

AMIRA fast Manual

Amira schedule

<https://nextcloud.cells.es/s/w2nwK5bxRfabZ7Y>

[Connecting remotely from home to CELLS](#)

<https://winanalysis.cells.es/>

<https://confluence.cells.es/display/ISS/Remote+connection+to+Amira>

Actions that will be done only once for your Home computer.

Download and install Workspace application from Citrix downloads.

For Windows home computer <https://www.citrix.com/downloads/workspace-app/windows/>

For MacOS home computer <https://www.citrix.com/downloads/workspace-app/mac/>

For Linux home computer <https://www.citrix.com/downloads/workspace-app/linux/>

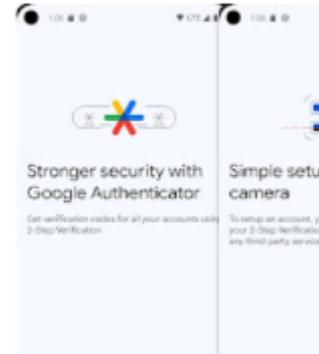
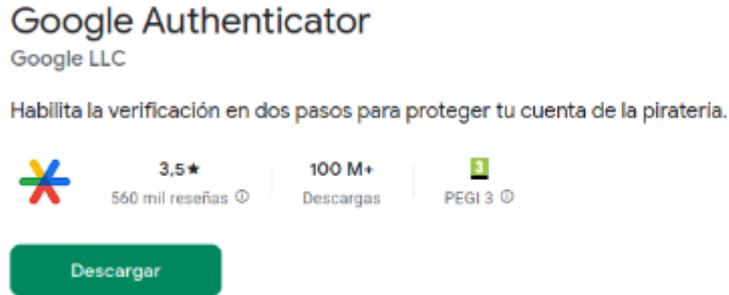
Close all instances of the browser you used and reopen it again (in order to ensure that the Citrix plugin is loaded)

Set up 2-Step Verification

2-SV will be needed and the users need to register their mobile phones. This is mandatory to all persons accessing Amira from outside Alba. Go to <https://winanalysis.cells.es/manageotp> to register your phones and setup the 2-SV.

Set up 2-Step Verification

1) Install the Google Authenticator app in your device (mobile phone or tablet)

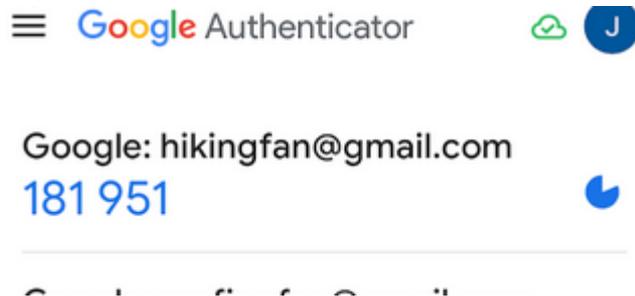


2) Then, from inside the Data Analysis desktop, go to the link <https://winanalysis.cells.es/manageotp/> to enroll your device (link it to your account):

3) Then go back to your phone, and in Google Authenticator click on the big plus sign (+) and then click on Escanear un código QR

Proceed to scan the QR code shown in the browser by pointing your phone to the PC screen:

If successful, the Google Authenticator app should show a random 6-digit number that will be changing every minute:

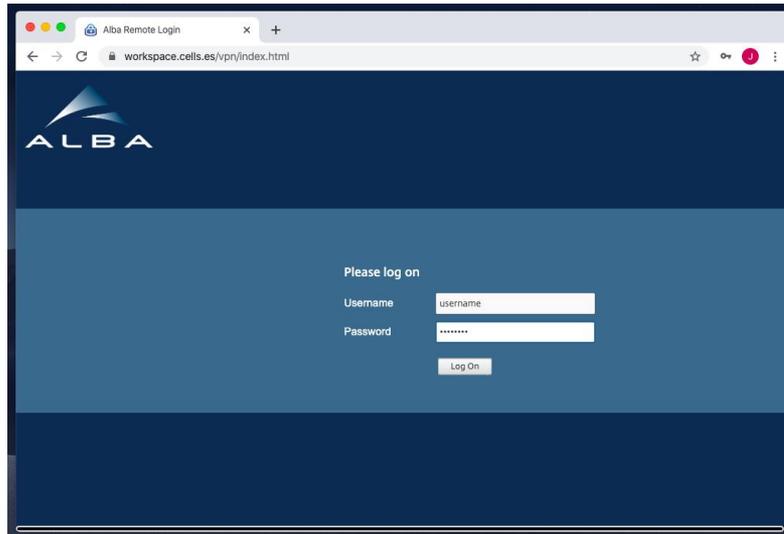


4) Now go back to the browser in the winanalysis desktop, you should your device listed as enrolled, and you can even test the connection:

Actions that should be done each time you want to connect.

1. Open a browser and go to <https://workspace.cells.es>

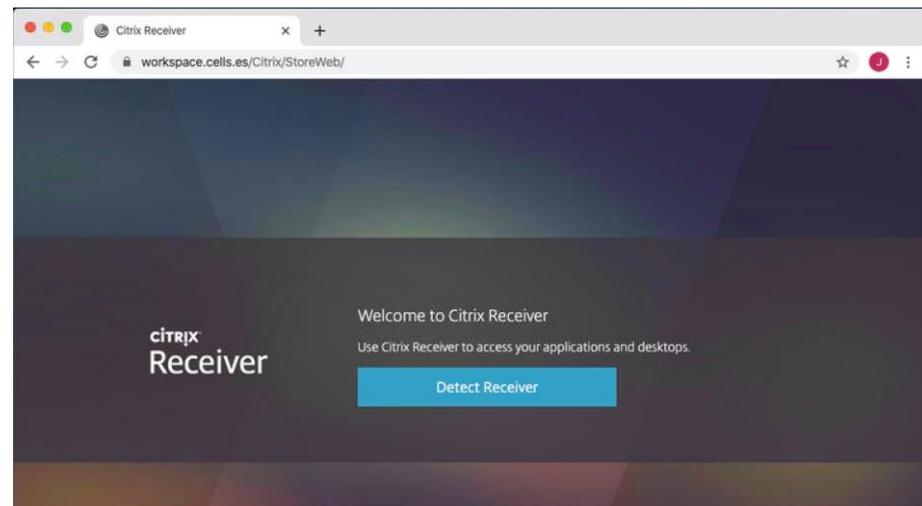
1.



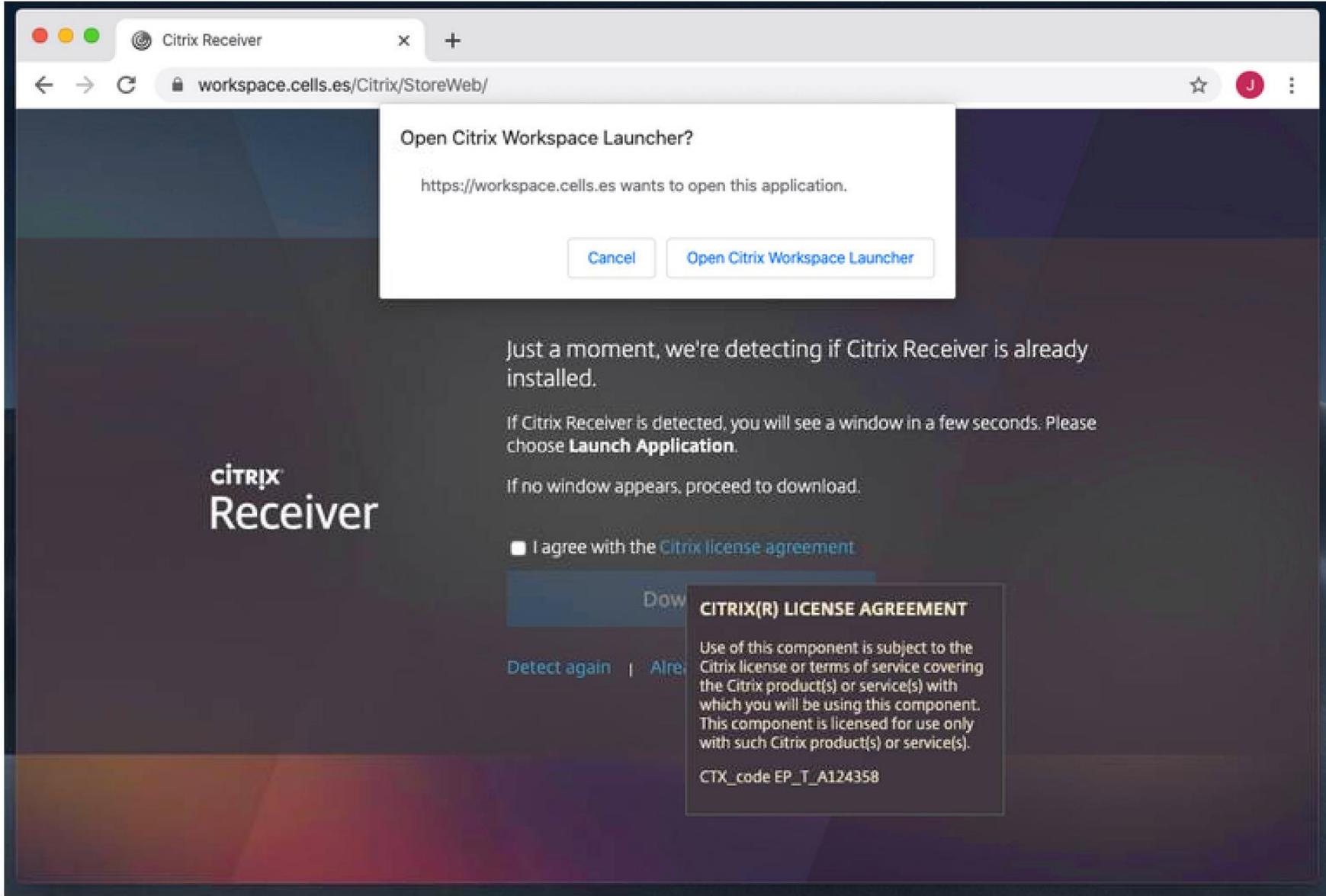
2. Type your username and password, remember that your username usually starts with u, ie u2018093095

3. Click Log On

4. Click Detect Receiver



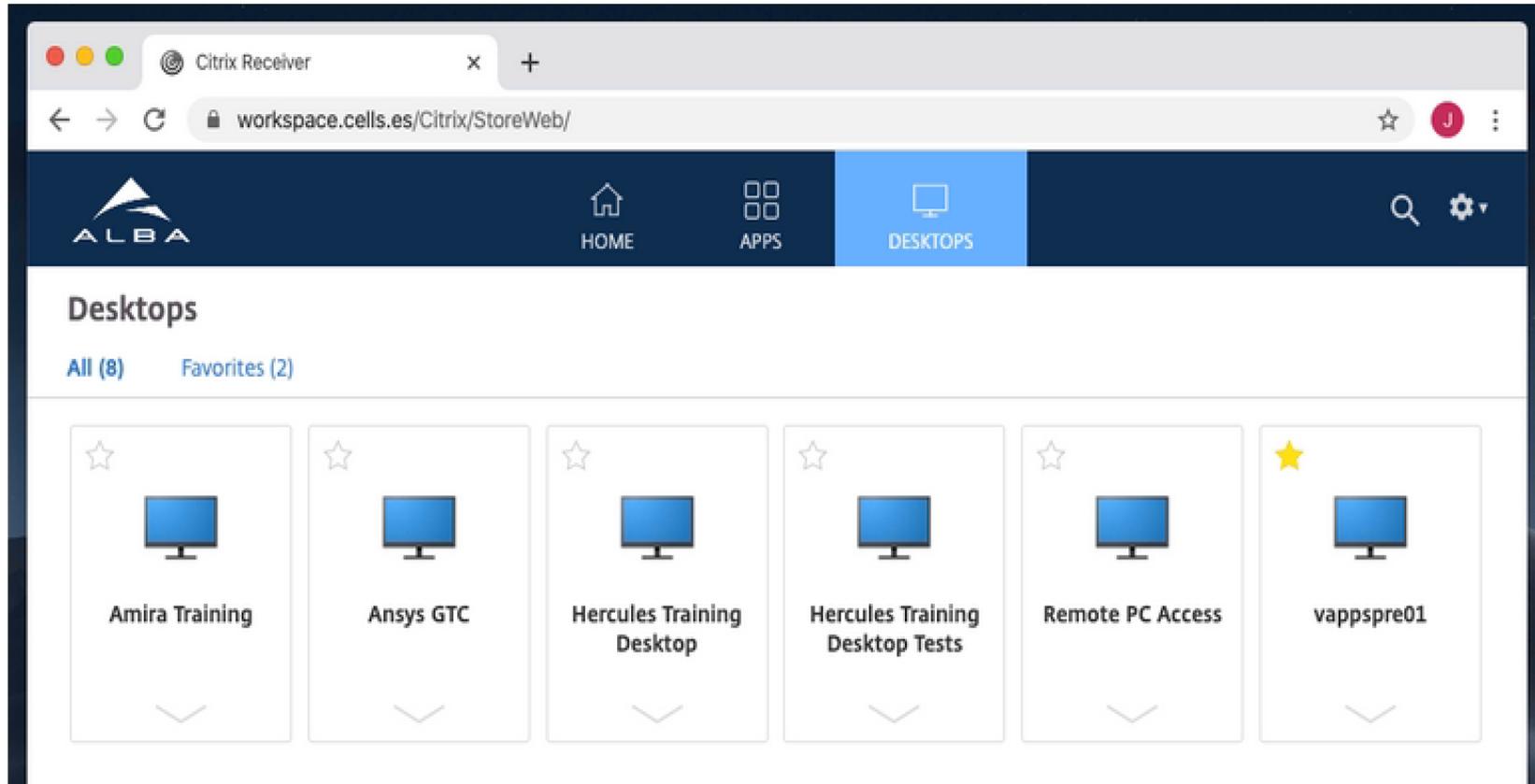
5. Allow any Citrix popup



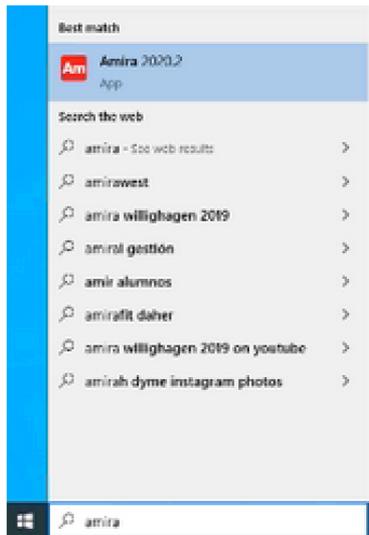
a.

6. Select the Desktops Section

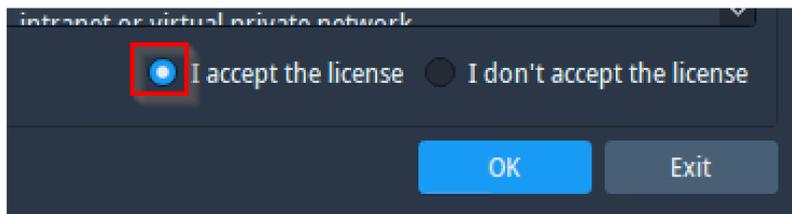
7. You will be presented with all your available virtual desktops. For your case click on **Amira Training**



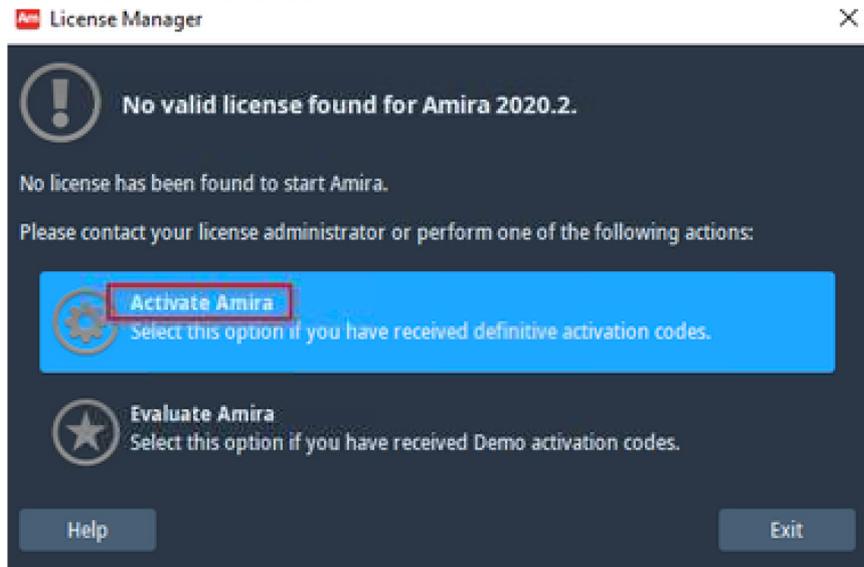
8. A .ica file will be downloaded, depending on your configuration it will ask you to open it with Citrix Workspace automatically or you should click on it to do so.
9. After a while you will be presented with your desktop
10. At some point it could appear a window about Citrix Workspace asking for an account just ignore it.
11. You can search "Amira" in the searching tab of Windows:



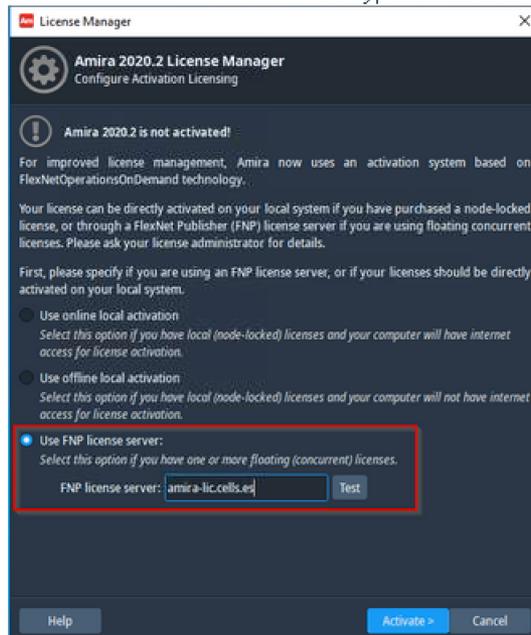
12. Accept the license



13. Activate Amira License



14. Select "Use FNP license server" and type "amira-lic.cells.es" into the FNP license server field.



15. If you want to browse your experimental data you will find it on Z: network unit.

16. Remember that everything, except the Z: contents, will be lost after your work.

Link for chedule Amira PC

https://drive.google.com/drive/folders/1j-ZLuzev2VD-4OrWcn4OaJIR_61C1i-u?usp=sharing

1) transmission signal

$$I = I_0 e^{-\int \mu_l(x,y,z) dz}$$

2) normalization

$$\frac{I}{I_0} = e^{-\int \mu_l(x,y,z) dz}$$

3) natural logarithm (has to be applied to the aligned stack)

$$-\ln\left(\frac{I}{I_0}\right) = \int \mu_l(x,y,z) dz$$

4) crop borders of the aligned ln stack symmetrically from the center of the projections (making sure that the borders are well cropped on all the stack projections): in ImageJ Image/Adjust/Canvas size keeping “center”)

5) 3D reconstruction of the absorbance (using tomo3d –w off or even better tomopy gridrec):

$$voxel = -\ln\left(\frac{I}{I_0}\right) = \mu_l$$

The value of each voxel is in 1/(pixel size) and the normal unit is μm^{-1} , if the pixel size used was for instance 10 nm, you need to divide the value by 0.01.

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)

After the alignment with IMOD

- If you are interested 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 series 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/software/TomoJ.html>

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 $1/(\text{pixel size})$ 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

The screenshot shows the Amira software interface. The main window displays the Amira logo and a "Welcome to Amira Software" message. Below this, there are sections for "RECENT DATA" and "RECENT PROJECTS". The "RECENT DATA" section lists several files, including "SXTSAM10547_B44_DMSOControlcells_dec...", "SXTSAM10548_2F4_DMSOControlcells_reco...", "SXTSAM10548_2F4_DMSOControlcells_reco...", "SXTSAM10552_6H2_Replicativecellstreated...", "SXTSAM10552_6H2_Replicativecellstreated...", and "20220709_tomo282_3DS30_IQM-PE-641_6h...". The "RECENT PROJECTS" section lists "b44.hx", "2Fbin2.hx", "2F.hx", "6Hbin2.hx", "Untitled.hx", "Untitled2.hx", and "amira_remdesivir.hx".

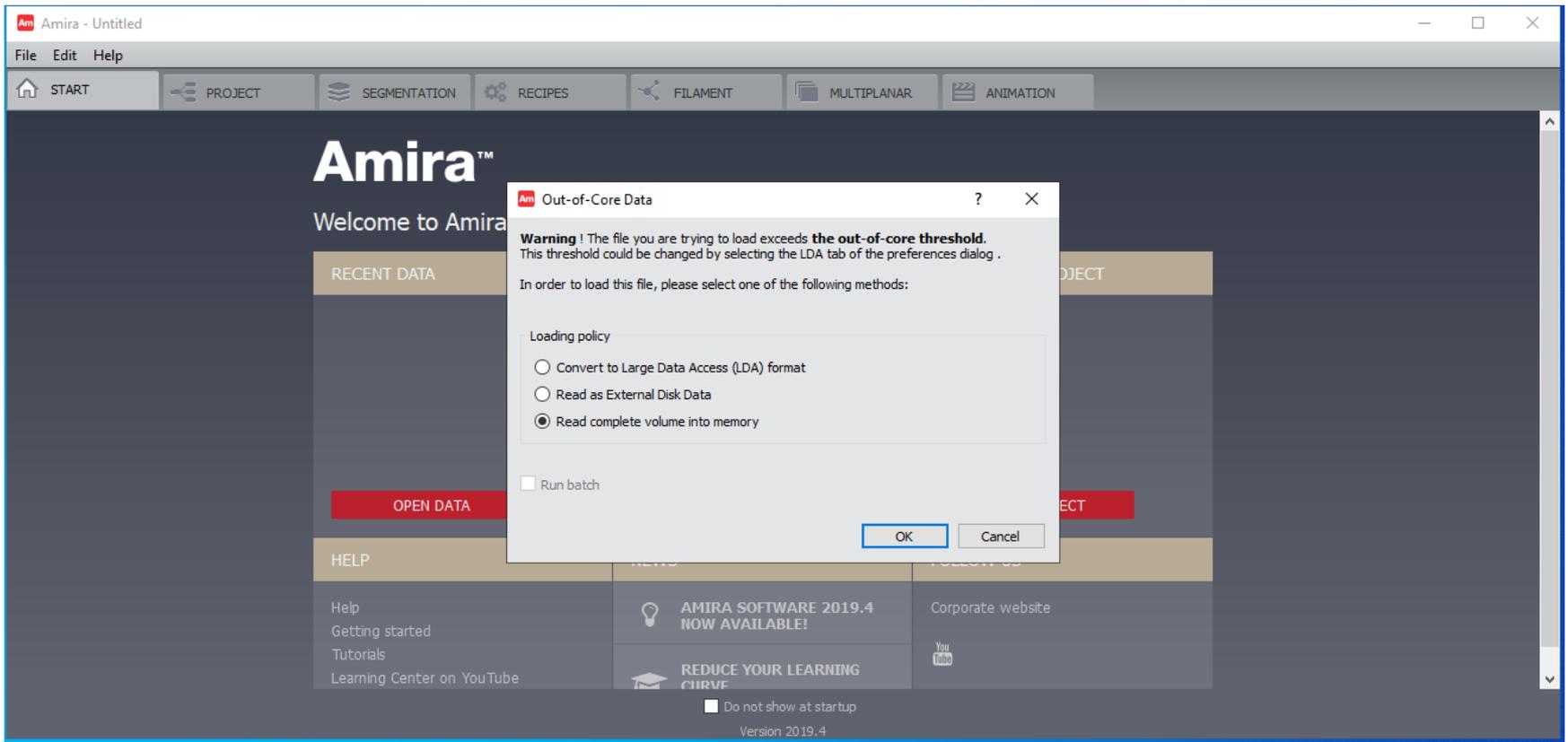
An "Open Data" dialog box is open over the software interface. The dialog box shows the file path: "Papers > drug_Sars_Cov-2 > Control_SARS-COV2 > tomo298". The file list contains the following items:

Name	Date modified	Type
Untitled-files	17/01/2024 12:33	File folder
20220710_tomo298_3DS30	14/12/2023 14:21	TIF File
20220710_tomo298_3DS30_8b	19/12/2023 11:51	TIF File
segmented_tomogram	19/12/2023 10:12	TIF File
Untitled	17/01/2024 16:19	Project

The "File name" field in the dialog box contains "20220710_tomo298_3DS30_8b". The "Open" button is highlighted with a red circle.

Select your pre-treated
DATA.tif

Load DATA 2



‘Big’ DATA needs to be loaded into memory.

Load DATA 3

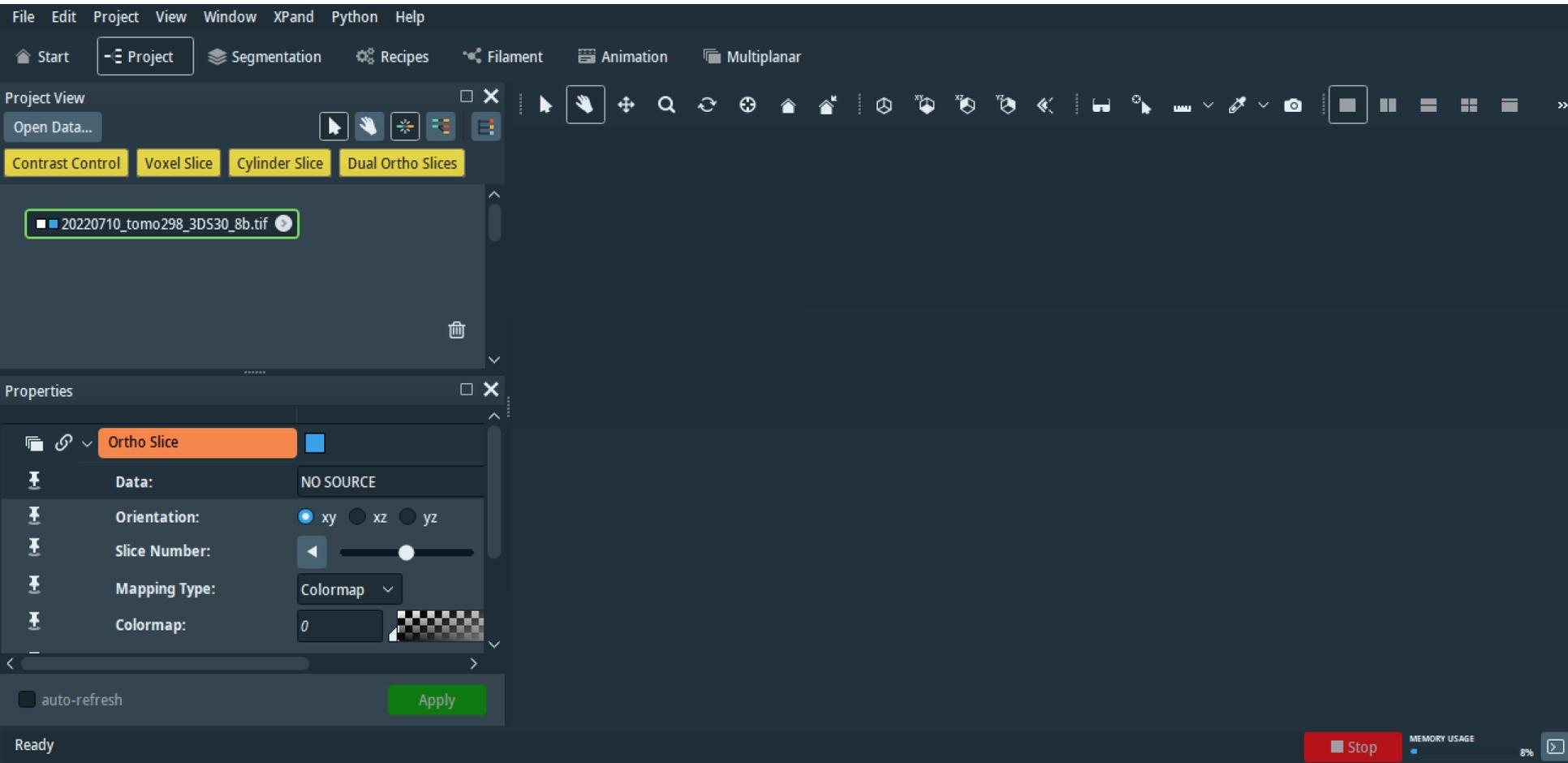
The screenshot displays the Amira software interface with the 'Image Read Parameters' dialog box open. The dialog box is titled 'Image Read Parameters' and contains the following sections:

- Info:** Files: 311, Image Size: 311 slices, 832x968, 1 channel, 1 time step
- Import:** Channel conversion: Channel 1, Object name: 20220710_tomo298_3DS30_8b.tif
- Resolution:** Define: bounding box, voxel size. The 'bounding box' option is circled in red. Below this are fields for Min coord (0, 0, 0) and Max coord (831, 967, 310).
- Read as multiple files:**
- Buttons:** OK, Cancel
- Footer:** Do not show at startup (checked)

The background shows the Amira software interface with the 'Amira' logo and 'Welcome to Amira Software' text. The 'RECENT DATA' section lists several files, and the 'HELP' section provides links to Help, Getting started, Tutorials, and Learning Center on YouTube.

