

# WHAT'S NEW IN SLIDEBOOK™ 5.0

We at 3i are pleased to announce the much anticipated release of SlideBook™ 5.0. In this latest installment of our cutting edge digital microscopy software, you will find innovative tools, improved workflow, and unprecedented flexibility. We listened to your feedback and worked diligently to put you, the user, more in control of your imaging system than ever before. Some highlights of SlideBook 5.0 include:

- Dynamic Data Management
- Offline 64-Bit Vista™ Compatibility
- Multidimensional Channel View
- Improved Interface in View Windows
- User-Configurable Toolbar
- Dual Z-Focus Drive Integration
- Advanced Unbiased Colocalization Module
- Macro Scripting Language
- User-Scriptable Perfusion System
- Super Resolution Particle Identification and Tracking Module
- Full Hardware Accelerated 3D/4D Volume Rendering in Base Package

## DYNAMIC DATA MANAGEMENT

By implementing an advanced virtual memory system, SlideBook 5.0 efficiently captures, visualizes, and analyzes data that exceeds the available computer memory. Users will notice faster image load/save time, faster mask creation on large data sets, and improved file management for 4D and spooled data types. Forget about slide size limits and monitoring RAM; users will now enjoy the convenience of virtually unlimited file sizes. Slides will display 4D data sets as a single file as shown below.

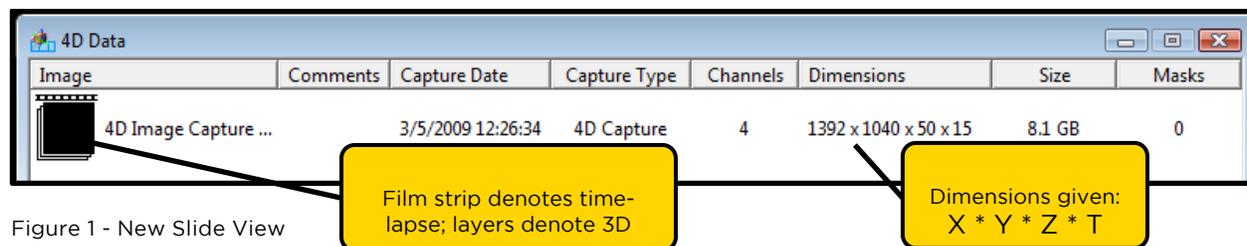


Figure 1 - New Slide View

## OFFLINE 64-BIT VISTA COMPATIBILITY

Users who routinely work with data sets larger than 1 GB will benefit significantly by running offline analysis on Windows Vista™ 64-bit operating system. Specifically, interactive analysis

and memory intensive processes such as deconvolution will speed up dramatically when the operating system of the computer is no longer subject to limited amounts of RAM. 3i strongly urges any offline users to upgrade their PCs to 64 bit Windows Vista, installing about twice as much RAM as your largest data sets. Online systems will be 64-bit compatible in the future but not all vendors currently have compatible drivers.

### MULTIDIMENSIONAL CHANNEL VIEW

The Multidimensional Channel View enables channel views of complex data over time. Individual channels can be pseudocolored or visualized in grayscale. Users have the option to hide or display any individual channel or the composite to aid in creation of figures for documentation. Multidimensional Channel View is accessed under the View menu; customized display is available under the View Settings icon in any View Window.

