

Intelligent  
Imaging  
Innovations

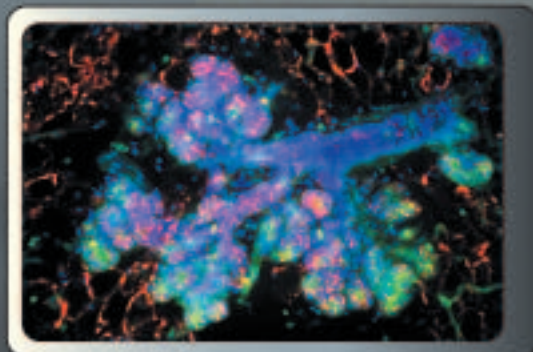
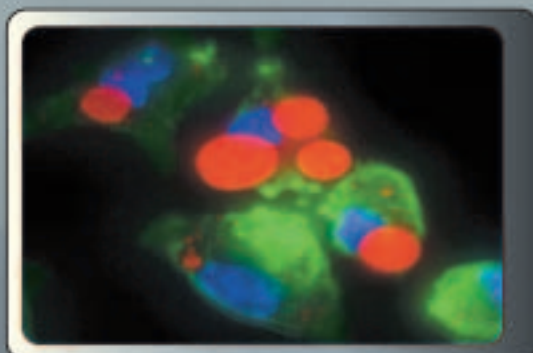
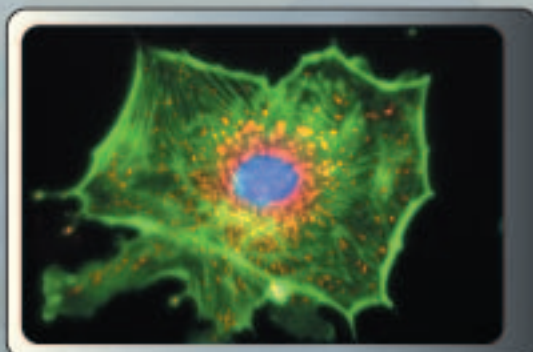
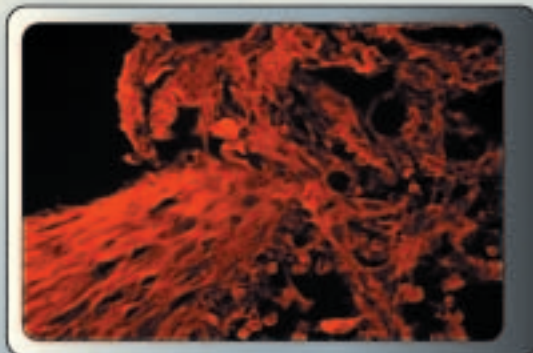
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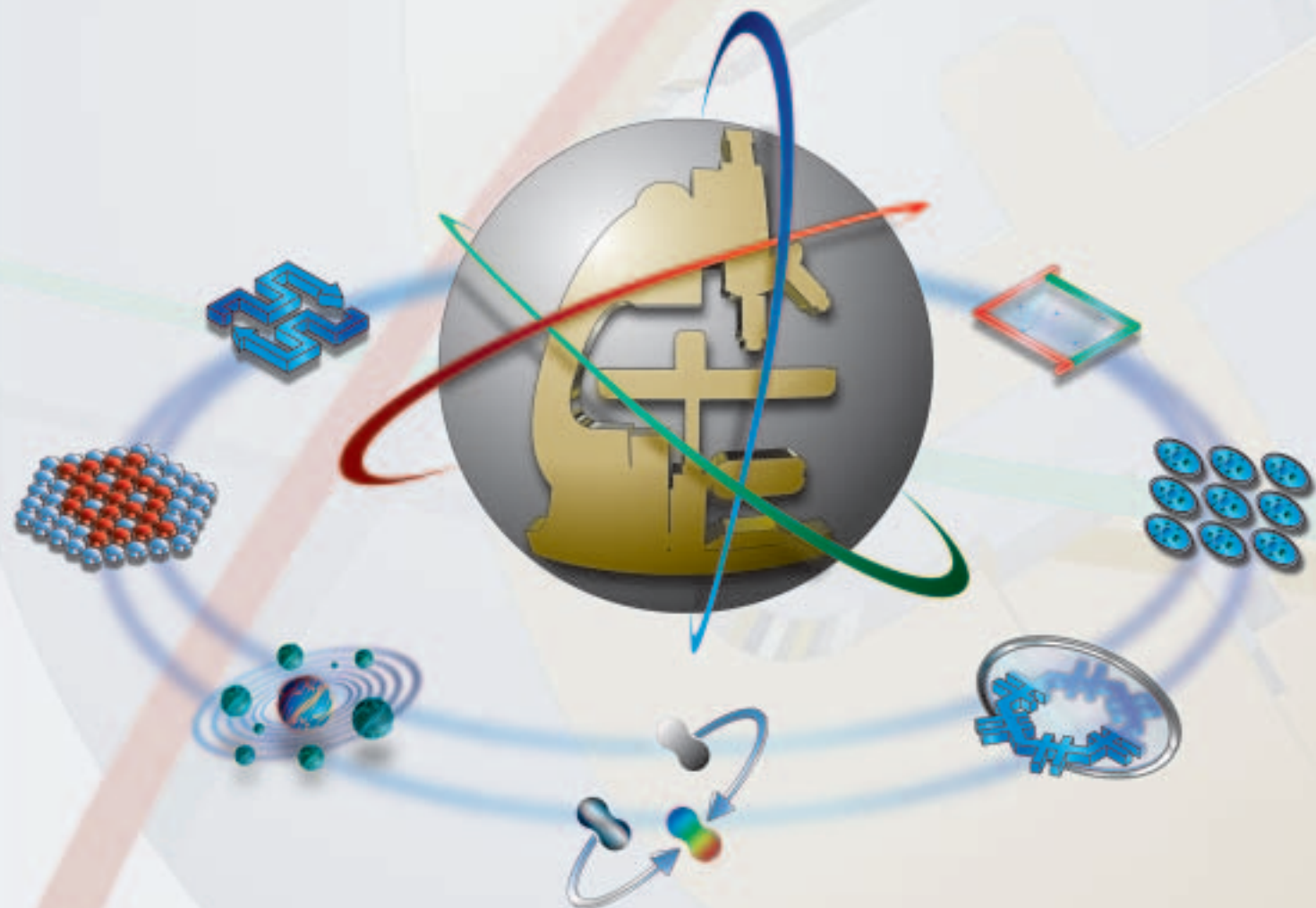


SLIDEBOOK™ 4.0

COMPLETE SOLUTIONS FOR ANALYTICAL MICROSCOPY

DIGITAL MICROSCOPY SOFTWARE FOR WINDOWS AND MAC OS

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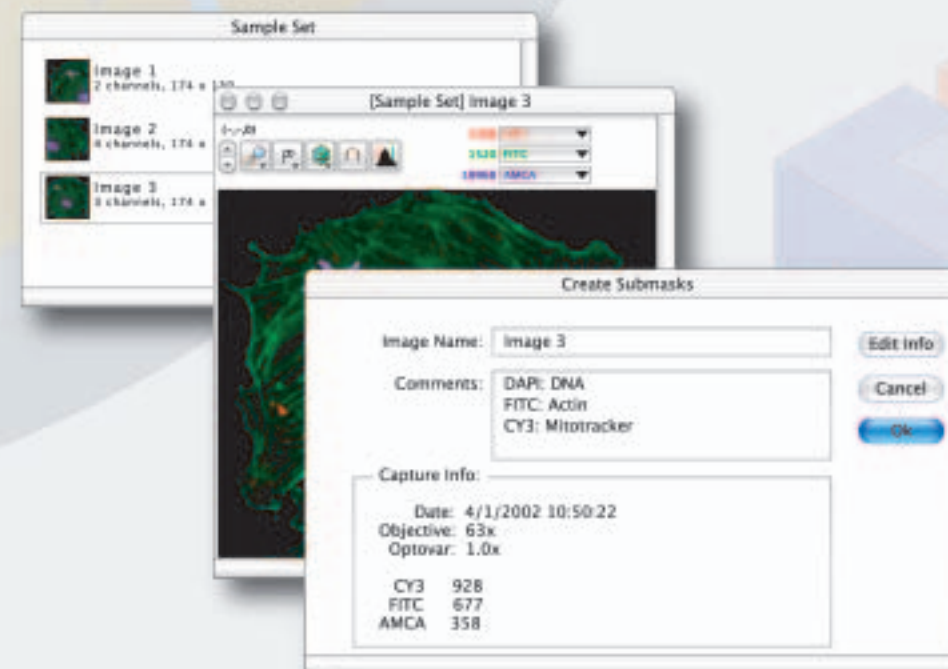
Digital microscopy has become an invaluable research tool for the study of complex cellular and molecular processes. Improvements in both cellular methods and imaging techniques have played important roles in the advancement of biological understanding. To keep pace with the fast-growing field of digital microscopy, one needs access to the cutting-edge tools of today and the flexibility to accommodate the novel tools of tomorrow. Powerful software is essential for capture and analysis of complex biological systems. Choosing appropriate software for digital microscopy is as important as choosing the correct conditions for an experiment. The software needs to be comprehensive in scope, flexible in ability, and analytically complete. While several software packages can provide some of these features, only one package integrates them all: SlideBook™ 4.0.

