



Brazilian Synchrotron
Light Laboratory



CNPem
Brazilian Center for Research
in Energy and Materials

Avizo Guide

A quick start

X-Ray Imaging Beamline (IMX)

Maio/2019

LNLS is part of the CNPem an organization certified by the Ministry of Science, Technology, Innovation and Communication (MCTIC).

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Sumário

1.	Using ImageJ to crop and change image type	2
a.	Change of image type.....	2
b.	Cropping the image at ImageJ.....	4
2.	How to create a 3D volume of your data.....	6
a.	Opening your data at Avizo	6
b.	Convert image Type	9
c.	Adding and checking the orthoslices.....	9
d.	Adding a scale bar	14
e.	Changing the background.....	15
f.	Correcting the units	17
g.	Alternatives ways to cropping (see Section 2 to open the data at Avizo)	20
3.	Filtering and Segmentation tools.....	22
4.	Segmenting the image	26
a.	Pick and Move.....	26
b.	Brush tool	28
c.	Lasso tool (2D and 3D)	30
d.	Magic Wand tool	31
e.	Propagation Contour	33
f.	Blow Tool.....	34
g.	Threshold tool	36
h.	TopHat tool	40
i.	Watershed.....	43
i.	Special application: Removing the background.....	43
ii.	Segmenting your image using a mask.....	49
5.	Image Stack Processing (2D Recipes).....	53
a.	Workroom	53
b.	Adding and removing tools	54
c.	Changing the reference.....	55
d.	How to save and apply to other data	57
6.	Didactic Images.....	58
a.	Setting the space.....	58
b.	3D Rendering	58
c.	Label Rendering.....	60
7.	Animations	61

a.	Orthoslice	61
b.	3D rendering and Clipping	62
c.	Camera Rotation.....	64
d.	Saving and exporting.....	65
8.	Registration of images.....	66
a.	Transform Editor	66
b.	Register Image.....	68
c.	Image Registration Wizard.....	70
d.	Resample Transformed Image	72
9.	Quantification.....	74
a.	Volume of a label	74
b.	Separate tool.....	76
c.	Label analysis.....	80
d.	Sieve Analysis	82
e.	Connectivity	83

Documentation History

Date	Revision	Description	Author
31/07/2016	1	Version based on Avizo 9.1	Nathaly L. Archilha
08/08/2017	2	Update to Avizo 9.3	Nathaly L. Archilha
30/04/2018	3	Update to Avizo 9.7	Paola R. R. Rosa

This is a **simplified version** of some tools available in Avizo. If you need further information, please consult:

- The Thermo Scientific EM & Spectroscopy channel in Youtube:
- Help Guide inside Avizo workspace,
- Technical support: fei-sw-support@fei.com
- Avizo web site: <http://www.thermofisher.com/amira-avizo>

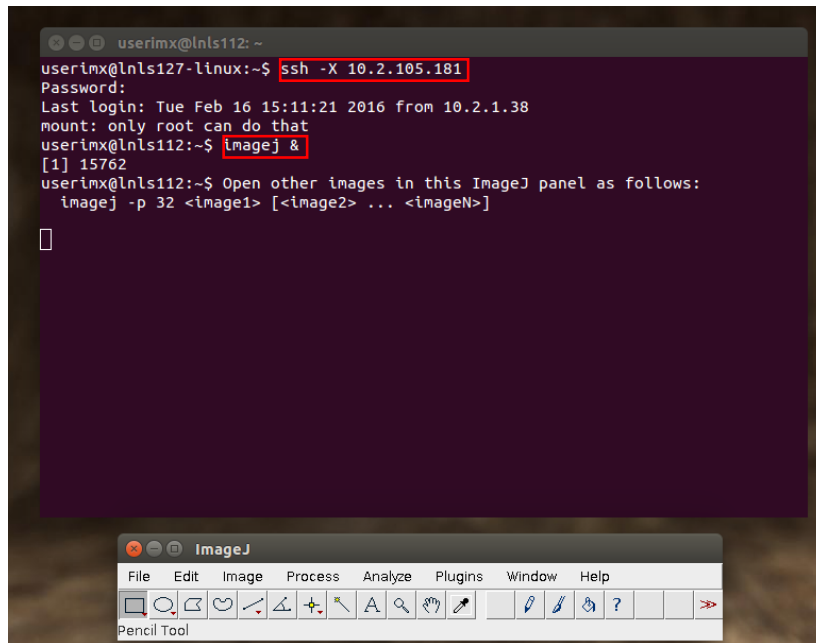
1. Using ImageJ to crop and change image type

a. Change of image type

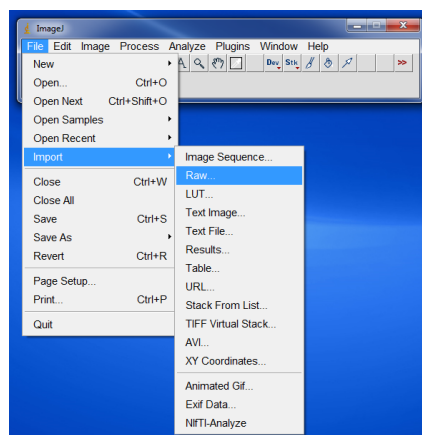
From any terminal connected to the Recon computer, **open ImageJ**. To do this, follow the commands:

- `ssh -X 10.2.105.181` (User password: ask your local contact or user support (SAU) team)
- `imagej &`

This will open the program and release the terminal for further use.



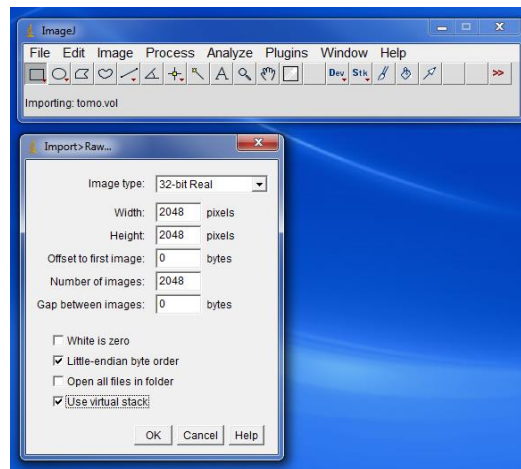
Import the raw file, created during the reconstruction. Go to **File > Open > Raw Data**, then go to the **Sample** folder and open the **Recon** folder. The largest file (more than 8GB), typically named as `tomo-2048x2048x2048_8bit.b`, is the raw volumetric file.



Fill the **Import>Raw** window with image information:

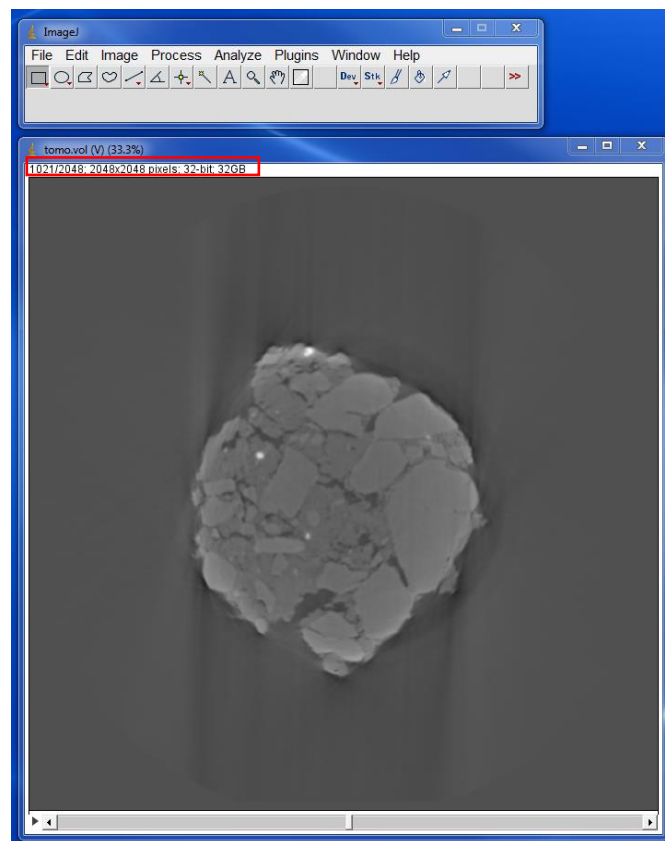
- Image type: choose the bit depth, typically 8 bit or 16 bit unsigned or 32. e.g. *32-bit Real*
- Width and Height: 2048

- Number of images: typically 2048, but it can be less if not all slices are reconstructed.
- Ensure *virtual stack* is enabled and little-endian byte order.

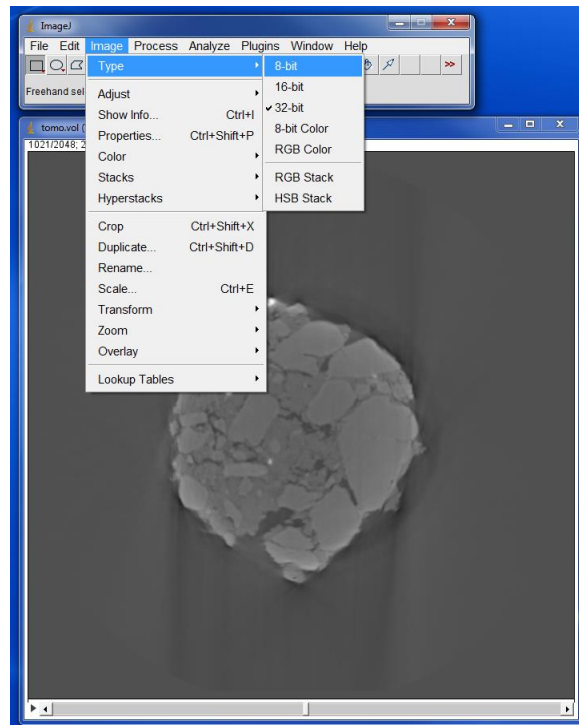


This is an example of a soil sample. First, check all slices to ensure the reconstruction is as expected. If strong rings are seen or the image contrast is not very good, (i.e. it is hard to separate the phases of interest), there are some parameters on the reconstruction process that can be changed. Talk to your local contact.

From the header (red square), you get information regarding the image: the first number is the slice number along the total number of slices; the following numbers are the size (in pixel) of the image in Z, X and Y directions, respectively. The next information is the image type (32-bit), followed by the image size (32 GB).

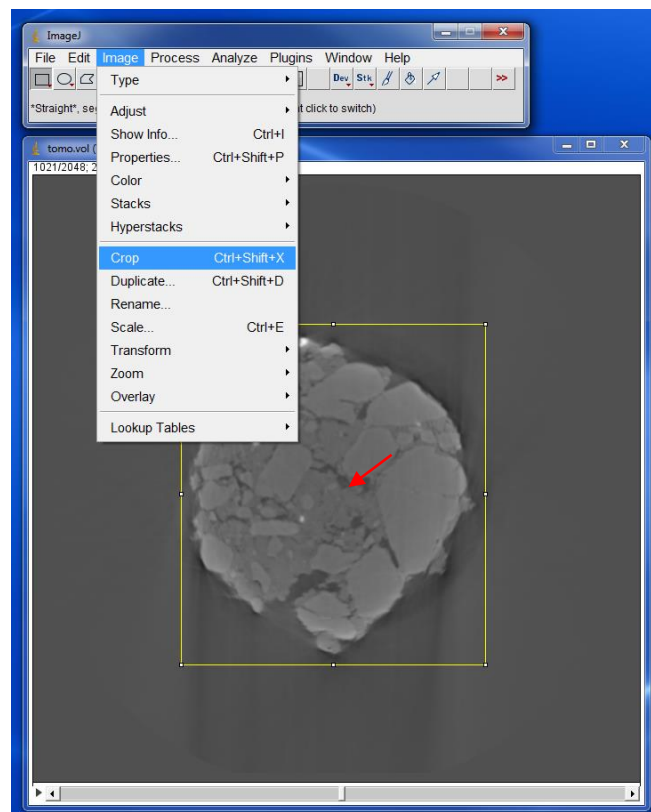


To reduce the image size, first change image type to 8-bit or 16-bit. It is desirable as Avizo uses RAM memory for most of the processes.