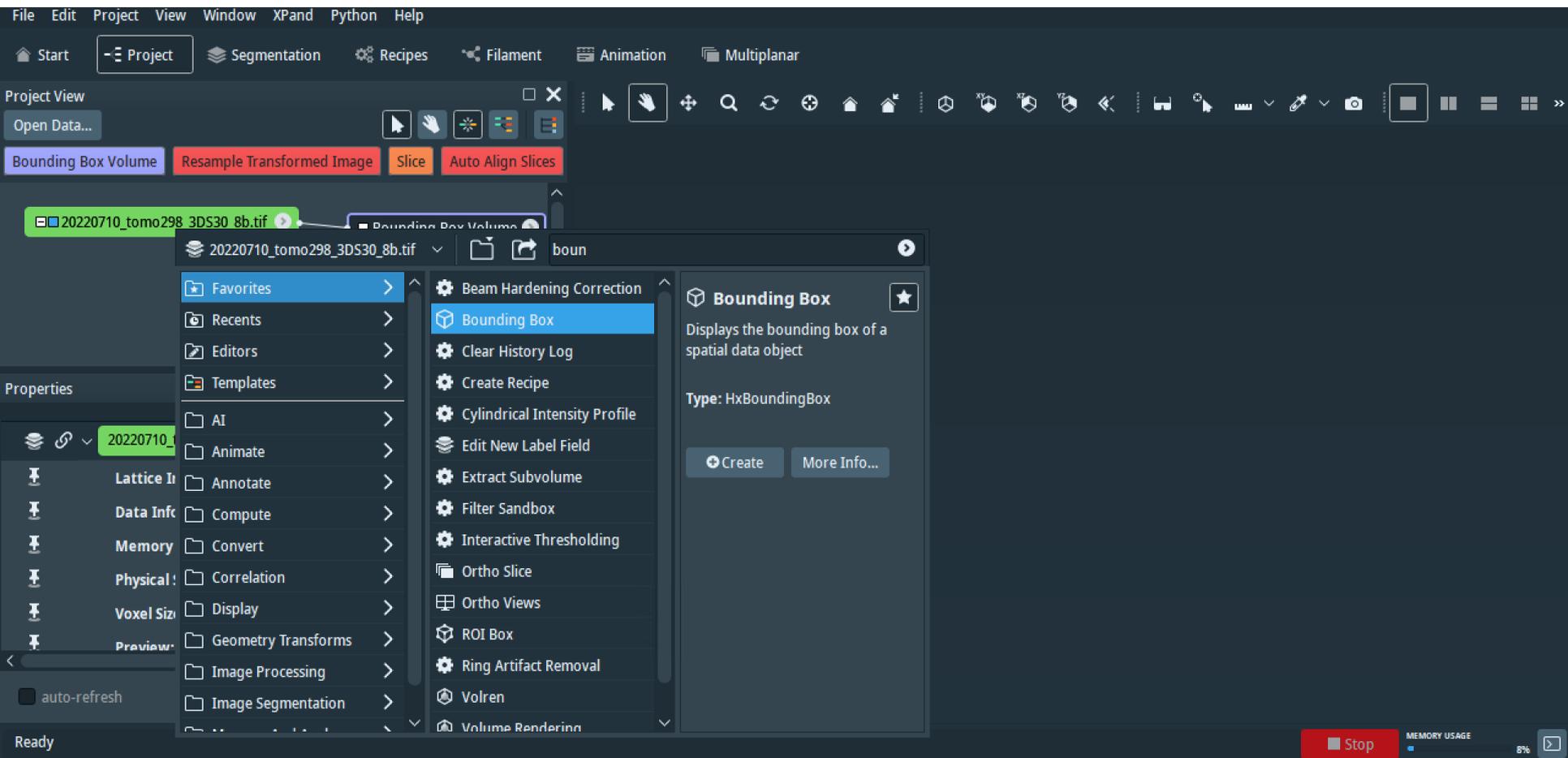
Select 'bounding box' option to forget about voxel size. Our unit volume/area/length is the 'voxel'

Visualize volume 1



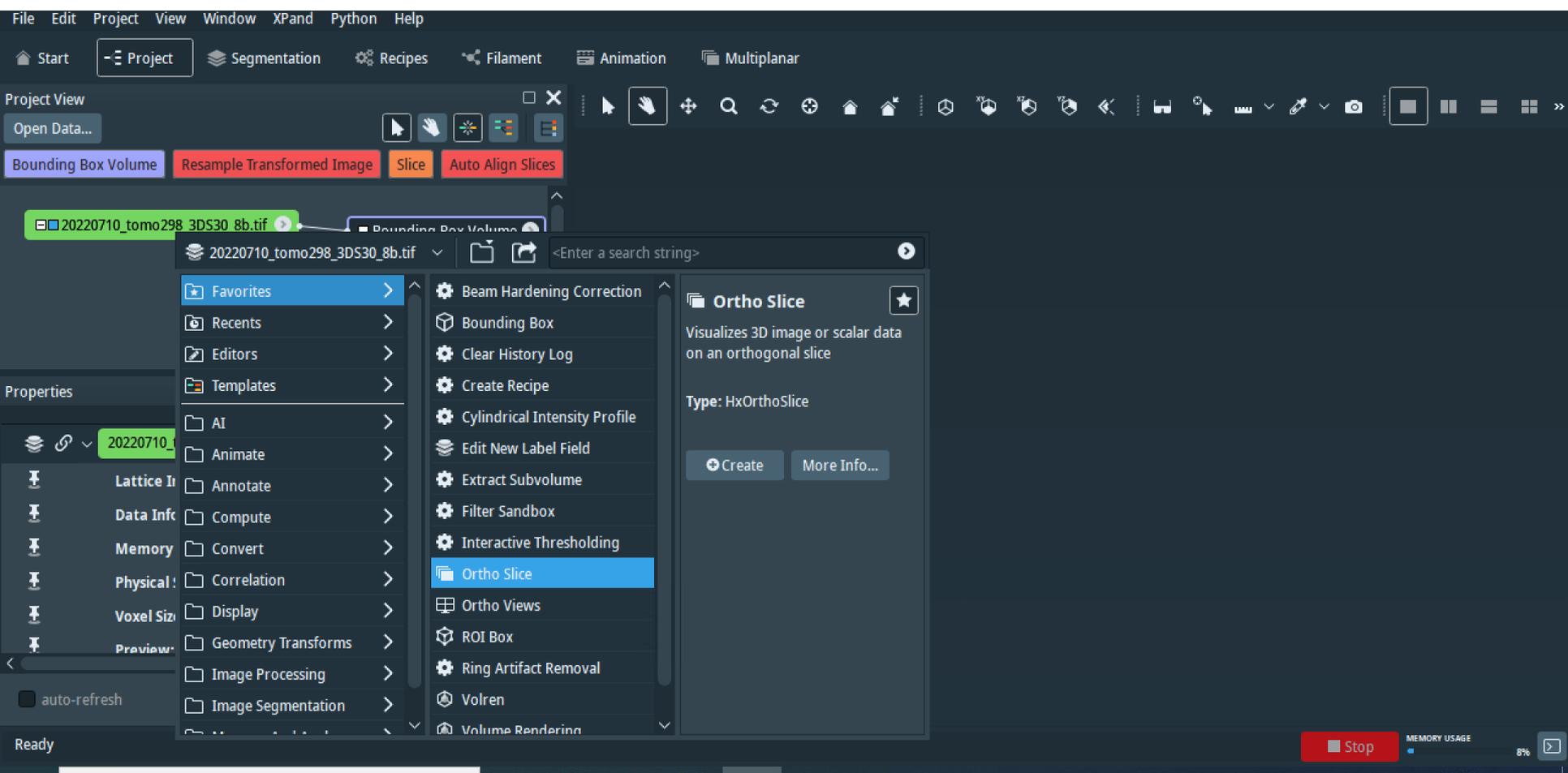
Your data are in the project window as a .tif object

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.

The screenshot displays the Amira 3D software interface. The main window shows a 3D visualization of a volume with an orange bounding box. The left sidebar contains the Project View and Properties panels. The Project View shows a tree structure with '20220710_tomo298_3DS30_8b.tif' selected, which is linked to 'Bounding Box' and 'Ortho Slice(2)'. The Properties panel for 'Ortho Slice(2)' is highlighted with a red box, showing the following attributes:

- Data:** 20220710_tomo298_3DS30_8b.tif
- Orientation:** xy (selected), xz, yz
- Slice Number:** 155
- Mapping Type:** Colormap
- Colormap:** 0 to 255
- Options:** adjust view, bilinear view, lighting
- Frame:** show, width: 1

The bottom status bar shows 'Ready', 'Stop', 'MEMORY USAGE 8%', and a refresh icon.

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

Visualize volume 5

Amira 3D - Untitled

File Edit Project View Window XPand Python Help

Start Project Segmentation Recipes Filament Animation Multiplanar

Project View

Open Data...

Contrast Control Voxel Slice Cylinder Slice Dual Ortho Slices

20220710_tomo298_3DS30_8b.tif Bounding Box Ortho Slice(2)

Properties

Ortho Slice(2)

Data: 20220710_tomo298_3DS30_8b.tif

Orientation: xy xz yz

Slice Number: 155

Mapping Type: Colormap

Colormap: 24 194 Edit

Options: adjust view bilinear view lighting

Frame: show width: 1

auto-refresh Apply

Ready Stop MEMORY USAGE 8%

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

Visualize volume 6

Amira 3D - Untitled

File Edit Project View Window XPand Python Help

Start Project Segmentation Recipes Filament Animation Multiplanar

Project View

Open Data...

Contrast Control Voxel Slice Cylinder Slice Dual Ortho Slices

20220710_tomo298_3DS30_8b.tif Bounding Box Ortho Slice(2)

Properties

Ortho Slice(2)

Data: 20220710_tomo298_3DS30_8b.tif

Orientation: xy xz yz

Slice Number: 155

Mapping Type: Histogram

Contrast Limit: 1

Options: adjust view bilinear view lighting

Frame: show width: 1

auto-refresh Apply

Ready Stop MEMORY USAGE 8%

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

Visualize volume 7

The screenshot displays the Amira 3D software interface. The main window shows a grayscale volume slice of a biological specimen, outlined with an orange border. The interface includes a menu bar (File, Edit, Project, View, Window, XPand, Python, Help), a toolbar with icons for Start, Project, Segmentation, Recipes, Filament, Animation, and Multiplanar, and a Project View panel on the left. The Project View panel shows a tree structure with the following items: 20220710_tomo298_3DS30_8b.tif (highlighted with a green box), Bounding Box (highlighted with a yellow box), and Ortho Slice(2) (highlighted with an orange box). The Properties panel on the right shows the settings for the selected Ortho Slice(2) object. The Orientation is set to xy (highlighted with a red box), and the Slice Number is 155. Other settings include Mapping Type: Histogram, Contrast Limit: 1, and Frame: show width: 1. The status bar at the bottom indicates 'Ready' and 'MEMORY USAGE 8%'.

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

Visualize volume 8

The screenshot displays the Amira 3D software interface. The top menu bar includes File, Edit, Project, View, Window, XPand, Python, and Help. Below the menu is a toolbar with icons for Start, Project, Segmentation, Recipes, Filament, Animation, and Multiplanar. The Project View panel on the left shows a tree structure with '20220710_tomo298_3DS30_8b.tif' selected, which is linked to 'Bounding Box' and 'Ortho Slice(2)'. The Properties panel for 'Ortho Slice(2)' is visible, with the 'Orientation' dropdown set to 'xz' (highlighted by a red box). Other settings include 'Data: 20220710_tomo298_3DS30_8b.tif', 'Slice Number: 546', 'Mapping Type: Histogram', 'Contrast Limit: 1', and 'Options: adjust view, bilinear view, lighting'. The 'Frame' section has 'show' checked and 'width: 1'. The main 3D view on the right shows a grayscale volume with an orange bounding box and a slice plane. The bottom status bar shows 'Ready', a 'Stop' button, and 'MEMORY USAGE 8%'.

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

Visualize volume 9

The screenshot displays the Amira 3D software interface. The main window shows a 3D visualization of a volume with three orthogonal slices (Ortho Slice(2), Ortho Slice, and Ortho Slice(3)) overlaid. The interface includes a menu bar (File, Edit, Project, View, Window, XPand, Python, Help), a toolbar with icons for Start, Project, Segmentation, Recipes, Filament, Animation, and Multiplanar, and a Project View panel with buttons for Contrast Control, Voxel Slice, Cylinder Slice, and Dual Ortho Slices. The Properties panel for 'Ortho Slice(2)' is highlighted with a red box, showing the following settings:

- Data: 20220710_tomo298_3DS30_8b.tif
- Orientation: xy xz yz
- Slice Number: 250
- Mapping Type: Histogram
- Contrast Limit: 1
- Options: adjust view bilinear view lighting
- Frame: show width: 1

The bottom status bar shows 'Ready', a 'Stop' button, and 'MEMORY USAGE 8%'.

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.

The screenshot displays the Amira 3D software interface. The top menu bar includes File, Edit, Project, View, Window, XPand, Python, and Help. Below the menu is a toolbar with icons for Start, Project, Segmentation, Recipes, Filament, Animation, and Multiplanar. The Project View panel on the left shows a tree structure with a file named '20220710_tomo298_3DS30_8b.tif' and three child items: 'Ortho Slice(2)', 'Ortho Slice', and 'Ortho Slice(3)'. The Properties panel at the bottom left is focused on the 'Ortho Slice(3)' module. It shows a red circle with the number '1' around a link icon. Other settings include Data: '20220710_tomo298_3DS30_8b.tif', Orientation: 'yz', Slice Number: '693', Mapping Type: 'Histogram', and Contrast Limit: '1'. An 'Apply' button is visible at the bottom right of the Properties panel. The main 3D view on the right shows a grayscale volume with three orthogonal slices (axial, sagittal, and coronal) outlined in orange.

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

Amira 3D - Untitled

File Edit Project View Window XPand Python Help

Start Project Segmentation Recipes Filament Animation Multiplanar

Project View

Open Data...

Contrast Control Voxel Slice Cylinder Slice Dual Ortho Slices

20220710_tomo298_3DS30_8b.tif

Ortho Slice(2)

Viewer Toggle (H)
The check boxes control the visibility of the object in viewers.
You can hide all visualizations from the active viewer excepted those associated with the selected objects clicking on H

Properties

Ortho Slice(2)

Data: 20220710_tomo298_3DS30_8b.tif

Orientation: xy xz yz

Slice Number: 250

Mapping Type: Histogram

Contrast Limit: 1

Options: adjust view bilinear view lighting

Frame: show width: 1

auto-refresh Apply

Ready Stop MEMORY USAGE 8%

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

Visualize volume 12

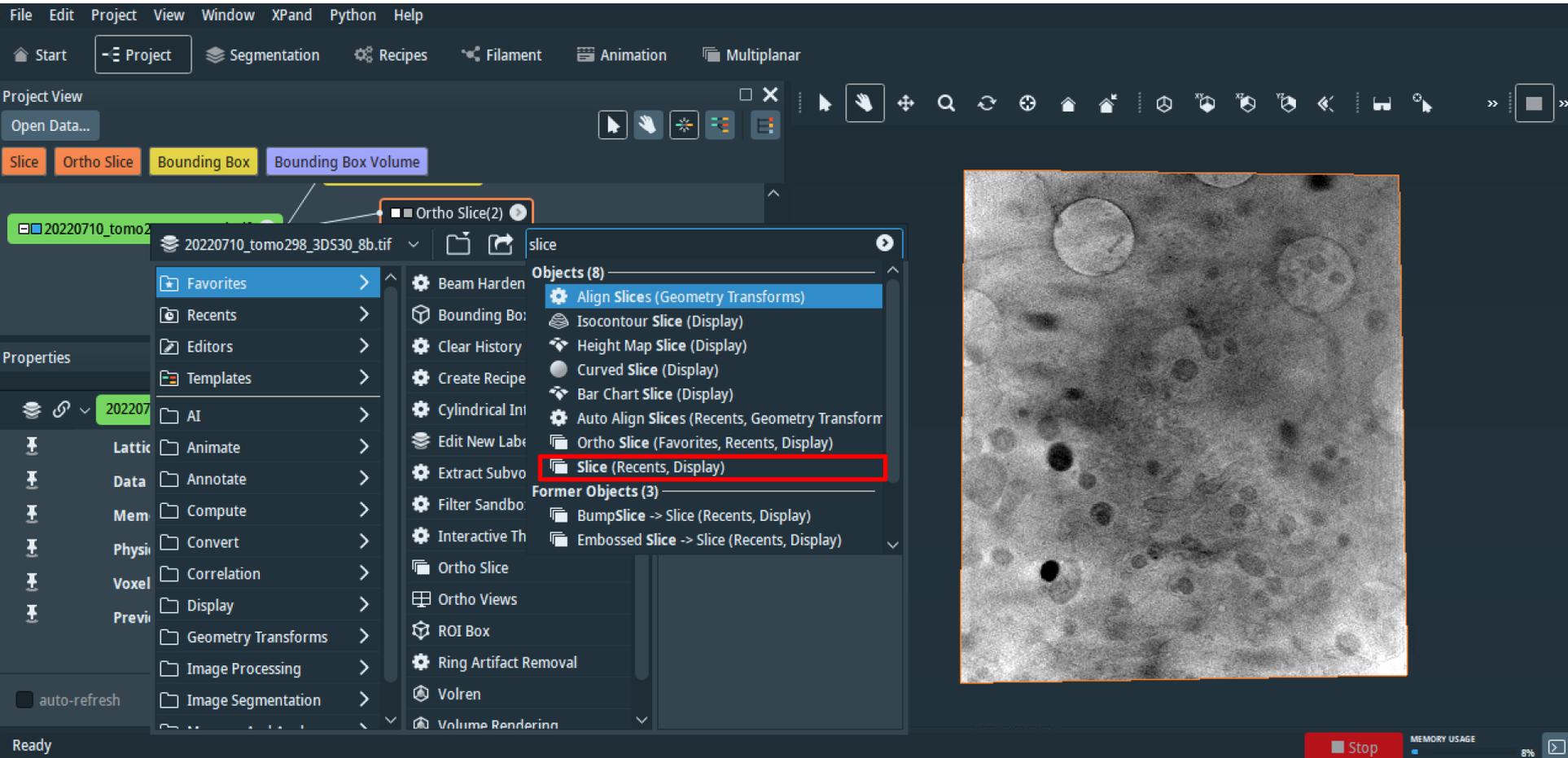
Change the visualization orientation using the 'slice' and 'Resample Transformed Image' module.

The screenshot displays the Amira 3D software interface. The top menu bar includes File, Edit, Project, View, Window, XPand, Python, and Help. Below the menu is a toolbar with icons for Start, Project, Segmentation, Recipes, Filament, Animation, and Multiplanar. The Project View panel on the left shows a tree structure with a file named '20220710_tomo298_3DS30_8b.tif' and three child nodes: 'Ortho Slice(2)', 'Ortho Slice', and 'Ortho Slice(3)'. The Properties panel on the left is configured for the 'Ortho Slice' module. The 'Data' field is set to '20220710_tomo298_3DS30_8b.tif'. The 'Orientation' is set to 'xy'. The 'Slice Number' is set to 0. The 'Mapping Type' is set to 'Histogram'. The 'Contrast Limit' is set to 1. The 'Options' section includes checkboxes for 'adjust view', 'bilinear view', and 'lighting', all of which are unchecked. The 'Frame' section includes a checkbox for 'show width' which is checked. The 'auto-refresh' checkbox is unchecked. The 'Apply' button is visible at the bottom right of the Properties panel. The main 3D visualization area on the right shows a grayscale volume with several circular features, viewed from an angle that is not perpendicular to the slice plane.

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

Visualize volume 14

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

Visualize volume 16

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

The screenshot displays the Amira 3D software interface. The top menu bar includes File, Edit, Project, View, Window, XPand, Python, and Help. The main toolbar contains icons for Start, Project, Segmentation, Recipes, Filament, Animation, and Multiplanar. The Project View panel on the left shows a tree structure with the file '20220710_tomo298_3DS30_8b.tif' selected. Below it, the 'Slice' module is active, showing two 'Ortho Slice' objects. The Properties panel for the 'Slice' module is visible, with the 'fit to points' option checked and highlighted by a red box. The 3D visualization on the right shows a grayscale volume with several circular features, representing the holes of a quantifoil.

Amira 3D - Untitled

File Edit Project View Window XPand Python Help

Start Project Segmentation Recipes Filament Animation Multiplanar

Project View

Open Data...

Contrast Control Cylinder Slice Dual Ortho Slices Isocontour Slice

20220710_tomo298_3DS30_8b.tif

Ortho Slice(2)

Ortho Slice

Slice

Properties

Data: 20220710_tomo298_3DS30_8b.tif

Orientation: xy xz yz

Translate: 155

Mapping Type: Colormap

Colormap: 0 255 Edit

Frame: show width: 1

Options: adjust view rotate immediate fit to points lighting

Sampling: fine interp. data interp. texture square texels move low res.

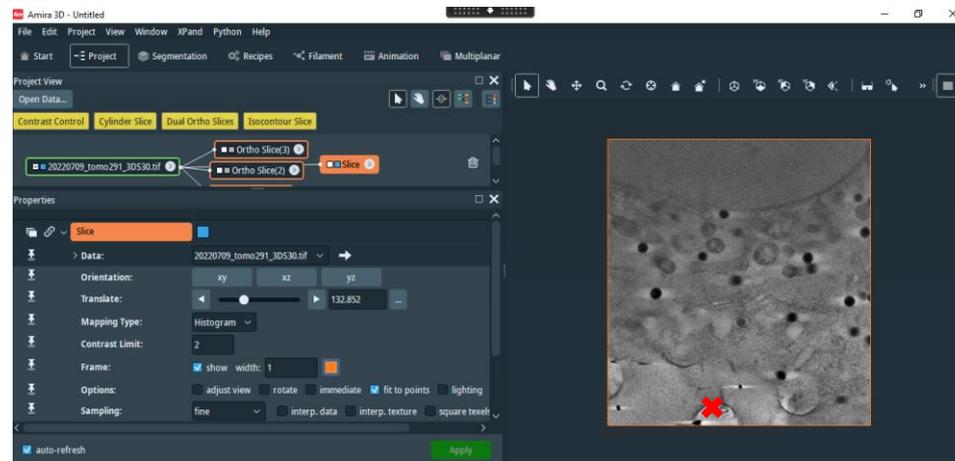
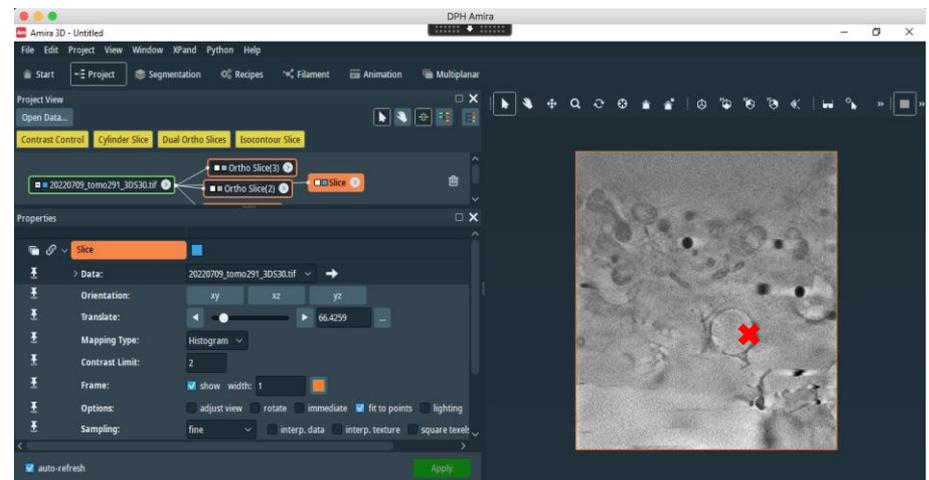
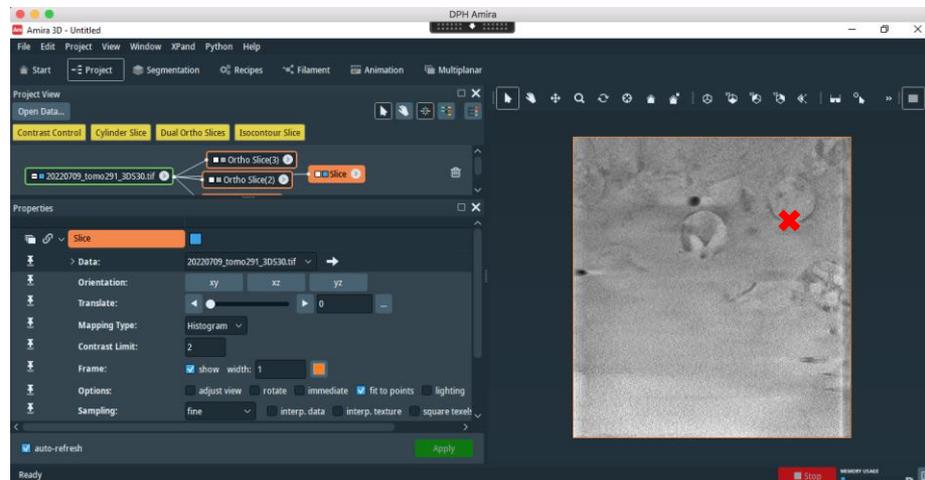
Transparency: None Binary Alpha

auto-refresh Apply

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.

The screenshot displays the Amira 3D software interface. The top menu bar includes File, Edit, Project, View, Window, XPand, Python, and Help. Below the menu is a toolbar with icons for Start, Project, Segmentation, Recipes, Filament, Animation, and Multiplanar. The Project View panel on the left shows a tree structure with a file named '20220709_tomo291_3DS30.tif' and its associated modules: 'Ortho Slice(3)', 'Ortho Slice(2)', 'Ortho Slice', and 'Slice'. The Properties panel at the bottom left provides detailed information for the selected file:

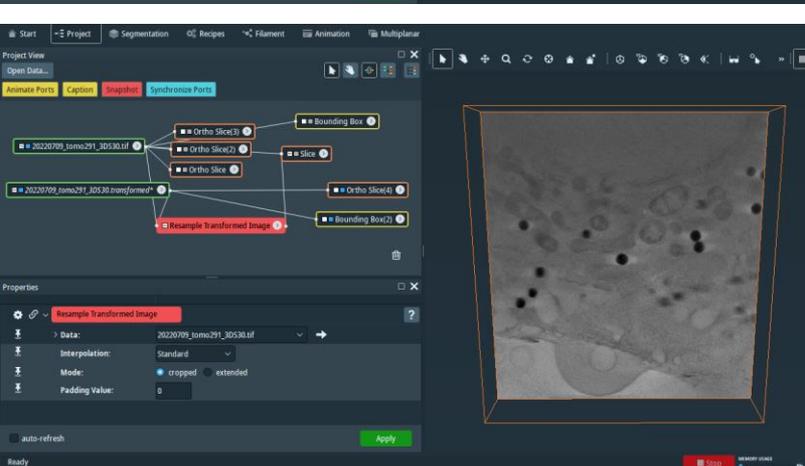
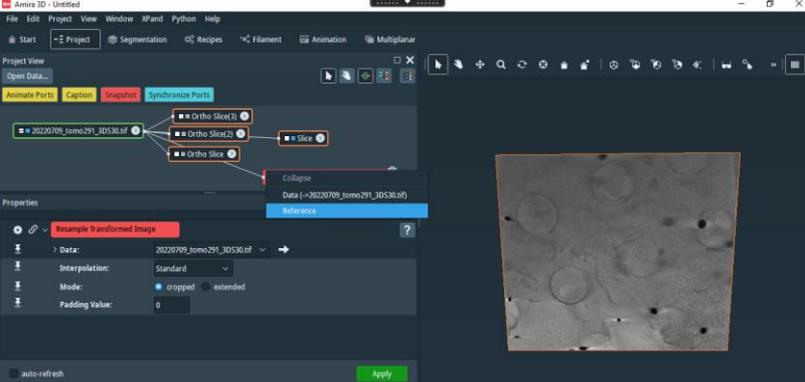
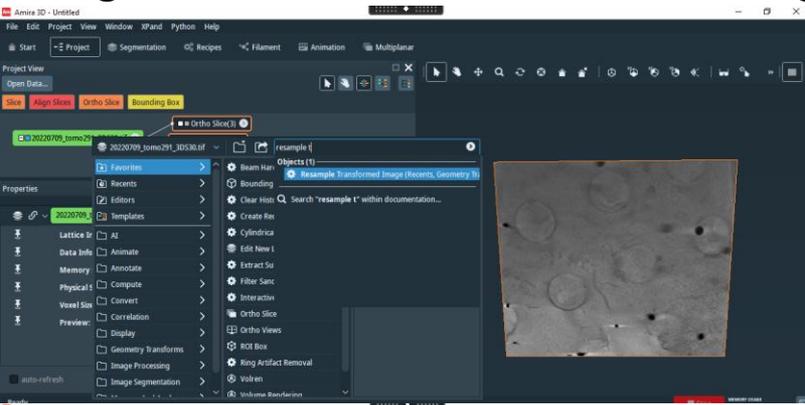
Property	Value
Lattice Info:	884 x 972 x 423, uniform coordinates
Data Info:	grayscale, 8-bit unsigned, min-max: 0...255
Memory Size:	346.6 MB
Physical Size:	883, 971, 422 from 0, 0, 0
Voxel Size:	1 x 1 x 1
Preview:	

The main visualization area on the right shows a large grayscale volume with a rectangular slice plane overlaid. The interface also includes a toolbar with navigation and manipulation tools, and a status bar at the bottom with a 'Stop' button, 'MEMORY USAGE' indicator, and a 8% CPU usage display.

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

Visualize volume 16

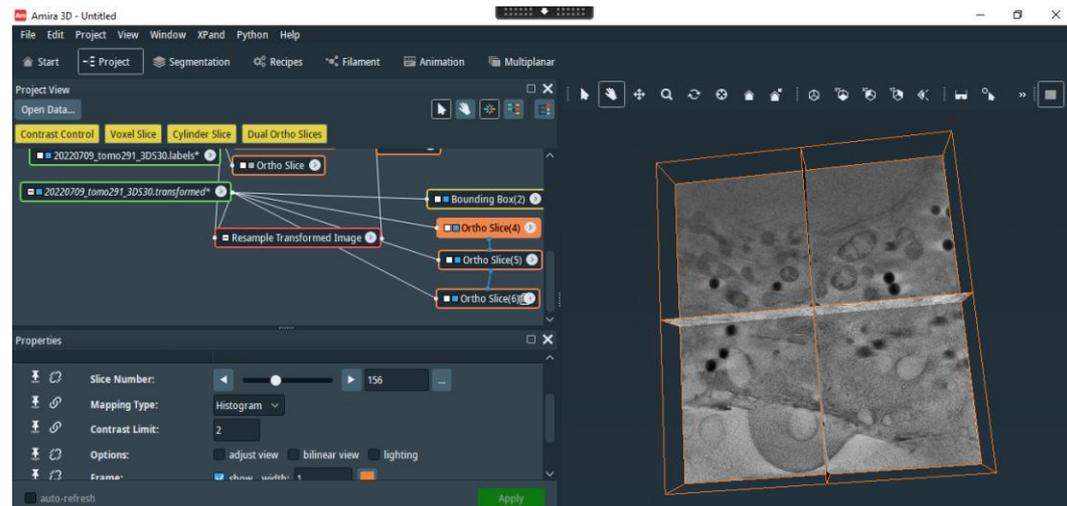
Change the visualization orientation using the 'slice' and 'Resample Transformed Image' module.



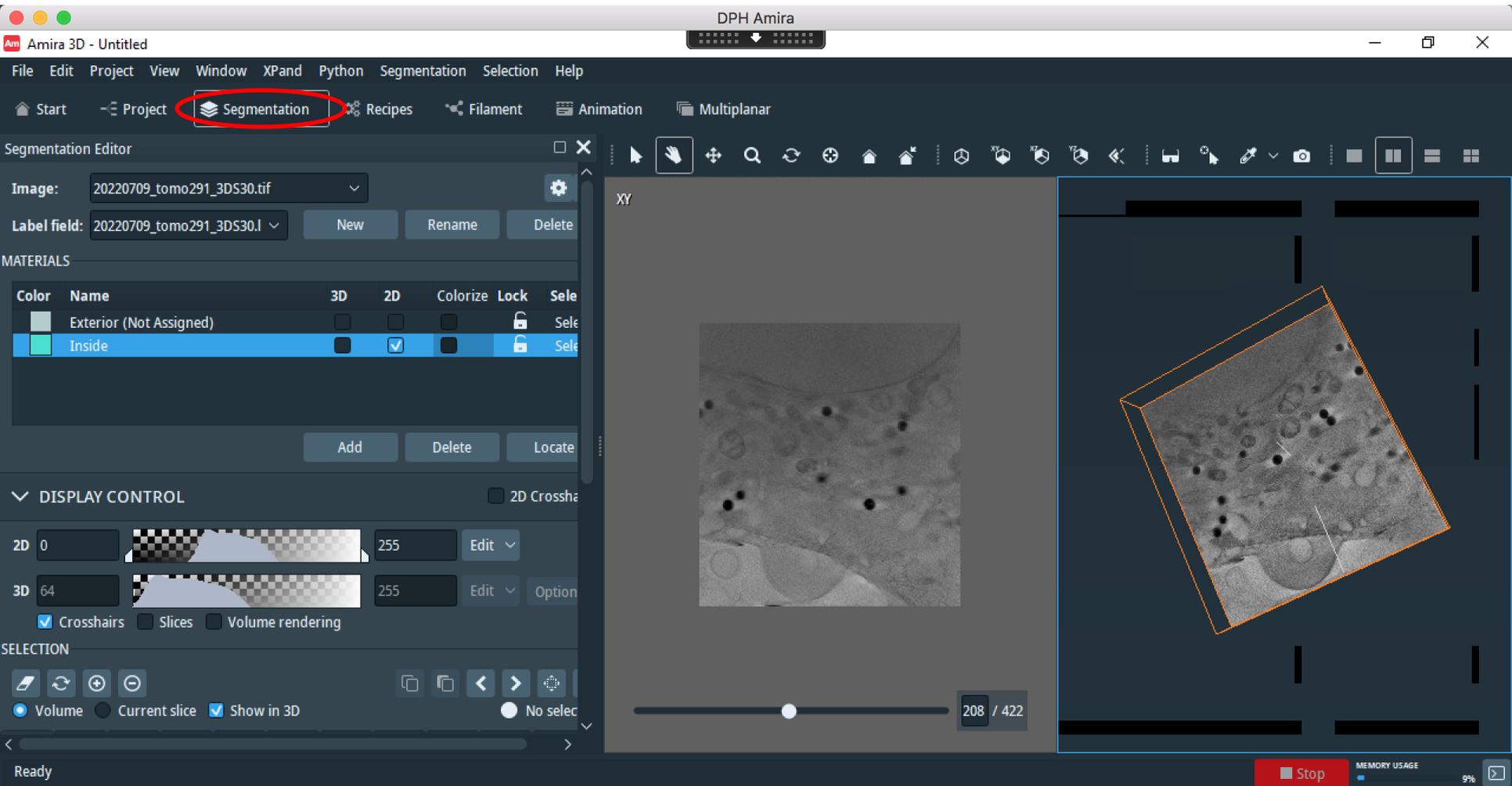
To apply the transformation to the data in such a way to use the newly oriented data in the SEGMENTATION EDITOR, create the computational module "Resample Transformed Image" (right click on data and search for it). Then left click on the white box and connect the "data" with the your data and the "reference" 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!)

