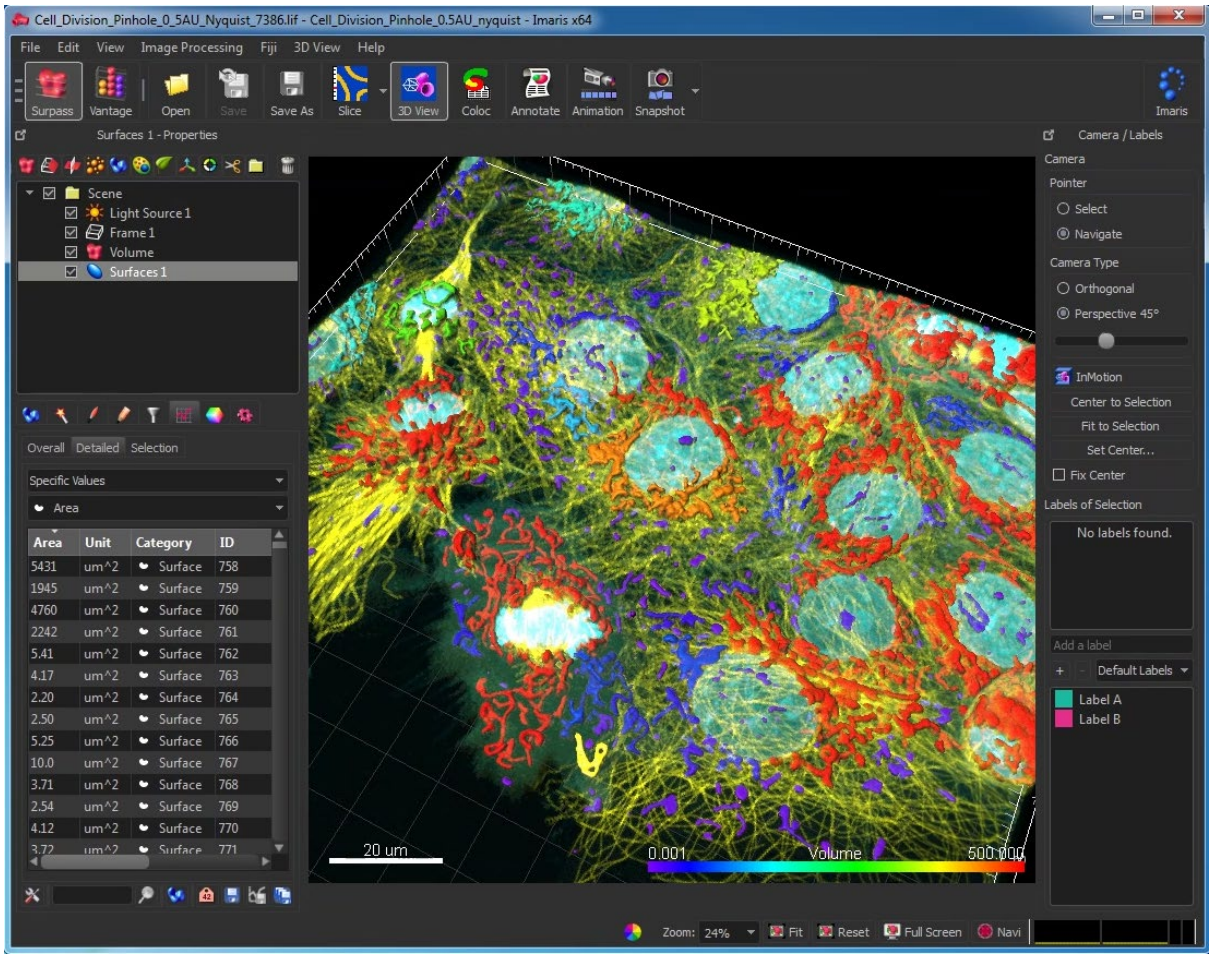
3D Visualization using Bitplane Imaris

Practical Examples



Center for Microscopy and Image Analysis
University of Zurich

2019

This script was written as a short summary for the users of Center for Microscopy and Image Analysis, University of Zurich, Switzerland

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This script was written using Imaris 9.2.0.

Contents

1. Abbreviations

ROI: Region of interest

LUT: Look-up table

Stack: Data structure with a set of related images of the same size and bit depth.

Slices: The images that make up a stack.

Voxel: In stacks, a pixel (which represents the smallest 2D image data) becomes a voxel (volumetric pixel), i.e. an intensity value on a regular grid in a three dimensional space.

2. Remarks

This script was written for practical training purposes by the Center for Microscopy and Image Analysis, University of Zurich. Theory is kept to a minimum.

The script should be used as a guideline for training.

