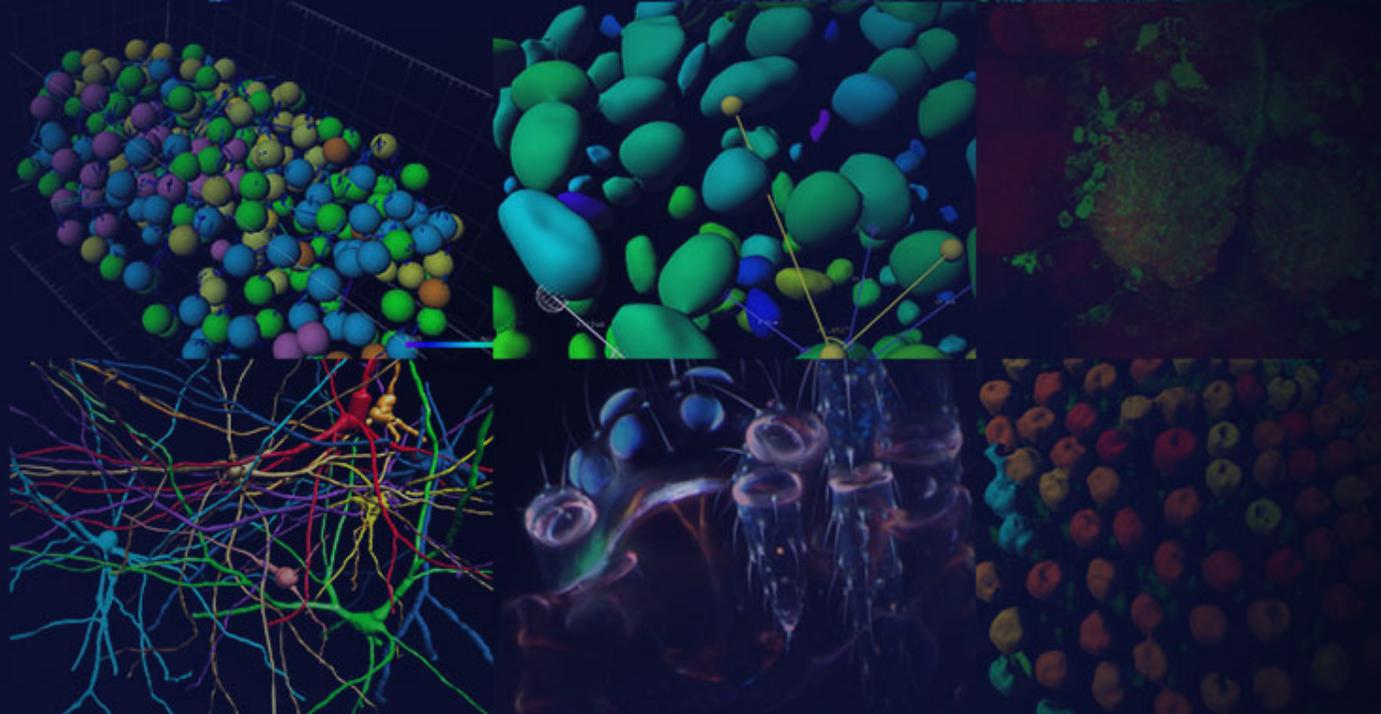


# BITPLANE

an **Oxford Instruments** company



## Visualization I: Basics

## 1. Object List

All components in the scene are represented here in a tree hierarchy

## 2. Object Properties

Analysis Parameters, Visualization Settings, Statistics....

## 3. View Area

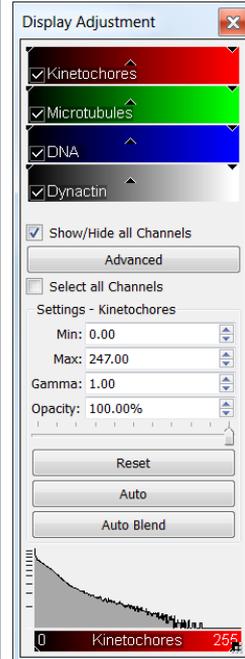
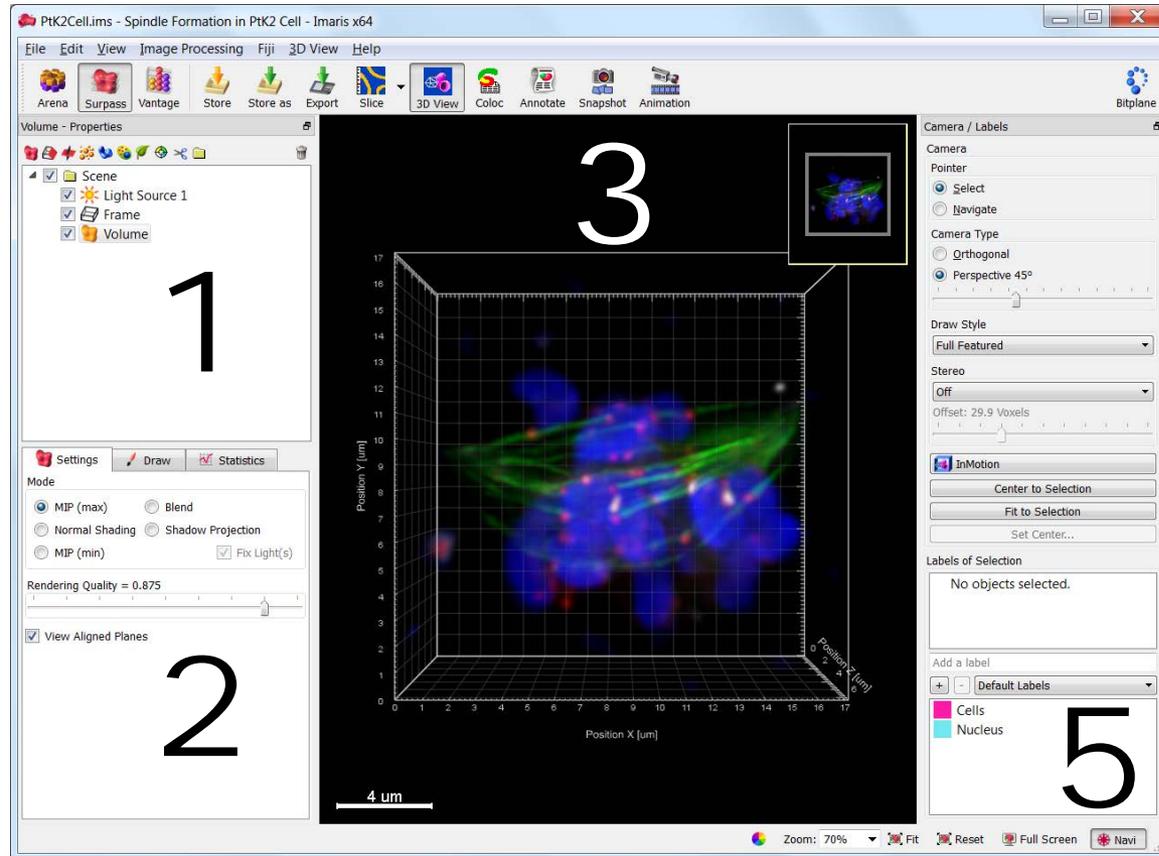
3D Rendering of Objects, and 3D Workspace for Navigation, Selection, Interaction, and Editing

## 4. Display Adjustment

Color, Max/Min Intensity, Opacity, Gamma

## 5. Object Labeling (for coloring and statistics export)

Add labels to 3D objects such as spots, surfaces, cells, vesicles, etc. The labels can be used to color code objects.



4

## Display Adjustment:

The 'Display Adjustment' dialog box contains the following elements:

- Channel selection:  Kinetochores (red),  Microtubules (green),  DNA (blue),  Dynactin (grey).
- Show/Hide all Channels
- Advanced section with buttons: **Advanced**,  Select all Channels
- Settings - Kinetochores:
  - Min: 0.00
  - Max: 247.00
  - Gamma: 1.00
  - Opacity: 100.00%
- Buttons: **Reset**, **Auto**, **Auto Blend**
- Histogram: A small histogram showing the intensity distribution for Kinetochores, with a value of 255 indicated.

## View Adjustment Buttons

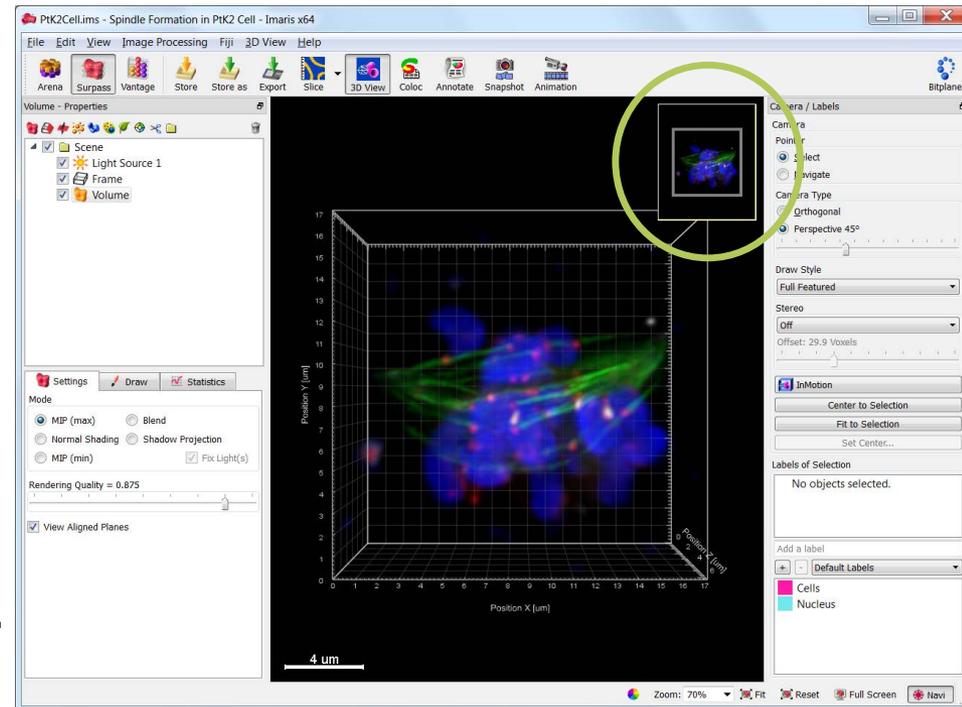
The screenshot shows the Bitplane software interface with the following components:

- View Adjustment Buttons (highlighted in green):** A toolbar containing a color wheel icon, a dropdown menu for 'Zoom: 55%', and buttons for 'Fit', 'Reset', 'Full Screen', and 'Navi'.
- Main Window:** Displays a 3D volume rendering of a cell. The axes are labeled 'Position X [um]', 'Position Y [um]', and 'Position Z [um]'. A scale bar indicates 4 um.
- Volume - Properties:** A tree view showing 'Scene' with sub-items 'Light Source 1', 'Frame', and 'Volume'.
- Settings:** Includes 'Mode' (MP (max), Blend, Normal Shading, Shadow Projection, MP (min)), 'Fix Light(s)', and 'Rendering Quality = 0.875'.
- Camera / Labels:** A panel on the right with settings for 'Camera' (Pointer, Select, Navigate), 'Camera Type' (Orthogonal, Perspective 45°), 'Draw Style' (Full Featured), 'Stereo' (Off), and 'Offset: 29.9 Voxels'. It also has buttons for 'Center to Selection', 'Fit to Selection', and 'Set Center...'. Below this is a 'Labels of Selection' section with 'No objects selected.' and a legend for 'Cells' (pink) and 'Nucleus' (cyan).
- Bottom View Adjustment Buttons (circled in yellow):** A secondary toolbar at the bottom of the window with 'Zoom: 70%', 'Fit', 'Reset', 'Full Screen', and 'Navi' buttons.