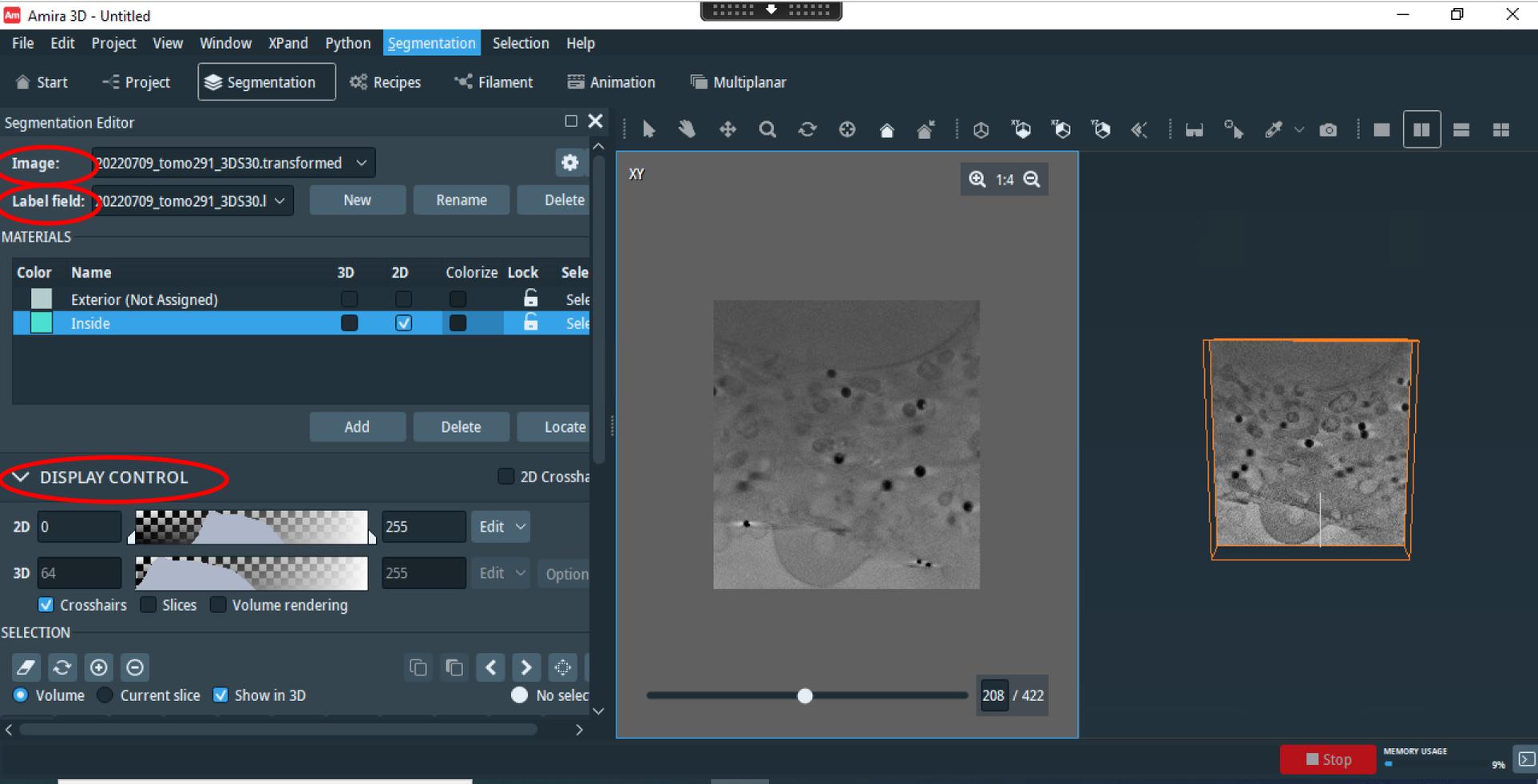
Computational modules are in general in RED.



Segmentation Editor 1



Segmentation Editor 2

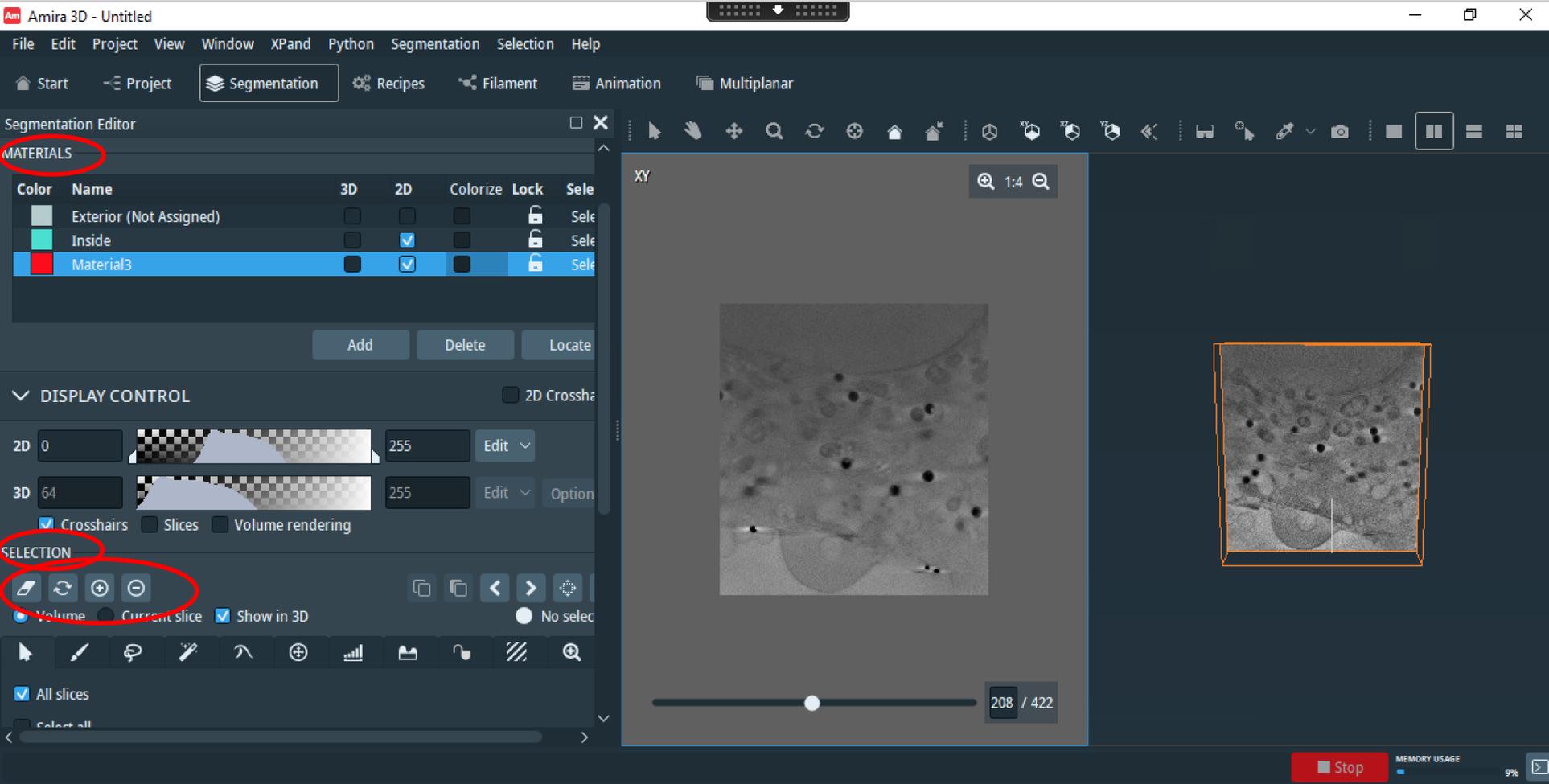


In *image* select the data you want to segment (in this case for instance the transformed one)

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



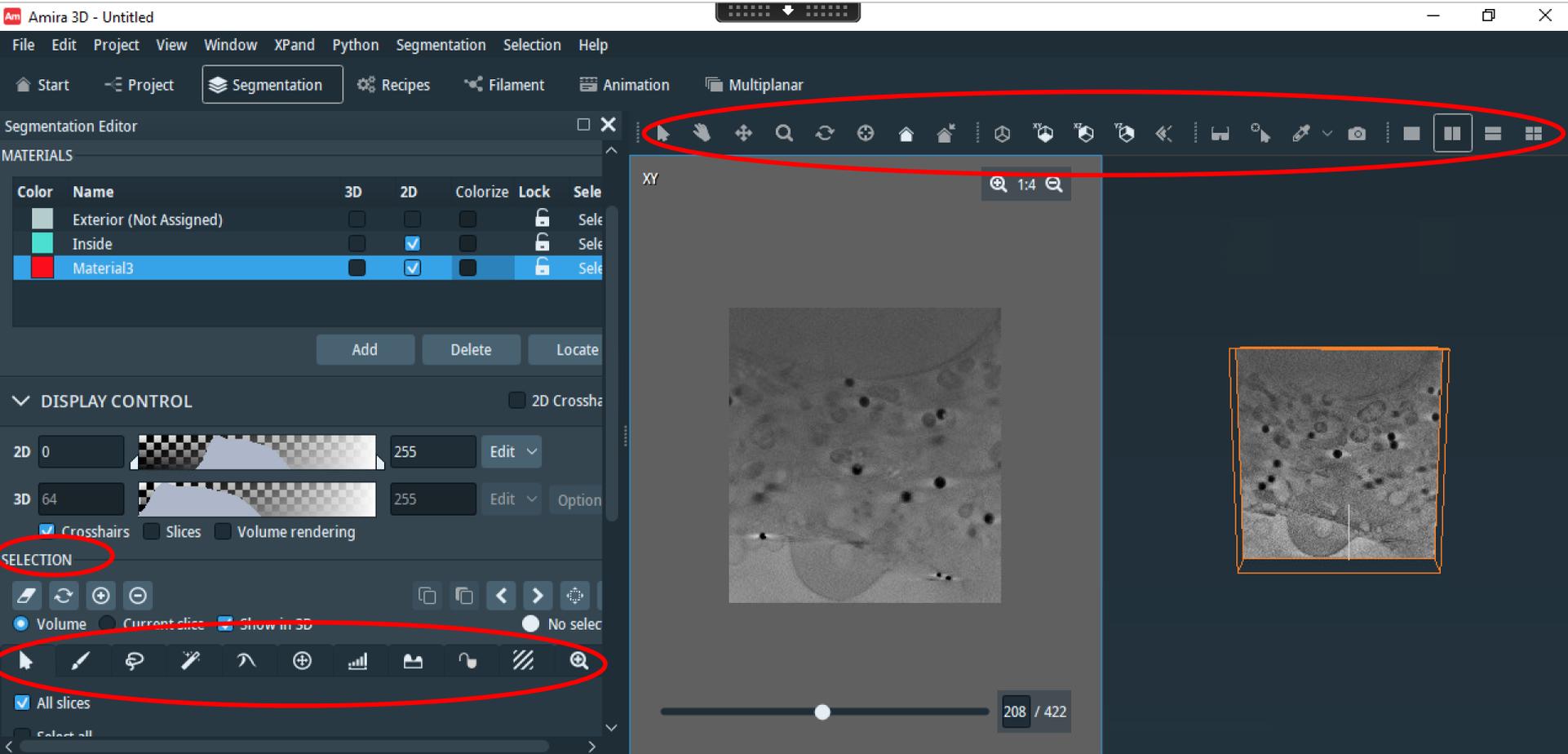
In *Materials* you have to press "add" to create a new material (Material3 in this case).

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

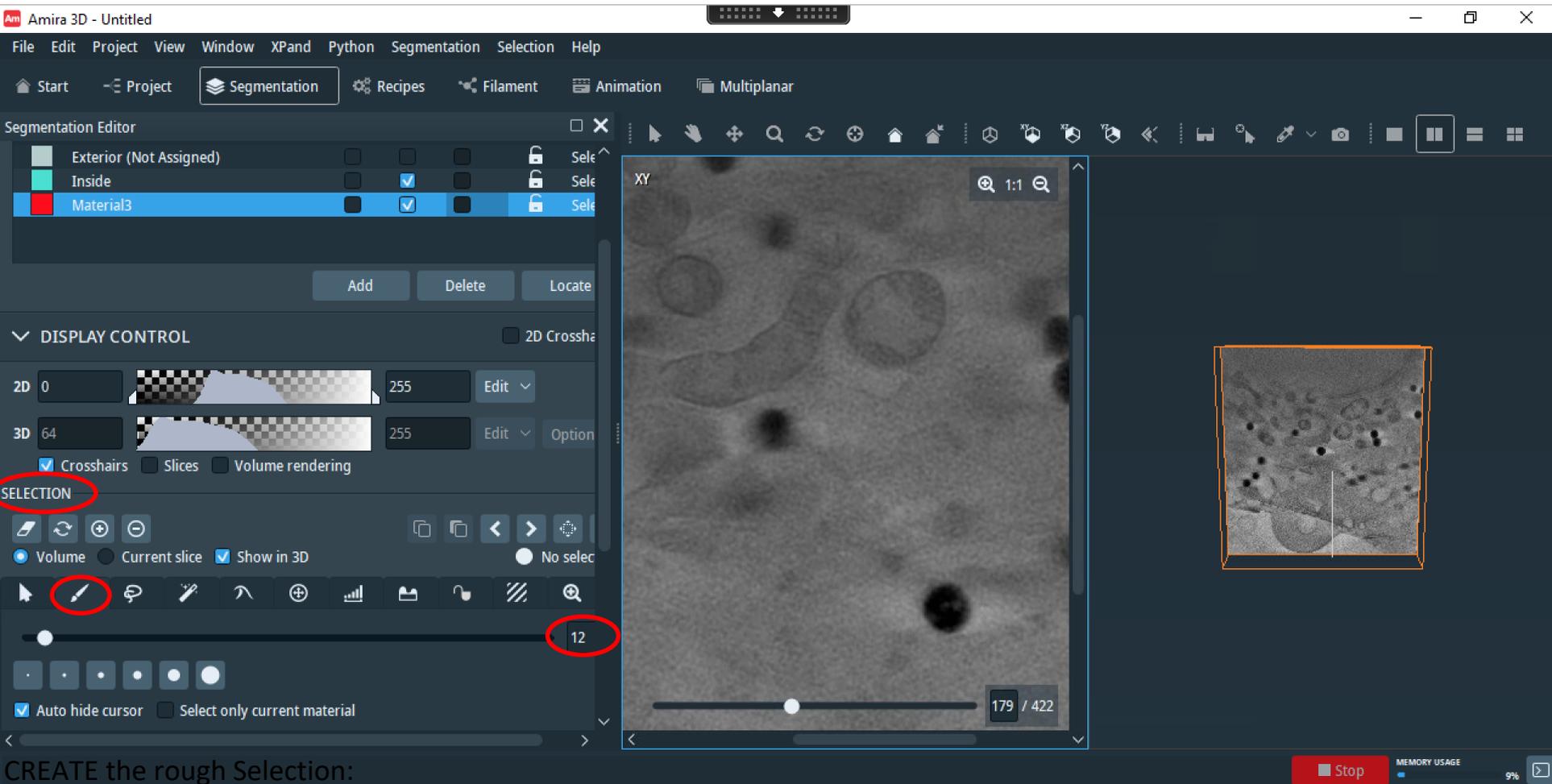


Use the tools in *Selection* to create it, i.e. to select the pixels you want to assign to some material.

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!

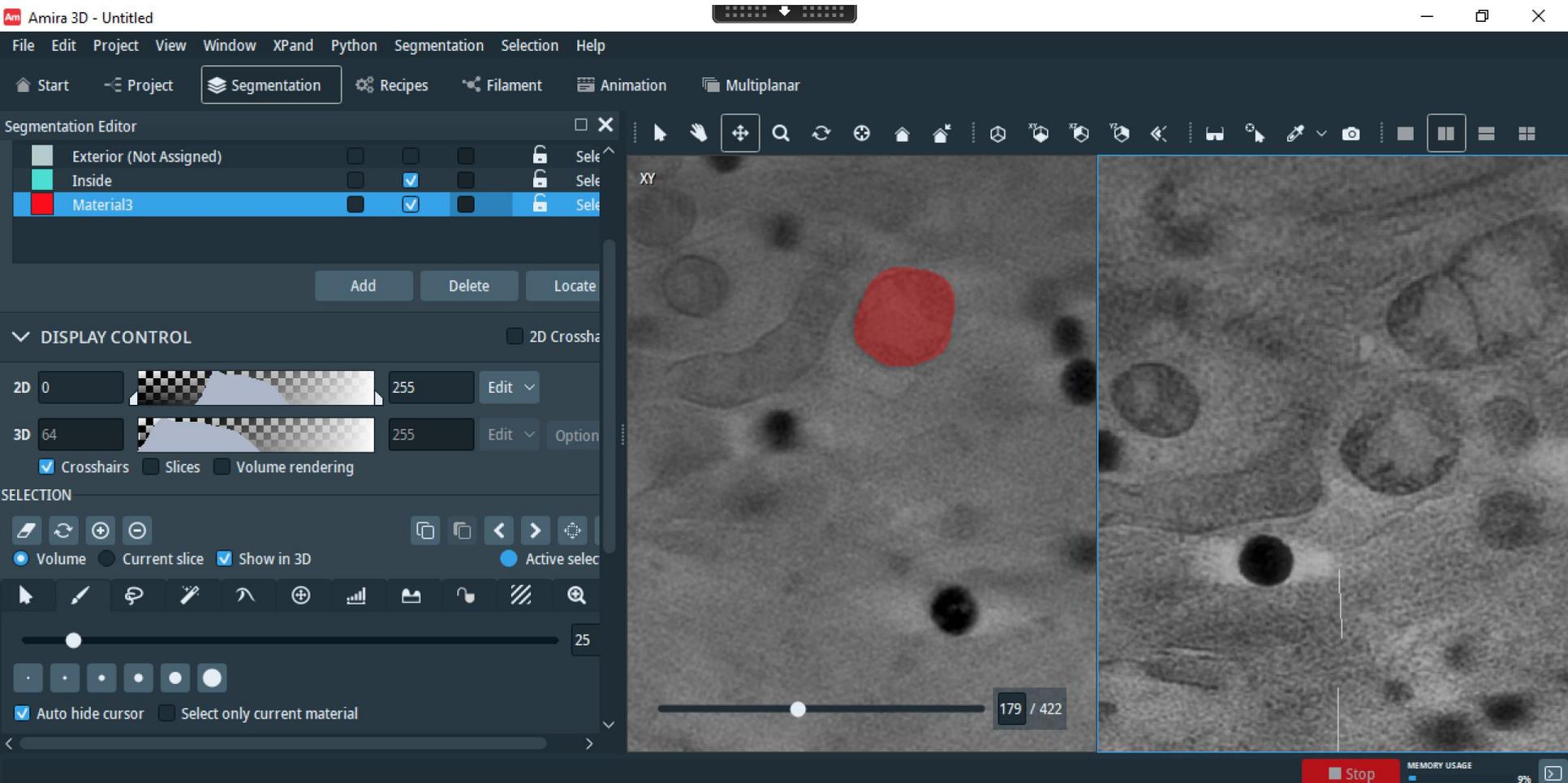
Semi-manual segmentation of a mitochondrion1



CREATE the rough Selection:

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

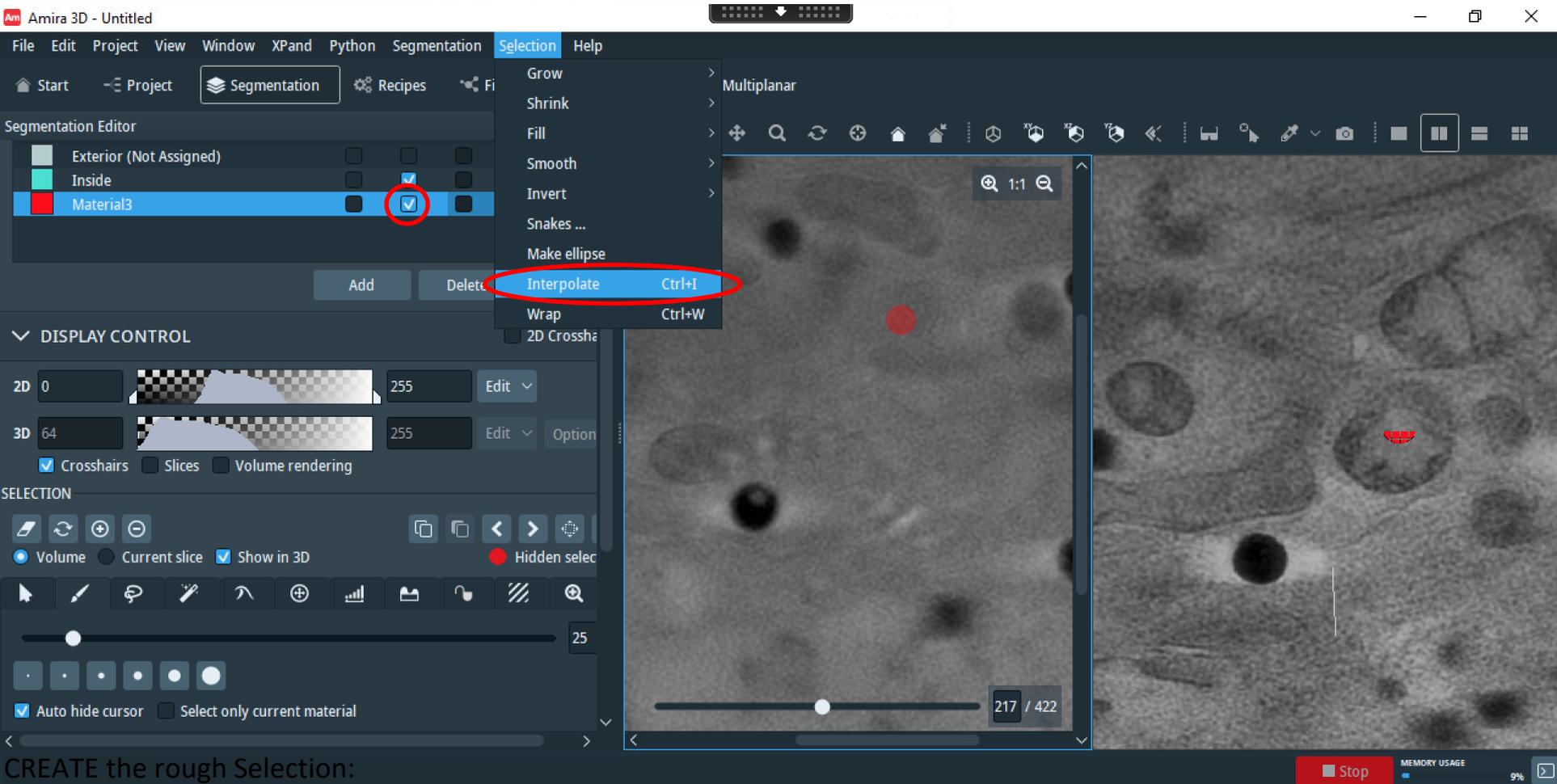
Semi-manual selection of a mitochondrion2



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

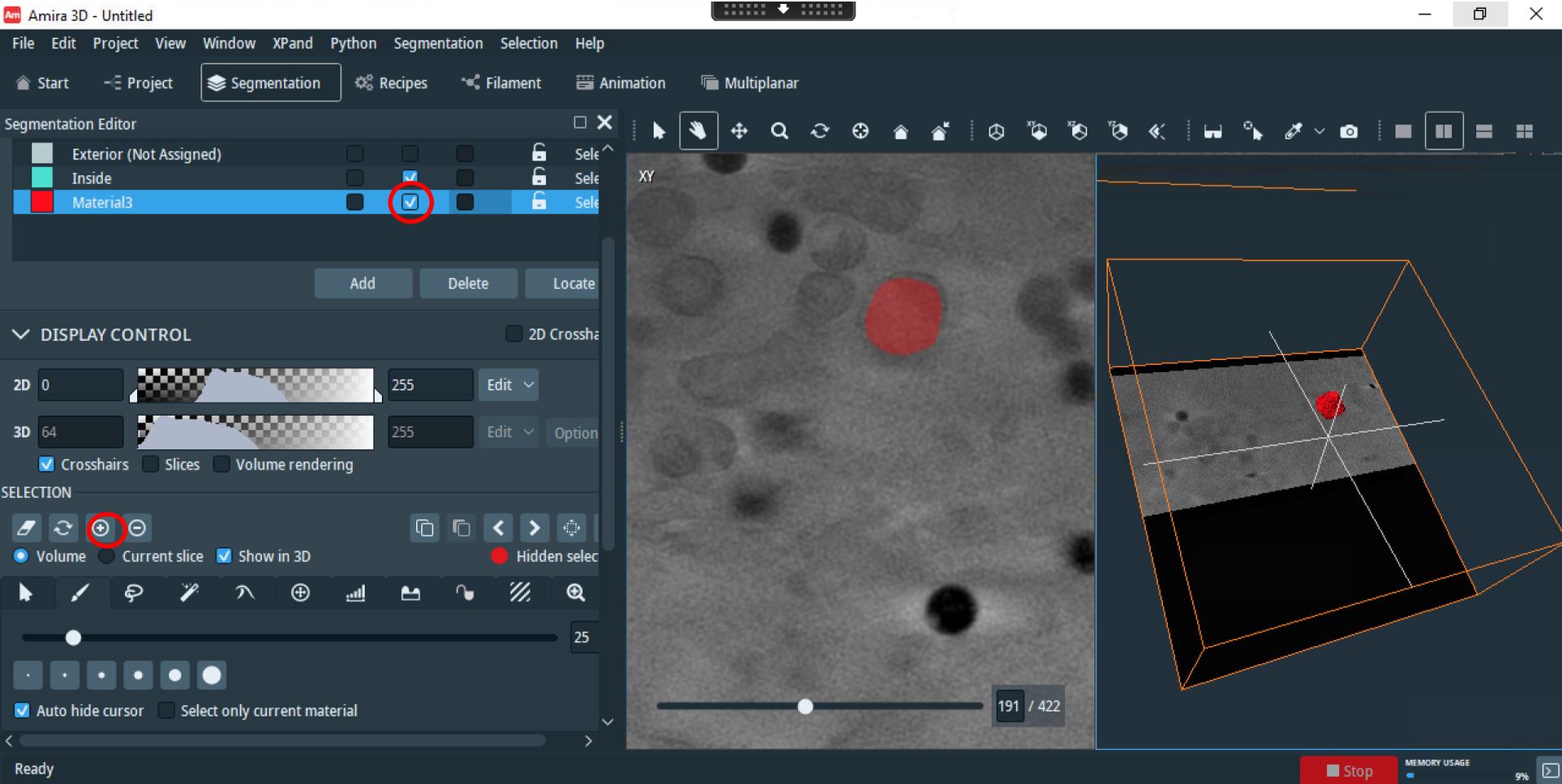


CREATE the rough Selection:

5) In Selection select *Interpolation*: it will extend the selection through all the slices

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

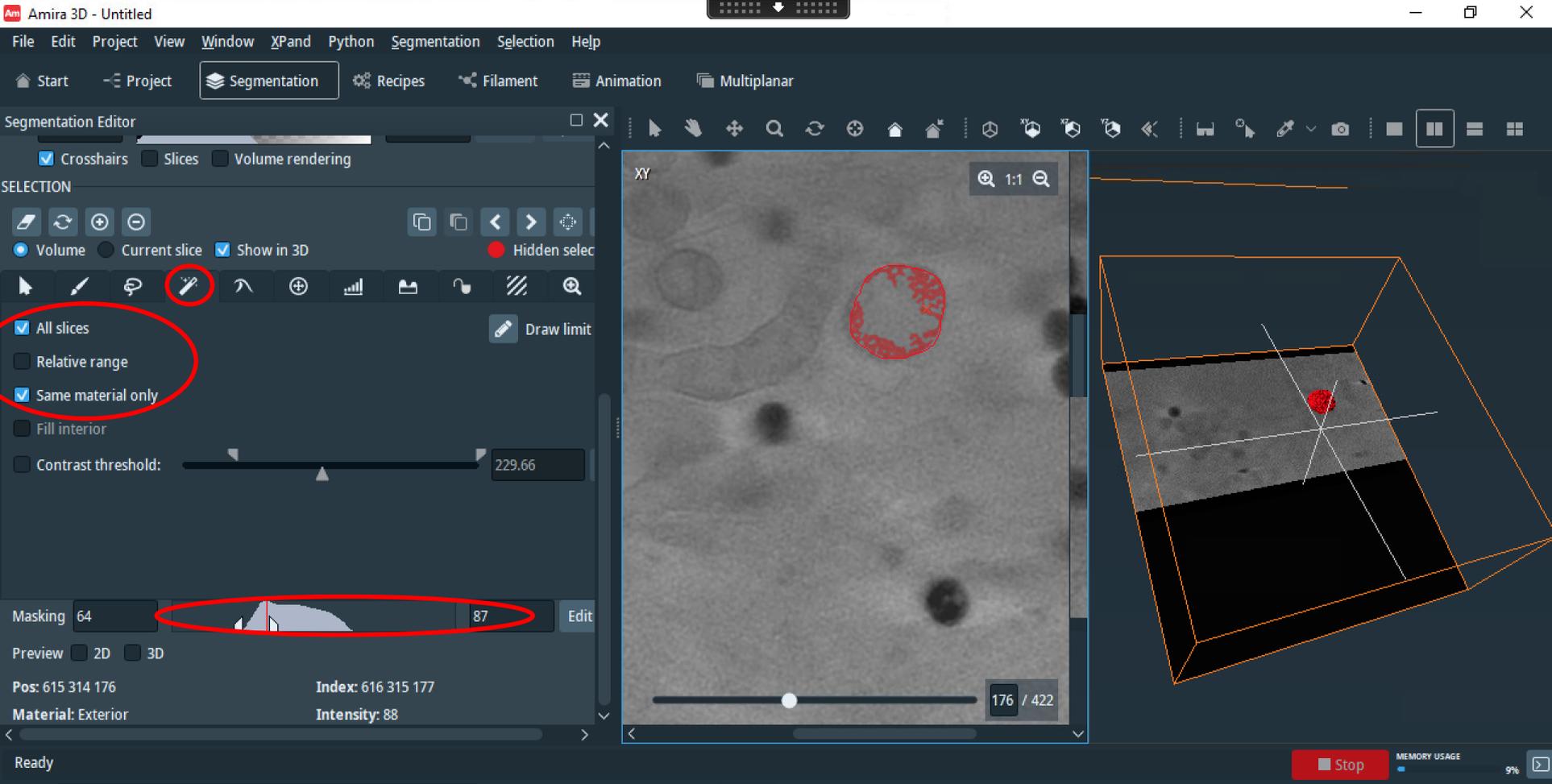
Semi-manual selection of a mitochondrion3



CREATE the rough Selection:

- 4) Take a look to it in the 3D viewer. Your selection are the pixel in the red.
 - 5) Add them to the selected materials (Material3 in this case) using +. Be sure that the Volume option is selected.
 - 6) 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).