## SlideBook™ 4.0

The redesigned cross-platform SlideBook™ 4.0 application comes with all of the tools necessary for imaging data, from simple 2D and timelapse to 3D, 4D, and 6D acquisition. SlideBook was designed as a native 3D application, which allows easy manipulation and analysis of multidimensional data. SlideBook™ 4.0 runs on Windows 2000®, Windows XP®, Macintosh OS 9®, and Macintosh OS X®.



SlideBook™ 4.0 features the slide architecture, which allows the user to store related images together. This simplifies both data analysis and archive retrieval. The advantage of this system is that metadata, details of the experiment such as optical configurations and exposure times, are always stored with the image data. The metadata is used to exactly reproduce experimental conditions, perform deconvolution, and obtain calibrated measurements.



In addition, SlideBook™ 4.0 features a suite of analysis tools specifically designed for 2D, 3D, and timelapse microscopy data. These include area/ volume, perimeter/ surface area, length along major axis, mean intensity, minimum and maximum intensity, integrated intensity, distance between objects and cross-wavelength correlation.

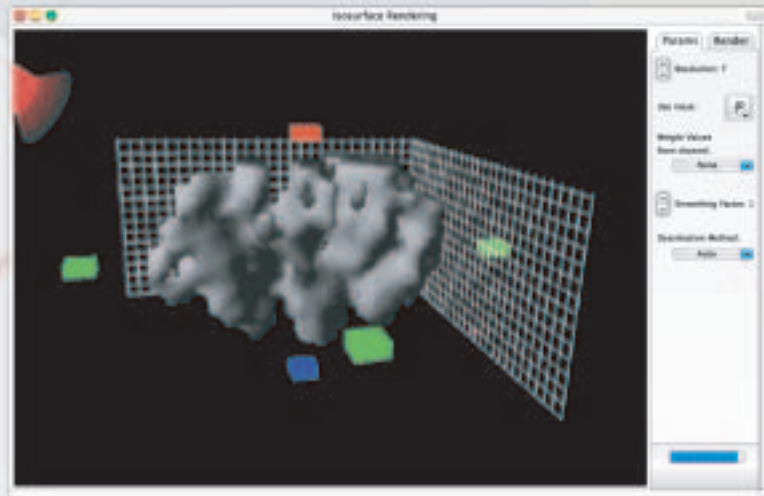
SlideBook's unique architecture facilitates the processing of 2D, 3D, and timelapse data by providing no neighbors deconvolution, common filters, photobleach correction, and custom convolution kernels. In addition to providing the tools for acquiring and analyzing 2D, 3D, 4D, and timelapse data, SlideBook controls all hardware associated with digital microscopy. Some of the features that are standard in SlideBook™ 4.0 are listed below.

### Image Capture Integration

The image capture integration features of SlideBook™ 4.0 allow the collection of everything from simple 2D images to advanced multi-wavelength 4D, and 6D data. Experiments can be seamlessly performed using a single flexible capture interface without the need for writing scripts or macros. This interface allows the user to optimize each imaging parameter, such as exposure time and frequency of collection. In addition, SlideBook™ 4.0 now allows the user to save and recall common capture schemes.

Types of capture that are supported include:

- 2D
- 3D
- Timelapse
- 4D (3D timelapse)
- Multipoint 2D, 3D, and Timelapse
- Montage Imaging
- Rapid 4D
- 6D (4D across multiple locations)

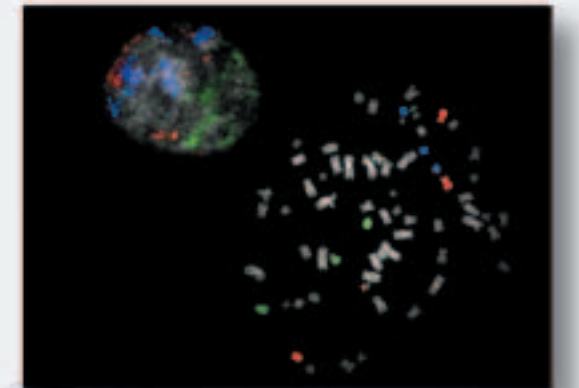
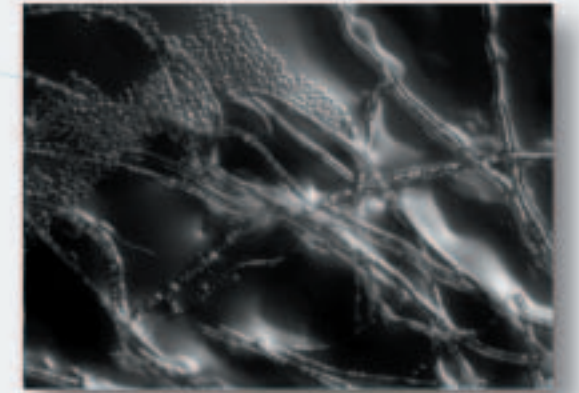


Further SlideBook™ 4.0 modules expand these capabilities with:

- *FRET (fluorescence resonance energy transfer)*
- *FRAP (fluorescence recovery after photobleaching)*
- *Photoactivation/uncaging*
- *Ratio Imaging*
- *Stereology*

Supported imaging techniques include:

- *Fluorescence*
- *Bright Field (monochrome and color)*
- *Dark field*
- *Phase Contrast*
- *DIC (differential interference contrast)*
- *IRM (internal reflectance microscopy)*
- *TIRF (total internal reflection fluorescence microscopy)*



SlideBook™ 4.0 automatically records all parameters used during image collection. These include the objective lens, magnification changing relay lens, optical filter configuration, xyz stage position, z spacing, capture time, capture interval, light source, etc. This metadata is permanently stored with the image data, allowing easy recall during analysis.

SlideBook™ 4.0 gives the experimenter control and live feedback during data collection. It can graph individual wavelength intensities, ratios, and calibrated ion concentrations for multiple adjustable regions, while continuously compensating for background. Live control allows capture pause, interactive refocusing, ROI adjustment, as well as event marking.

## Camera control

SlideBook™ 4.0 comes standard with drivers for most available scientific grade CCD cameras and frame grabber boards. SlideBook supports sub-frame readout, variable exposure times, binning, and advanced camera features.

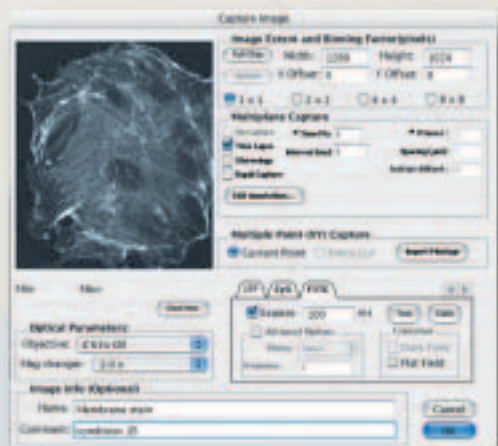
## Multi-Camera control

SlideBook™ 4.0 simultaneously supports up to four cameras, which allows the optimal detector to be used for a variety of imaging conditions. Single collections can be split across detectors to optimize collection speed or detector sensitivity.

Time resolution between related images such as FRET donor and acceptor pairs is critical for accurate image processing and analysis. Emission-based filter switching mechanisms can be slow relative to the cellular processes being studied. SlideBook™ 4.0 has been designed to capture images on multiple detectors simultaneously. This removes the necessity to move filters, maximizing collection speed and enabling precise comparison of two emission wavelengths simultaneously. This feature is used for high-speed FRET acquisition, multi-label live cell imaging, and emission-based ratio imaging.

## Z axis control

Z axis control enables SlideBook™ 4.0 to control a variety of axis positioning mechanisms, including stepper motors, piezoelectric focusing collars, and internal z axis microscope motorization.



## Filter and Shutter control

Filter control enables SlideBook™ 4.0 to direct a variety of filter switching devices. These include filter wheels, tunable liquid crystal devices, rapid wavelength switchers and monochromators. SlideBook also automatically controls multiple transmitted light and fluorescence shutters.

## Automated scope control

SlideBook™ 4.0 controls all modern motorized microscopes (Leica, Nikon, Olympus, and Zeiss). Additionally, SlideBook can control of the z axis position, internal filter turret, objective nosepiece, transmitted light lamp intensity, motorized condenser, motorized camera port prism, neutral density filters, motorized diaphragms, and shutters.

## Image Splitter support

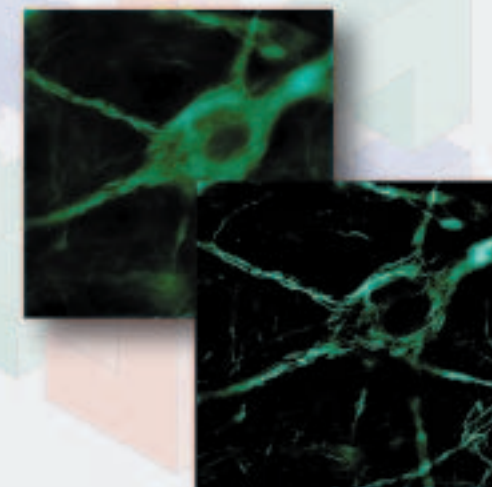
SlideBook™ 4.0 supports the both two- and four-way image splitting devices, such as those manufactured by Optical Insights and Hamamatsu. SlideBook automatically combines the split images and overlays them on-the-fly for real time analysis of data.