Highlighting styles used in this script:

File names are set in green (e.g. Cell_Division_Pinhole_0_5AU_Nyquist_7386.lif)

Step 1.
Practical steps are set in italic. ***Keyboard shortcurts here***

3. Software: Imaris

Bitplane Imaris is a commercial software package for the visualization, segmentation and analysis of multidimensional microscopy datasets.

As confocal microscopes were first becoming commercially available, the founders of Bitplane, Marius Messerli, Karl-Hermann Fuchs, and Jürgen Holm, realized that there was no suitable way to visualize and analyze the images provided by this more modern equipment. While pursuing their research at the Institute for cell Biology at the ETH in Zurich the first productive version of Bitplane's core product, Imaris, was developed and released in 1993 (Adapted from Wikipedia).

Today Imaris is still developed and sold by Bitplane:

http://www.bitplane.com/

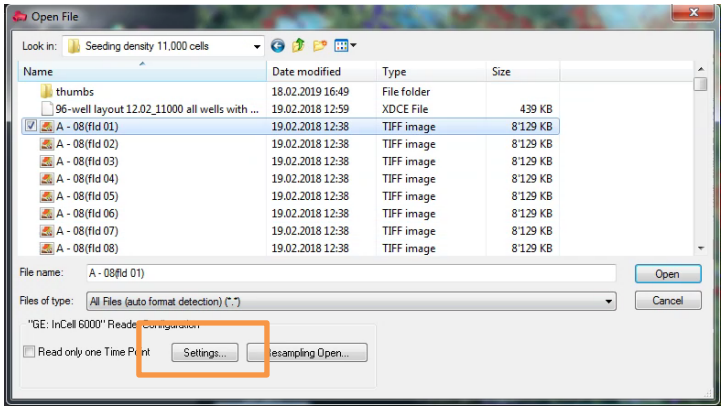
Besides the open source tools such as Fiji, Imaris is the most used software at the ZMB for exploring and analyzing microscopy images. The main reasons are the **simplicity and the capability of easily visualizing data, segmentations and also quantifications in 3D or 4D.** Especially for the 3D visualization there are as of now no open source tools with a similar performance and therefore Imaris and similar tools such as Thermo Scientific Amira or Arivis Vision4D are used in universities all around the globe.

By Imaris XT, a software interphase for Python and Matlab, Imaris also allows you to write custom programs which can interact with the data and the visualizations.

4. Opening Standard File Types

In the background Imaris uses a version of the OME bioformats reader (https://www.openmicroscopy.org/bio-formats/). Therefore it should be able to open a very similar set of microscopy image file formats such as Fiji. You can open a file in Imaris using

