AMIRA fast Manual

After the alignment with IMOD

- If you are interest just in the morphological description of your cell you can directly reconstruct the transmission using the SIRT algorithm implemented in tomo3d. You will obtain a 3D volume with a good contrast BUT the numeric values of the voxels will have no physical meaning (SIRT is rescaling the values during the iterations into an arbitrary scale of contrast).
- If you are interested also in the 3D values of linear absorption coefficient you have to apply –ln() to the tilt serie and then reconstruct. Because in this case we want to preserve the "real" numbers and not just the contrast, we will use the ART algorithm. It is implemented in the plugin "TomoJ" of imageJ:

http://www.cmib.fr/en/download/softwares/TomoJ.html

Transmission: $T = \frac{I}{I_0} = e^{-\int \mu_l(z)dz}$ Absorbance: $\int \mu_l(z)dz$ (it is additive!)

Linear absorption coefficient : $\mu_l(z) = \sigma n = \mu_m \rho$ (what we get from the reconstruction)

σ photoelectric cross section, n atoms per unit volume

$\mu_m = \sigma/m$, $\rho = m/V$ (if you prefer a mass density)

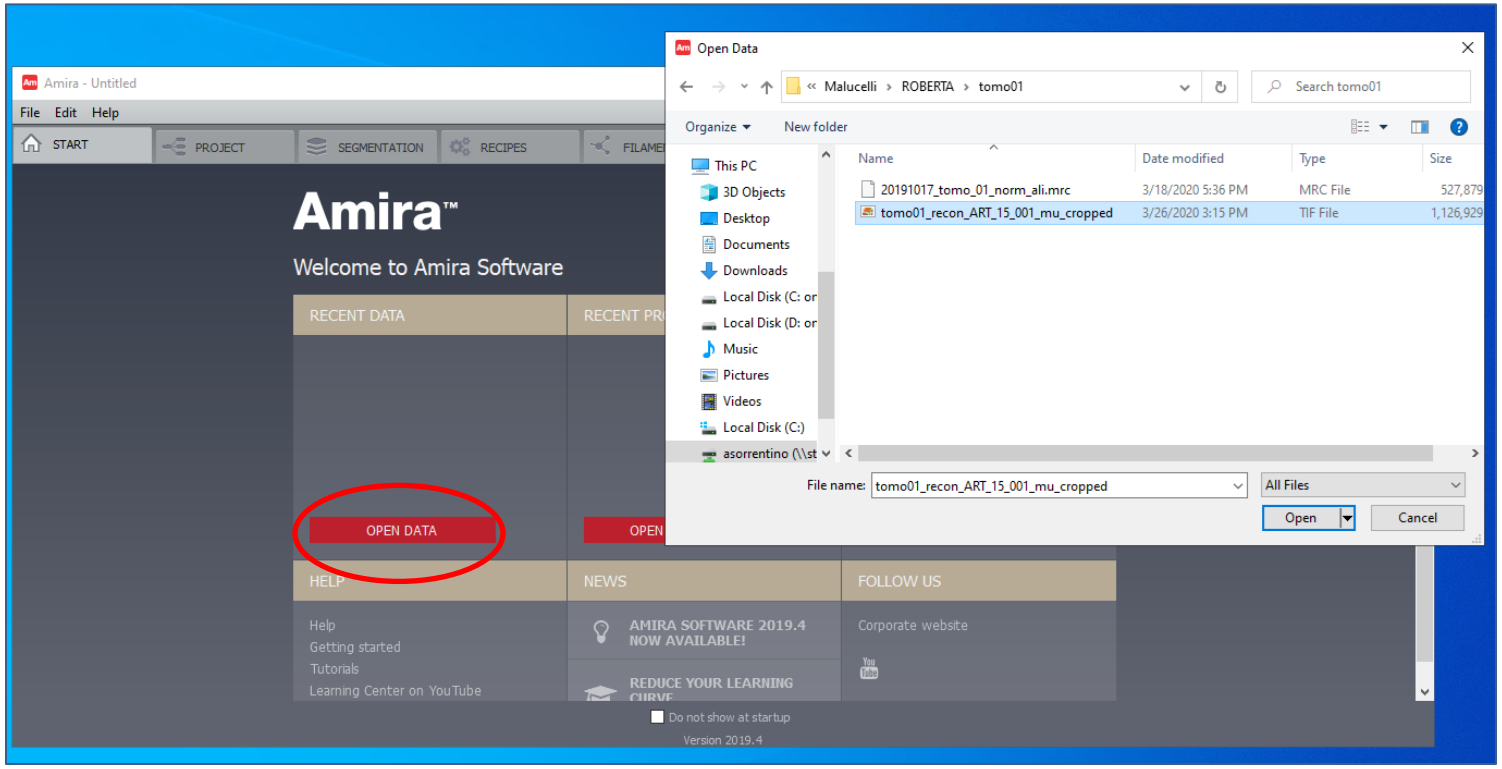
A tip that might be useful:

sometimes our eyes are better looking at the highest absorbance in black because we are more used to it. For instance, people doing classical EM. So one trick would be to reconstruct with ART and then multiply in ImageJ all the voxels by -1 to invert the contrast. In this case, all the voxel values would be preserved (keeping the physical mean) and you just need to disregard the minus sign when doing analysis with Amira.

Reconstruction pre-treatment

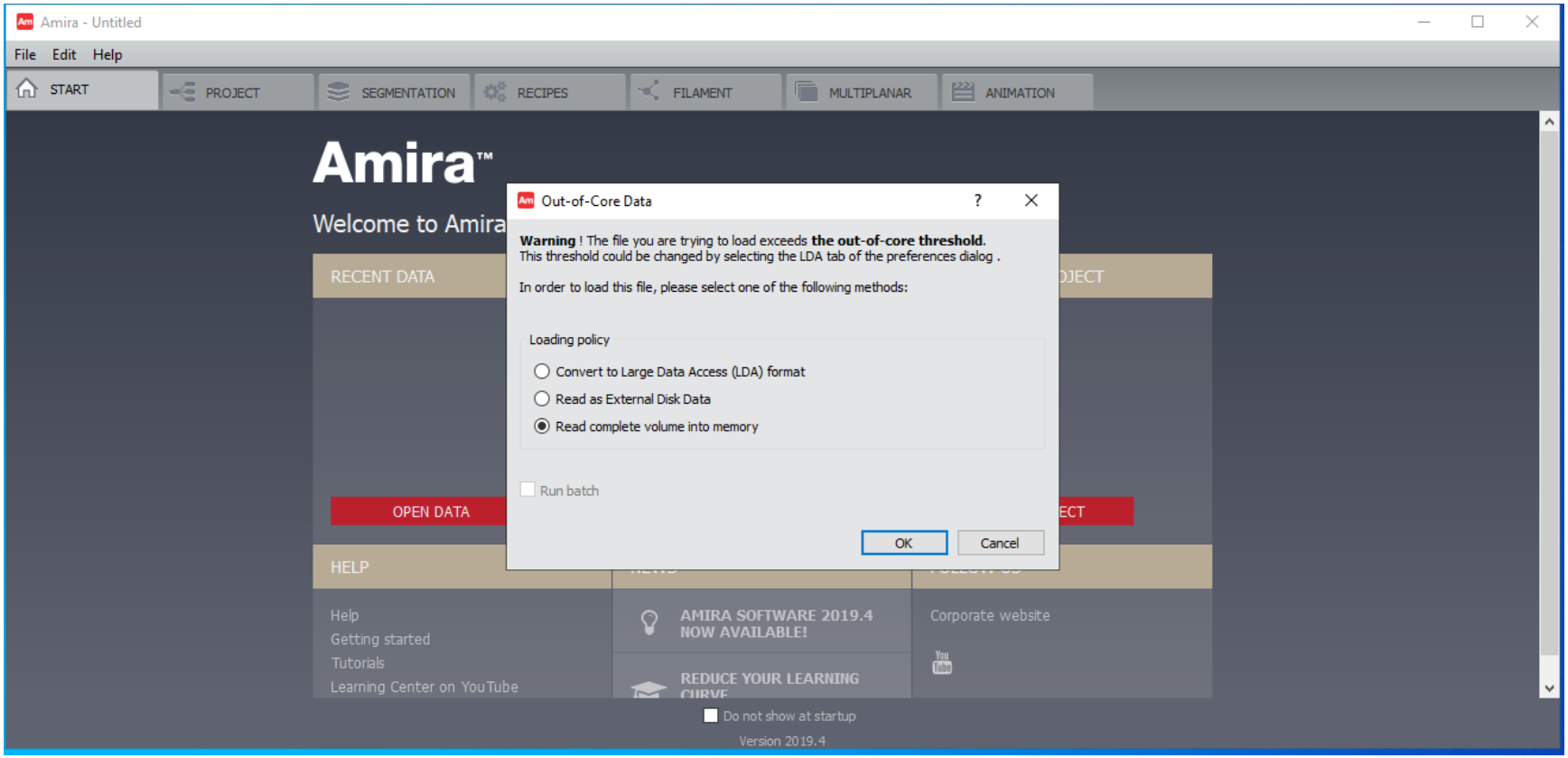
- CHANGE OF UNIT: the value of the linear absorption coefficient in each voxel in the reconstruction is in 1/(pixel size) and the normal unit for it is μm^{-1}, so you need to divide the value by 0.01 if the pixel size used was for instance 10 nm. You can do this in ImageJ.
- CROPPING: As Amira calculations are time consuming, we recommend to crop your volume as much as possible in all directions using the cropping function of ImageJ for X and Y and the “make sub-stack” tool for the Z (=number of slice).

Load DATA 1



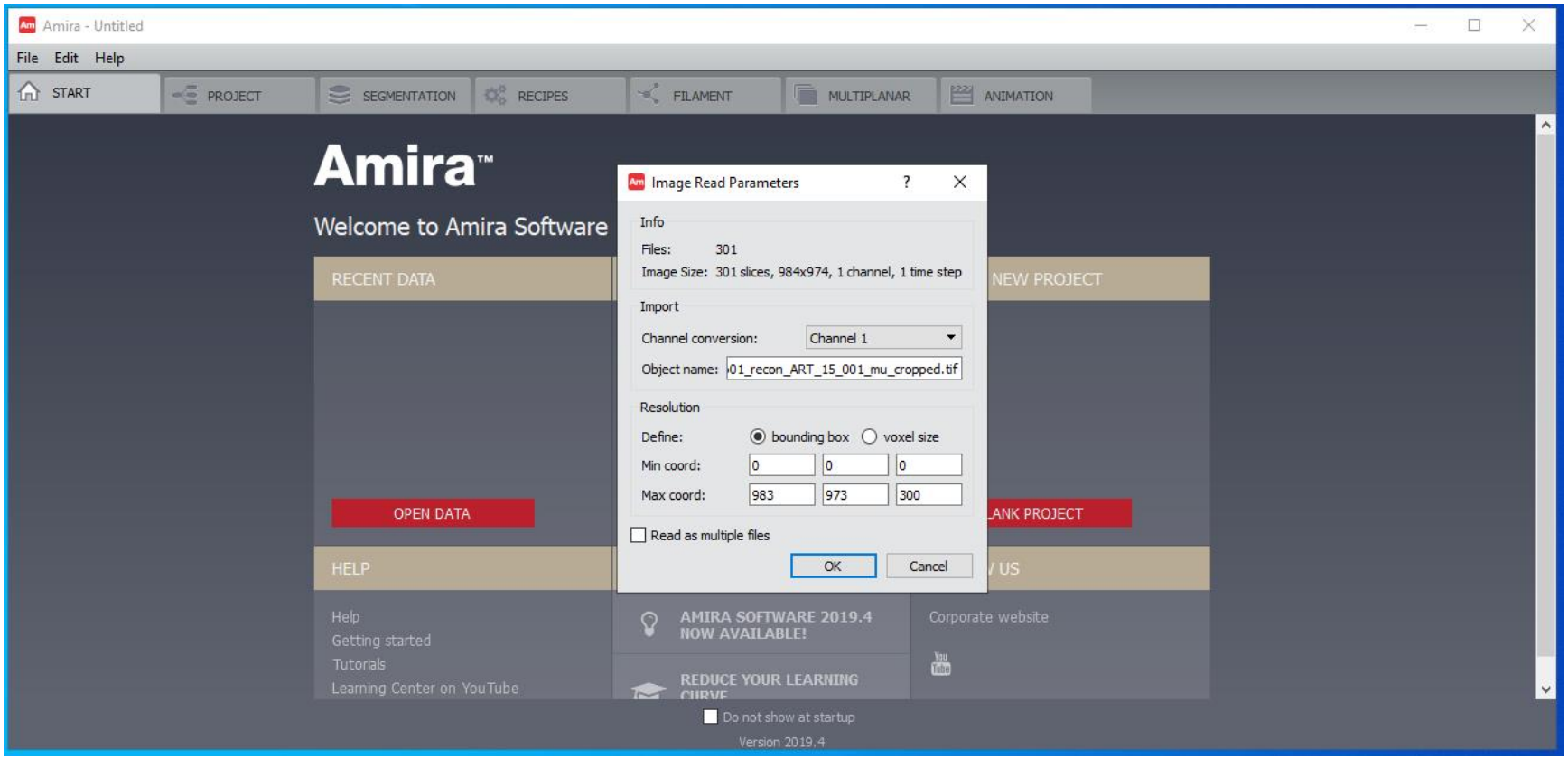
Select your pre-treated DATA.tif

Load DATA 2



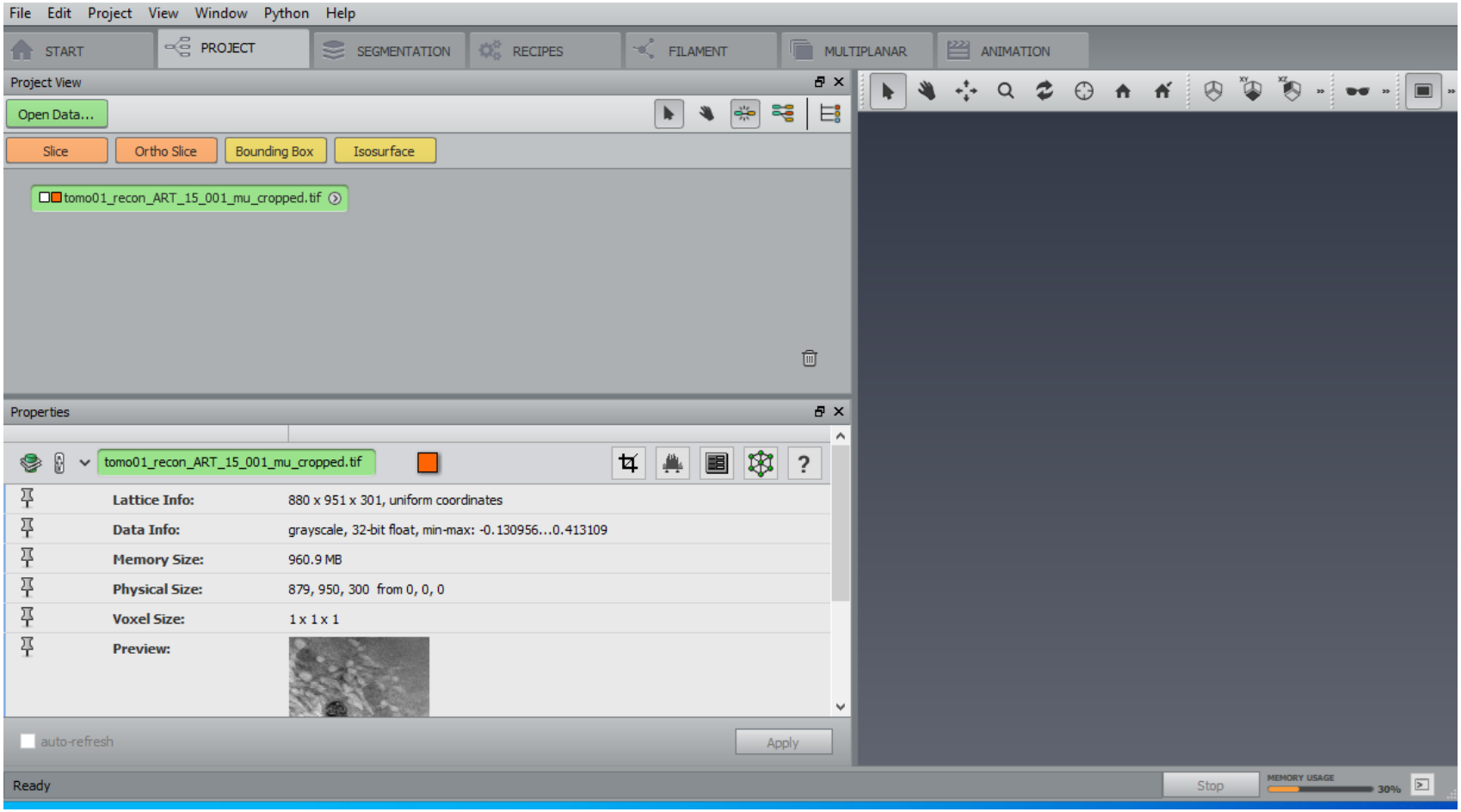
‘Big’ DATA needs to be loaded into memory.

Load DATA 3



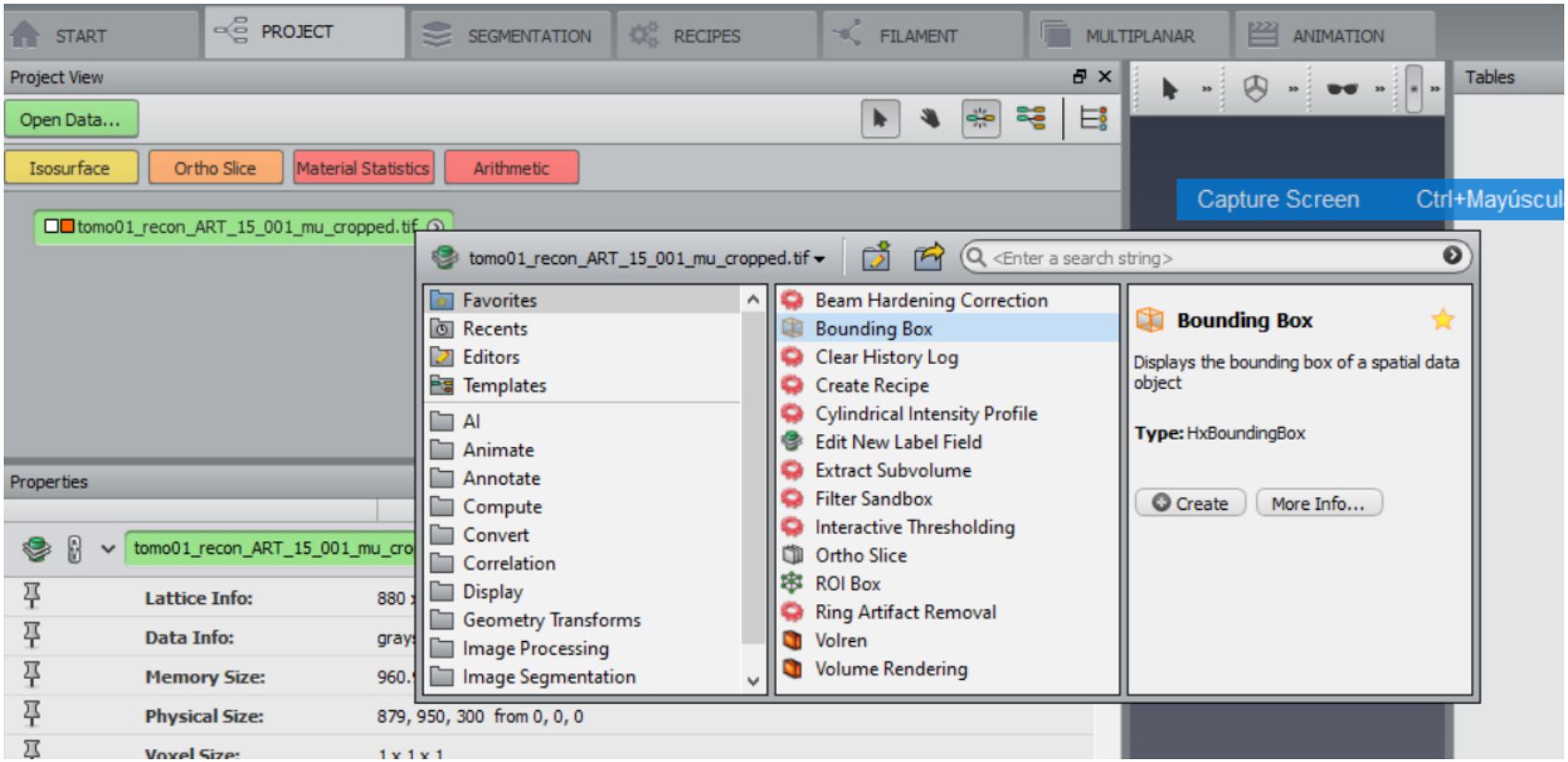
Select ‘bounding box’ option to forgot about voxel size. Our unit volume/area/length is the ‘voxel’

Visualize volume 1



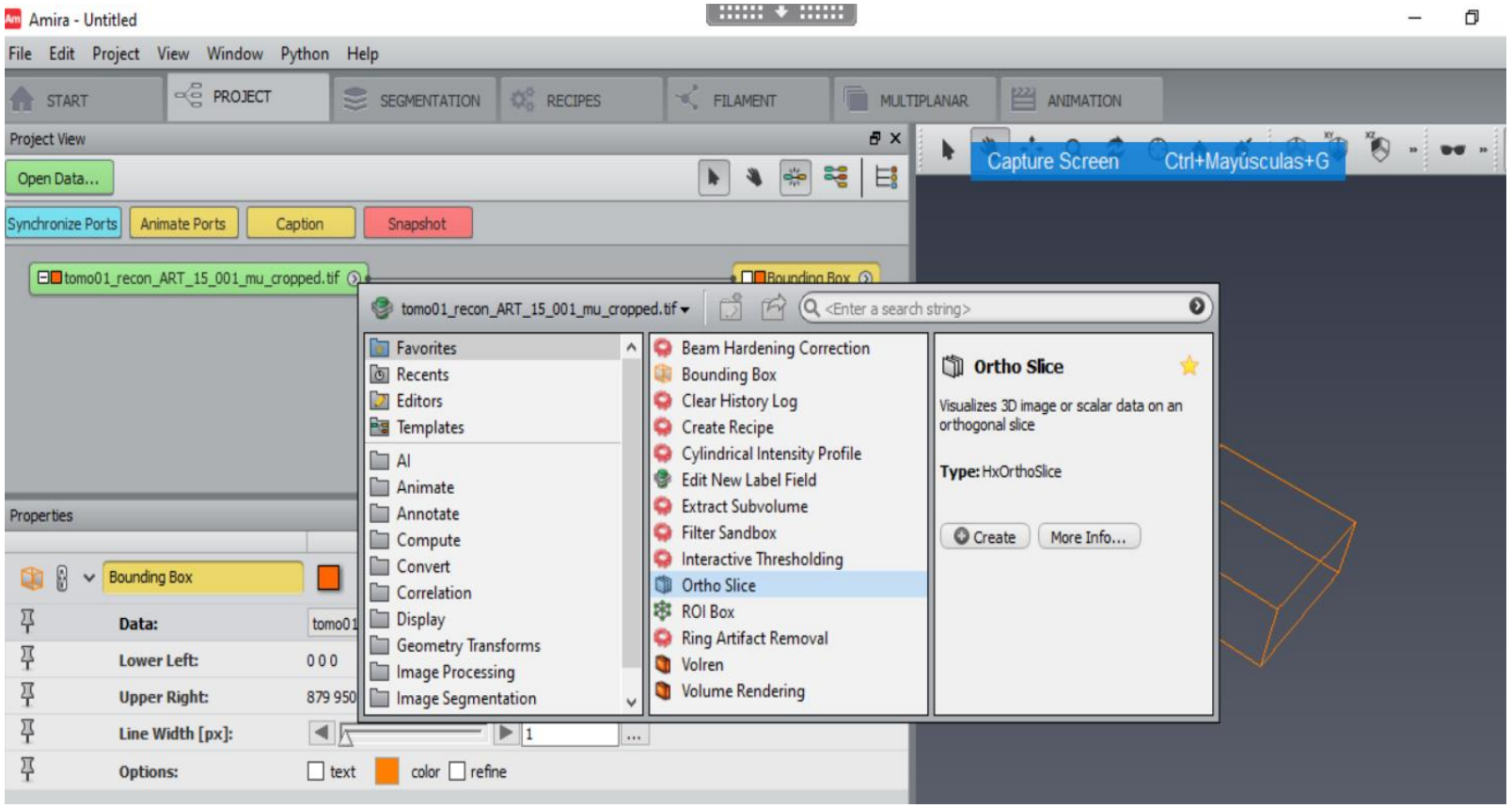
Your data are in the project window as a .tif object

Visualize volume 2



Create a bounding box and an orthoslice modules: right click on the data and select in the window

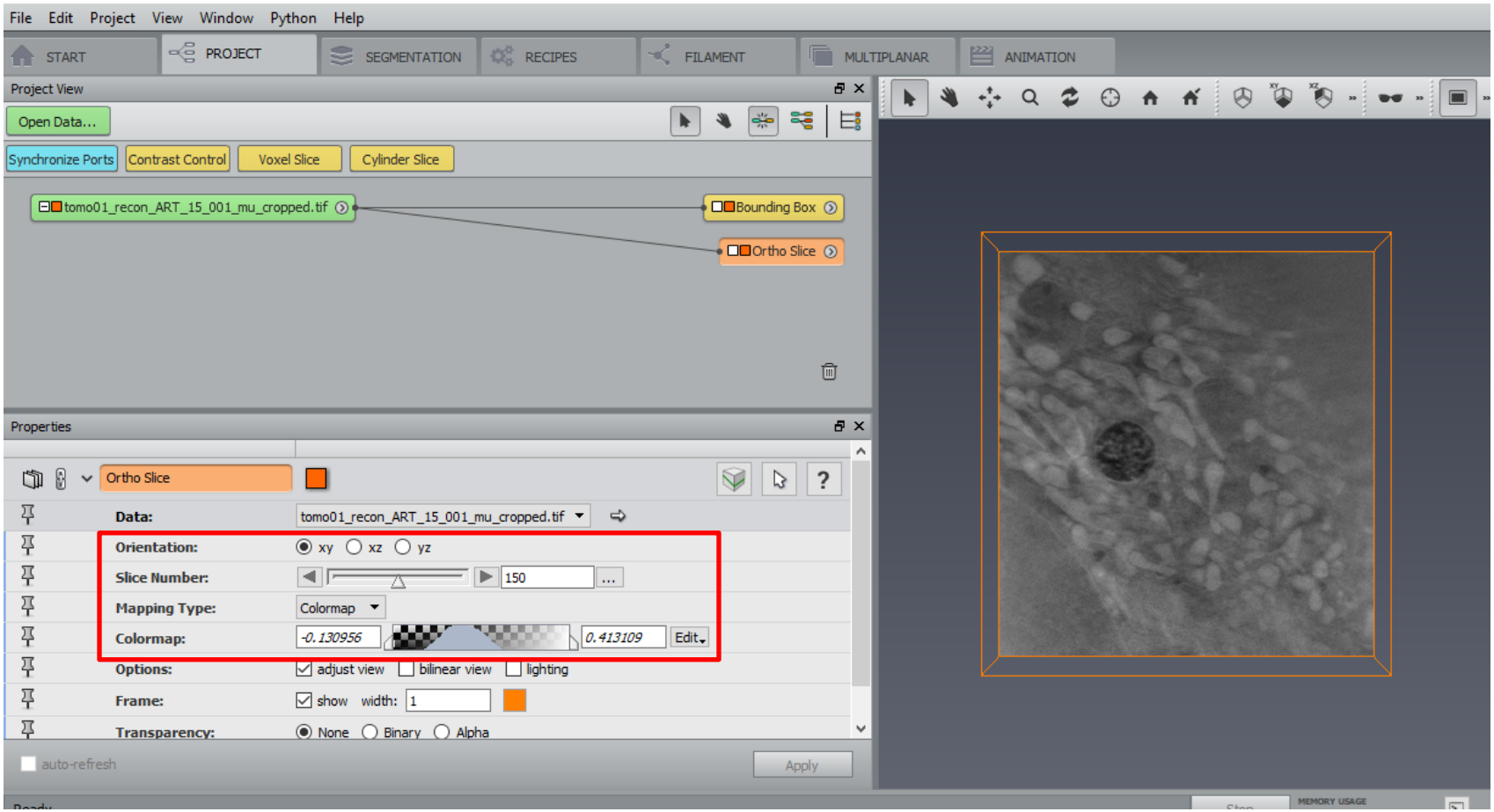
Visualize volume 3



Create a bounding box and an orthoslice modules: right click on the data and select in the window

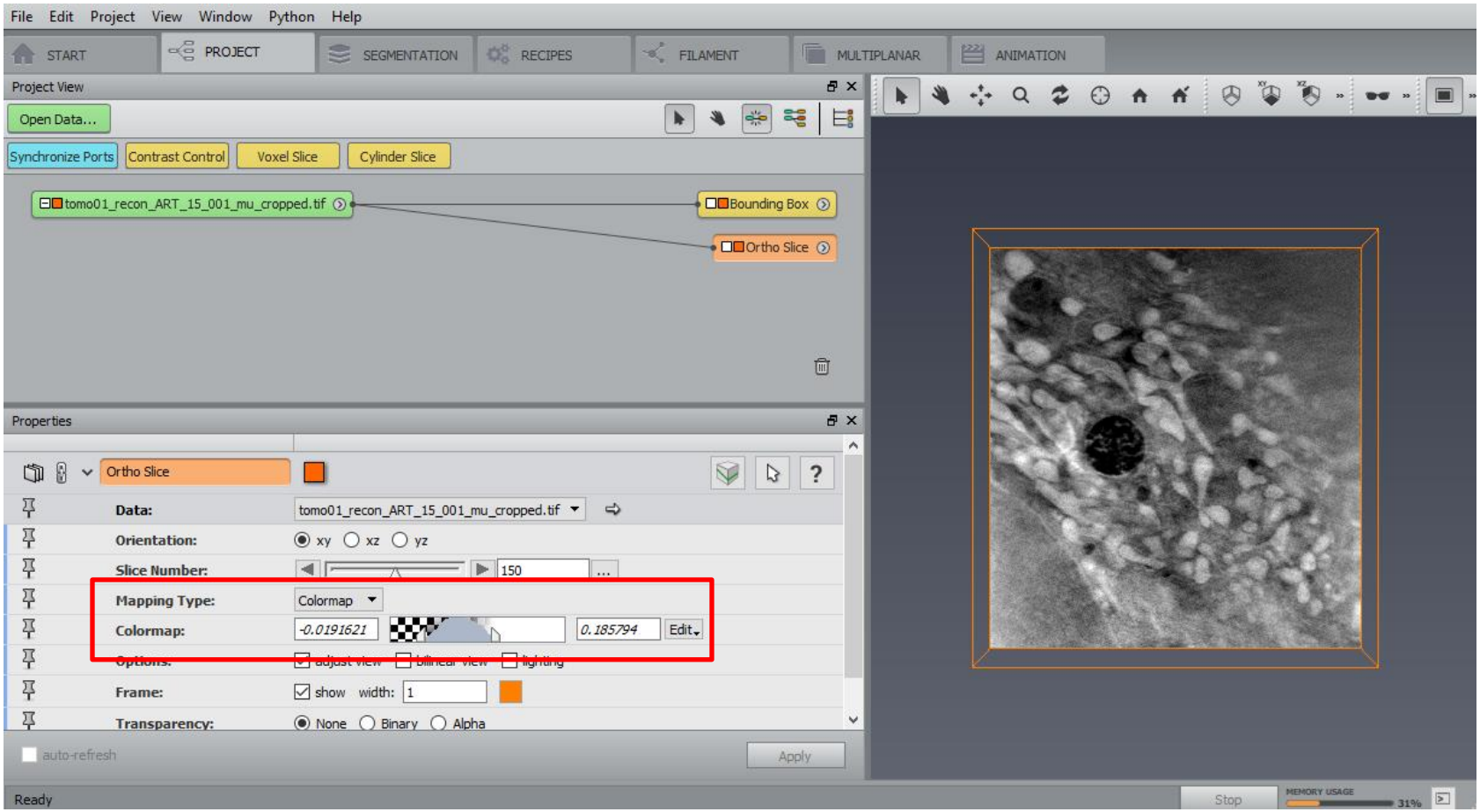
Visualize volume 4

Click on the module to visualize its attributes.