## Cell Robotics LaserScissors® and LaserTweezers®

SlideBook™ 4.0 includes support for Cell Robotics LaserScissors® and LaserTweezers® technology. SlideBook can coordinate control of laser power, xy stage position, cutting ROI, Cell Selector state, and more.

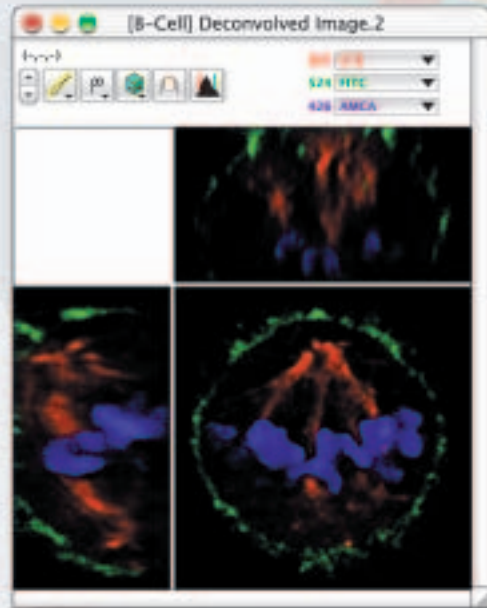
## No Neighbors Deconvolution

The No Neighbors Deconvolution algorithm provides the user with basic deblurring functionality, which is suitable for 2D and timelapse data. The algorithm has been optimized for both speed and performance. No neighbors deconvolution seamlessly interacts with images captured using SlideBook by reading image metadata. This makes no neighbors deconvolution a simple one-click operation.



## 3D image collection, visualization and analysis

Biological samples do not live in two dimensions, so why should your analysis and exploration tools? The 3D features of SlideBook™ 4.0 extend all analysis, rendering and exploration tools into the third dimension.

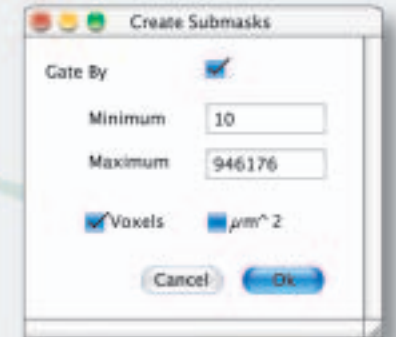


SlideBook™ 4.0 provides a variety of views for exploring data, including the tile view, channel view, and three view. These views allow intuitive exploration of 3D and 4D data within a single window. SlideBook™ 4.0 adds several new views, including QuickTime® VR rendering, isosurface generation and rendering, surface plots, and enhanced 3D movie generation.

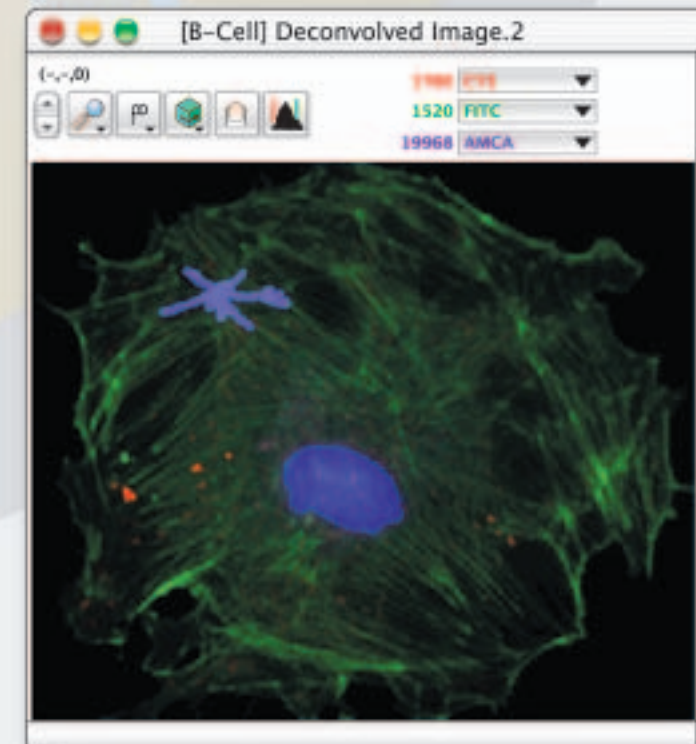
SlideBook™ 4.0 contains many tools to segment data for analysis. These include 3D segmentation by intensity, gradient, or edge, 3D erosion and dilation, and manual selection delineation. New segmentation features include 3D “magic” tools for interactive selection of multiple objects, as well as 3 dimensional skeletonization and medial axis determination.



SlideBook supports classification of data into individual objects, which can be analyzed in 3D. Objects can be classified by morphometric properties. These features can be selected from the standard library or developed by the user.



SlideBook™ 4.0 can quantify data using a variety of standard metrics. In addition, users can write their own metrics. SlideBook™ includes numerous measurement tools for 3D data. Some of the statistics available are volume, surface area, center of volume, center of intensity, 3D mean intensity, 3D line intensity plot, 3D distance (linear and non-linear), major axis length, and 3D object-to-object distance.



SlideBook™ 4.0 supports advanced techniques for remapping data to an object-centered coordinate system, which is useful when a feature of interest does not lie along any of the three canonical axes. SlideBook allows curvilinear analysis to profile intensity variation along smooth curves through the remapped image space.

## Statistics Engine

Modern digital microscopy is becoming increasingly focused on quantitative data analysis. The Statistics Engine for SlideBook™ 4.0 provides researchers with a new suite of analytical tools designed to quantify complex data. Features of the new statistics engine include:

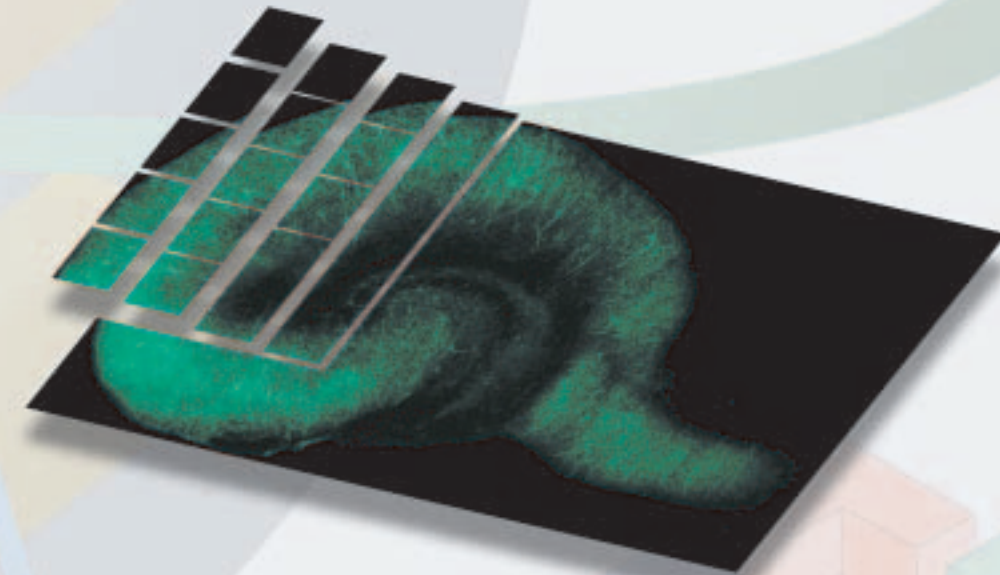
- A flexible plug-in architecture
- Automatic methods for generating and storing statistics to aid in gathering large amounts of data
- Advanced clustering techniques to help define populations of objects and images
- Many new statistical metrics, including multiple label colocalization
- Skeleton-based features, such as branching analysis



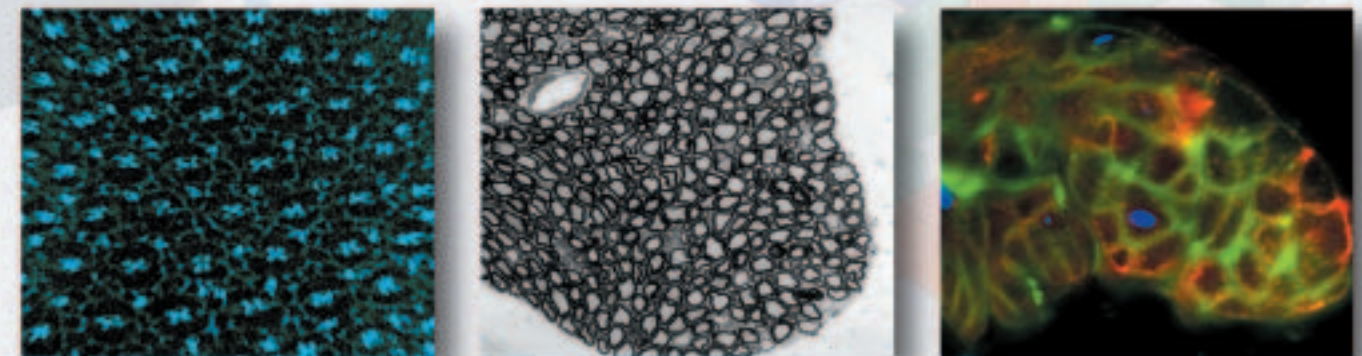
## Montage

SlideBook's Montage feature allows researchers to capture images larger than a single camera field. This capability is often needed to image large tissue sections and to perform quantitative analysis on entire organs.

