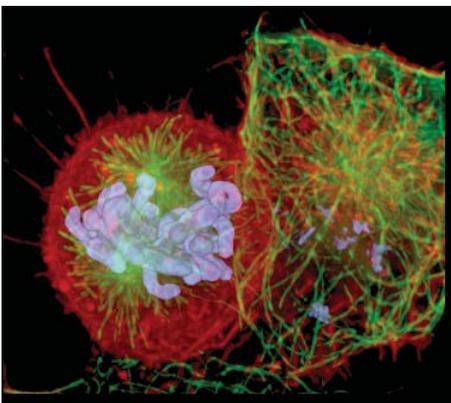
Differential partition of components after separation of a Cajal body. A plasmid containing YFP-fibrillarin was transfected into HeLaGFP-coilin cells. Maximum intensity projections from time-lapse three-dimensional data sets are shown. Top two rows show GFP-coilin (a-c) and YFP-fibrillarin (d-f). The boxed region is enlarged in the bottom three rows to show a CB splitting that generates an unequal distribution of YFP-fibrillarin to the two resulting Cajal bodies (GFP-coilin, g-i; YFP-fibrillarin, j-l; overlay, m-o). Image courtesy of Angus I. Lamond, University of Dundee, Dundee, Scotland.



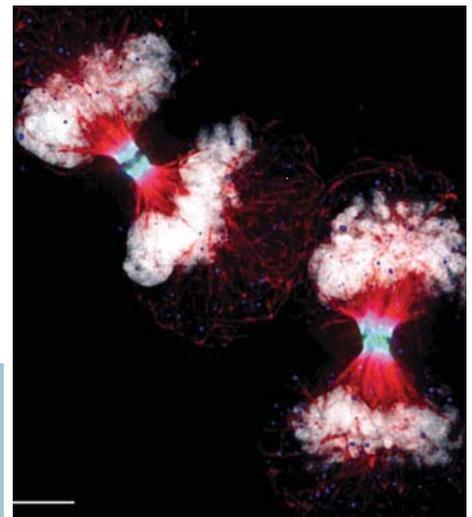
High resolution imaging of yeast. Image courtesy Lu Helinski, Emory University, Atlanta, GA.



Mitochondria trafficking in Arabidopsis root hairs - Image courtesy Naohiro Kato, 3D Microscopy of Living Cells Workshop.



Chinese Hamster Ovary - Image courtesy Paul Goodwin, Applied Precision, Inc.



Microtubules are shown in red, inner-centromere protein (NIECP) in blue, Aurora B_GFP in green and DNA in white. This is a maximum-intensity projection of a deconvoluted 3D data set. Nature Cell Biology, February 2003 Vol 5 issue 2 pg 101. - Image courtesy Paul Andrews, Wellcome Trust Biocentre, University of Dundee, Scotland, U.K.

product comparison chart

	DeltaVision Core	personalDV
base system		
microscope base	Olympus IX71	
illumination sources	250 W Xenon LED transillumination	
stage	NanoMotion III Control 25mm (X) x 50mm (Y) x 1mm (Z) patent pending flexure design stage	NanoMotion III Control 25mm (X) x 25mm (Y) x 1mm (Z) patent pending flexure design stage
filter sets	DV quad filter set DAPI FITC TRITC & Cy3 Cy5 5 element neutral density filter set	
filter wheel specs	10-position ND filter wheel 10-position EX filter wheel 6-position EM filter wheel	
objectives	qualified 60x oil 1.42 NA	
camera	CoolSnap HQ2 (20M/10MHz)	CoolSnap ES2 (20MHz)
computer hardware specs	dual processor/dual core CPU 4GB RAM 1TB RAID Storage DVD-R 24" LCD monitor	dual processor 2GB RAM 400GB RAID Storage DVD-R 19" LCD monitor
OS	Cent OS (Linux)	
image processing and analysis	softWoRx 3.7.0 (Linux)	
data management	DMS 1.1 (Win, MAC, Linux) softWoRx Suite 1.3	softWoRx Explorer (Win, MAC, Linux)
footprint	3 ft x 6 ft (90 cm x 180 cm) (includes airless vibration isolation table and electronics rack)	3 ft x 5 ft (90 cm x 150 cm) (includes vibration isolation platform)
options/upgrades		
filters	CFP/YFP AND EGFP/mCherry	
objectives	API certified 40x, 60x, 100x oil API certified 40x, 60x water	
QLM	FRAP/FLIP 488nm (base) PA-GFP 405nm (option 1) FRET 514nm (option 2) FLIP 561nm (option 3)	n/a
TIRF	available	n/a
multiplex	EGFP/mCherry Polychroic and Dual Band EM CFP/YFP Polychroic and Dual Band EM	
environmental control	integrated opaque chamber with CO ₂ , humidity and temperature controls	
data management	n/a	softWoRx Suite DMS 1.1 (powered by Glencoe)
stage	n/a	25mm (X) x 50mm (Y) x 1mm (Z) patent pending flexure design stage
table	n/a	airless vibration isolation table
camera	Cascade II EMCCD	CoolSnap HQ2
monitor	30" LCD monitor	24" LCD monitor