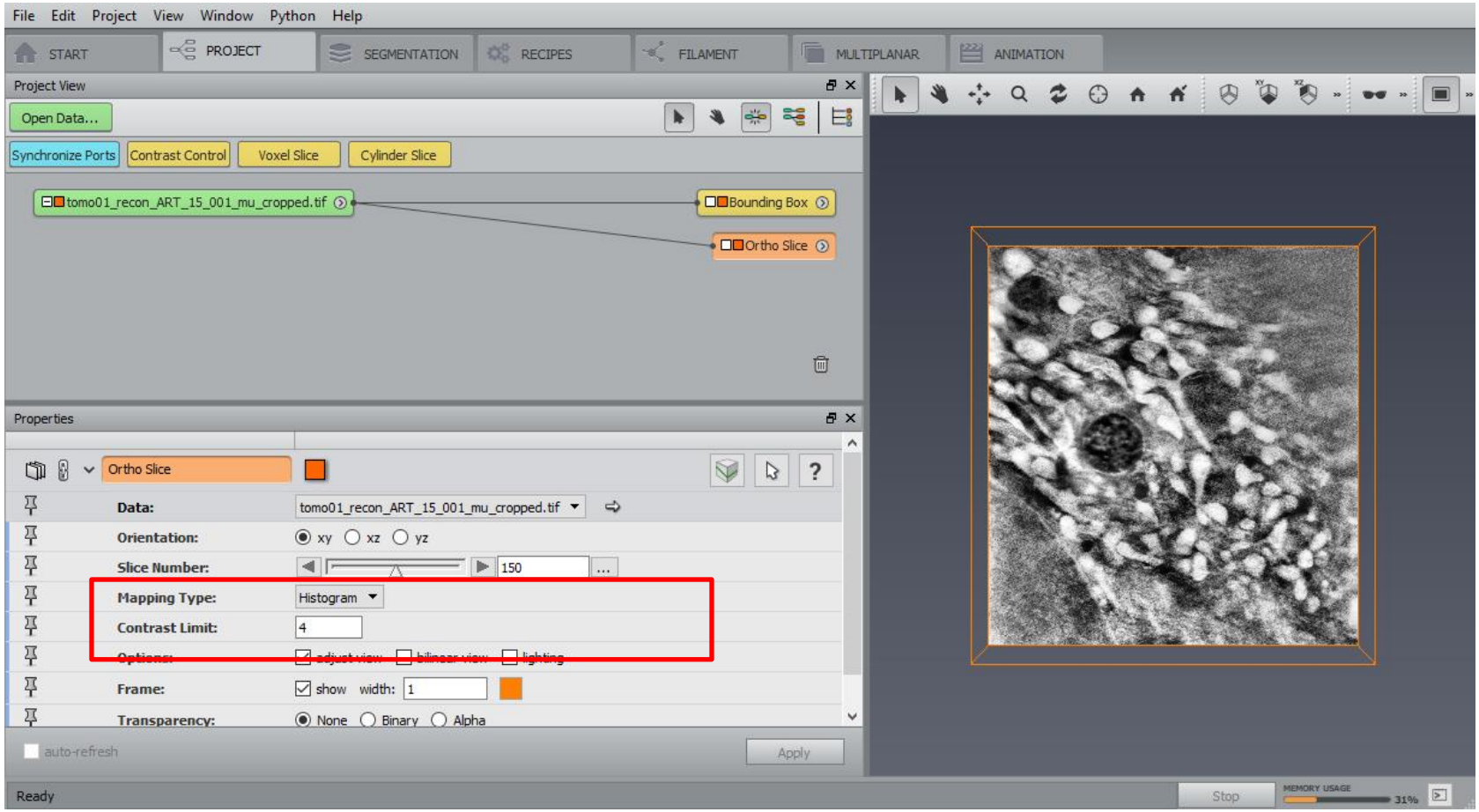
Orthoslice Main attributes: Orientation-Slice Number and Mapping Type-Colormap.

Visualize volume 5



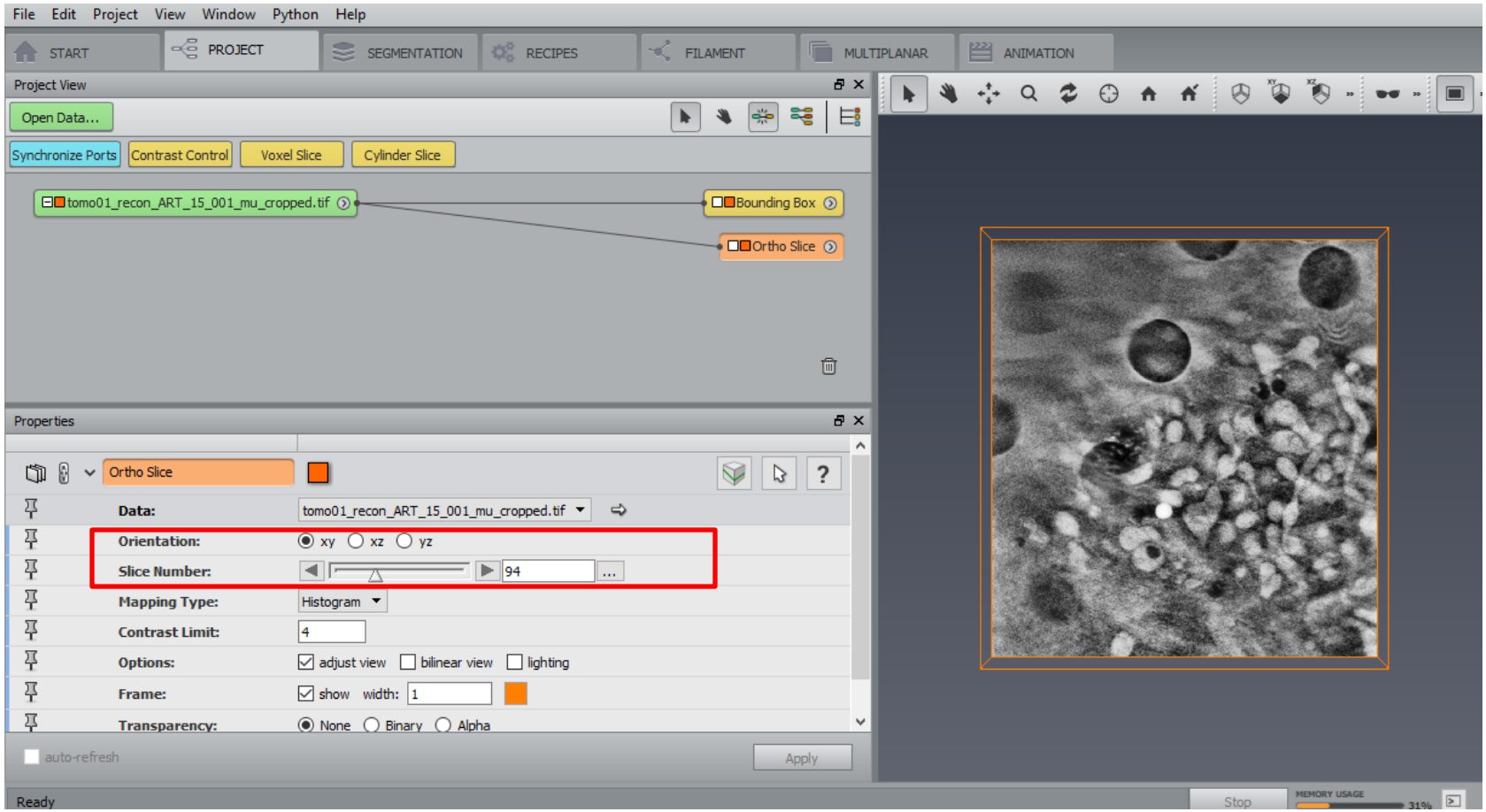
Mapping Type-Colormap: changing the contrast manually selecting different areas of the contrast histogram.

Visualize volume 6



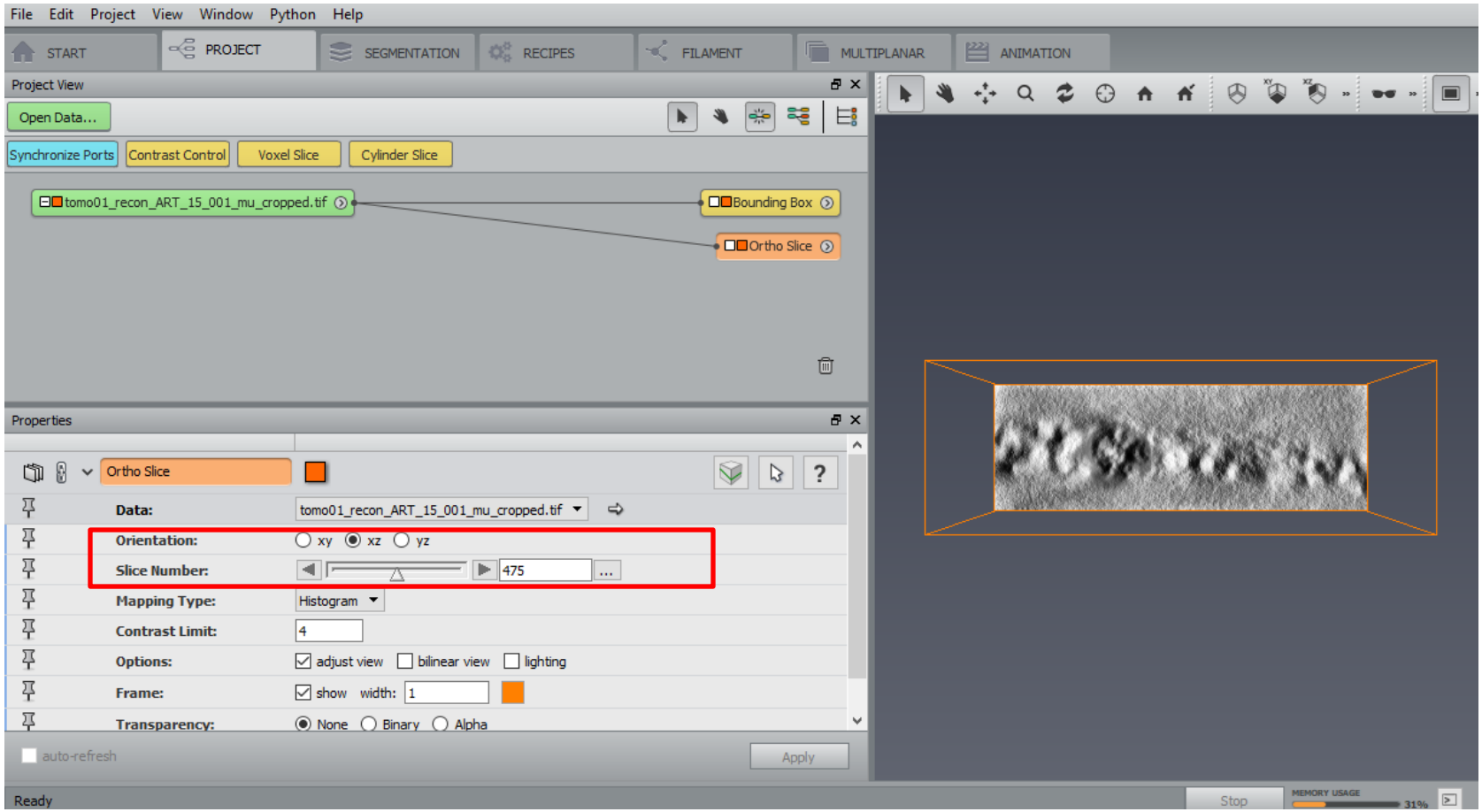
Mapping Type-Histogram: changing the contrast with different automatic selection of the contrast histogram.

Visualize volume 7



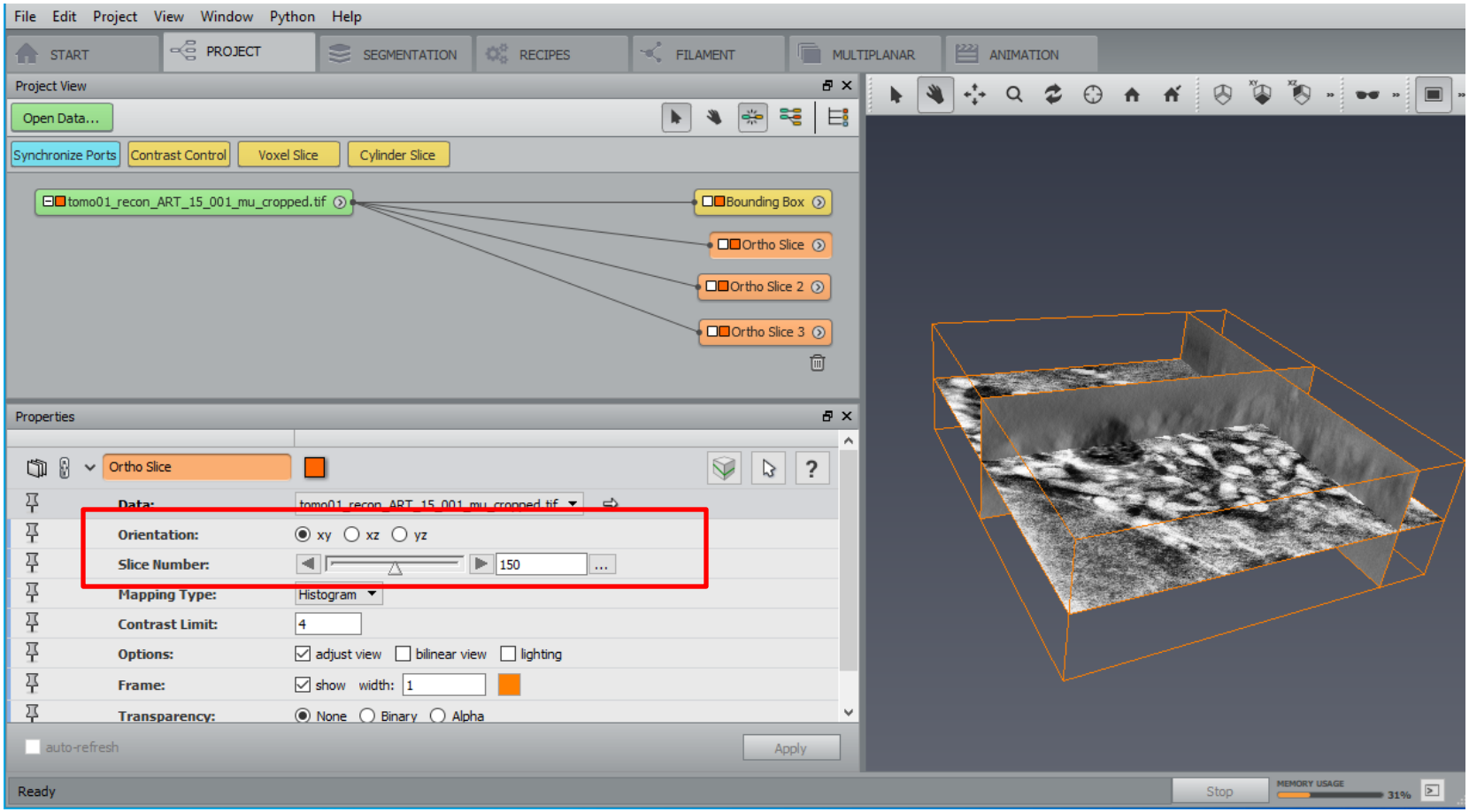
Orientation-Slice Number : selecting slice along a perpendicular direction (respect to the data)

Visualize volume 8



Orientation-Slice Number : selecting slice along a *another* perpendicular direction (respect to the data)

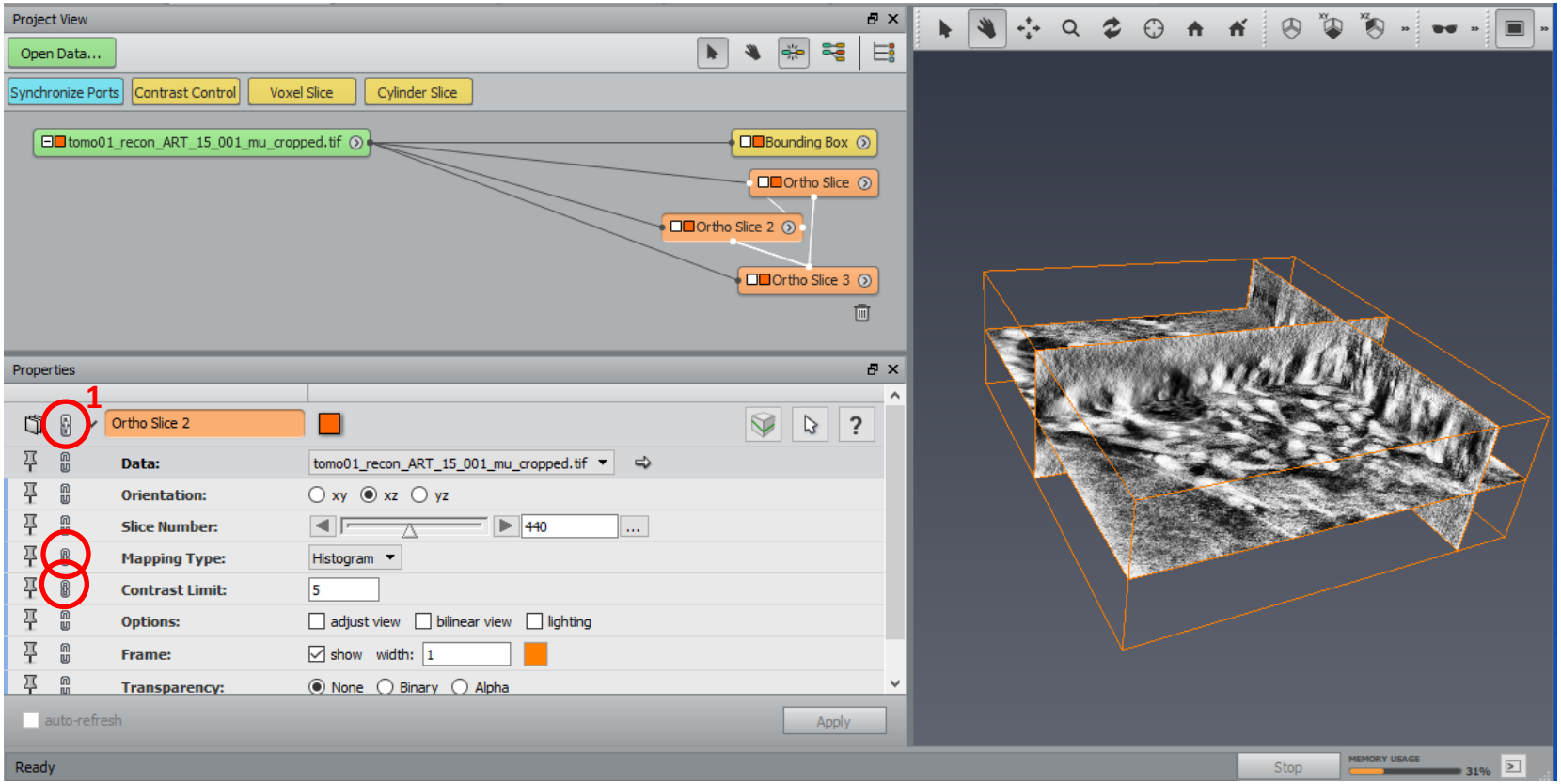
Visualize volume 9



Orientation-Slice Number : with 3 orthoslices modules, the 3 projections can be visualized altogether selecting for each orthoslice a different orientation

Visualize volume 10

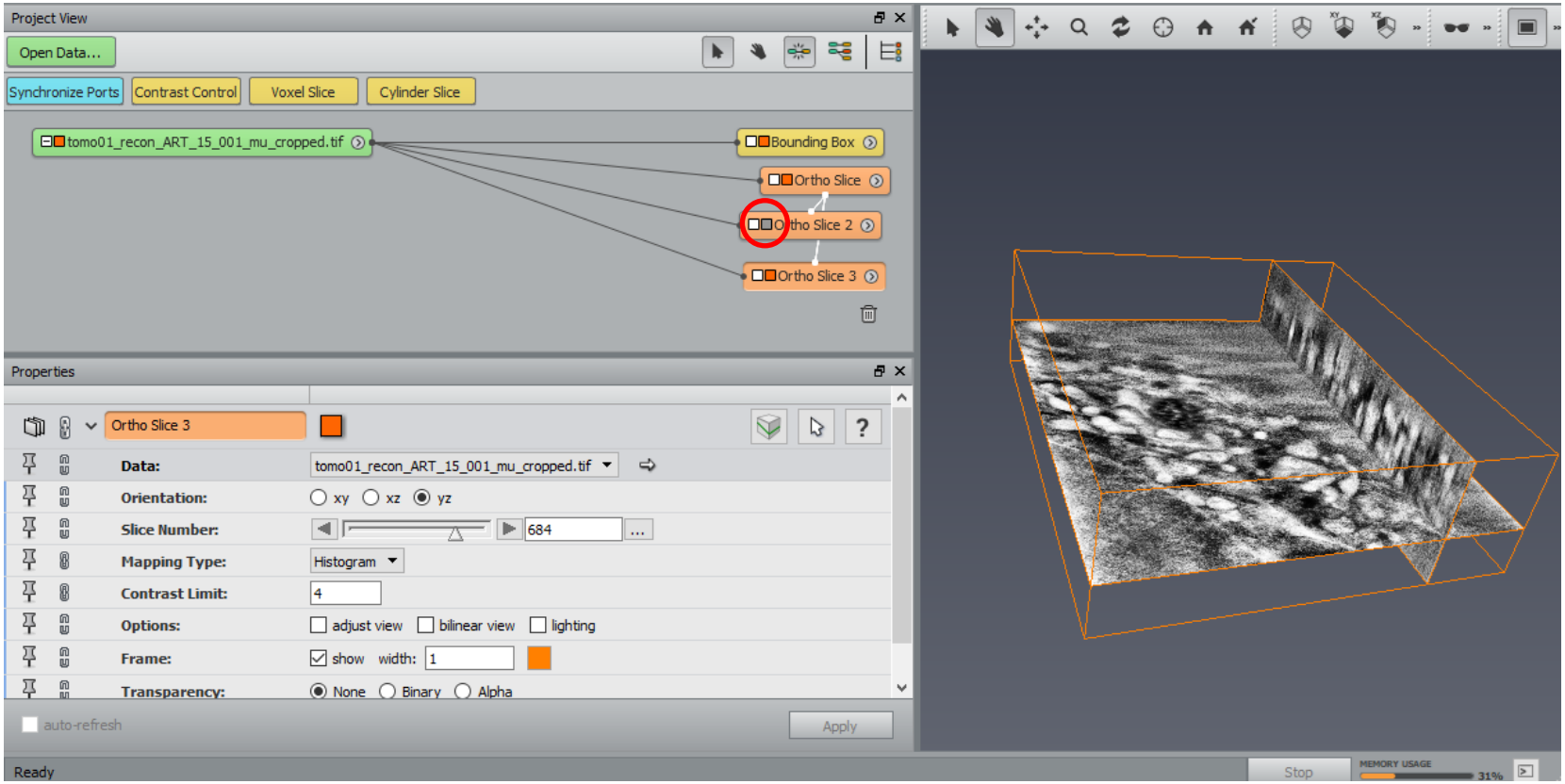
Synchronizing attributes.



Synchronizing the contrast on the 3 projections clicking on the icon ‘1’ and then drag the corresponding attribute on the other modules.
You can synchronize in this way any attribute.

Visualize volume 11

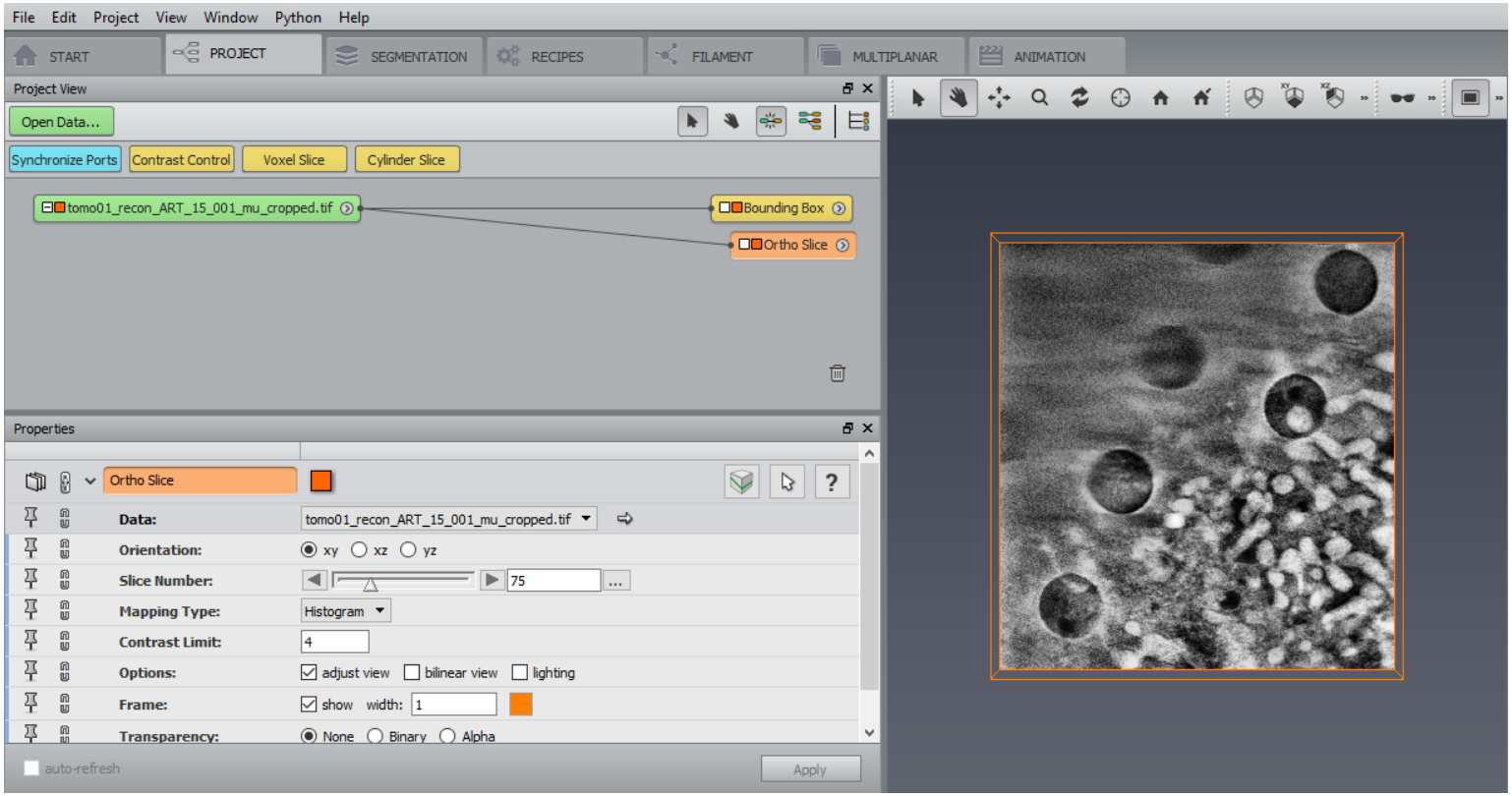
Hide the visualization of any modules



Hide the visualization of any modules (orange = visualization module) by de-selecting the orange box.

Visualize volume 11

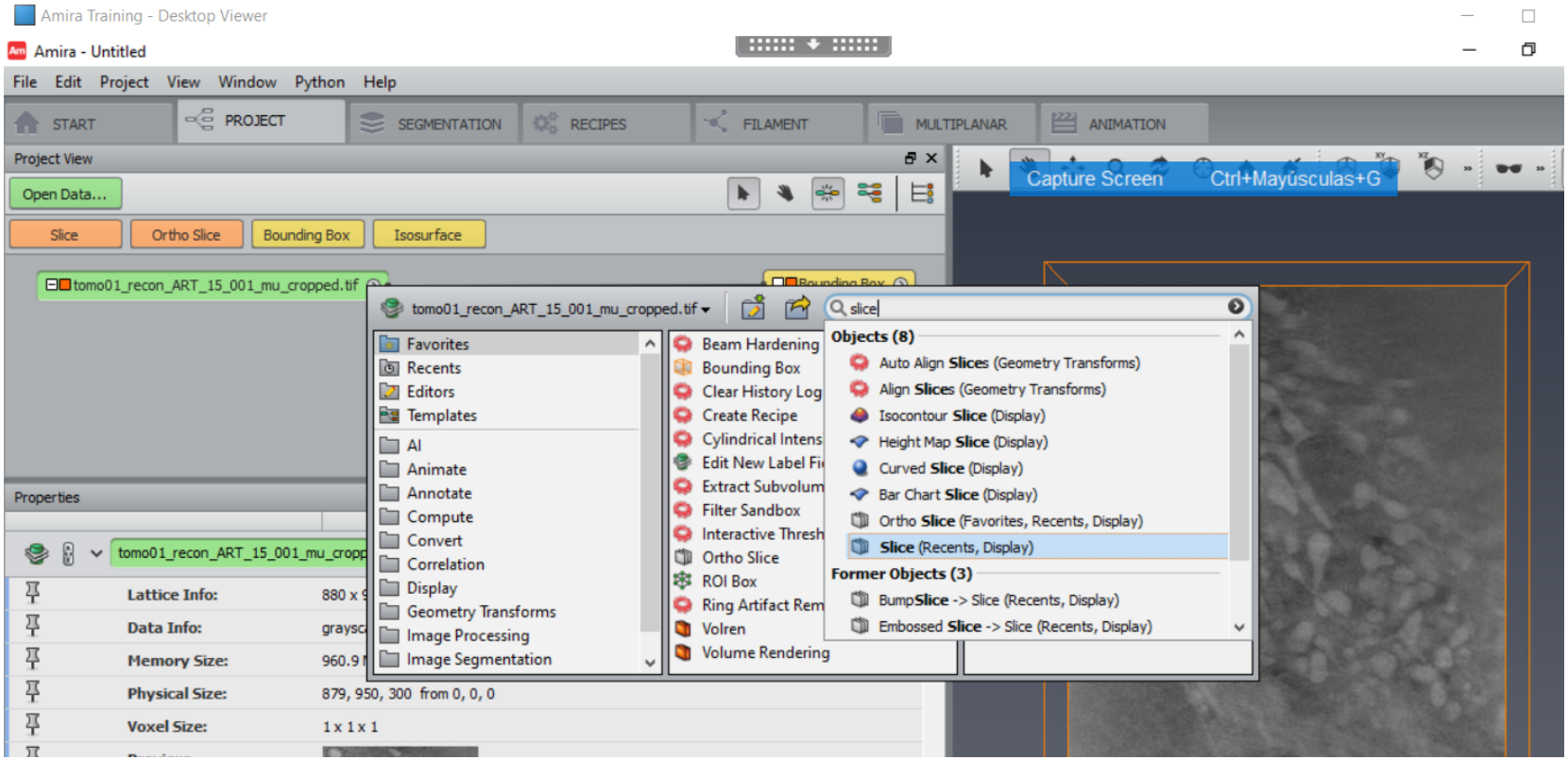
Change the visualization orientation using the ‘slice’ and ‘Resample Transformed Image’ module.