Step 1.
File>Open

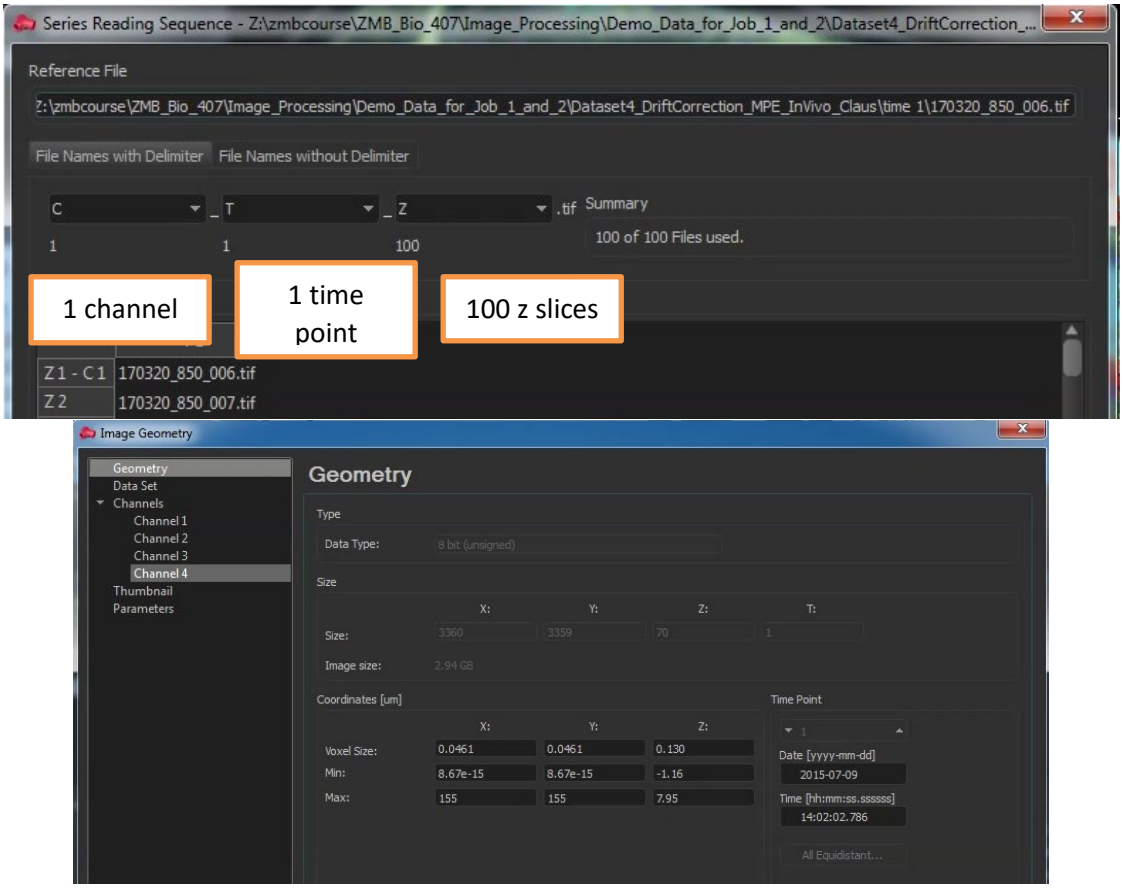


If you open multidimensional .tif files where each slice/channel/timepoint etc. is stored as a single file it might be necessary to instruct Imaris in how to interpret the filenames in a folder. This can be done in the file open dialog under “Settings”

5. Explore Metadata and Calibrate Images

You can explore the metadata of the opened file by using

Step 2.
Edit>Image Properties

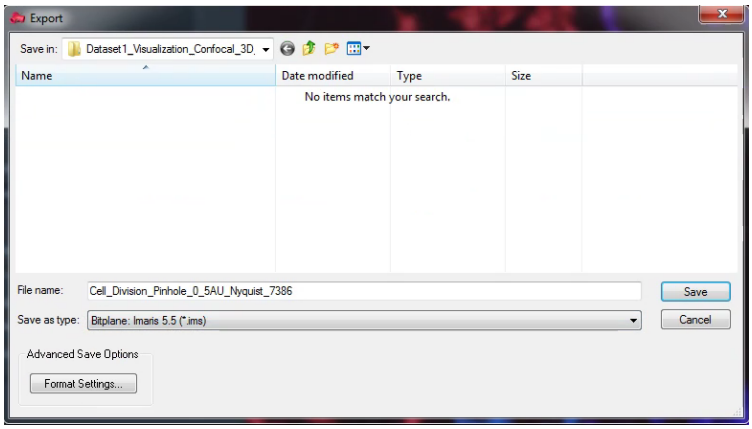


Depending on your source file format (e.g. tif) the pixel/voxel size might not be calibrated. Be sure to check and correct the values here if needed.

6. Resaving in the Native Imaris File Format

In order to have the maximum visualization performance as well as software stability, we recommend resaving the data in the native Imaris file format (.ims) before doing any further image analysis.

Step 3.
File>Save As



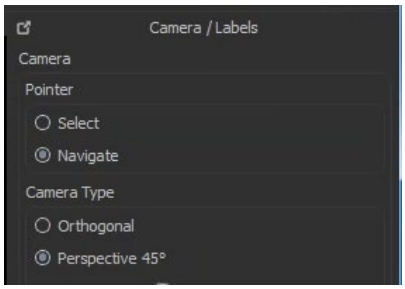
7. Adjusting the Image Visualization

In order to adjust LUTs of the different channels open

Step 4.
Edit>Show Display Adjustment

Click on “Advanced” to manually set the min/max values as well as the gamma. Play with these settings and see how the image representation changes.

By clicking on the channel names you can change the names as well as the LUTs. Turning off and on channels by using the checkbox is also very useful for adjusting the values of one channel after another.

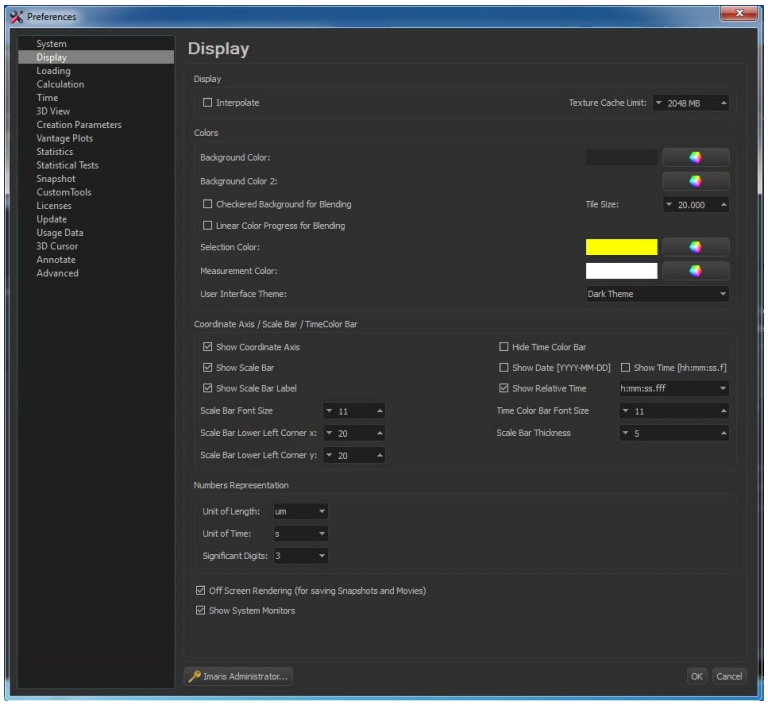


Click into your image and see how you can change the view including zooming in and out using the mouse wheel. Make sure that your pointer selection on the right pane is on “Navigate”:

Now put it on “Select” and try to manipulate the appearance of your scale bar. If you are not happy with the color of your scale bar or want to deactivate completely, you can go to

Step 5.

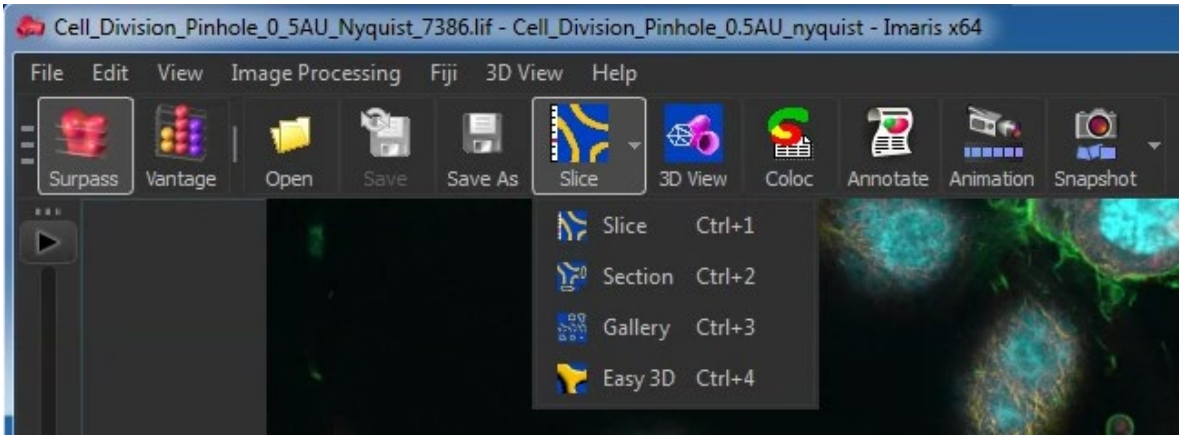
File>Preferences>Display



Here you can adjust the image representation to your liking. Keep in mind that these settings are global and will be kept when you next time start and use Imaris.

8. Slice Representations

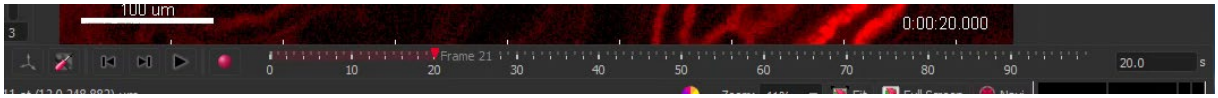
Explore the different slice image representations under the **“Slice”** icon such as the **“Section”** (Normally called ortho viewer).



The “Slice” representation also allows you to do a maximum intensity projection of your dataset (Easy 3D).

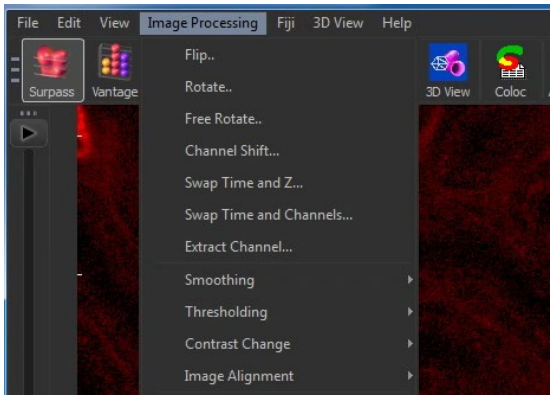
9. Time Series

If you open 2D or 3D time series either directly from a microscope format or by importing a .tif series with the proper “Settings” during opening, you should see a time bar which allows you to navigate the time dimension of your series



If you imported your data without specifying the dimensionalities, and as an example a 2D time series is loaded as a single 3D volume you can also change this by