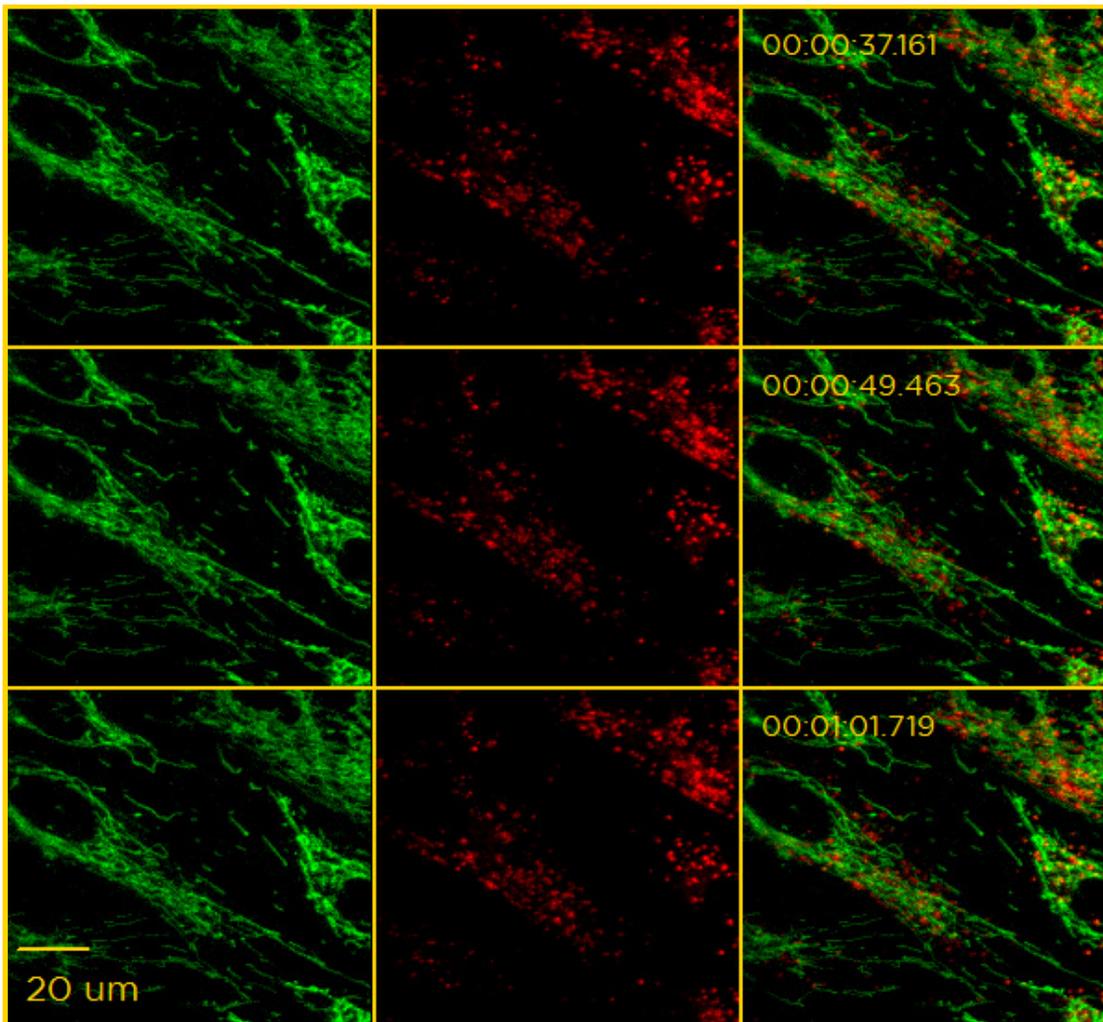


Figure 2 - Tiff Export from Multidimensional Channel View

**IMPROVED INTERFACE IN VIEW WINDOWS**

Now you can generate masks, open ratio graphs, and alter annotation settings directly from any View window. In addition to improved access to these popular tools, the zoom interface has been redesigned to enhance usability and time-lapse images can be looped with intuitive playback controls.

- 1 - Zoom Interface
- 2 - Playback Controls
- 3 - Generate Mask Icon
- 4 - Graph Icon
- 5 - View Settings Icon
- 6 - Playback Rate