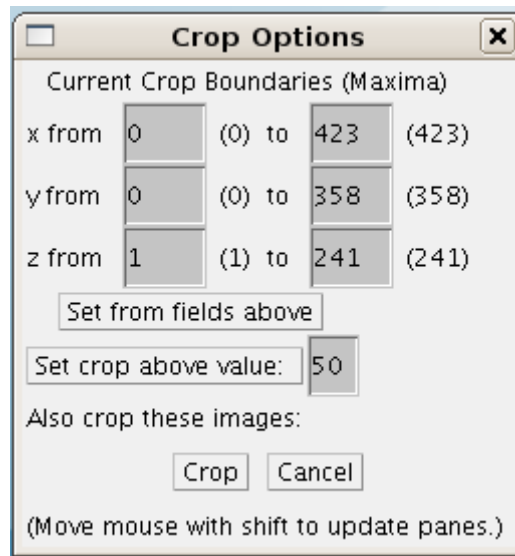


b. Cropping the image at ImageJ

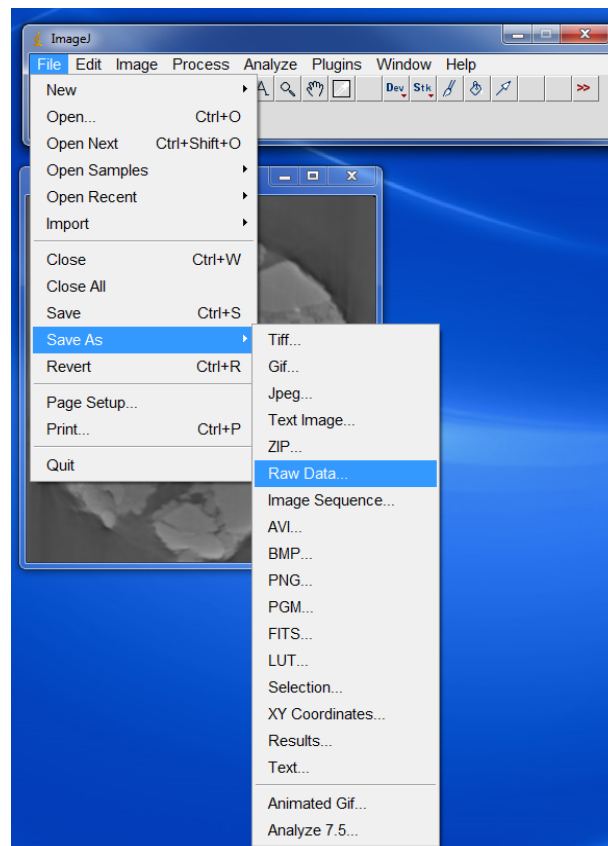
After the conversion is finished (it will take a while), use the square tool to choose the area of interest, i.e. the area to crop. Be sure that the whole image is inside the square. Check all slices by moving the bar (red arrow). Go to **Image > Crop**.



Tip: at the menu option, select: Plugins -> Stacks -> Crop (3D) > ok. This is a plugin that allows cropping 3D image stacks in all three dimensions, from a three window view of the image stack. The three window view demands three times more memory. So, in the case of lack of memory, uncheck the "three pane view" option on startup.



Save the cropped image as a raw data and use the following termination: **tomoXxYxZsizes_nbit.b**. An example of name is tomo980x1132x2048_8bit.b. Image dimension and type are required information to load the data in Avizo. If you want to open the image automatically use tiff.



2. How to create a 3D volume of your data

a. Opening your data at Avizo

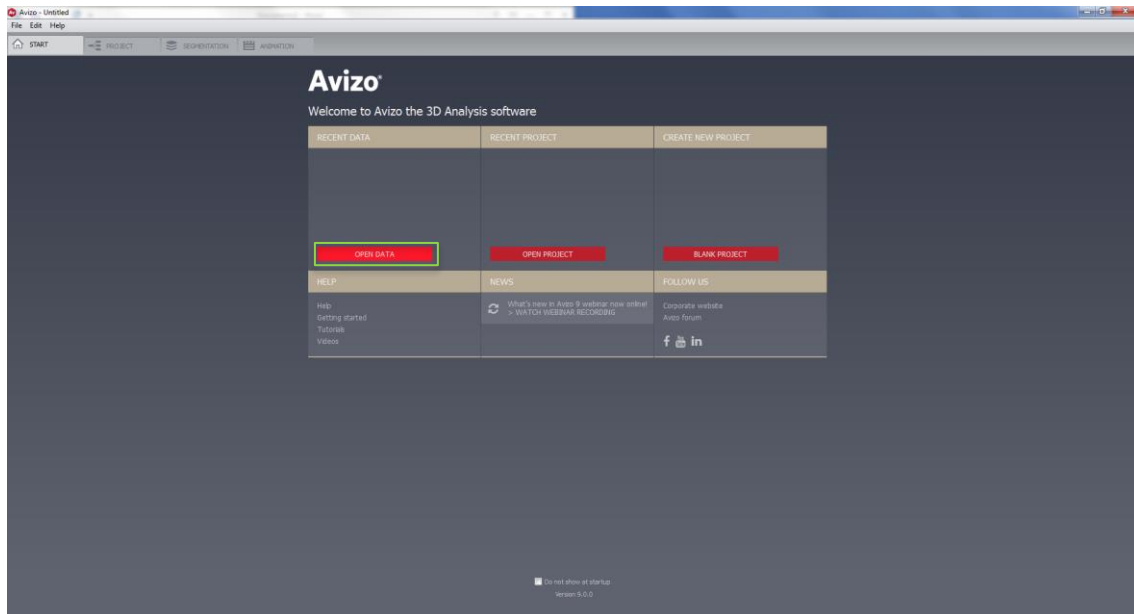
Open Avizo 9.7: you will find a shortcut at your desktop.

Click on **Open Data** and load your image (green rectangle). Here you have two options:

- (Not recommended): Load the original data (~32GB).
- (Recommended): Load the cropped image. The file name contains almost all required information to load the data. The only missing information is pixel size, which is fundamental for quantitative analysis. Knowing the objective you have used in your experiment, you can get the pixel size.

Observation: *If you cropped your image, use the new dimensions. If you saved your file in TIFF after you cropped it, Avizo will open it automatically.*

If you are not sure about the dimension or the pixel size of your file, you can also get it from the information file (*raft.par* or *sample.txt*) and look for "IMAGE_PIXEL_SIZE" - check the next few steps for this.



In this example, we will open "tomo.vol" (but the recent samples always are written as the following: tomo2048x2048x2048_8bit.b), all required information is inside *raft.par* file.

First, open the information file (*raft.par*), using Notepad++ or gedit.

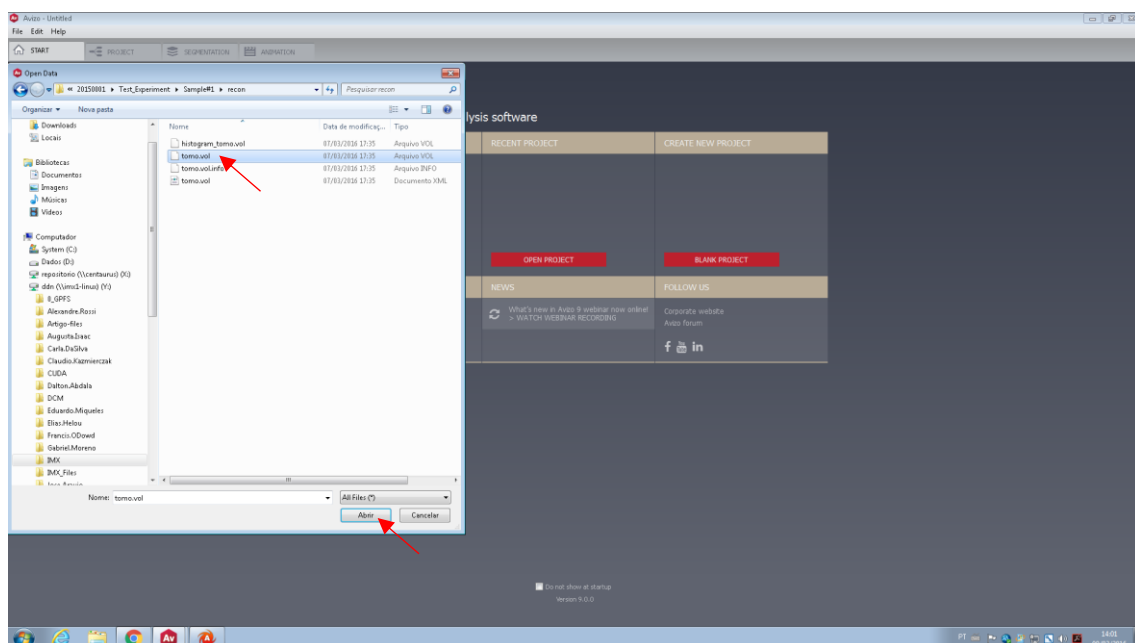
A new window will open. Here, you will find information regarding X and Y dimensions of your image (red box), Z dimension is always 2048, if all slices were reconstructed (green arrow). Pixel size (red arrow) is always shown in micrometers.

```

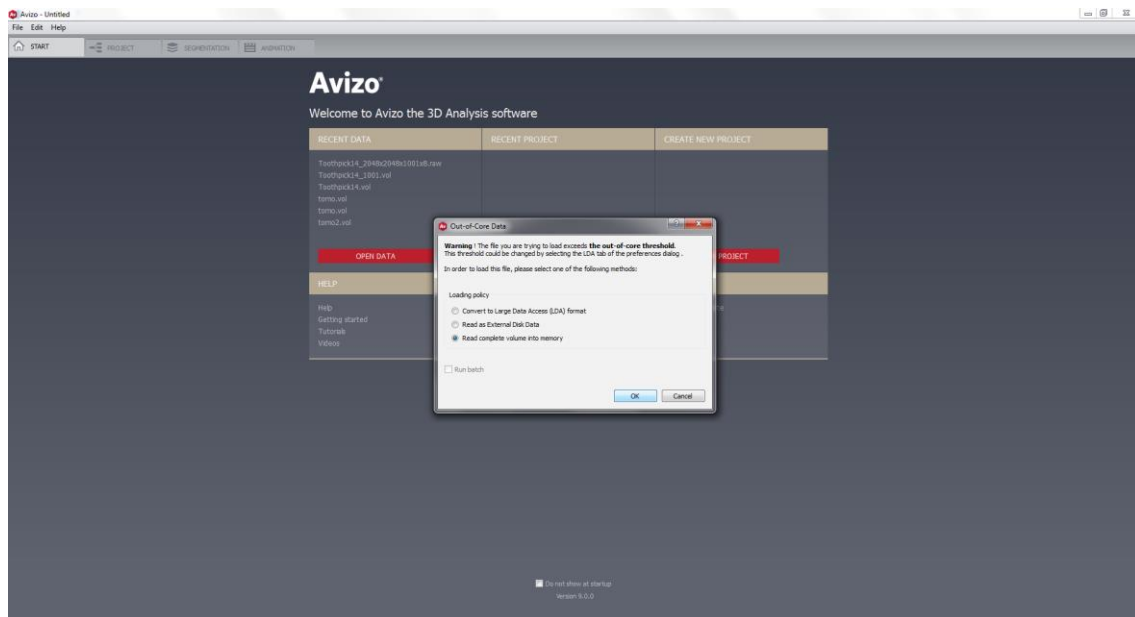
1 #Run: pyhst2_33beta2 input.par lns112,0
2 #Sample Path = /IMX/Sample#1/
3
4 #Angle Range = 180.0
5 #Number of Projections = 1001
6 #Exposure Time = 250 ms
7 #No Filters
8 #Detector Position = 200
9
10 #####
11 #Parameters to modify...
12 ROTATION_AXIS_POSITION = 1127.8174
13 DO_AXIS_CORRECTION = NO
14 AXIS_CORRECTION_FILE = correct.txt
15
16 #Select Slice Number(s)... 1 - 2048
17 START_VOXEL_3 = 1
18 END_VOXEL_3 = 2048
19
20 #####
21
22 # Parameters defining input file / format
23 FILE_PREFIX = tomo.h5
24 PROJ_DS_NAME="images"
25
26 FLATFIELD_FILE = tomo_flat_before.h5
27 FF_DS_NAME = "flats"
28
29 BACKGROUND_FILE = tomo_dark_before.h5
30 BACKGROUND_DS_NAME = "darks"
31
32 # Parameters defining output file / format
33 OUTPUT_FILE = recon/tomo.vol
34
35 # Parameters defining projection file format
36 NUM_FIRST_IMAGE = 0
37 NUM_LAST_IMAGE = 1000
38 NUM_IMAGE_1 = 2048
39 NUM_IMAGE_2 = 2048
40
41 IMAGE_PIXEL_SIZE_1 = 0.82
42 IMAGE_PIXEL_SIZE_2 = 0.82
43
44 #####
45
46 # Parameters defining background treatment
47 SUBTRACT_BACKGROUND = YES
48

```

Then, open the volume file.