# View Adjustment Buttons



- ❑ “Zoom”
  - ❑ preset zoom factors
- ❑ “Fit”
  - ❑ Fit image to window size
- ❑ “Reset”
  - ❑ **Rotates the image to the original position**
  - ❑ centers the image
  - ❑ Fit image to window size
- ❑ “Full Screen”
  - ❑ Enters Full Screen mode.
  - ❑ **Click again to exit full screen mode.**
- ❑ “Navi” (Navigation window)
  - ❑ Inset image of entire dataset
  - ❑ Yellow box shows current zoom position



## Shortcut to Keyboard & Mouse Guide:

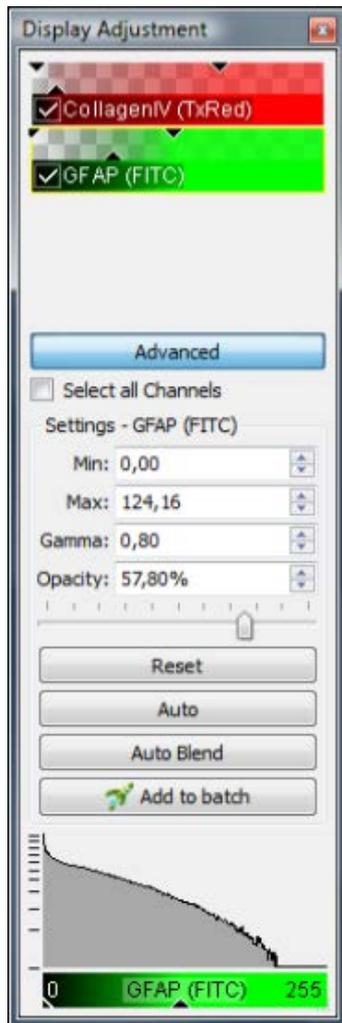
- Click Help -> Keyboard and Mouse

### Main Mouse Interactions

- Scroll Mouse wheel:
  - Zoom in/out
- Right-click & drag:
  - Pan image
- Left-click & drag:
  - Rotate image

### Frequent Commands and their Keyboard Shortcuts

- “Esc” key
  - Toggles Surpass pointer between Select / Navigate
- “Ctrl + D” (OSX: Command + D)
  - Show / Hide Display Adjustment Window
- “Ctrl + I” (OSX: Command + I)
  - Opens the Image Properties window
- “Ctrl + P” (OSX: Command + P)
  - Opens the Imaris Preferences



## Controls:

- Channel visibility
- Channel color
- Intensity range min/max
- Blend Opacity

## Displays:

- Channel intensity histogram

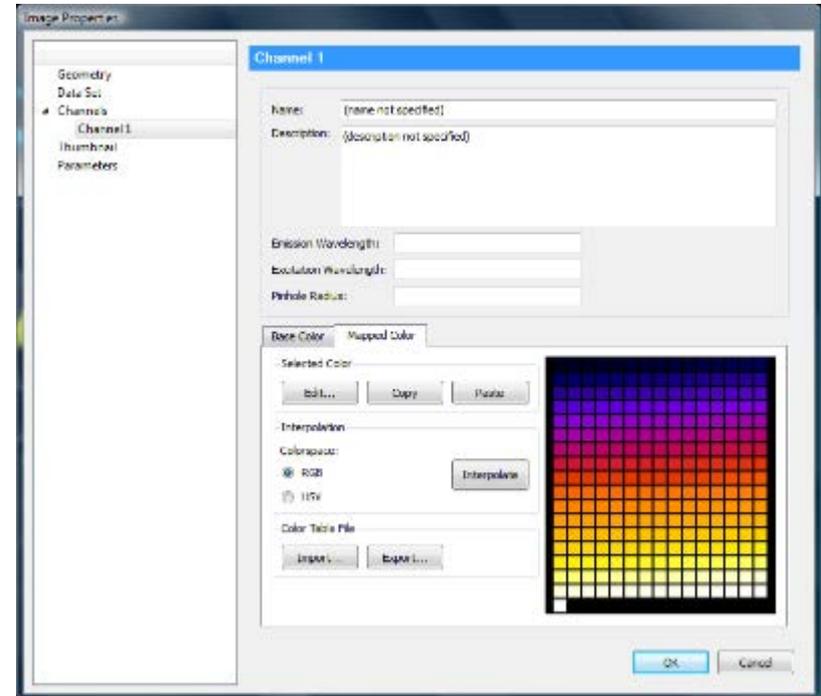
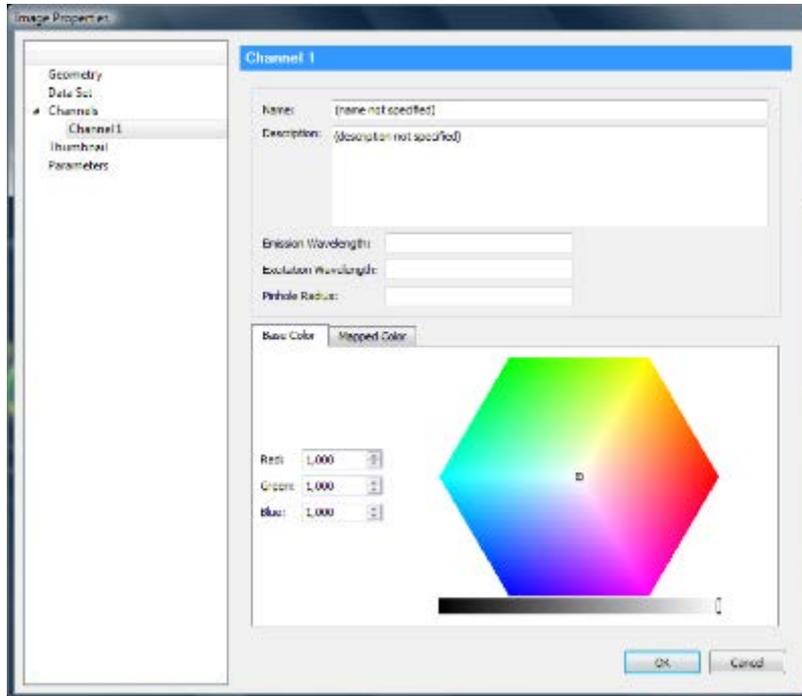
Open the Display Adjustment Window:

\* **Short cut: Ctrl-D**

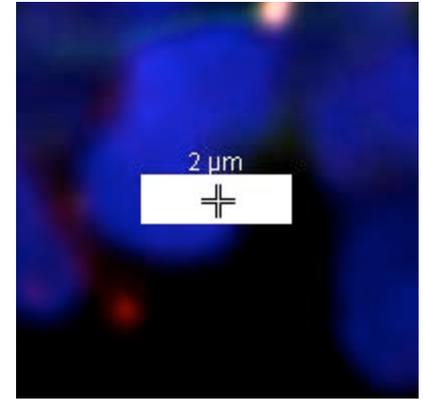
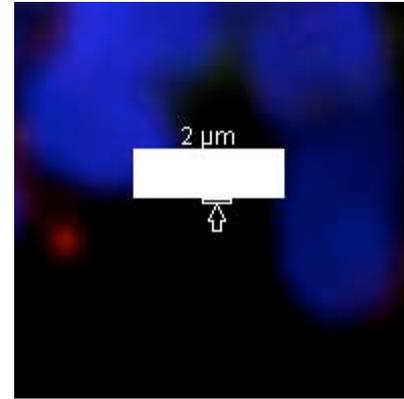
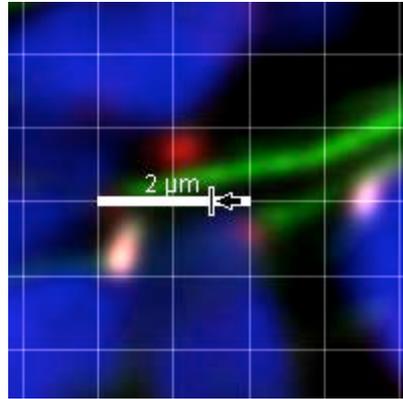
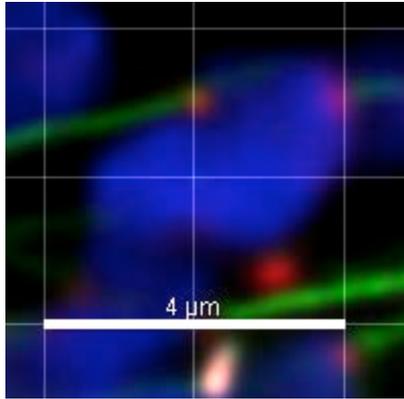
\* More menu -> Edit menu > Show Display Adjustment

- **Click on checkbox to control channel visibility**
- **Click on triangles to control min/max/gamma**
- Drag on colour bar to control intensity (left/right) or contrast (up/down)
- **Click on channel name to get to colour dialogue**
- Right click drag on colour bar or use slider to adjust blend opacity (left/right)
- “Reset” reverts to default
- **“Auto” selects min and max values in each channel**
- “Auto Blend” selects “optimized” min/max values for blending

# Display Adjustment: The Color dialogue



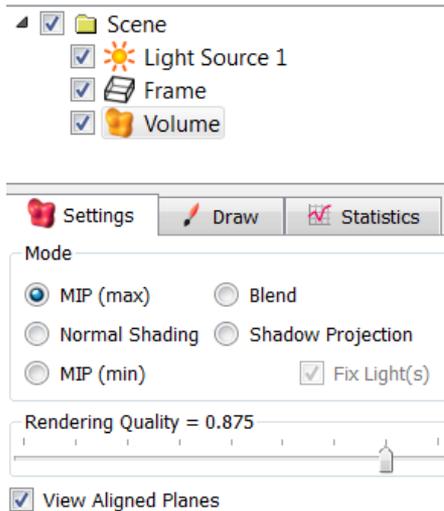
- Select Base Color to show the channel with one specific color
- Select Mapped Color to map intensities of one channel to multiple colors
- Create your own color map by editing the color for specific intensities and Interpolate the colors for the intensities in between



- To adjust the length of the scale bar, drag on one end of the scale bar
- To adjust the height of the scale bar, drag the top or bottom of the scale bar