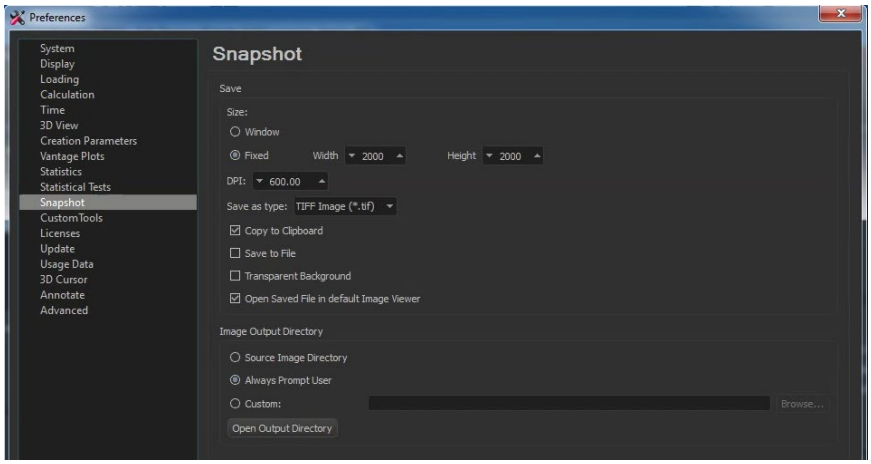
Step 6.
Image Processing>Swap Time and Z



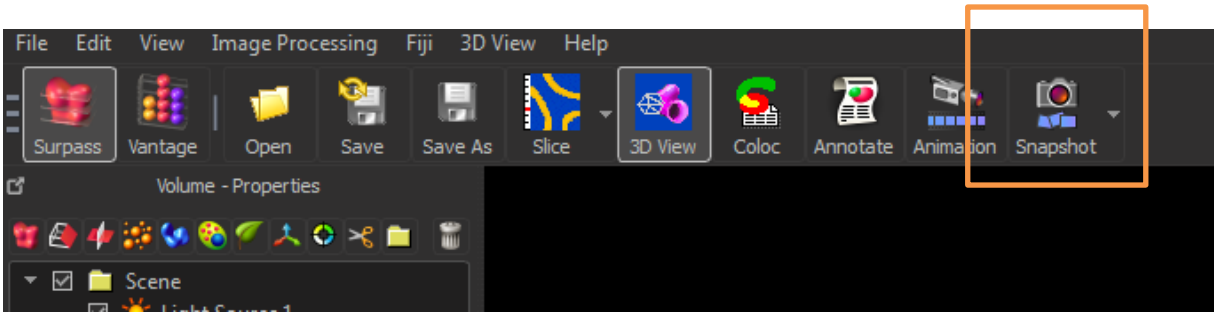
10. Snapshots

Go back to the **“3D View”** and explore the settings for the “Snapshot”

Step 7.
File>Preferences>Snapshot



Configure these settings according to your liking and make a few snapshot of your dataset by clicking on the “Snapshot” icon.

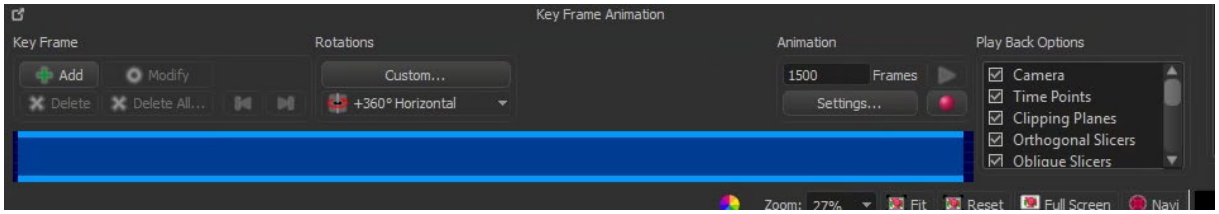


Be sure to check where your snapshots are going to be saved. You can use this approach for making publication ready images using Imaris.

11. Movie Animations

If you have 3D or 4D datasets you might want to show those in a presentation as a rendered movie sequence. This definitely is one of the strengths of Imaris and can be done very easily.

Activate **“3D View”** as well as **“Animation”** and you should see the **“Key Frame Animation”** pane.

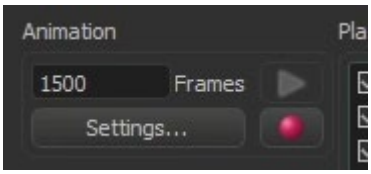


To make a movie sequence you can define “Key Frames”.

Keyframes in a movie animation are frames/views that define the start and the end of a smooth transition. Use navigation to find a perspective/view and zoom factor you like and **"+ Add"** a keyframe. Go to another position and add the next keyframe. Everything in between will be interpolated by the software.