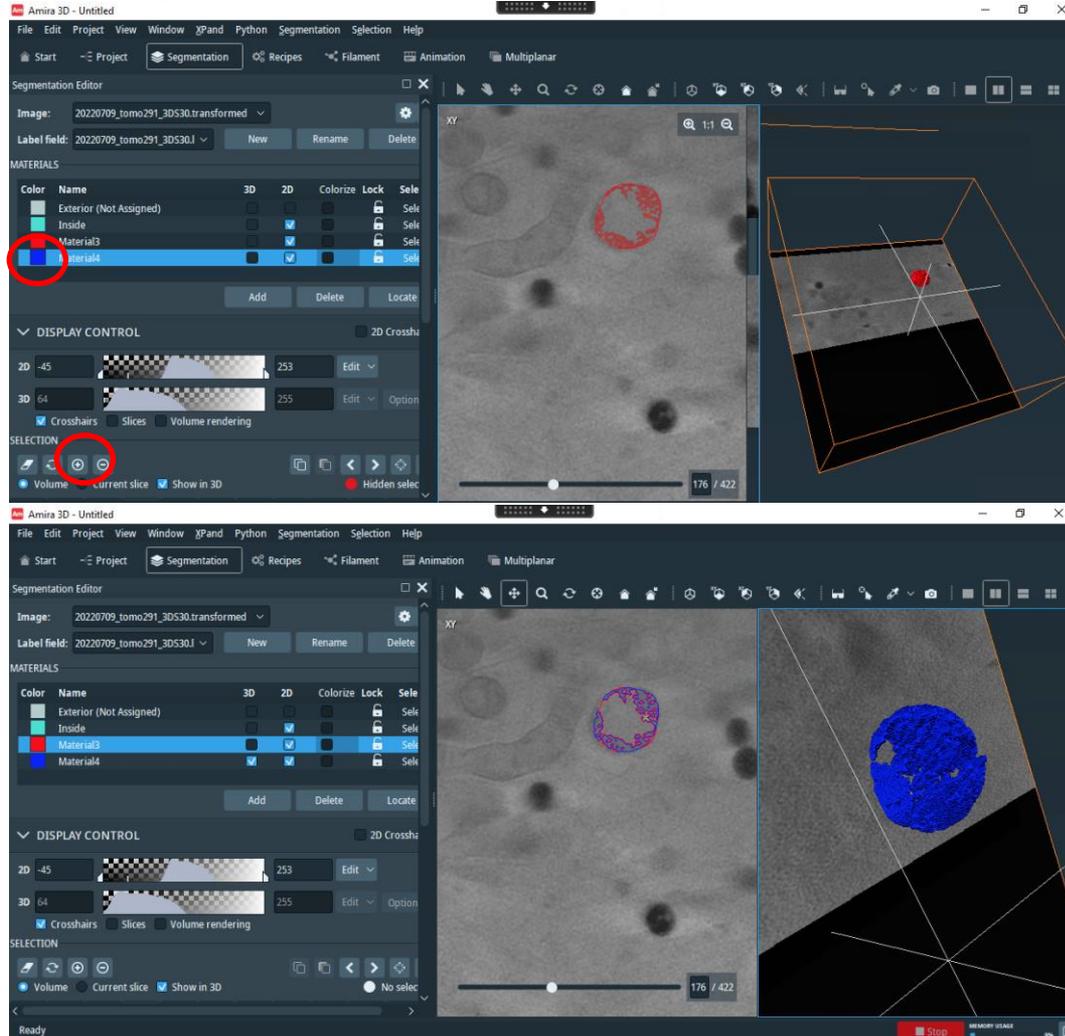
Semi-manual selection of a mitochondrion4



CREATE the *refined* Selection/Material for instance the mitochondrial cristae:

- 1) Use the magic wand tool with the All Slices option and Same Material only.
- 2) Using the threshold of the histogram to 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 +

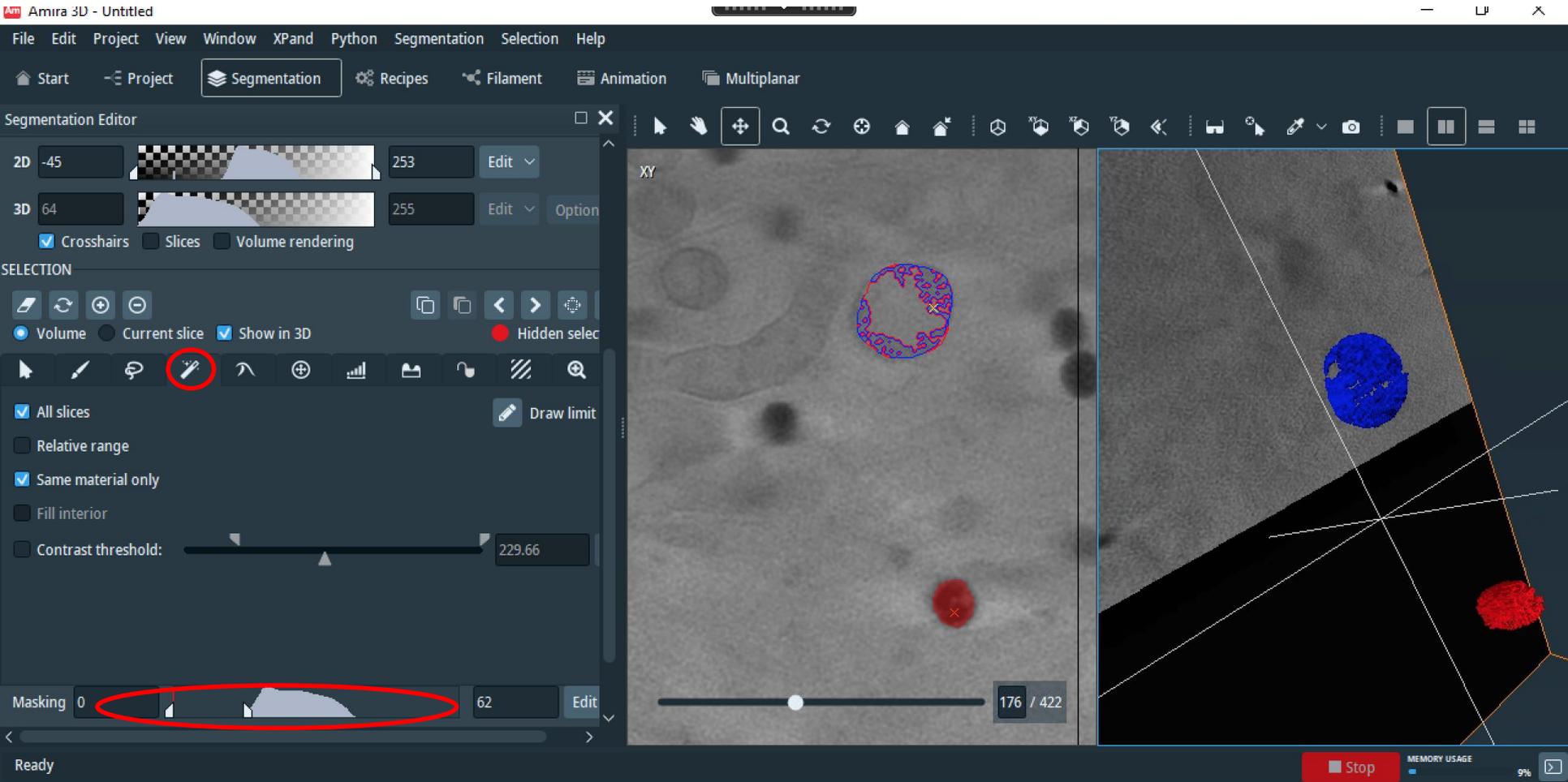
Semi-manual selection of a mitochondrion4



CREATE the refined Selection/Material:

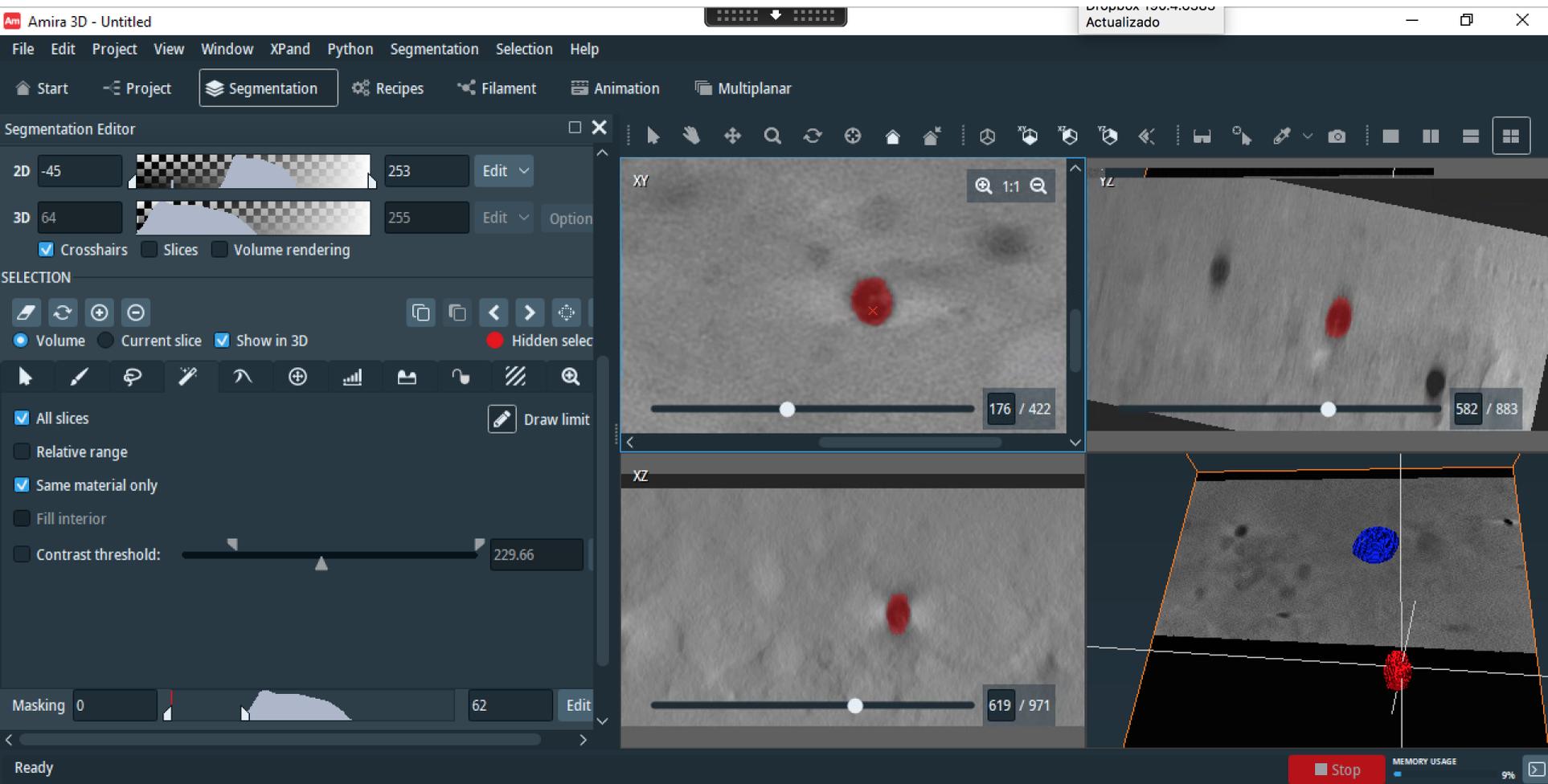
- 4) Create a new material (Material4) with *add* and add the new selection to it with *+*
- 5) Again selected pixels will disappear in the 3D viewer. To see them again select the option 3D in the selected material. The refined selection (in blue) will appear, both with the rough one (in red) as far 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 particle.

Ending the segmentation



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

Label Analysis1

The screenshot displays the Amira 3D software interface. The main window shows a grayscale tomographic slice of a sample with several circular features. A yellow tooltip is visible over the 'Label Analysis (Measure And Analyze-Indivi)' option in the 'label a' context menu. The tooltip text reads: 'Computes measures on connected components of label/ binary images' and 'HxAnalyzeLabels Former Name: l_analyze/1_analyzeseq/HxShapeAnalysis'. The software's Project View on the left lists the loaded data: '20220612_tomo271_3DS30_Remdesivir_24h.tif', '20220612_tomo271_3DS30_Remdesivir_24h.transformed', and '20220612_tomo271_3DS30_Remdesivir_24h.labels'. The Properties panel on the left shows details for the selected '20220612_tomo271_3DS30_Remdesivir_24h.labels' object, including Lattice Info, Data Info, Memory Size, Physical Size, Voxel Size, and a color map. The Windows taskbar at the bottom shows the system tray with the date 22/01/2024 and time 9:58.

Create the computational module "Label Analysis" (right click on the Labels and search for it).

Label Analysis2

The screenshot displays the Amira 3D software interface. The top menu bar includes File, Edit, Project, View, Window, XPand, Python, and Help. Below the menu is a toolbar with icons for Start, Project, Segmentation, Recipes, Filament, Animation, and Multiplanar. The Project View panel on the left shows a tree structure with the following items:

- 20220612_tomo271_3DS30_Remdesivir_24h.tif
- 20220612_tomo271_3DS30_Remdesivir_24h.transformed
- 20220612_tomo271_3DS30_Remdesivir_24h.labels

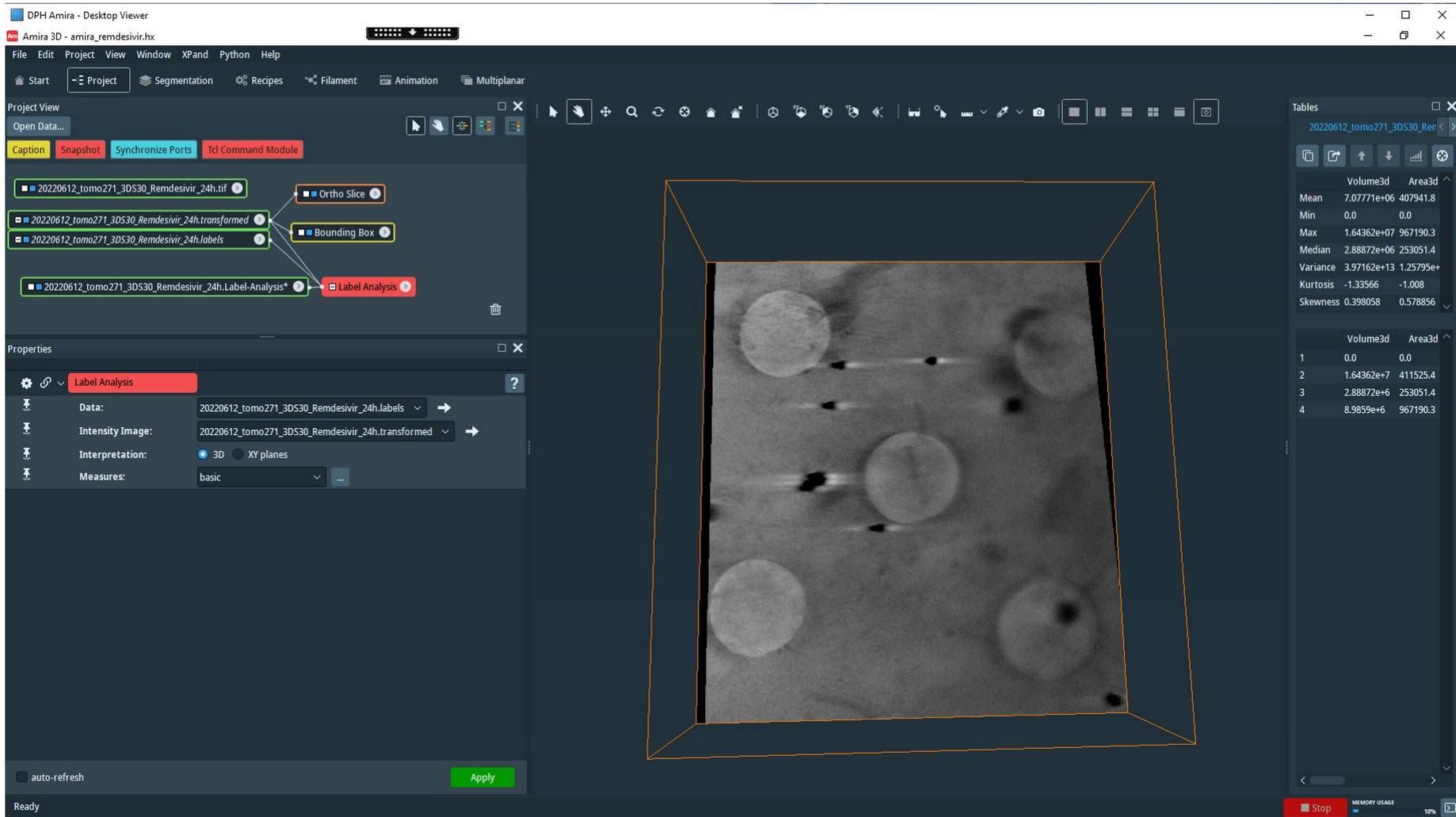
Two items are highlighted with yellow boxes: "Ortho Slice" and "Bounding Box". A context menu is open over the "Bounding Box" item, showing options: Collapse, Data (->20220612_tomo271_3DS30_Remdesivir_24h.labels), and Intensity Image (highlighted in blue). The Properties panel at the bottom left shows the "Label Analysis" settings:

- Data: 20220612_tomo271_3DS30_Remdesivir_24h.labels
- Intensity Image: NO SOURCE
- Interpretation: 3D (selected), XY planes
- Measures: basic

The main 3D view shows a grayscale tomogram slice of a sample, with a white rectangular bounding box overlaid on the central region. The Windows taskbar at the bottom shows the system tray with a Stop button, memory usage (10%), and the date/time (10:00, 22/01/2024).

Right click on the white square and connect "Intensity Image" to the DATA (the transformed one of course).

Label Analysis 3



DPH Amira - Desktop Viewer

Amira 3D - amira_remdesivir.hx

File Edit Project View Window XPand Python Help

Start Project Segmentation Recipes Filament Animation Multiplanar

Project View

Open Data...

20220612_tomo271_3DS30_Remdesivir_24h.tif

Ortho Slice

20220612_tomo271_3DS30_Remdesivir_24h.transformed

Bounding Box

20220612_tomo271_3DS30_Remdesivir_24h.labels

20220612_tomo271_3DS30_Remdesivir_24h.Label-Analysis*

Label Analysis

Properties

Label Analysis

Data: 20220612_tomo271_3DS30_Remdesivir_24h.labels

Intensity Image: 20220612_tomo271_3DS30_Remdesivir_24h.transformed

Interpretation: 3D XY planes

Measures: basic

auto-refresh Apply

Ready

Tables

20220612_tomo271_3DS30_Remdesivir_24h.Label-Analysis*

	Volume3d	Area3d
Mean	7.07771e+06	407941.8
Min	0.0	0.0
Max	1.64362e+07	967190.3
Median	2.88872e+06	253051.4
Variance	3.97162e+13	1.25795e+
Kurtosis	-1.33566	-1.008
Skewness	0.398058	0.578856

	Volume3d	Area3d
1	0.0	0.0
2	1.64362e+7	411525.4
3	2.88872e+6	253051.4
4	8.9859e+6	967190.3

Stop MEMORY USAGE 10%

Then click apply. Wait.

Label Analysis 4

The screenshot displays the Amira 3D software interface. In the Project View, a workflow is shown with the following objects: '20220612_tomo271_3DS30_Remdesivir_24h.tif', 'Ortho Slice', '20220612_tomo271_3DS30_Remdesivir_24h.transformed', 'Bounding Box', and '20220612_tomo271_3DS30_Remdesivir_24h.Label-Analysis*'. The 'Label Analysis*' object is highlighted with a red oval. The Properties panel for 'Label Analysis' shows: Data: 20220612_tomo271_3DS30_Remdesivir_24h.Labels, Intensity Image: 20220612_tomo271_3DS30_Remdesivir_24h.transformed, Interpretation: 3D (selected), and Measures: basic. A central 3D view shows a grayscale tomogram with a yellow bounding box around a region of interest. On the right, a 'Tables' panel displays the numerical results for '20220612_tomo271_3DS30_Remdesivir_24h.Label-Analysis', which is also circled in red. The table includes statistical measures for the volume and a list of materials.

	Volume3d	Area3d	BaryCenterX	BaryCenterY	BaryCenterZ	Mean	index	Materials
Mean	7.07771e+06	407941.8	469.362	420.567	97.591	40.8335	2.5	--
Min	0.0	0.0	0.0	0.0	0.0	0.0	1.0	--
Max	1.64362e+07	967190.3	744.58	781.054	143.046	63.0041	4.0	--
Median	2.88872e+06	253051.4	556.819	511.891	125.014	55.8729	3.0	--
Variance	3.97162e+13	1.25795e+11	78769.0	79035.9	3238.22	599.556	1.25	--
Kurtosis	-1.33566	-1.008	-0.805741	-1.06925	-0.713303	-0.862758	-1.36	--
Skewness	0.398058	0.578856	-0.912269	-0.301668	-1.08511	-0.915116	0.0	--

	Volume3d	Area3d	BaryCenterX	BaryCenterY	BaryCenterZ	Mean	index	Materials
1	0.0	0.0	0.0	0.0	0.0	0.0	1	Inside
2	1.64362e+7	411525.4	744.58	781.054	122.304	63.0041	2	Material3
3	2.88872e+6	253051.4	556.819	511.891	125.014	55.8729	3	Material4
4	8.9859e+6	967190.3	576.047	389.324	143.046	44.457	4	Material5

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 Analysis 5

The screenshot displays the Amira 3D software interface. The central 3D view shows a grayscale volume rendering of a sample with a yellow bounding box. The 'Label Analysis' module is active, and its properties are visible in the bottom-left panel. The 'Measures' dropdown menu is open, showing a red circle around the '...' option. A tooltip below it reads: 'The measures are computed in the working unit.'

The 'Tables' panel on the right shows the results of the label analysis. The table is titled '20220612_tomo271_3DS30_Remdesivir_24h.Label-Analysis' and contains two tables of data.

	Volume3d	Area3d	BaryCenterX	BaryCenterY	BaryCenterZ	Mean	index	Materials
Mean	7.07771e+06	407941.8	469.362	420.567	97.591	40.8335	2.5	--
Min	0.0	0.0	0.0	0.0	0.0	0.0	1.0	--
Max	1.64362e+07	967190.3	744.58	781.054	143.046	63.0041	4.0	--
Median	2.88872e+06	253051.4	556.819	511.891	125.014	55.8729	3.0	--
Variance	3.97162e+13	1.25795e+11	78769.0	79035.9	3238.22	599.556	1.25	--
Kurtosis	-1.33566	-1.008	-0.805741	-1.06925	-0.713303	-0.862758	-1.36	--
Skewness	0.398058	0.578856	-0.912269	-0.301668	-1.08511	-0.915116	0.0	--

	Volume3d	Area3d	BaryCenterX	BaryCenterY	BaryCenterZ	Mean	index	Materials
1	0.0	0.0	0.0	0.0	0.0	0.0	1	Inside
2	1.64362e+7	411525.4	744.58	781.054	122.304	63.0041	2	Material3
3	2.88872e+6	253051.4	556.819	511.891	125.014	55.8729	3	Material4
4	8.9859e+6	967190.3	576.047	389.324	143.046	44.457	4	Material5

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

Label Analysis 6

Selection of measure groups

Choose a measure group: basic

Create a new measure group.

Measures selected in the group: **This list can not be modified.**

Name	Formula
Volume3d	Native
Area3d	Native
BaryCenterX	Native
BaryCenterY	Native
BaryCenterZ	Native
Mean	Native

Native measures:

Name	Formula
Histogram	
HistoKurtosis	Native
HistoMean	Native
HistoPeak	Native
HistoQuantile10	Native
HistoQuantile25	Native
HistoQuantile50	Native
HistoQuantile75	Native
HistoQuantile90	Native

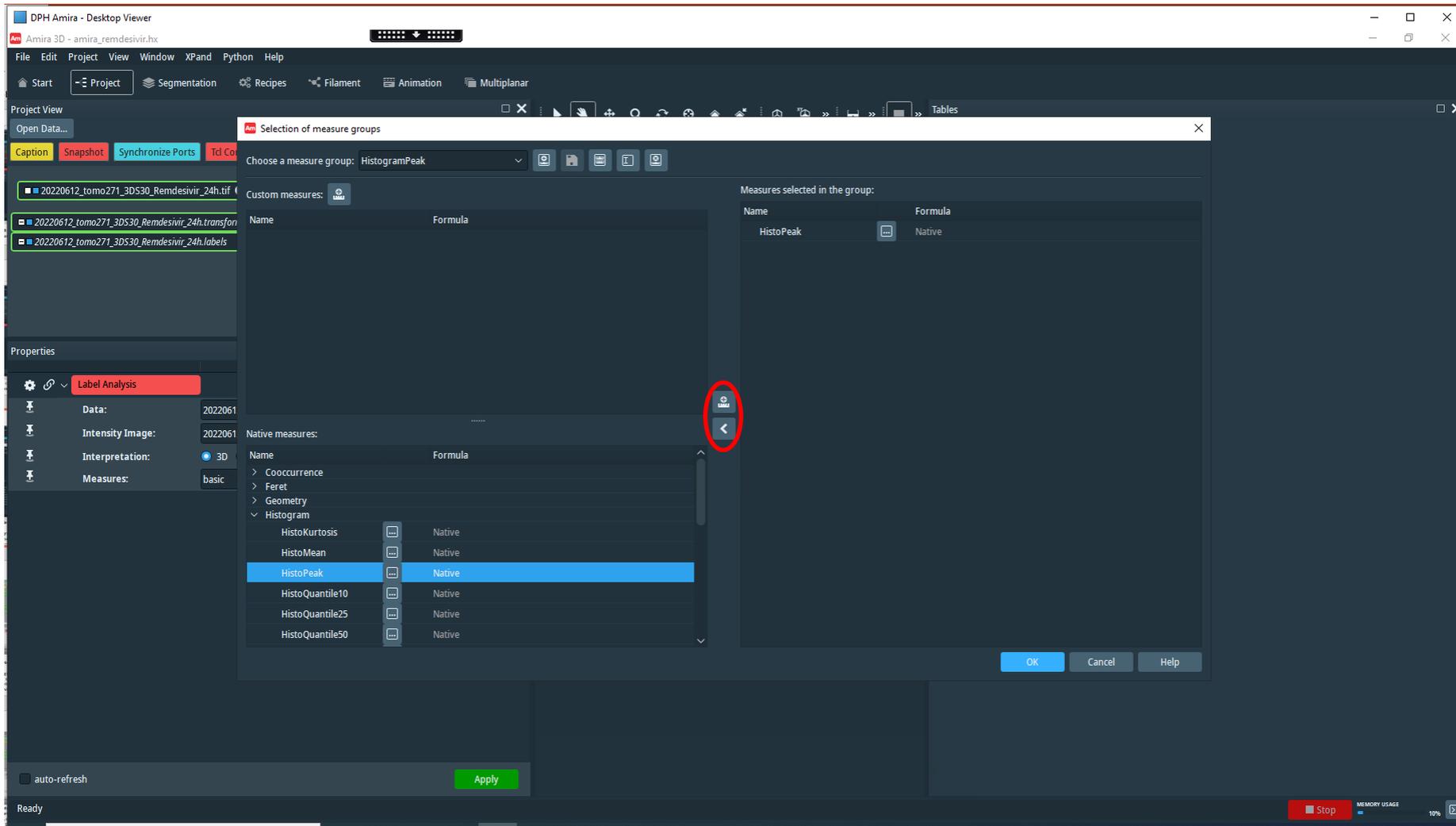
Volume3d	Area3d	BaryCenterX	BaryCenterY	BaryCenterZ	Mean	index	Materials
41.8	469.362	420.567	97.591	40.8335	2.5	--	
0.0	0.0	0.0	0.0	0.0	1.0	--	
90.3	744.58	781.054	143.046	63.0041	4.0	--	
51.4	556.819	511.891	125.014	55.8729	3.0	--	
795e+11	78769.0	79035.9	3238.22	599.556	1.25	--	
18	-0.805741	-1.06925	-0.713303	-0.862758	-1.36	--	
3856	-0.912269	-0.301668	-1.08511	-0.915116	0.0	--	

ea3d	BaryCenterX	BaryCenterY	BaryCenterZ	Mean	index	Materials
0.0	0.0	0.0	0.0	0.0	1	Inside
25.4	744.58	781.054	122.304	63.0041	2	Material3
51.4	556.819	511.891	125.014	55.8729	3	Material4
90.3	576.047	389.324	143.046	44.457	4	Material5

Create a NewGroup using + 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

Label Analysis7



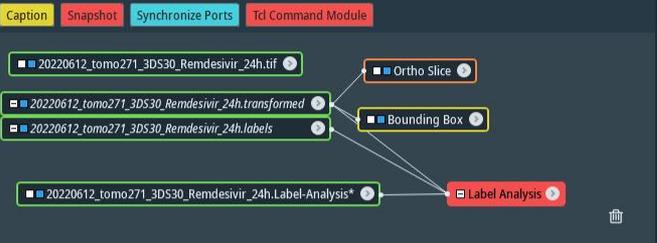
The screenshot shows the Amira 3D software interface. The 'Selection of measure groups' dialog box is open, displaying the 'HistogramPeak' group. The 'Native measures' list is expanded, and the 'HistoPeak' measure is selected. A red circle highlights the 'Add' button (a plus sign in a square) next to the 'HistoPeak' measure. The 'Measures selected in the group' table shows 'HistoPeak' with a 'Native' formula.

Name	Formula
HistoPeak	Native

Name	Formula
HistoKurtosis	Native
HistoMean	Native
HistoPeak	Native
HistoQuantile10	Native
HistoQuantile25	Native
HistoQuantile50	Native

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 \neq mode.

Project View
Open Data...
Caption Snapshot Synchronize Ports Td Command Module



Properties

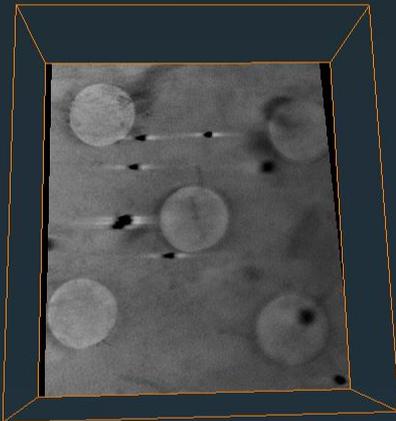
Label Analysis

Data: 20220612_tomo271_3DS30_Remdesvir_24h.labels

Intensity Image: 20220612_tomo271_3DS30_Remdesvir_24h.transformed

Interpretation: 3D XY planes

Measures: HistogramPeak



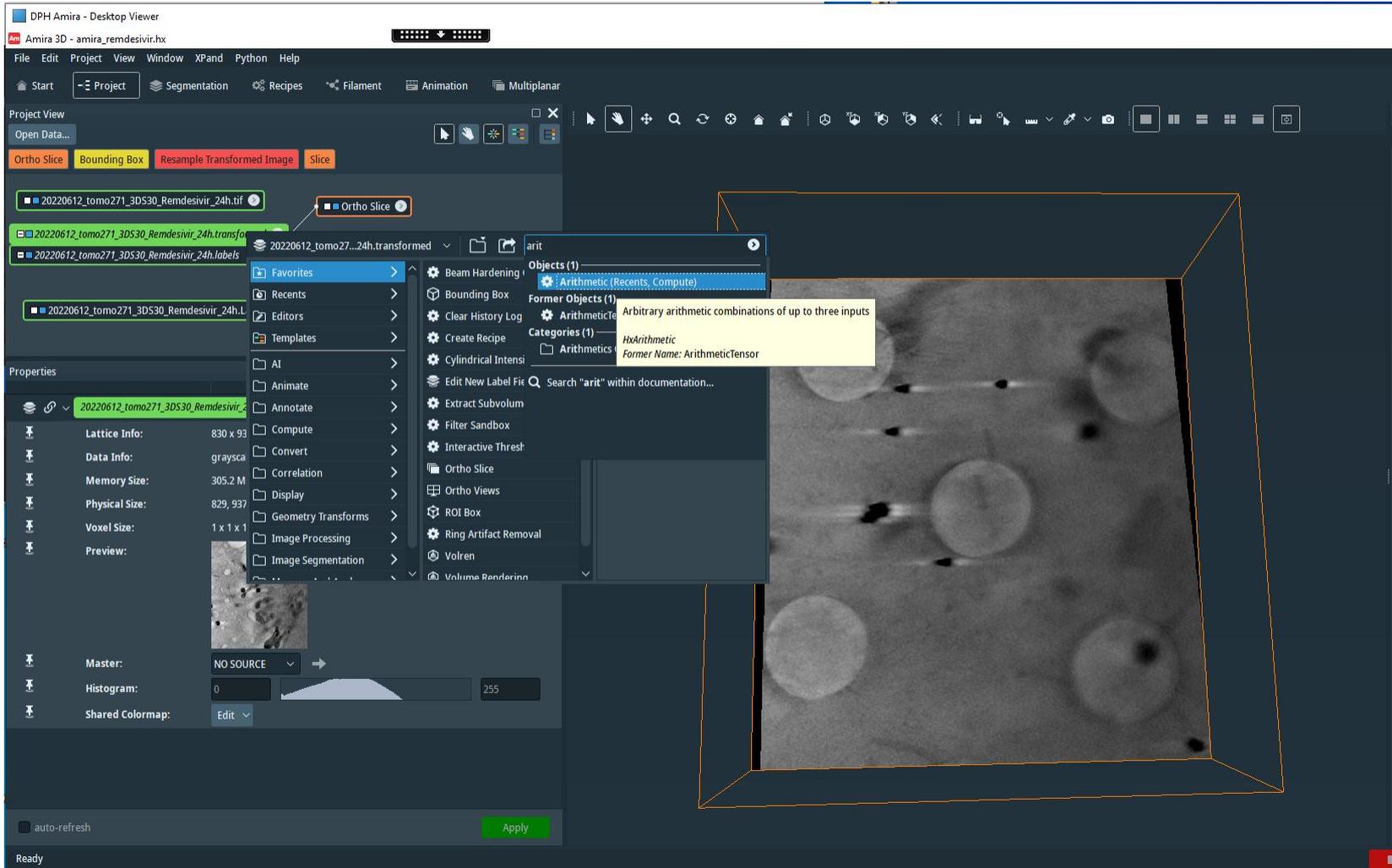
Tables
20220612_tomo271_3DS30_Remdesvir_24h.Label-Analysis

	HistoPeak	index	Materials
Mean	23.1011	2.5	--
Min	0.0	1.0	--
Max	52.959	4.0	--
Median	52.959	3.0	--
Variance	549.35	1.25	--
Kurtosis	-1.83033	-1.36	--
Skewness	0.128485	0.0	--

	HistoPeak	index	Materials
1	0.0	1	Inside
2	0.365234	2	Material3
3	52.959	3	Material4
4	39.0801	4	Material5

auto-refresh Apply

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).