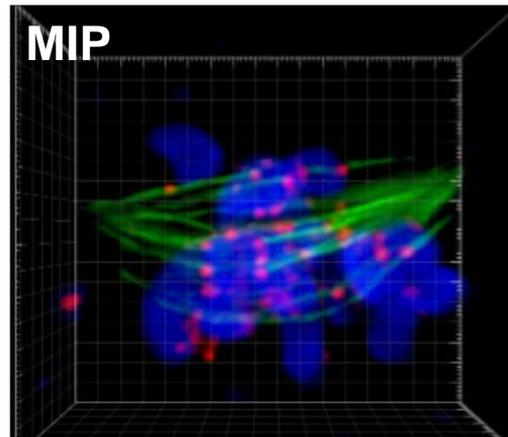
- To move the scale bar position, drag the center of the scale bar
- To adjust the size of the font, drag the top of the text

In the 3D Perspective rendering mode of Surpass, the bar is scaled for the center of the dataset



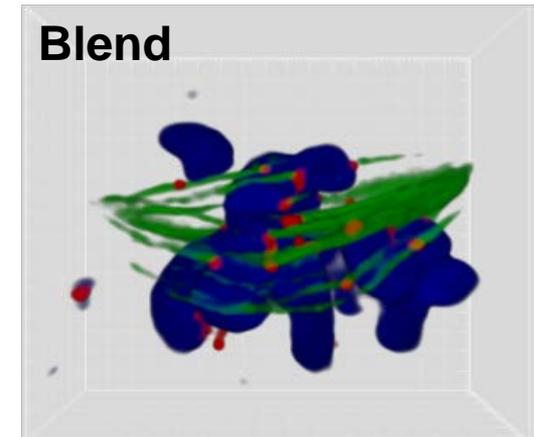
## MIP

- Only the brightest point along the viewing direction displays
- Combination colour of different channels is displayed
- Opacity settings are ignored



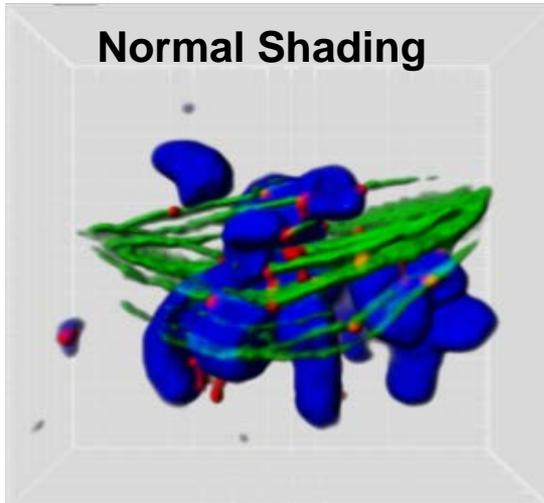
## Blend

- All values along the viewing direction including their transparency are used
- depth effect, edges appear dark
- Effect: data closest to the view is visible while the data further away is obscured



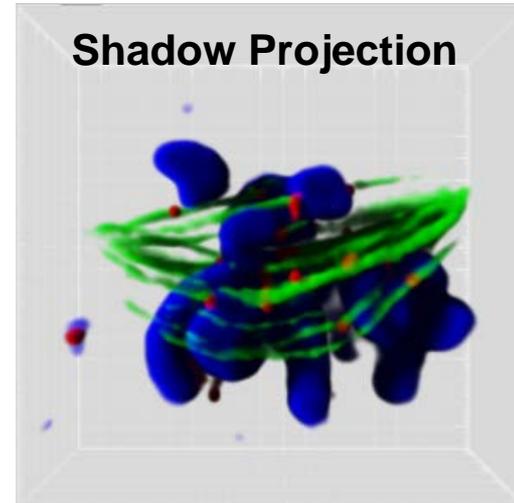
## Normal Shading

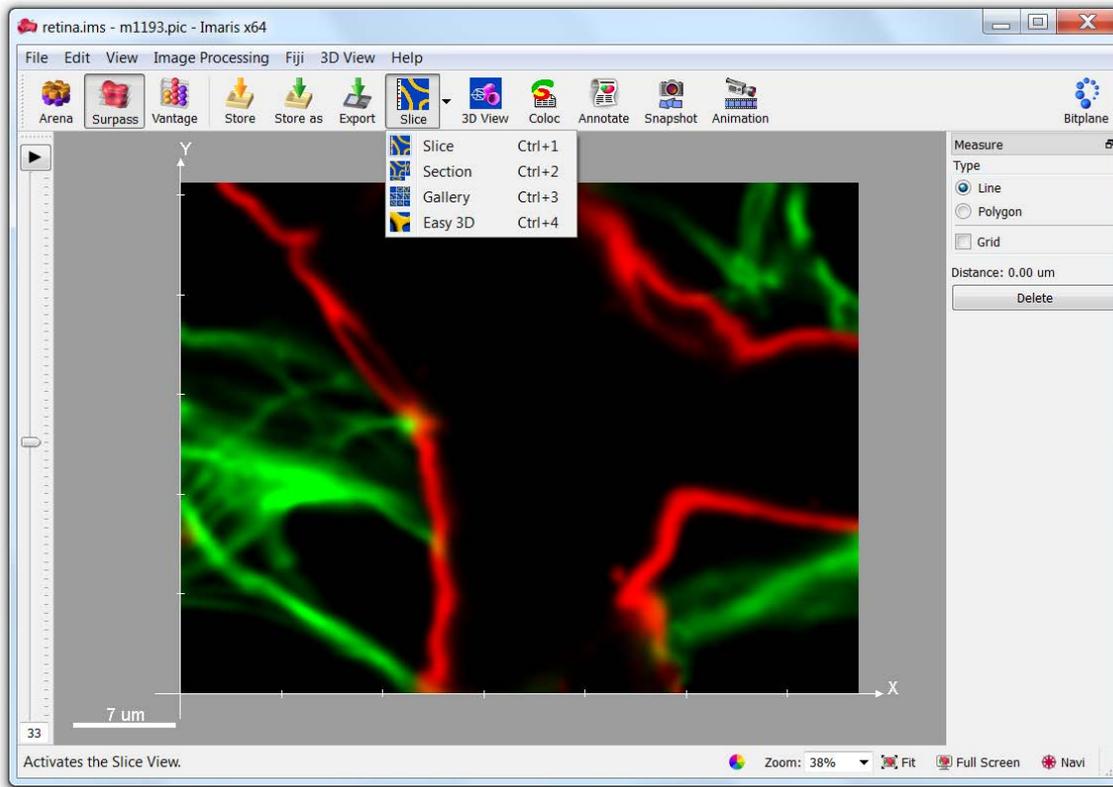
- Uses artificial light source
- Surfaces turned away from light source appear darker
- Depth effect
- Light source can be interactively positioned,
- Light intensity not adjusted



## Shadow Projection

- Uses artificial light source
- An object between light source and the displayed object projects a shadow to the object
- Not an interactive mode

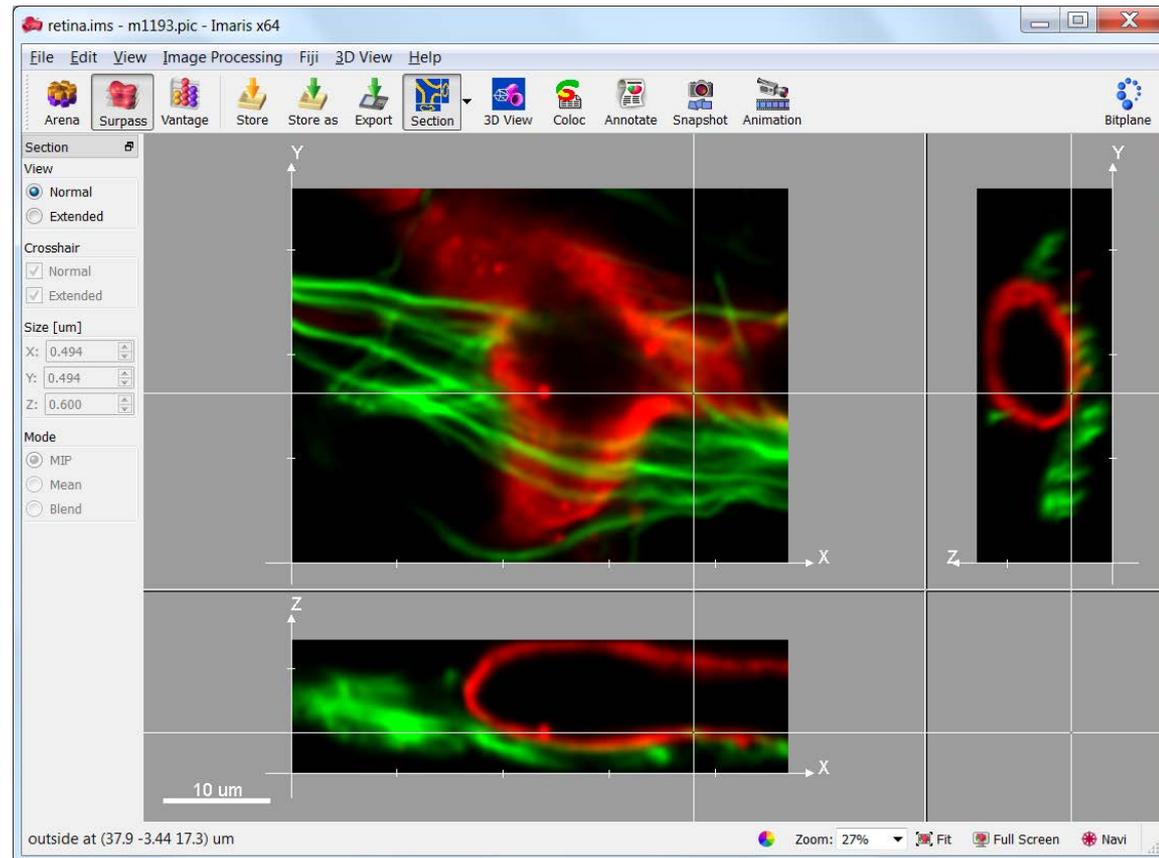




- View individual Z-slices
- Browse through your Slices using a Slider
- Inspect intensities under mouse pointer