Using orhtoslice I’m entering the volume in a direction which is clearly not perpendicular to the quantifoil.

Visualize volume 12

Change the visualization orientation using the ‘slice’ and ‘Resample Transformed Image’ module.

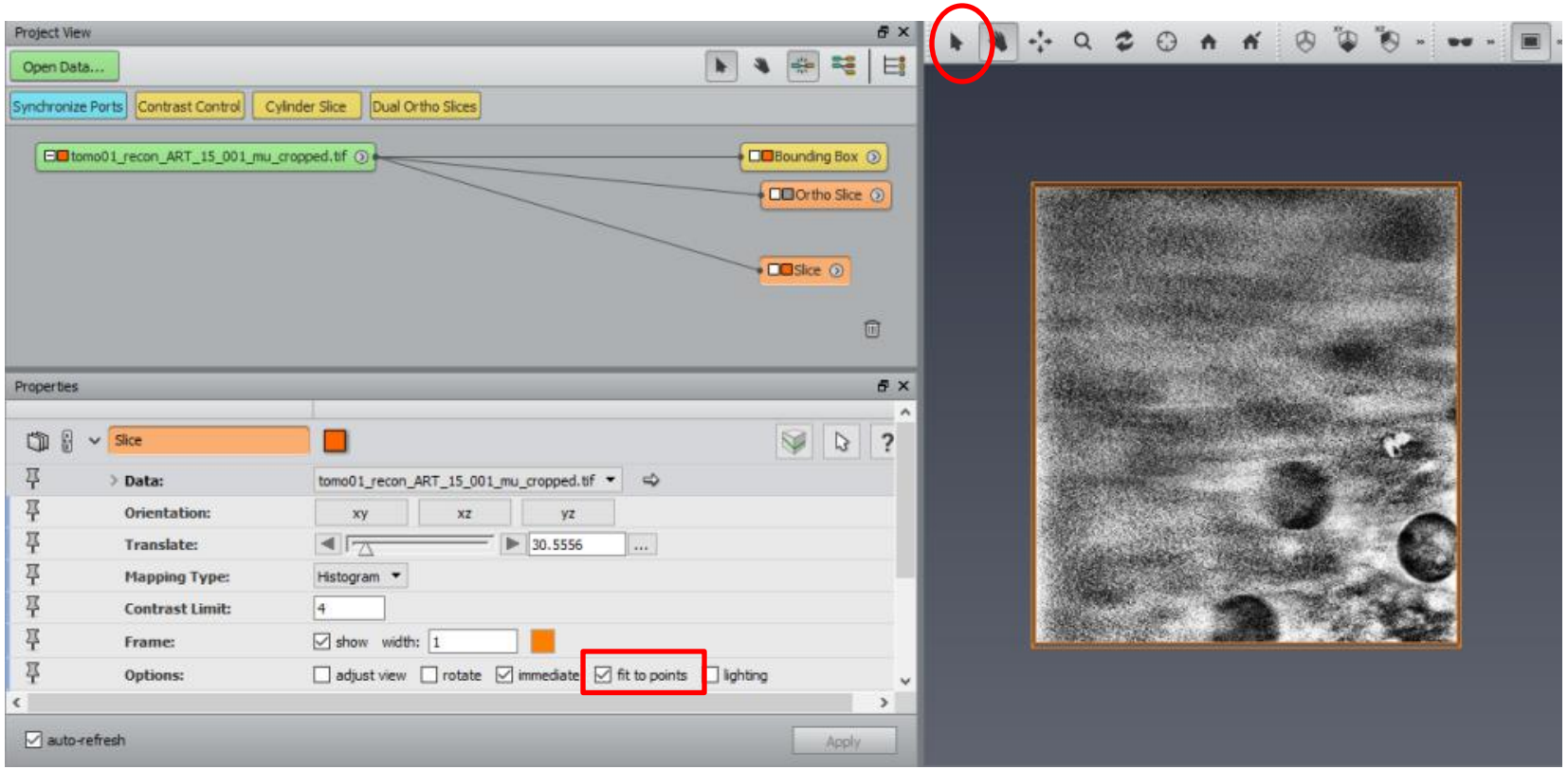


Create the ‘slice’ module: right click on the data.

You can find any module that doesn’t appear in the list using the searching option.

Visualize volume 13

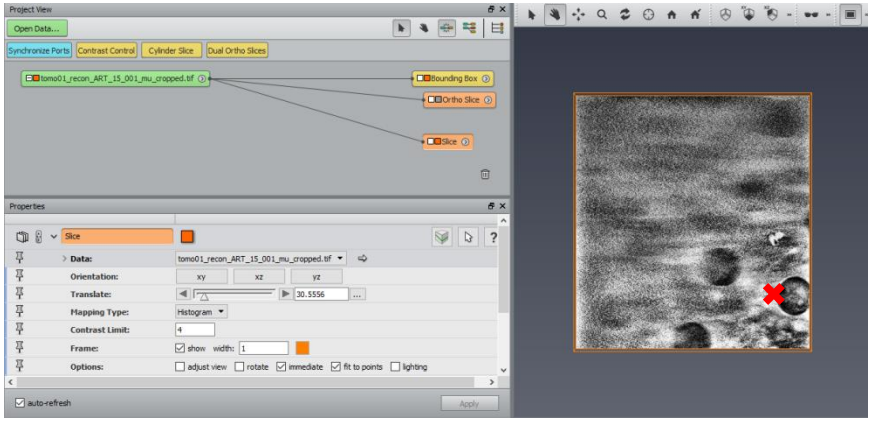
Change the visualization orientation using the ‘slice’ and ‘Resample Transformed Image’ module.

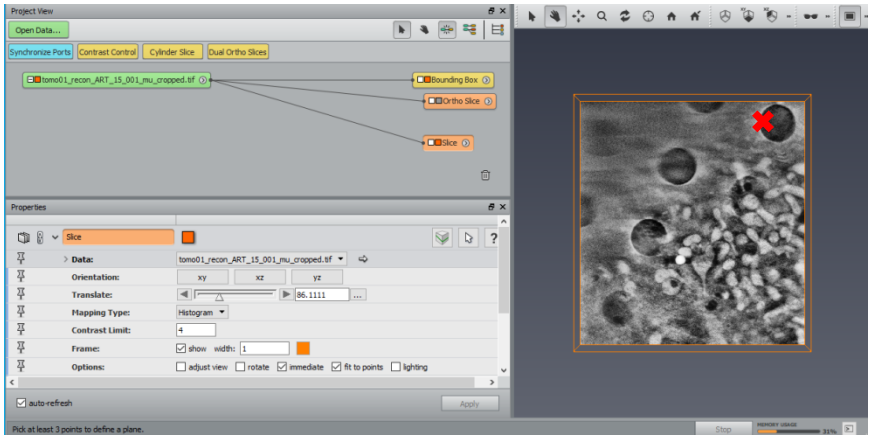


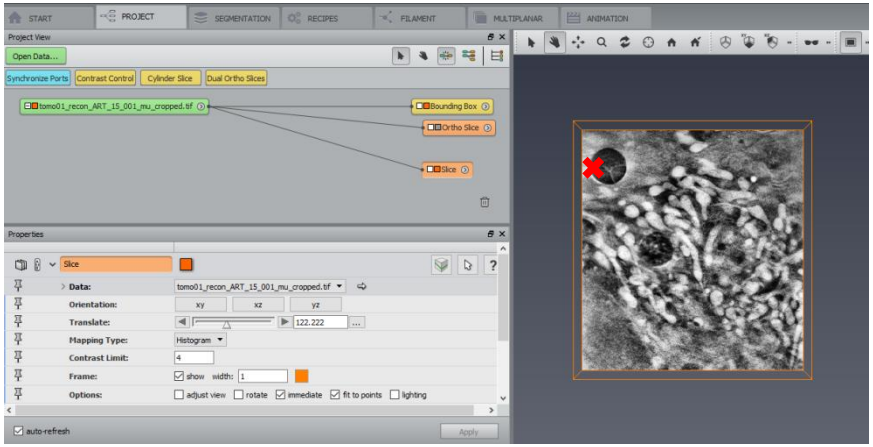
In slice, with the option ‘fit to points’, click on 3 point you want on the same plane. In this case the border of different holes of the quantifoil.

Visualize volume 14

Change the visualization orientation using the ‘slice’ and ‘Resample Transformed Image’ module.



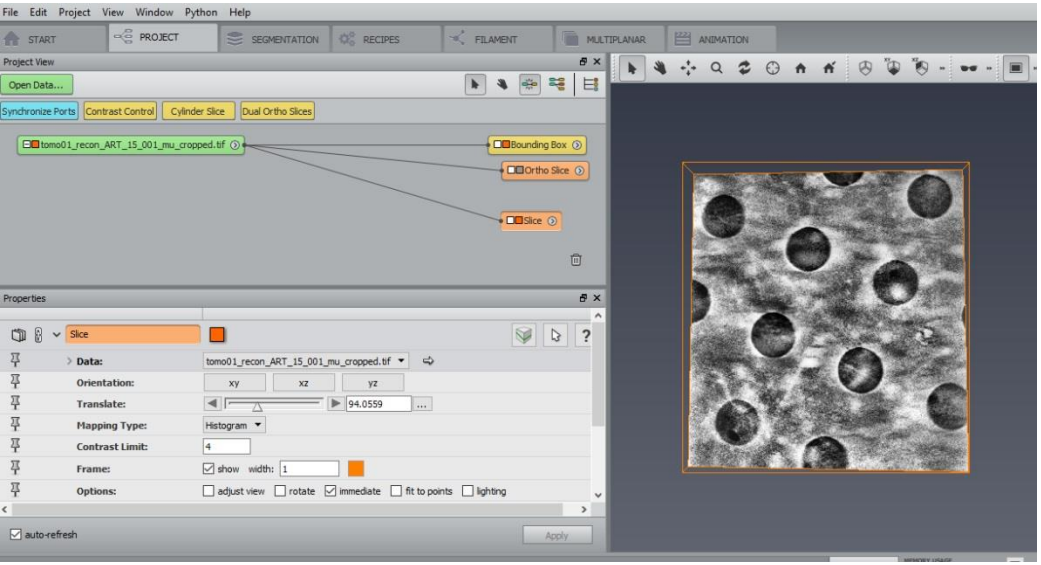


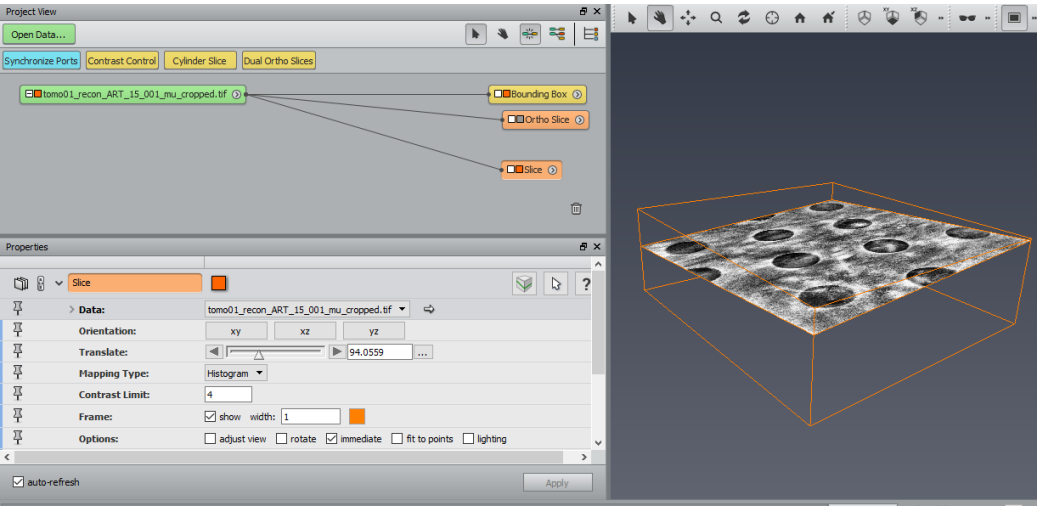


In slice, with the option ‘fit to points’, click on 3 point you want on the same plane. In this case the border of different holes of the quantifoil.

Visualize volume 15

Change the visualization orientation using the ‘slice’ and ‘Resample Transformed Image’ module.

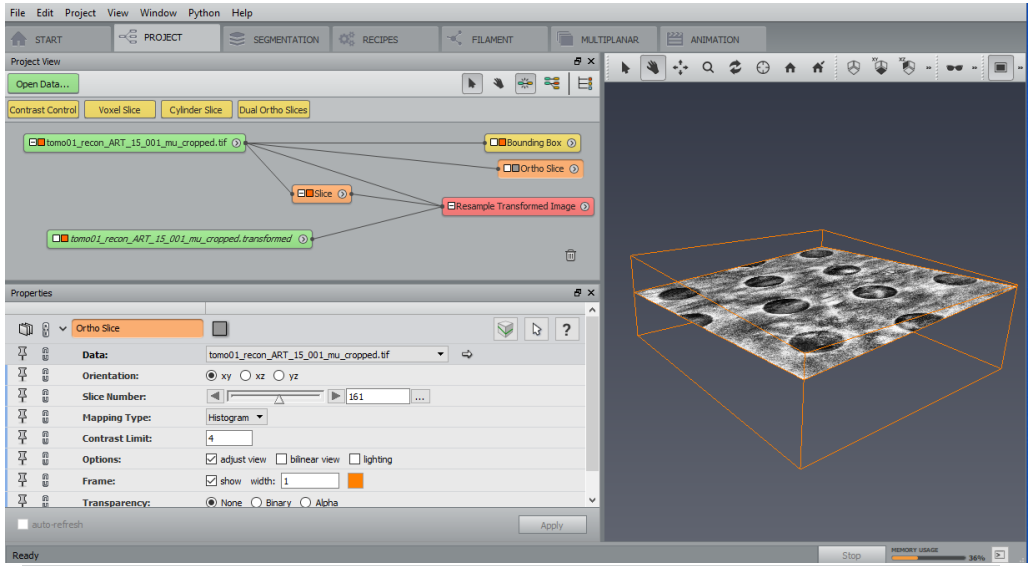


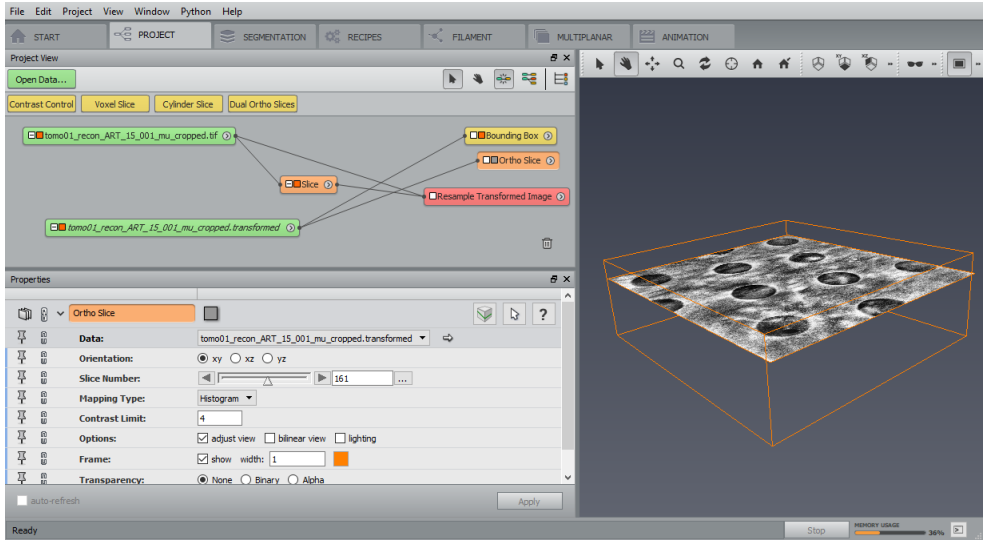


Now you enter through the volume in a direction which is perpendicular to the quantifoil …

Visualize volume 16

Change the visualization orientation using the ‘slice’ and ‘Resample Transformed Image’ module.

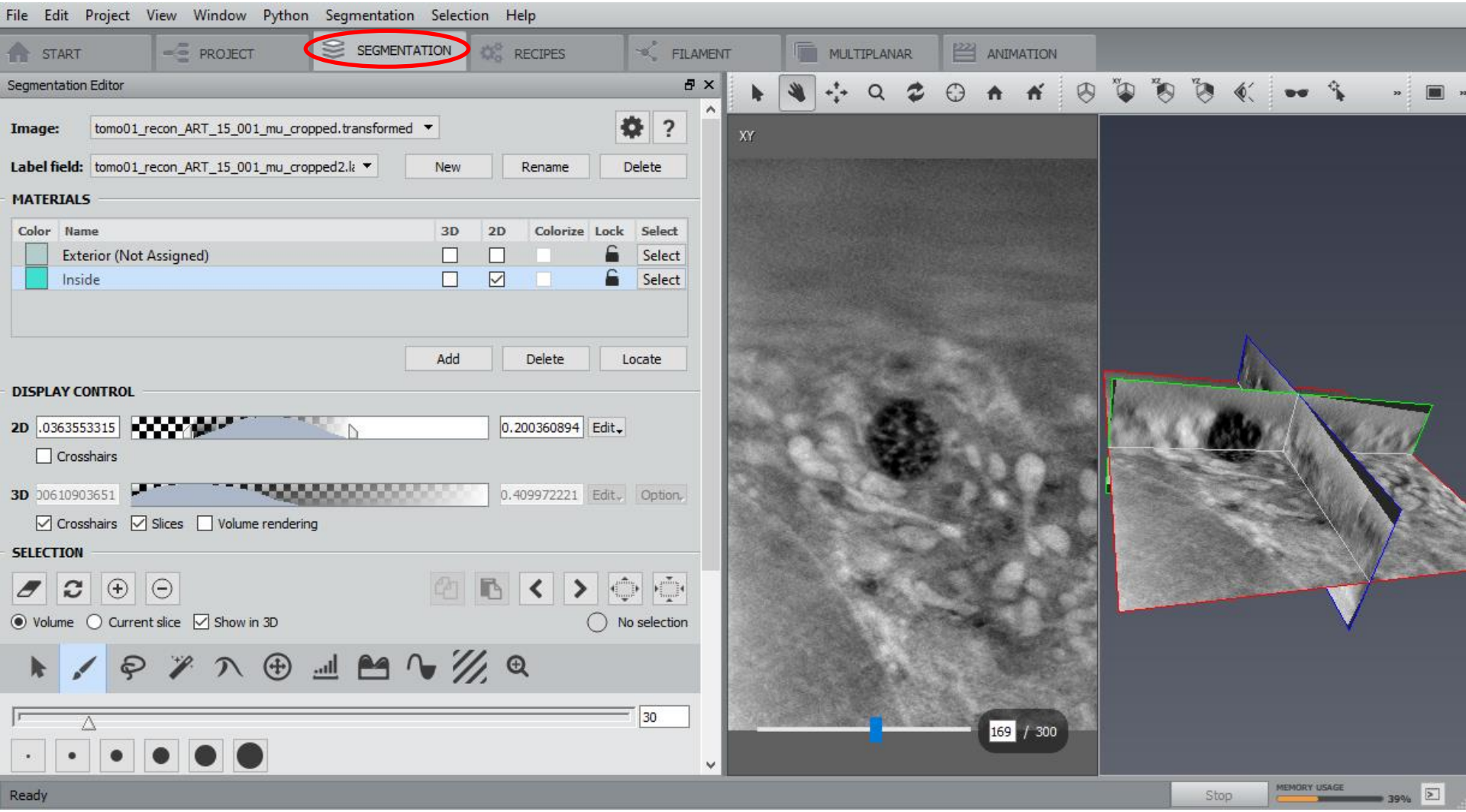




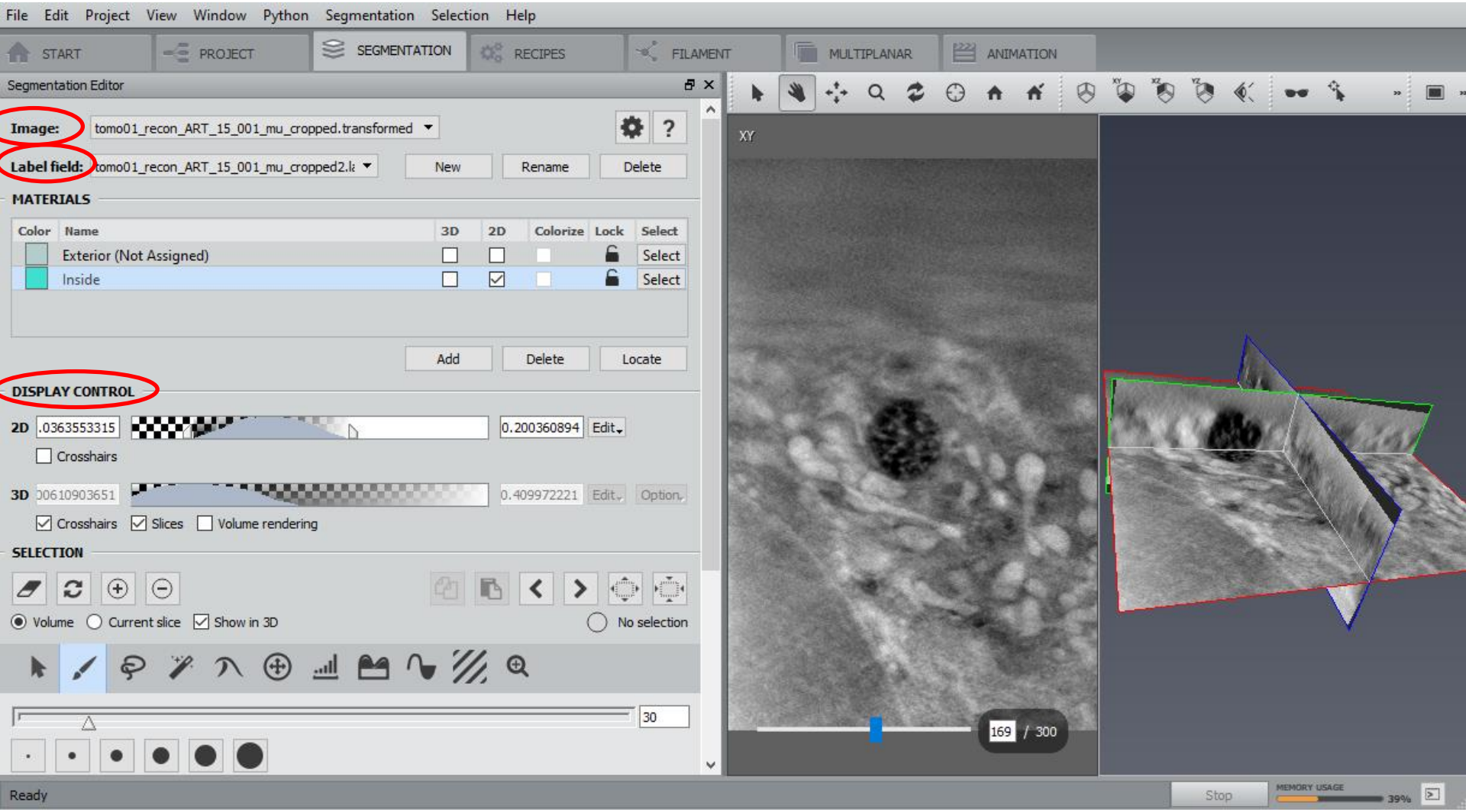
To apply the transformation to the data in such a way to use the newly oriented data
in the SEGMENTATION EDITOR, create the computational module ¨Resample Transformed Image¨ (right click on data and search for it). Then left click on the white box and connect the ¨data¨ with the your data and the ¨reference¨ with the slice module. Finally press apply to obtain the data transformed.
If you now visualize the transformed data using the orhtoslice module you still have the ¨right orientation¨ defined with the slice one. We have performed the equivalent of the re-slice transformation in ImageJ (but in a more efficient way!)

Computational modules are in general in

Segmentation Editor 1



Segmentation Editor 2

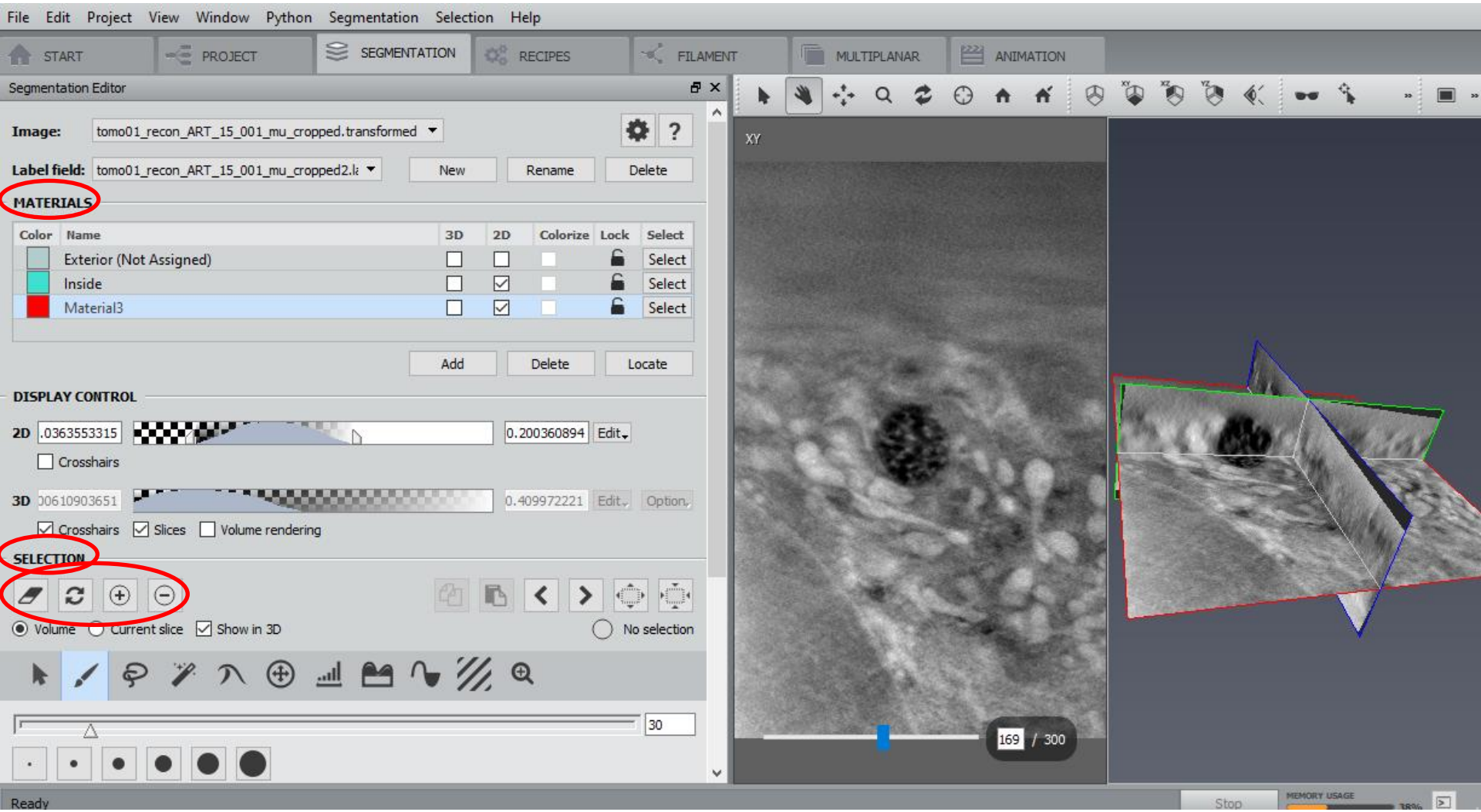


In *image* select the data you want to segment (in this case for instance the transformed one)

In *label field* you have the name of the segmentation you are going to perform. Select ¨ new ¨ if you want to start a new one.

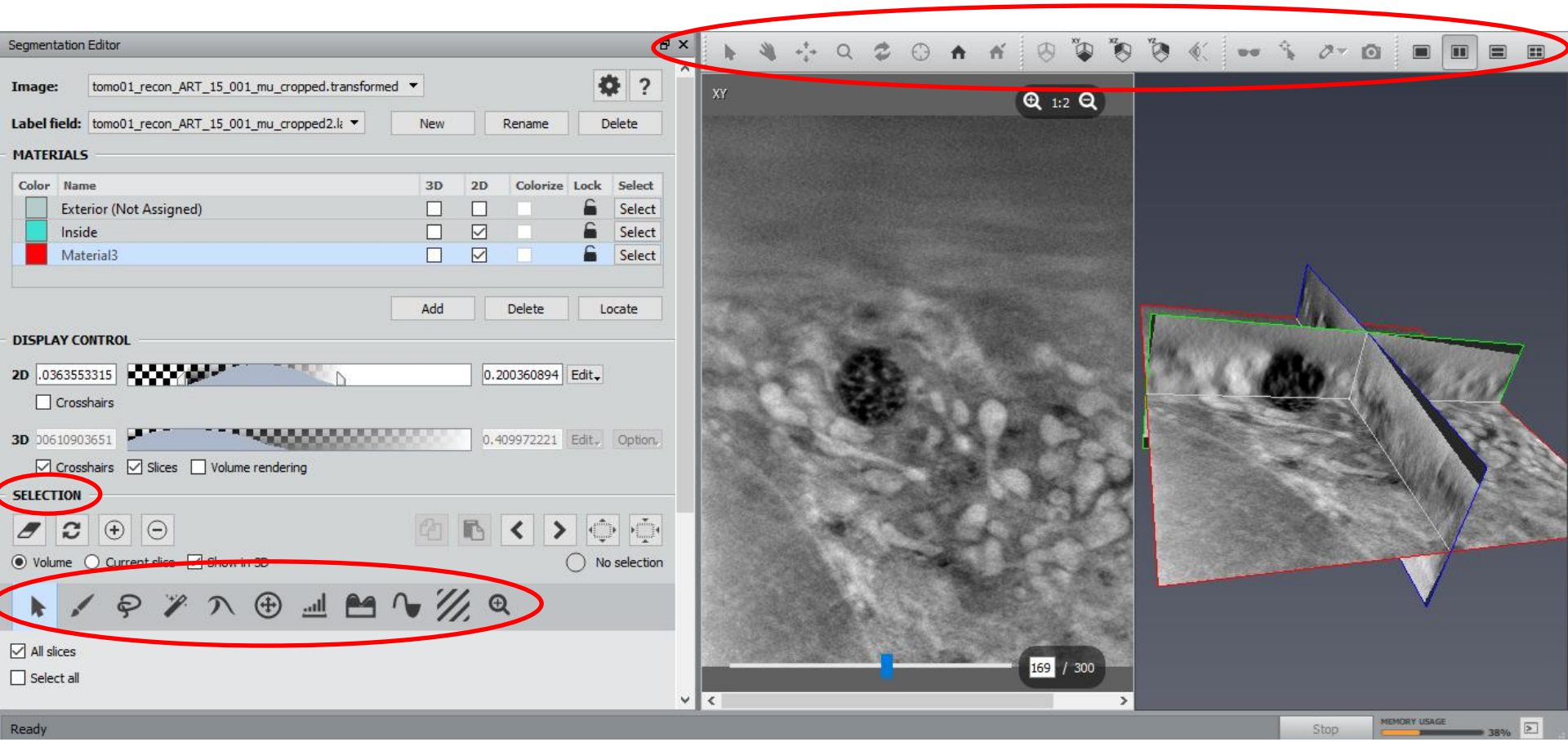
In *display control* re-adjust the histogram selection to optimize the contrast.