During montage capture, SlideBook™ 4.0 controls a motorized xy stage to collect individual camera frames at adjoining locations. These frames are assembled into a single, seamless full-size image.



Montage collection can be performed using both fluorescent and transmitted light imaging modes.



## Additional Modules



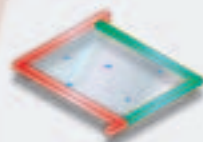
### Ratio Imaging/FRET/Densitometry Module

This module provides easy-to-use tools for both on-line and post-capture analysis and exploration of ratiometric data. This includes support for fura-2 imaging, fluorescence resonance energy transfer (FRET) experiments, and densitometry measurements. The module adds real-time graphing of ratios and intensities, background subtraction, and tracking of multiple objects. It also incorporates post-capture ratio generation, a ratio calibration guide, and both direct sensitization FRET and acceptor photobleaching FRET capture protocols and transfer computations.



### TTL Synchronization

The TTL Synchronization module provides software control of an analog/digital I/O board. The module can tightly control device timing via TTL pulses, improving system performance. This module can also be used to synchronize external stimulation and data collection instruments, such as electrodes, patch clamp recording devices, and perfusion systems.



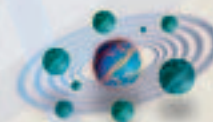
### Stereology

The Stereology module employs unbiased stereological techniques for accurate estimation of total number, volume, surface area, and length of objects in a biological structure. Stereology systematically samples 3D volumes from a series of tissue sections in a random fashion. The module supports stereology for both transmitted light and fluorescence microscopy, and includes an offline mode that permits acquisition and counting to be performed separately.



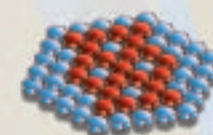
### 3D Deconvolution

The 3D Deconvolution module adds a 3D deblurring and an image restoration algorithm to SlideBook. The nearest neighbor and constrained iterative deconvolution algorithms have been optimized for speed and performance. The module includes an interactive guide for measuring point spread functions (PSF) and a PSF database. The database can store multiple PSFs for each configuration, allowing easy selection of the appropriate PSF.



### 4D Particle Tracking

The 4D Particle Tracking module offers tools for tracking and quantifying 4D data. The module enables the user to follow multiple 3D objects over time through an intuitive interface. Object paths can be generated through standard routines or customized approaches. Once object paths have been established, the 4D analysis tools allow the user to quantify changes in each object over time.



### CellNet™

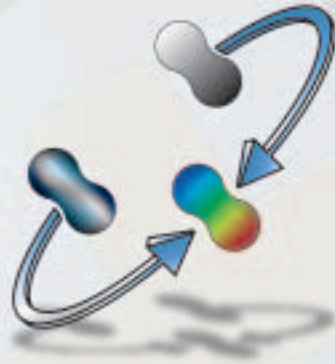
The CellNet™ Pattern Recognition module automatically identifies 2D or 3D objects through proprietary neural network technology. CellNet™ can be taught to recognize many types of objects. This segmentation system rapidly identifies objects from both simple and complex data sets. In addition, CellNet™ can simultaneously recognize a number of different types of objects within the same data.



### Multi-Well Plate Screening

The Multi-Well Plate Screening module enables SlideBook to automatically scan and image multi-well plates. This module coordinates x,y,z stage control, filter control, auto-focusing, and image capture for rapid screening of multi-well plates. Image data can be automatically processed by advanced analysis routines and summarized in reports.

## Ratio Imaging/FRET/Densitometry

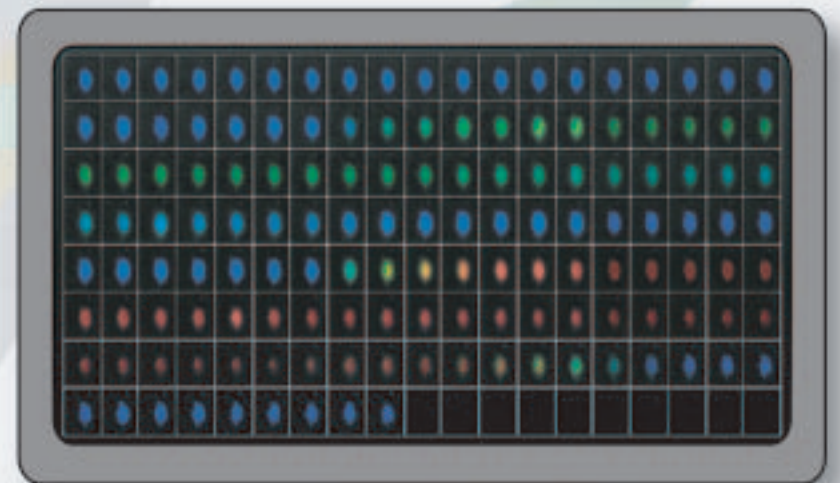
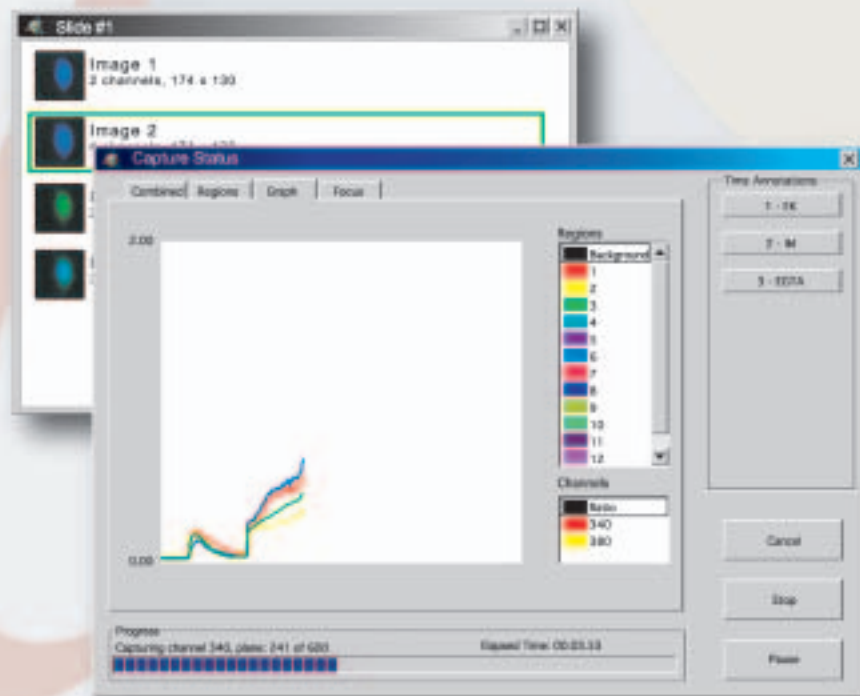


Certain experimental imaging techniques require two wavelengths to be displayed as a ratio. The Ratio Imaging/FRET/Densitometry module for SlideBook™ 4.0 is designed for these applications.

### Ratio Imaging/Densitometry

The measurement of free ion concentrations in living cells provides insight into cellular processes such as activation and compartmentalization. Ratiometric ion indicators change their excitation or emission profiles when bound to specific ions. These indicators allow measurement of ion concentrations in living cells. This module supports a wide array of ion indicators including fura-2, indo-1, and BCECF. The module includes:

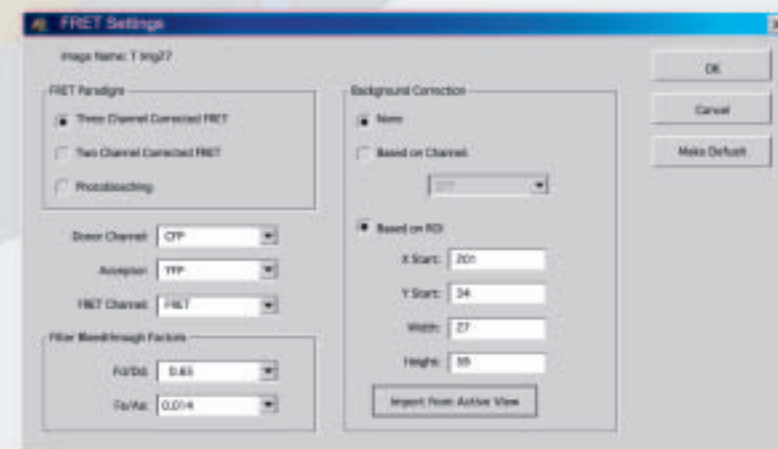
- Real-time ratio computation during image acquisition
- Real-time graphing of ratios individual wavelengths during acquisition
- Real-time tracking of up to 20 ROIs
- Simultaneous imaging of two ratio indicators
- Calibration guide for computing nM ion concentration
- Analytical graphing tools



### FRET Analysis

The Ratio Imaging/ FRET/ Densitometry module is the first commercially available software specifically designed for FRET analysis. This module was developed in collaboration with a number of leading FRET biologists. The module contains analysis tools for both sensitized emission FRET and acceptor photobleaching FRET:

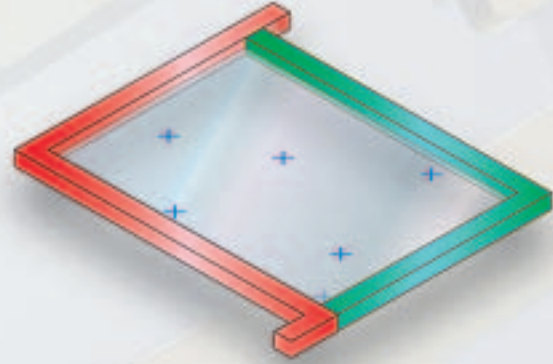
- Channel bleedthrough calculation
- Background subtraction
- Corrected FRET image generation (Herman equation)
- Single pixel to whole object energy transfer measurement
- Support for 2D, 3D, and 4D data