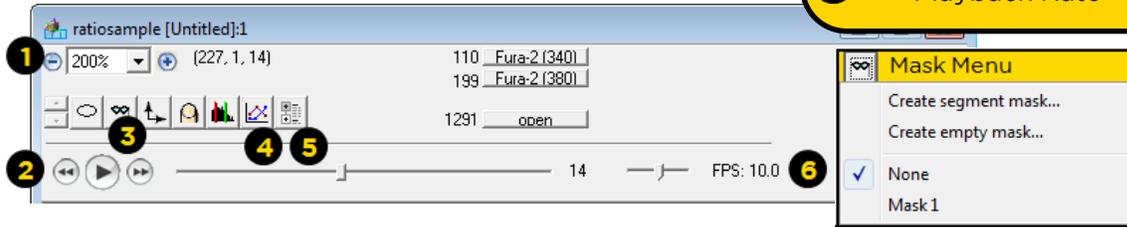


Figure 3 - Enhanced View Interface with Mask Menu

**USER-CONFIGURABLE TOOLBAR**

SlideBook 5.0 now allows users to customize the toolbar with icons for over 30 of the most frequently applied functions. In order to create a personalized workspace, these icons are saved to each individual user account. Scripted macros can be assigned icons to execute complex image analysis routines with a single button click. The toolbar is configured by going to Edit > Setup Guides > Configure User Toolbar.

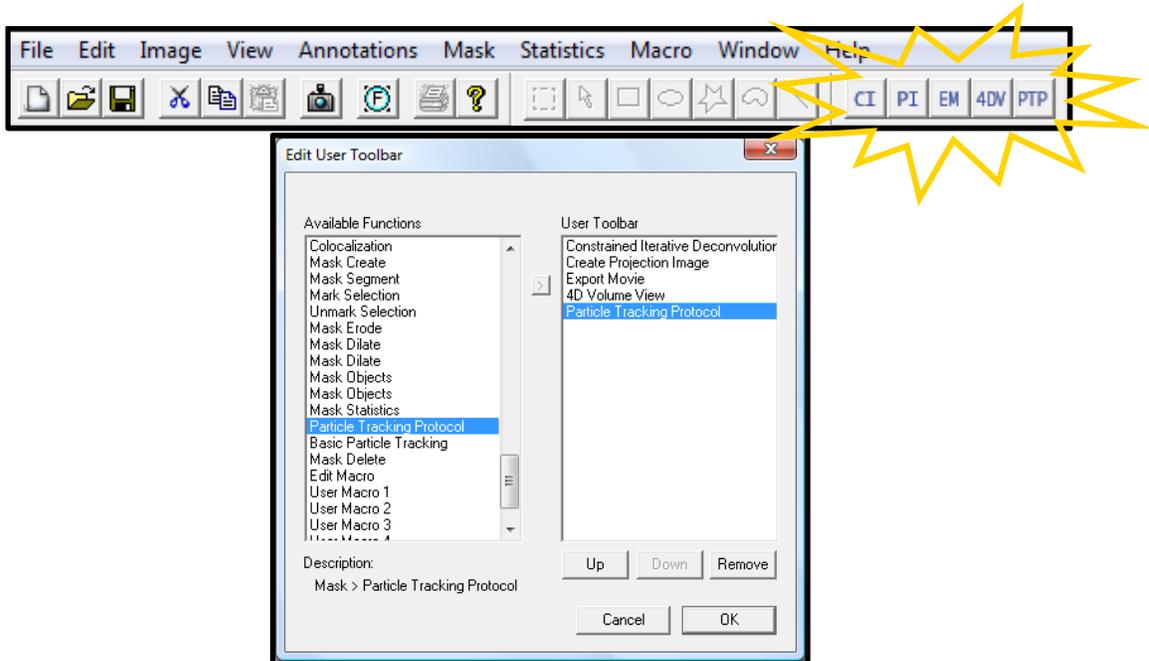


Figure 4 - Customized Toolbar with Configuration Dialog

## DUAL Z-FOCUS DRIVE INTEGRATION

SlideBook 5.0 allows you to take better advantage of piezo focus devices by enabling real time z-drive switching from the focus window. Users with piezo focus devices often become frustrated with a limited, 100 micron range of travel. By integrating dual z-drive control in SlideBook, this frustration is eliminated. Setting up experiments using the microscope z-drive will ensure you never reach the travel constraints of a piezo z drive; this is particularly useful when performing point-visiting time-lapse captures. Point lists generated from the Multiwell dialog or within the XY tab of the focus window will set z points using the main (microscope) z-drive. Stacks will then be collected efficiently by utilizing the piezo z drive which will reset between XY points.