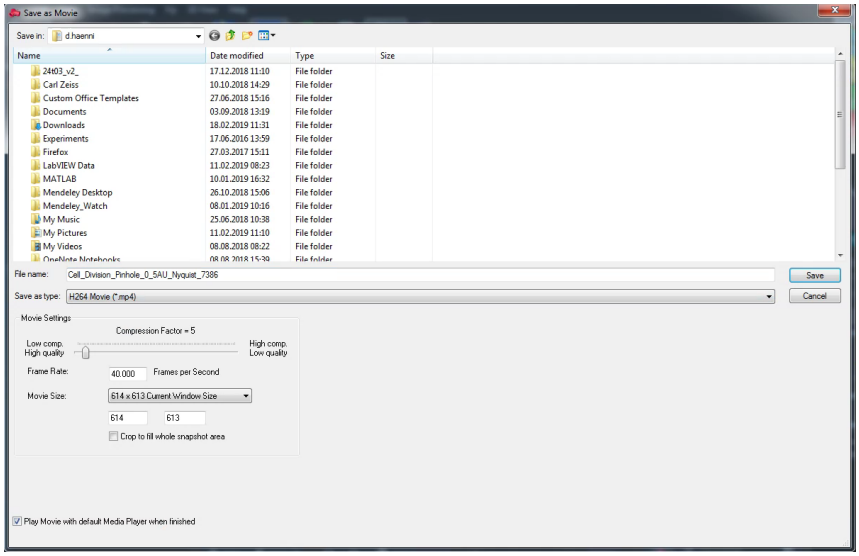
In order to achieve good animations it is recommended that you avoid changing the view too much in between two keyframes. Also the displacement distance of your transitions should be more or less homogenous between keyframes, otherwise your audience might become seasick. You can add as much keyframes as you like. Operations such as changing the brightness, show or hide certain channels, shown time points can also be included as transformations in between keyframes.

You can review your animation using the play button



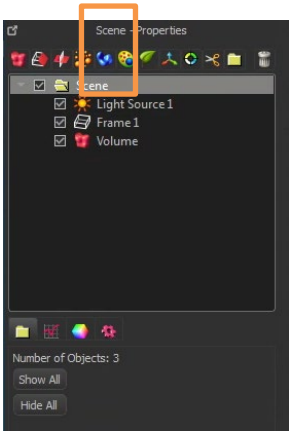
If you have too many keyframes, the animation might start to stutter. In this case you might try increasing the number of frames. Please consider that more frames also means longer movies and more data to put into you presentation. Under **"Settings"** in the animation pane you can adjust the frame rate. Values above 25 are recommended in order to get smooth movies. The number of frames divided by the framerate gives you the playing time of your movie in seconds.

You can press the red record button to render and save the movie

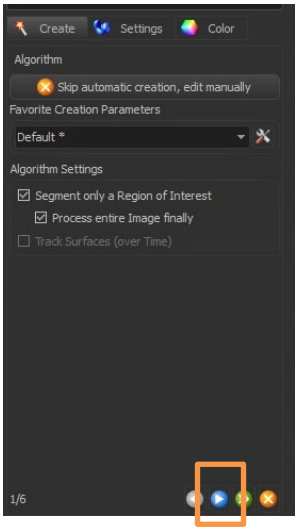


Be sure to use a movie file format that is supported by your operating system and presentation software such as Microsoft PowerPoint (Test this before defending your PhD!). If you are done, close the "Animation" pane.

12. 3D Segmentation and Surfaces



Next we will try to make an intensity based 3D segmentation and visualization. In the “3D View” go to the **“Scene – Properties”** pane and click the blue Icon “Add new Surface”.



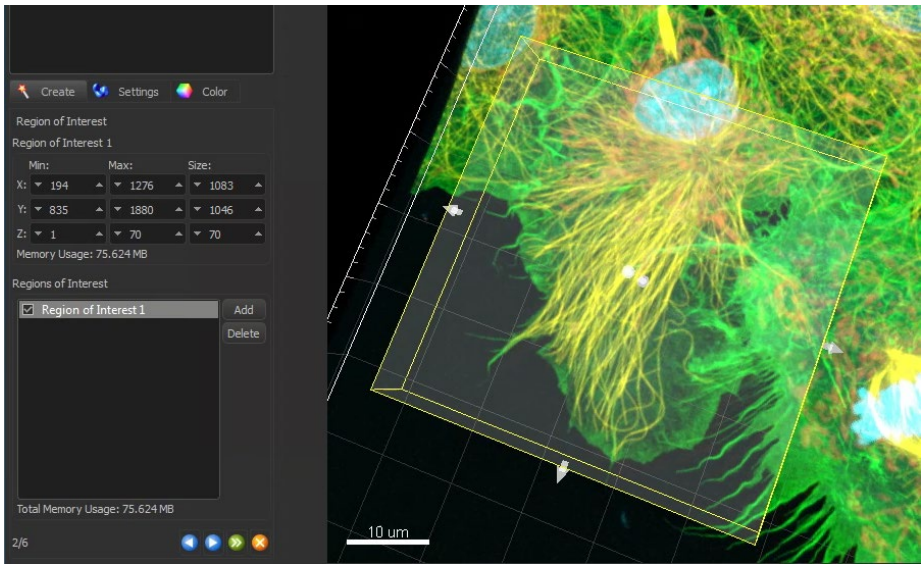
Then a surface creation wizard starts.