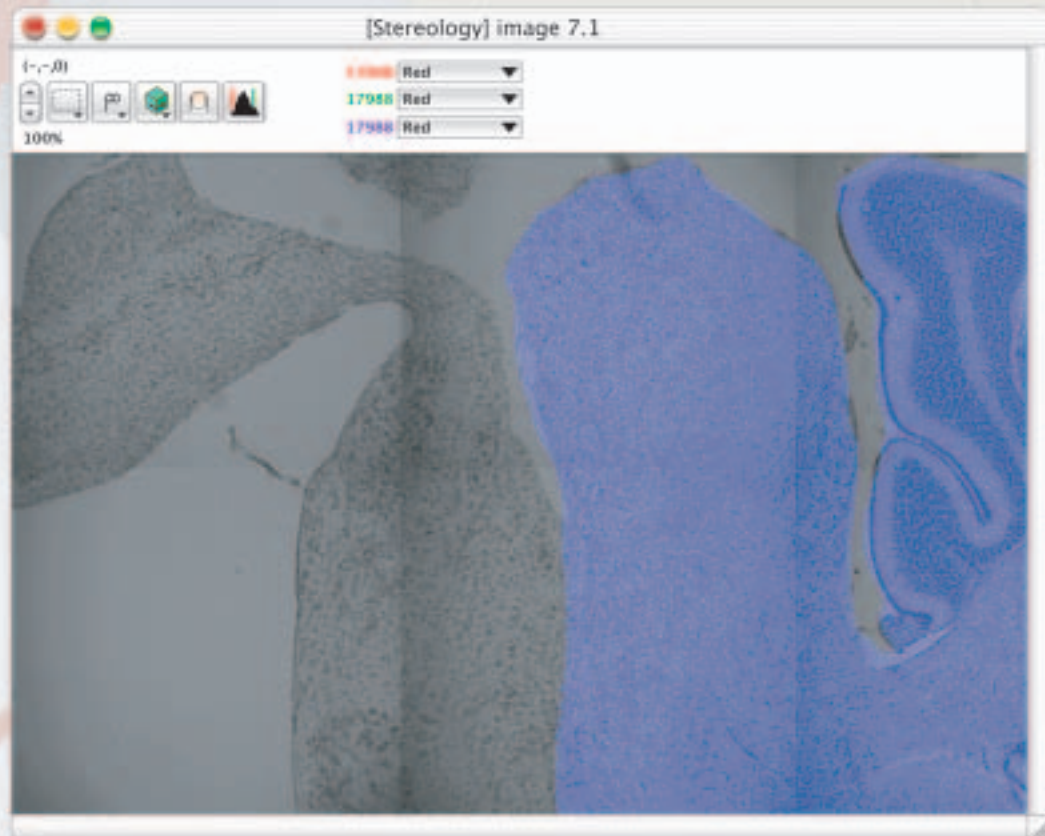
This module enables the user to perform real-time FRET analysis by supporting dual camera capture and image splitting devices.



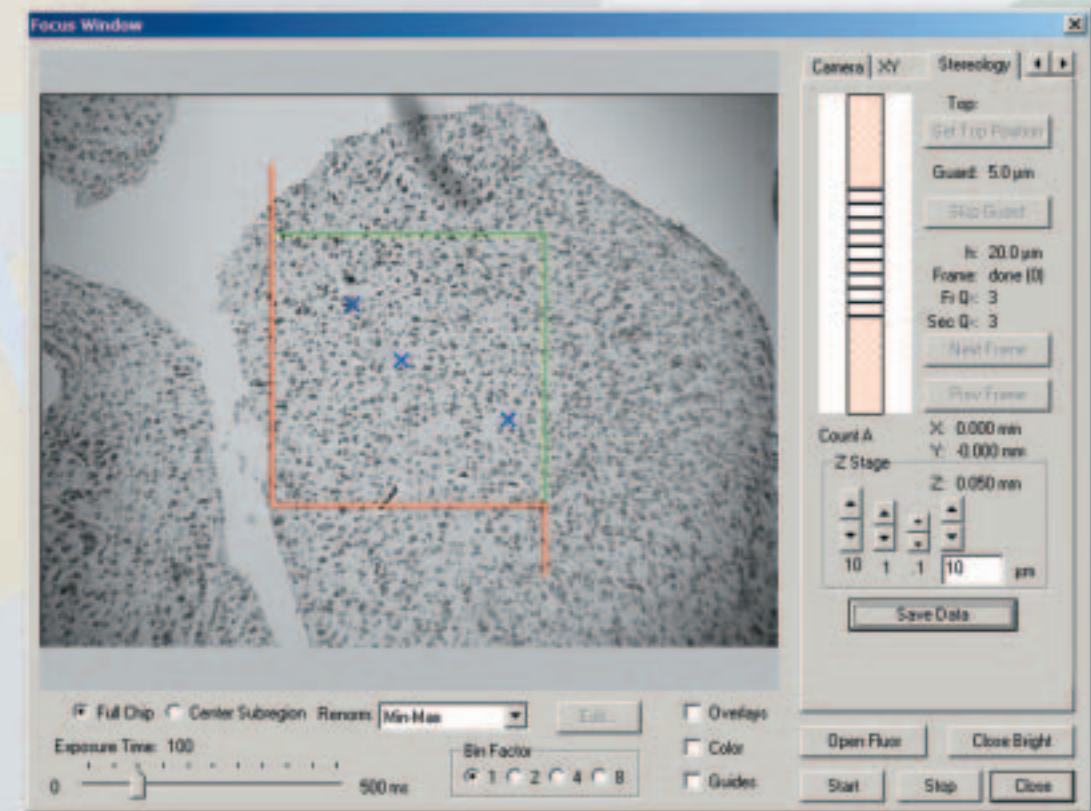
# Stereology



As quantitative questions move from the cellular to the organ level, direct measurement is often best replaced by efficient sampling. The field of unbiased stereology has made many recent advances, allowing researchers to accurately estimate from a small sample the number of cells of interest in an organ or other anatomically delineated structure. Stereological tools can also be used to estimate total surface area, volume, or length (for instance, the total length of vasculature within an organ).



SlideBook™ 4.0 integrates support for a number of stereological tools within its standard capture framework. Stereology works in concert with montage capture to outline the boundaries of a structure within a tissue section at low magnification. After a scoring region has been defined, a count can be performed at high magnification with the Stereology module controlling the stage position and the counting grids.



Stereology support in SlideBook™ 4.0 has been seamlessly integrated with fluorescence as well as transmitted light capture. Many dedicated stereology systems have been designed solely for transmitted light microscopy, where photobleaching is not an issue. SlideBook, however, supports offline stereology. This permits multiple counting volumes to be collected quickly for later analysis in order to minimize photobleaching.

## 3D Deconvolution



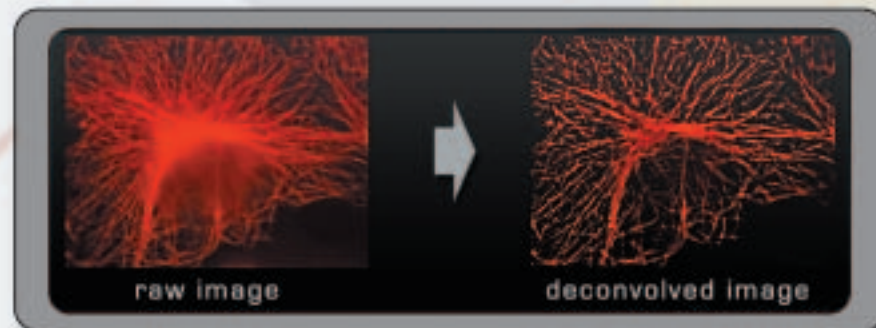
SlideBook™ 4.0 features an expanded and improved 3D Deconvolution module that extends the base application's ability to remove out-of-focus information from 3D fluorescence data. This module includes two algorithms:

### *Nearest Neighbors Deconvolution*

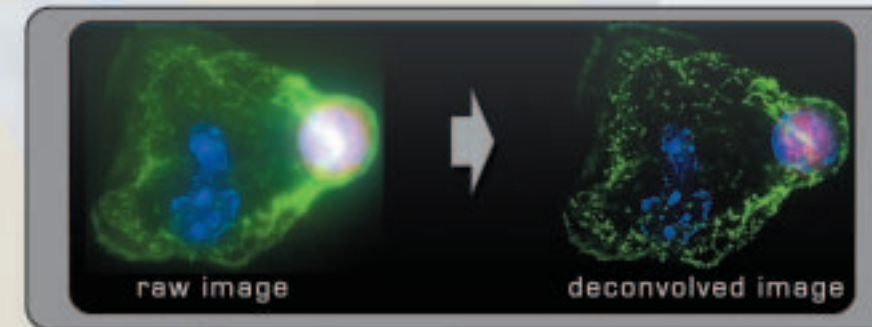
Our nearest neighbors deconvolution is a rapid way to deblur fluorescence data. The algorithm uses the plane above and below the plane of interest to compute and subtract the fraction of the data that is out-of-focus.