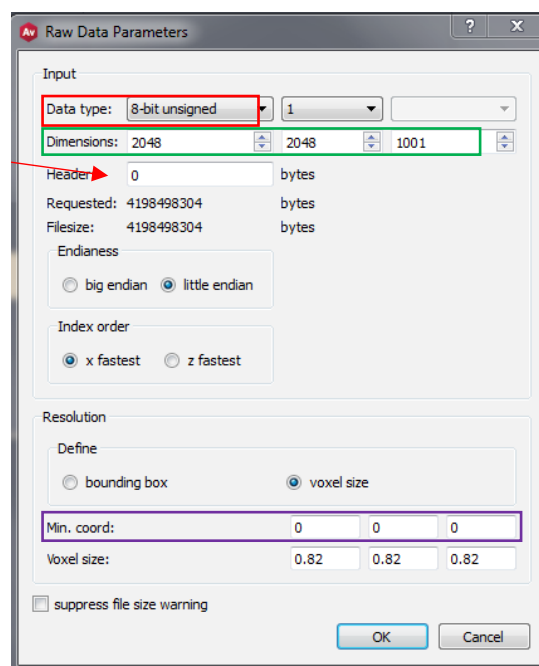


The **Out-of-Core Data** window will open; select *Read complete volume into memory*.



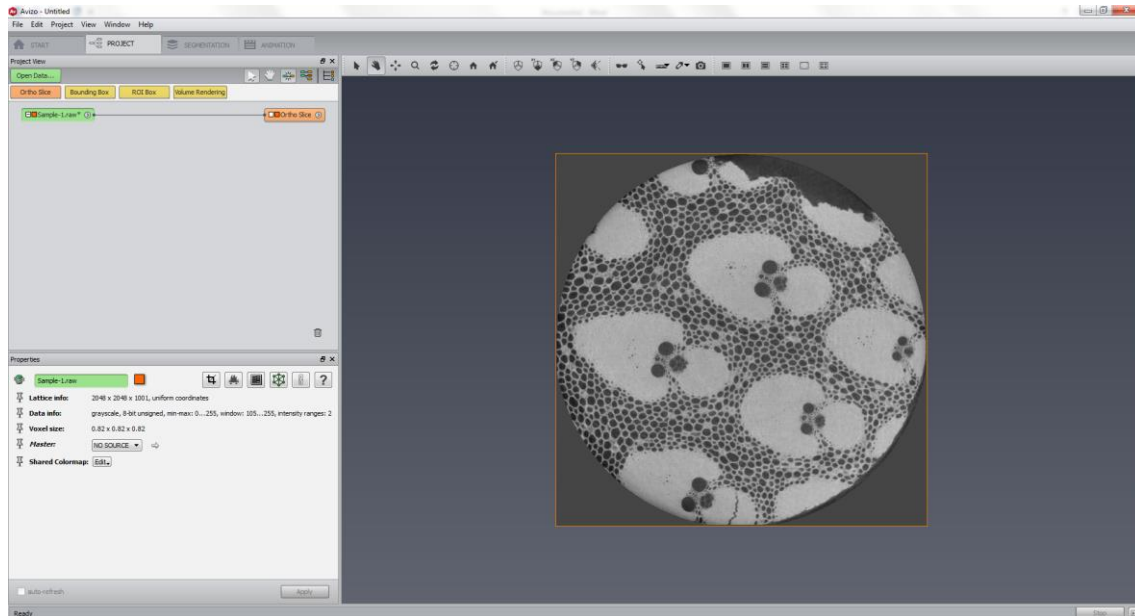
The **Raw Data Parameters** window will open and you have to fill it with the following information:

- *Data type*: the output of the reconstruction is 32-bit; however, it is possible to transform the image into 16- or 8-bit using ImageJ (Image menu > Type Submenu > determine the type of the active image or to convert it to another type).
- *Dimensions*: you will find this in the information file (*raft.par*) or if you cropped your image, this information will be at the file name.
After adding these parameters, the **Header** value (red arrow) should be zero, as it subtracts the file size of the requested size. If some information is wrong, the **Header** will be different of zero – double check the information you added before you load the image.
- *Voxel size*: also defined in the information file. You will find just two values, but it is the same for the three axes, as the voxel is actually a cube.



Click **OK** and your image will be loaded. Depending on its size, it can take a couple of minutes! At this point, it is very likely that the software requests the **voxel size unit** – choose *micrometers*. If Avizo doesn't request this right now, it is still possible to change it later. (see section f.)

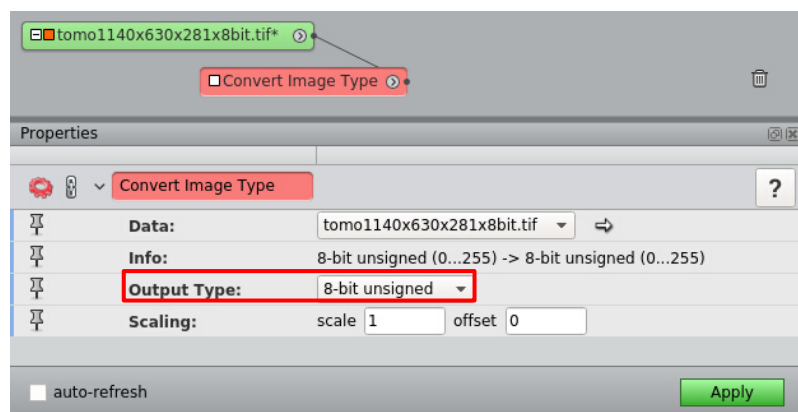
This is an example of a toothpick.



b. Convert image Type

Attach this module to the data by right clicking it and typing “Convert Image Type”. In output type you can determine if you want to transform your data to a different type of a regular 3D scalar field or an RGBA color field or convert a uniform scalar field of bytes into a Label Field (red rectangle).

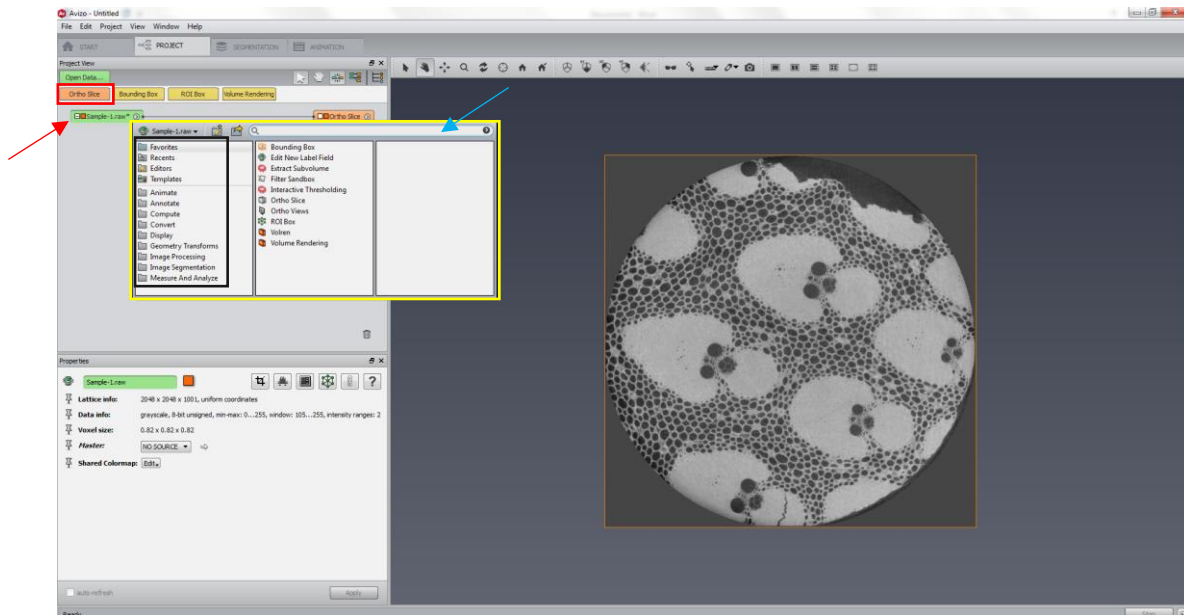
All of this transformations will have the same dimensions and coordinates. And it will generate a new data after you select apply.



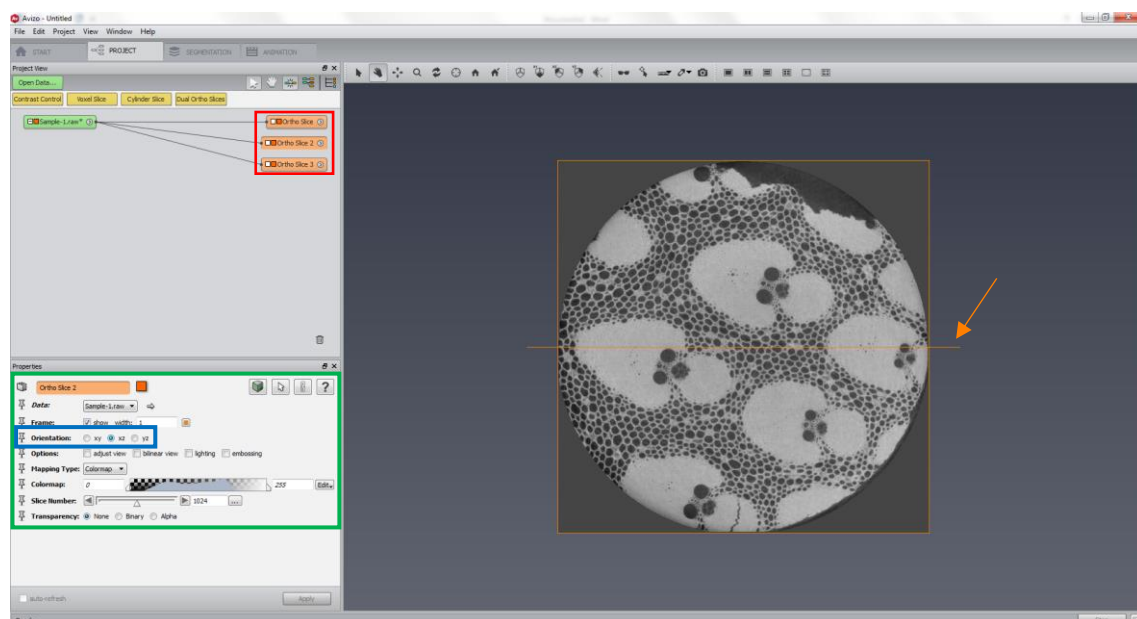
c. Adding and checking the orthoslices

The image above shows an orthoslice of a toothpick sample. This view is automatically loaded when you open a new image. To add more orthoslices, you have to click on your raw image (red arrow) and then click on **Orthoslice** (red box). Not all commands will be available as **Orthoslice** was. For these cases, you will need to open the dialog box (yellow