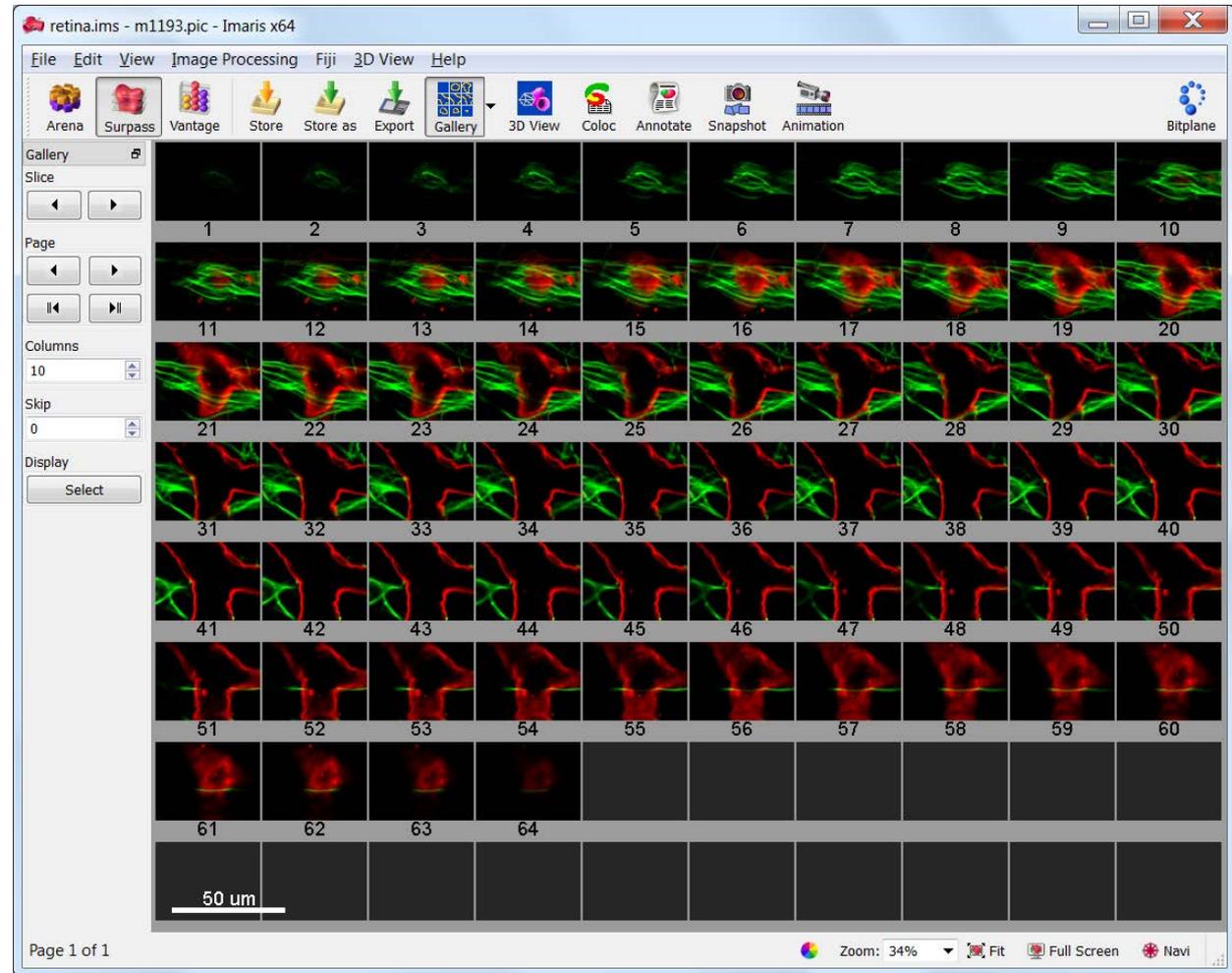


- Interactive view on XY, XZ and YZ sections
- Define the sections you want to see by moving the crosshair
- Get an impression of the organization in all dimensions
- Render Extended Sections in 3 different projection modes
  - Adjust thickness with yellow lines

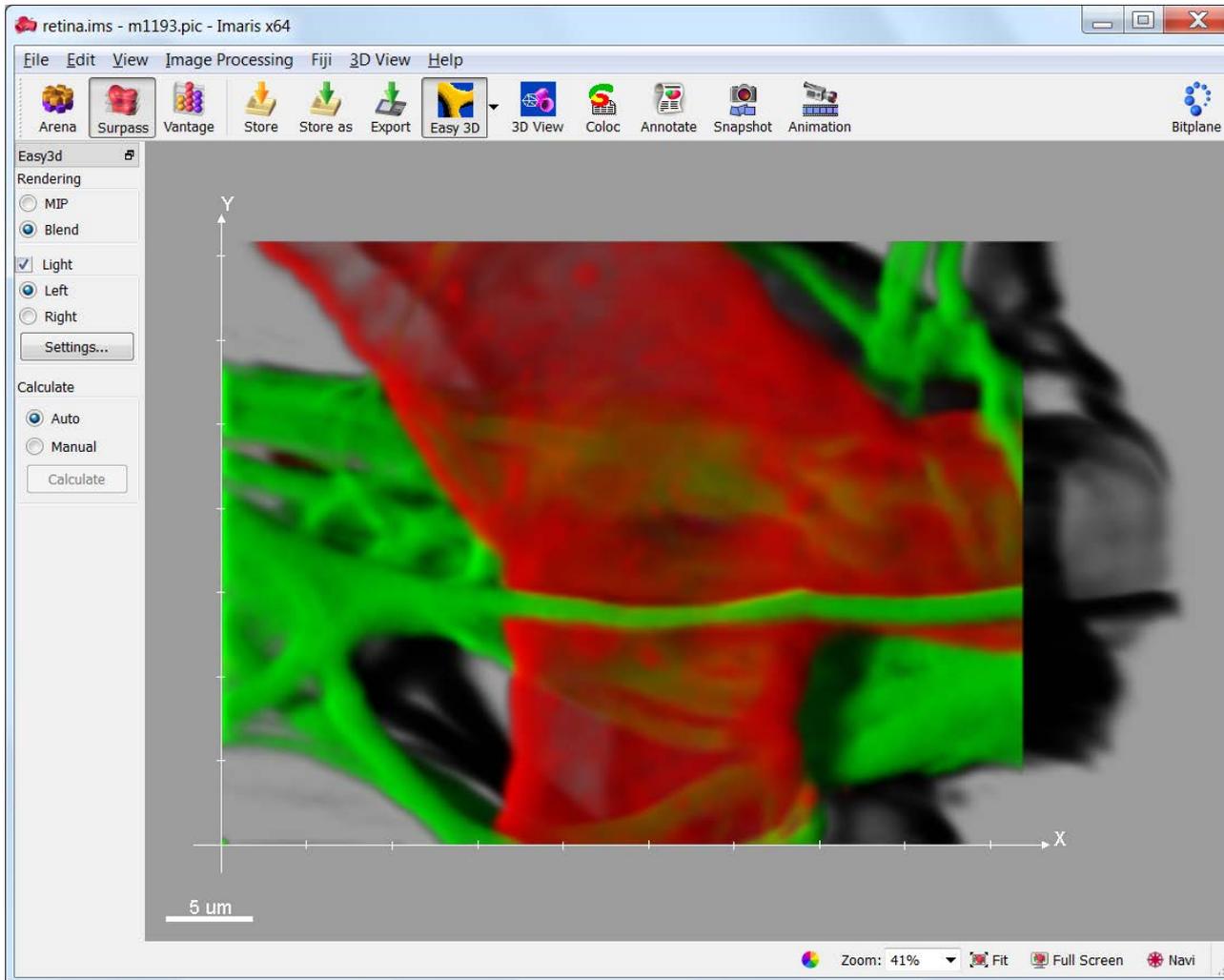
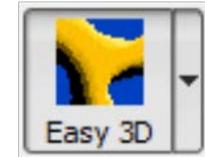




- Get an overview of multiple z-slices at once
- Choose a subset of slices you want to display from the gallery with Select...
- Organize the columns of your gallery



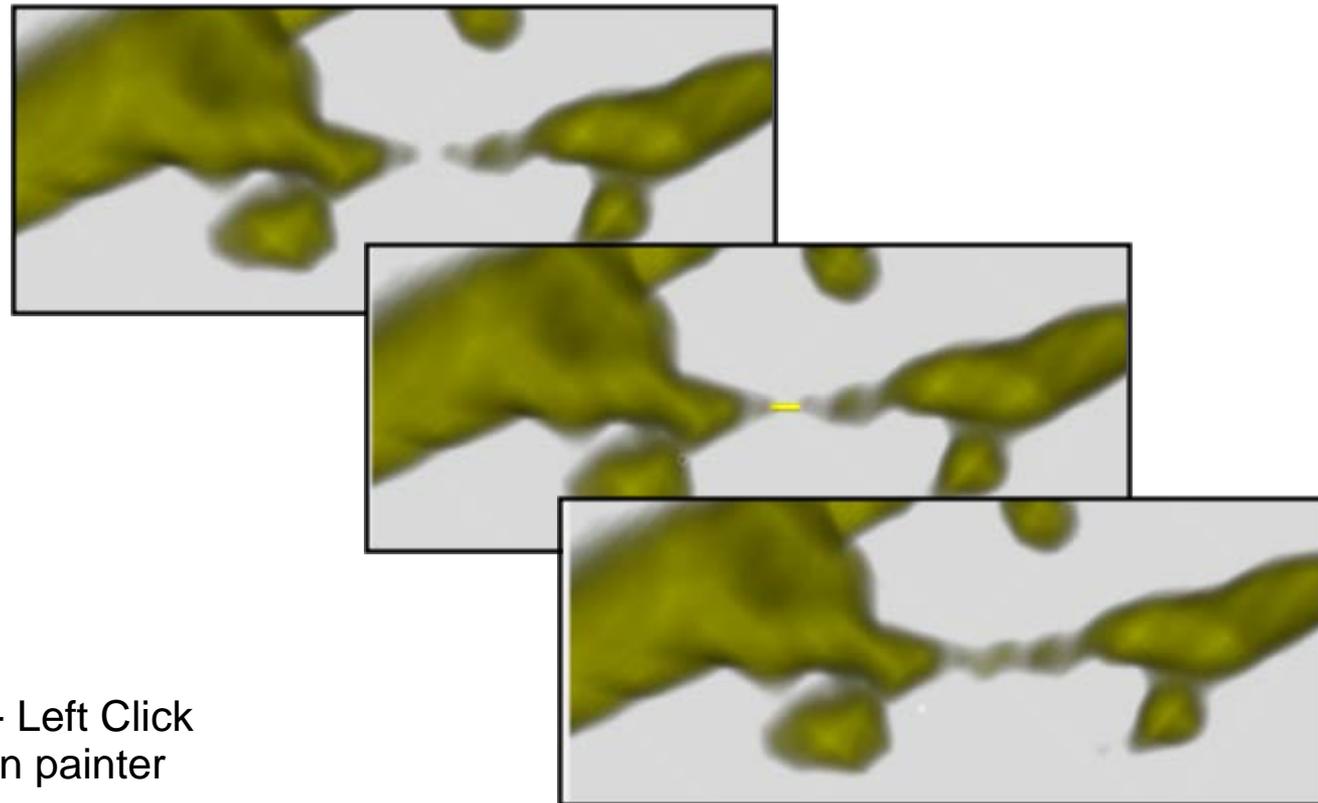
# Basic Views III: Easy 3D View



- Get a simple 3D projection of your dataset along Z
- Choose between two different projection modes
- Illuminate the scene from 2 different directions to project shadows
- Great for quick look at data (though slow for large datasets)

- Fill a small region with high intensity to force a known connection
- Fill a small region with 0 intensity to force a known break, or create a visualization “window” to the inside of a structure

The screenshot shows the 'Volume Draw' tab in the software. It includes a 'Scene' panel with 'Light Source 1', 'Frame', and 'Volume' checked. The 'Settings' panel has tabs for 'Settings', 'Draw', and 'Statistics'. Under 'Settings', the 'Selected Channel' is 'Channel 1 - (name not specified)'. The 'Painter Shape' is set to 'Disk'. The 'Selection' parameters are: Diameter: 0.909 um, Width: 0.227 um, and Intensity: 0.00. There are 'Set Intensity' and 'Delete All' buttons. The 'Selected Point' section shows Position: X: 0.00 um, Y: 0.00 um, Z: 0.00 um, with a 'Delete' button.