Arithmetic module

The screenshot displays the Amira 3D software interface. The top menu bar includes File, Edit, Project, View, Window, XPand, Python, and Help. The Project View panel on the left shows a hierarchy of data: '20220612_tomo271_3DS30_Remdesivir_24h.tif' is connected to 'Ortho Slice', which is connected to 'Bounding Box'. Below it, '20220612_tomo271_3DS30_Remdesivir_24h.transformed' and '20220612_tomo271_3DS30_Remdesivir_24h.labels' are connected to 'Label Analysis'. A context menu is open over the 'Label Analysis' node, with 'Input B' highlighted in blue and circled in red. The Properties panel at the bottom left shows the 'Arithmetic' module configuration:

Property	Value
Input A:	20220612_tomo271_3DS30_Remdesivir_24h.transformed
Input B:	NO SOURCE
Input C:	NO SOURCE
Result Type:	<input checked="" type="radio"/> input A <input type="radio"/> regular
Options:	<input type="checkbox"/> ignore errors
Result Channels:	like input A
Expression:	0

The main 3D view on the right shows a grayscale tomogram slice of a cell, with a white rectangular bounding box overlaid on the central region. The 'Ortho Slice' node in the Project View is also highlighted with an orange box.

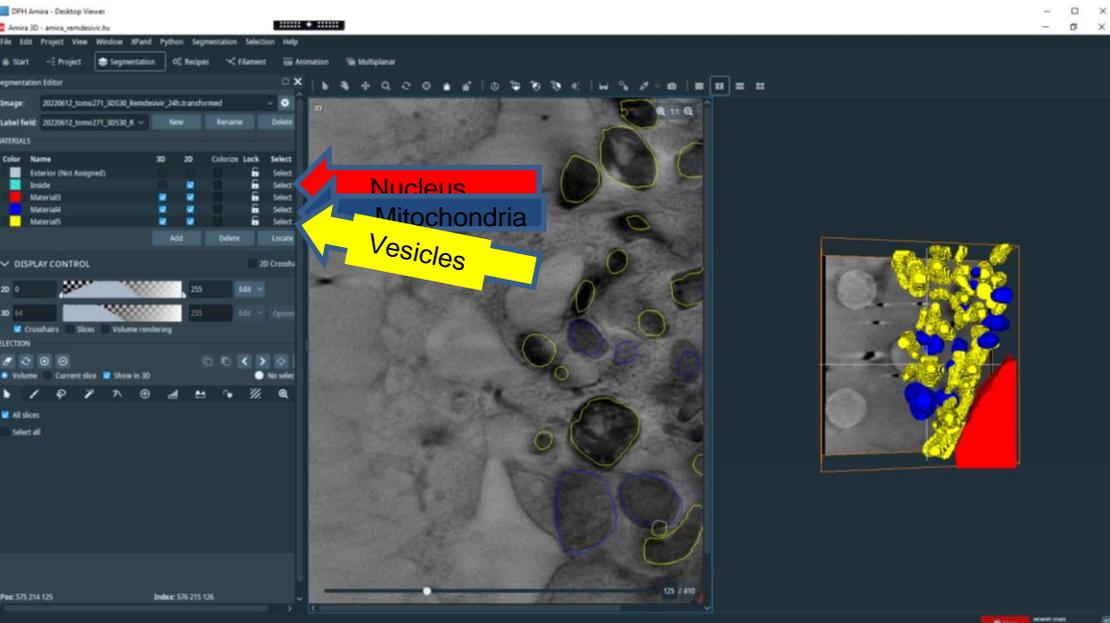
Right click on the white box and connect Input A with the DATA and Input B with the labels file.

Arithmetic module

The screenshot displays the Amira 3D software interface. The top menu bar includes File, Edit, Project, View, Window, XPand, Python, and Help. The Project View panel on the left shows a hierarchy of data: 20220612_tomo271_3DS30_Remdesivir_24h.tif, 20220612_tomo271_3DS30_Remdesivir_24h.transformed, 20220612_tomo271_3DS30_Remdesivir_24h.labels, and 20220612_tomo271_3DS30_Remdesivir_24h.Label. A tooltip for the labels file reads: "20220612_tomo271_3DS30_Remdesivir_24h.labels Labeled volume with uniform coordinates HxUniformLabelField3 F1: Help Ctrl-Right Click: Favorites". The Properties panel shows the Arithmetic module configuration: Input A is set to 20220612_tomo271_3DS30_Remdesivir_24h.transformed, Input B is NO SOURCE, Input C is NO SOURCE, Result Type is input A, and Options include ignore errors. The Expression field is empty. The main 3D view shows a grayscale volume rendering of a cell with several bright spots, enclosed in a white bounding box. The interface also includes buttons for Animate Ports, Caption, Snapshot, and Synchronize Ports, and a status bar at the bottom indicating "Ready".

Right click on the white box and connect
Input A with the DATA and Input B with the labels file.

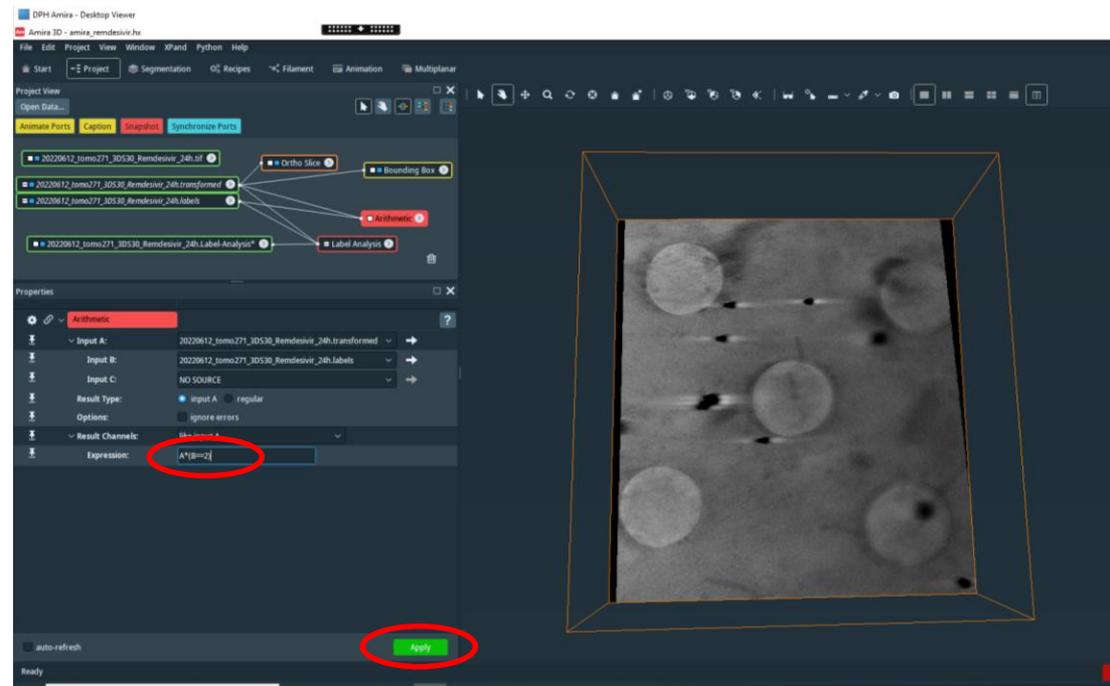
Arithmetic module



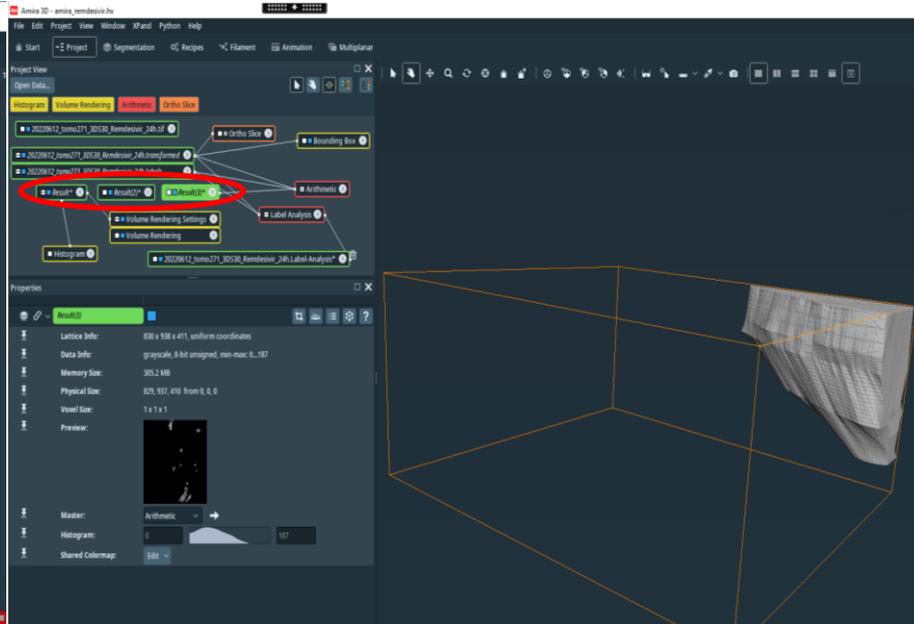
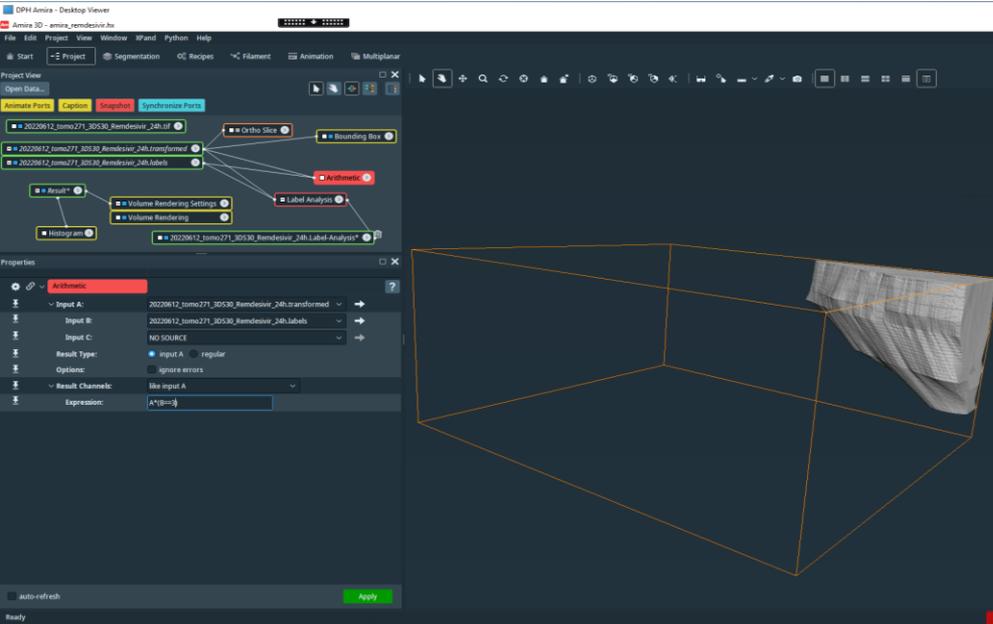
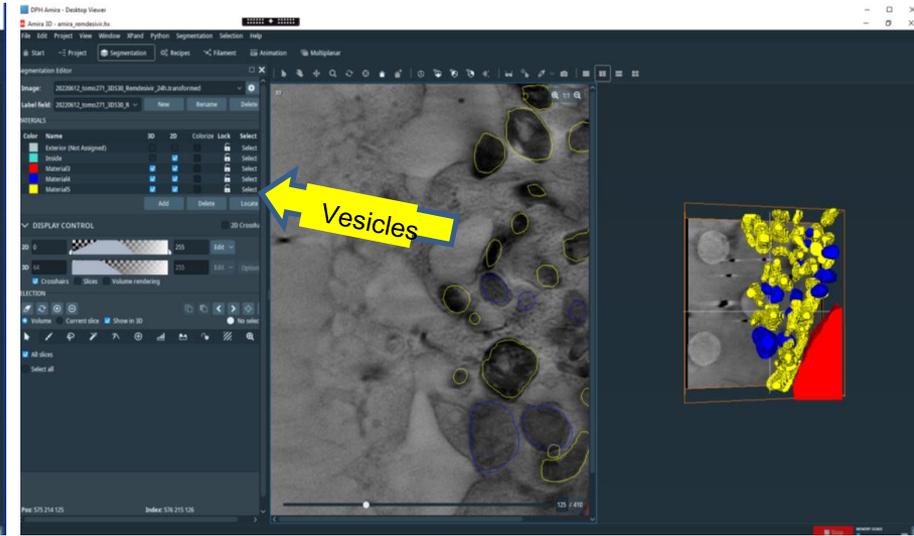
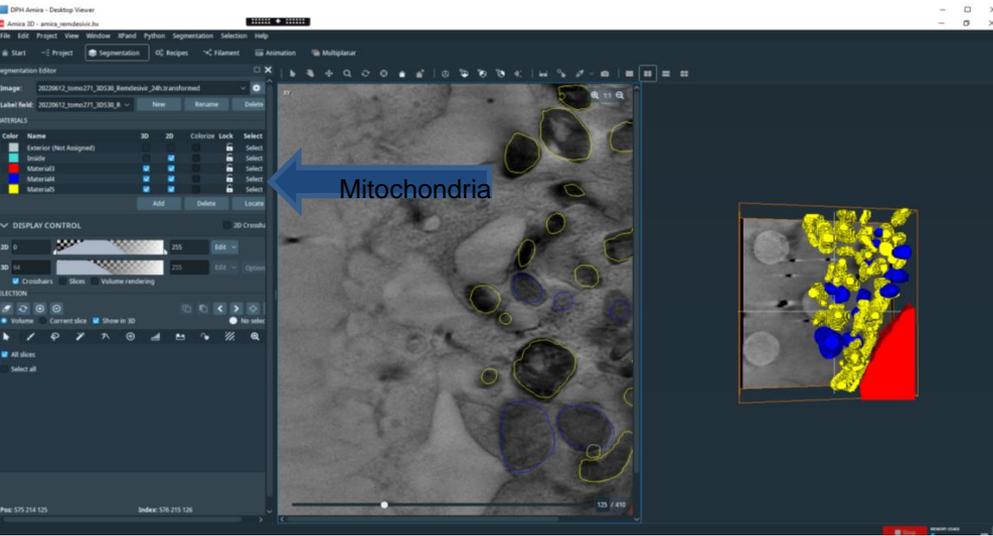
Set the Expression as $A*(B=3)$. This will multiply the data by the selection we have created in the segmentation editor corresponding to the Label=3 which is Material 4 (the Nucleus).

It is always Material number = Label number + 1.

With Apply and the DATA "Result" will be created. General operation can be performed.

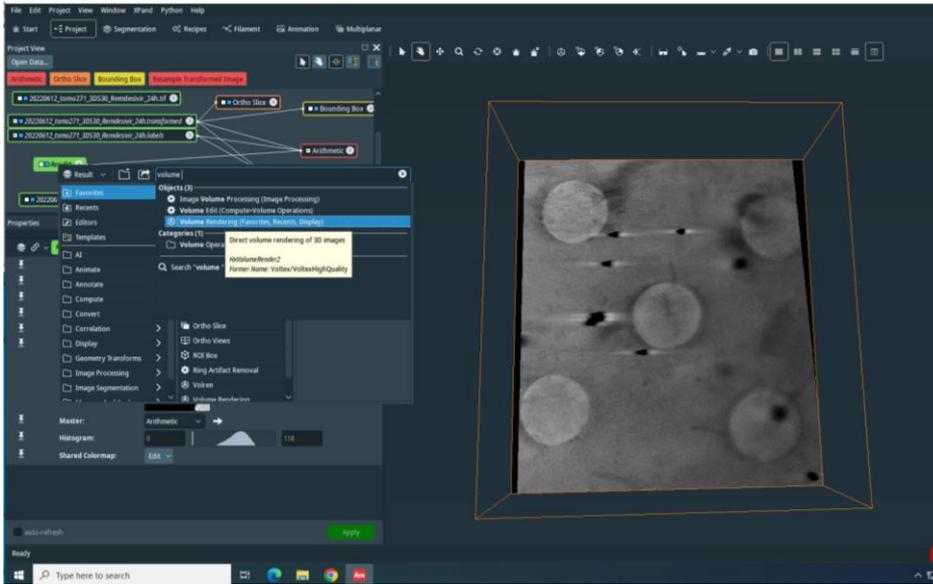


Arithmetic module



Create the others materials using the arithmetic module

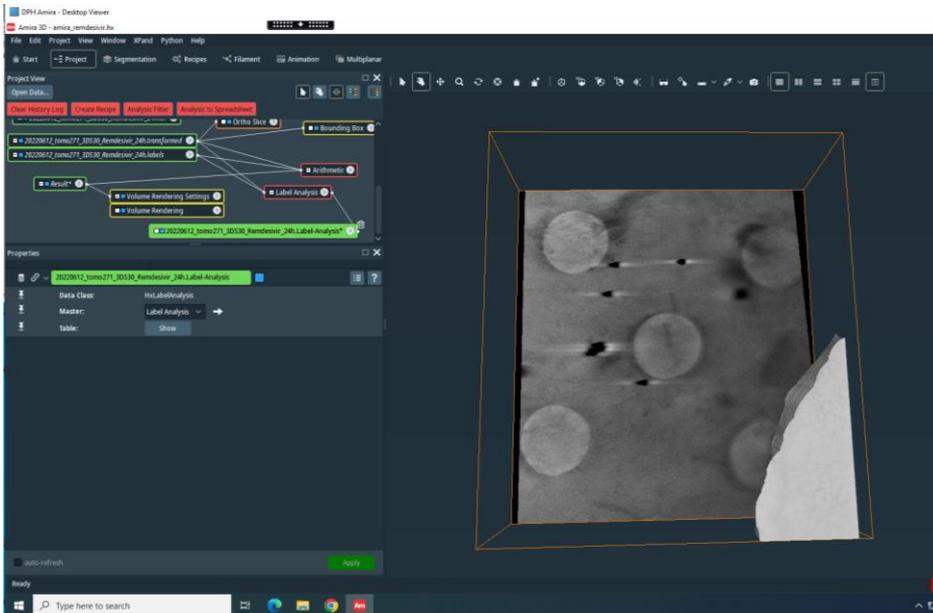
Arithmetic module



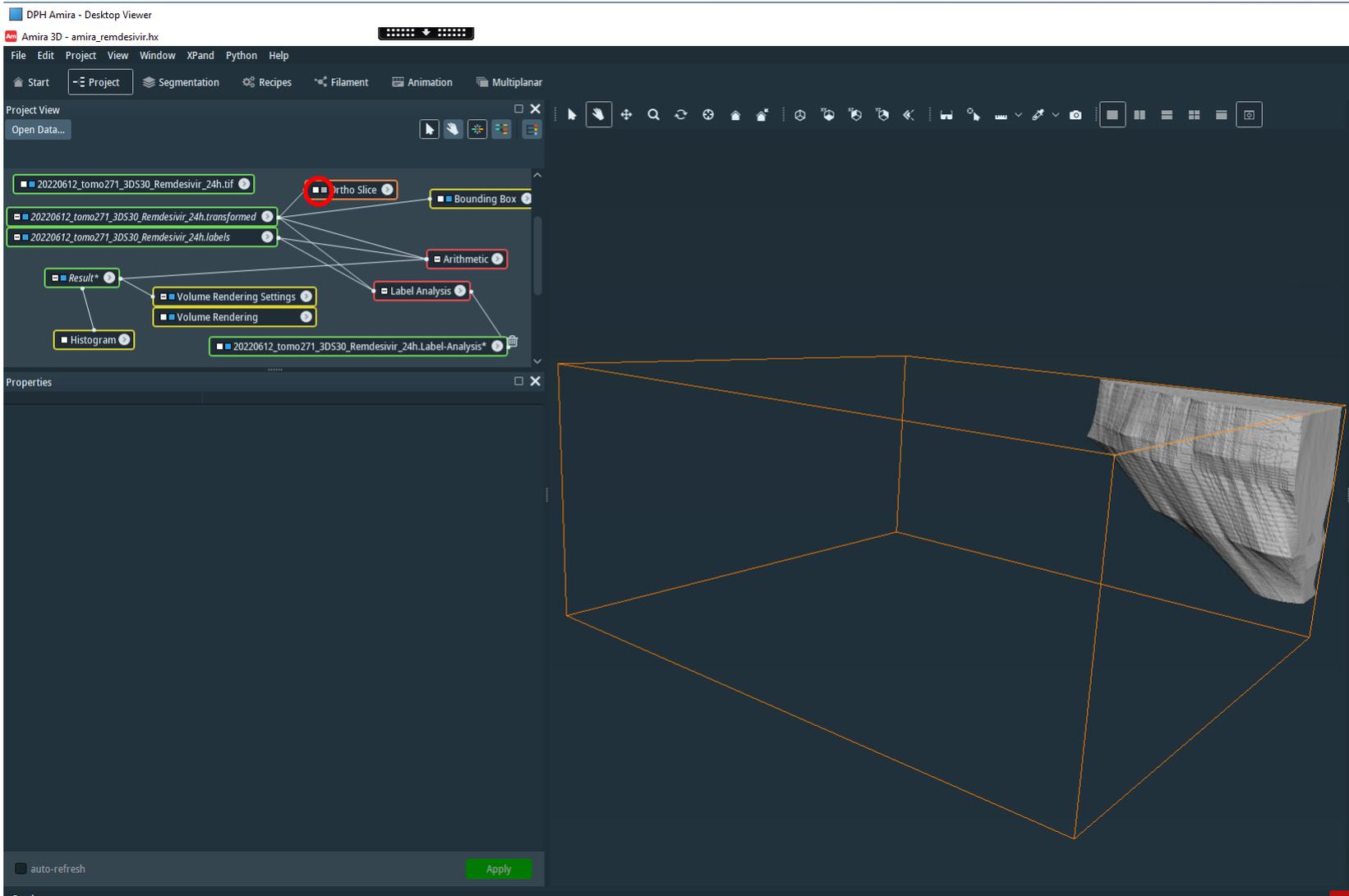
With Apply and the DATA "Result" will be created.

General operation can be performed

You can take a look to it in 3D using the Volume rendering module (right click on result DATA and create it).

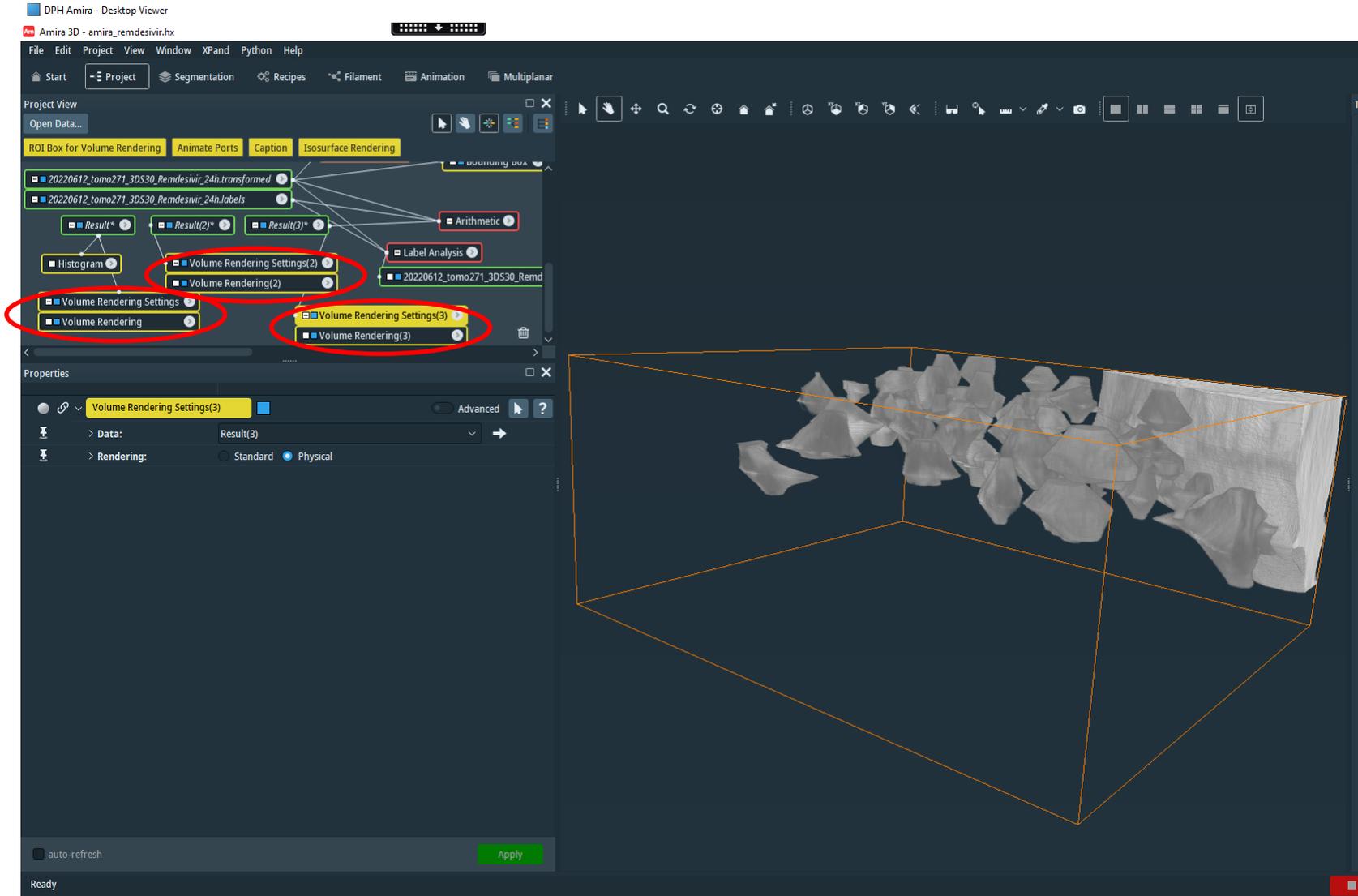


Volume rendering



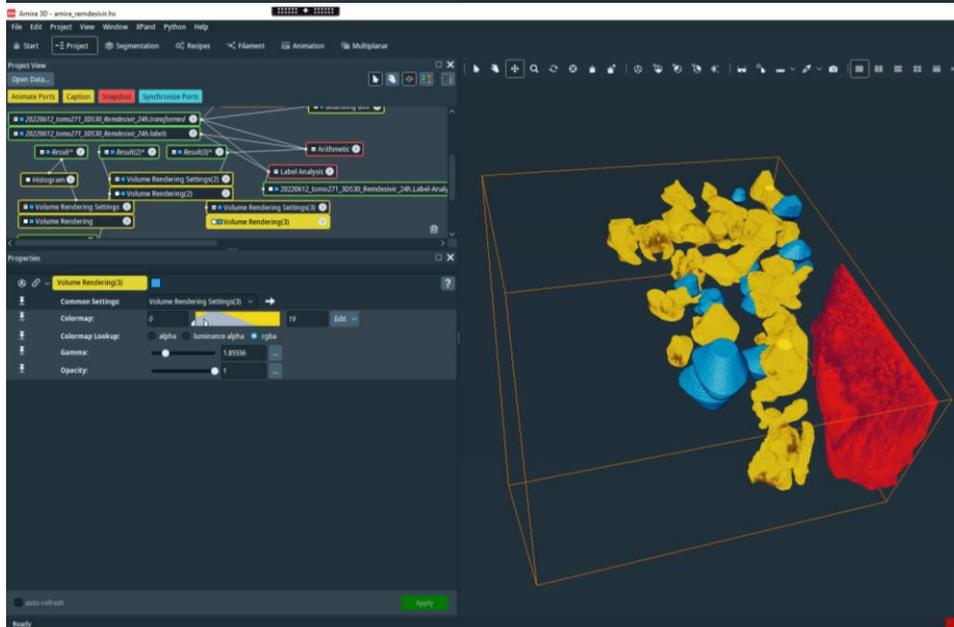
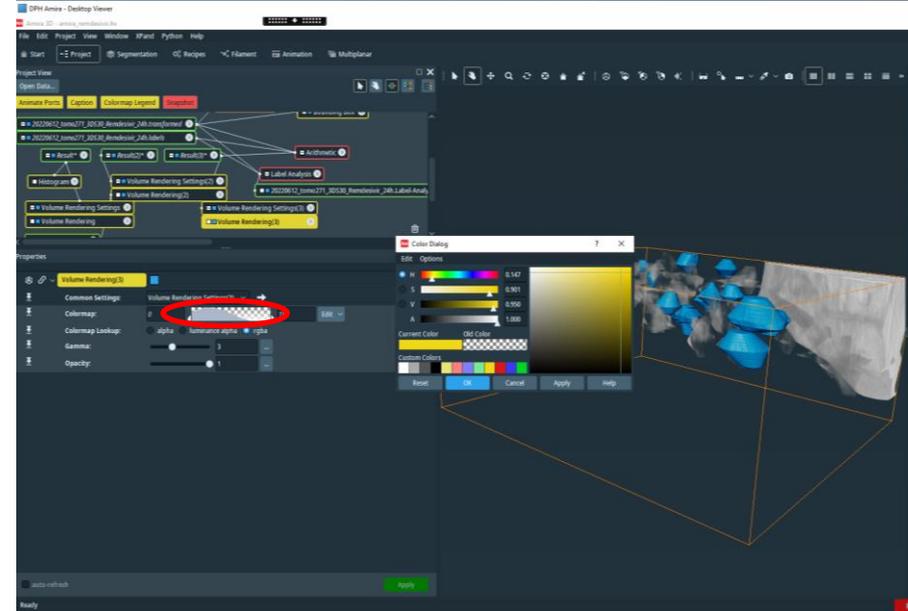
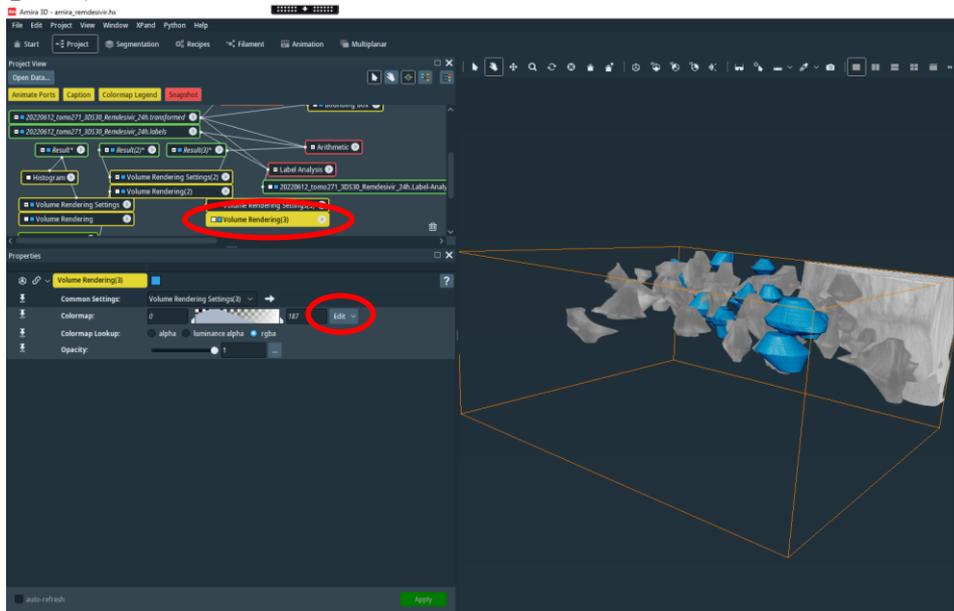
Deselect the blue box in any orthoslice or slice module to remove the stack visualization.