### *Constrained Iterative Deconvolution*

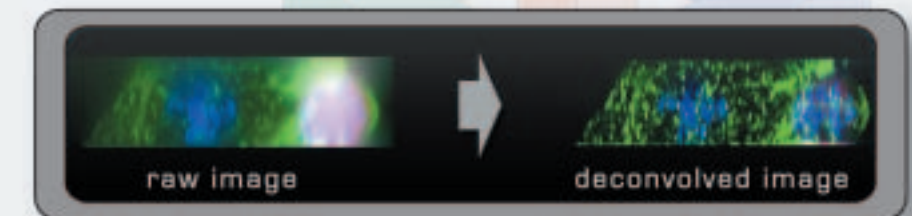
Our constrained iterative (CI) deconvolution is a true image restoration tool. Based on an algorithm developed by David Agard and John Sedat at UCSF, our CI deconvolution can quantitatively reassign out-of-focus information in 3D data while improving both axial and lateral resolution.



The module can use either measured point spread functions (PSFs) or, when a measured PSF is unavailable, theoretical computed PSFs. The module includes a PSF collection guide and a PSF database. SlideBook™ can use image metadata stored during capture to automatically select the appropriate PSF.



With SlideBook™ 4.0, CI deconvolution includes extensive speed improvements by taking advantage of advances in computational algorithms, multiple processors, and hardware acceleration for both Macintosh and Windows platforms. CI deconvolution has been improved so that it can handle deconvolution of very large data sets (up to 350 MB per wavelength for a system with 2 GB RAM). In addition, CI deconvolution includes a number of advanced user options to compensate for a variety of imaging conditions so that an optimal deconvolution can be achieved for a particular experiment.



## Multi-Well Plate Module



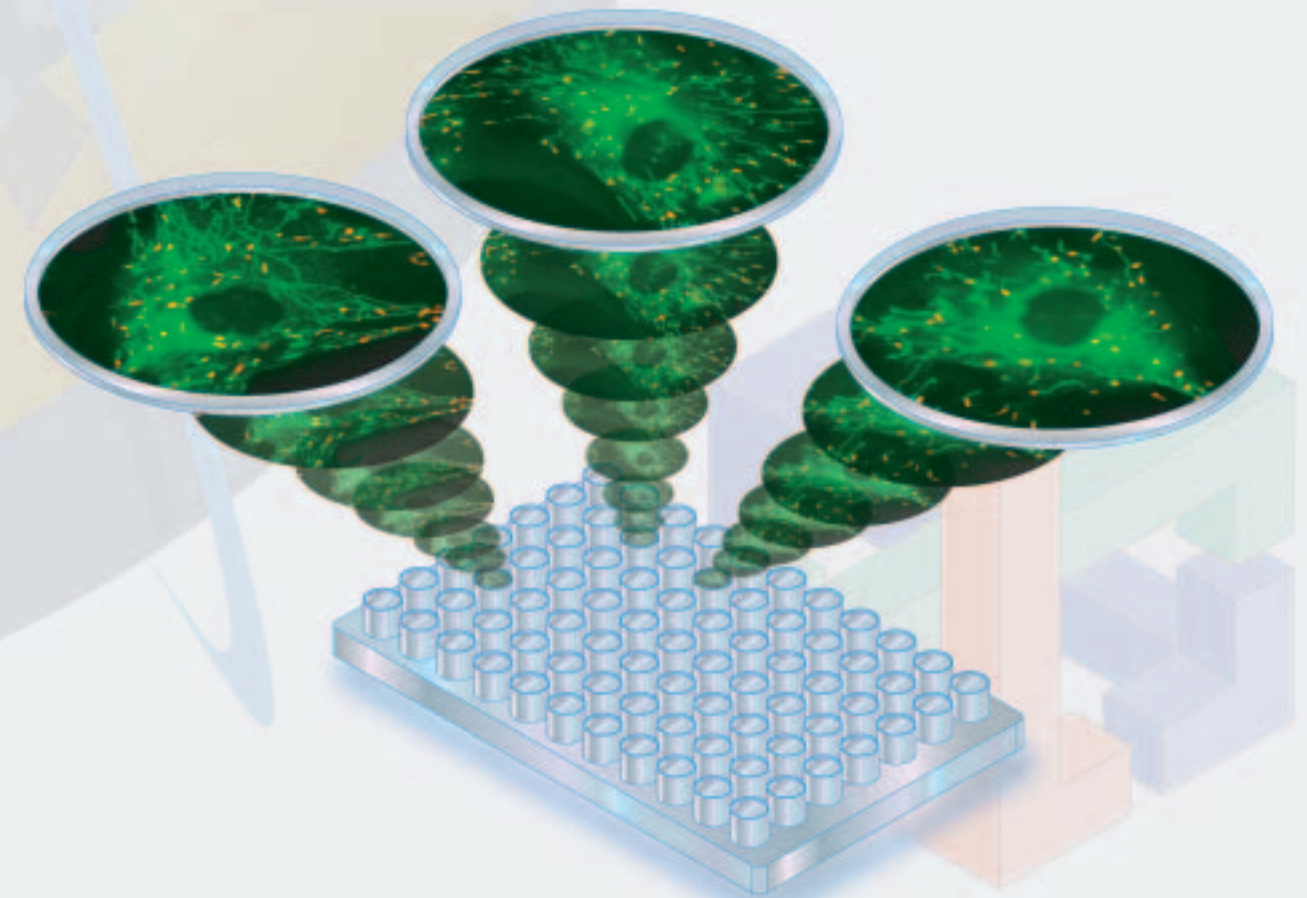
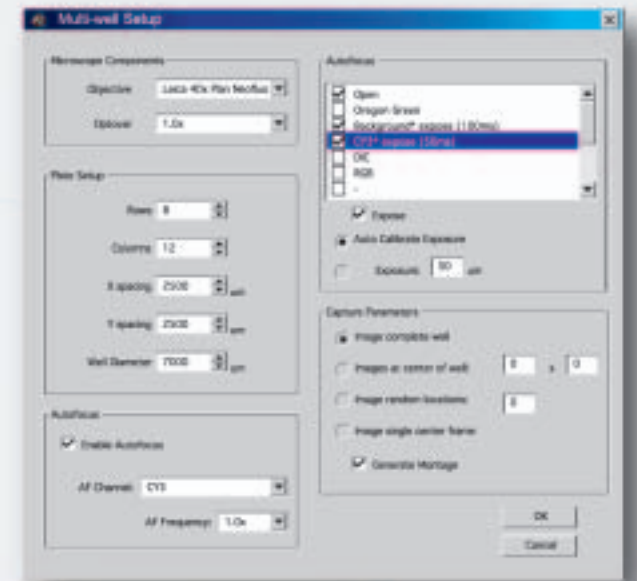
SlideBook™ 4.0 features a new, easy-to-use plate reading module. This module enables SlideBook™ to capture data from a wide range of multi-well plates, including 24, 96, and 384-well plates.

This module gives the researcher complete control over all imaging parameters. An experiment can include any combination of SlideBook-supported imaging modes, including multi-spectral fluorescence and combination fluorescence/transmitted light imaging. In addition, this module includes an optimized autofocus algorithm to ensure high quality image data. The researcher can select the range of wells to be imaged, along with the location and number of images to be taken per well. These parameters can be saved as a template, making it fast and easy to image multiple plates under identical conditions.

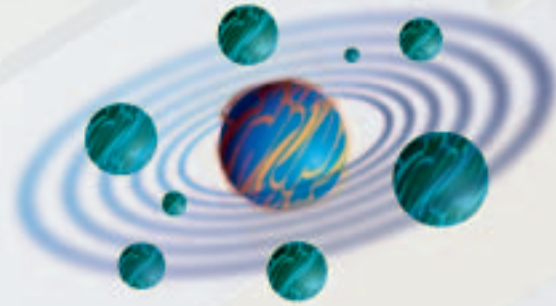


Designed to facilitate both automated and manual review of image data, the software enables the researcher to quickly evaluate and annotate images captured from a particular well or an entire plate. The resulting image data can also be analyzed using SlideBook's quantitative image analysis features.

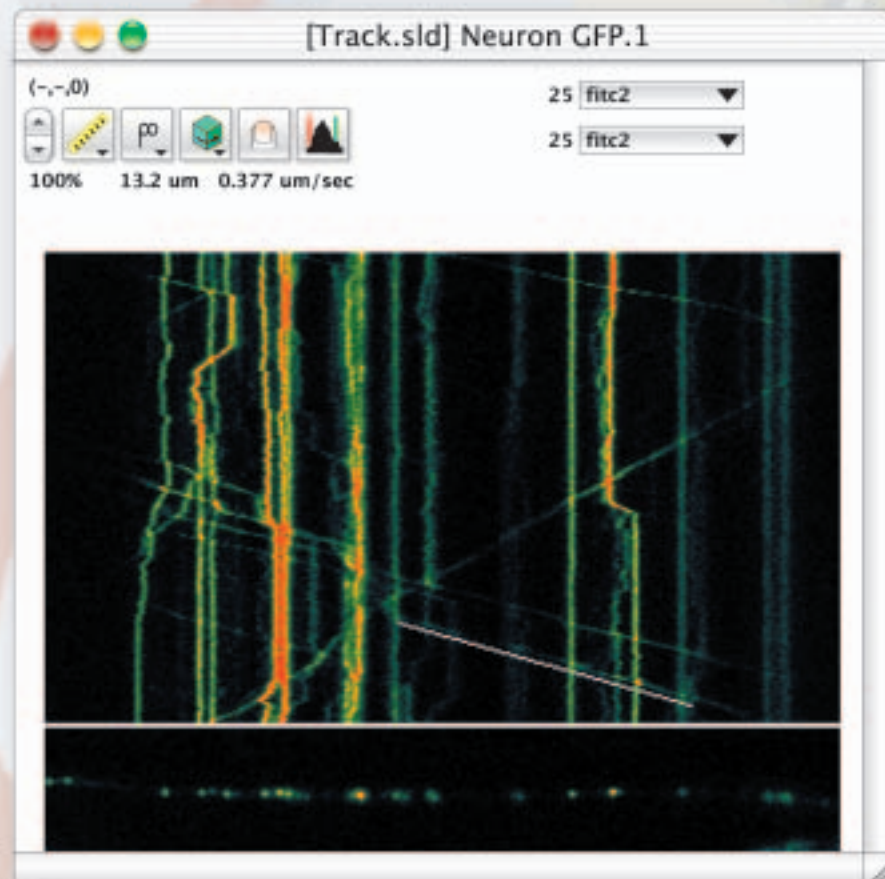
The Multi-Well Plate Reading module leverages the integrated power of SlideBook to provide a flexible and economical way to conduct medium-throughput, high content screening experiments.