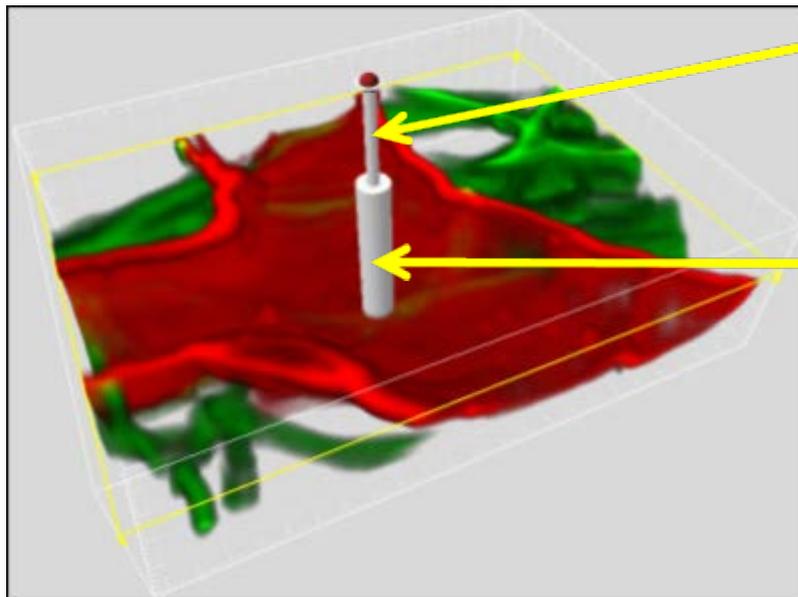


1. Choose Painter Shape
2. Place painter by Shift + Left Click
3. Set intensity of voxels in painter region

- Scene
- Light Source 1
- Frame
- Clipping Plane 1
- Volume

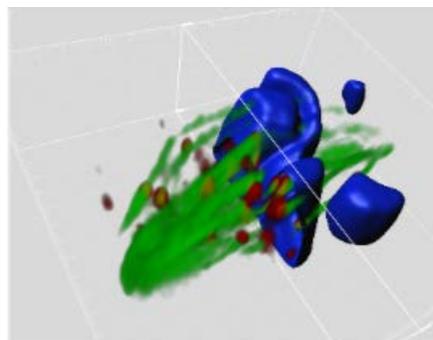
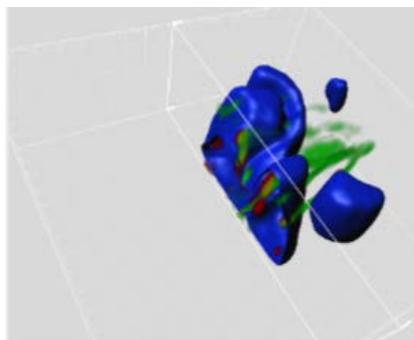
Only cuts objects lower in Objects List

- Clipping Plane 1
- Volume
- Surfaces 1



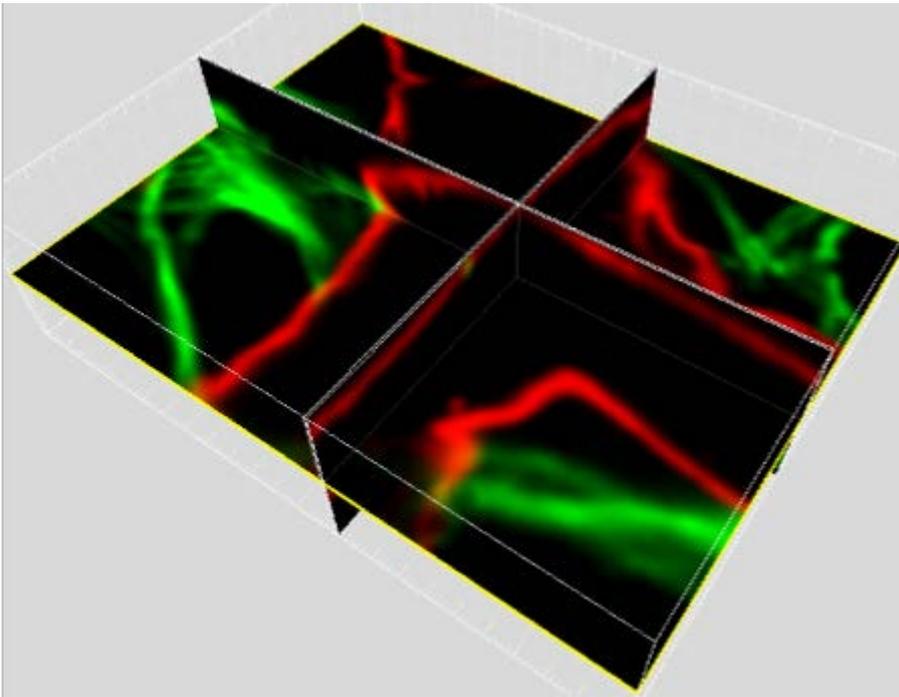
Use this rod to freely rotate the plane

Use this rod to change the position of the plane

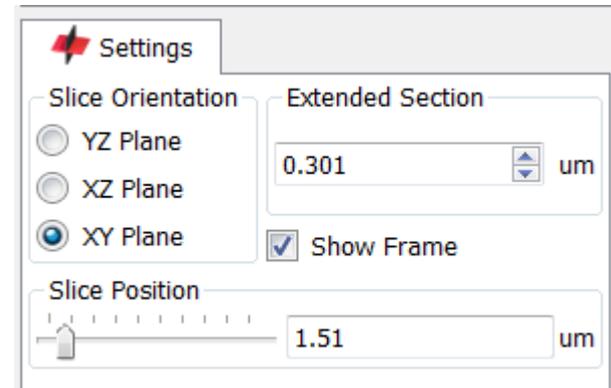


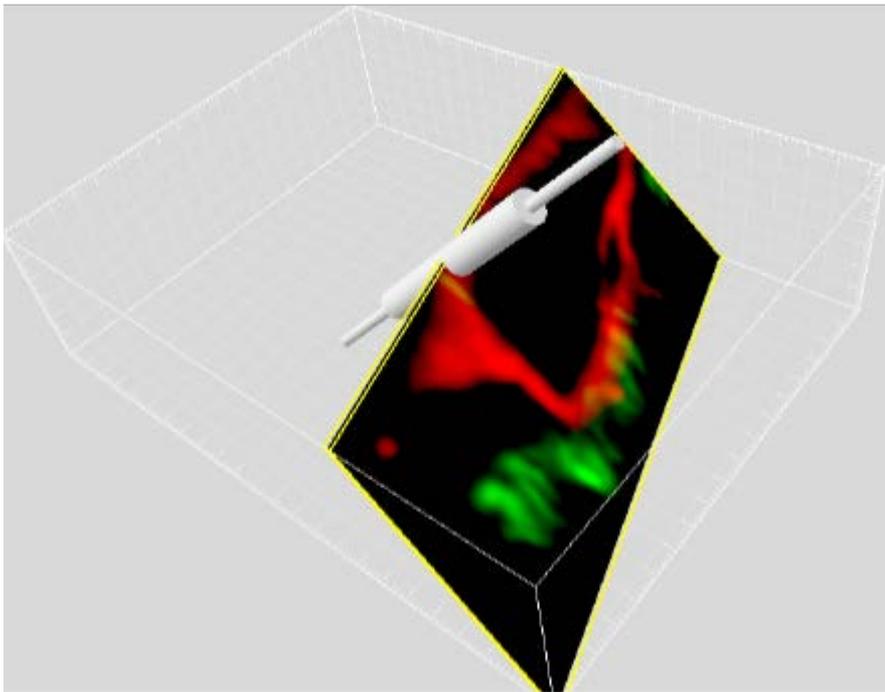
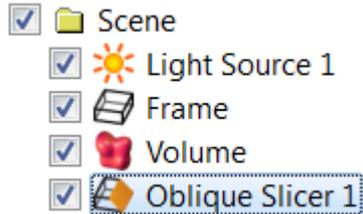
- Volume
- Clipping Plane 1
- Surfaces 1

- Scene
- Light Source 1
- Frame
- Volume
- Ortho Slicer 1
- Ortho Slicer 2
- Ortho Slicer 3

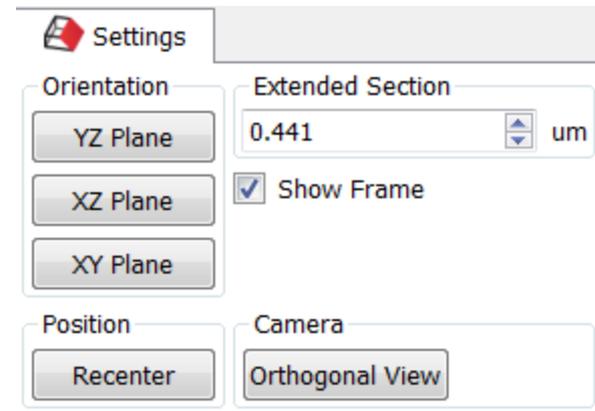


- Shows only data intersecting that plane
- Can be moved within the dataset
- “Extended section” adjusts slice thickness. Additional data projected onto plane as MIP





- Shows only data intersecting that plane
- Can be freely moved and rotated within the dataset (similar to Clipping Plane)
- “Extended section” adjusts slice thickness. Additional data projected onto plane as MIP



# BITPLANE

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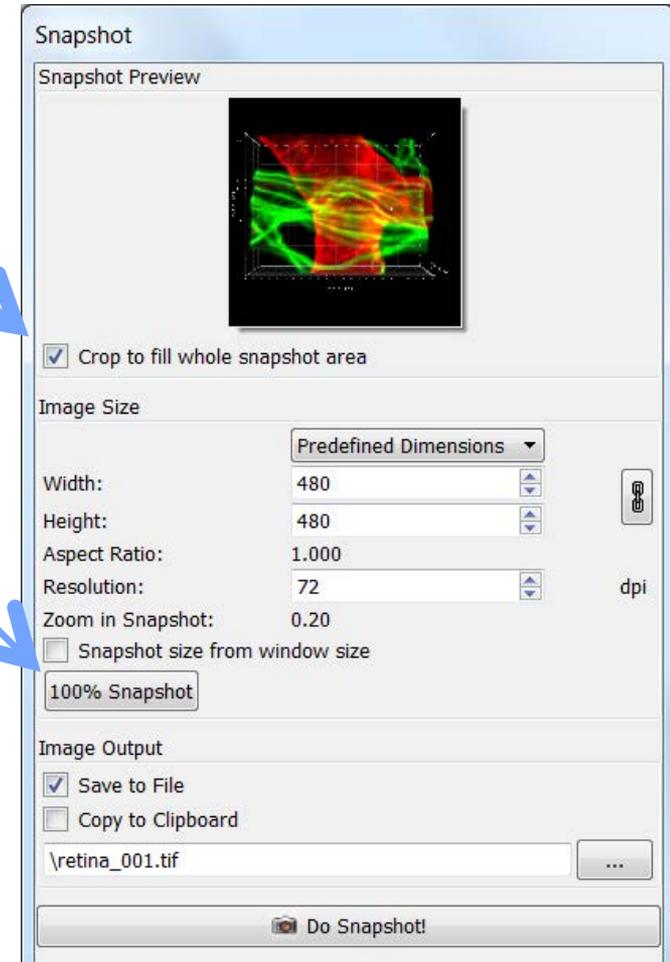
## Visualization II: Export and Animation