Volume rendering



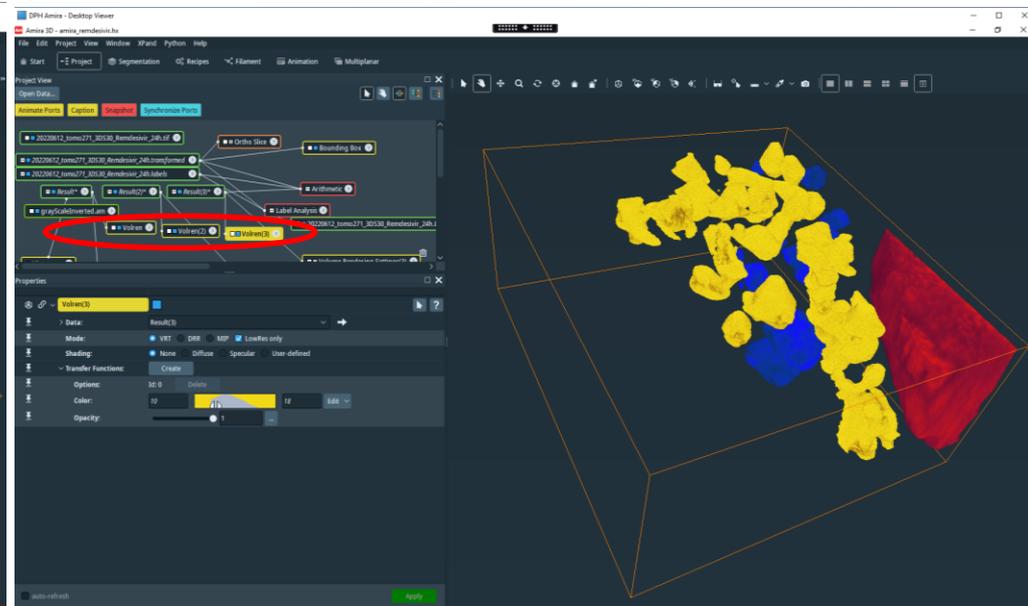
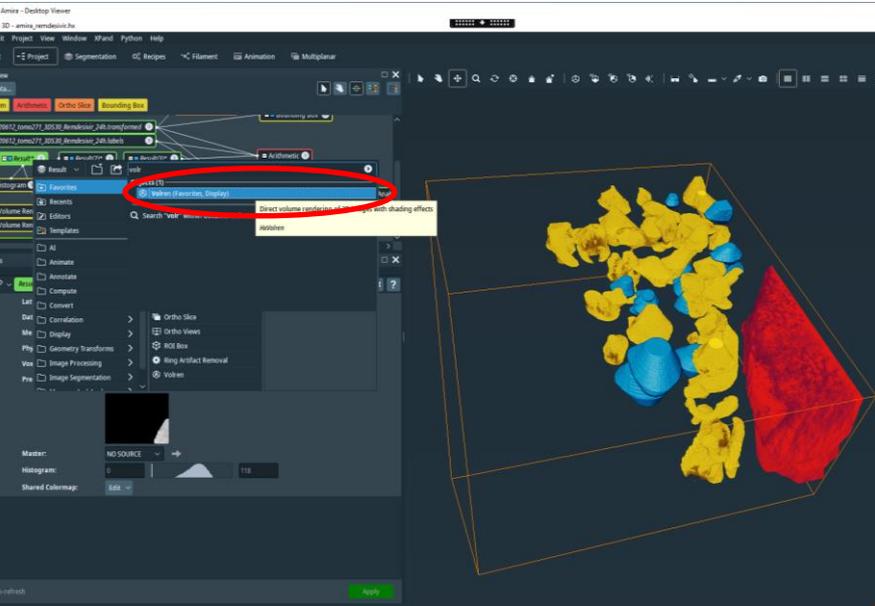
Create the others materials using the arithmetic module and visualize them using 3 different Volume rendering modules.

Volume rendering



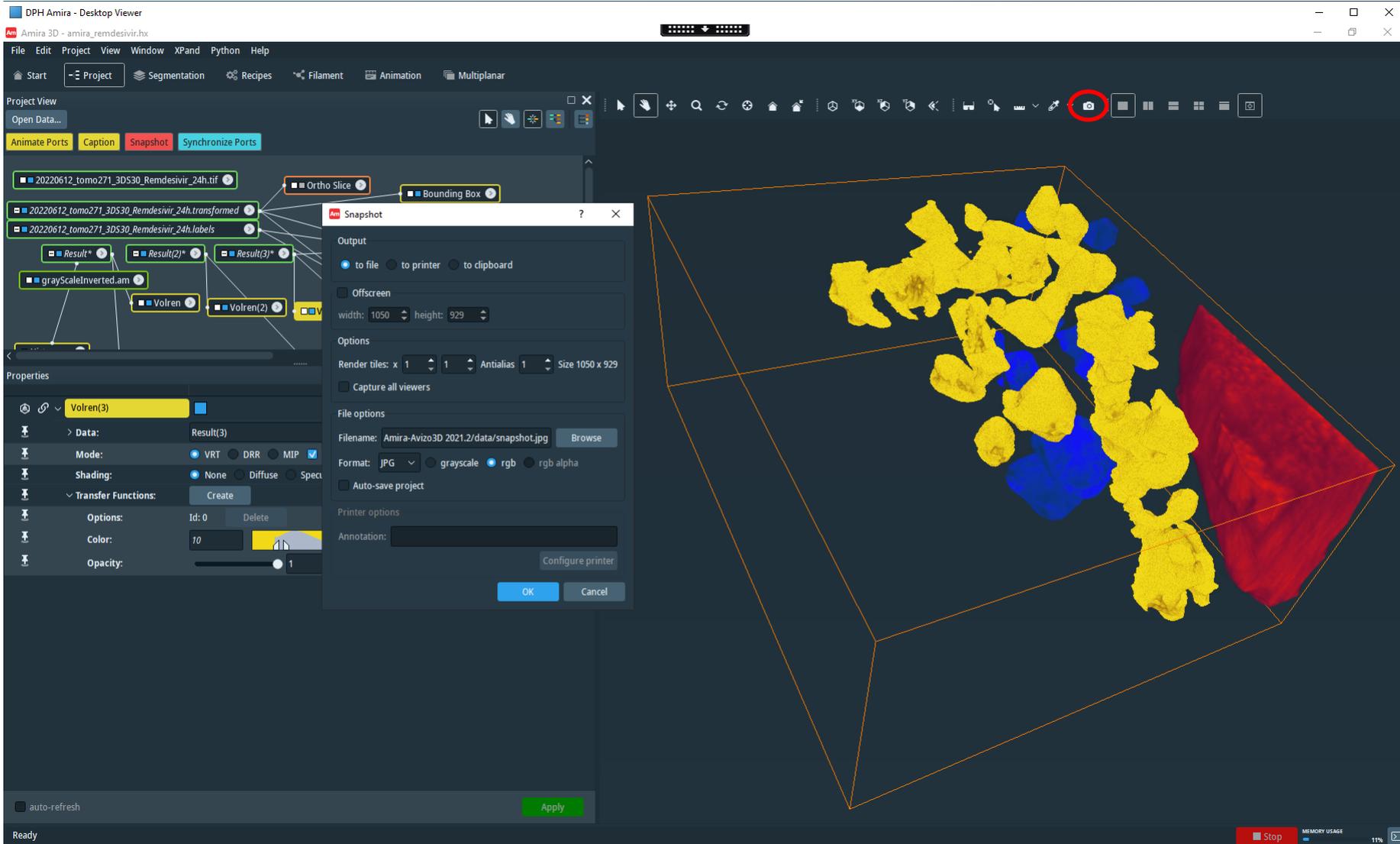
You can also manage the color option.
Double click inside colormap.
Here in red is the nucleus
In Blue the mitochondria
In yellow the vesicles

Volume rendering



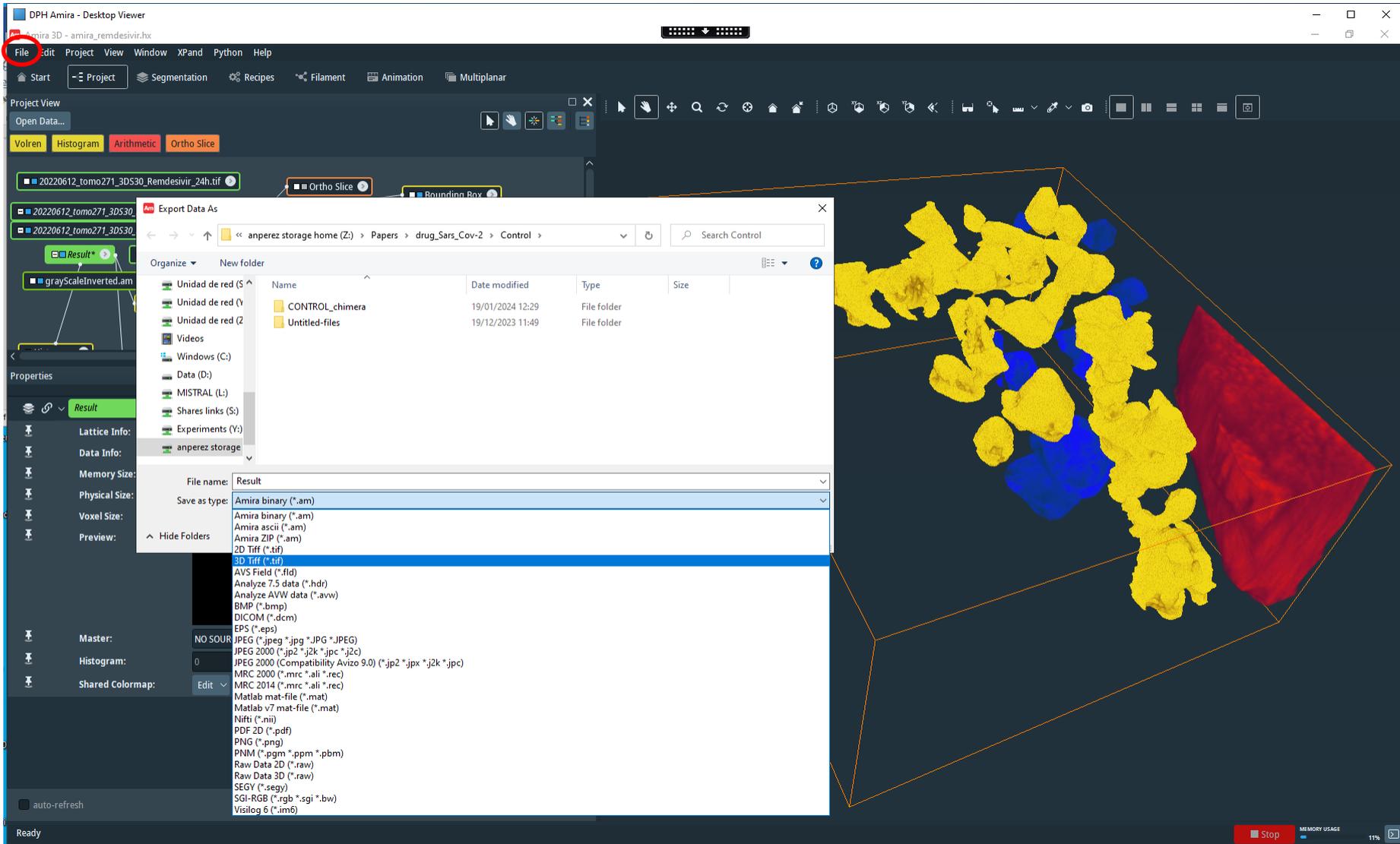
The module Volren can also be used. It seems to use much less memory...

Volume rendering



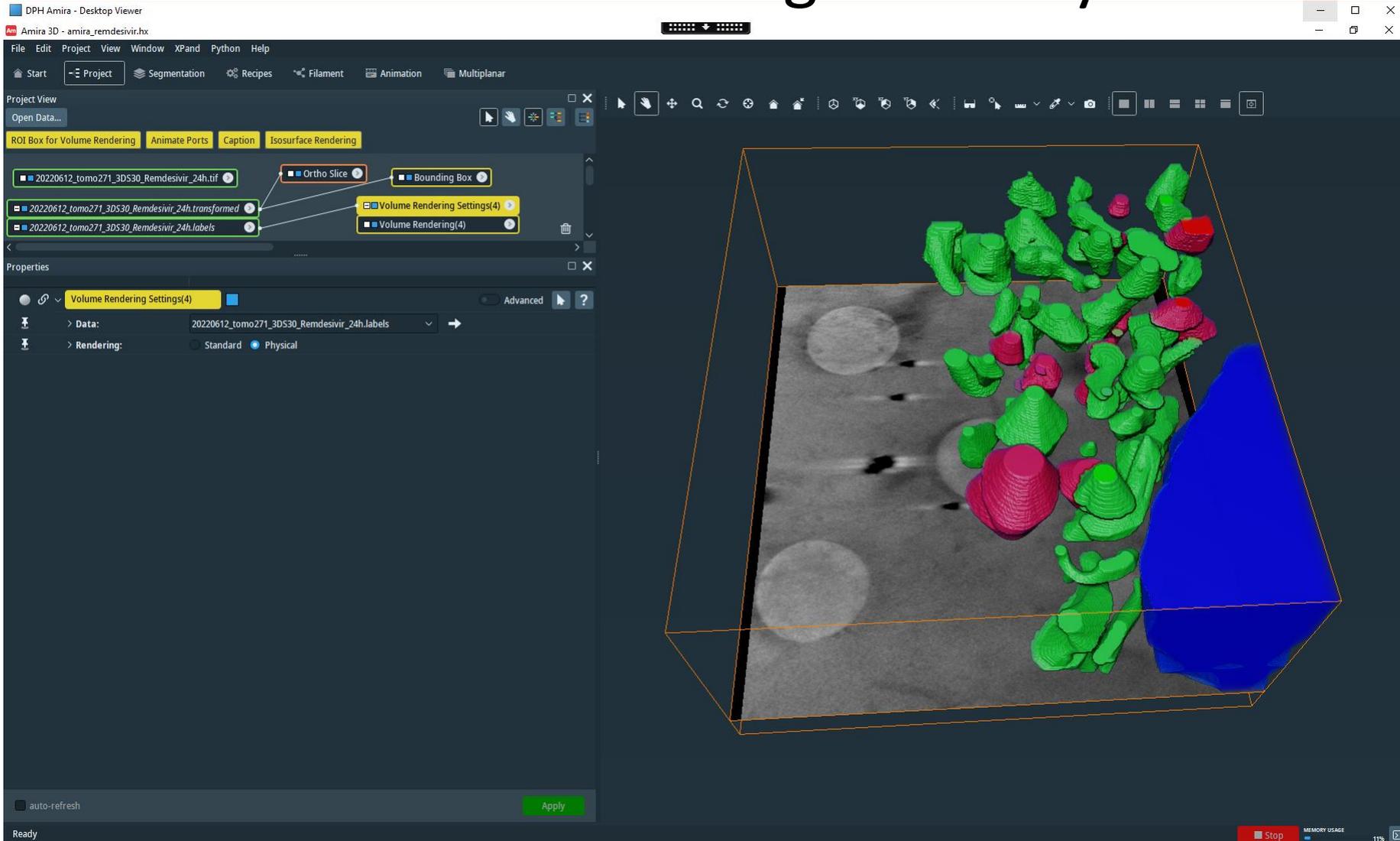
You can take a snapshot of the viewer and create a high resolution figure in many different formats.

Volume rendering



The Results can be exported: file export data as (.mrc 3d tiff .tif 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.

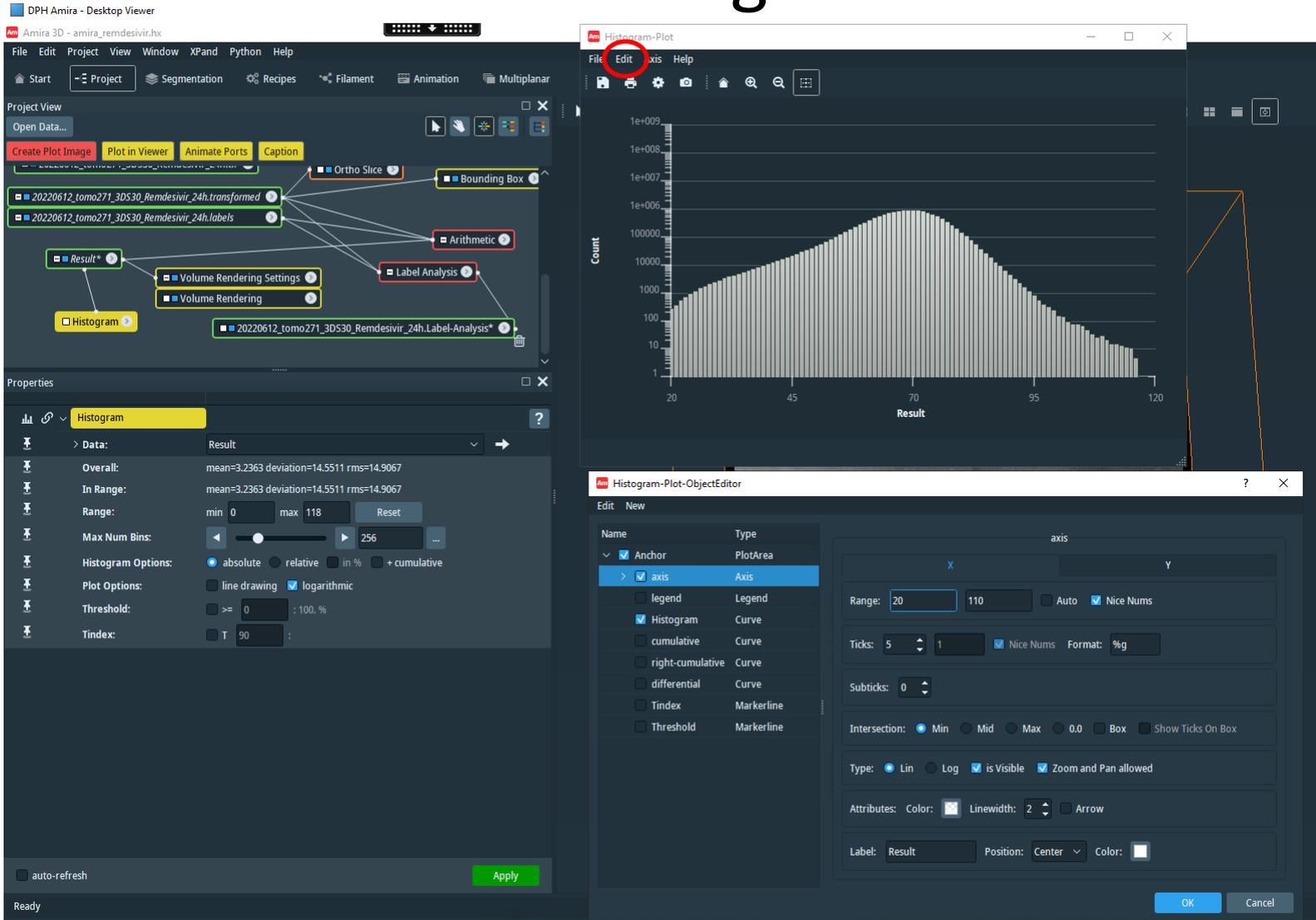
Histogram1

The screenshot displays a software interface with a project view on the left, a main image window in the center, and a histogram plot window on the right. The project view shows a workflow with nodes for 'Ortho Slice', 'Bounding Box', 'Arithmetic', and 'Histogram'. The main image window shows a grayscale image of a textured surface with a bounding box. The histogram plot window shows a distribution curve with a peak at approximately 112.5. The histogram plot window also displays a table of statistics:

Mean	Min	Max	Median	Variance	Kurtosis	Skewness
23.921	0.0	1.0	52.959	52.959	540.35	-1.36

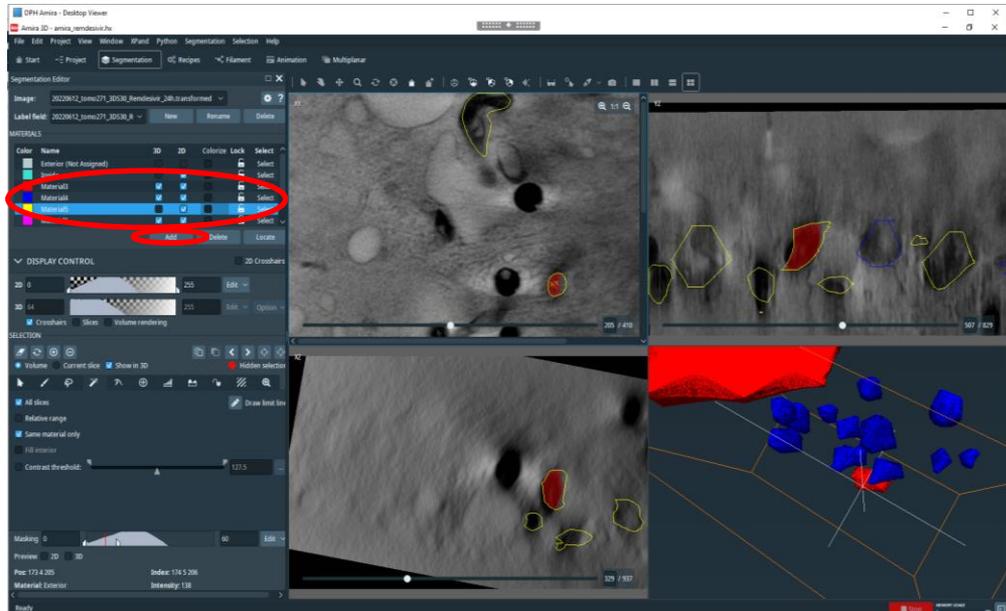
You can also take a look to the actual distribution of the pixels values using the Histogram module.

Histogram2



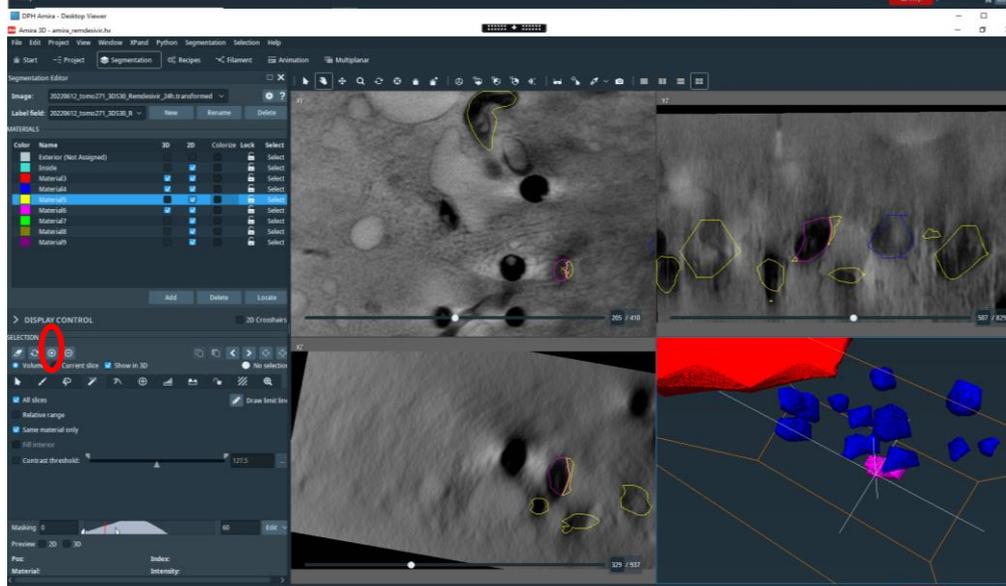
To change the default settings (the axis in particular, to optimize the visualization) click on edit axis.

Volume rendering: fast way2a

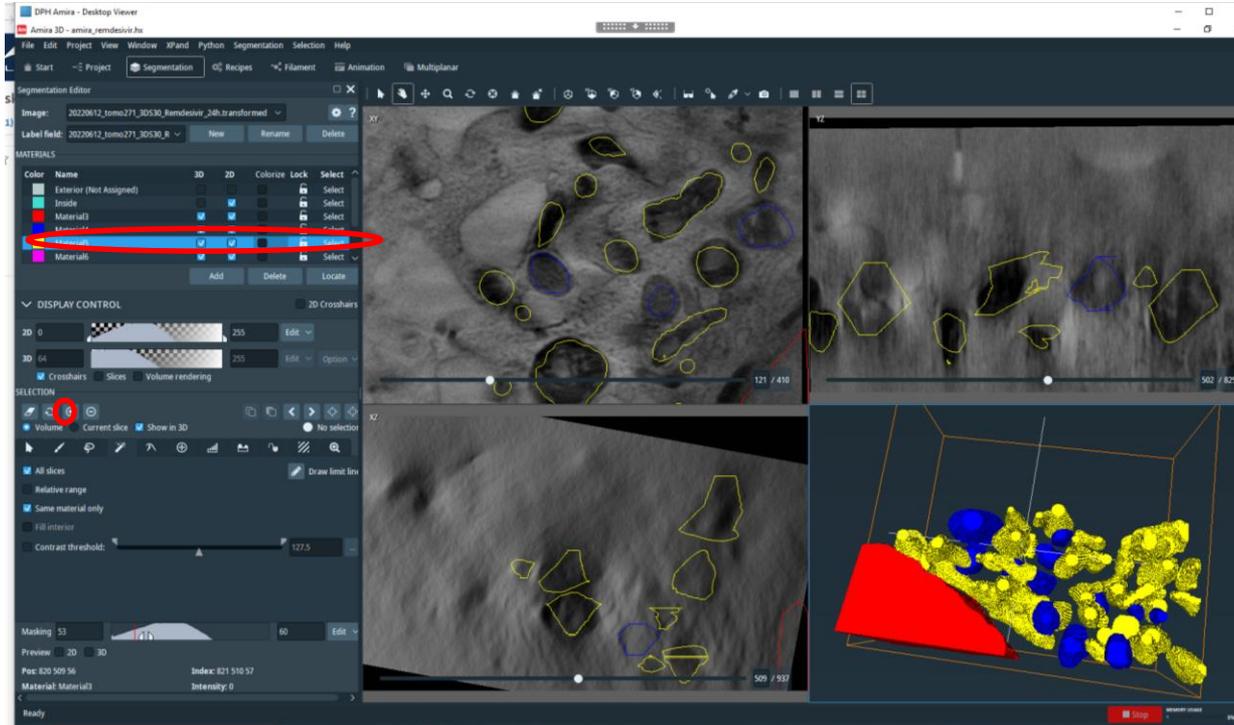


You can create in the segmentation editor a new material (with “Add”) and then you can transfer all the voxels from a material to the new one using the corresponding “select” button and then the “+”.

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 material5 to material7).



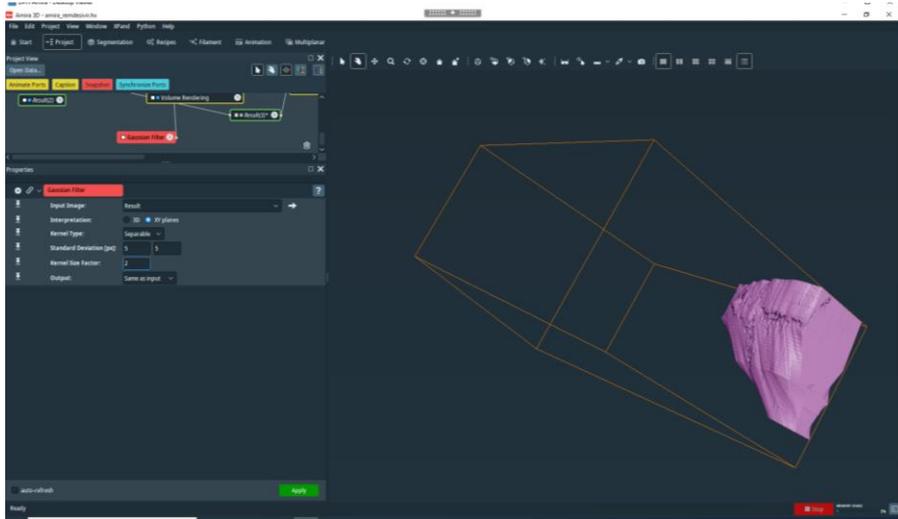
Volume rendering: fast way2b



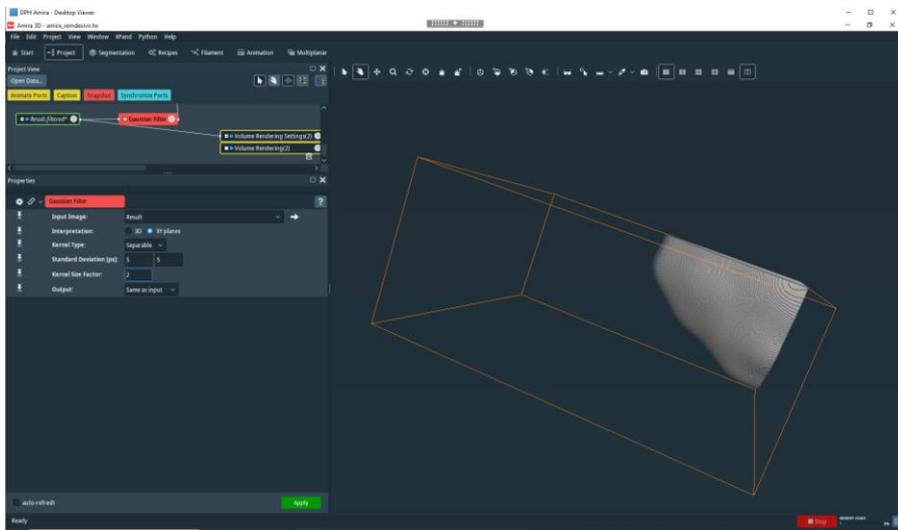
If you do it for all the mitochondria, at the end you will end with a single (big) material containing all the mitochondria and you can give to it the color you want in the project editor. This is a smart way to have all the same kind of object with the same color without creating them one by one using 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 lose 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



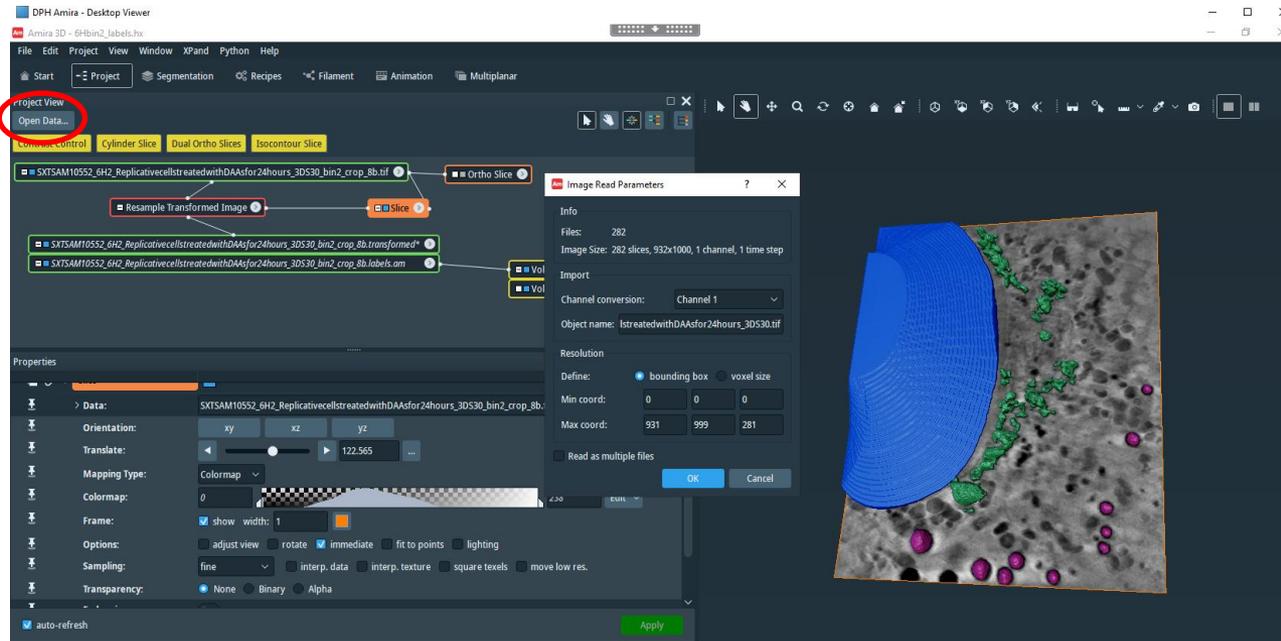
You can improve the appearance of you material using a filter. Right click on the “Result” and search for “filter”: you have a lot of them. Here I used the Gaussian Filter module for example, with those parameters...



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

...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.

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 "32b"

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.