## 4D Particle Tracking

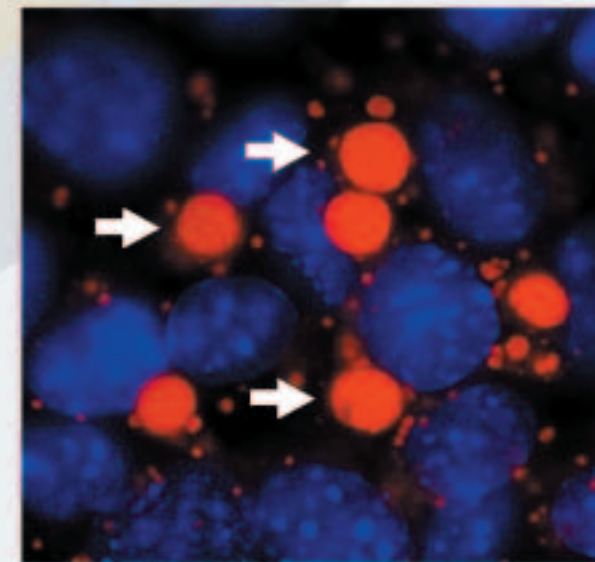
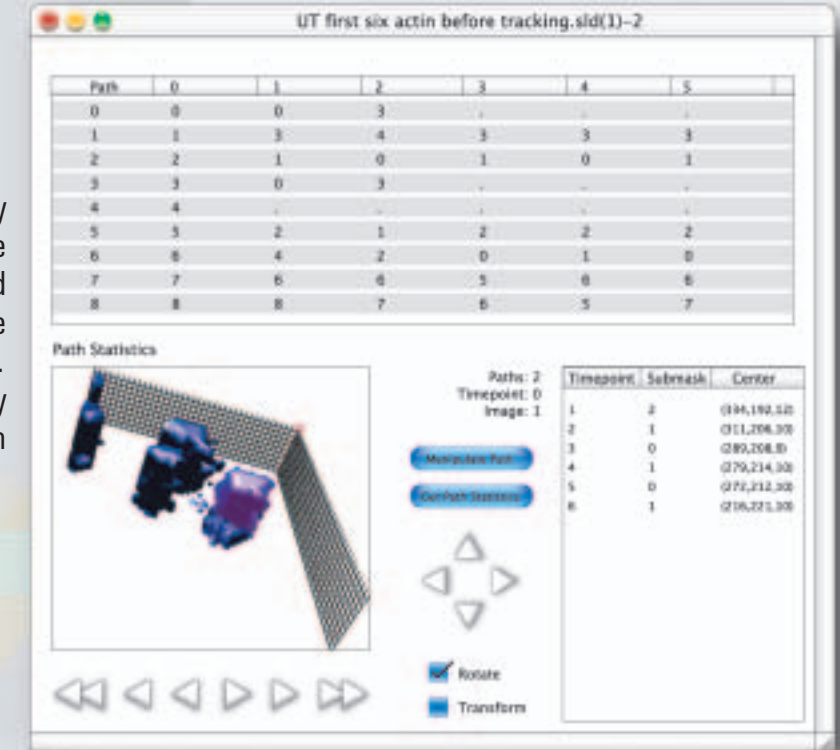


The new 4D Particle Tracking module contains tools to track individual 3D objects through time. The Particle Tracking engine is based on individual object paths, which can be followed through time. The Particle Tracking module can also correlate objects with respect to one another and generate statistics about each object as it evolves.



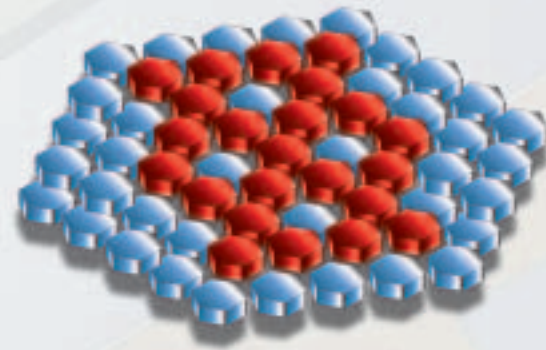
Object paths can be traced automatically or by manual delineation. The Particle Tracking module offers flexible tools to review and edit paths as well as combine both automatically generated and manually selected object paths.

The module includes a variety of visualization tools to help explore object paths. In the OpenGL-based 3D path view, a trajectory can be selected and rendered over time. This rendering can be rotated freely and viewed as a continuous animation or in single frame increments.



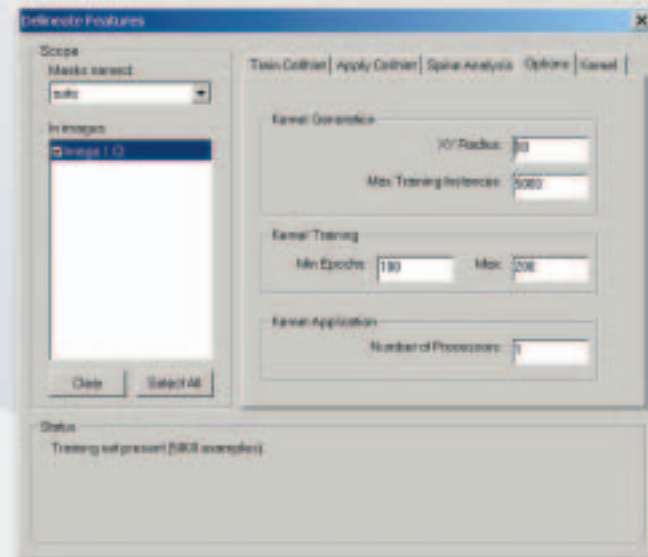
In addition to generating statistics about each object at each time point, the Particle Tracking module can compute descriptive statistics about an object's path through time, including average and instantaneous velocity, acceleration, and level of randomness in an object's motion. These statistics can be automatically generated for all paths or on a path-by-path basis.

## Cell Net™ Pattern Recognition

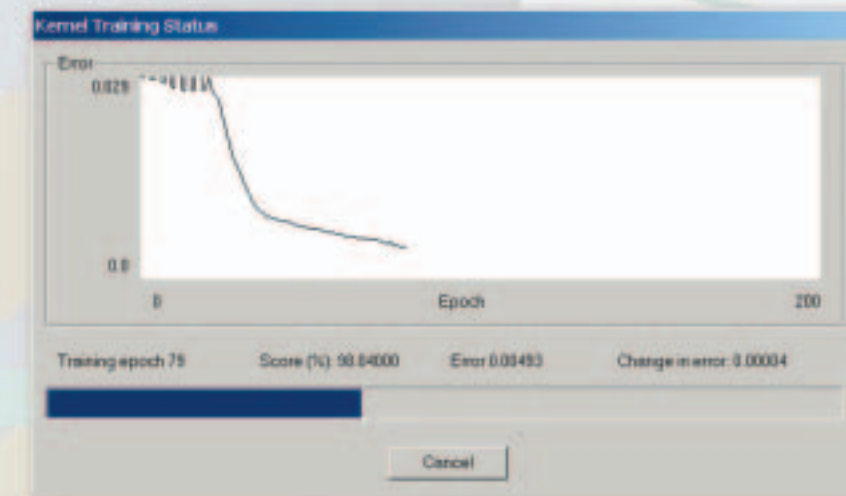


As our ability to collect multidimensional data outpaces our ability to manually analyze it, automated analysis tools have become essential. We designed CellNet™ as a tool that allows the researcher to teach the computer specific analysis by example. The computer can then rapidly apply the analysis to a large amount of data. This allows a significant increase in analysis throughput.