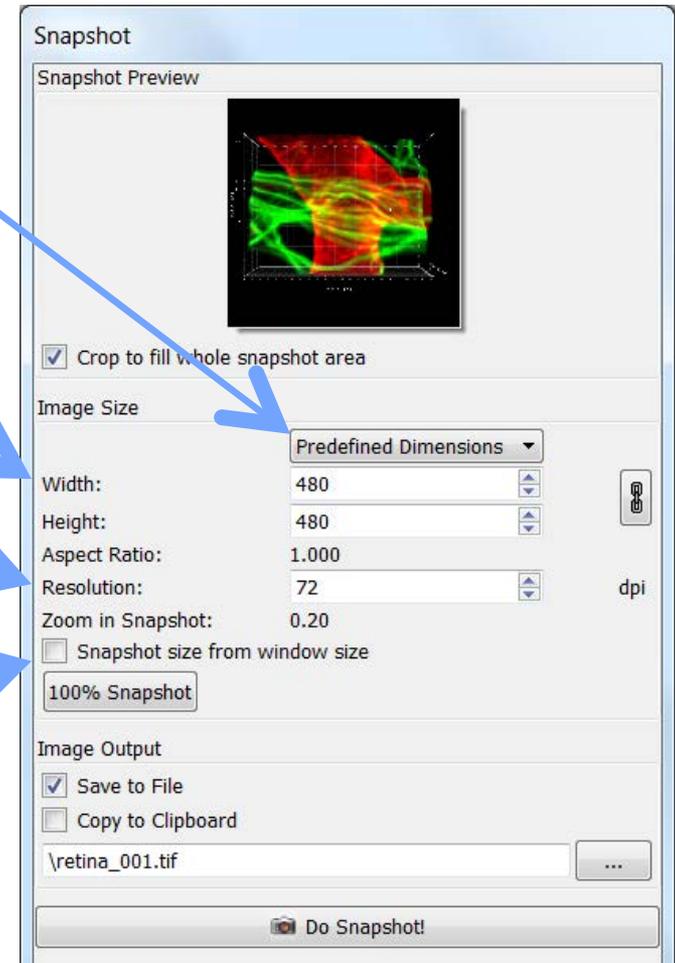
- Press the Snapshot Button or Press Ctrl T (Command-T for Mac).
- Captures the image in the viewing window of the current view.
- Captures any items in the viewing area including scale bars, time codes, or measurements.
- Saves the image as a TIFF file
- Works in all Imaris views and in full screen mode (must use Ctrl T)

# Snapshot

- ❑ The checkbox “Crop to fill whole snapshot area”. If checked, the image will be scaled and positioned in the snapshot so that the whole area of the snapshot is covered by the image. Parts of the image will then be cropped.
- ❑ “100% Snapshot” when clicked, the snapshot width and height will be set to sizes so that the zoom in the resulting snapshot will be 100% (1.0), *i.e.* one snapshot pixel will correspond to one dataset voxel

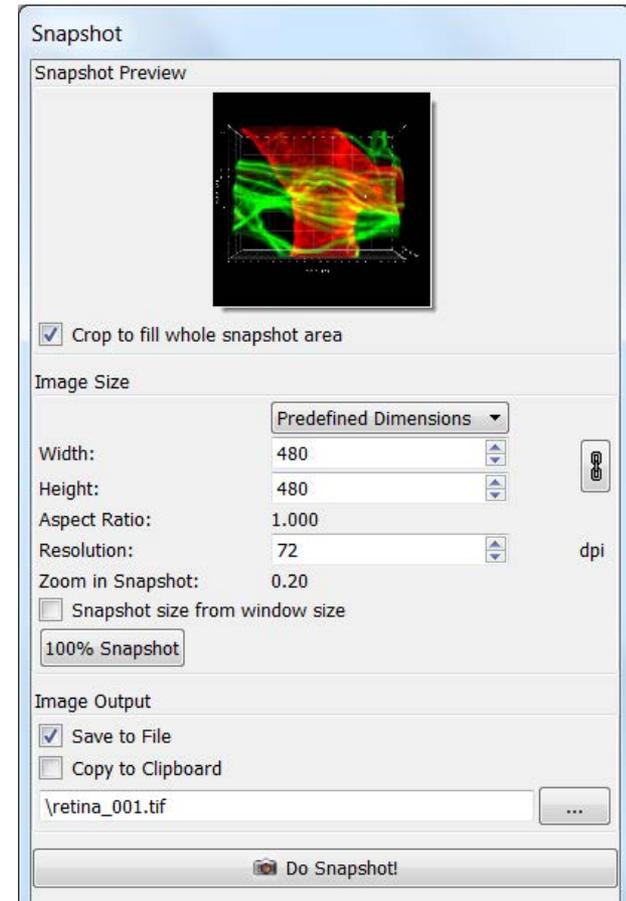


- ❑ The size of the resulting snapshot image can be selected from a predefined list of dimensions (even much larger than the screen size)
- ❑ You can manually enter the size you would like for the resulting snapshot
- ❑ You can choose the DPI of the resulting image
- ❑ The size of the resulting snapshot image can be the same as the window size of Imaris by clicking “Snapshot size from window size”

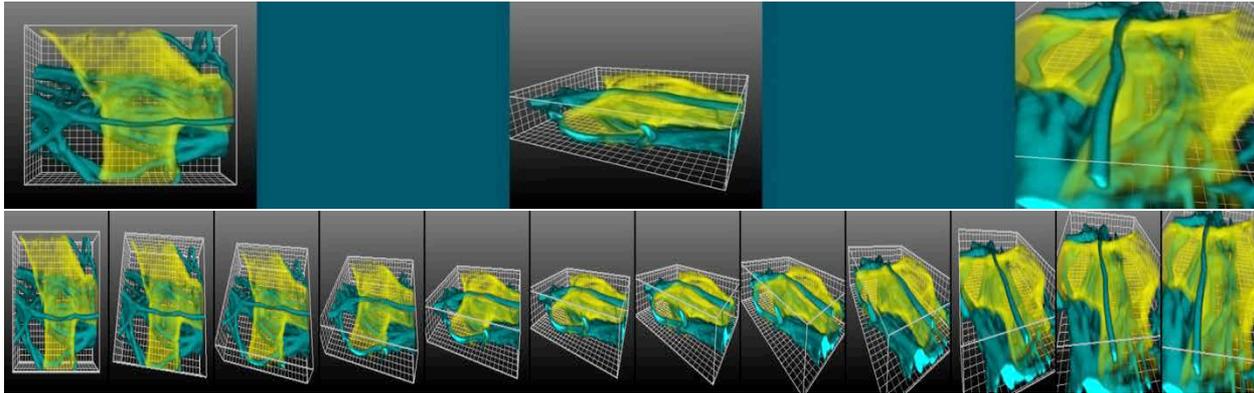


# Snapshot

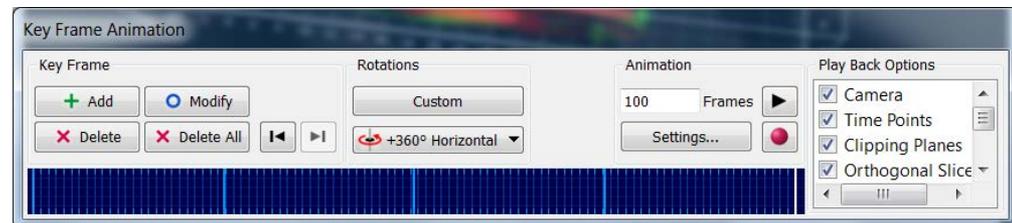
- Automatic naming takes the data set name and adds a running number for the snapshot name
- Must choose [...] where you want the Image Output to go or nothing will happen
  - Save to File
  - Copy to Clipboard
  - Combination of both
- After determining save location, remember to click **Do Snapshot!**