## ADVANCED UNBIASED COLOCALIZATION MODULE

SlideBook's new Colocalization Module allows users to easily visualize colocalization across any two channels. Unbiased analysis automatically sets thresholds according to cross-channel correlation; randomized simulation can determine statistical likelihood of true colocalization. The SlideBook Colocalization Module includes Manders', Pearson's, and Costes' algorithms for colocalization values. An interactive scatterplot and Excel export round out the module. This module is found under View > Colocalization.

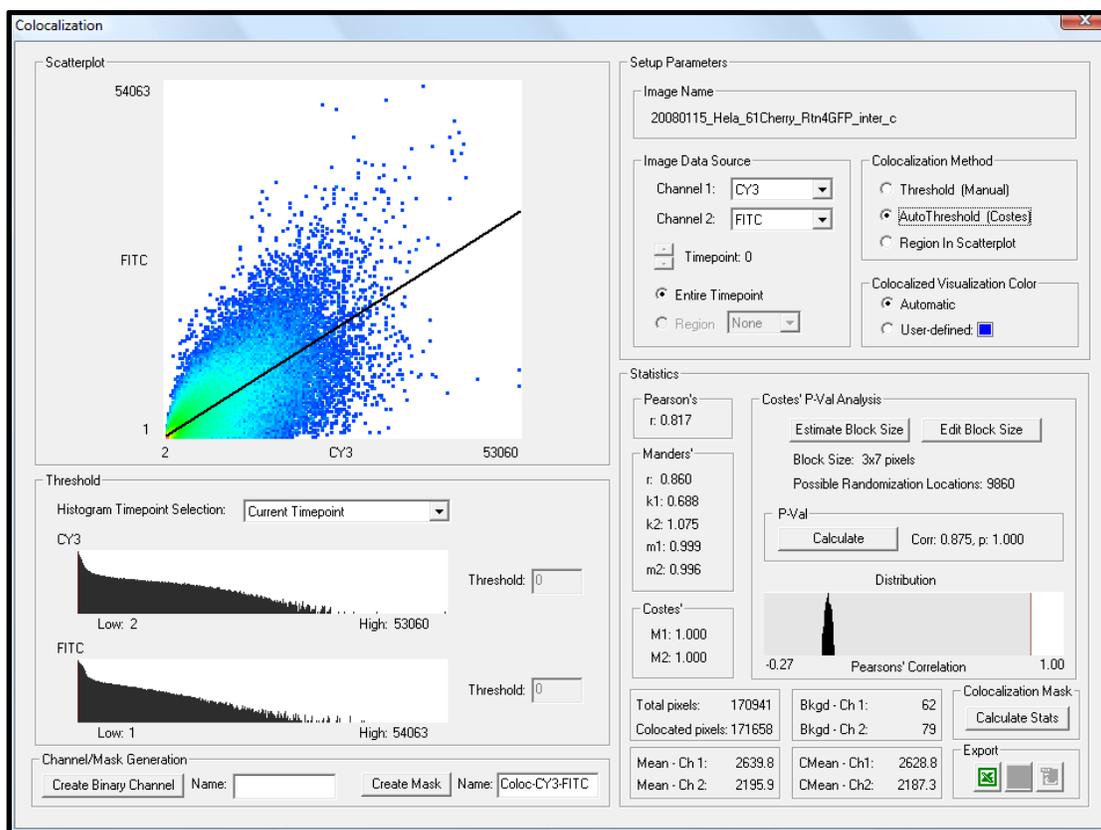


Figure 5 - Colocalization Dialog

**MACRO SCRIPTING LANGUAGE**

Macro scripting language allows users to string together sequences of analysis commands to work on specific images or entire slides. Functions, including image processing, mask operations, and data management, can be linked in a macro scripting environment. For example, a user can create a script to deconvolve an image, open the deconvolved image, generate a mask by thresholding on specific values, exclude objects smaller than 10 voxels, and then export the number of objects with their mean intensity and volume. Scripts can be saved and loaded or linked to icons for a smoother workflow in analysis of future captured images. The scripting dialog is found under Macro > Edit Script.