Segmentation Editor 3



In *Materials* you have to press ¨add¨ to create a new material (Material3 in this case).
In *selection* you can add (+) or remove (-) the selection to/from the selected material.
The option ¨Volume¨ or ¨Current slice¨ allows you perform this operation considering all the selected pixels in the volume or in just 1 slice (the one you are visualizing on the right).
Use the rubber to remove pixels form the selection (again use ¨Volume¨ or ¨Current slice¨ options).

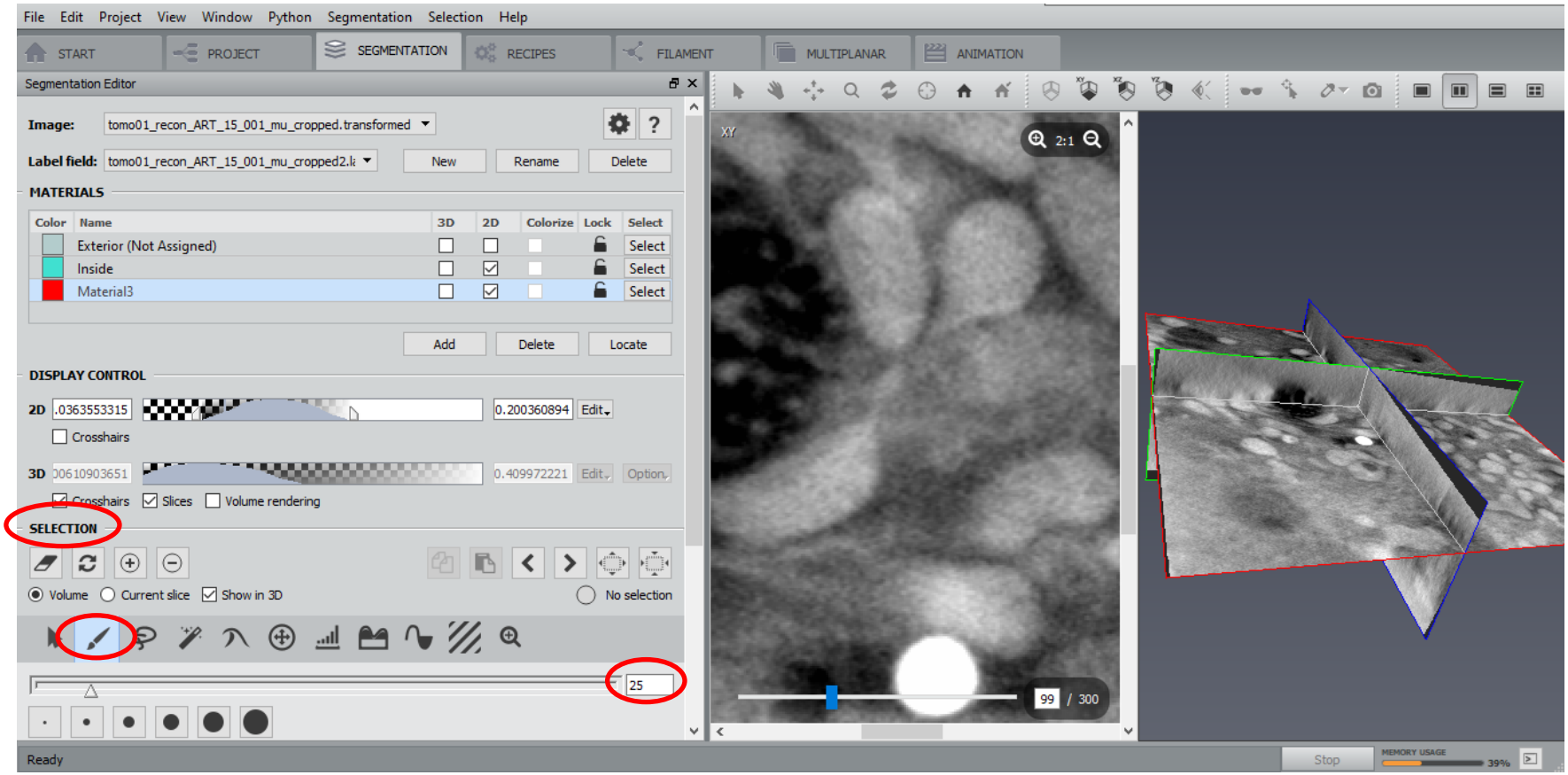
Segmentation Editor 4



Use the tools in *Selection* to create it, i.e. to select the pixels you want to assign to some material.
Use the tools in the right upper side to modify both the 2D and the 3D visualization of the DATA.

We will never use the *Exterior* and the *Inside* materials: don`t add or remove pixels from them, i.e. don’t select them!

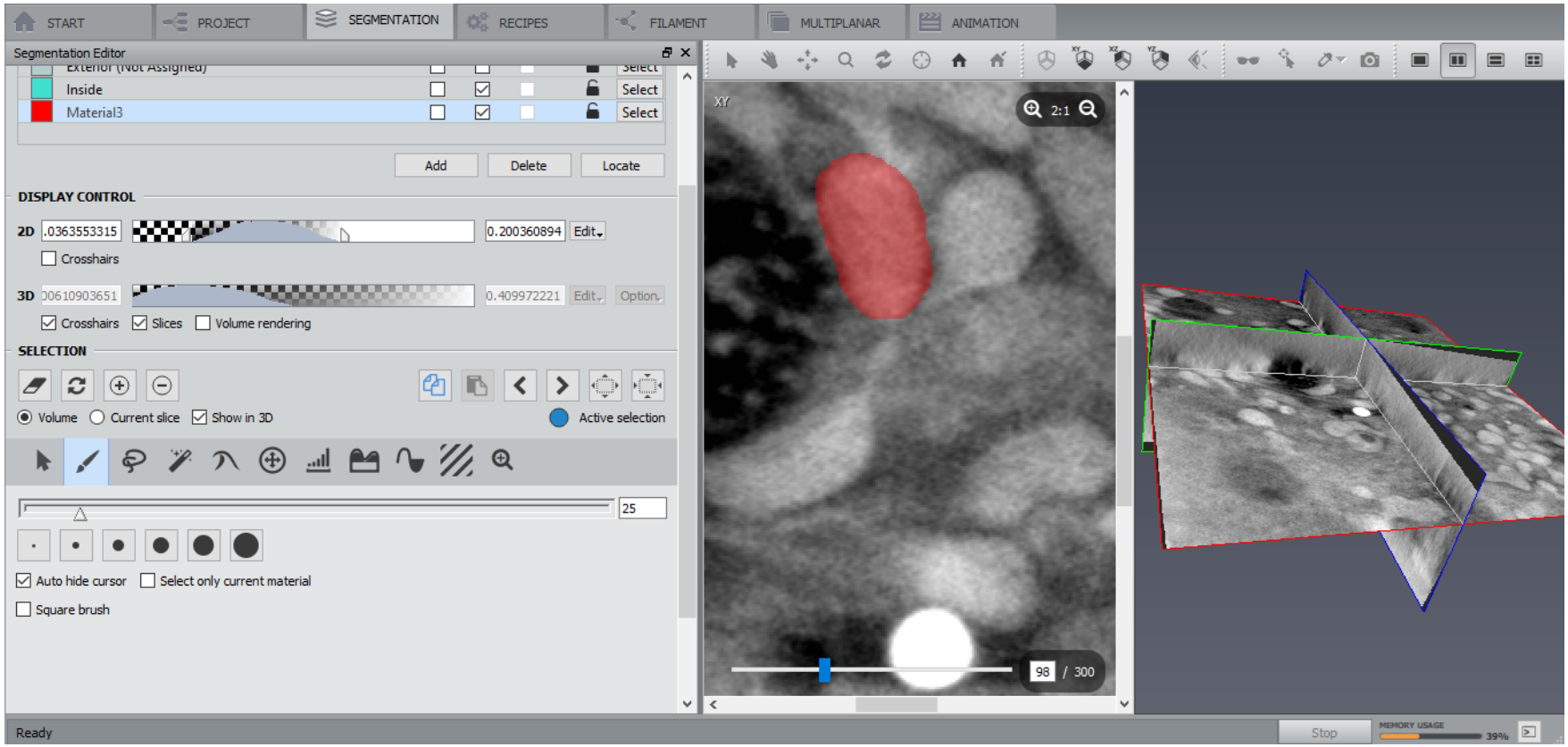
Semi-manual segmentation of a mitochondrion1



CREATE the rough Selection:

1) Zoom on the mitochondrion.
2) Select the Brush tool and its dimension

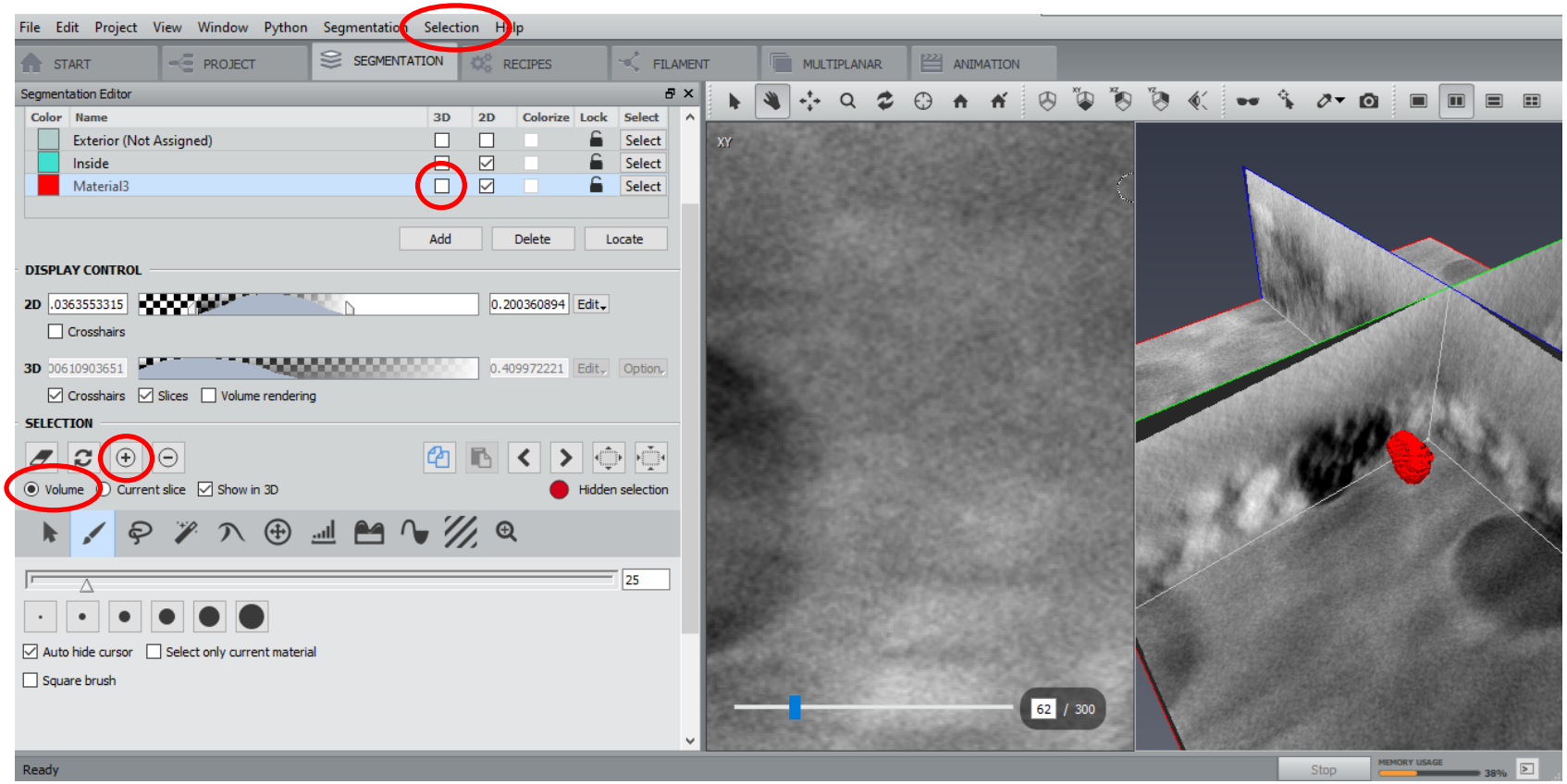
Semi-manual selection of a mitochondrion2



CREATE the rough Selection:

3) Paint the pixels using the right button of the mouse. The selection can be fast and not precise, approximate it *por exceso.* You can remove very wrong pixels using the control key.
4) Do it every 5 slices.

Semi-manual selection of a mitochondrion3

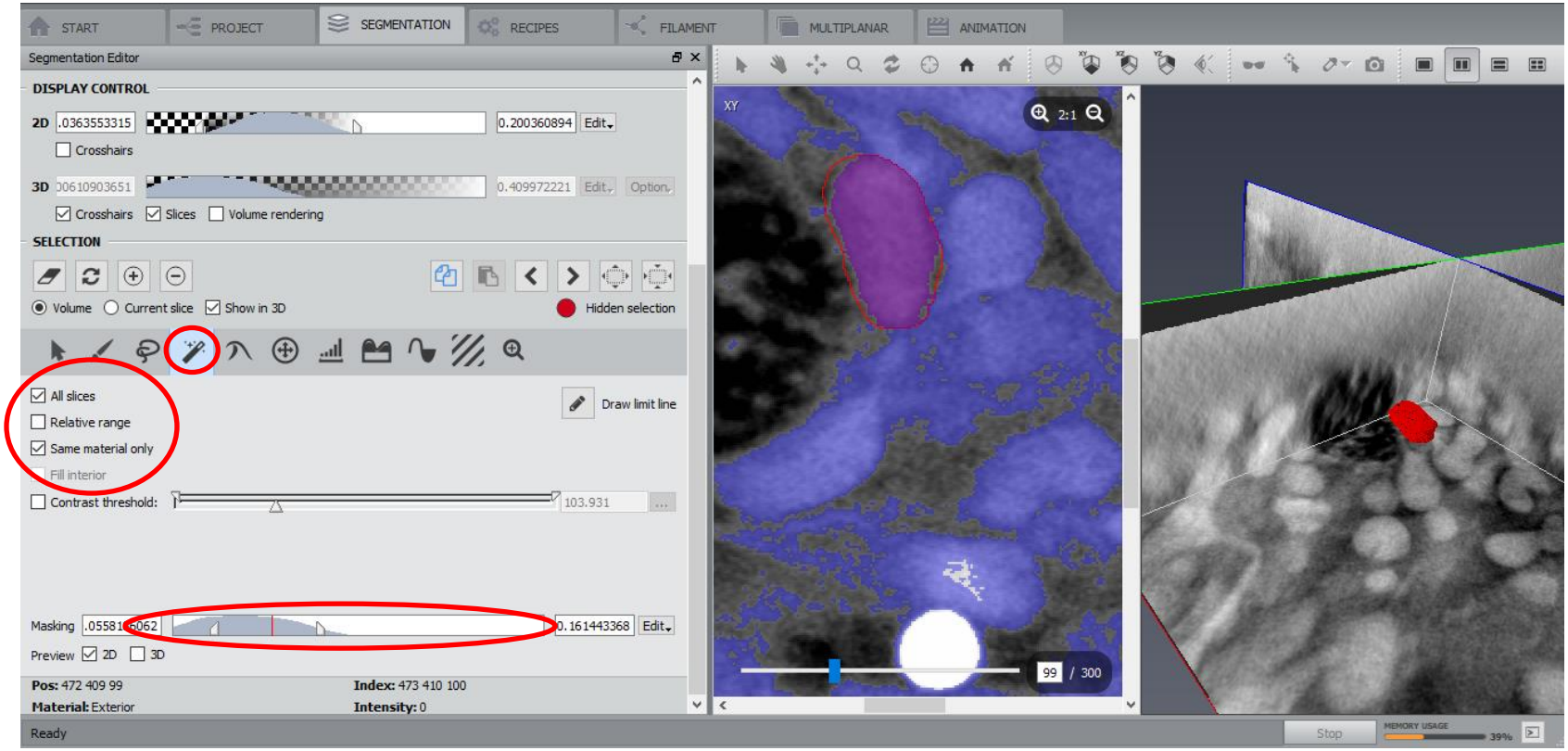


CREATE the rough Selection:

5) In *Selection* select *Interpolation*: it will extend the selection through all the slices
6) Take a look to it in the 3D viewer. Your selection are the pixel in the red.
7) Add them to the selected materials (Material3 in this case) using +. Be sure that the Volume option is selected.
8) The selected pixels will disappear. To see them again select the option 3D in the selected material .

OBS: the interpolation could miss some slices: ADD them manually! (Look for them in the 2D viewer).

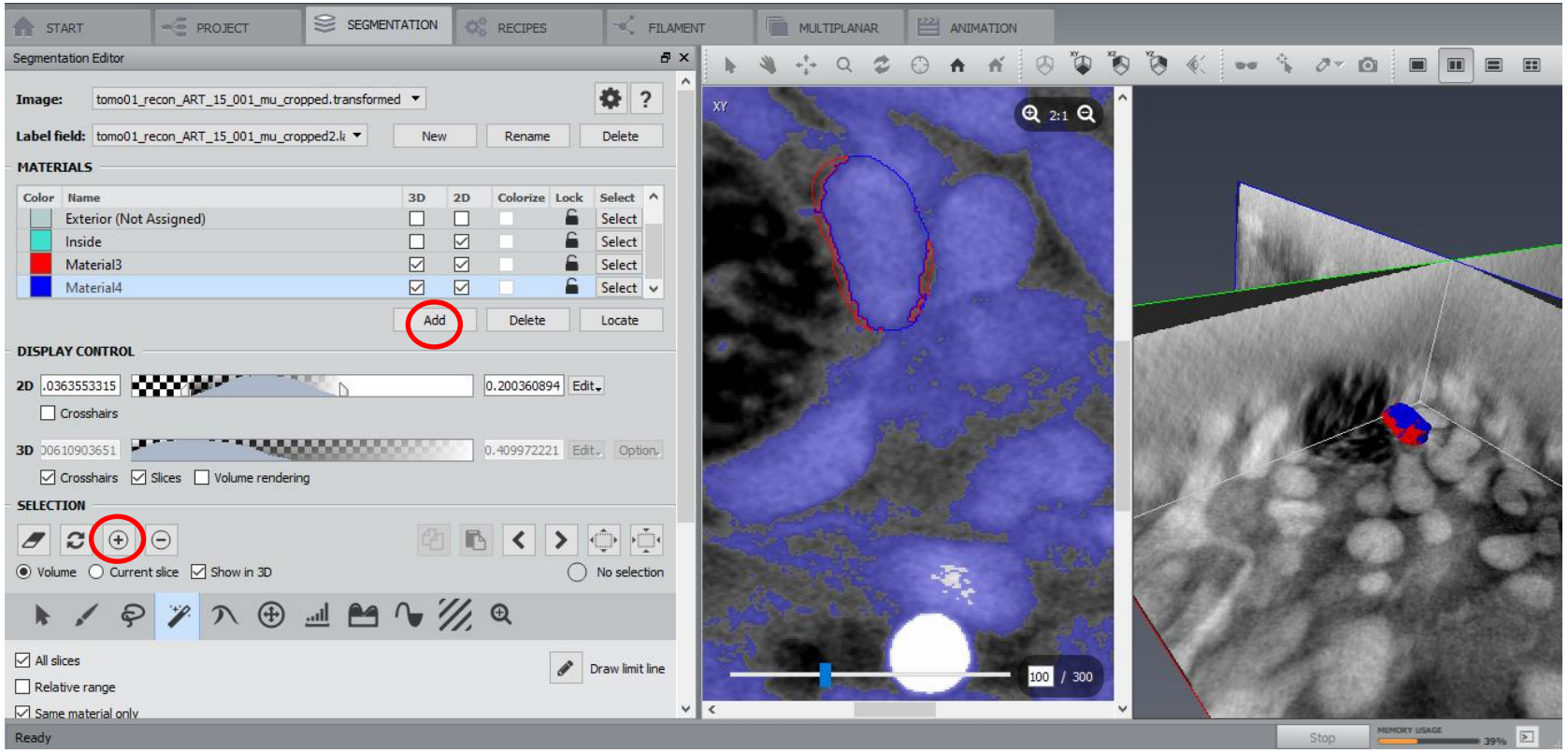
Semi-manual selection of a mitochondrion4



CREATE the *refined* Selection/Material:

1) Use the magic wand tool with the All Slices option and Same Material only.
2) Using the threshold of the histogram to the adjust the selection in violet. Optimize it, i.e. refine the shape of the mitochondrion.
3) Add them to the selected materials (Material3 in this case) using +

Semi-manual selection of a mitochondrion5



CREATE the refined Selection/Material:

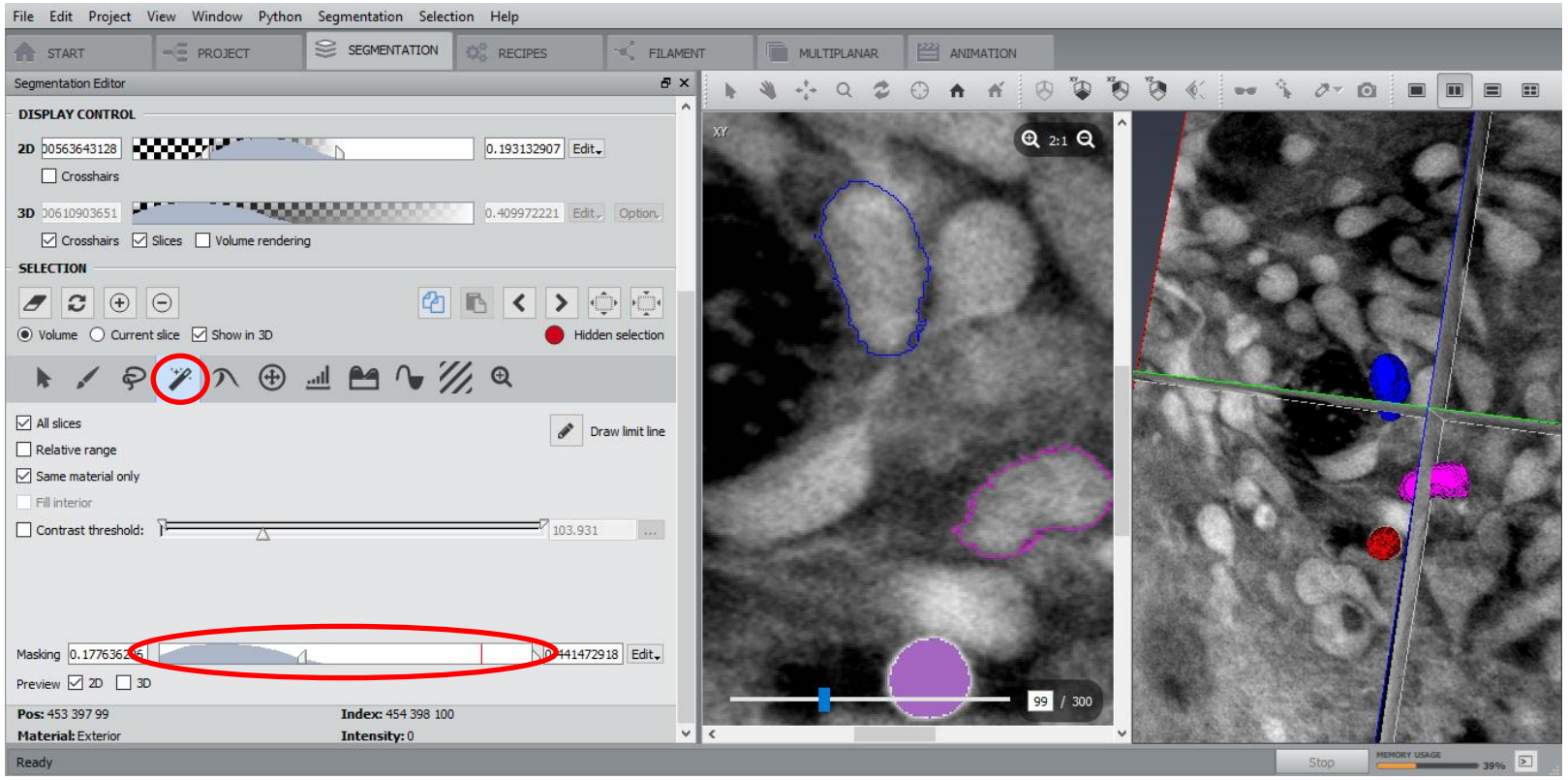
4) Create a new material (Material4) with *add* and add the new selection to it with +

5) Again selected pixels will disappear in the 3D viewer. To see them again select the option 3D in the selected material.

The refined selection (in blue) will appear, both with the rough one (in red) as far the 3D option is selected for material 3.

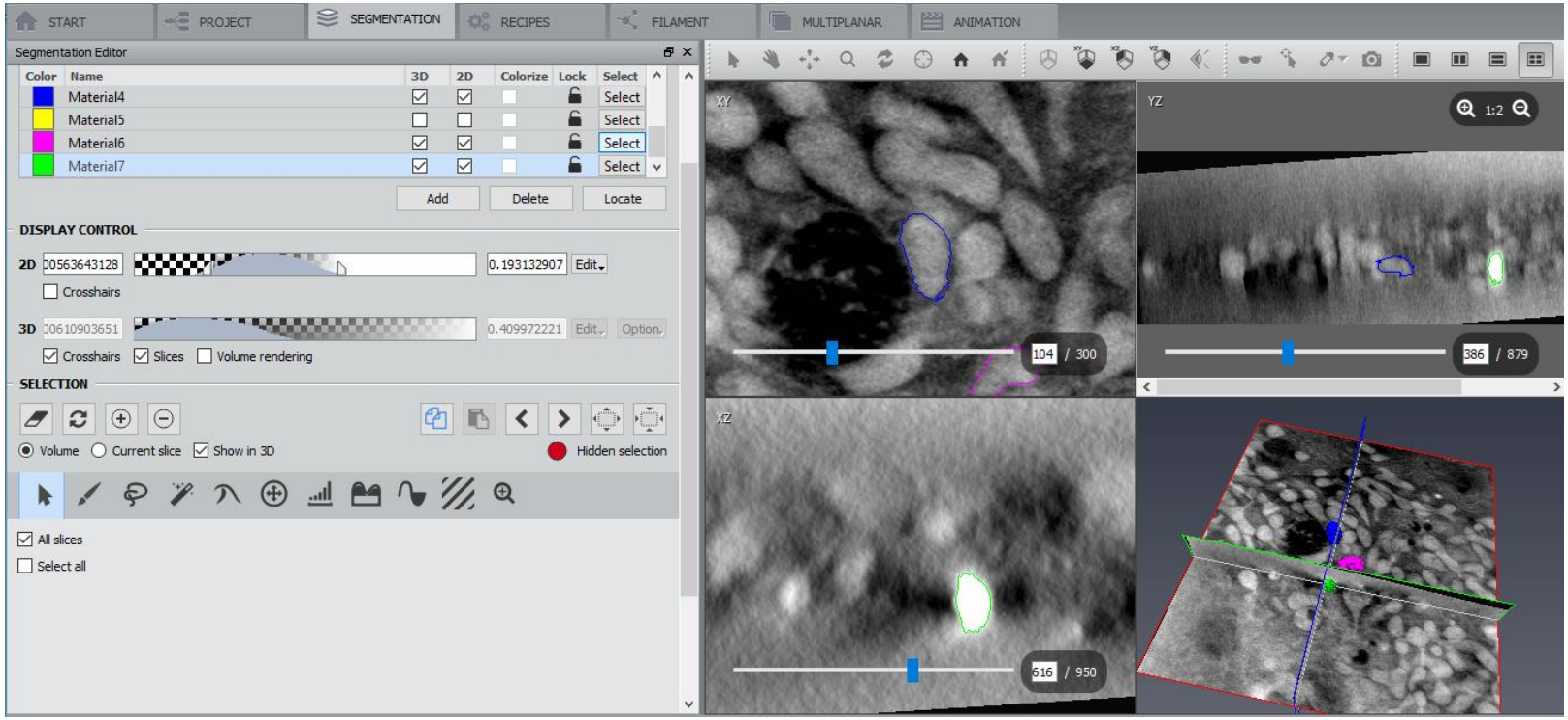
The blue volume should be smaller than the red one. Can you re-use the same thresholds with the magic wand on another mito? In general no, but sometimes yes. In principle this is the appropriate choice….