# Key Frame Animator

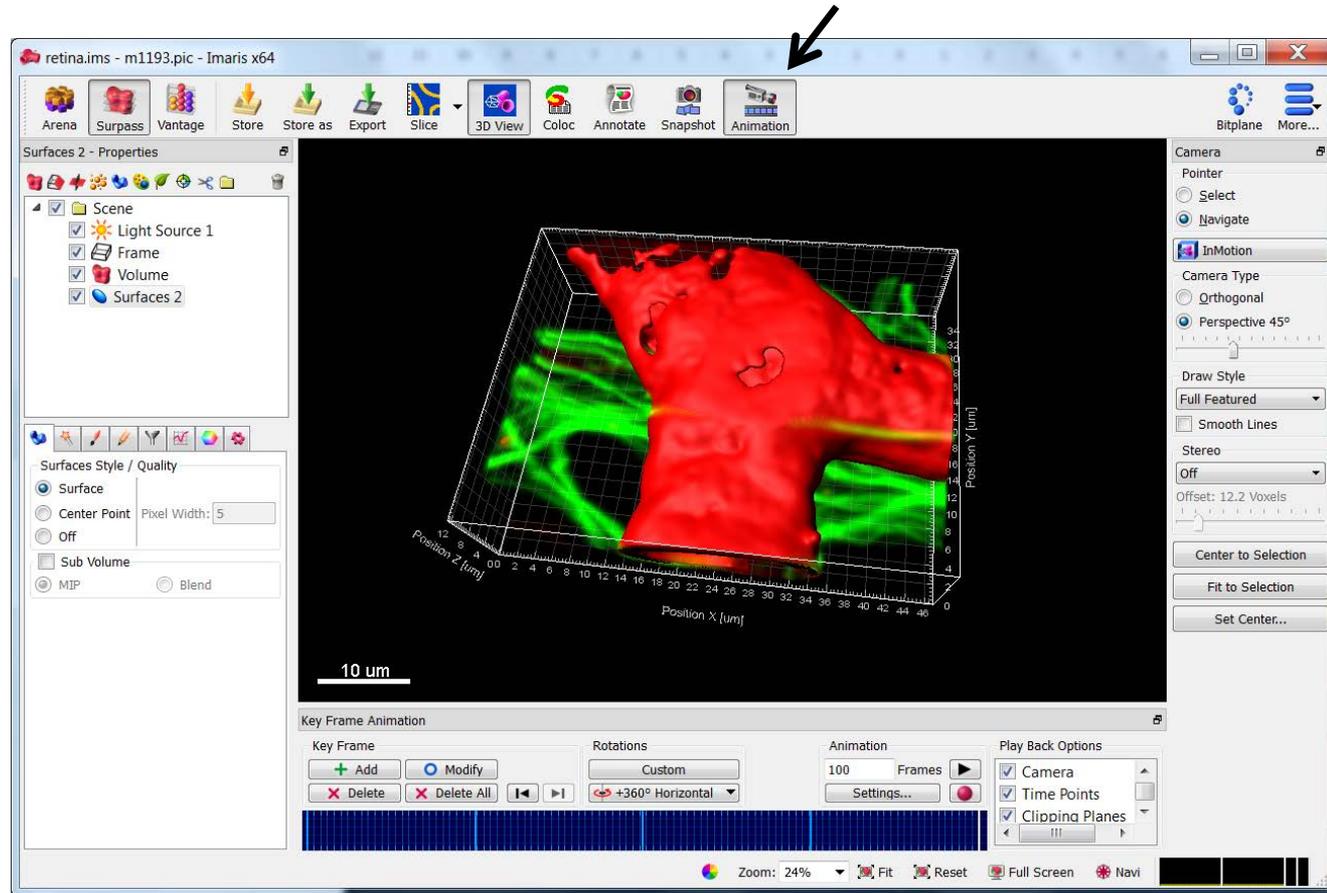


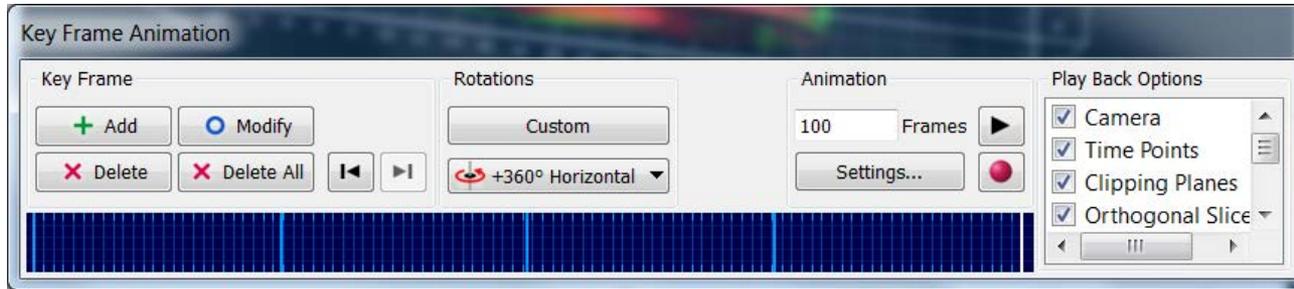
- Subsystem to generate movies for animated presentation
- Define some key frames
- Key frames are views and time points through which the movie must pass.
- Store Key Frames
  - Camera (position, angle, zoom)
  - Time point of data set
  - Clipping Planes (position, angle)
  - Orthogonal Slices (position)
  - Oblique Slices (position, angle)
  - Objects Show/Hide
  - Objects Colors
  - Display Adjustments



# How to get to Animations

- ❑ To get to the animator, simply click on the Animation Icon on the top panel.
- ❑ This is essentially the Surpass view with the animation toolbar at the bottom of the screen

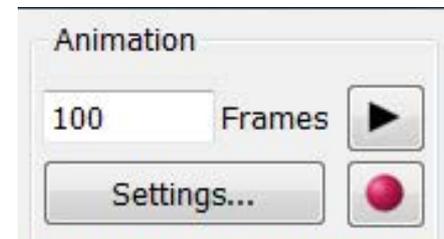
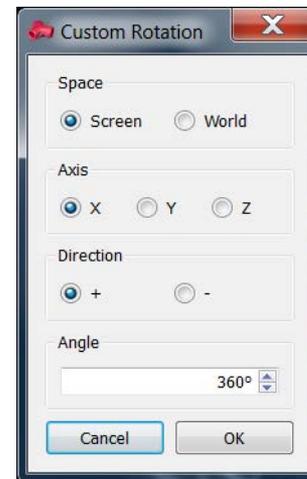
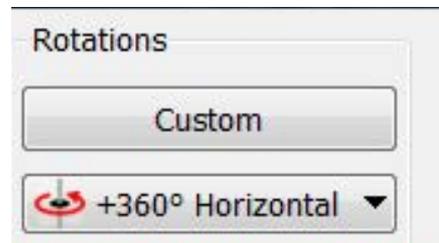
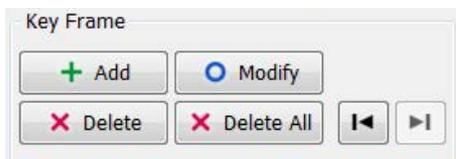


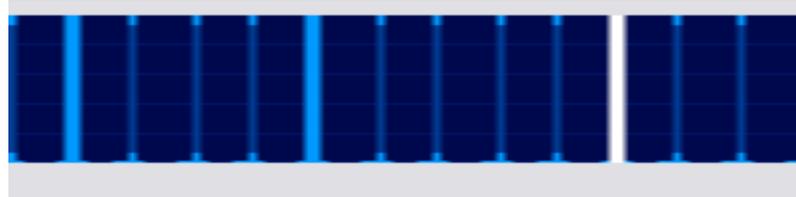


- ❑ Key Frame Panel – where your key frames are added and modified
- ❑ Rotations Panel – where Automatic and Custom rotations are inserted
- ❑ Animation Panel – where frames and play state are determined
- ❑ Play Back State Panel – where you determine what is allowed to appear in your animation
- ❑ Film Strip Panel – where you can see the visual representation of your key frames in a film strip

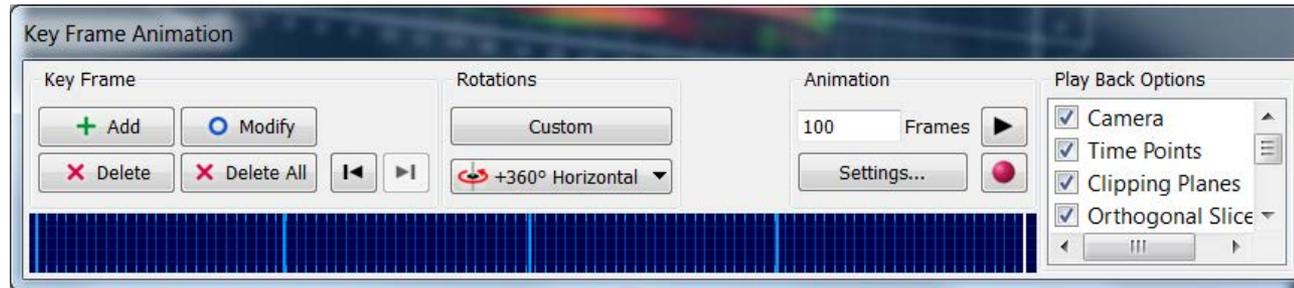
# Your Tools: Key Frame Panel

- ❑ Click **Add** and the image present on your main screen will appear as a key frame (highlighted in light blue on the film strip)
- ❑ To **modify** a key frame highlight the frame in the film strip and adjust the frame to your modified version, then click the Mod. button.
- ❑ The **rotation's** pull-down menu will allow you to scroll through the pre-set rotations
- ❑ The '**Custom**' button allow you to insert you own degree of rotation in any direction
- ❑ In the Animation window you can enter the **number of movie Frames**
- ❑ There is the button to open the Animation **Settings...**
- ❑ Use the **play button** to play a preview of the movie - Please note to **pause** the animation re-click on play button
- ❑ Use the **record** (red dot) button to record the movie. **THIS IS NOT A STOP BUTTON!!!**





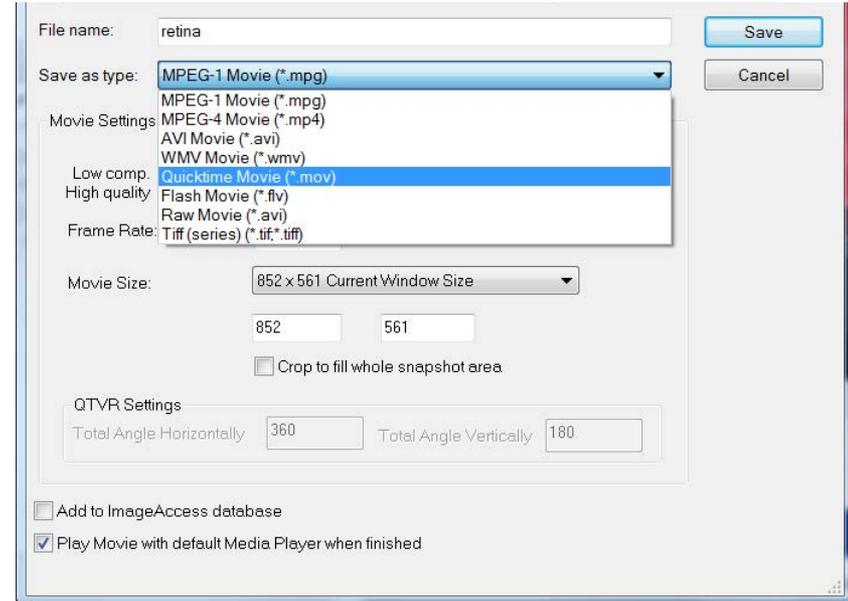
- ❑ The Strip window provides a working area to create your animation
- ❑ The thick light blue line indicates the user-defined key frames
- ❑ The white line indicates the active/selected frame
- ❑ The thin blue lines represent the interpolated frames
- ❑ The spacing between key frames can be adjusted and the animation will play faster or slower according to the spacing
- ❑ Left-Click on a line to display that frame in the Surpass view area
- ❑ Right-Click on a line to select that frame, without changing the Surpass view



1. Load a data set and compose a Surpass scene
  2. Adjust viewing angle (camera) and time point of data set
  3. Click Add Key Frame
  4. Repeat steps 2 & 3 for any other interesting views
  5. Define the total number of frames (duration of animation)
  6. Press the play button to see a preview
  7. Record animation and store it as a movie
- \* Note: When Add is pressed the first time the same key frame is added to the start and the end of the filmstrip . This ensures a smooth rotation back to the beginning of the movie.

# Animation: Recording the Movie

- ❑ To save the animation as a movie, click in the Animation window on the Record button (red dot). Then Save As Movie dialog window is displayed on screen.
- ❑ Animations may be saved as AVI, QuickTime, or a numbered TIFF Series of each frame
- ❑ The frame rate (the play back speed) of the movie can be selected
- ❑ The compression level can be adjusted.
- ❑ You can choose to have the movie played back or not immediately after recording.
- ❑ Once the save button is pressed, Imaris will begin to play through the movie (this is part of the recording process)
- ❑ A screen shot is taken as each frame is played and then tied together as a movie once all frames are taken.



# Saving Your Work : The Imaris File



- The Imaris file (\*.ims) contains the image pixel/voxel data, the Imaris-specific scene data and the statistics
- Combined Image + Scene saving is activated by Default
- Imaris files that contain scenes can be opened in the free Imaris Scene Viewer
- Saving files in version 8.2 is significantly faster (up to a factor of 5) than previous versions due to multithreaded gzip compression of image data. This applies to both Imaris and ImarisFileConverter.



# Saving Your Work : The Imaris File



- To **save** a created Imaris file that contains both image and scene data **on your local storage space** click on the **Export** option in the File menu or Export icon Export Save.
- Saving in Imaris format (\*.ims) is recommended whenever the data set is cropped or the parameters changed.
- Saving a data set in Imaris file (\*.ims) format provides the advantage of a faster loading process and the possibility of using thumbnails. In addition, parameters are saved with the images.
- You can save the file when you are done or if you want you can stop in the middle and resume work later. Simply, choose Export from the File menu and give the file a name you will remember. If you then close the file and reopen it at a later time, you can resume work from where you left off.
- When the work is done, it is recommended to save the dataset by using '**Store as**'. Selecting the 'Store as' option could take longer time but will give you the smallest file size possible.