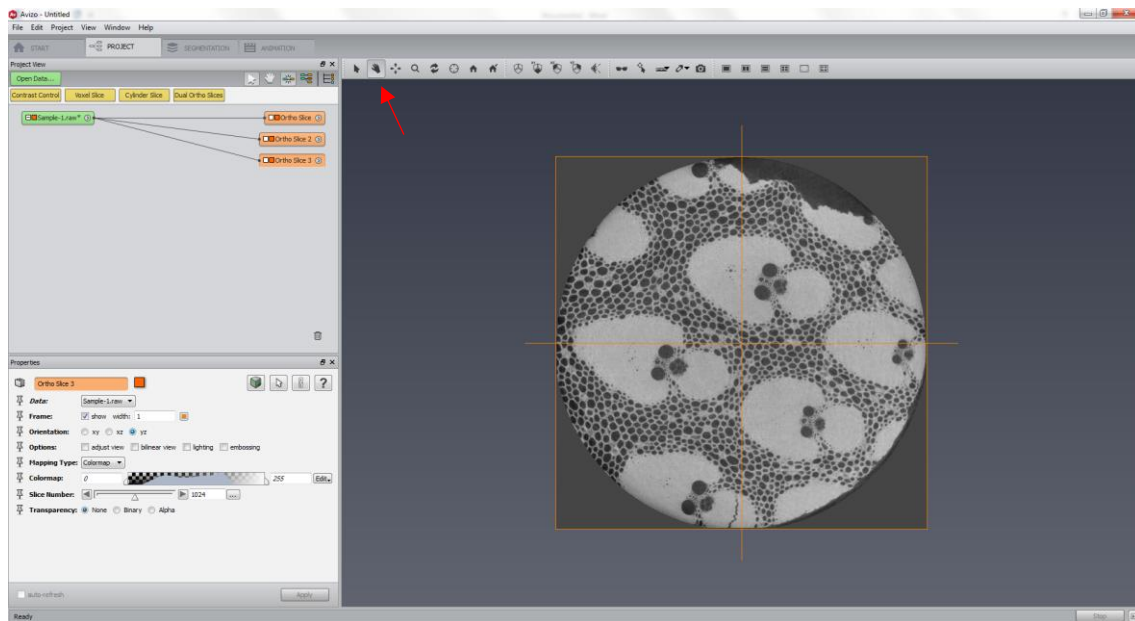
box), by right clicking the raw image. You can either look for all the options, by opening each folder (black box), or write the command (i.e. orthoslice) at the search place (blue arrow).



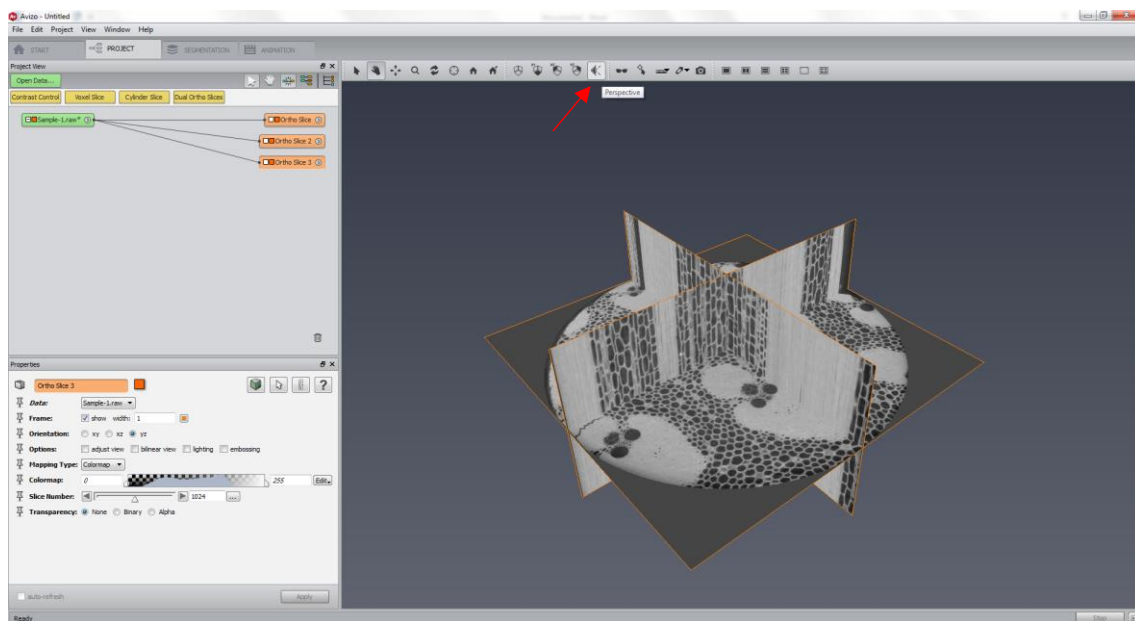
Create two more orthoslices (red box). They will have the same orientation as the first Orthoslice. For another view of your sample, click on Orthoslice 2 and its **Properties** (green box) will appear. Change its orientation to **xz** (blue box) and an orange line will appear in your image (orange arrow).



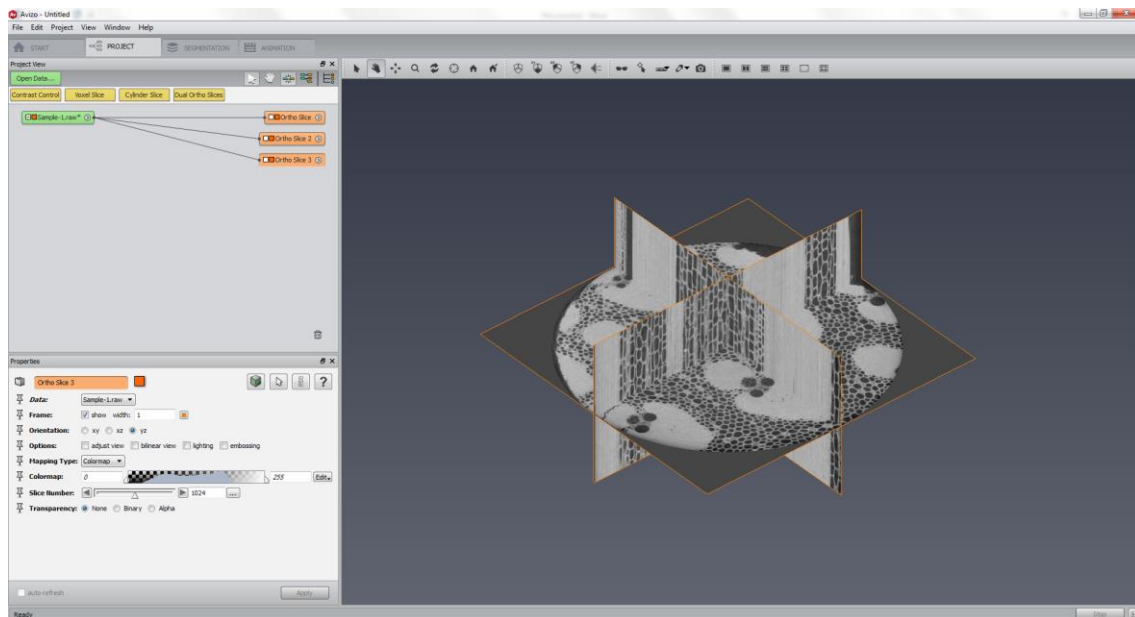
Make the same for Orthoslice 3, but choose orientation **yz** for a complete view of your image. To rotate the sample, click on the **hand icon** (red arrow), then click on the toothpick image and drag the mouse.



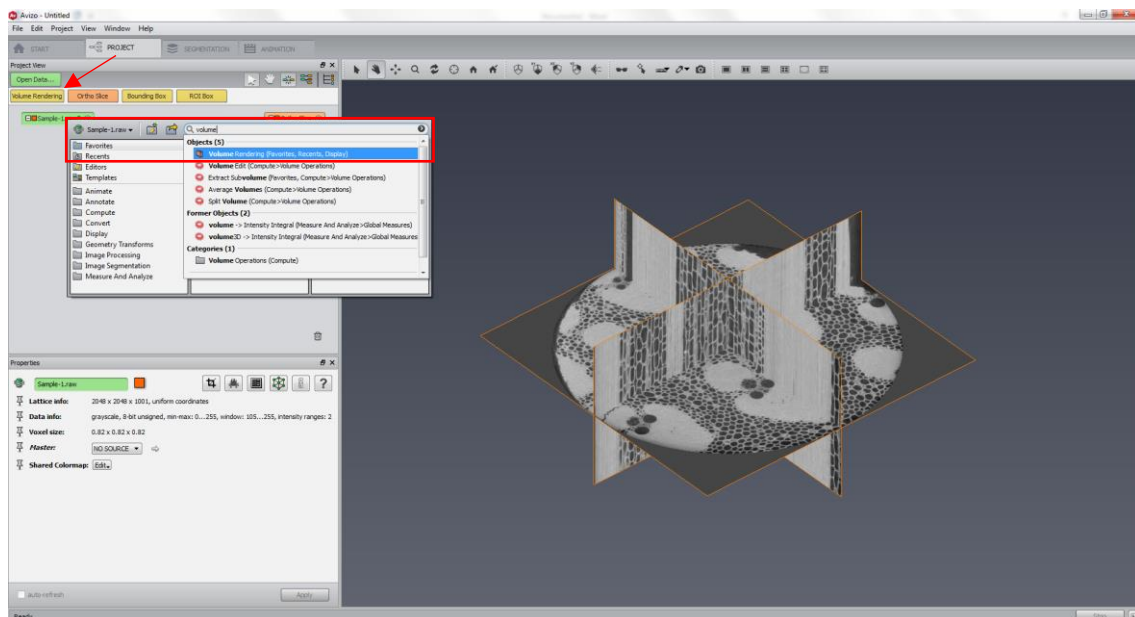
You'll see an image like the following one. If you want to change perspective, click on the *perspective icon* (red arrow).



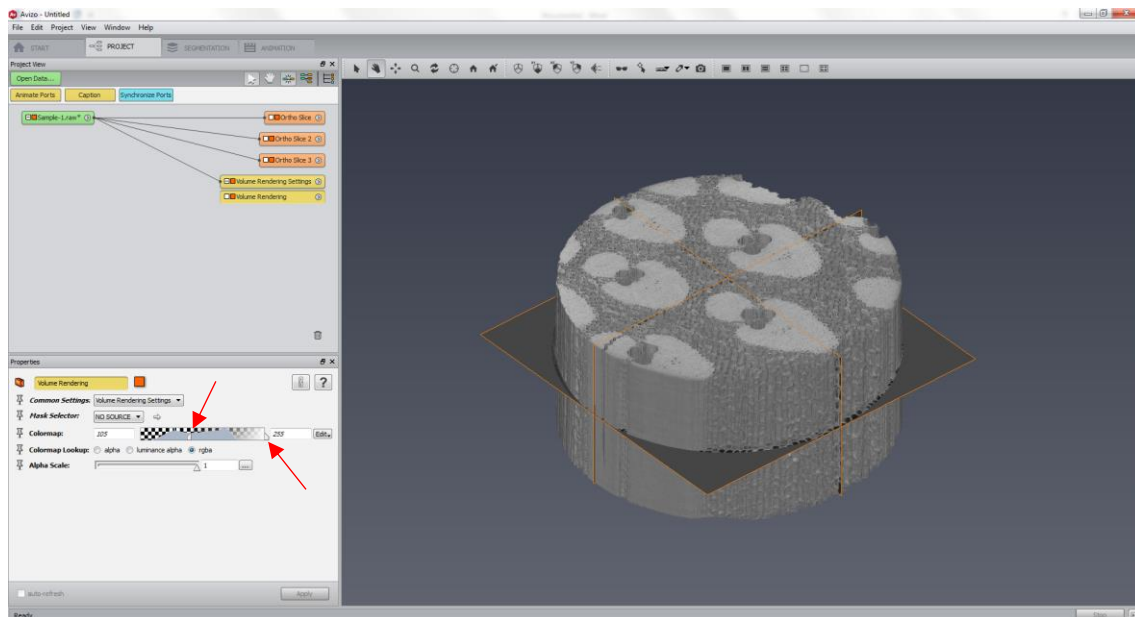
The new image is slightly different.