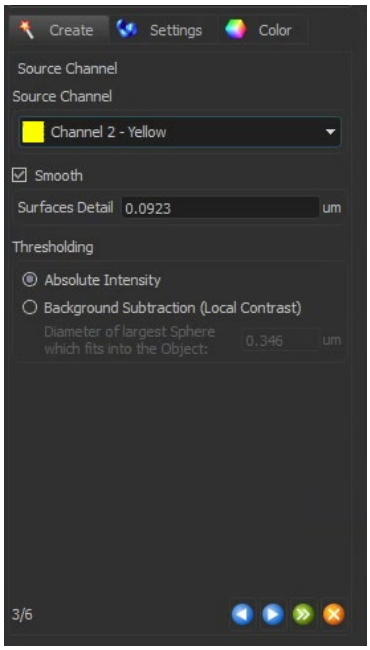
Section 1/6

Select **“Segment only a Region of Interest”** and **“Process entire Image finally”**. This allows you to do your parametrization on sub-volumes of your dataset and therefore everything is much more fluid and responsive. If you then set everything up correctly, the whole dataset can be processed at the end using your defined parameters.

Section 2/6

Press next and Switch the “Pointer” between “Navigate” for adjusting the view and “Select” for adjusting the “Region of Interest” box (You can also switch between “Navigate” and “Select” by pressing the Escape button). If your image is very heterogeneous you can also place multiple “Region of Interest” Boxes for parametrization by pressing “Add”. Then press next

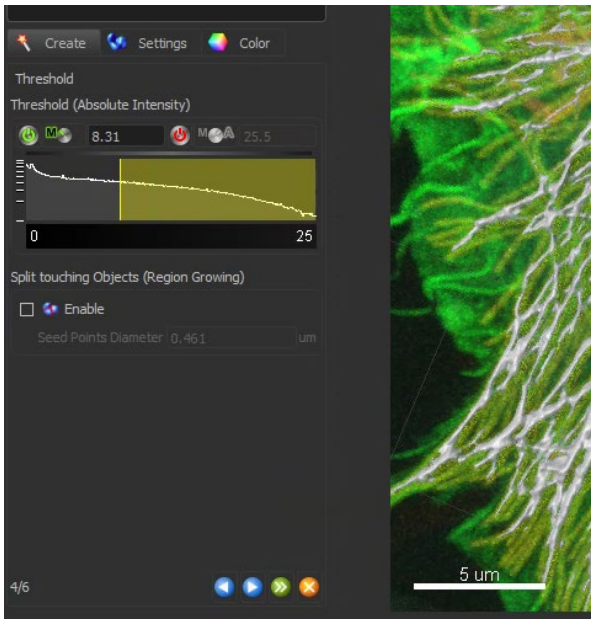




Section 3/6

In step 3 you can select the channel you want to segment and also decide if you want to apply a feature smoothing as well as an adaptive thresholding with a local background subtraction. If you are unsure try with the default values and if you are not happy you can always go back. Also evaluate the effect off different settings (try to use extreme values, then the effect should become more obvious)

Section 4/6



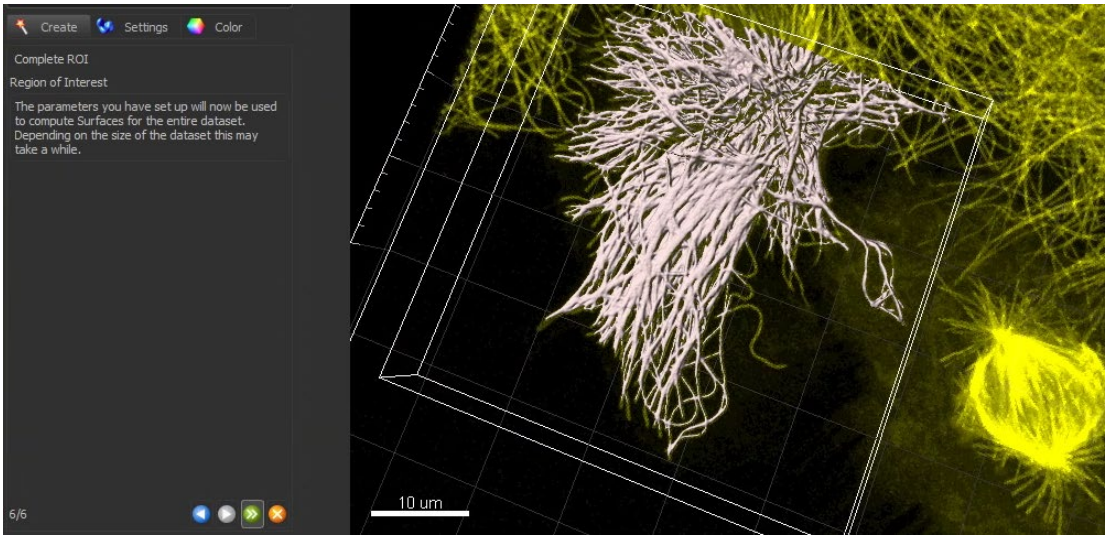
In step 4 you can adjust the intensity threshold for segmentation. For the way the wizard in Imaris works it's usually better to include a bit more of your intensity (Lower threshold) and then in the following steps to work with filters. If you have difficulties to interactively see your segmentation results, you can also toggle off and on the different channels in the "Display Adjustment" window.