Automatic selection of a lipid droplet



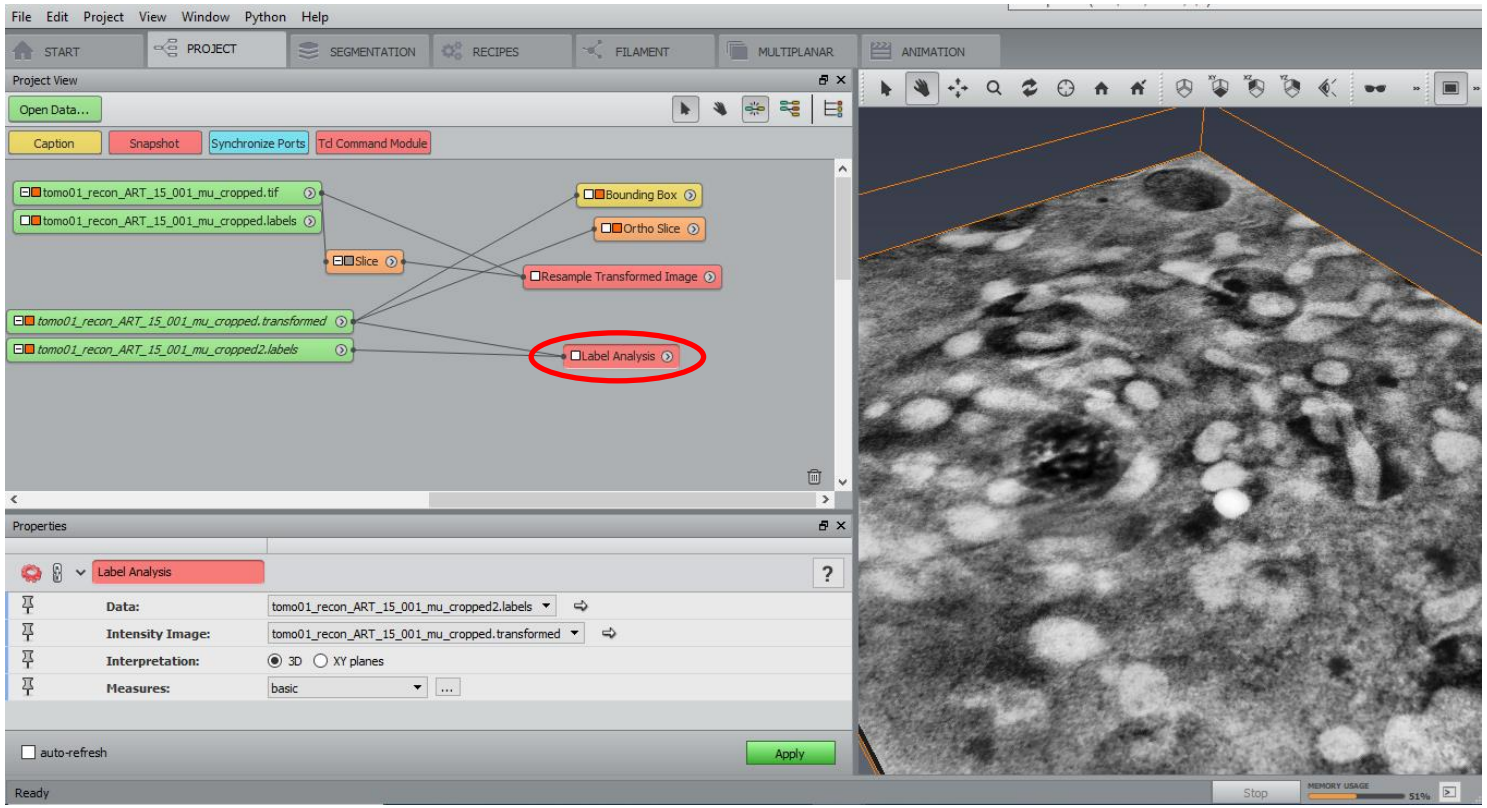
If the object is well in contrast respect to the background (different intensity, i.e. absorption coefficient) respect with the surroundings) you can directly select it well with the magic wand.
Just define the thresholds appropriately.
In the figure I have already created a second mitochondrion.

Ending the segmentation



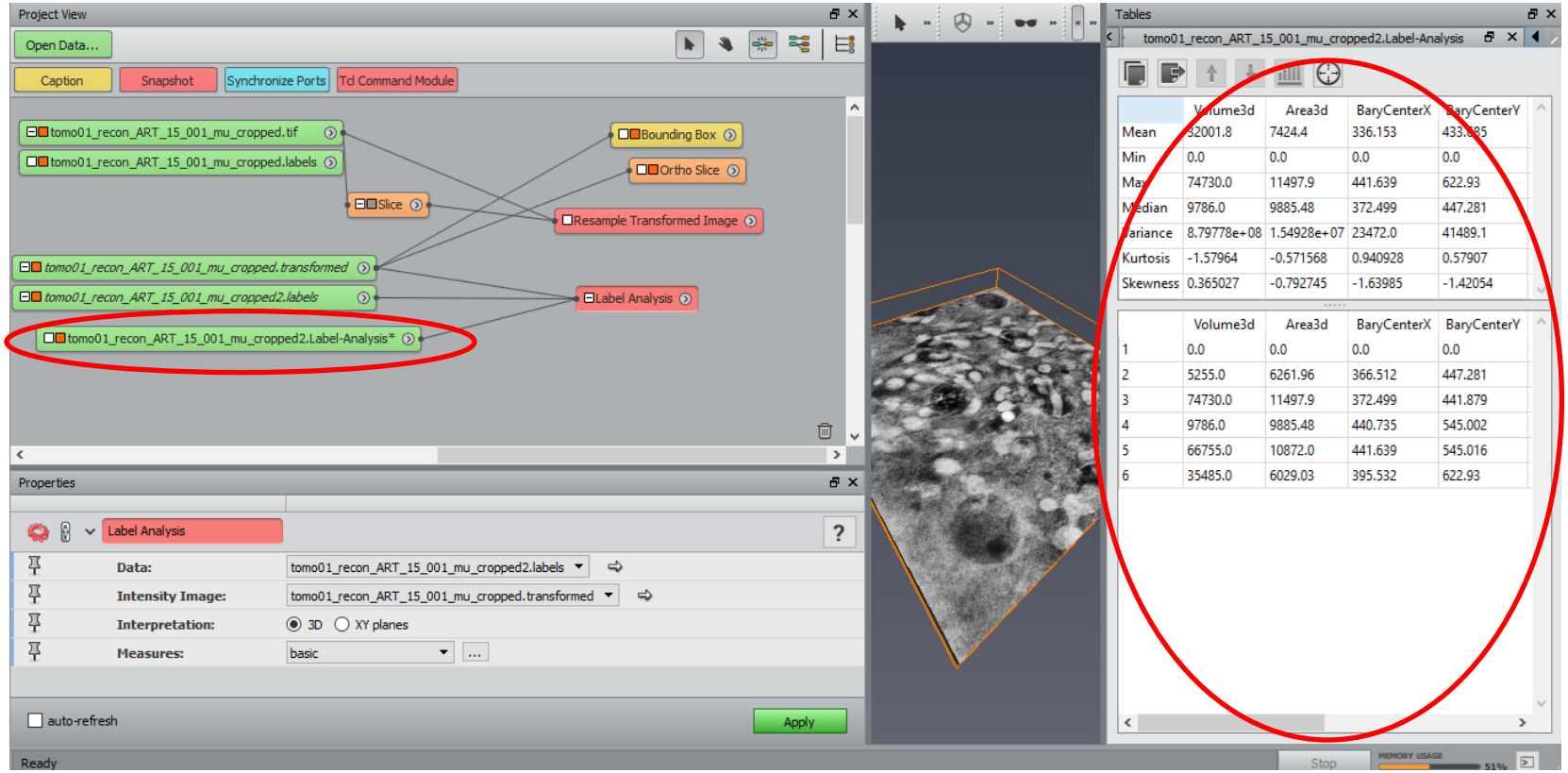
You should check the quality of your segmentation looking also to the other planes.

Label Analysis1



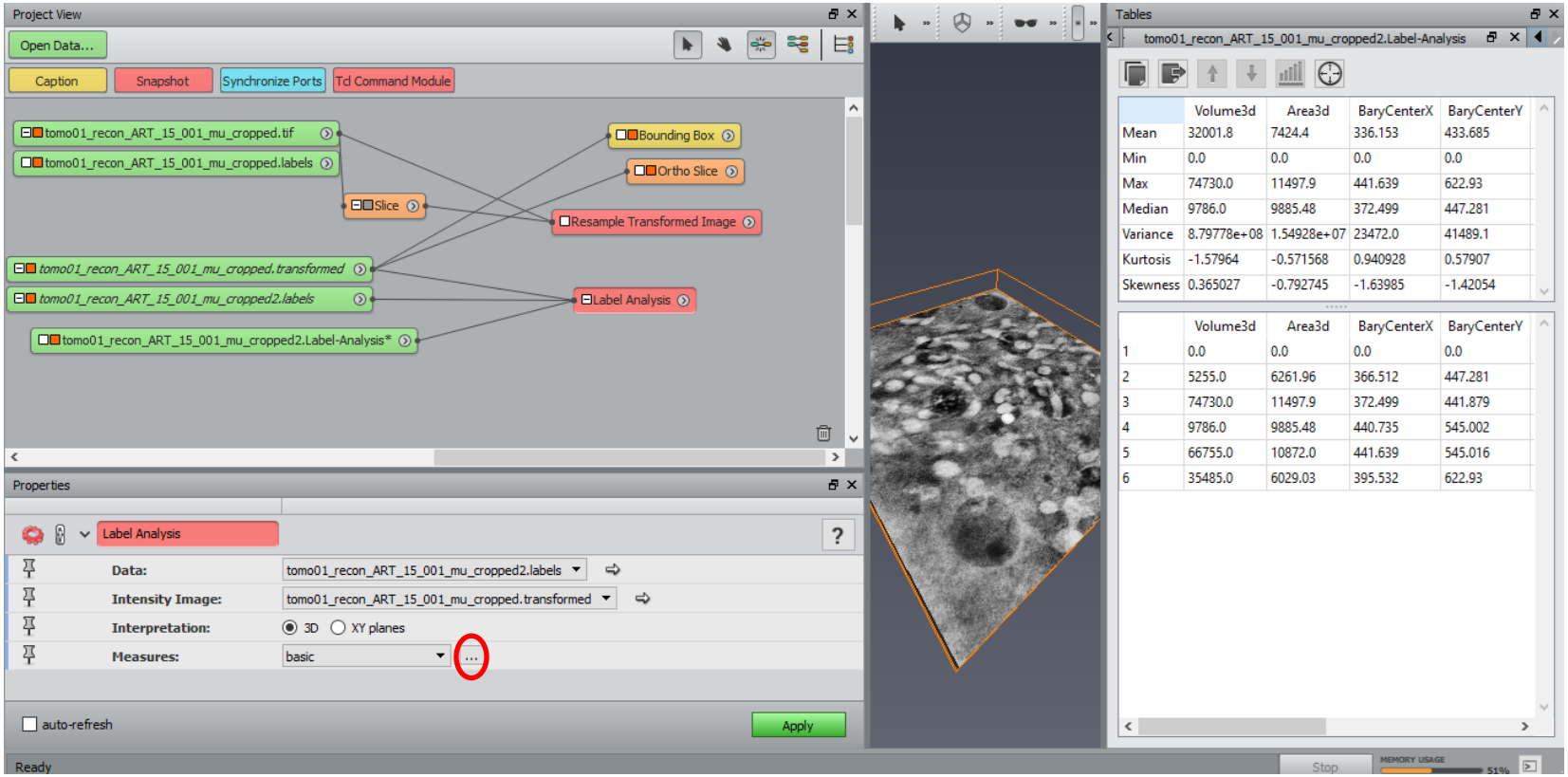
Create the computational module ¨Label Analysis¨ (right click on the Labels and search for it). Right click on the white square and connect ¨Intensity Image ¨ to the DATA (the transformed one of course), then click apply. Wait.

Label Analysis2



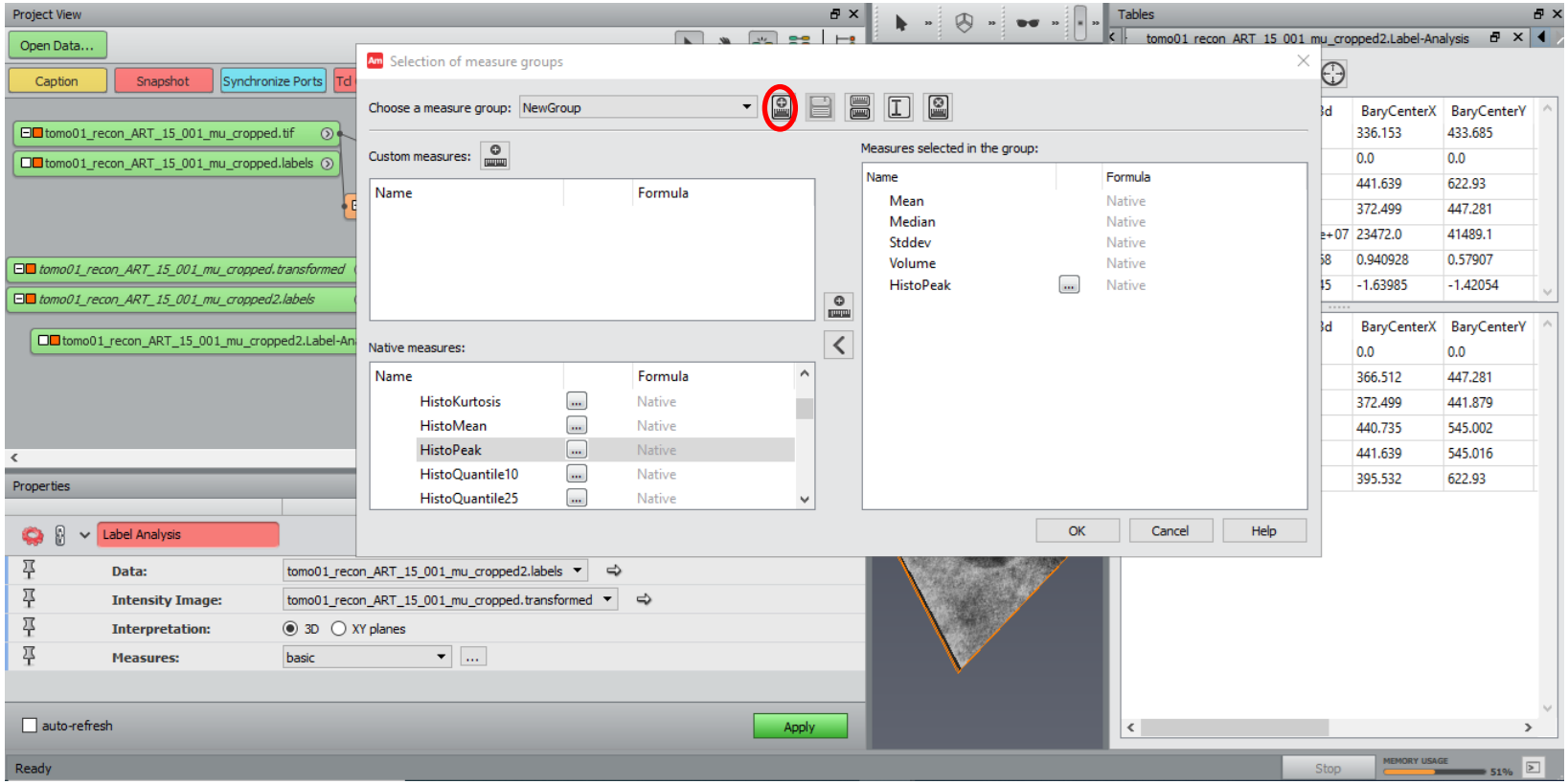
The result of the Label Analysis will appear both as a new object connected the Label Analysis and as a TAB on the right with the numerical results.

Label Analysis3



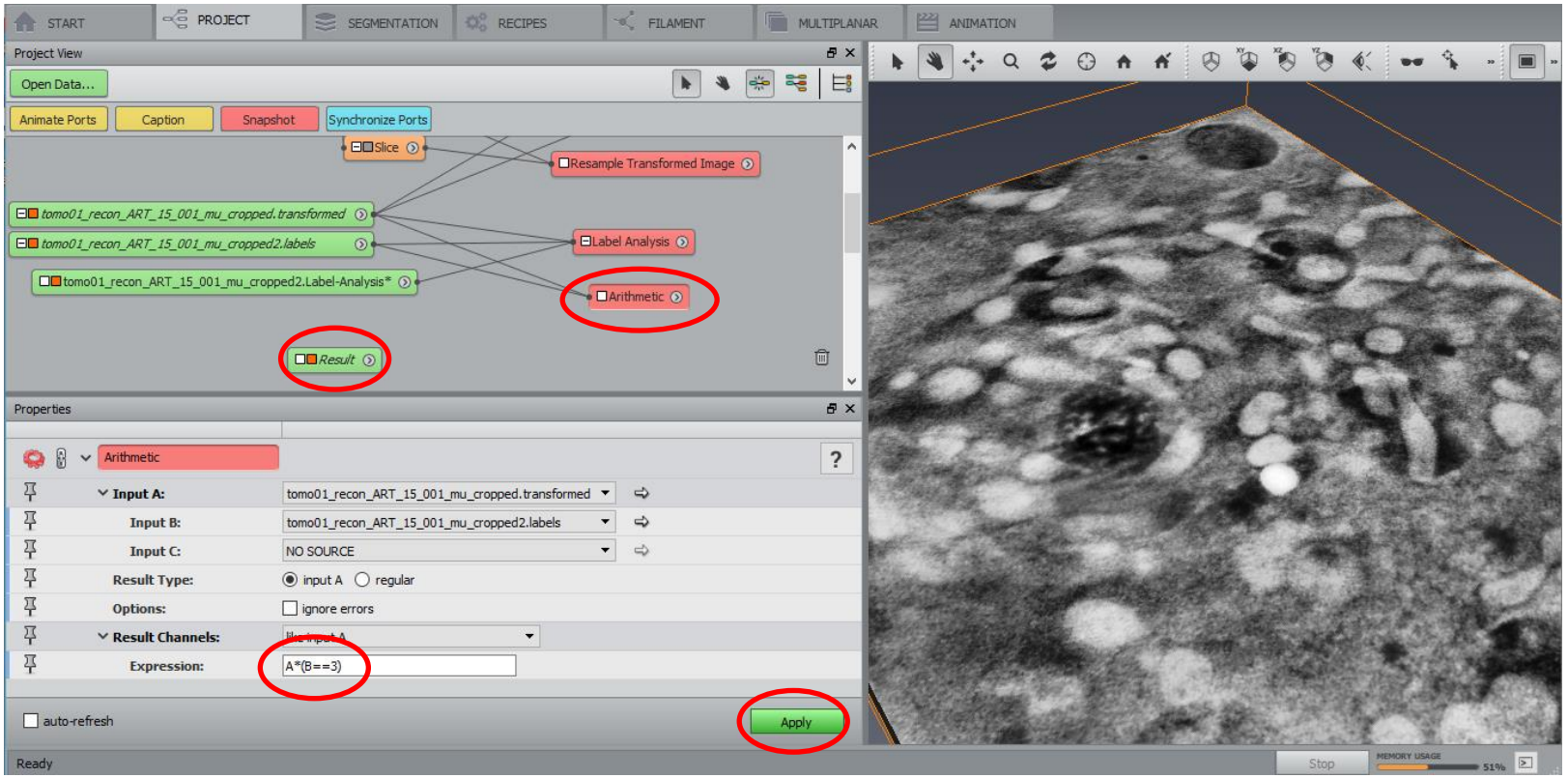
You can change the computed quantities and define a new measurements group clicking on … in *Measures*

Label Analysis4



Create a NewGroup unidng + and add the quantities you want to compute.
Click OK.
The Histogram Peak is an interesting value: is the Mode
as we will see in the following slides, the intensity distribution (i.e. the values of the linear absorption coefficient) is not Gaussian. So that the mean value is not the most probable value, i.e, the mean ≠ mode.

Arithmetic module

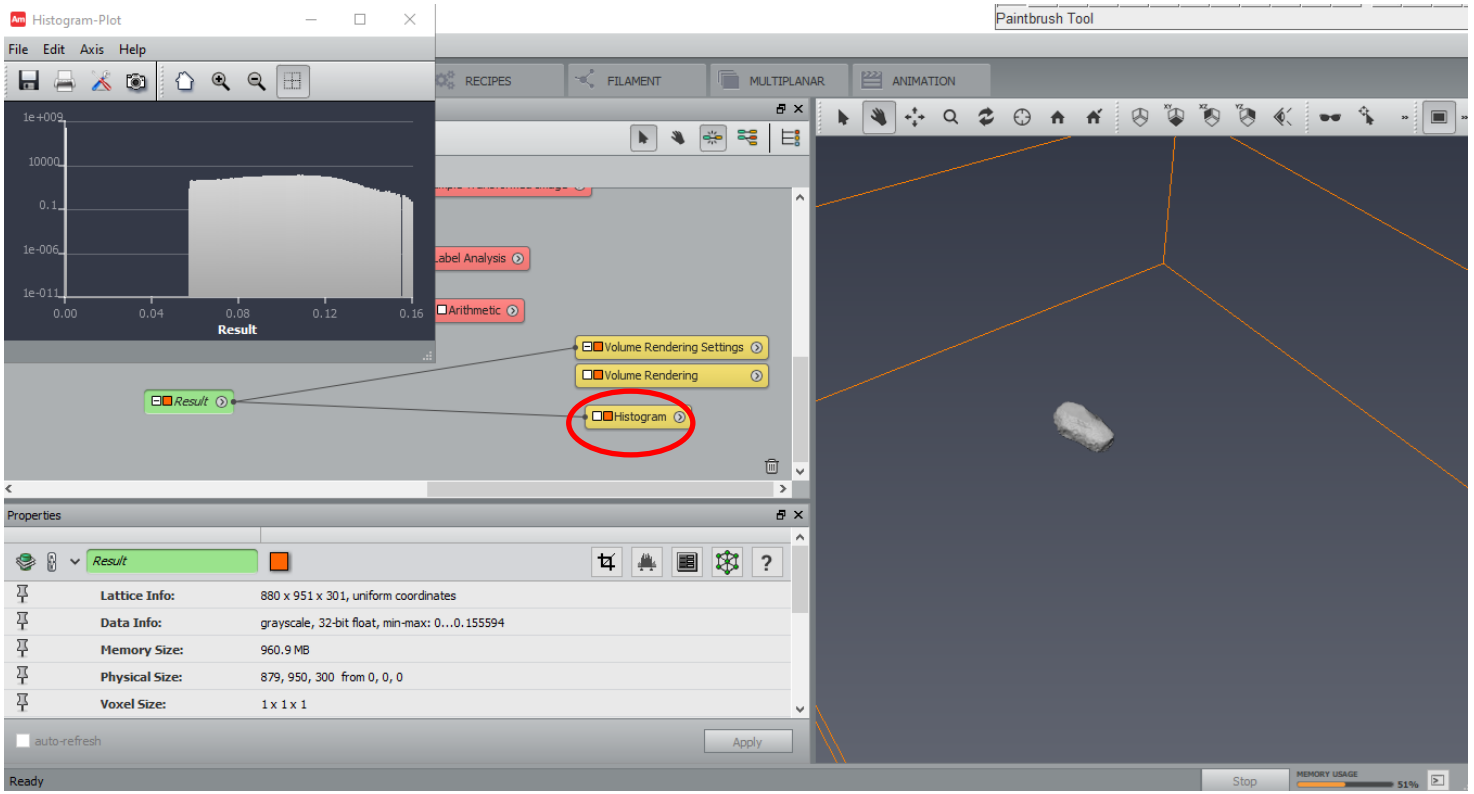


To create a material from the segmentation we have performed we can use the Arithmetic module (right click on the data for it). Right click on the white box and connect Input A with the DATA and Input B with the labels file.

Set the Expression as A*(B==3). This will multiply the data by the selection we have created in the segmentation editor corresponding to the Label=3 which is Material4 (the first refined

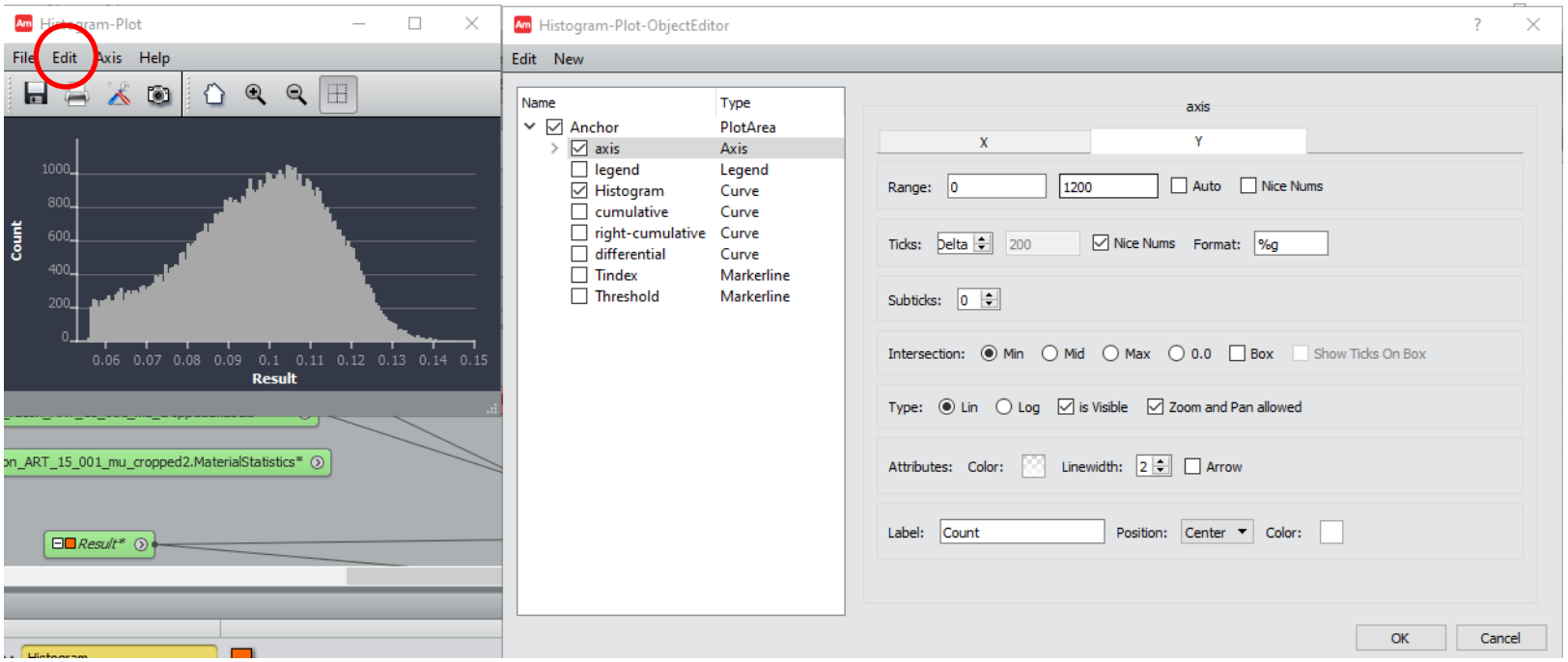
mitochondrion). It is always Material number = Label numebr +1.

Histogram1



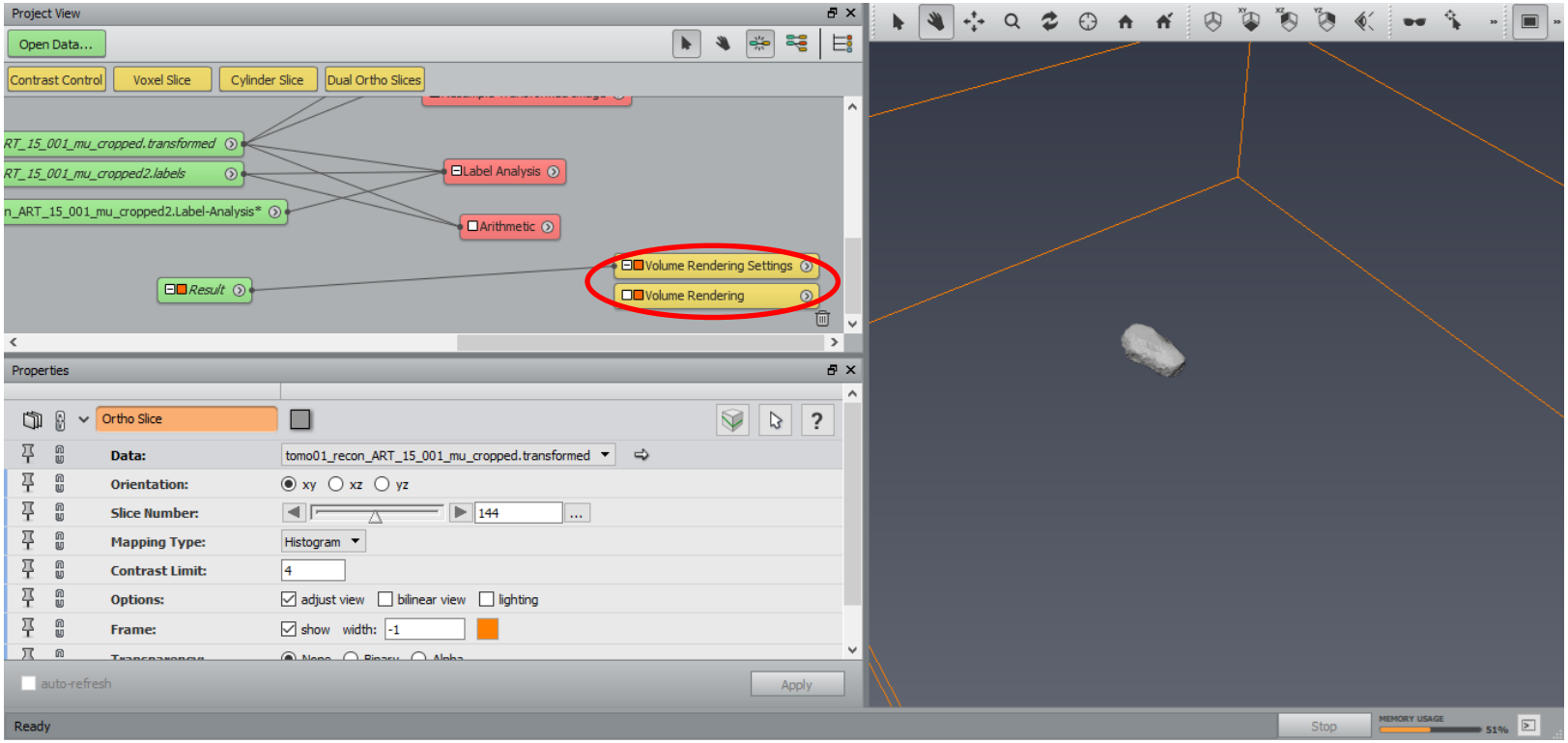
You can also take a look to the actual distribution of the pixels values using the Histogram module.

Histogram2



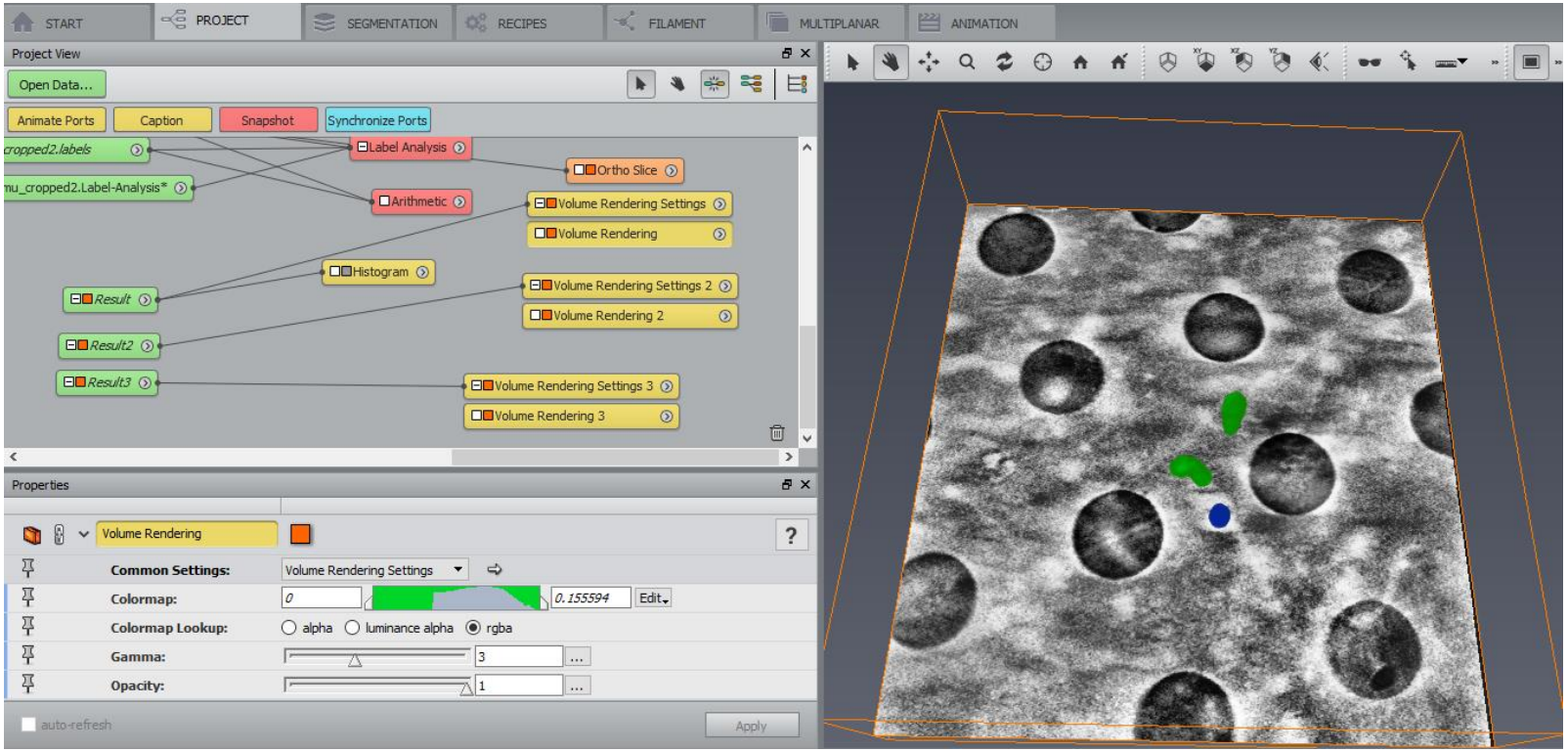
To change the default settings (the axis in particular, to optimize the visualization) click on edit axis.