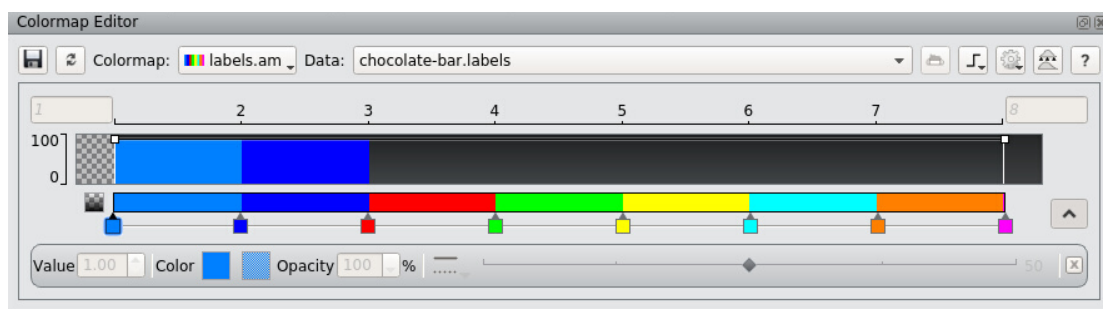
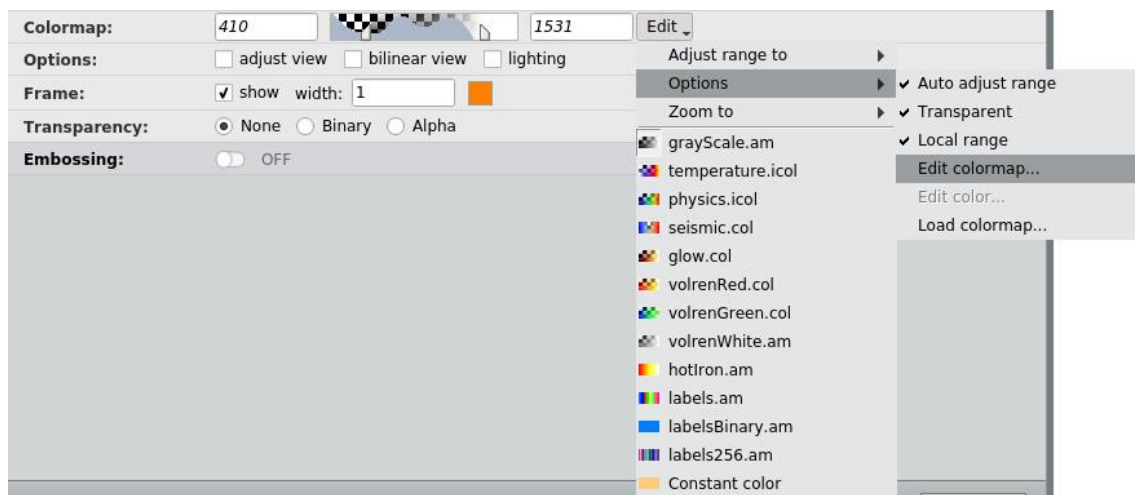
Next step is to create the 3D volume rendering of your sample. You can right click on the raw image and look for Volume Rendering (red box) or you can click on the Volume Rendering button (red arrow).



The result is the following image. If the image you create is not satisfactory, try to change the threshold of your colormap (red arrows) until you find the best representation of your sample.

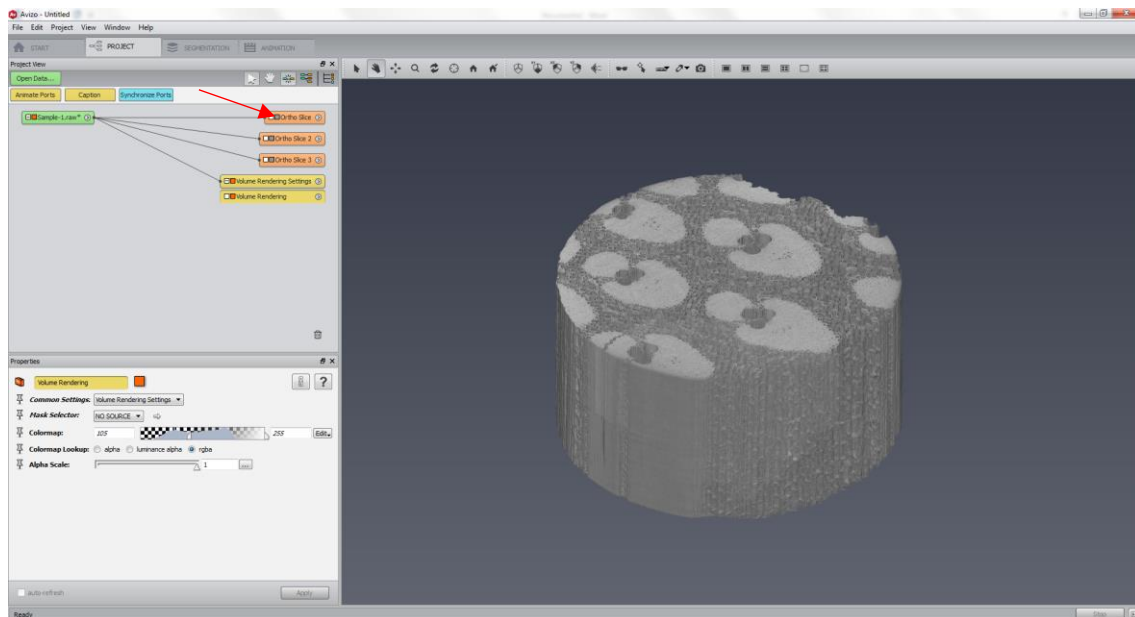


There are plenty of options of colormap. Click on **Orthoslice > Colormap Edit > Options > Edit colormap** to modify the colors or transparency.



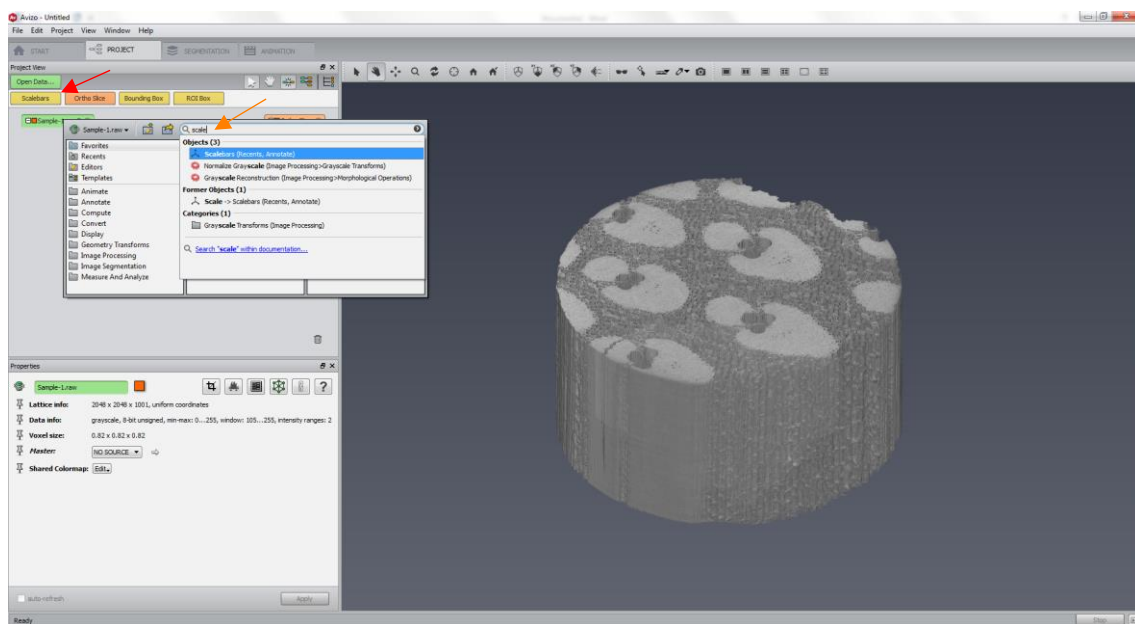
Observation: You can add the colormap module to the project view. Right-click on the **colormap icon** and select **Annotate>ColormapLegend**.

You can hide the orthoslices and show only the volume rendering of your sample. Just click on the orange box in the raw image and it will turn it to gray (red arrow).



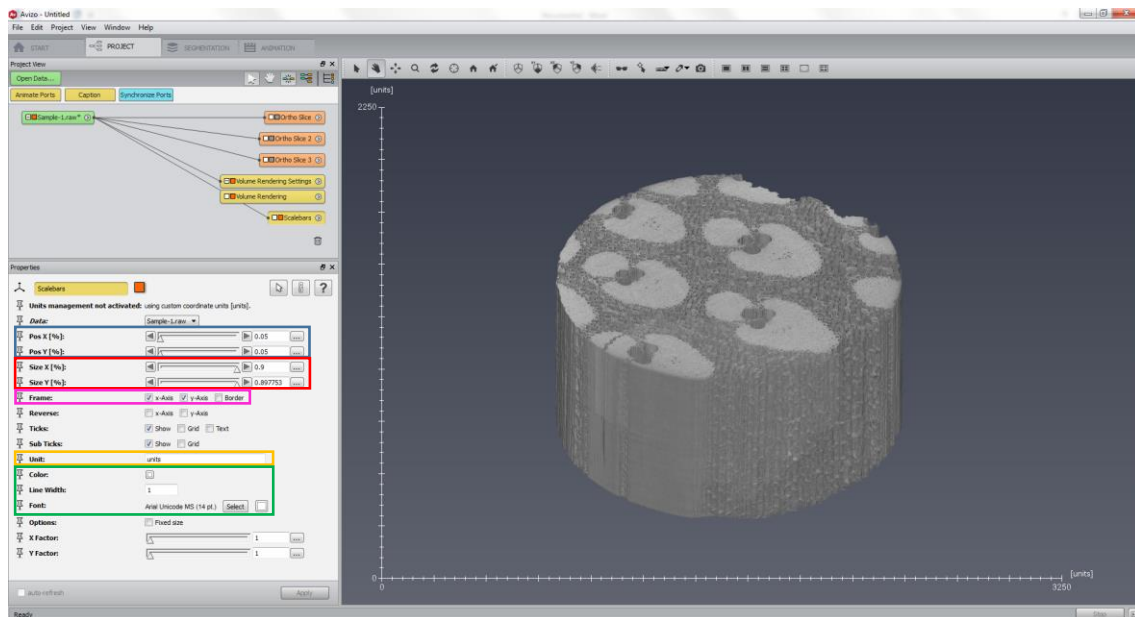
d. Adding a scale bar

To add a scale bar, you just need to click on *Scalebars* (red arrow) or search this in the searching area (orange arrow)



A scale bar will appear on the left corner of your image. There are many options to edit it, some of them are:

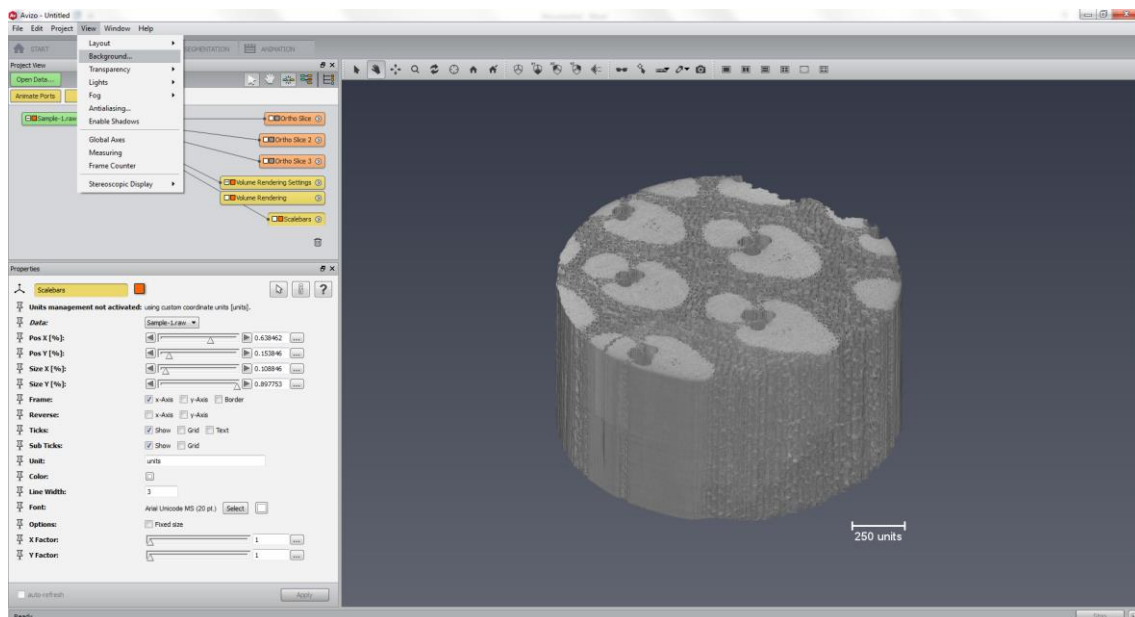
- 1) X and Y spatial positions (blue box).
- 2) X and Y sizes (red box).
- 3) X and Y frames (pink box): you enable or disable the axis.
- 4) Units (yellow box): if this box is unavailable/locked (not the case here), Avizo already knows the units (i.e. you had included this information when you loaded your data).
***Suggestion:** for quantitative analysis, always include this information prior to your analysis (Section f shows how to change this).*
- 5) X and Y color and width, font color and type (green box).



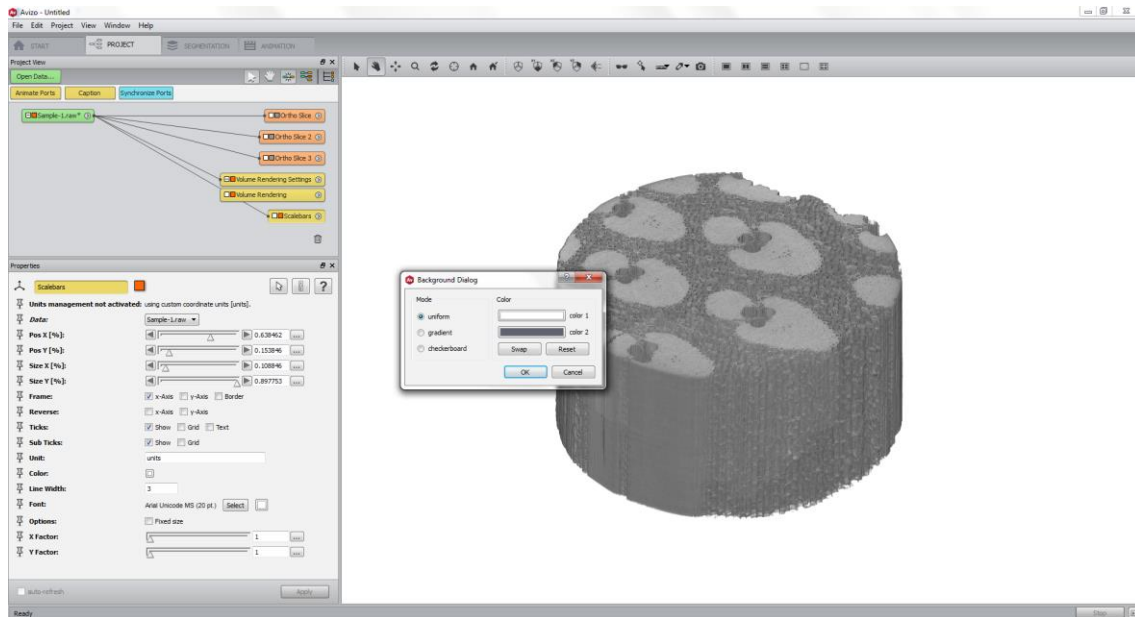
Important observation: Scalebars only make sense for a scene viewed with an orthographic camera (not perspective).

e. Changing the background

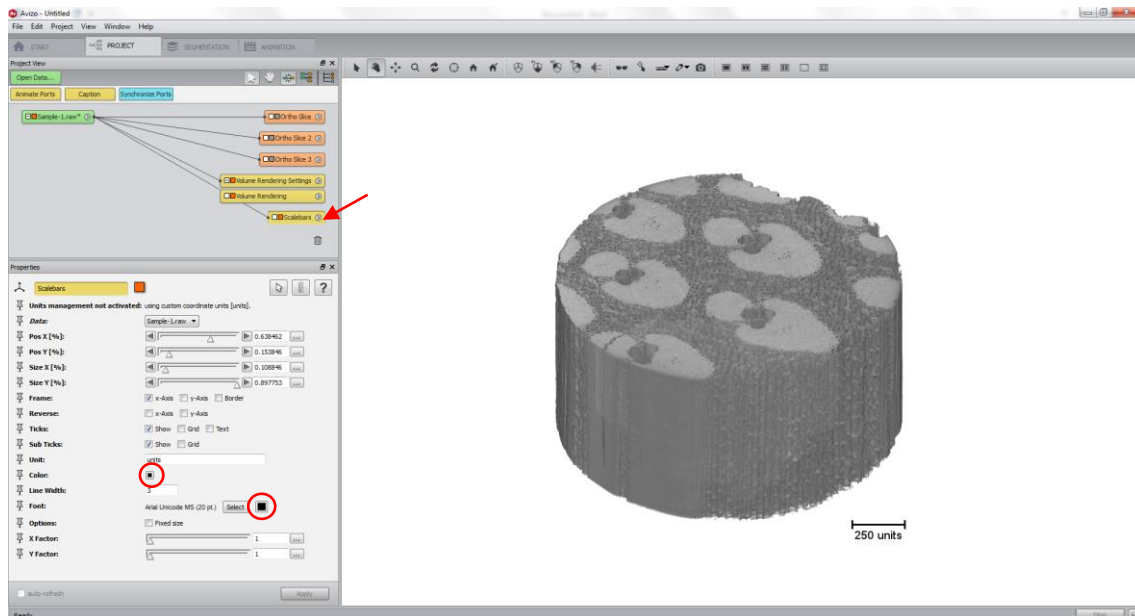
The default gray background is not appropriate for publications and reports. To change this, go to **View > Background**.



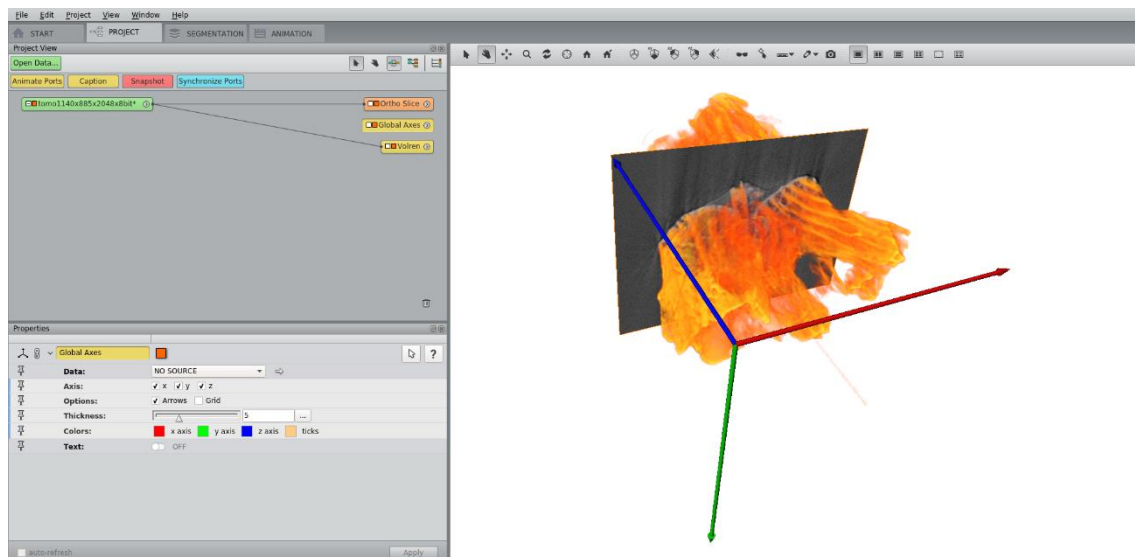
Change **color 1** to white and click on **uniform**. If the scale bar is white, it will disappear.



You simply need to click on **Scalebars** and change its color.

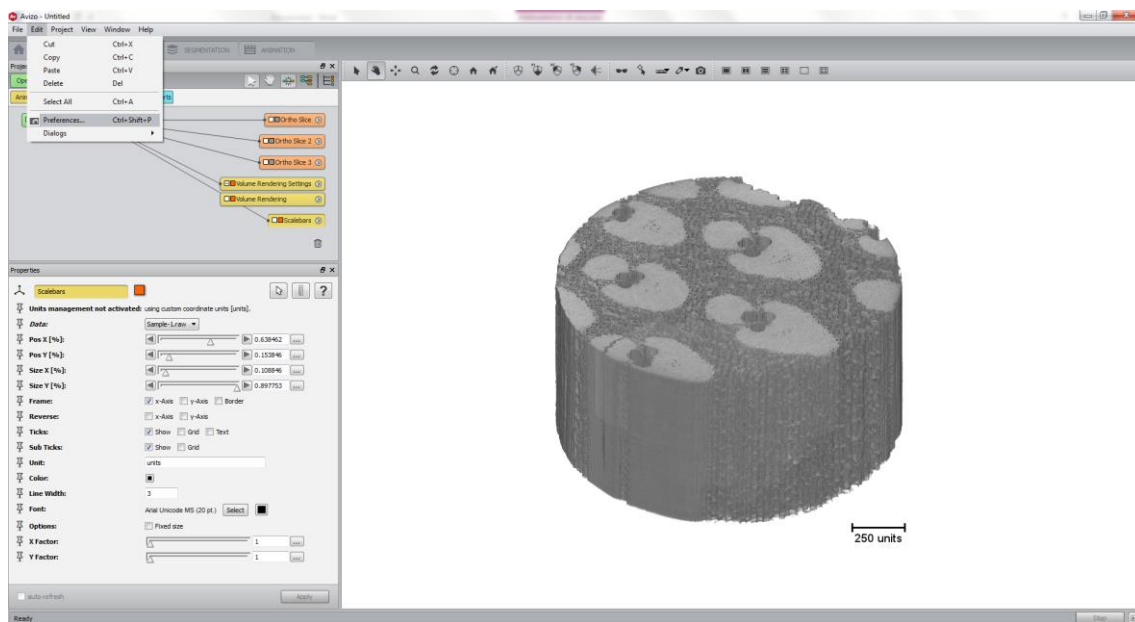


Axes are also available. Right click on the module of the data and attach **Local Axes**. This will give the coordinate frame of the input object. The other option is available at **View > Global Axes**. In this case, the axes are at the center of the coordinate system of Avizo.