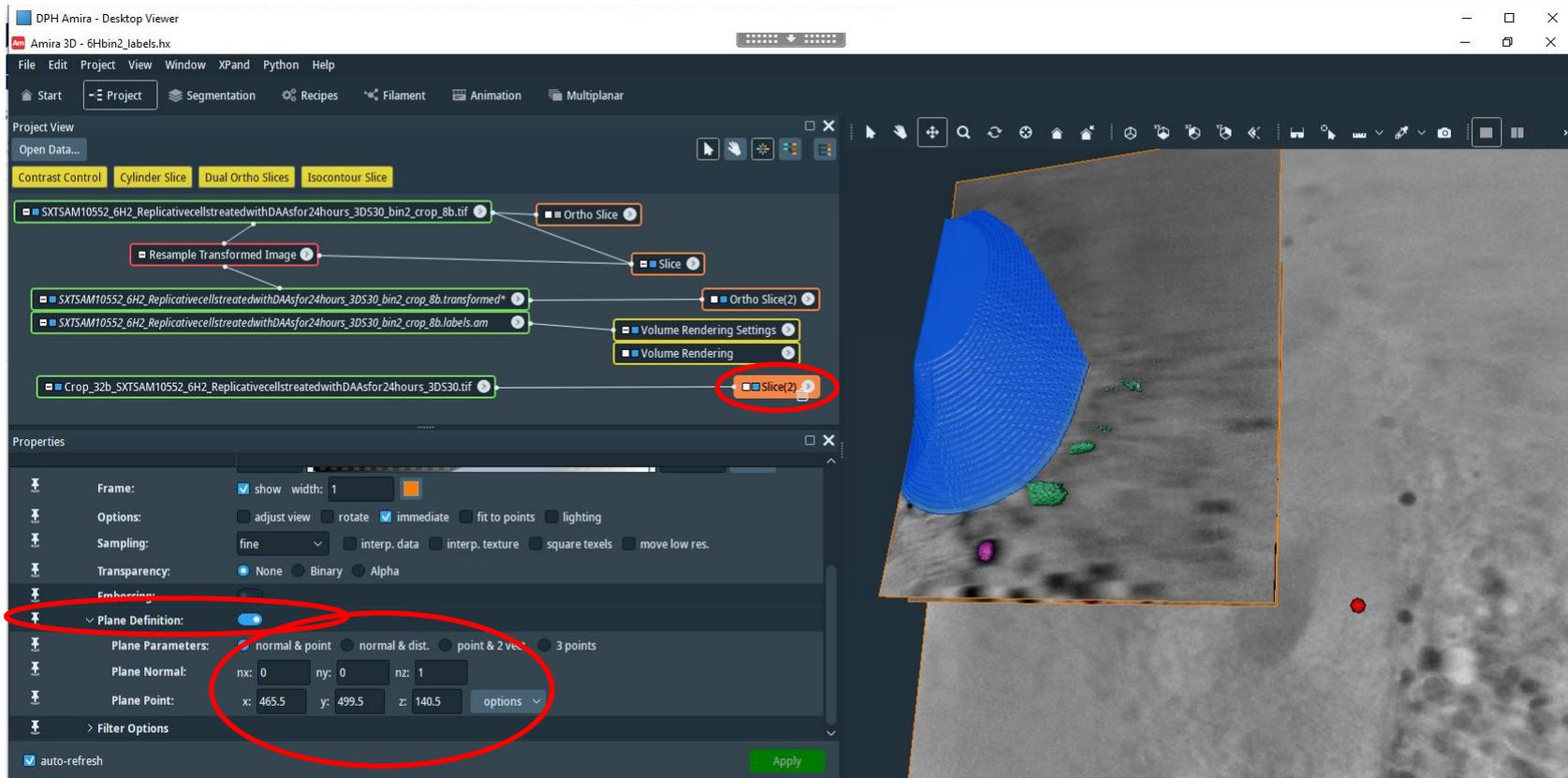
Apply the same segmentation to different DATA

The screenshot displays the DPH Amira Desktop Viewer interface. The main window shows a project view with a workflow graph. The workflow starts with a data source: "SXTSAM10552_6H2_ReplicativecellstreatedwithDAAsfor24hours_3DS30_bin2_crop_8b.tif". This data is processed through a "Resample Transformed Image" step, which then feeds into a "Slice" step. The output of the "Slice" step is used to generate "Ortho Slice" and "Vol" (Volume) outputs. The "Ortho Slice" output is currently selected, showing a 3D visualization of a blue, curved structure on a grayscale background. The "Vol" outputs are shown as green and purple structures. The "Image Read Parameters" dialog box is open, showing the "Info" tab with the following details:

- Files: 282
- Image Size: 282 slices, 932x1000, 1 channel, 1 time step
- Channel conversion: Channel 1
- Object name: !streatedwithDAAsfor24hours_3DS30.tif
- Resolution: Define: bounding box (selected), voxel size
- Min coord: 0 0 0
- Max coord: 931 999 281
- Read as multiple files:

The "Properties" panel at the bottom left shows the current data source and various settings, including Orientation (xy, xz, yz), Translate (122.565), Mapping Type (Colormap), Colormap (0), Frame (show width: 1), Options (adjust view, rotate, immediate, fit to points, lighting), Sampling (fine, interp. data, interp. texture, square texels, move low res.), and Transparency (None, Binary, Alpha). The "auto-refresh" checkbox is checked, and an "Apply" button is visible at the bottom right.

Apply the same segmentation to different DATA

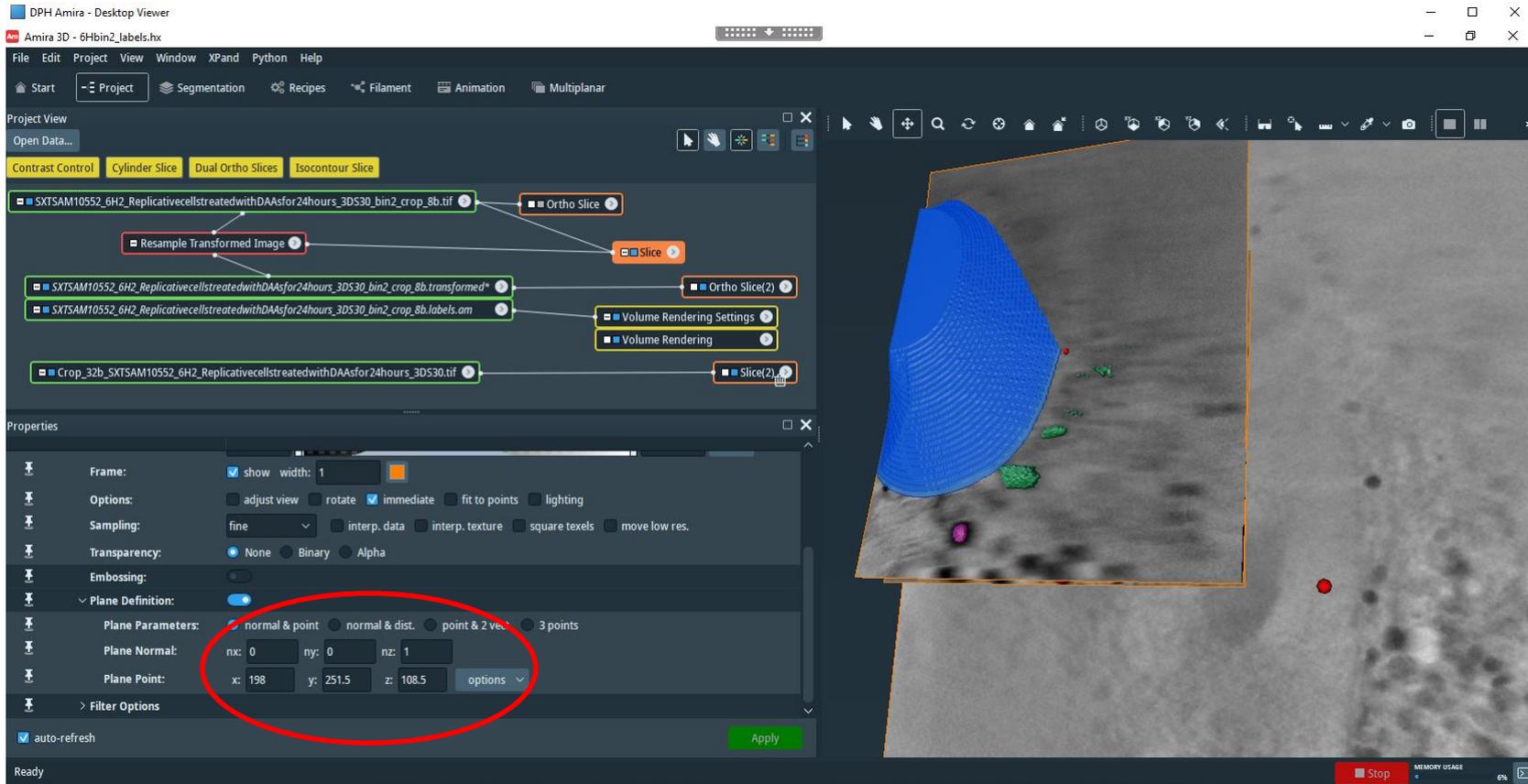


In this case we want to re-apply the transformation defined by the module “slice”.

In the new data create a module “slice”.

Activate the plane definition option

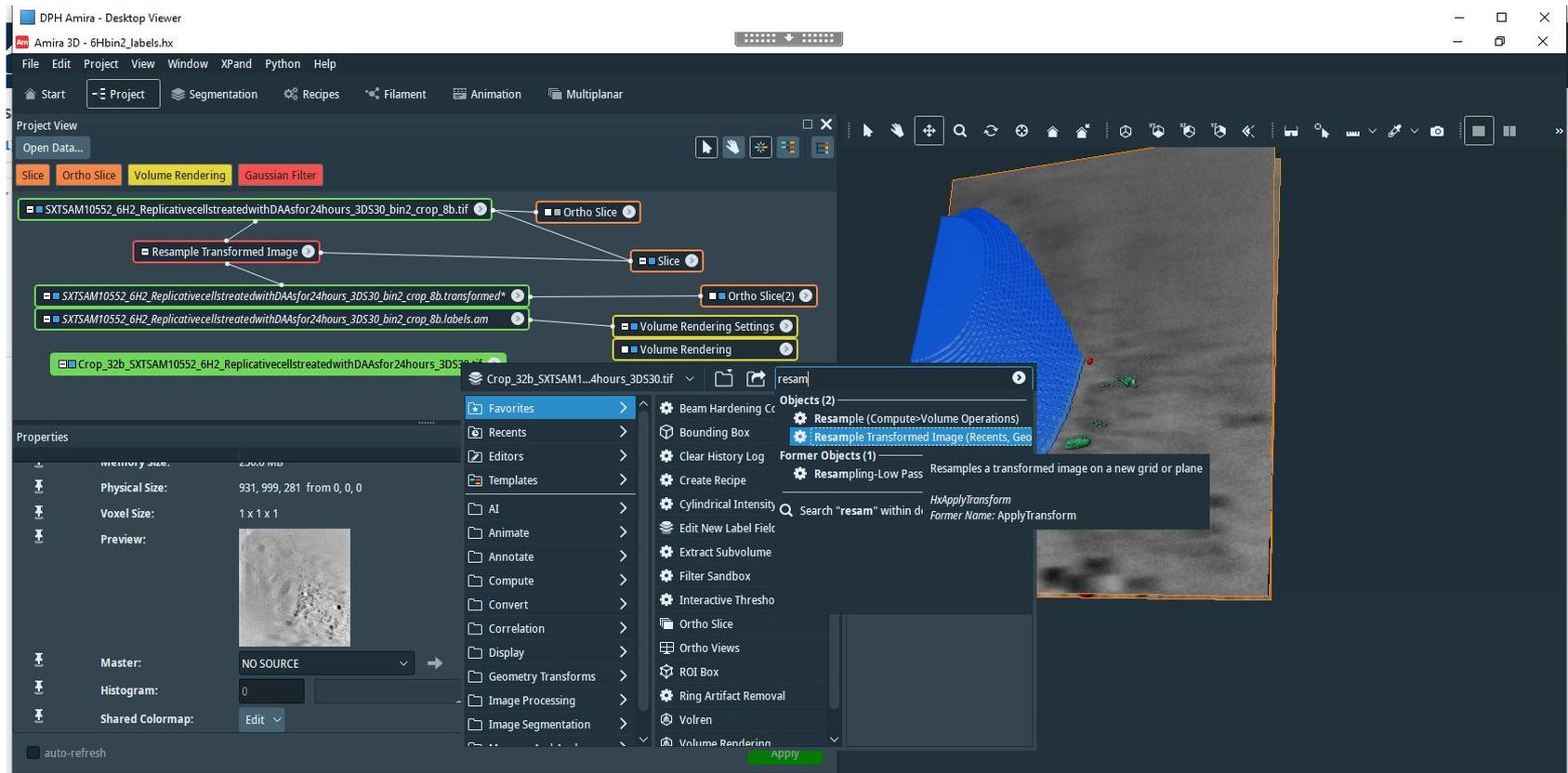
Apply the same segmentation to different DATA



Create another slice module and then to apply exactly the same orientation.

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).

Apply the same segmentation to different DATA



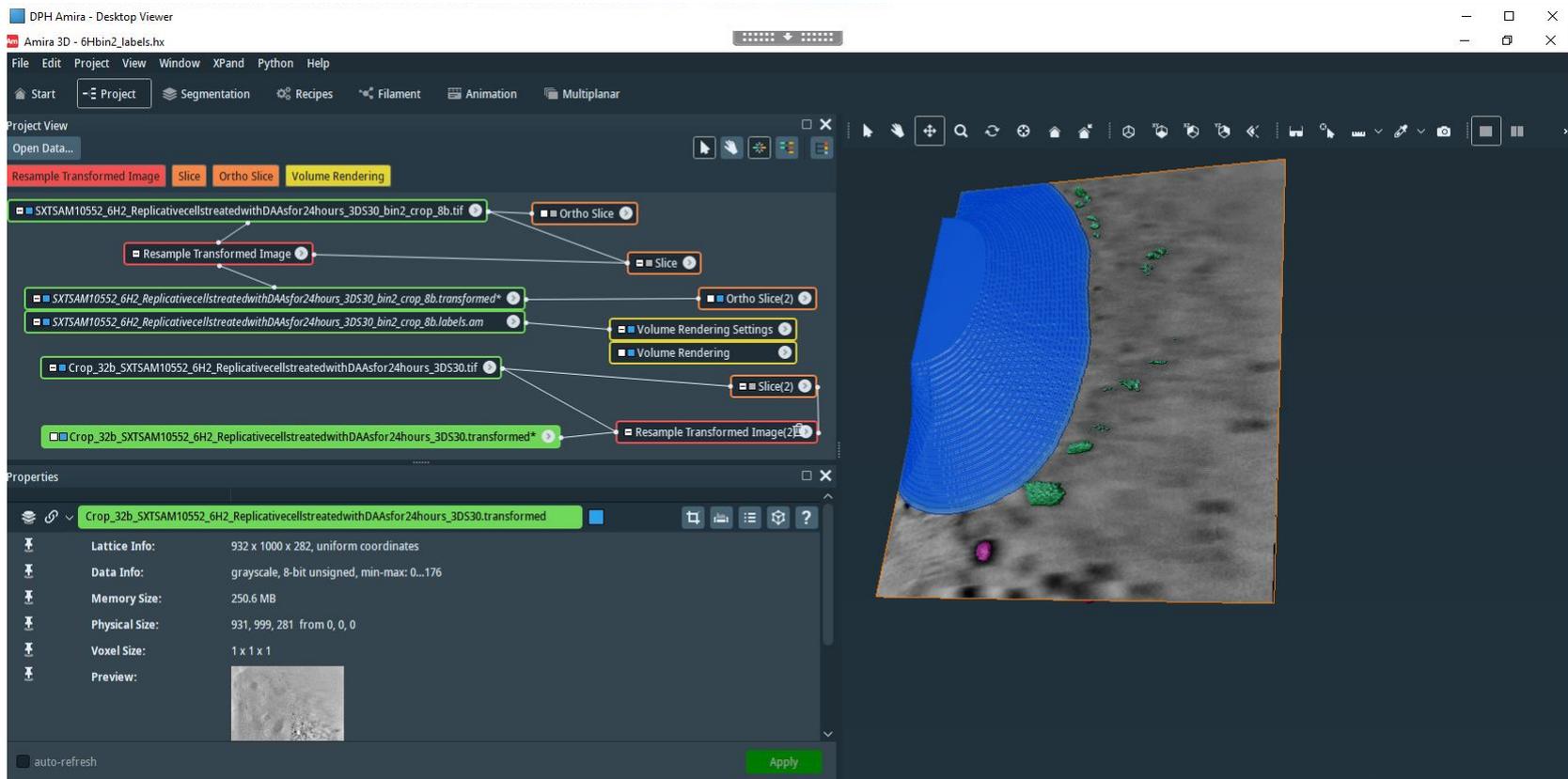
Now the new DATA set has the right orientation and you can re-apply the transformation using the “Resample Transformed Image” module.

Apply the same segmentation to different DATA

The screenshot displays the Amira 3D software interface. The top menu bar includes File, Edit, Project, View, Window, XPand, Python, and Help. The Project View panel on the left shows a workflow diagram with several modules: 'SXTSAM10552_6H2_ReplicativecellstreatedwithDAAsfor24hours_3DS30_bin2_crop_8b.tif', 'Resample Transformed Image', 'SXTSAM10552_6H2_ReplicativecellstreatedwithDAAsfor24hours_3DS30_bin2_crop_8b.transformed*', 'SXTSAM10552_6H2_ReplicativecellstreatedwithDAAsfor24hours_3DS30_bin2_crop_8b.labels.am', and 'Crop_32b_SXTSAM10552_6H2_ReplicativecellstreatedwithDAAsfor24hours_3DS30.tif'. The 'Resample Transformed Image' module is highlighted with a red box. The Properties panel at the bottom left shows the settings for 'Resample Transformed Image(2)', including Data, Interpolation, Mode, and Padding Value. A 'Collapse' dialog box is open over the 'Crop_32b_SXTSAM10552_6H2_ReplicativecellstreatedwithDAAsfor24hours_3DS30.tif' module, showing 'Data (->Crop_32b_SXTSAM10552_6H2_ReplicativecellstreatedwithDAAsfor24hours_3DS30.tif)' and 'Reference (->Slice(2))'. The 3D visualization on the right shows a blue segmented volume on a grayscale background.

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.

Apply the same segmentation to different DATA



If you now visualize the transformed data using the orthoslice module you still have the "right orientation" defined with the slice one.

Computational modules are in general in RED.

Apply the same segmentation to different DATA

The screenshot shows the Amira 3D software interface with a workflow for applying segmentation to different data. The workflow includes steps like 'Resample Transformed Image', 'Slice', 'Volume Rendering', and 'Label Analysis'. The 'Label Analysis' properties are shown in the bottom left, and a 3D visualization of a segmented object is shown in the center. A 'Tables' panel on the right displays statistical data for the segmented object.

Label Analysis Properties:

- Data: SXTSAM10552_6H2_ReplicativecellstreatedwithDAAsfor24hours_3DS30_bin2_crop_8b.labels.am
- Intensity Image: SXTSAM10552_6H2_ReplicativecellstreatedwithDAAsfor24hours_3DS30_bin2_crop_8b.transformed
- Interpretation: 3D
- Measures: basic

Tables:

	Volume3d	Area
Mean	1.15344e+06	84321.
Min	0.0	0.0
Max	4.26069e+06	19119.
Median	81863.0	22830.
Variance	3.22801e+12	5.9574
Kurtosis	-0.674832	-1.607
Skewness	1.14441	0.2434

	Volume3d	Area
1	0.0	0.0
2	4.26069e+6	19119.
3	81863.0	22830.
4	271217.0	12325.

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. Even complex 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.

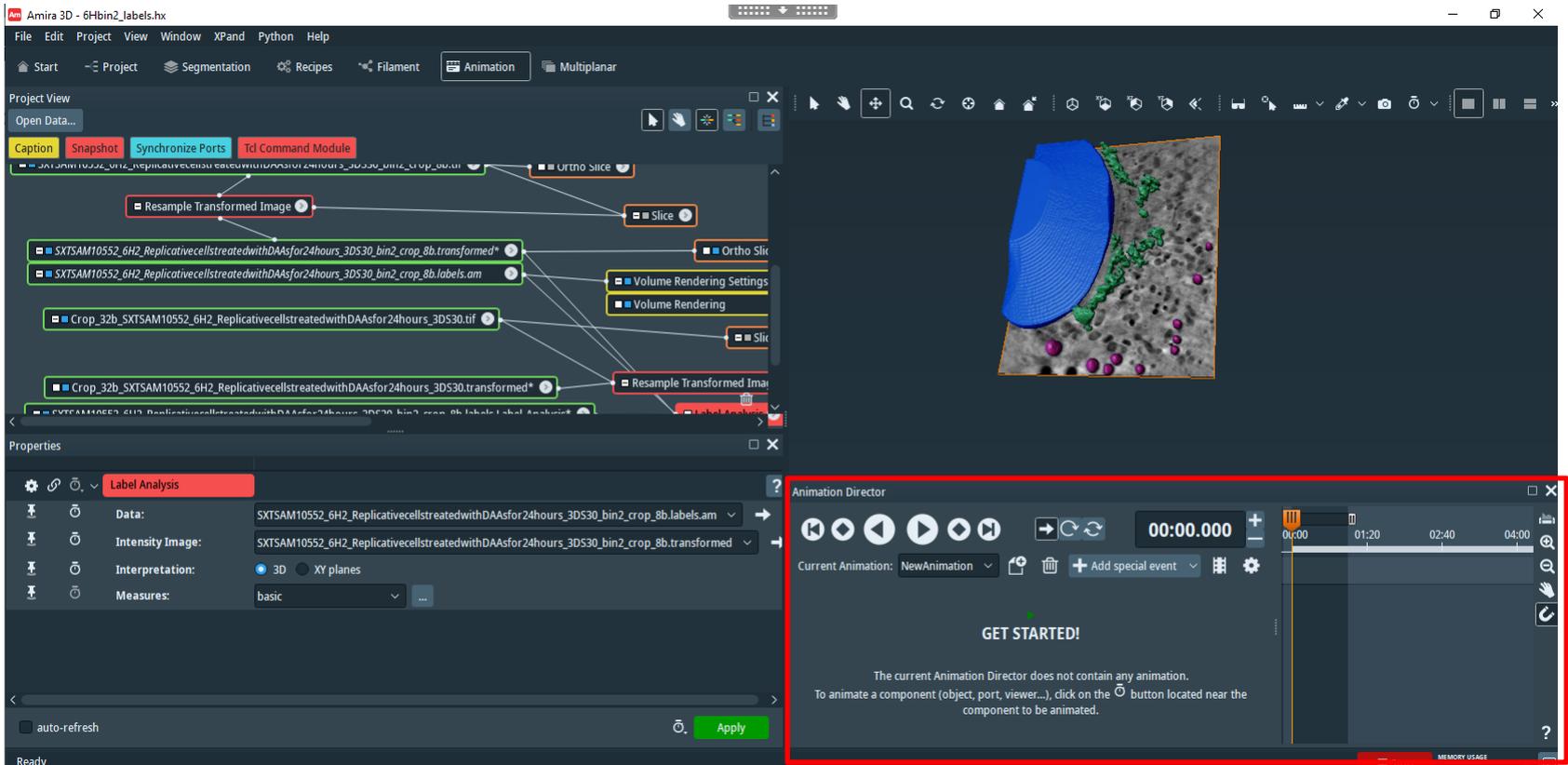
The Animation Director module is activated or deactivated by pressing the Animation button in the toolbar, or by selecting **Window > Animation**

The screenshot displays the Animation Director software interface. The top menu bar includes File, Edit, Project, View, Window, XPand, Python, and Help. The main toolbar features buttons for Start, Project, Segmentation, Recipes, Filament, Animation (highlighted with a red circle), and Multiplanar. The Project View panel on the left shows a workflow diagram with modules such as Resample Transformed Image, Slice, Ortho Slice, Volume Rendering Settings, Volume Rendering, and Label Analysis. The central 3D visualization shows a blue, curved structure on a textured surface with green and purple particles. The Properties panel at the bottom left shows the Label Analysis module settings, including Data, Intensity Image, Interpretation (3D), and Measures. The Tables panel on the right displays statistical data for Volume3d and Area.

	Volume3d	Area
Mean	1.15344e+06	84321.
Min	0.0	0.0
Max	4.26069e+06	19119.
Median	81863.0	22830.
Variance	3.22801e+12	5.9574
Kurtosis	-0.674832	-1.607
Skewness	1.14441	0.2434

	Volume3d	Area
1	0.0	0.0
2	4.26069e+6	19119
3	81863.0	22830.
4	271217.0	12325

Animations and Movies



There are different perspectives when it comes to animation:

Animate data object

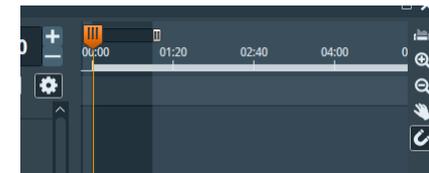
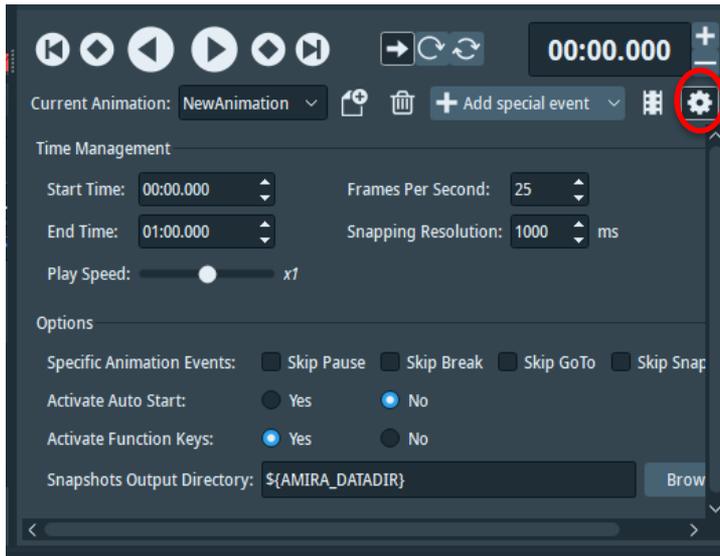
Animate camera

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



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 activates 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.

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.

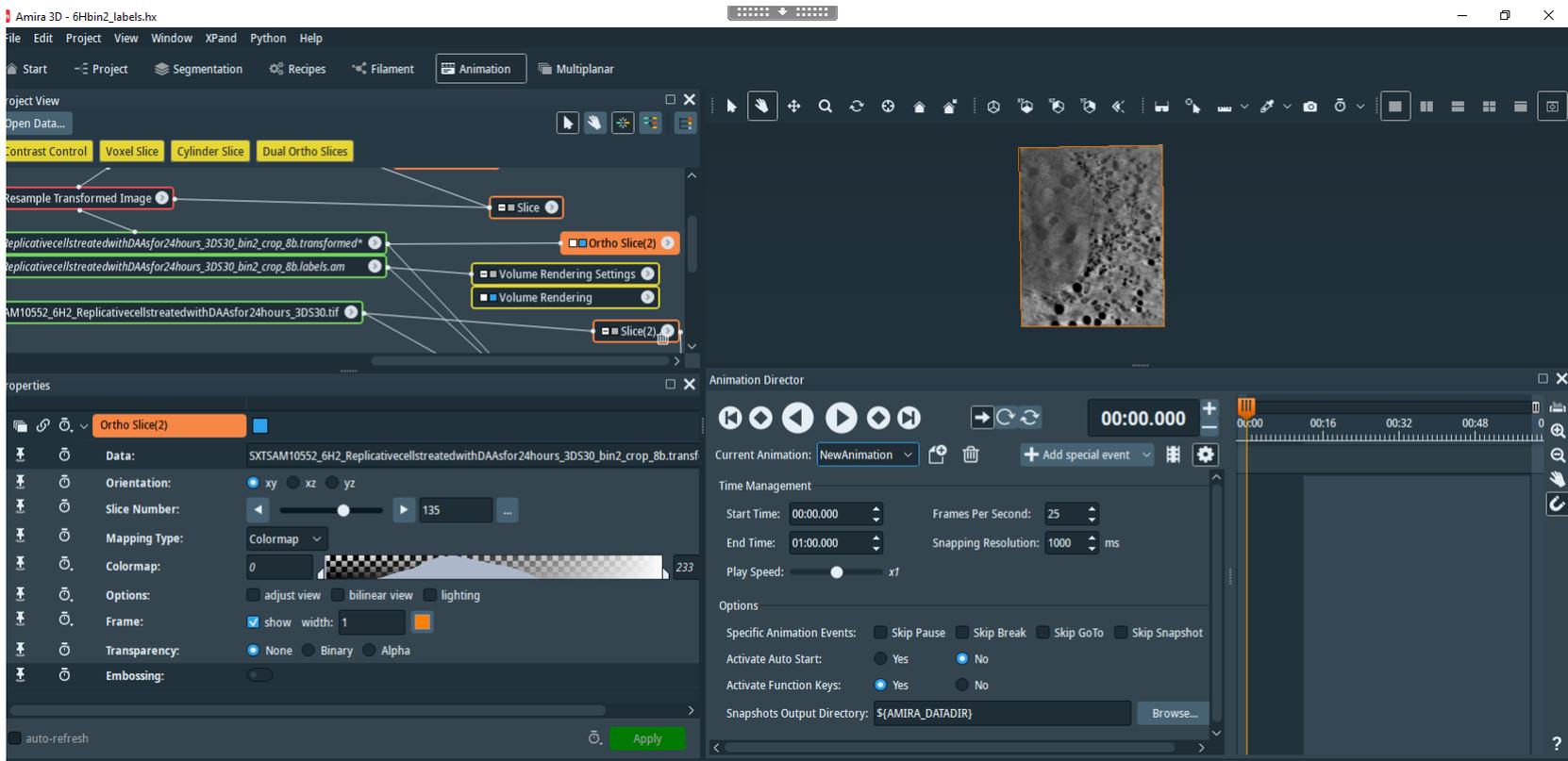
Animating an Ortho Slice module

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.



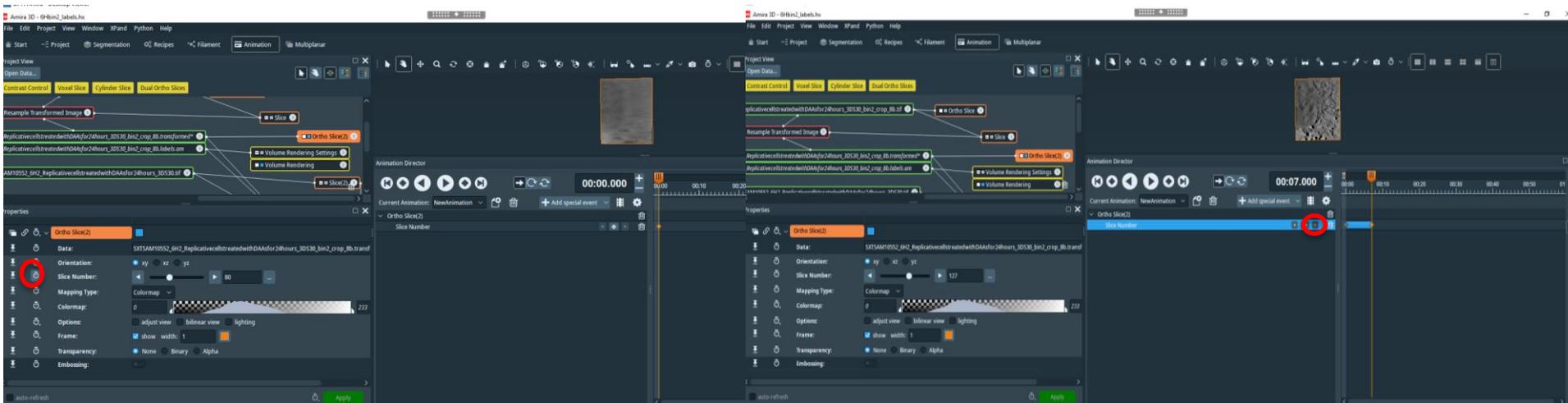
Like the other widgets, this widget is also dockable and you can place it at a convenient position within the Amira user interface. After activating the Animation Director by clicking on the related button in the toolbar, all ports of the currently available modules that can be animated are extended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

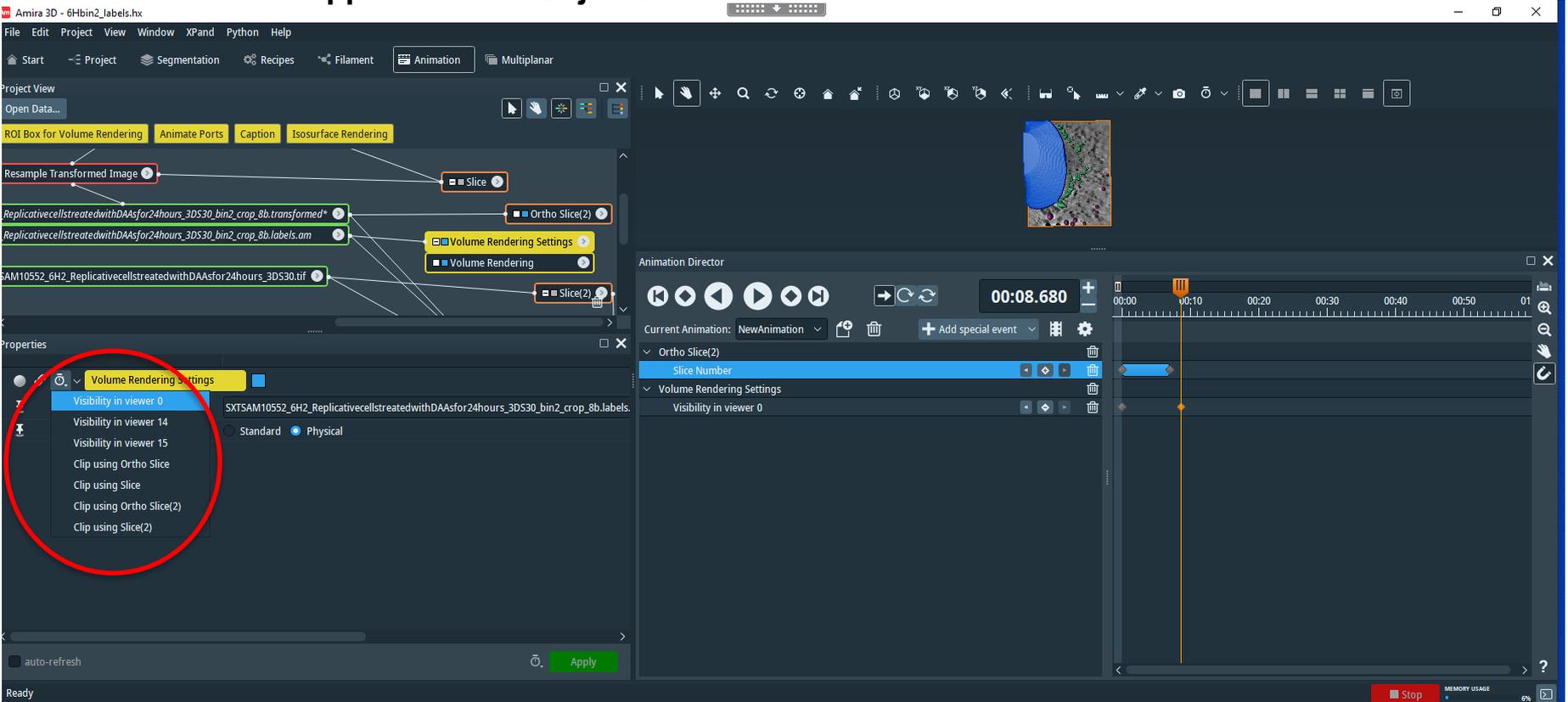
If you hold the mouse cursor above the small orange diamond symbol in the timeline panel, this will activate a small input field where you can adjust the time and the accompanying value for the port with which it is associated. In order to adjust the schedule, you can simply drag the diamond icon to 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 131. After you click the stopwatch button again, the keyframe is created in the timeline.