The screenshot displays several overlapping dialog boxes in the Slidebook 5.0 software interface:

- Constrained Iterative Deconvolution:** Shows optical parameters such as Microns/pixel (0.106), Num aperture (1.32), Working distance (70), and Index of refraction (1.518).
- Segment Image:** Shows a histogram of intensity values for a selected channel (CY3).
- Define Objects:** Shows a size filter configuration with "Gate Objects by Size" checked and a "Minimum size" of 10.
- Mask Statistics:** The primary dialog shown, with "Image Scope" set to "Current 2D/3D Image" and "Mask Scope" set to "Object". It lists features to compute, including Volume (voxels), Surface Area, and various axis measurements. A description for "Volume (voxels)" is provided: "The number of voxels in an object, in cubic microns (3D) or square microns (2D)".

At the bottom of the Mask Statistics dialog, a table displays the results of the analysis:

Object	Volume (microns <sup>3</sup> )	Mean Intensity (ADU), ch1:CY3	Mean Intensity (ADU), ch1:FITC	Mean Intensity (ADU), ch1:DAPI
0	191.23	912.66	324.38	4321.85
1	0.23	1432.71	324.56	2872.35
2	0	1203	261	2726
3	0	1277	377	2732
4	0	1489	343	2768
5	0	1685	411	2744
6	0	1199	321	2783
7	0	1053	348	2711
8	0	1268	287	2812
9	0	1279	222	2755
10	0	2057	281	2724
11	0	1737	337	2832
12	0	1333	166	2755
13	0	1676	286	2713

Figure 6 - Example of Macro Functionality

**USER-SCRIPTABLE PERFUSION SYSTEM**

Complex experiments utilizing TTL driven perfusion devices are effortless in SlideBook 5.0. A new Perfusion Configuration Utility provides manual control of up to 24 channels and a scripting dialog for more complex experiments. Manual control of pumps is linked to the “Notes” buttons during capture execution. Setup of perfusion scripting is found by clicking on the “Advanced” button in the Capture dialog and navigating to the Perfusion tab.

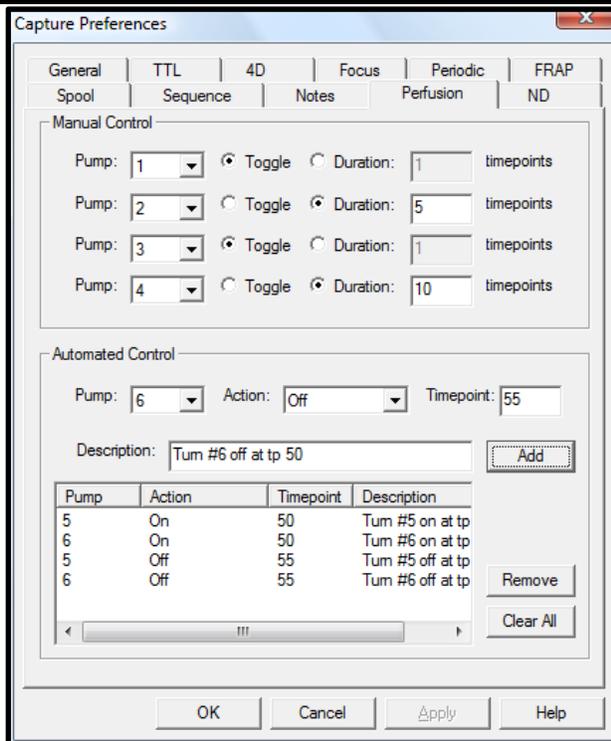


Figure 7 - Perfusion Configuration Interface

**SUPER-RESOLUTION PARTICLE IDENTIFICATION AND TRACKING MODULE**

This is a new module designed specifically for single molecule identification and tracking. Tracking employs a more advanced approach that allows for gaps in trajectories accounting for such phenomena as “blinking” Quantum Dots. Within this module, objects are automatically identified, their positions are tracked through time, and both individual object statistics and path statistics are reported. Trajectories are displayed in SlideBook and can also be exported to MATLAB for further analysis.