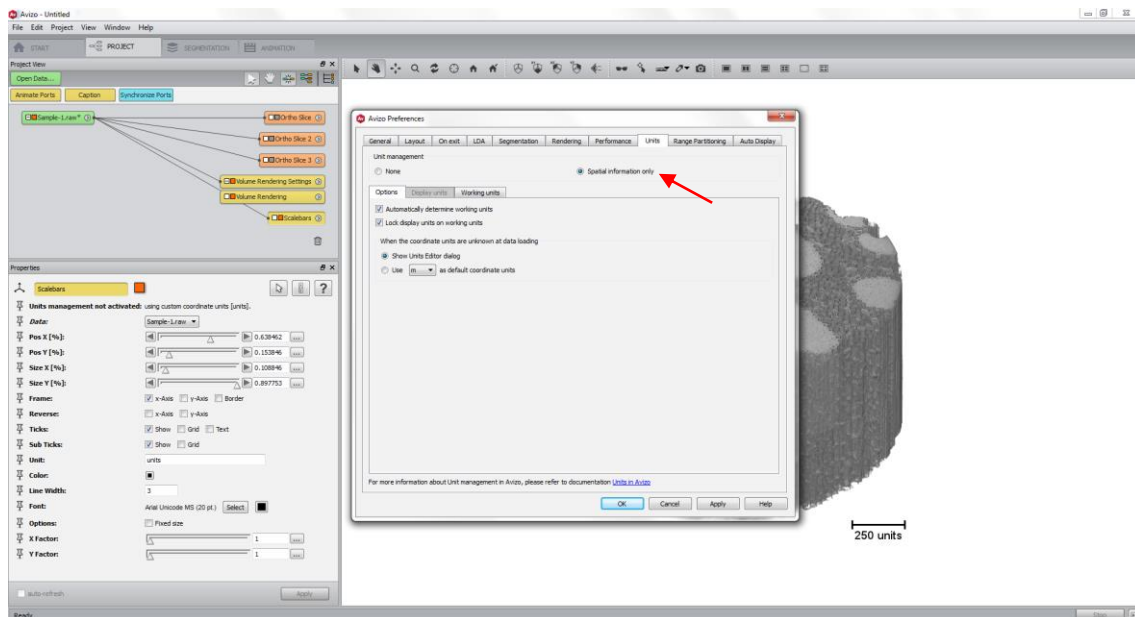


f. Correcting the units

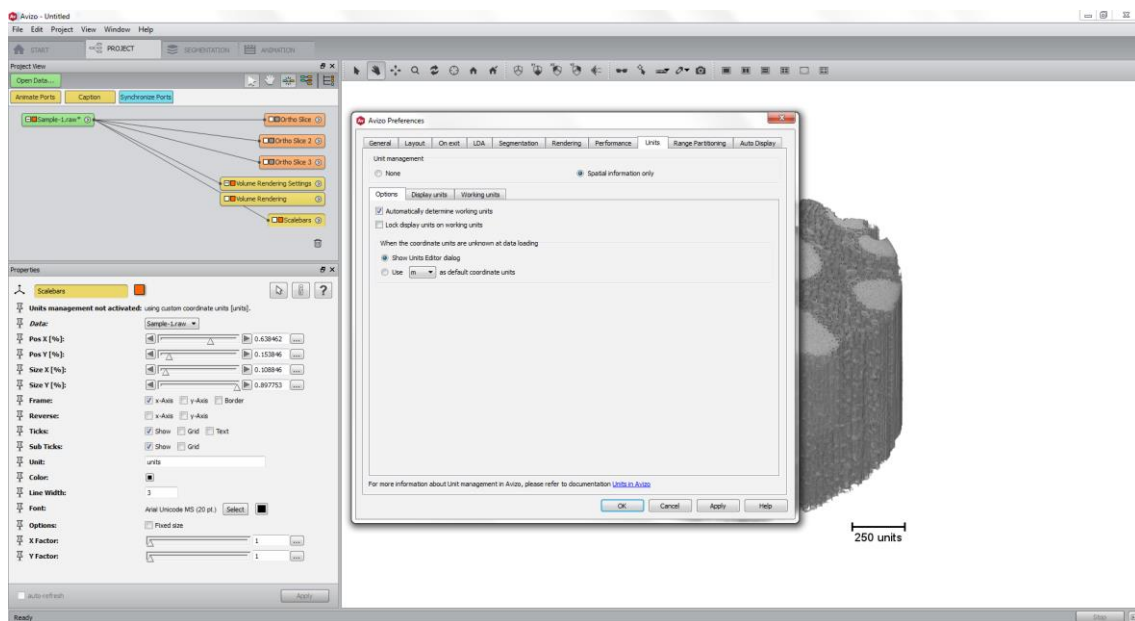
Click on **Edit > Preferences**.



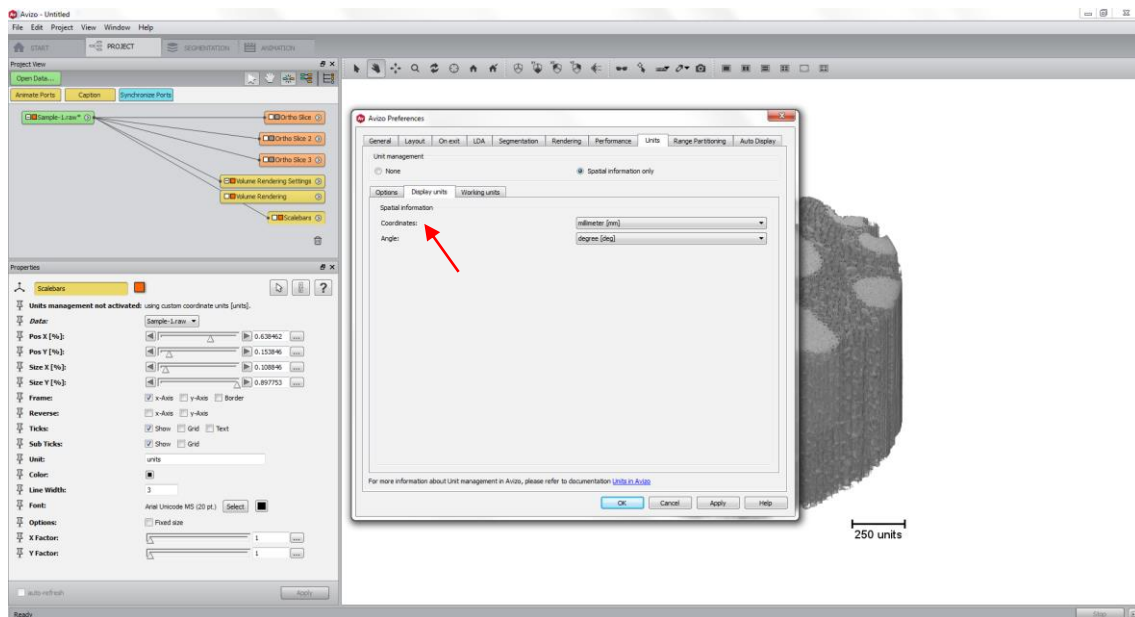
Then, click on **Units** tab and click on **Spatial information only** (red arrow).



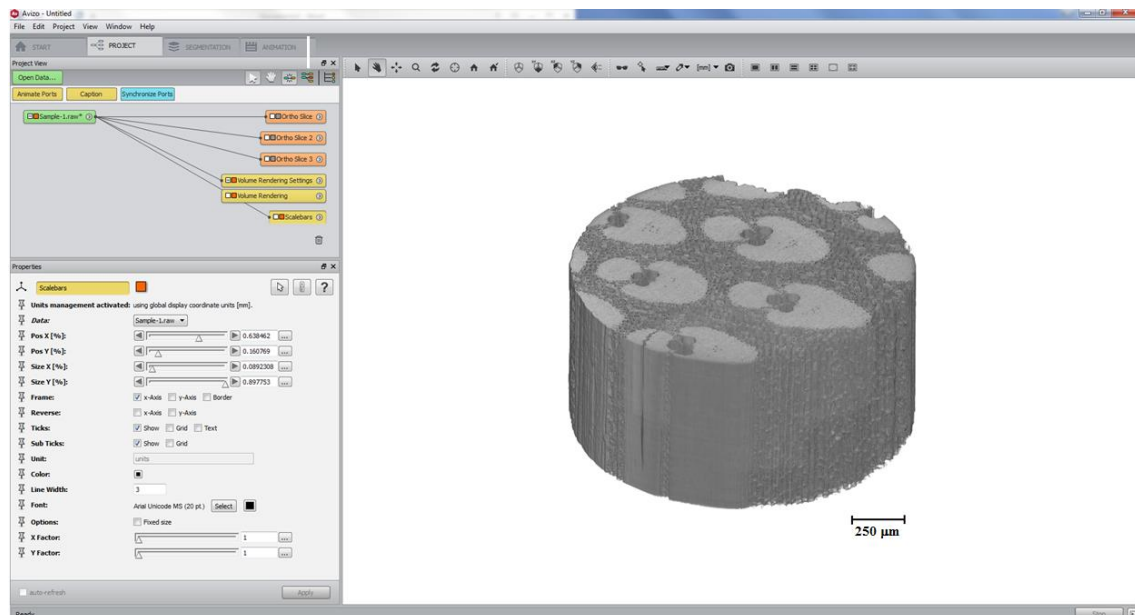
Unclick **Lock display units on working units**. Avizo working unit is meter, which is huge comparing to our scale.



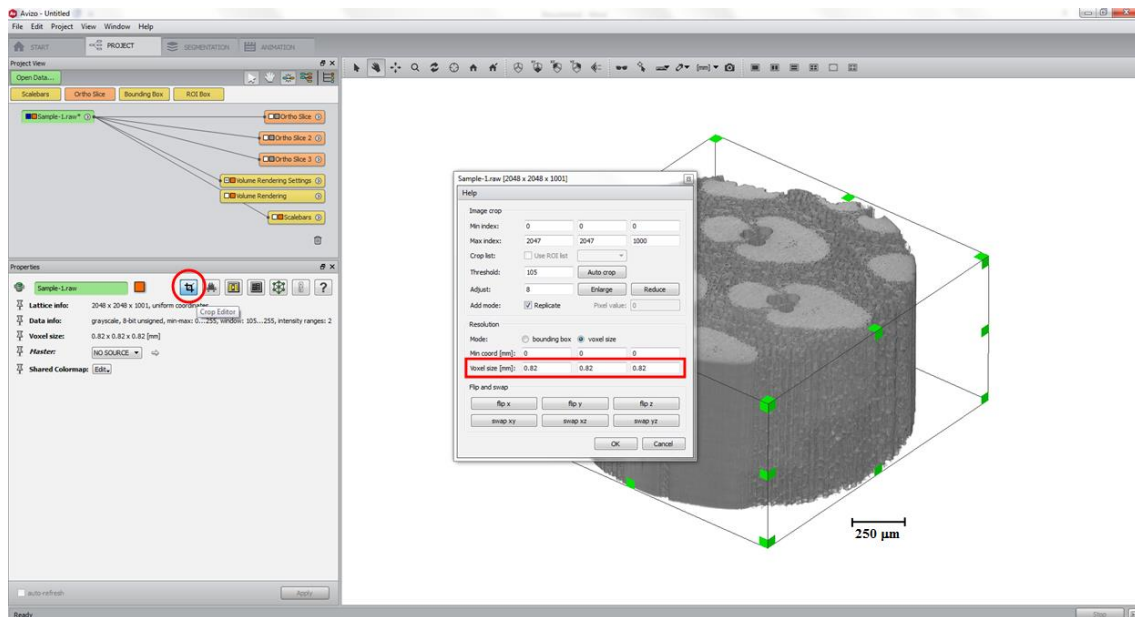
Click on **Display unit** and change **Coordinates** (red arrow) to an appropriated unit (normally micrometer).



The final image is showed below.