Volume rendering



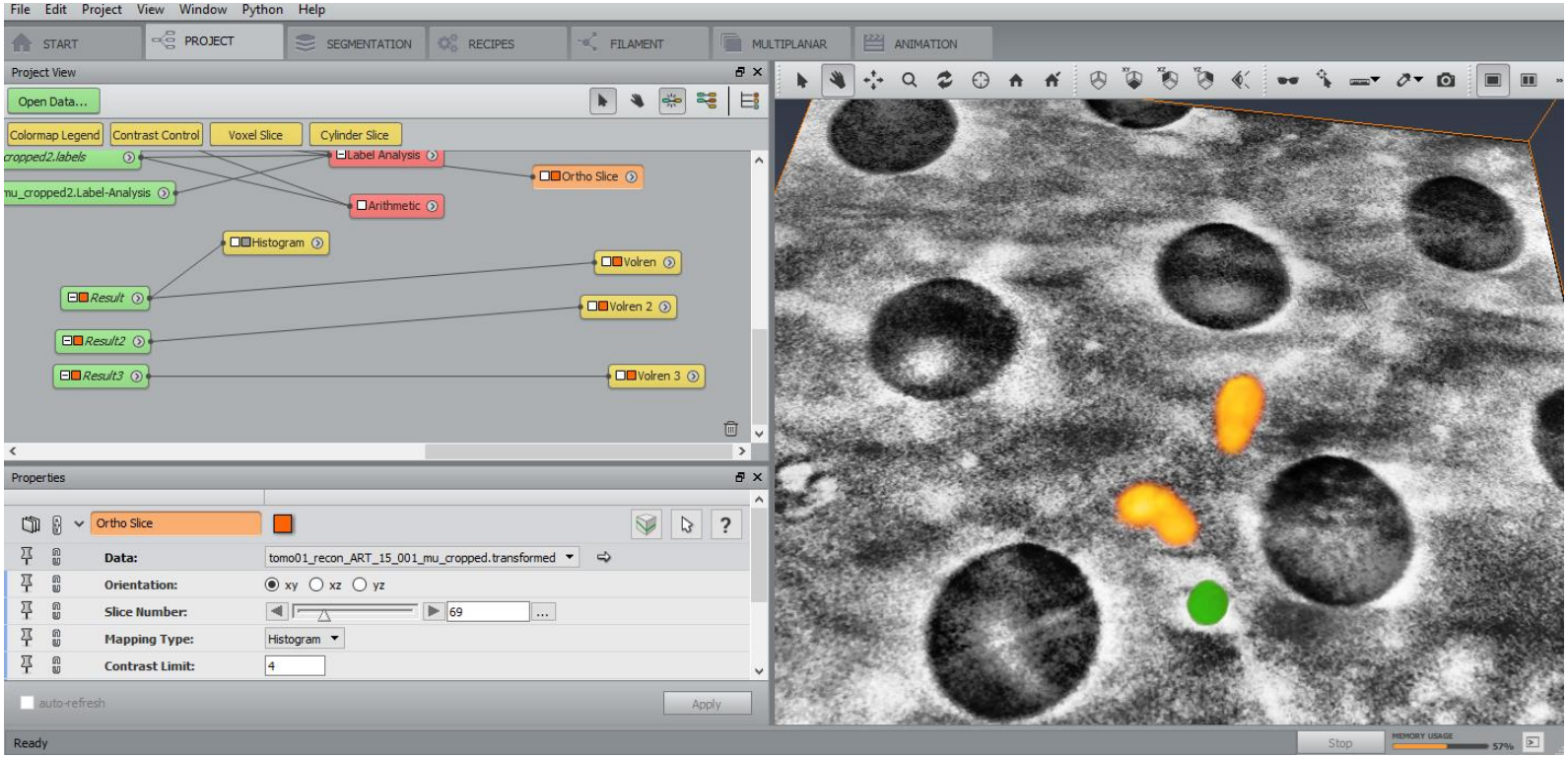
You can take a look to it in 3D using the Volume rendering module (right click on result DATA and create it). Deselect the orange box in any orthoslice or slice module to remove the stack visualization.

Volume rendering2



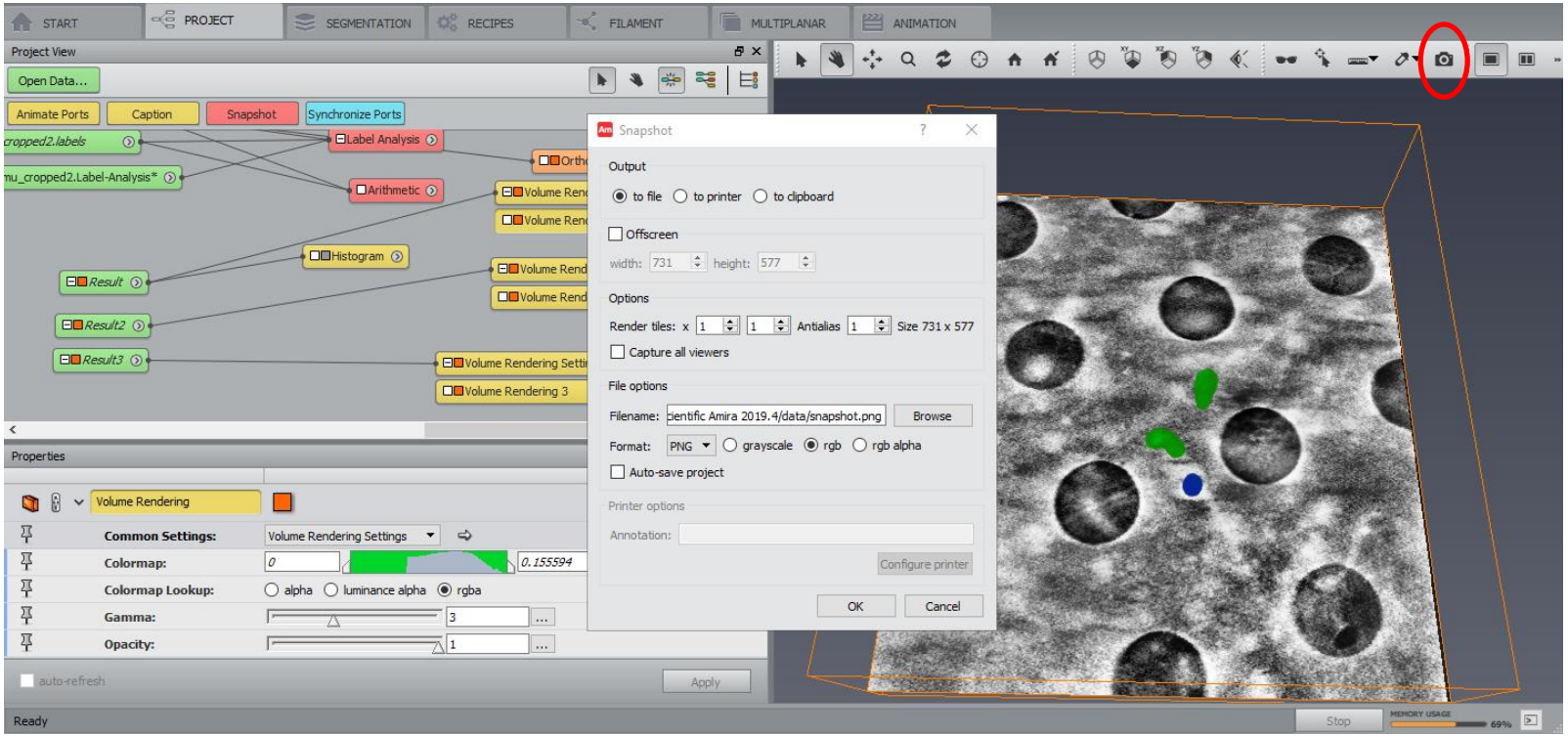
Create the others materials using the arithmetic module and visualize them using 3 different
Volume rendering modules. You can also manage the color option.
Here in green are the 2 mitos and in blue the lipid droplet.

Volume rendering3



The module Volren can also be used. It seems to use much less memory…

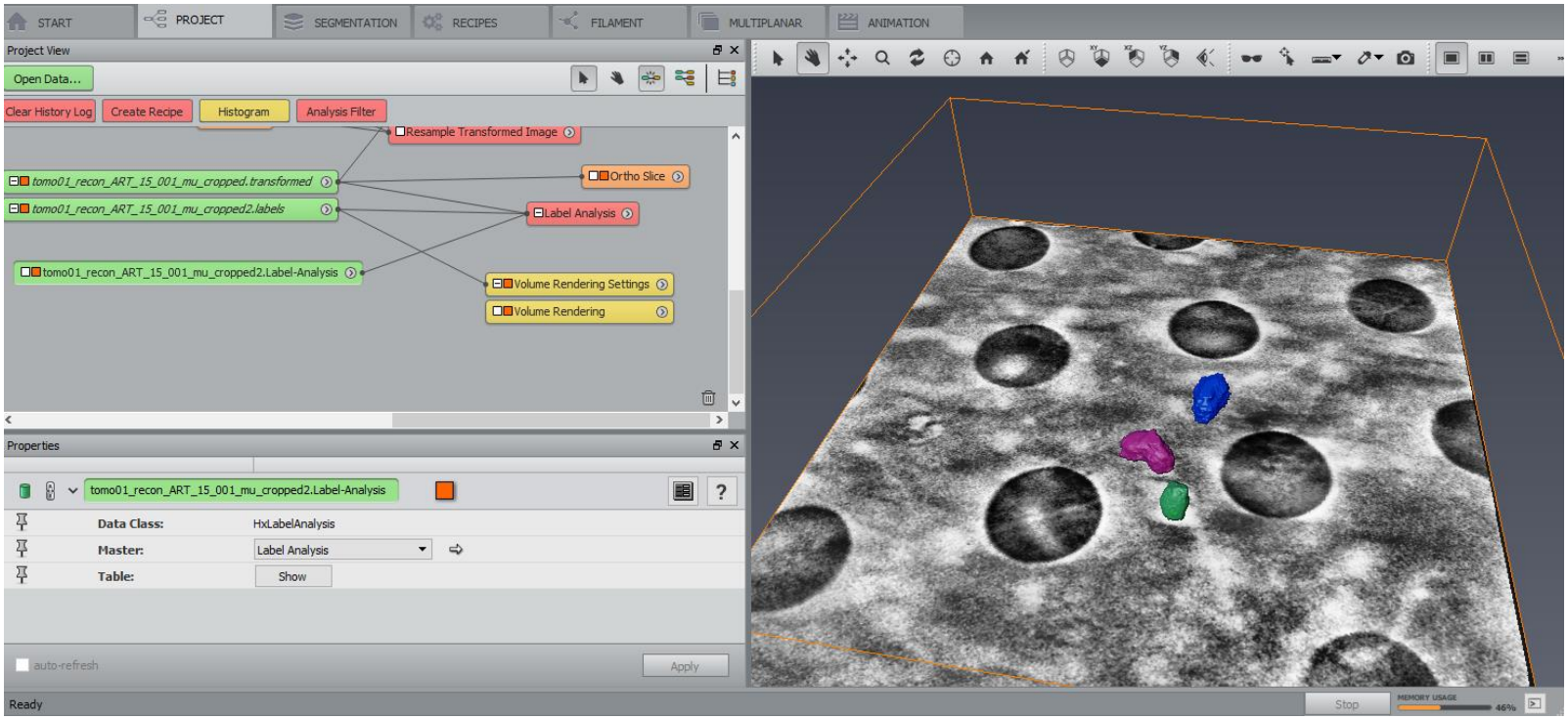
Volume rendering4



You can take a snapshot of the viewer and create a high resolution figure in many different formats.

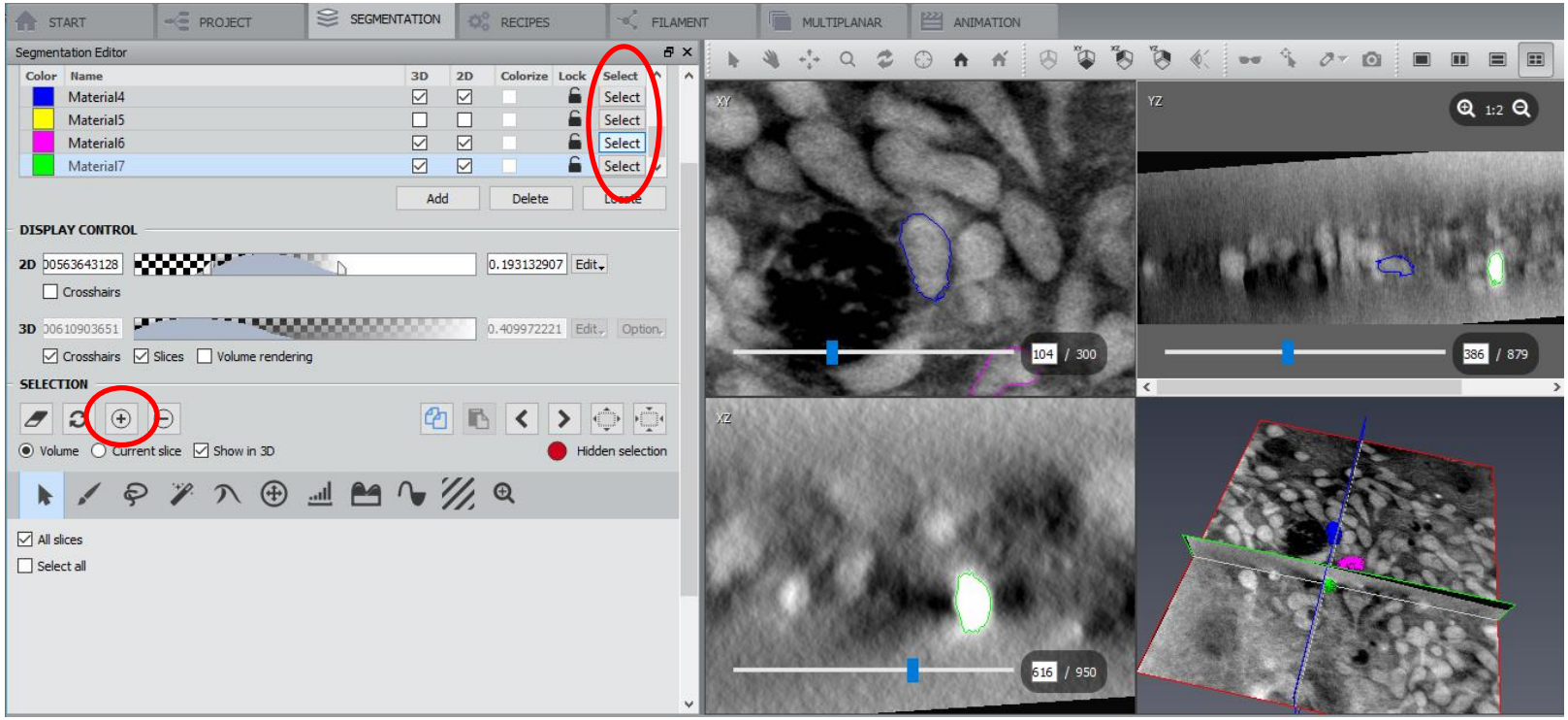
The Results can be exported: file export data as (.mrc for instance) and be opened, visualized and analyzed using another software.

Volume rendering: fast way



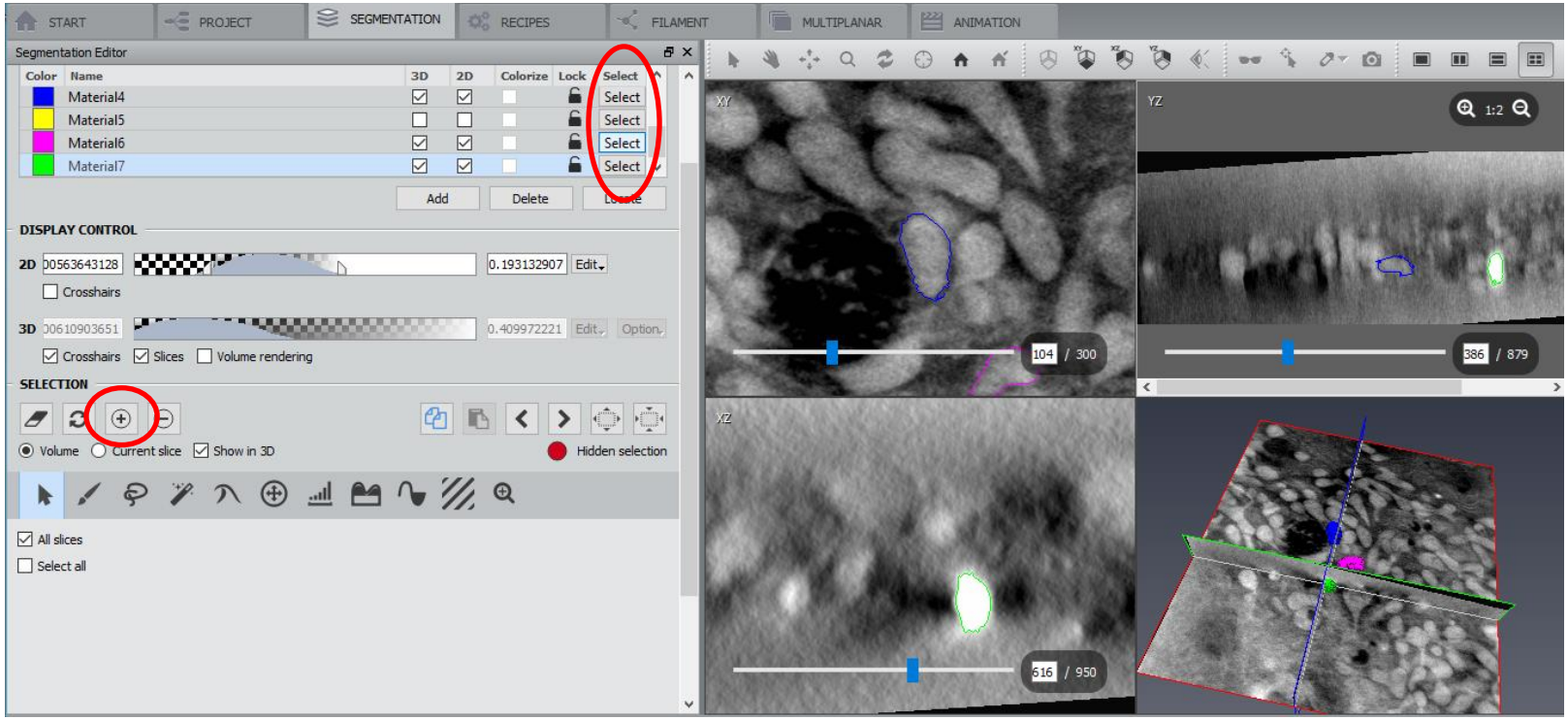
You can directly create a Volume Rendering module from the labels without using the 'arithmetic' and create 3 separate results.
But in this case just different colors can be used (1 per label = material).
Also, you cannot export them.

Volume rendering: fast way2a



You can create in the segmentation editor a new material (with “Add”) and then you can transfer all the voxels from a material to the new one using the corresponding “select” button and then the “+”. Pay attention to select with the mouse the new material to transfer the pixels to it (in figure for instance I’m moving the voxels from material6 to material7).

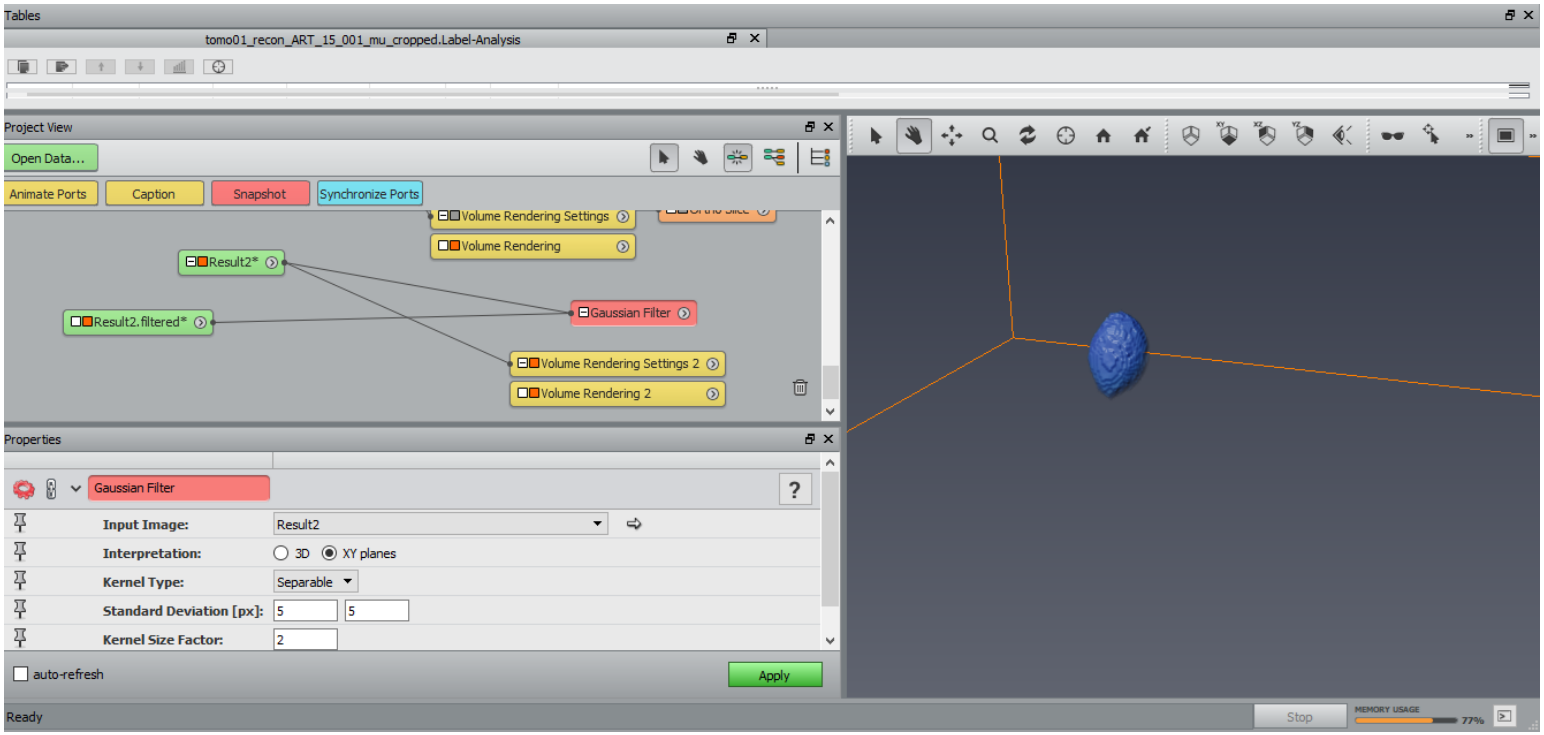
Volume rendering: fast way2b



If you do it for all the mitochondria, at the end you will end with a single (big) material containing all the mitochondria and you can give to it the color you want in the project editor. This is a smart way to have all the same kind of object with the same color without creating them one by one using the arithmetic module (which is still needed if you want to export them). You will have to use arithmetic just once!

NOTE that in this way you will loose the other materials so that if you are interested in the statistics on the single object (in this case mitochondrion) you have to save it as an another project (Save Project as in File).

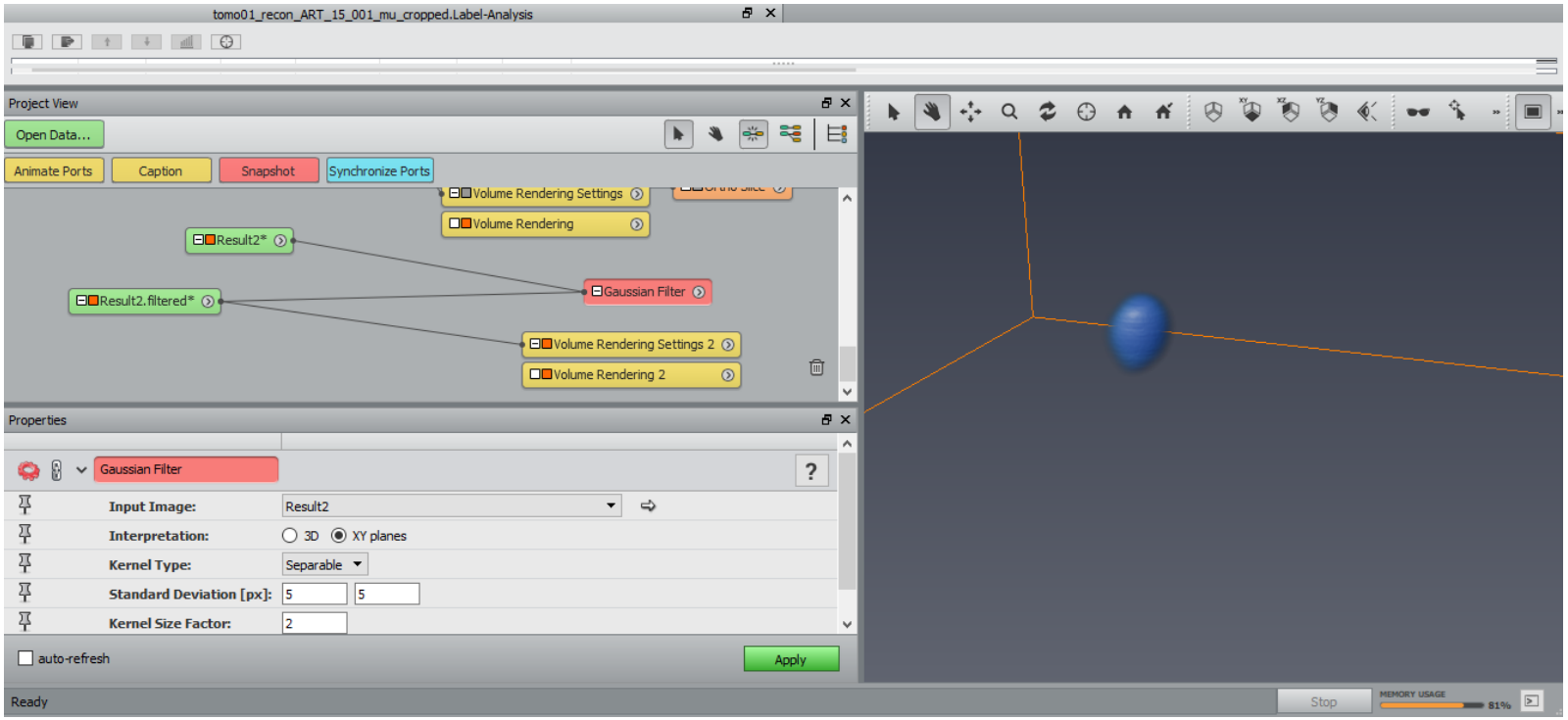
Volume rendering: filters



You can improve the appearance of you material using a filter. Right click on the “Result” and search for “filter”: you have a lot of them. Here I used the Gaussian Filter module for example, with those parameters…

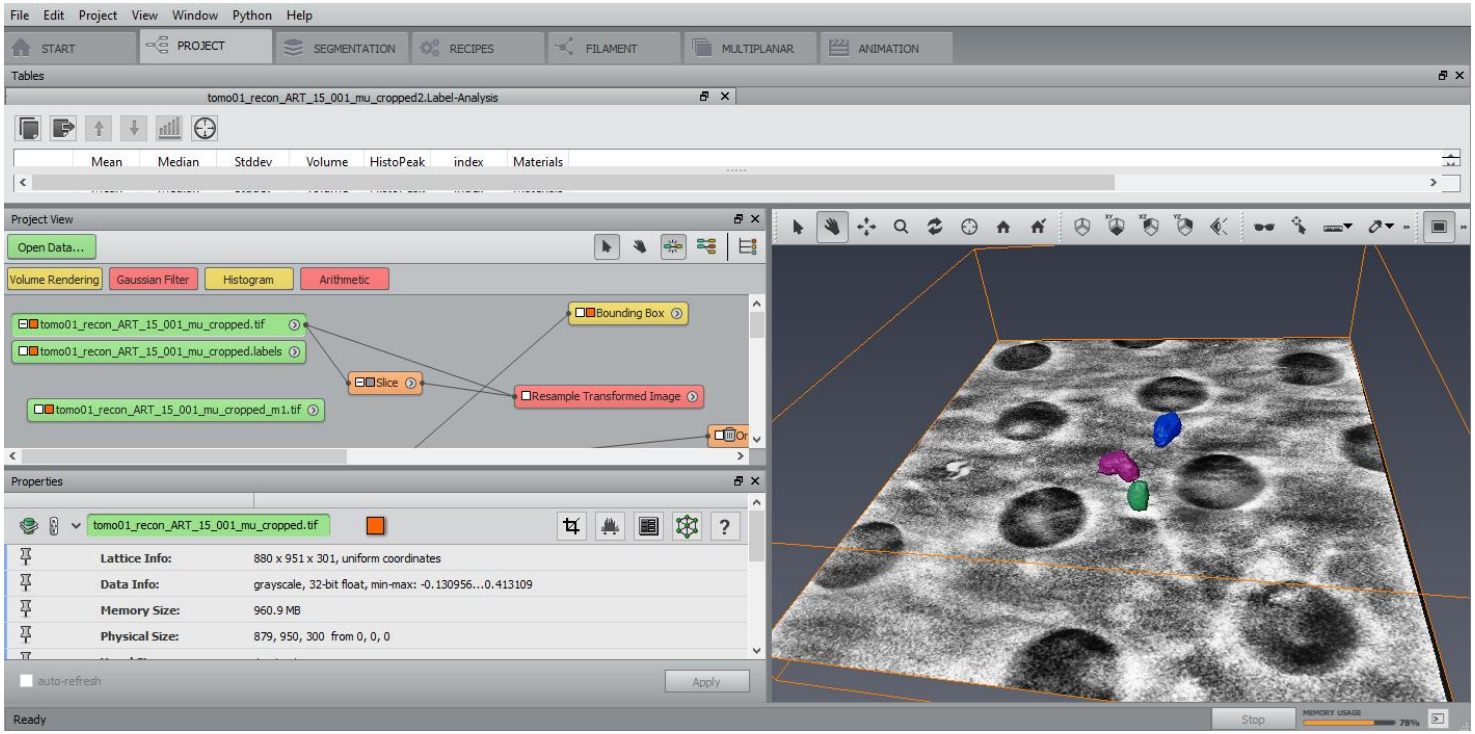
Our experience is that the “Non Local Mean” (to preserve average values) and is good one.