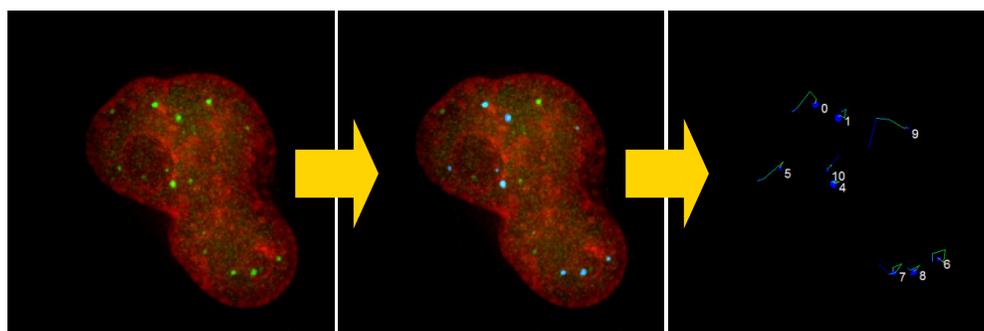


Figure 9 - Particles are identified according to a Gaussian or autocorrelation-based profile

**FULL HARDWARE ACCELERATED 3D/4D VOLUME RENDERING INCLUDED IN BASE PACKAGE**

In order to allow all users to take advantage of the powerful rendering engine in SlideBook, 3i is now including Hardware Accelerated Volume Rendering in the base package of SlideBook. This module takes advantage of the latest graphics card technology to produce real-time rendering of 3D and 4D data. All computing is performed on the graphics card itself, freeing up the computer processors to perform other functions such as deconvolution in the background without slowing down the rendering. Images can be renormalized and re-sized while playing through time and spinning. Full QTVR, .avi, and .mov export is included. 3D and 4D Volume Rendering are found under the View menu.

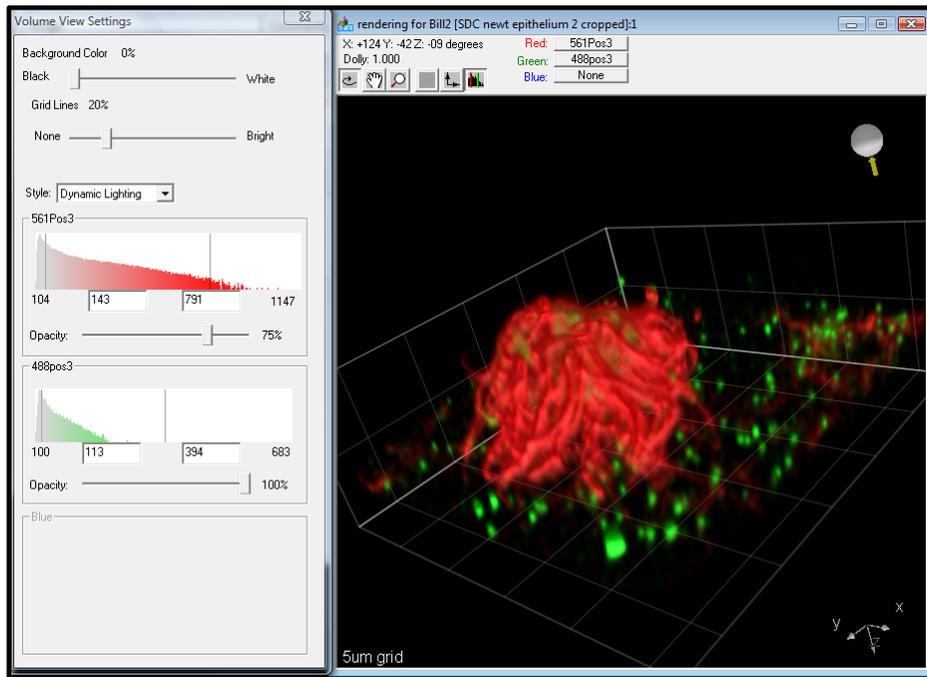


Figure 8 - 3D Volume Rendering Interface

**THANK YOU**

3i would like to thank our customers for their continuous support and contributions. Your feedback helps to shape current and future versions of SlideBook and will continue to do so. We expect you will enjoy the enhanced control and customization of SlideBook 5.0 and the improvements to your workflow. Keep your suggestions coming as they make SlideBook what it has always been: cutting-edge software designed by scientists for scientists with cutting-edge research.

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