- To save an existing data set click Store in the File menu or Store icon on the Main toolbar.
- Selecting this option **stores** the image data and (any) created objects within the selected **Arena Group**. The icon overlaying the image is updated to reflect the newly created surpass objects. The newly created object is added under Properties in the Surpass tab.
- Please note: Store functionality is implemented to be performed at maximum speed. **This functionality overwrites the existing file without warning and only writes changed or new data without deleting obsolete ones.** As a result, the resulting Save file could actually be larger than the original.

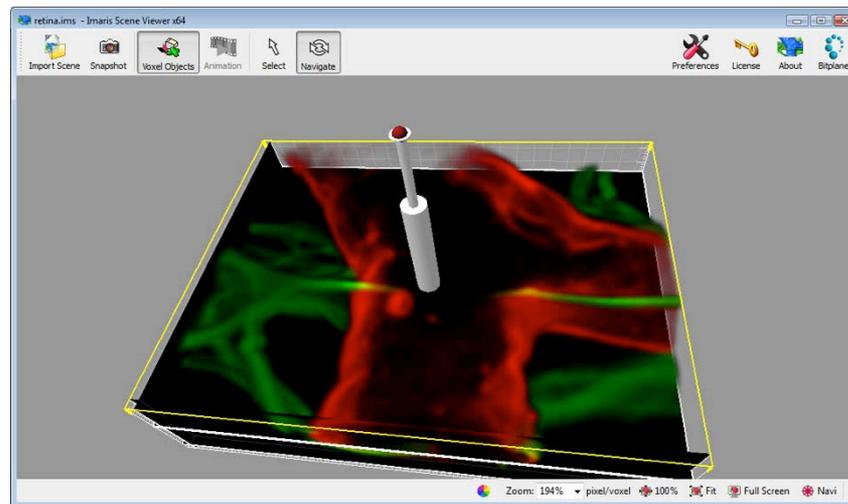
# Store As – a modified image in Arena



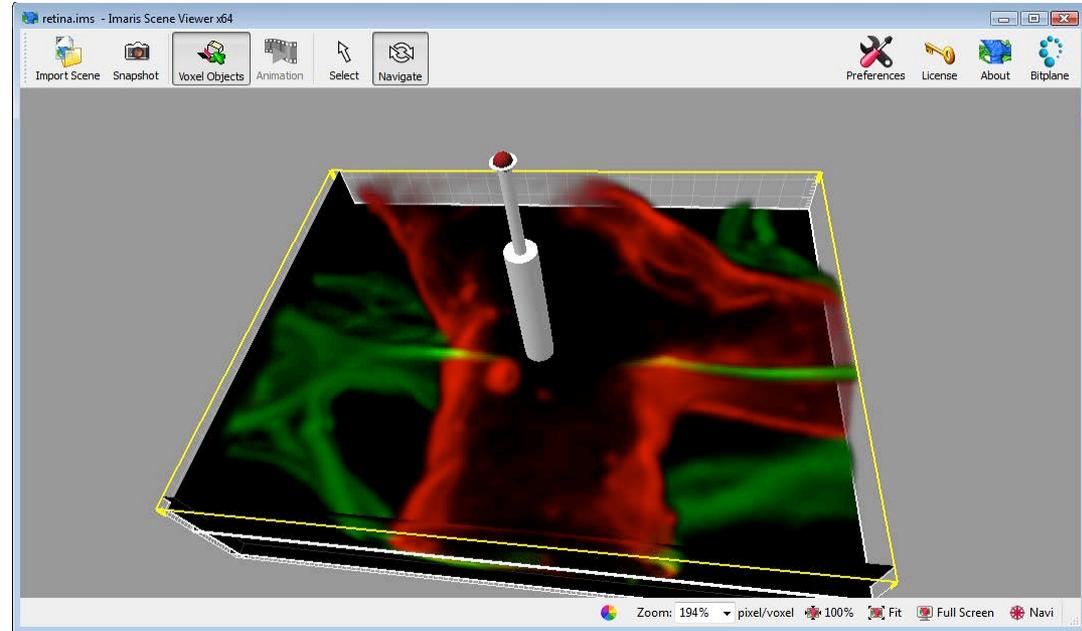
- To store the changes made to the image in a different file whilst maintaining the original file click on the **Store as** option in the File menu or Store as icon.
- This option enables you to **modify the image name and creates a new image item within the Arena**. The selected group now contains one original image file which remains unchanged and the new image file with the additionally segmented object. This newly created image item has an overlaid icon indicating the image type and segmented surpass objects. Under the Objects tab the newly created object is added.
- Enter the name for the file to be saved or confirm the suggestion.
- Select the requested file format and click OK

# Free Scene Viewer (up to version 7.7.2) Discontinued with Imaris8

- Installed and licensed with the Imaris installation (up to version 7.7.2)
- Floating licenses have unlimited instances, so you can always view your files, even if the full Imaris license is in use
- Can be installed for free on any other computer that you like.
- Must obtain a license by registering on the Bitplane website by going to Downloads and then to the free SceneViewer
- <http://www.bitplane.com/go/download/free-sceneviewer>



- It is essentially the Surpass viewer of Imaris without any of the controls.
- It opens Imaris scene files only when full dataset file is available and is capable of displaying any element that the scene file contains, including the volume images.
- Snapshots may be taken
- Existing animations can be played back

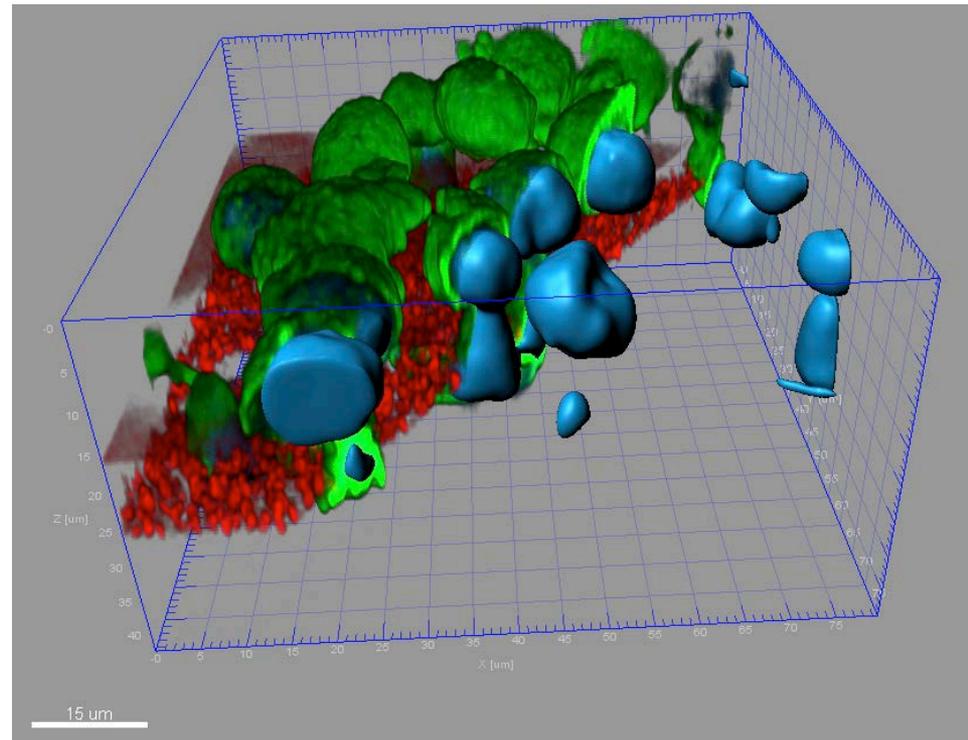


- Ideal for sharing data with colleagues who don't own Imaris
- Nice for creating figures for publication outside of Imaris

# Exercise 1



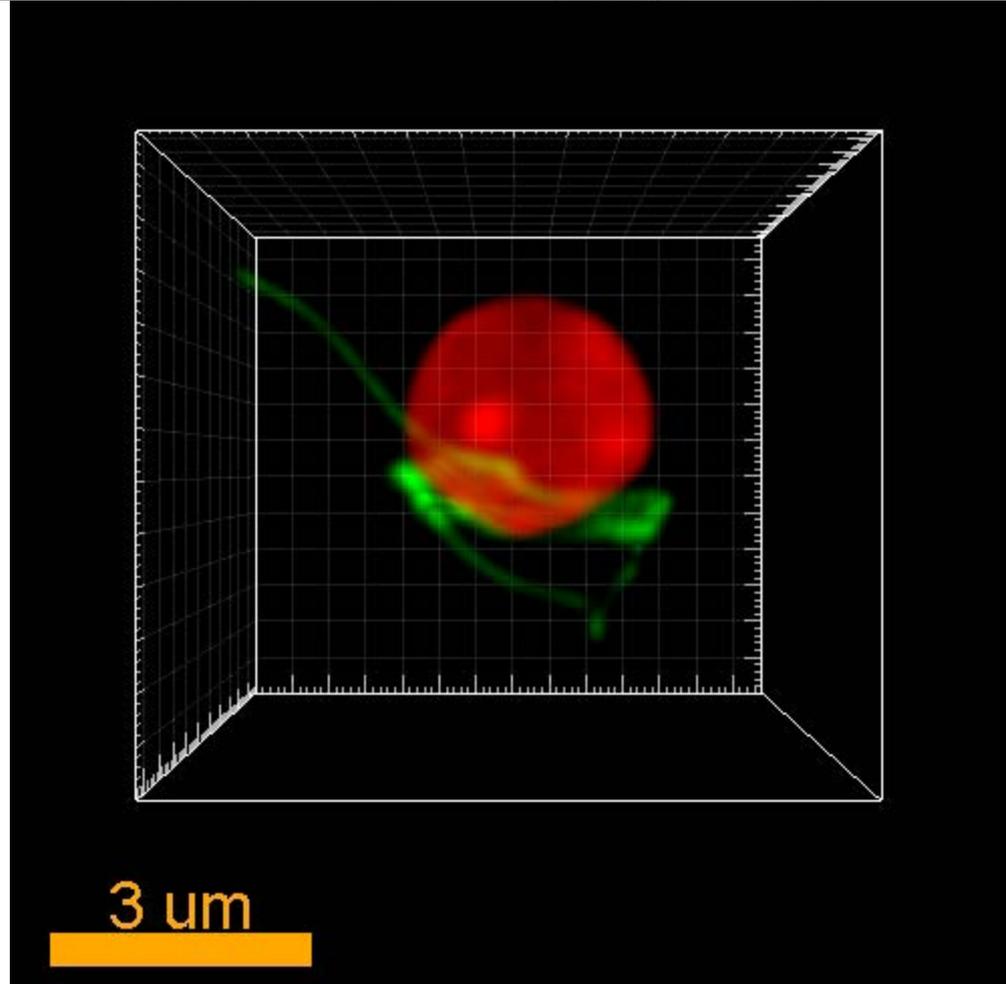
In Surpass mode reproduce the following view with the **ShibutaniAttCorr.ims** dataset and *Store As* the file in Arena



# Exercise 2



1. Create this animation from [spDSADecon-crop.ims](#):
2. Export the movie as an avi file



# Exercise 3



1. Create animation from [ShibutaniAttCorr.ims](#) like this:
2. Export the an IMS file