Volume rendering: filters



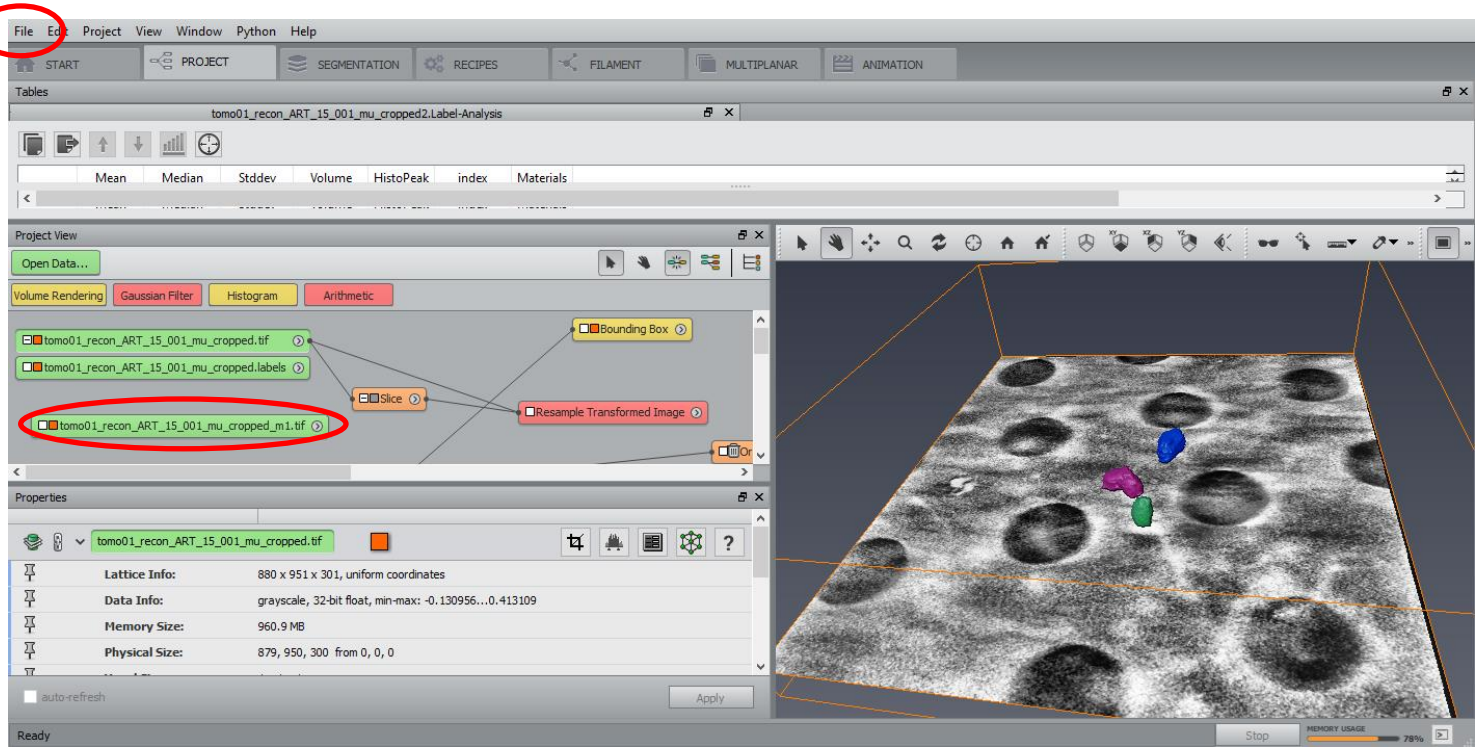
…and I got this: “pixelling” of the object surface is removed.

Apply the same segmentation to different DATA



It has sense of course only if your DATA are the same, but with different numbers.
Typically you would like to re-apply the same segmentation you performed on the linear absorption coefficient obtained using ART on the volume you get using SIRT with the transmission (or vice versa).
We have to assume that the 2 reconstructions obtained with 2 different algorithms are the same in terms of morphology.

Apply the same segmentation to different DATA



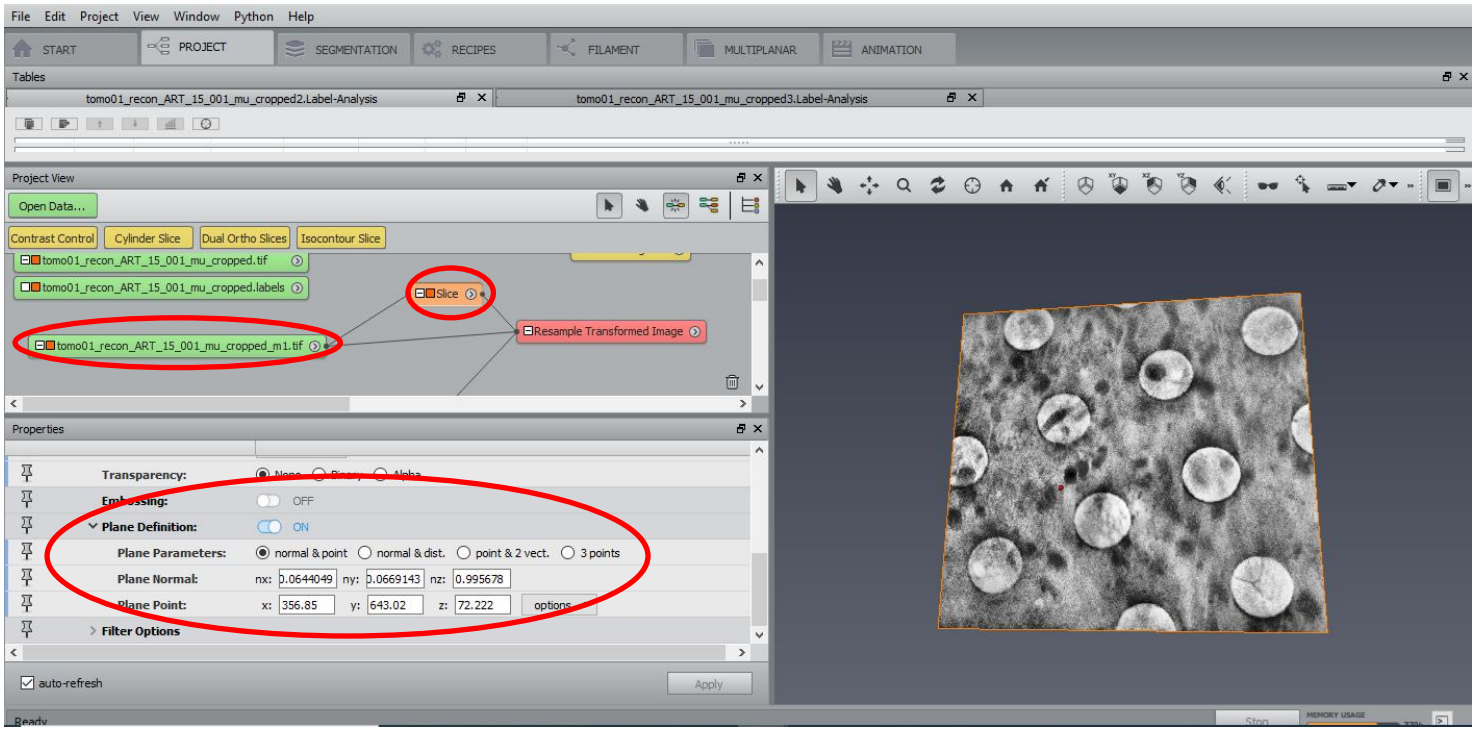
Then:

1- Load your amira project containing the segmentation.

2- In the project, open new data from file. I called it like the original one with "_m1"

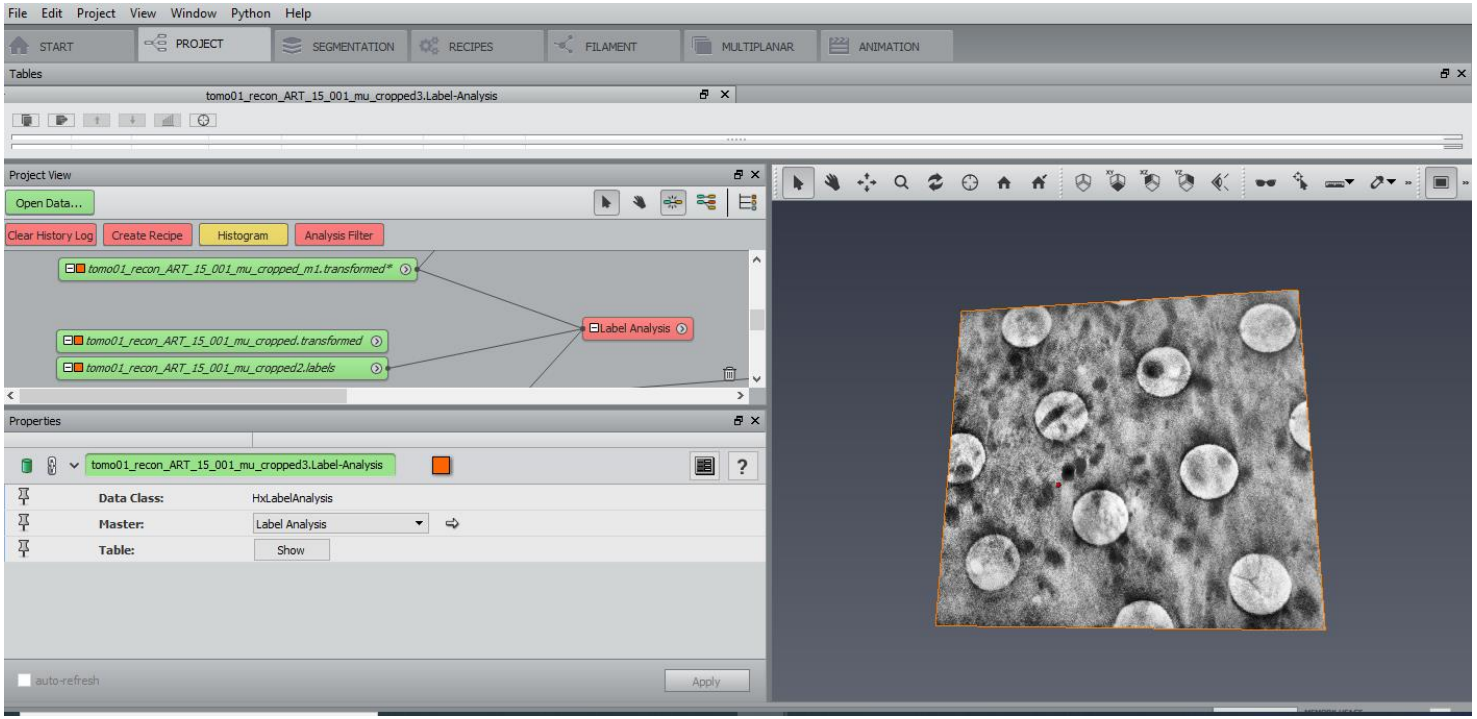
3- If you have applied some transformation on your data BEFORE creating the materials in the segmentation editor, we have to apply the same transformation to our new data set.

Apply the same segmentation to different DATA



in this case we want to re-apply the transformation defined by the module “slice”. Create another slice module and then to apply <u>exactly the same</u> orientation, activate the plane definition option and insert the same parameters you had in the first slice module, i.e. the one you used on the original DATA set. If you move the same “slice” module from one to the other data you will lost in any case the “plane parameters” (write down them somewhere at the real beginning, just in case). Now the new DATA set has the right orientation and you can re-apply the transformation using the “Resample Transformed Image” module.

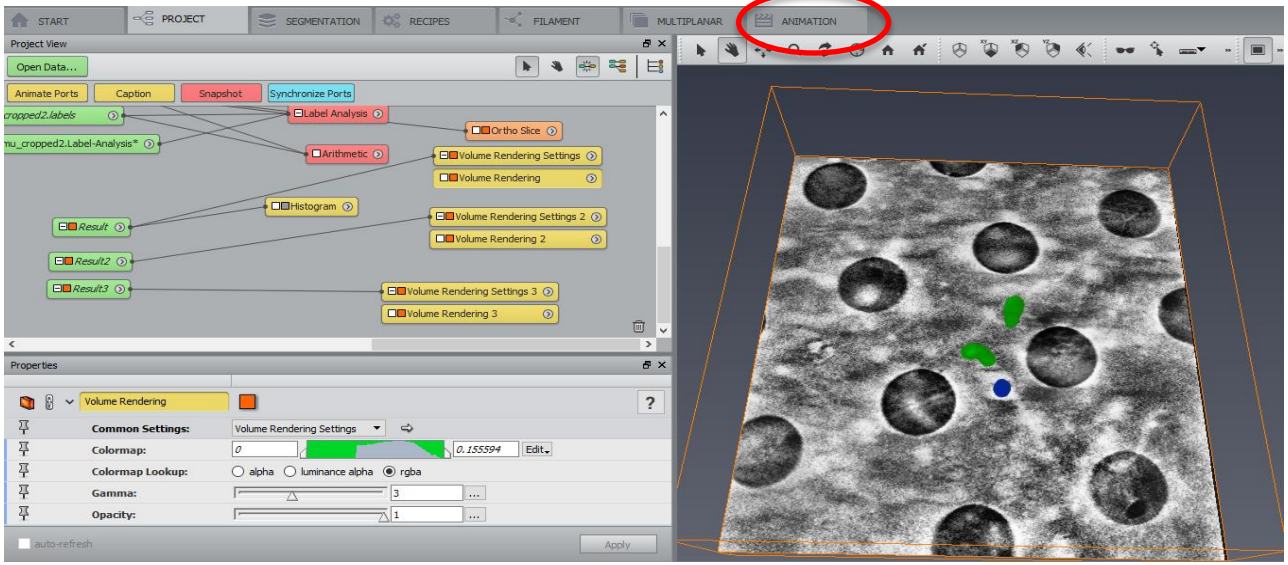
Apply the same segmentation to different DATA



4 – Use the Label Analysis module for your calculations:
Left click on the white square, the DATA is your already existing label field, the “Intensity image” is the new transformed DATA on which you want to apply the old label field.

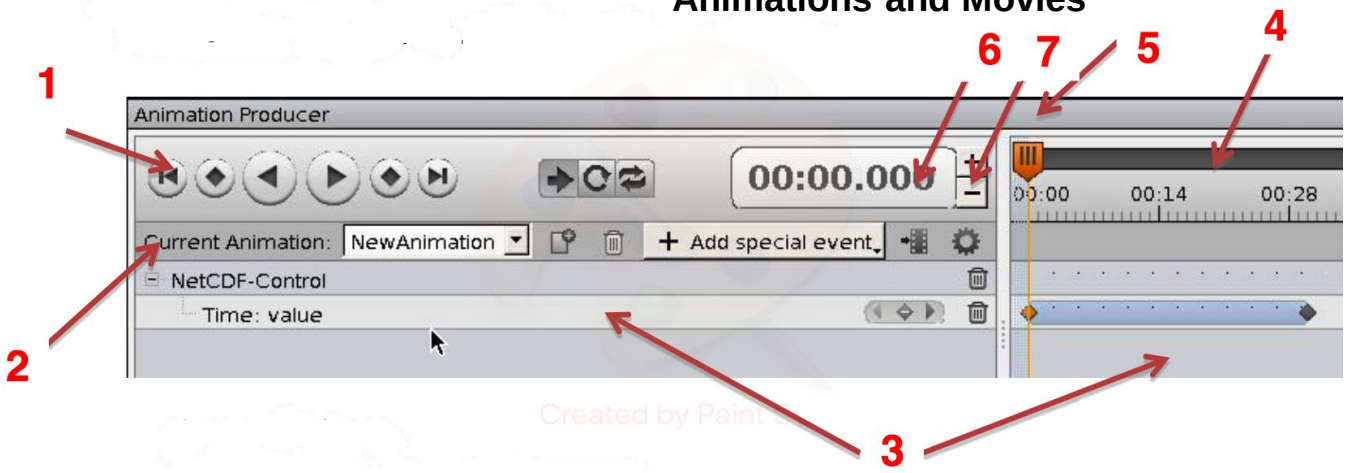
Animations and Movies

If you want to produce a simple video with a visualization of time-dependent data with a fixed view, you will only need the MovieMaker.
More complex animations or series of animations can be set up with the Animation Director module. With the Animation Director, you can combine and synchronize time animations, camera rotations, and movements of 2D slices etc., as well as switch modules on or off.
Basically, all parameters of the active modules can be changed. Evencomplex animations, like time animation combined with rotation of the view or a moving camera position can be accomplished by using the Camera Orbit or the Camera Path modules.
After you have finalized the choreography of your animation in the Animation Director, the result can be saved in form of an MPEG-1 video or as a sequence of single image files.
The Animation Director module is activated or deactivated by pressing the Animation button in the toolbar or by selecting Window > Animation

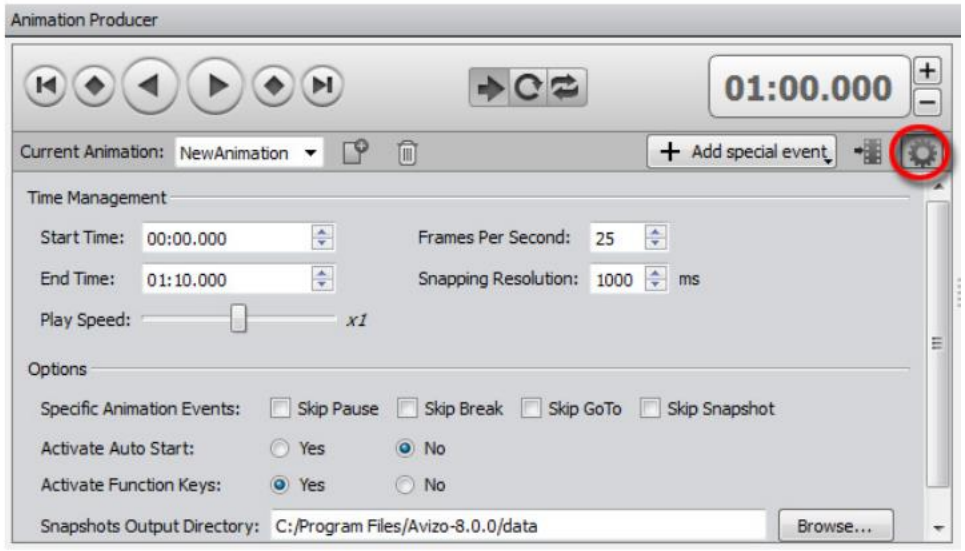


There are different perspectives when it comes to animation:
Animate data object
Animate camera
Animate visualization property: Animate orthoslice, clipping plan, or volume rendering transparency

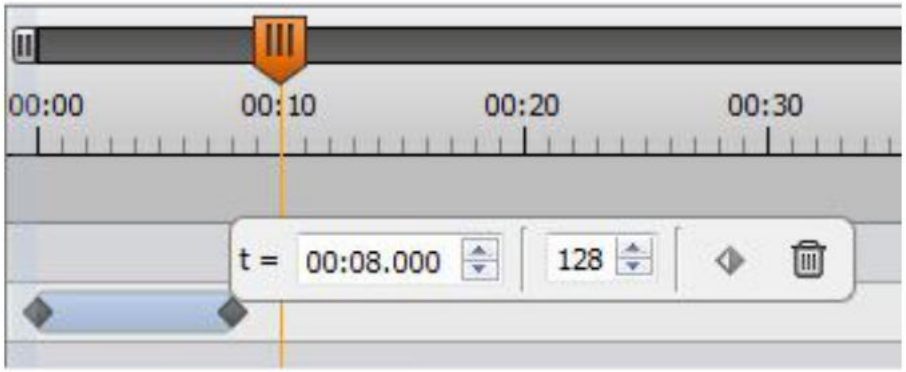
Animations and Movies



1. Control bar
2. Animation Director toolbar
3. Event list
4. Timeline
5. Master Time Slider
6. “Movie Creation” Menu
7. More Options” Menu



Time Management: In order to define the length of your animation storyboard, you can open the “More Options” menu (7.) and set parameters such as the start and end time and number of frames per second.

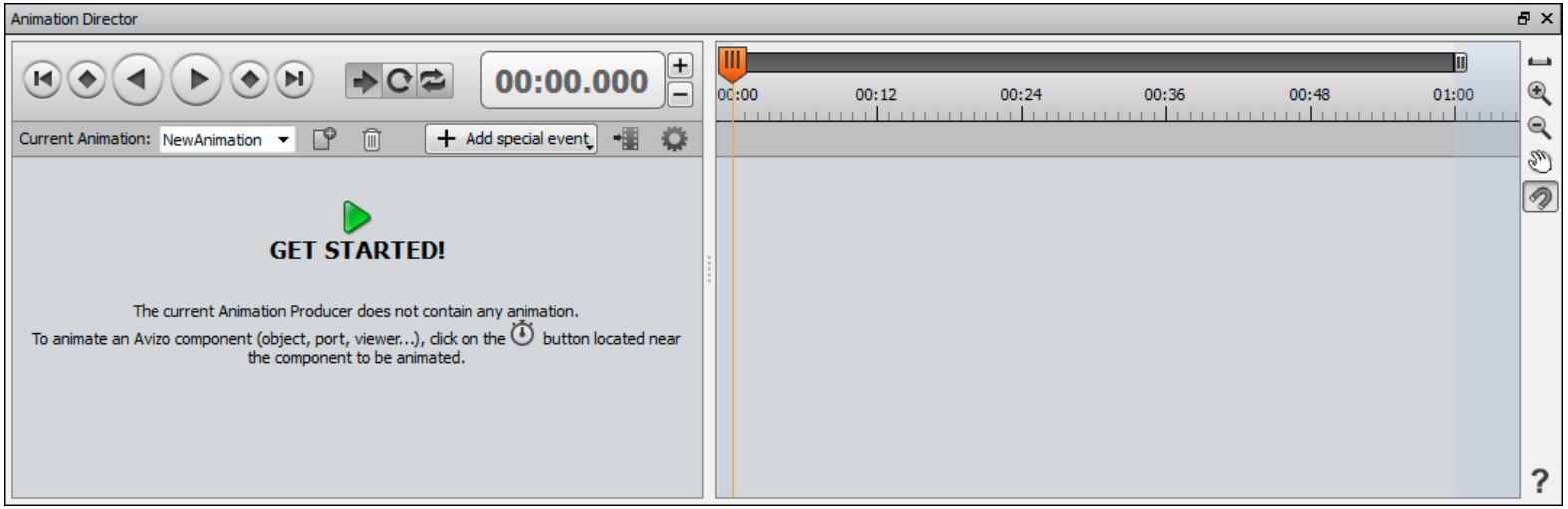


A new widget becomes visible, hosting the Animation Director’s user interface.
Clicking on the stopwatch button in the Properties of a module creates a new keyframe in the Animation Director timeline and the event is listed in the left panel of the user interface. If you hold the mouse cursor above the small orange diamond symbol in the timeline panel, it activate a small input field where you can adjust the time and the accompanying value for the port that it’s associated with.
In order to adjust the schedule, you can simply drag the diamond icon to the desired position on the timeline.

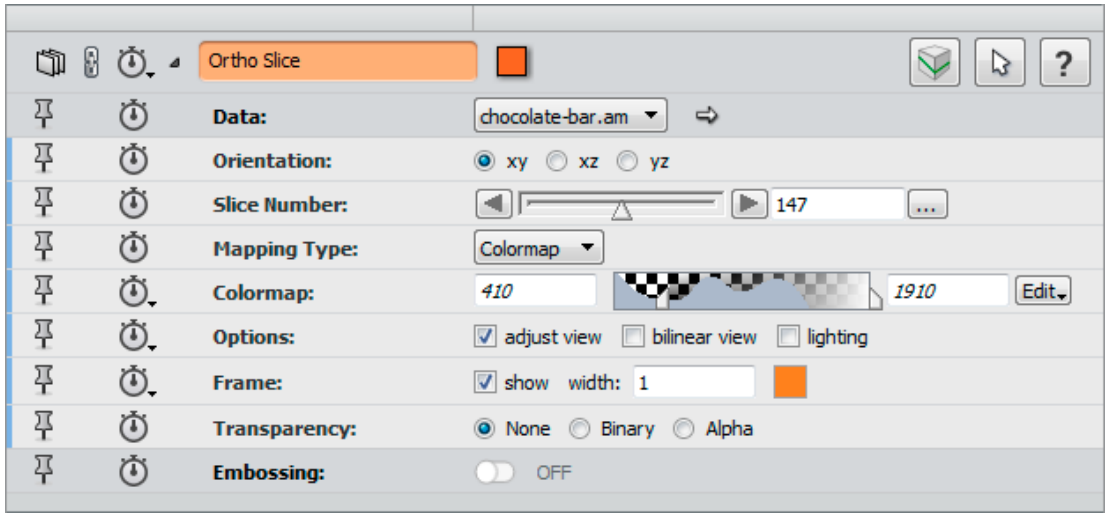
Animating an Ortho Slice module

Moving the Ortho Slice: plane up and down to show what the data looks like.
The Ortho Slice module has a port called Slice Number . If you change the value of that slider, you see the plane move in the viewer.

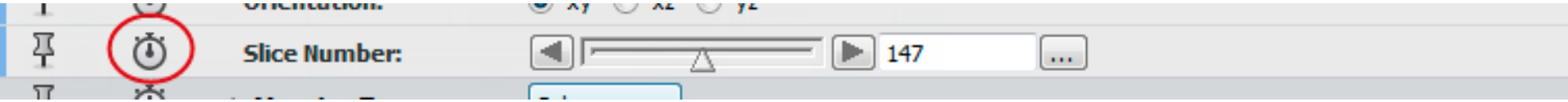
From the toolbar, click on the Animation Director button.
A new widget becomes visible hosting the Animation Director user interface.



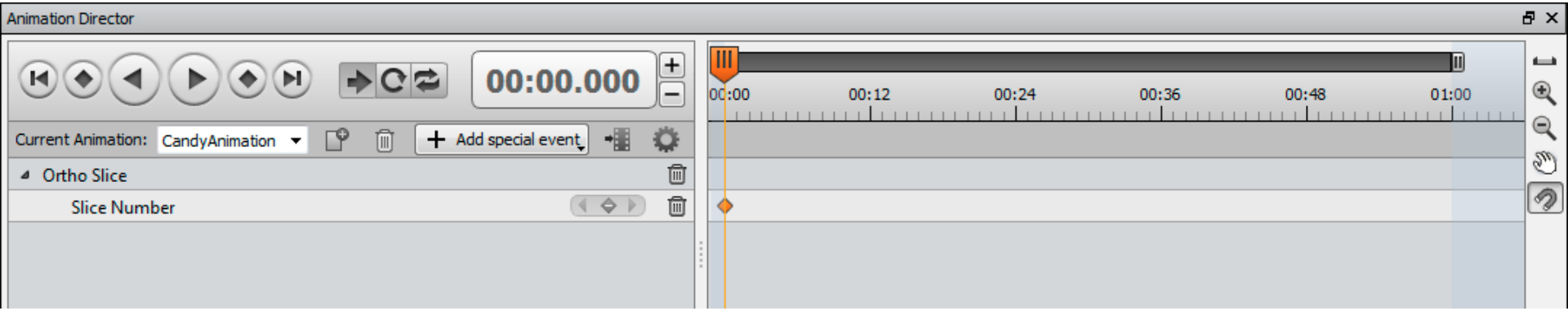
Like the other widgets, this widget is also dockable and you can place it at a convenient position
within the Amira user interface.
After activating the Animation Director by clicking on the related button in the toolbar, all ports of the currently available modules that can be animated are extended by
an additional button representing a stopwatch .



We can now animate the Ortho Slice position. We do this by clicking on the stopwatch button of the Slice Number port in order to schedule the start event:



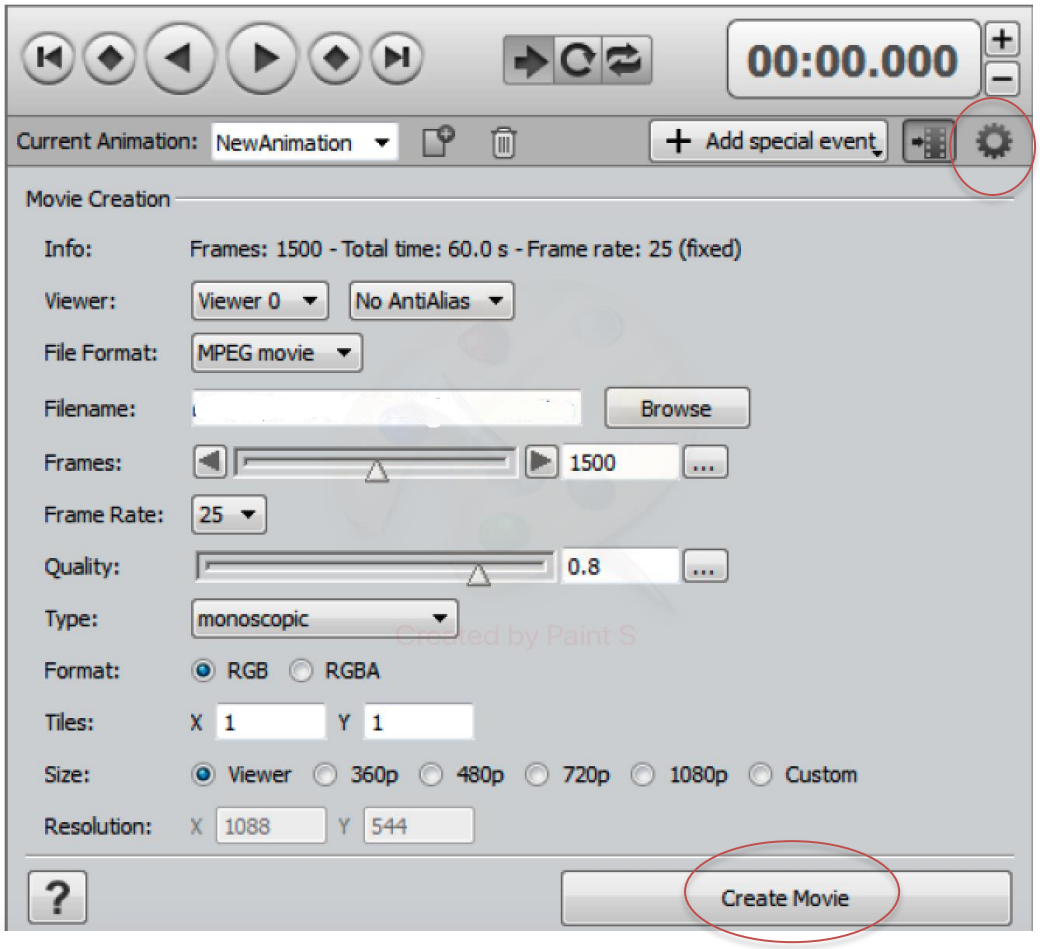
Clicking on the stopwatch button creates a new keyframe in the Animation Director timeline and the event is listed in the left panel of the user interface.
If you hold the mouse cursor above the smal orange diamond symbol in the timeline panel, this will activate a small input field where you can adjust the time and the accompanying value for the port with which it is associated. In order to adjust the schedule, you can simply drag the diamond icon to the desired position on the timeline.