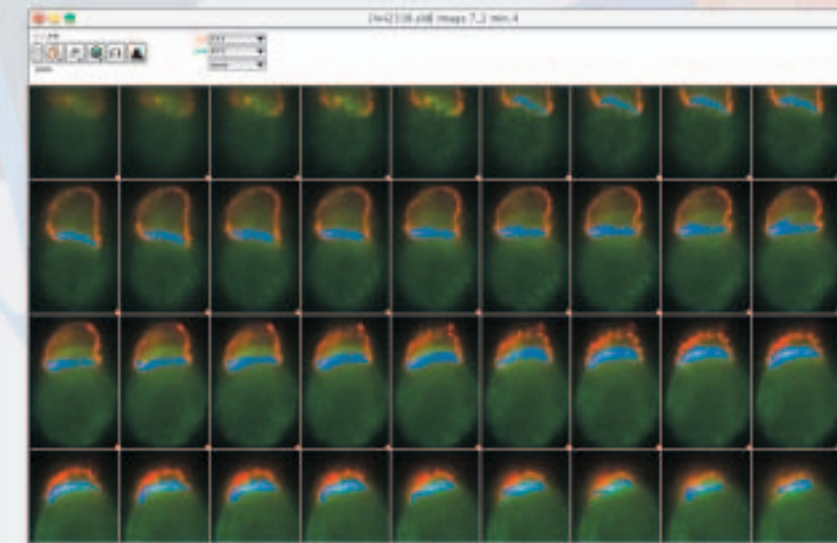
CellNet™ gives SlideBook the ability to recognize cells and subcellular features based on morphology. Besides making it possible to locate objects that are hard to describe using only intensity information, CellNet™ is able to trace the extent of individual objects for subsequent statistical analysis.



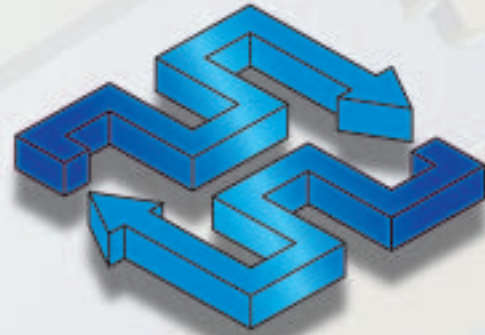
Employing proprietary neural network technology, CellNet™ builds a typical representation that identifies persistent features from a set of example images that the user provides. It can then find objects that possess similar features in new images.



CellNet™ can work on 2D and 3D data and can also provide the basis of segmentation for timelapse and 4D particle tracking. It is particularly valuable where object identification is difficult.

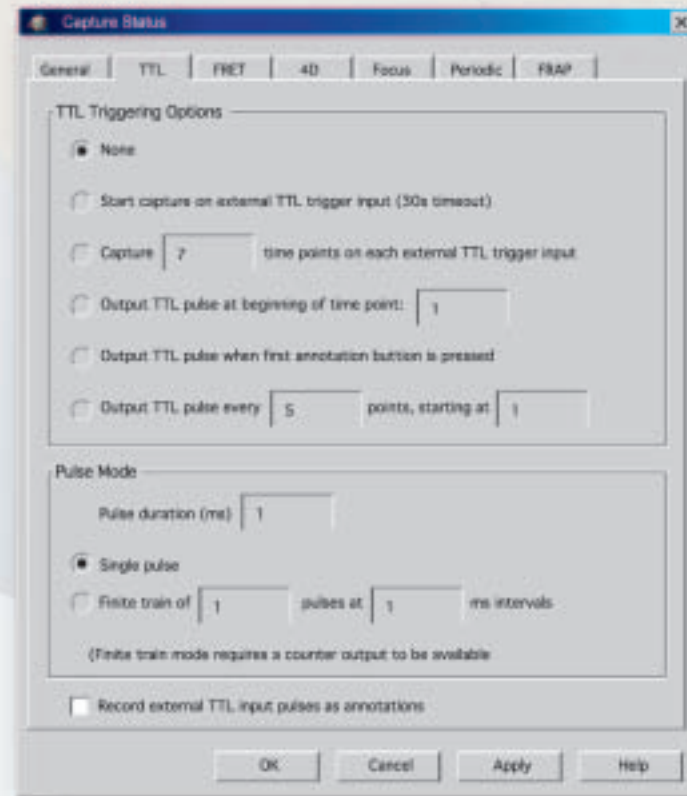


## TTL Synchronization



The TTL Synchronization module for SlideBook™ 4.0 is a digital I/O board and software specifically designed to optimize the synchronization of hardware. Normally, hardware device control relies on slower, RS-232 serial communication. This module utilizes TTL (transistor-transistor logic) pulses to simultaneously trigger multiple devices. This module provides the precise timing necessary for applications such as rapid 4D image acquisition. TTL Synchronization comes complete with all hardware necessary for controlling devices through the digital I/O board, including a breakout box, PCI board, and cables.

TTL synchronization can be used in concert with other devices, such as electrophysiology instruments and perfusion systems. It can coordinate image capture to trigger external devices at specific times. TTL pulses can be generated by user input during an experiment or after a predefined number of frames. In addition, image capture can be triggered by TTL pulses generated by external systems, such as patch clamps or signal generators.



## SlideBook™ 4.0 Feature List

### Image Database and File I/O

- Intuitive slide interface
- Open
- Close
- Save

### Hardware Control

- automated microscopes
- fluorescence shutters
- bright field shutters
- filter wheels, turrets and monochrometers
- objective turrets
- x,y stage
- z axis control systems
- mag changer/optovar
- prism turrets
- port controls
- lamp intensity
- LCD filters
- imaging splitter

### Camera Control

- Controls most scientific-grade digital cameras
- Simultaneous dual-camera capture
- Interactive control of speed, gain, and intensification (when possible)

### Image Capture

- 2D (single and multi-channel)
- 2D timelapse
- 3D (single and multi-channel)

- 4D (3D time lapse)
- Automatic objective, filter, x, y, z location detection and capture time
- Image annotation and naming
- Interactive annotation buttons
- Autofocus
- Live region tracking
- Multipoint (x, y, z)
- Mid-volume capture
- Mixed brightfield / fluorescence capture
- User controlled capture feedback
  - Single channel
  - Color composite view
  - Histogram

### Image Processing

- image renormalization
- linear filtering
- 2D deconvolution
- channel math
- image math
- flat field correction
- white balance
- FFT
- Photobleach correction

### Stereology and Montage Capture

- interactive montage boundary selection
- automatic stage movement
- automatic image alignment

### Spinning Disk Confocal Control

- User definable park channel for spinning disk
- Laser power control
- Laser sleep mode control

#### CRI Micro-dissection Hardware Control

- *ROI selections*
  - rectangle
  - oval
  - free hand
- Laser Power control
- Laser Pulse rate control
- *Hardware control during image capture*
  - Cell Selector Module
  - Automated Fragment Retrieval (AFR) Module

#### Views

- RGB, grayscale, inverse grayscale, pseudocolor, gated pseudocolor
- Tile view (3D, time lapse, 4D)
- Three view (3D, time lapse, 4D)
- Main view (axis selection)
- Channel view
- QTVR volume rendering
- QuickTime movie generation (timelapse, 3D and 4D)
- Isosurface rendering
- Surface plot
- 3D prism generation
- 3D and 4D data rotation
- Path sectioning (timelapse)
- 4D view
- 4D tile view

#### Data Segmentation

- Intensity segmentation

- Gradient segmentation
- 3D higher order feature segmentation
- Magic Tools for local segmentation
- Manual mask editing
- Binary operations (AND, OR, NOT)
- Erode
- Dilate
- Skeletonize

#### Image Analysis and Statistics

- Area
- Center of area
- Volume
- Center of volume
- Surface area
- Colocalization
- Velocity
- Intensity deviation
- 2D and 3D angles
- 2D and 3D integrated intensity
- 2D and 3D mean intensity
- 2D and 3D center of mass
- 2D and 3D center of intensity
- 2D and 3D object counting
- 2D and 3D distance measurement (linear and nonlinear)
- 2D and 3D object distance (edge to edge)
- 2D and 3D object distance (center to center)
- 2D and 3D intensity plot
- 2D and 3D line plot

#### Data Import/Export

- TIFF, PICT, raw, QuickTime, FluoView, Zeiss 510LSM, BioRad PIC, and more

## Additional Modules Feature List

#### Three-Dimensional Deconvolution

##### \* *Nearest Neighbor*

- Multiple processor optimization
- speed optimized
- Programmable batch deconvolution

##### \* *Constrained Iterative*

- Noise smoothing
- Edge padding

- Individual channel programmability
- PSF guide
- PSF database
- Uses calculated or measured PSFs
- speed optimized
- Programmable batch deconvolution

#### Ratio Imaging/FRET/Densitometry Module

##### \* *Ratio Capture and Analysis*

- Real-time ratio region tracking and graphing
- regions to mask transfer
- supports simultaneous dual ratio capture (e.g. FURA-2 and BCECF)
- FURA-2 calibration guide
- Immediate display of automated ratio values or ion concentration
- analytical graphing tools
- multiple camera / MultiSpec Imager enabled

##### \* *Acceptor Photobleach FRET Capture and Analysis*

- automatic bleach capture script
- automatic FRET computation
- gated pseudocolor display
- track FRET by object or pixel
- Background subtraction

##### \* *Sensitized Emission FRET Capture and Analysis*

- Live corrected FRET image generation
- Background subtraction
- Channel bleedthrough calculation
- FRET calibration guide
- Gated pseudocolor display
- Track FRET by object or pixel

##### \* *Densitometry Capture and Analysis*

- interactive calibration guide
- multiple curve fits
- calibrations can be saved and applied to multiple images

#### TTL Synchronization Module

- TTL control of hardware
- TTL start of image capture
- Pushbutton control of TTL out during image capture
- Automatic TTL pulse during image capture
- Record external TTL pulses during image capture
- Includes hardware for pulse generation and recording