You can test your first animation ▶

How to Animate the Appearance of Objects with the Animation Director



We do this by selecting Volume Rendering and clicking on the stopwatch button

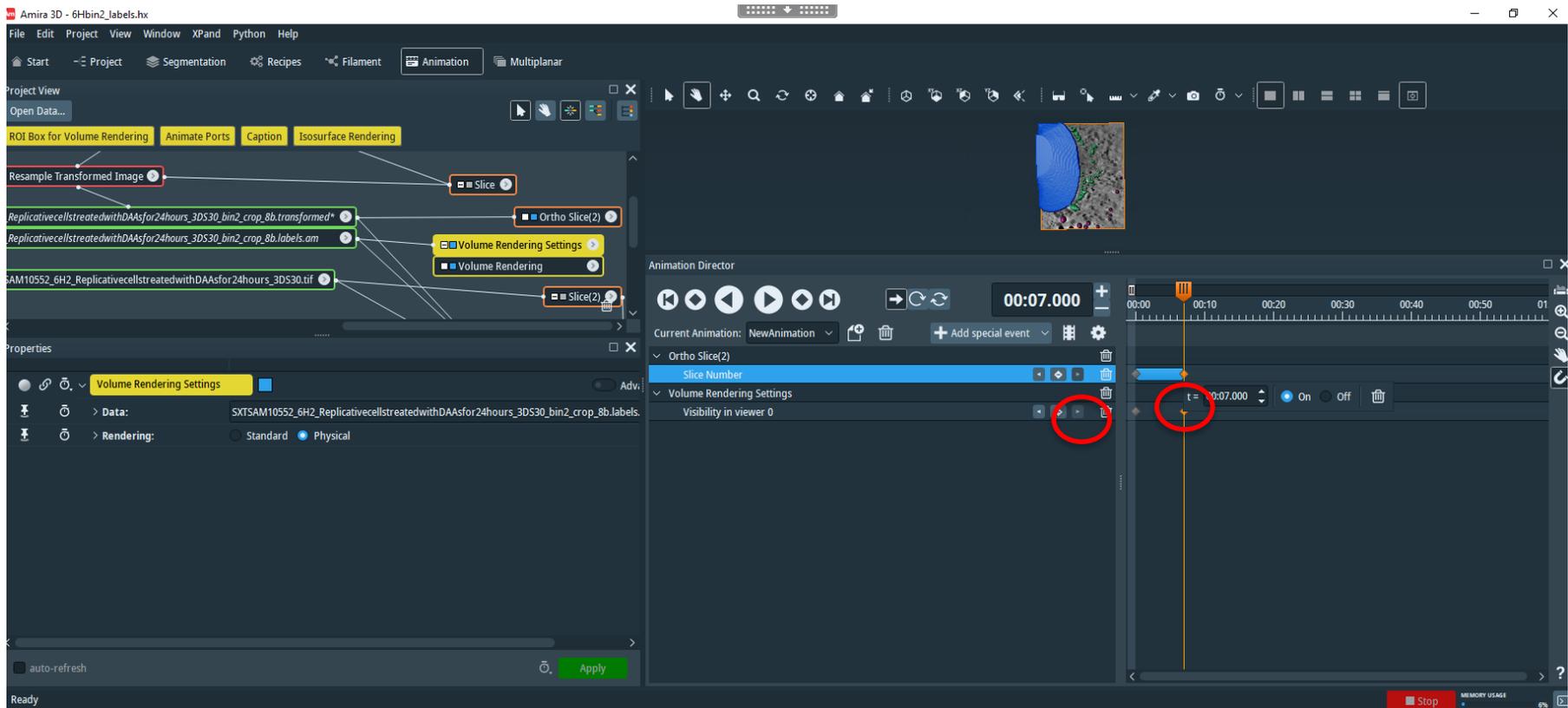
Select visibility 0

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, 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.

How to Animate the Appearance of Objects with the Animation Director

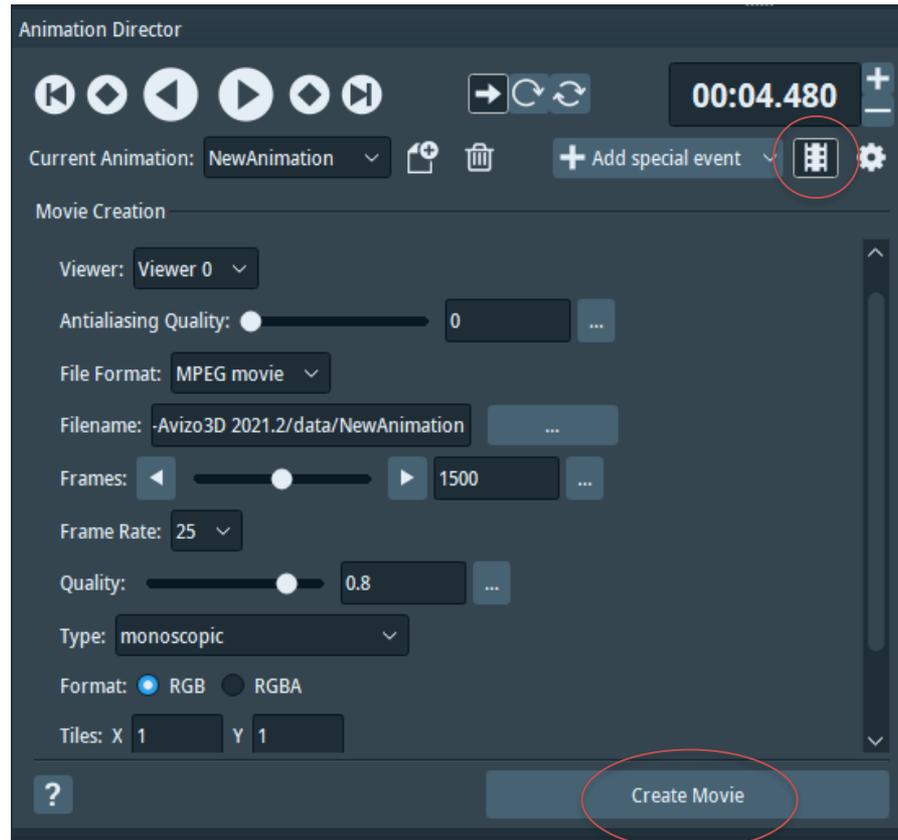


Press for adding a new keyframe: new keyframe is created in the timeline
Double click in the Orange diamond and selected ON and the volumen rendering would be now visible.

You can test your animation clicking

Creating a movie from an animated demonstration

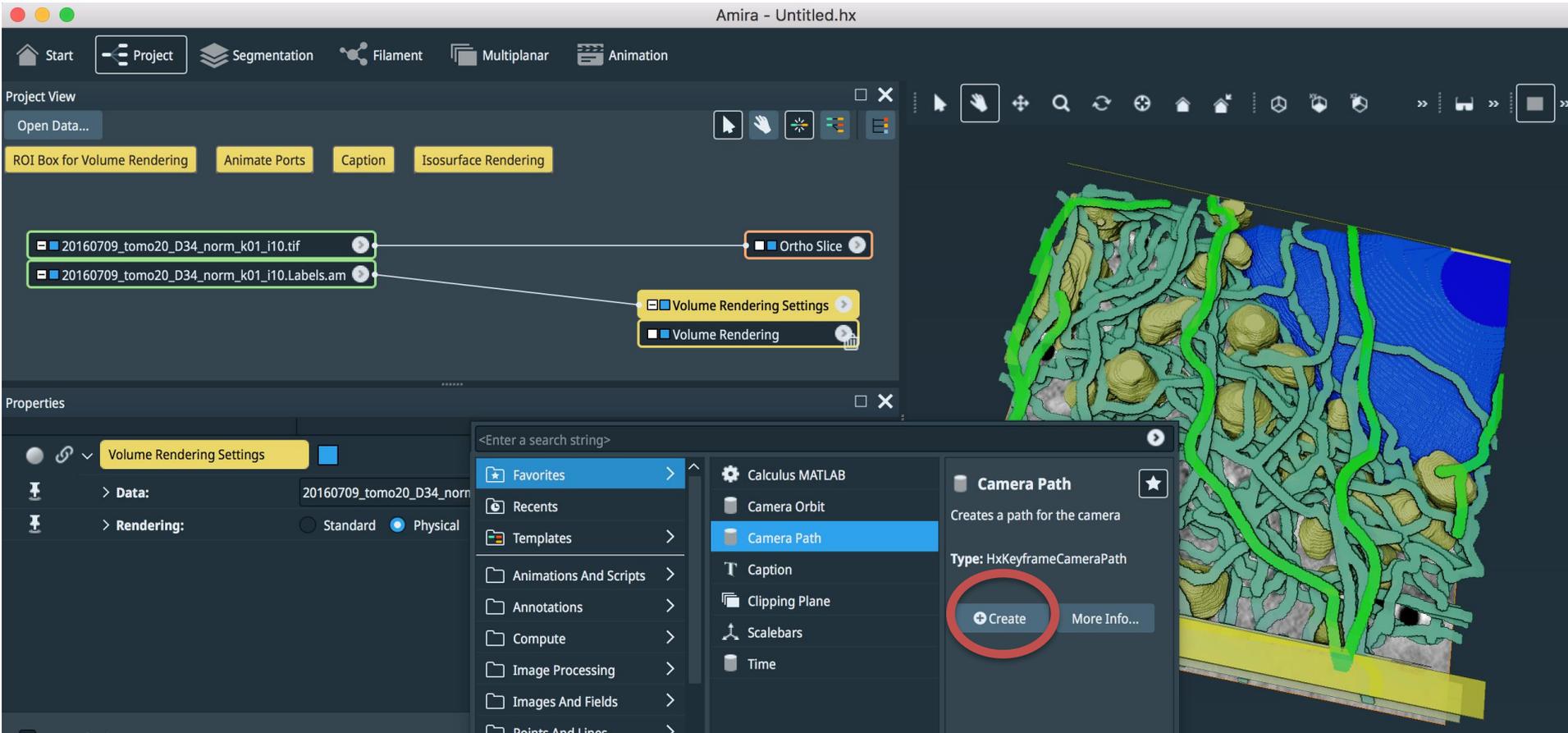
To create a movie from an animation defined with the Animation Director, simply click  on the Movie Creation button of the Animation Director panel. The following panel will appear:



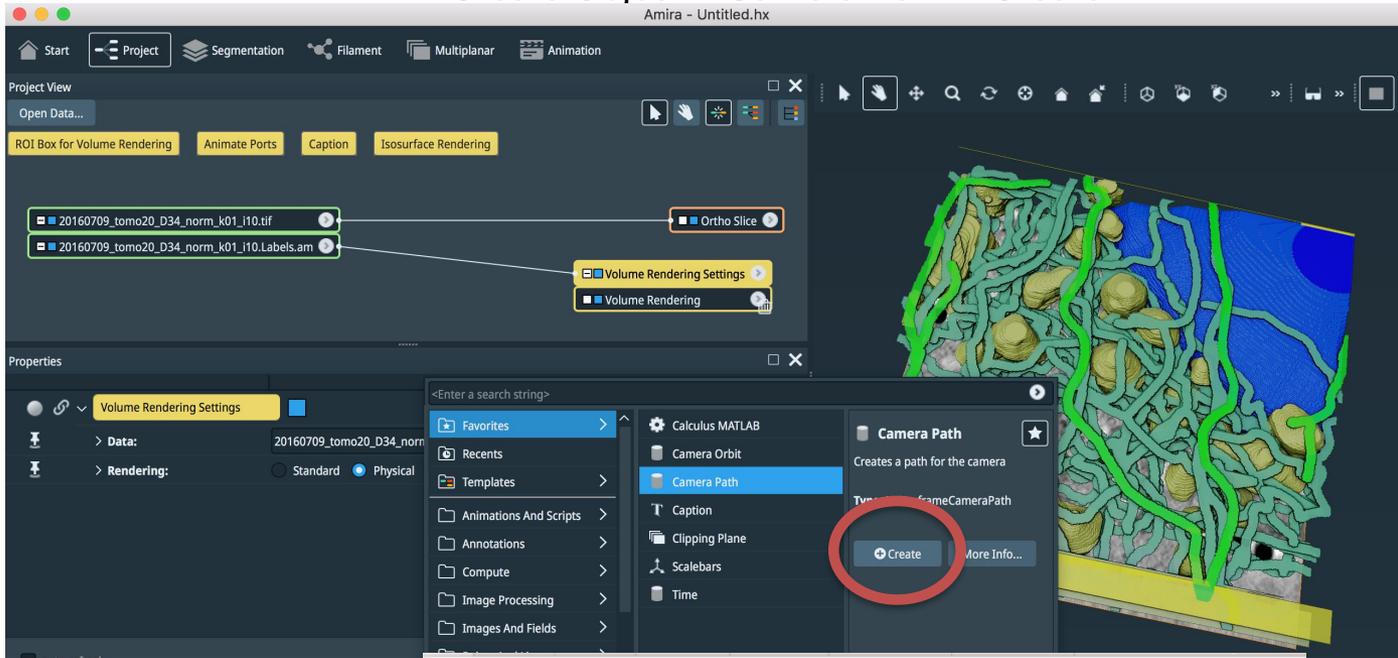
This module is already pre-configured to create a movie that respects the animation settings (duration, frame rate, filename...) that are defined by the Animation Director module. However, you can adjust these parameters, if needed. Just click on the Create Movie button to generate the movie.

Movie Maker using Camera Path

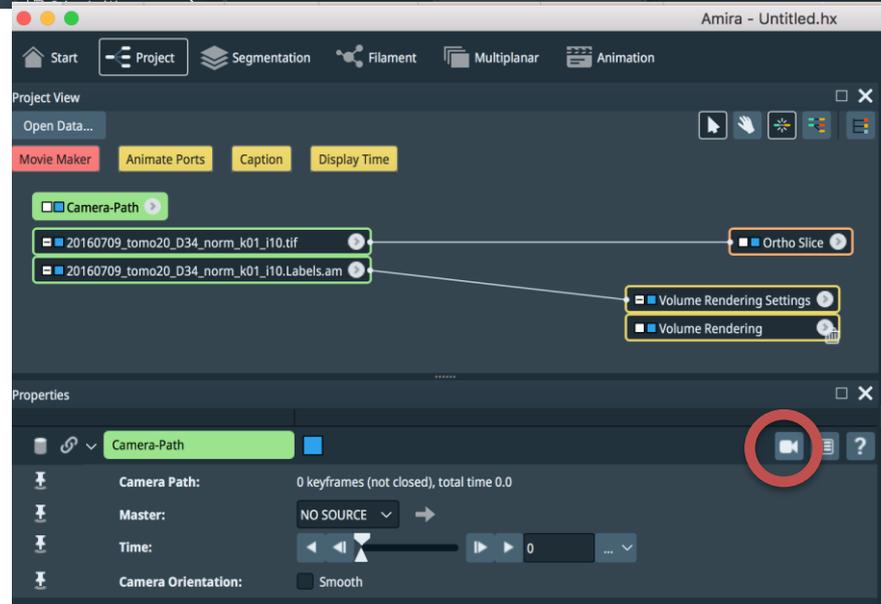
Right click in empty space, choose
Create Object ->Camera Path-> Create



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



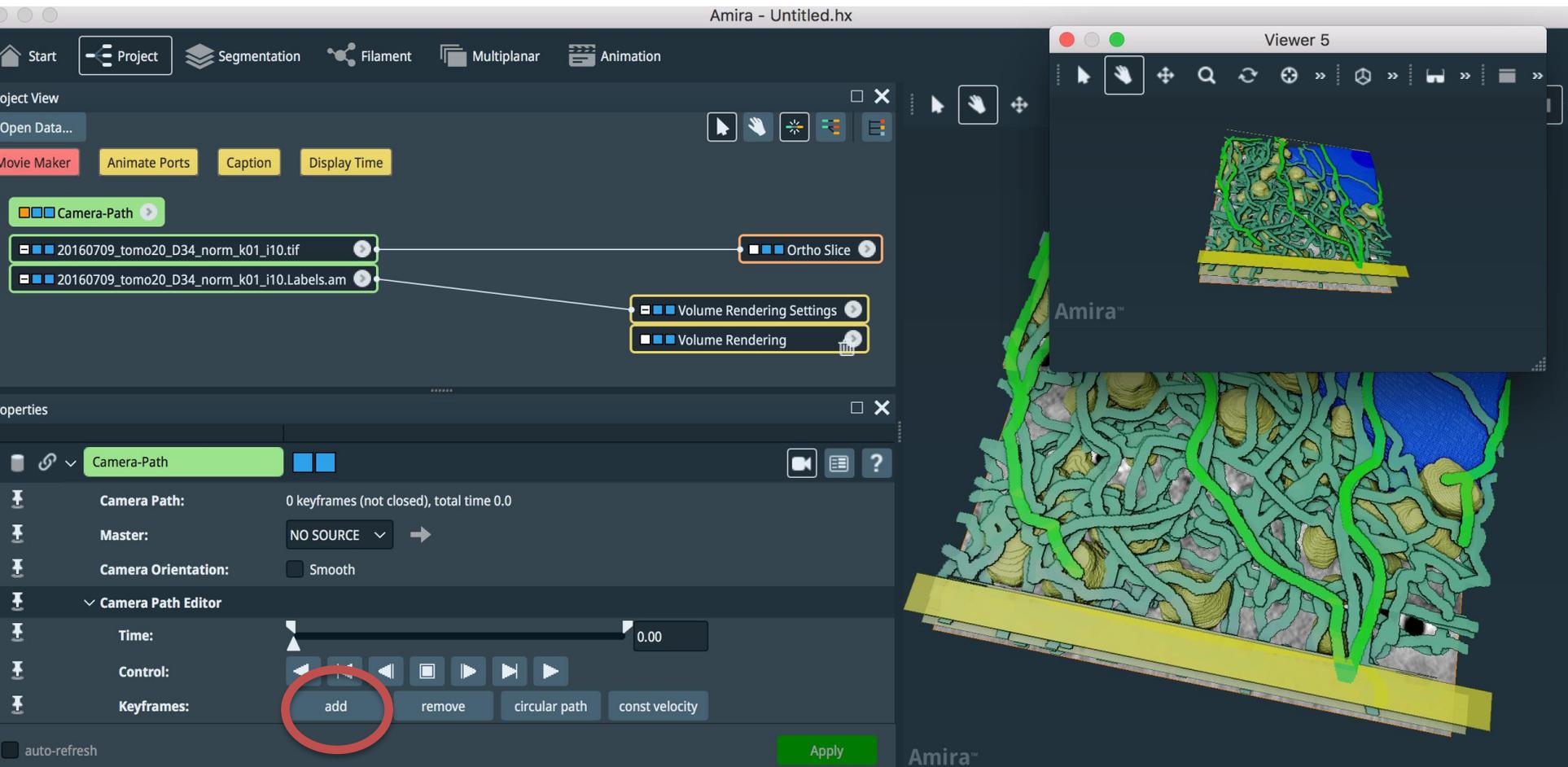
Open camera path editor,
Click camera Path editor



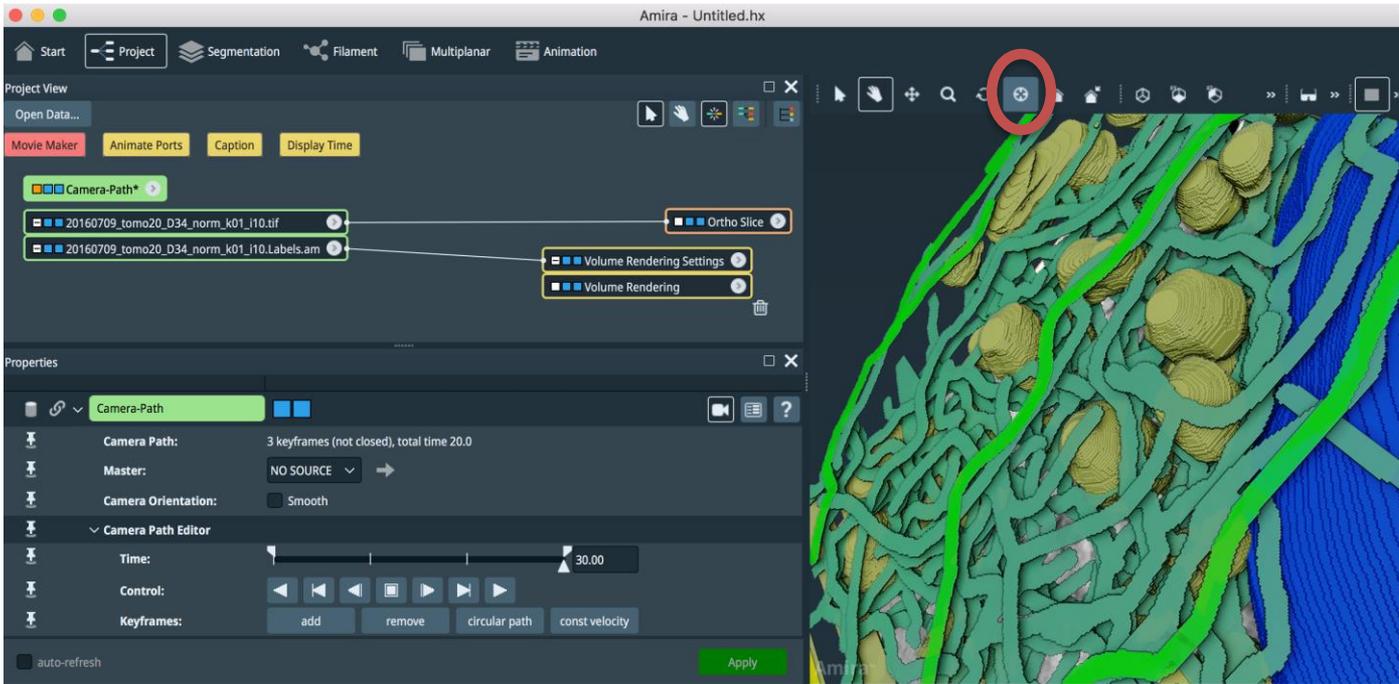
A separate camera path edit/view window will open.

Click camera Path editor.

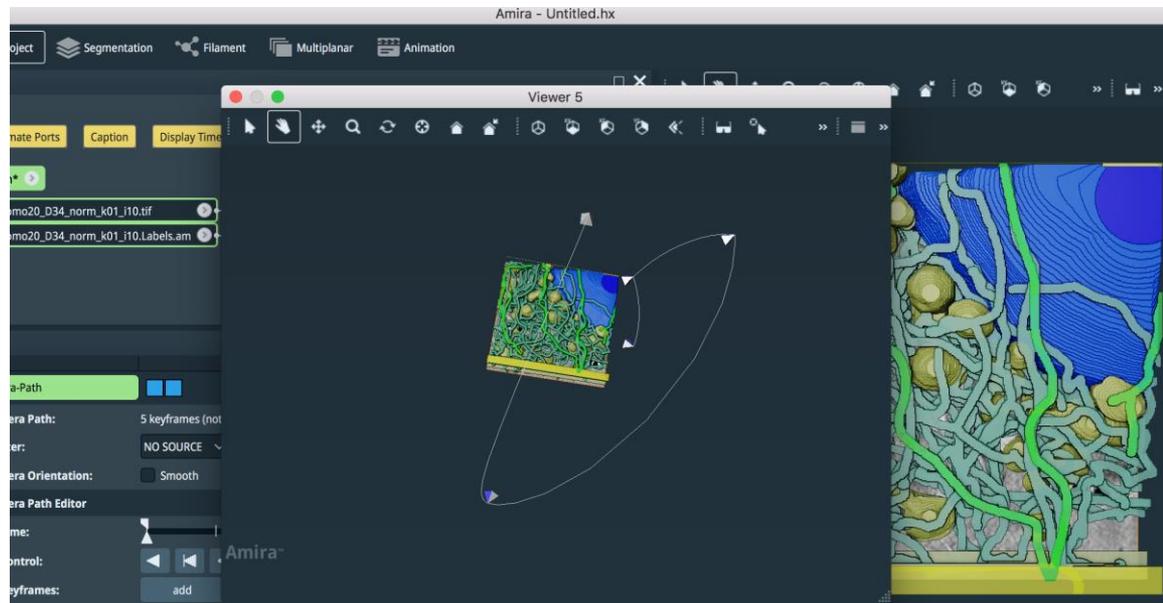
Change view position in main window, then click “add” button to add a keyframe.



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

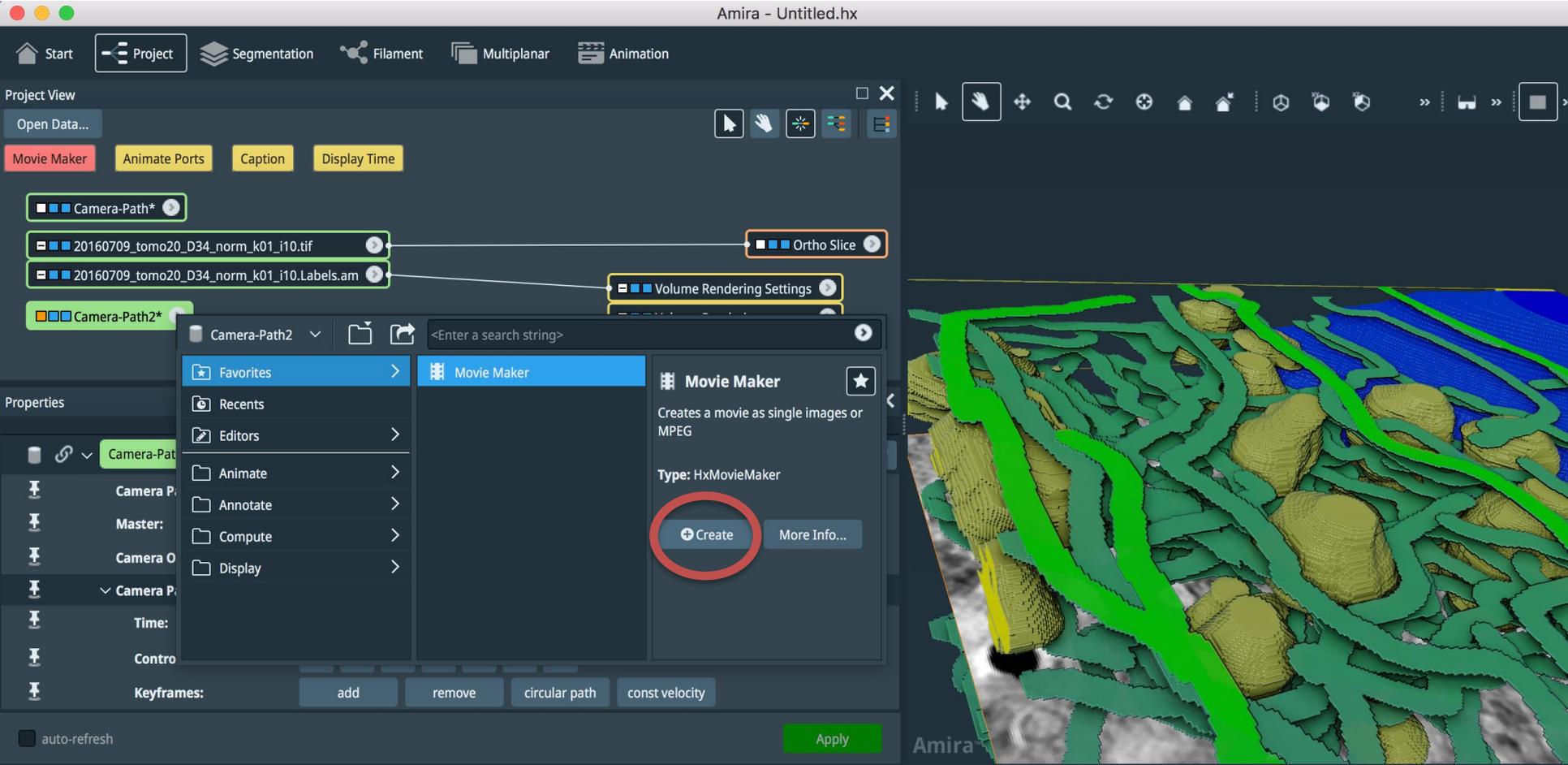
The screenshot displays the Amira software interface for a 3D volume rendering project. The main window is titled "Amira - Untitled.hx".

Project View: Located at the top left, it contains navigation icons (Home, Start, Project, Segmentation, Filament, Multiplanar, Animation) and a toolbar with icons for navigation and rendering. Below the toolbar are buttons for "Movie Maker", "Animate Ports", "Caption", and "Display Time". A list of objects is shown, including "Camera-Path*", "20160709_tomo20_D34_norm_k01_i10.tif", "20160709_tomo20_D34_norm_k01_i10.Labels.am", "Ortho Slice", "Volume Rendering Settings", and "Volume Rendering".

Properties Panel: Located at the bottom left, it shows the selected object "Camera-Path2". The "Camera Path" section indicates "4 keyframes (not closed), total time 30.0". The "Master" is set to "NO SOURCE". The "Camera Orientation" is set to "Smooth". The "Camera Path Editor" section includes a "Time" slider set to 28.50, a "Control" section with navigation buttons (Back, Forward, Home, Play, Stop), and a "Keyframes" section with buttons for "add", "remove", "circ path", and "const velocity". The "Play" button is circled in red.

3D Visualization: The main area on the right shows a 3D volume rendering of biological tissue. The tissue is rendered in green and yellow, with a blue background. A camera path is visible as a yellow line on the surface of the volume.

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



Select your output file and the file format-> APPLY

The screenshot displays the Amira software interface with the following components:

- Project View:** Contains buttons for 'Open Data...', 'Animate Ports', 'Caption', 'Snapshot', and 'Synchronize Ports'. It shows a hierarchical tree of objects including 'Camera-Path*', '20160709_tomo20_D34_norm_k01_i10.tif', '20160709_tomo20_D34_norm_k01_i10.Labels.am', 'Camera-Path2*', 'Ortho Slice', 'Volume Rendering Settings', and 'Volume Rendering'.
- Properties Panel:** Configured for the 'Movie Maker' object. It includes:
 - Time:** Set to 'Camera-Path'.
 - Viewer:** Set to 'Viewer 0'.
 - Antialiasing Quality:** A slider set to 0.2.
 - Format Options:**
 - Info:** Frames: 200 - Total time: 8.3 s - Frame rate: 24 (fixed)
 - Filename:** 'aper_FTIR/tomo20/tomo_pell.mpg' with a file selection button.
 - File Format:** 'MPEG movie'.
 - Resolution Options:**
 - Size:** 'Viewer' selected, with options for 360p, 480p, 720p, 1080p, and Custom.
 - Resolution [px]:** X: 528, Y: 576.
- 3D Viewport:** Shows a 3D reconstruction of a biological sample with green and yellow structures. A yellow rectangular plane is visible in the foreground.
- Toolbar:** Located at the top right, containing various navigation and tool icons.
- Footer:** The 'Amira' logo is visible in the bottom right corner.

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>