With this operation, we have defined the beginning of the animation of the slice position. Next we want to define the time where the animation should end. To do this, we drag the master time slider to the desired time on the timeline, e.g., to 00:04.000, which means 4 seconds. As a next step, we set the slice position of the Ortho Slice module by either setting the Slice Number port in the properties of the module or by positioning the slice interactively in the viewer window. Using either method, set the value of the Slice Number port should be set to 294. After you click the stopwatch button again, the keyframe is created in the timeline.
You can test your first animation

Creating a movie from an animated demonstration

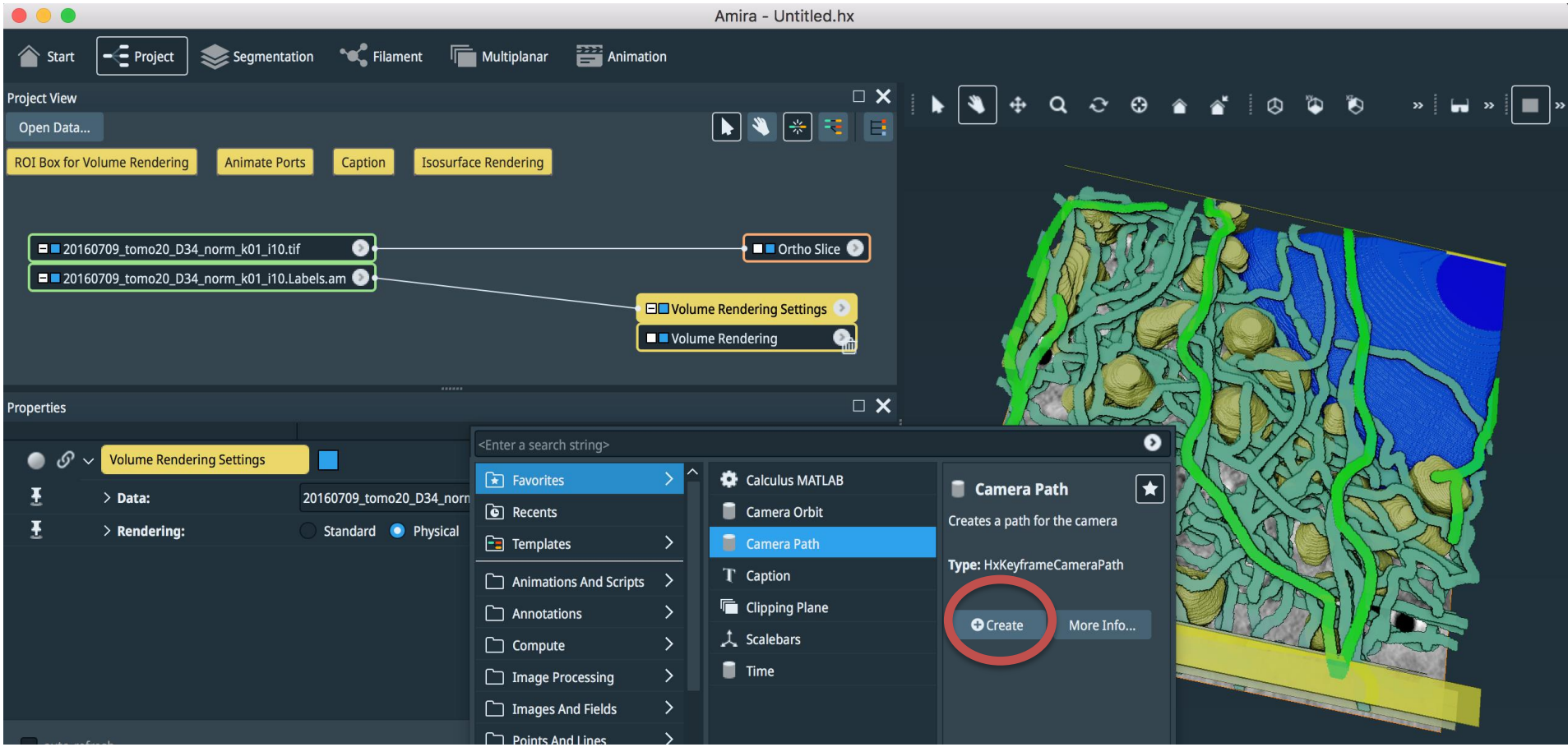
To create a movie from an animation defined with the Animation Director, simply click on the Movie Creation button of the Animation Director panel. The following panel will appear:



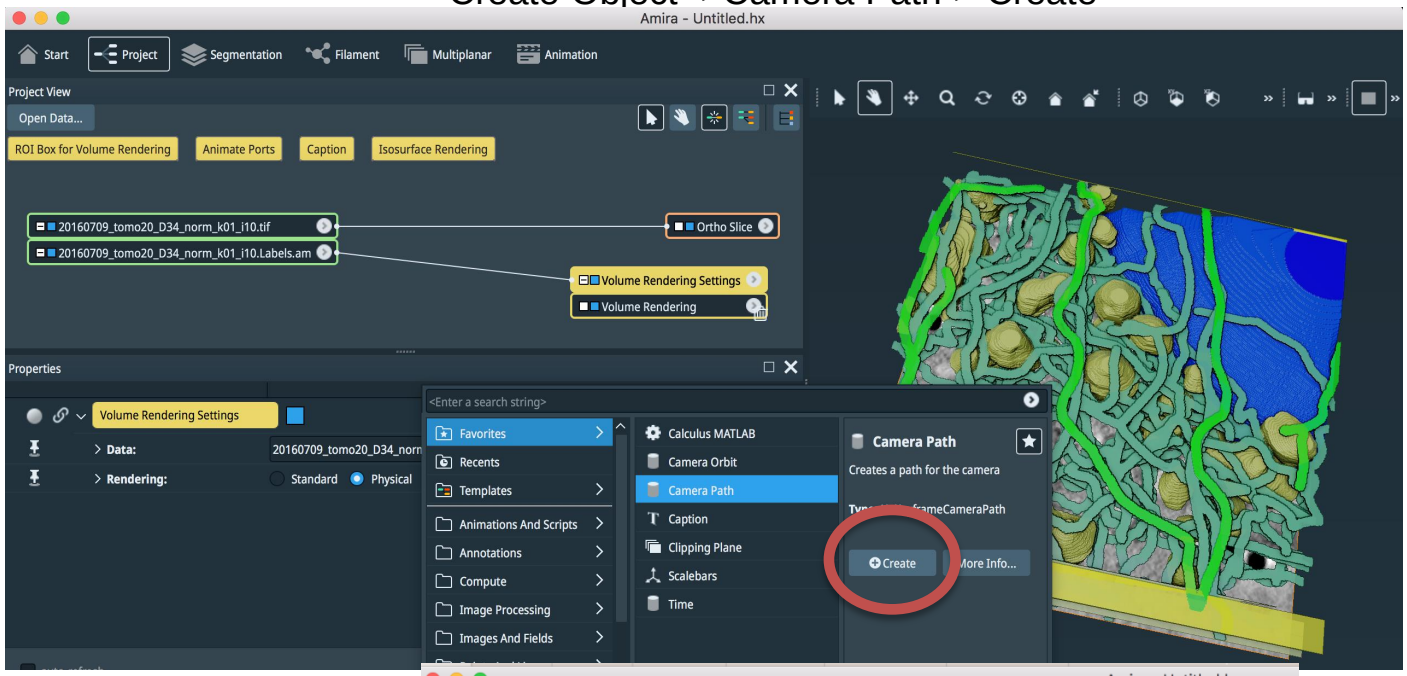
This module is already pre-configured to create a movie that respects the animation settings (duration, frame rate, filename...) that are defined by the Animation Director module. However, you can adjust these parameters, if needed. Just click on the Create Movie button to generate the movie.

Movie Maker using Camera Path

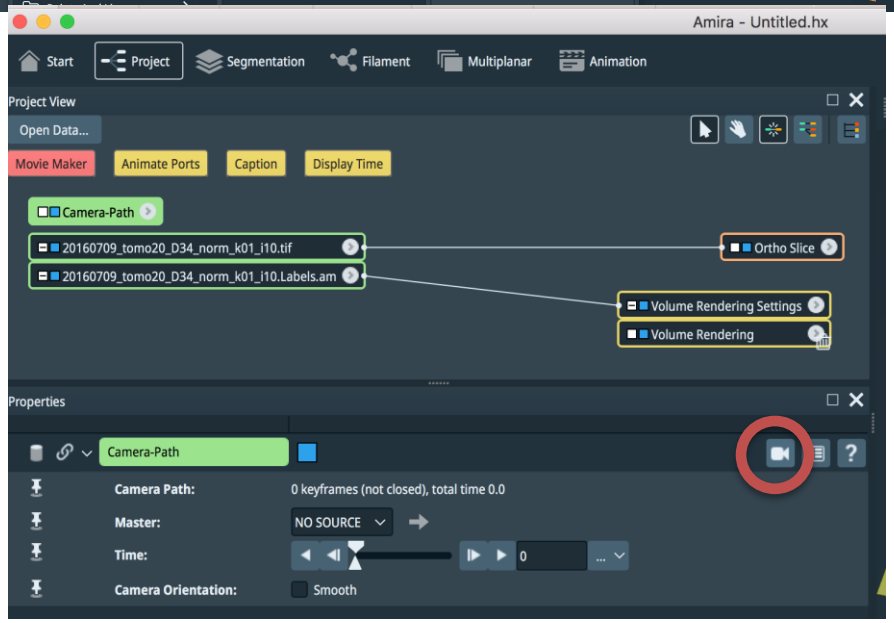
Right click in empty space, choose
Create Object ->Camera Path-> Create



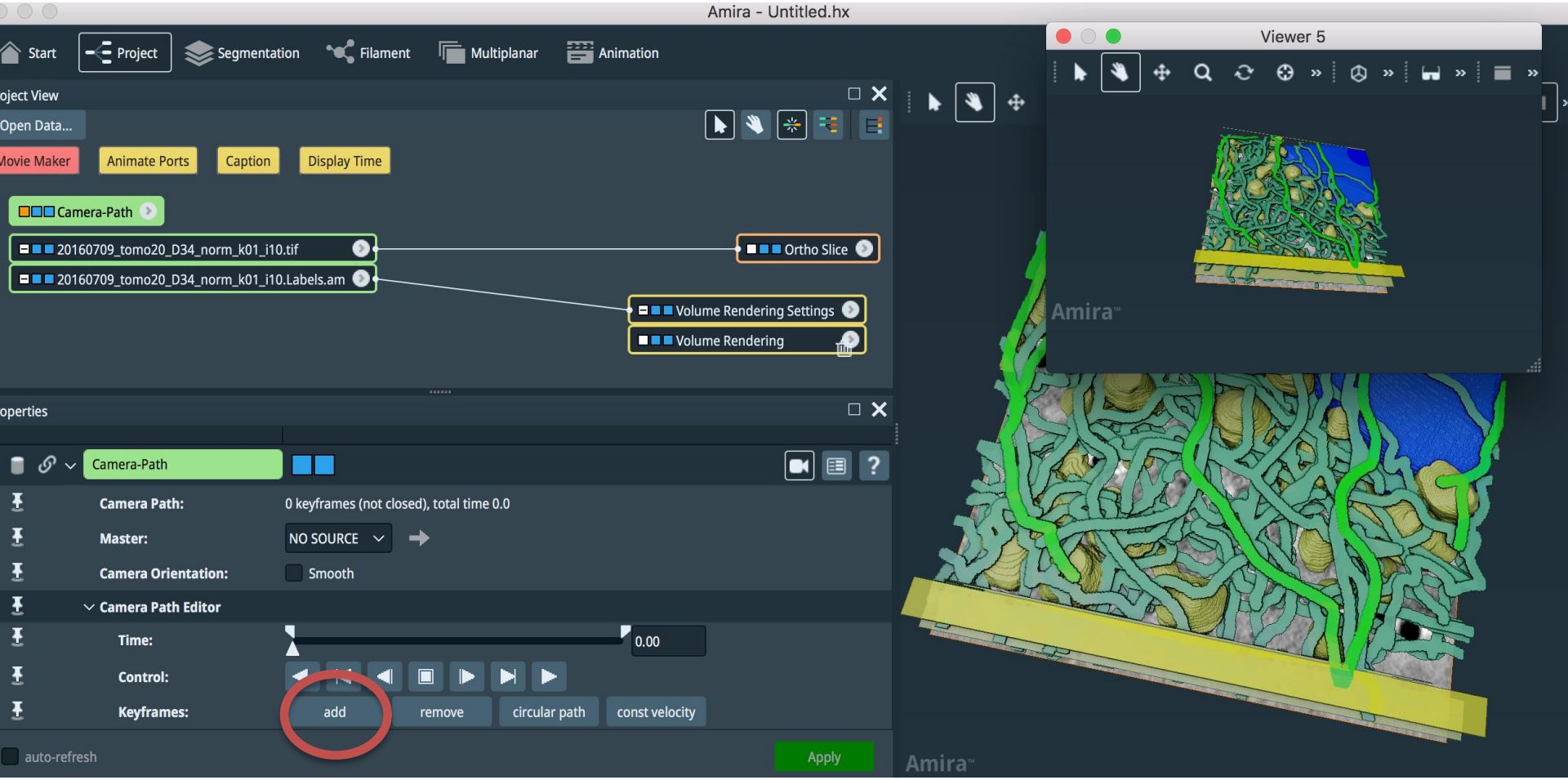
Right click in empty space, choose
Create Object ->Camera Path-> Create



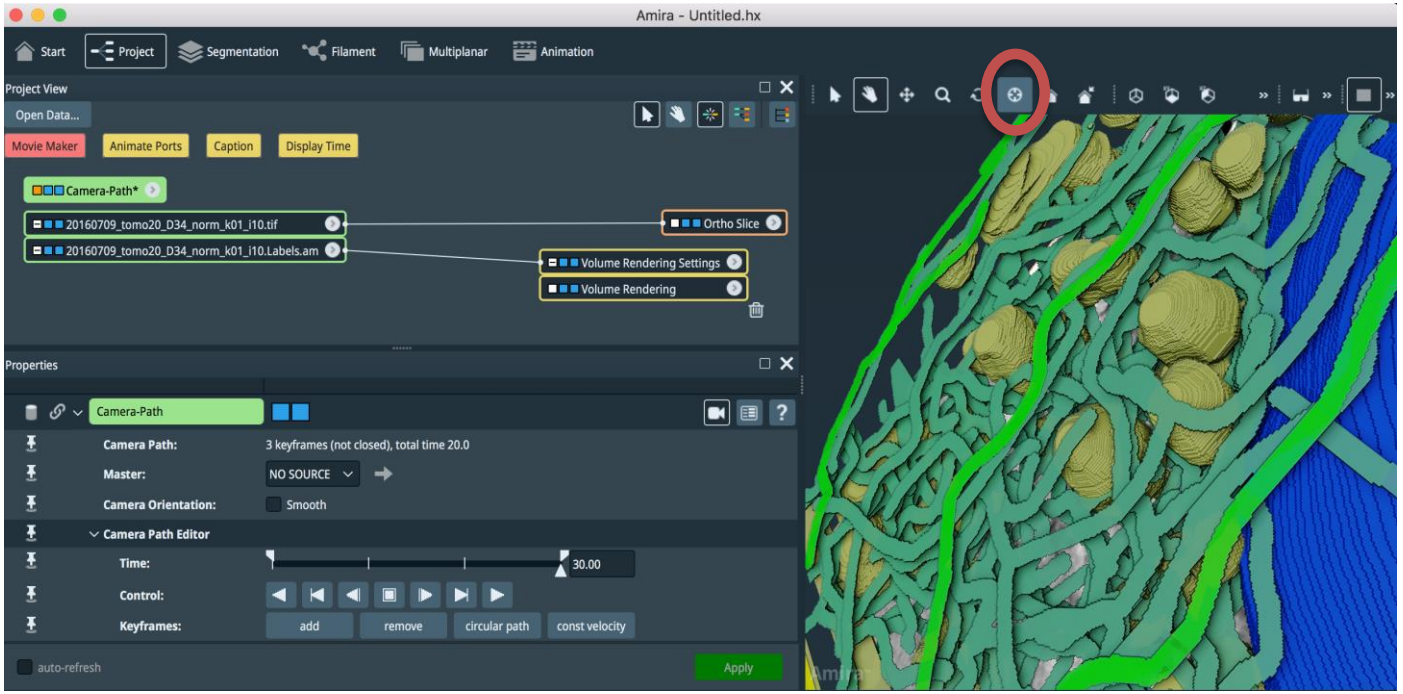
Open camera path editor,
Click camera Path editor



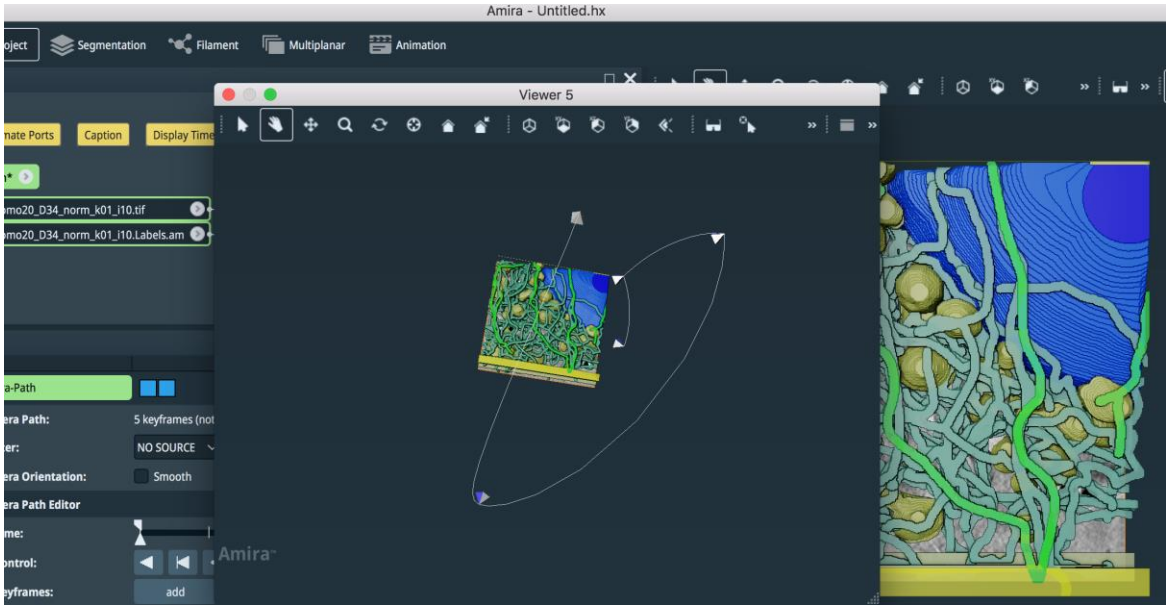
A separate camera path edit/view window will open.
Click camera Path editor.
Change view position in main window, then click “add” button to add a keyframe.



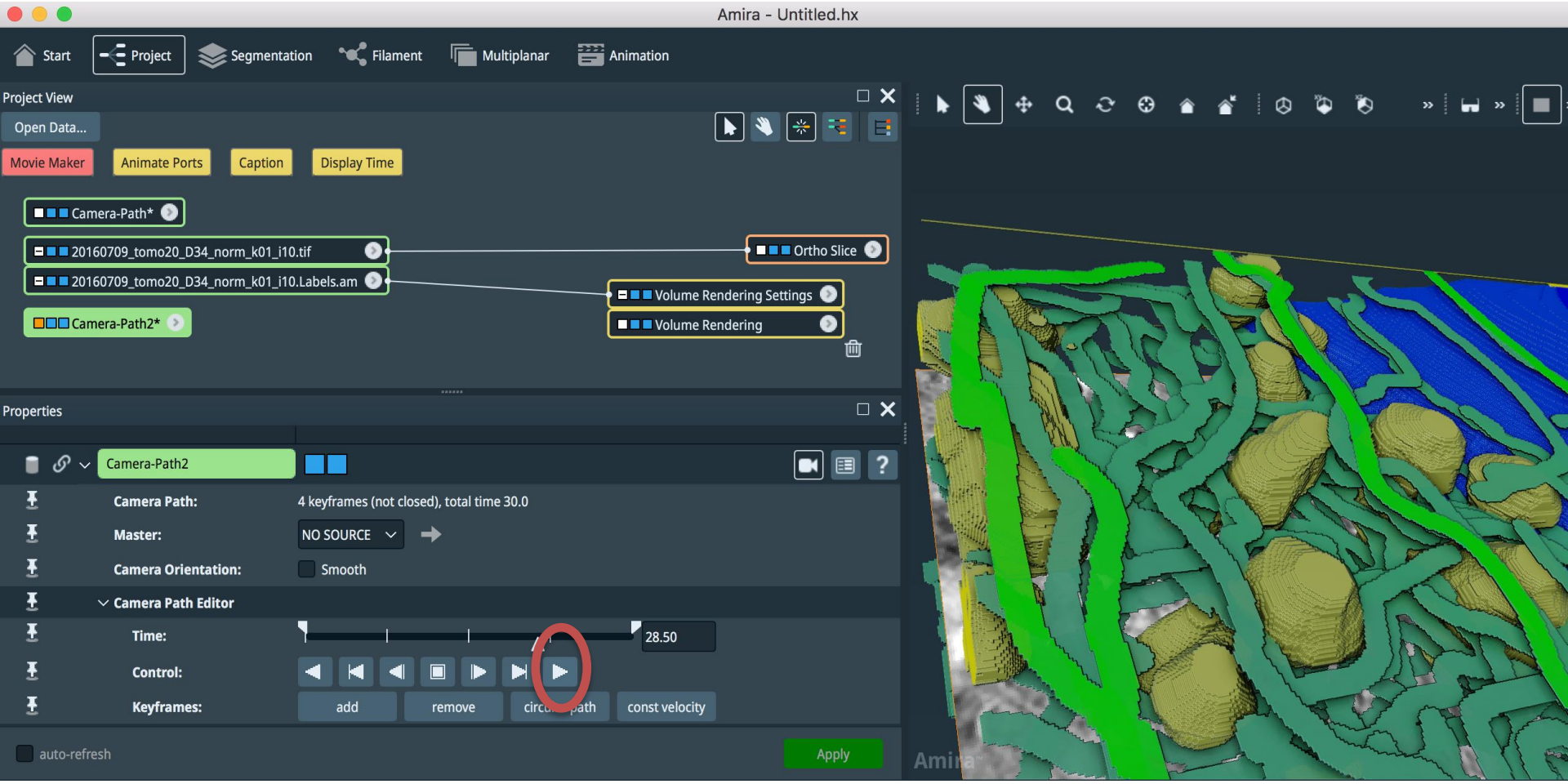
Change zoom level in main window, then click “add” button to add a keyframe.



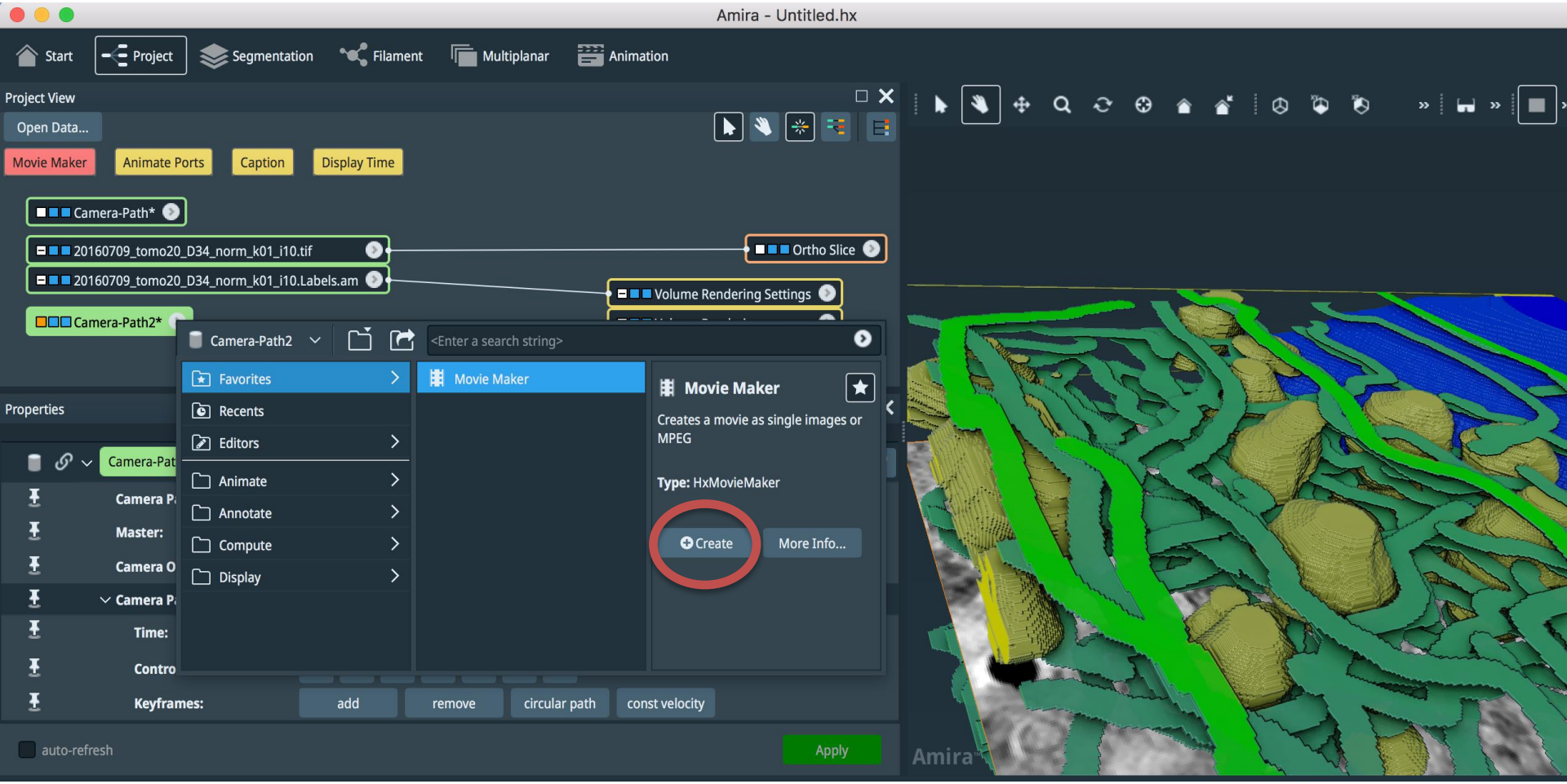
In the small window you can move the visualization camera of your volume



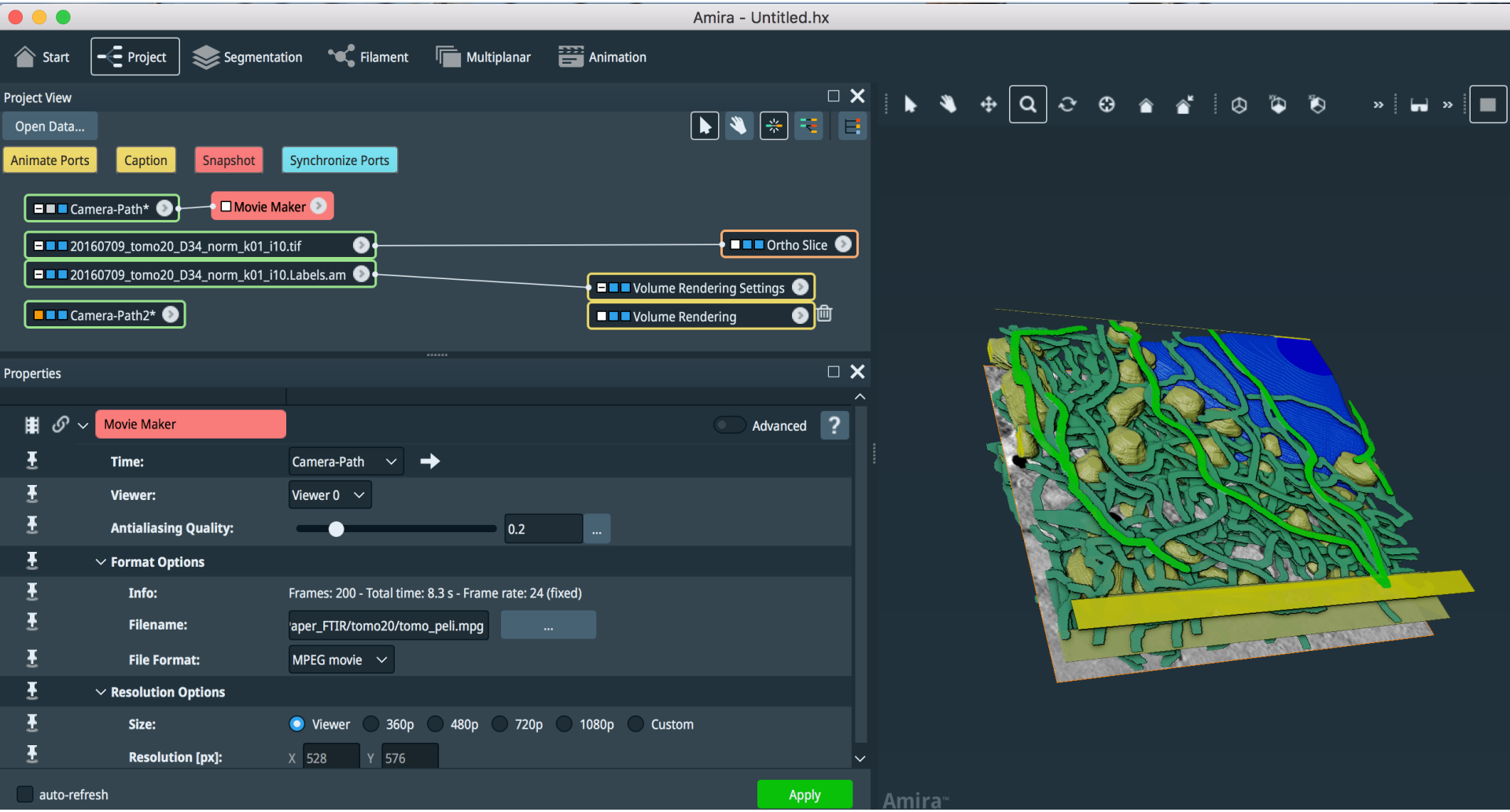
Check your movie



Click on empty space and select -> Favorites -> Movie Maker



Select your output file and the file format-> APPLY



AMIRA-AVIZO learning center on YouTube

For many more tutorials…..

https://www.youtube.com/playlist?list=PLoxdPzacxPYjDVMD4tPCaVbuQjxYizr_g

For example this is an interesting one (with some instruction to separated objects that cannot be separate by a simple threshold):
https://www.youtube.com/watch?v=YsOc5R80MFM