Section 5/6

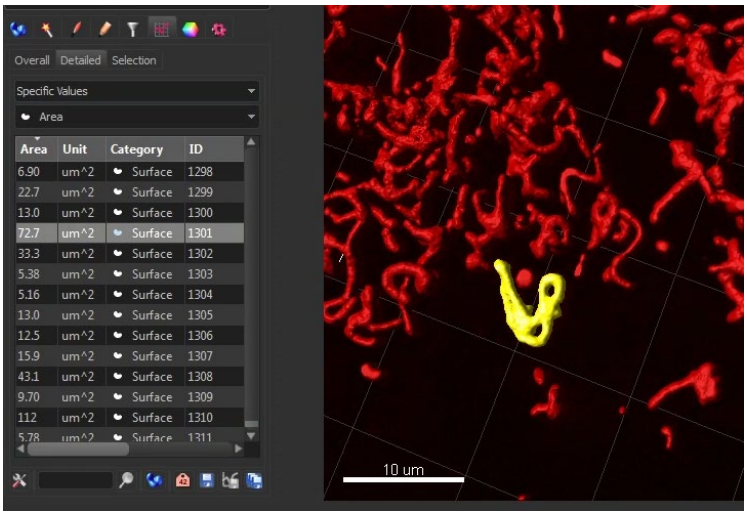
In step 5 you can classify/filter your surfaces using a combination of various filters. As an example, this can be useful to remove small dot like structures, which might not be of interest. Also this allows to use the intensity information of other channels and therefore as an example find structures which have an intensity in two different channels.

Section 6/6

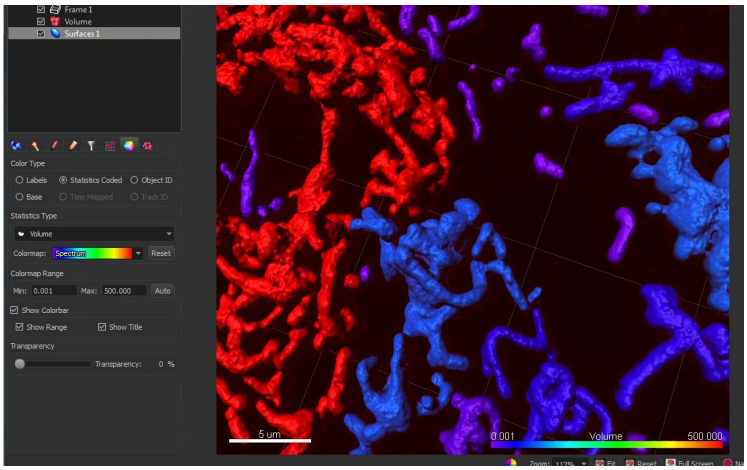
Since you have now adjusted all parameters, you can apply the segmentation of your ROI to the whole dataset in step 6. This can take a bit of time. If you have a time series, the surface generation will be performed for all time points.



When your segmentation is done, you can adjust the visualization of your surfaces, manually delete objects which are not of interest and explore and export (e.g. to excel or matlab) the statistics for each individual object or globally.



Something simple but very helpful to see patterns and trends in your data is the statistical coding of objects. Here you can assign a color according of one of the objects variables such as size or roundness.



You can make as many surfaces as you want. The surface objects are different from your intensity data since they consist of a 3D mesh and not voxels. This allows for advanced rendering using a virtual light source. You can adjust the position and intensity of your virtual light by clicking on “Light Source 1” in the “Scene” pane.

13. Literature and Further Information

- The Imaris reference manual

http://www.bitplane.com/download/manuals/ReferenceManual9_2_0.pdf

- The Imaris learning center where you can find many nice examples of how the software can be used

http://www.bitplane.com/learning

- Community extensions for Imaris

http://www.bitplane.com/download

http://open.bitplane.com/

- Two alternatives to Imaris

https://www.arivis.com/de/imaging-science/arivis-vision4d

https://www.thermofisher.com/ch/en/home/industrial/electron-microscopy/electron-microscopy-instruments-workflow-solutions/3d-visualization-analysis-software.html