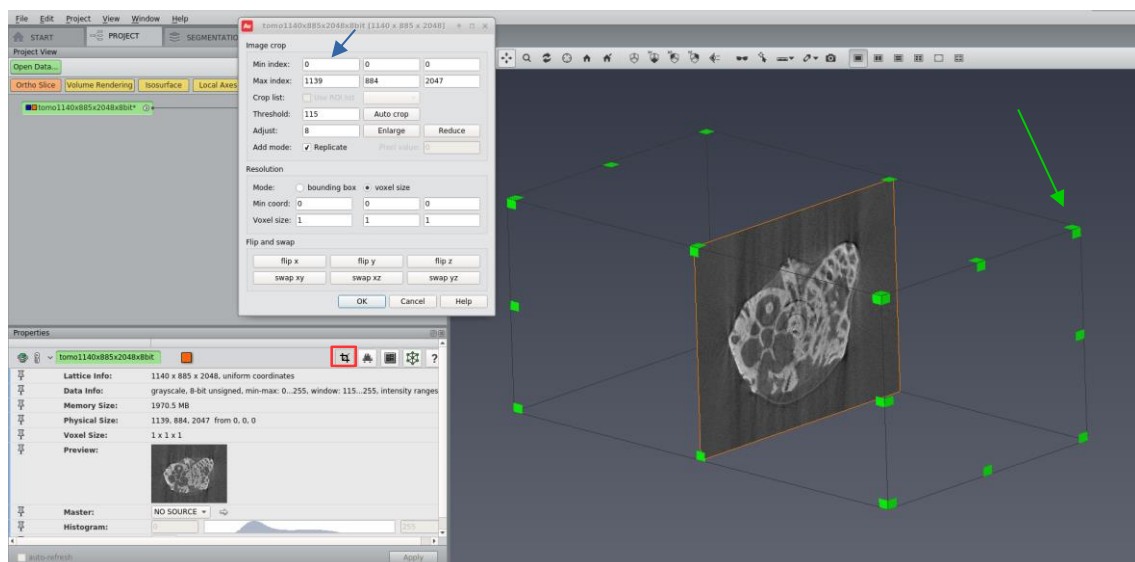


To check or include the pixel size, you can click on **Crop editor** and check the red box. If it is wrong, you can update these values.

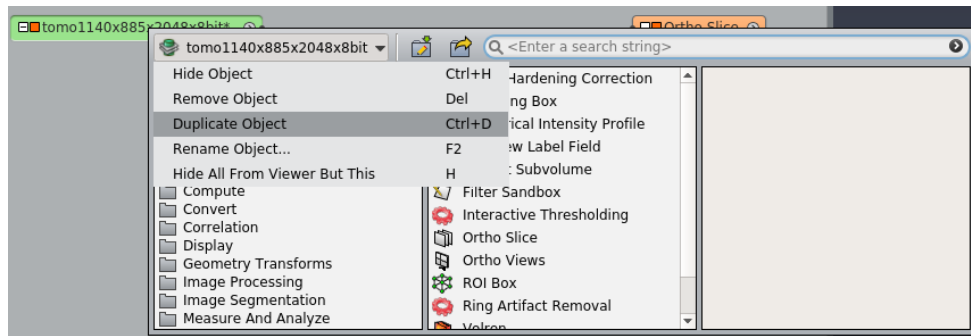


g. Alternatives ways to cropping (see Section 2 to open the data at Avizo)

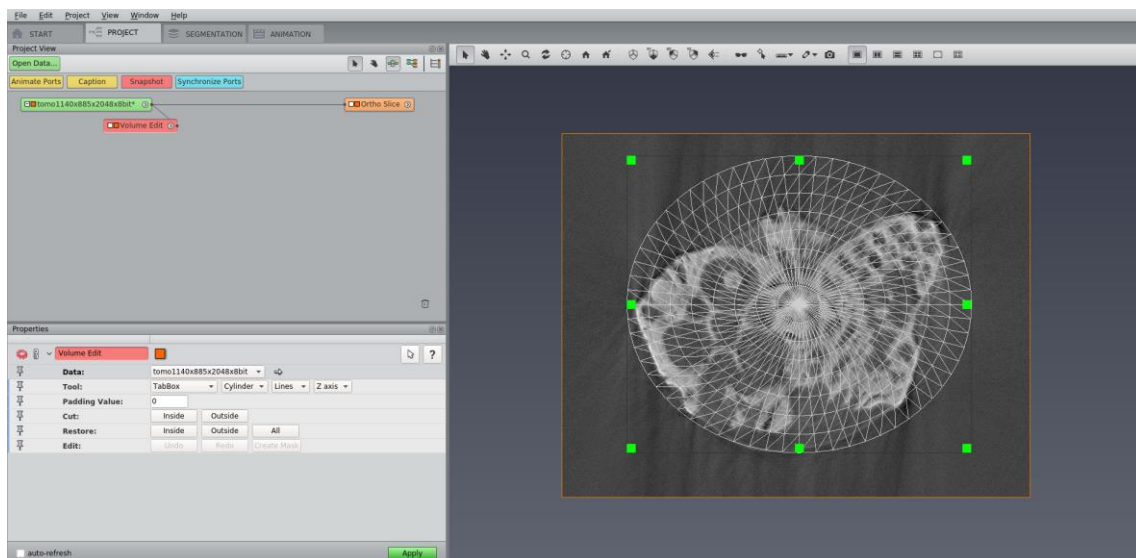
i. Select the data (green module) and at the properties section is situated the **Crop Editor Button** (red rectangle). When you activate the crop editor, the dialog shown in the figure pops up. Observe that there are two mechanisms to define the new cropp. The first option is to define the exact slices on the X, Y and Z axis (blue arrow). The second alternative is drag and move the box that appears around the data (green arrow). In that case, values in the text fields of the editor change according to your manipulations.



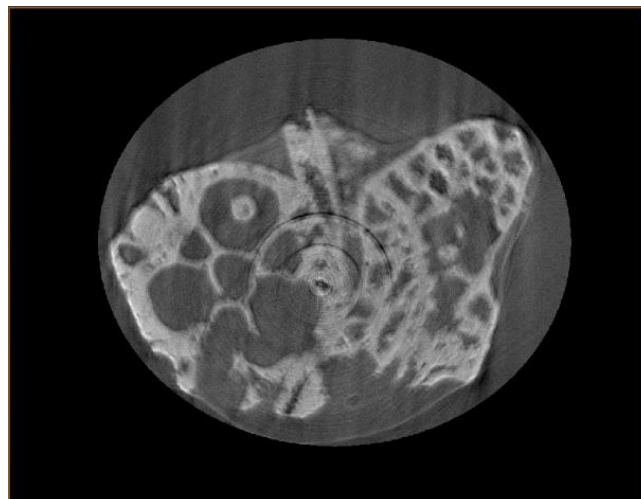
Observation: There is no option to return to the original data once it's cropped. If you are not sure about the result, please duplicate your data by right clicking on it and selecting the option **Duplicate object** at the top left of the dialog box. In the same part, there is the options of **remove** and **rename objects**.



ii. Select the data and right click on it. Type **Volume edit** in the search area of the dialog box. At the tool section change to **Tabbox** (this will create a box that you can easily drag) and to the shape that makes more sense in your data (box, ellipsoid, cone, cylinder or pyramid). If the button interior is pressed, voxels inside the region selected are replaced with the value(s) given by the **Padding Value port**. If the button exterior is pressed, voxels outside this region are replaced. In this case we are using Cylinder, outside (the background will receive a zero value) and manipulating at the Z axis.



Observation: In this situation the image is not actually cropped, but the background will receive a zero value, which can make the segmentation a more efficient process. If you have a 1024x1024x1024 data, the size will remain the same after the apply.



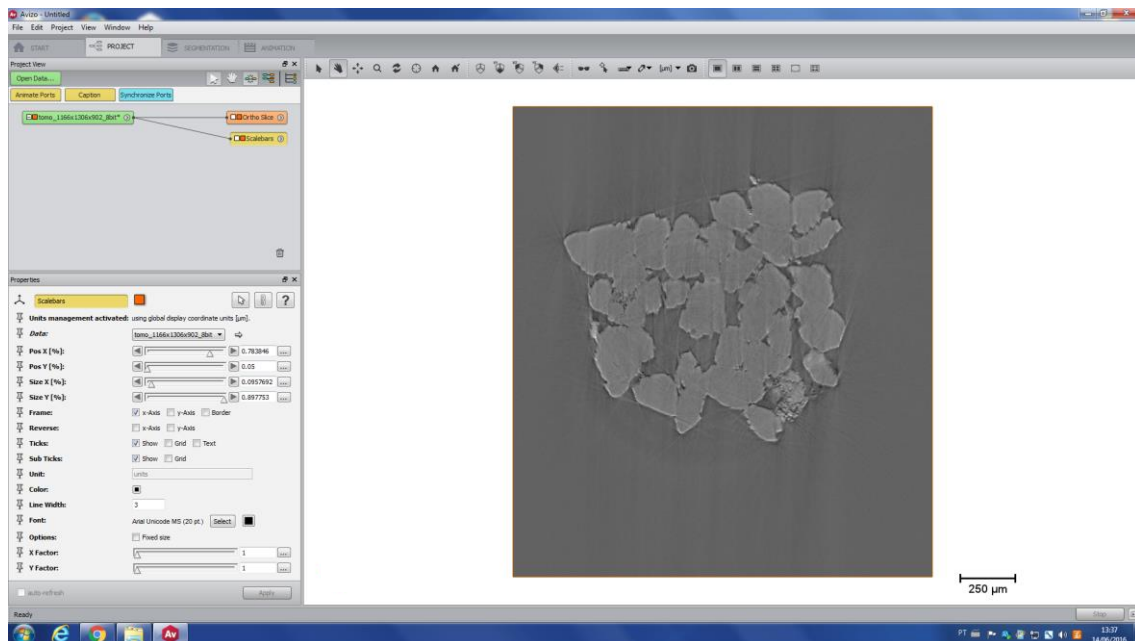
3. Filtering and Segmentation tools

Even the best measurement and the most robust reconstruction method generate an image with a noise. From this image, quantitative and qualitative information can be obtained. Prior to image analysis, it is very common to take advantage of filters to improve the image quality and, consequently, facilitate the post processing.

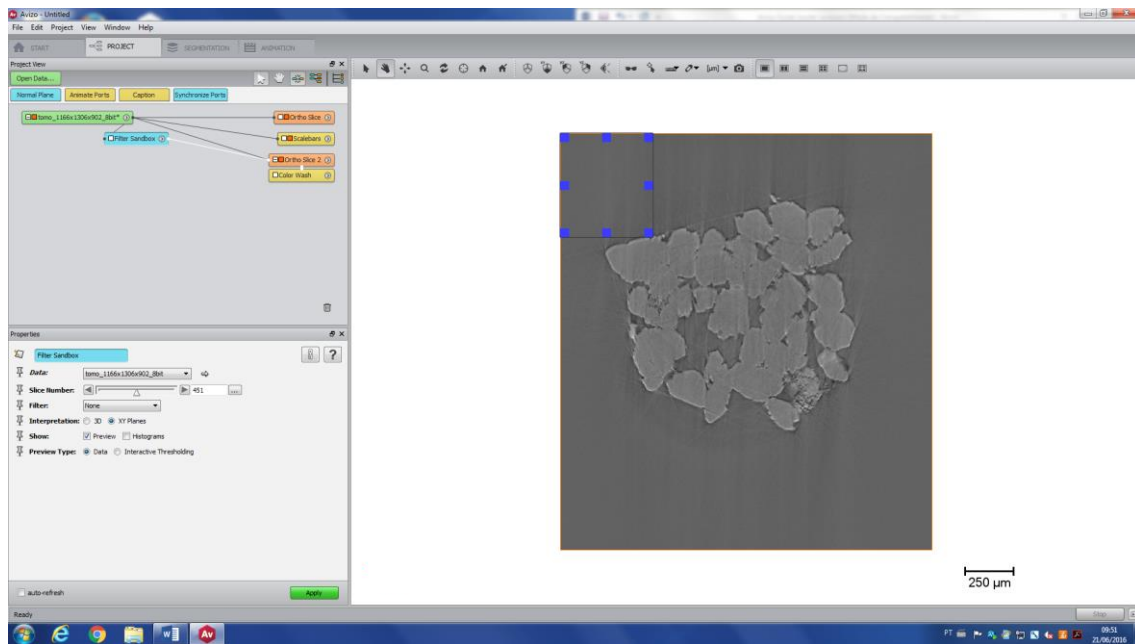
The main goal of filtering an image is to remove noise and this process consists on an image transformation, where the new image is obtained by neighborhood operations. The next step is the image segmentation, which is the partitioning of an image (R) into regions ($R_1, R_2, R_3, \dots, R_n$) that are homogeneous with respect to some characteristic, for example, intensity or texture. The idea is to study each label separately from the others.

See below some filtering and segmentation tools available in Avizo.

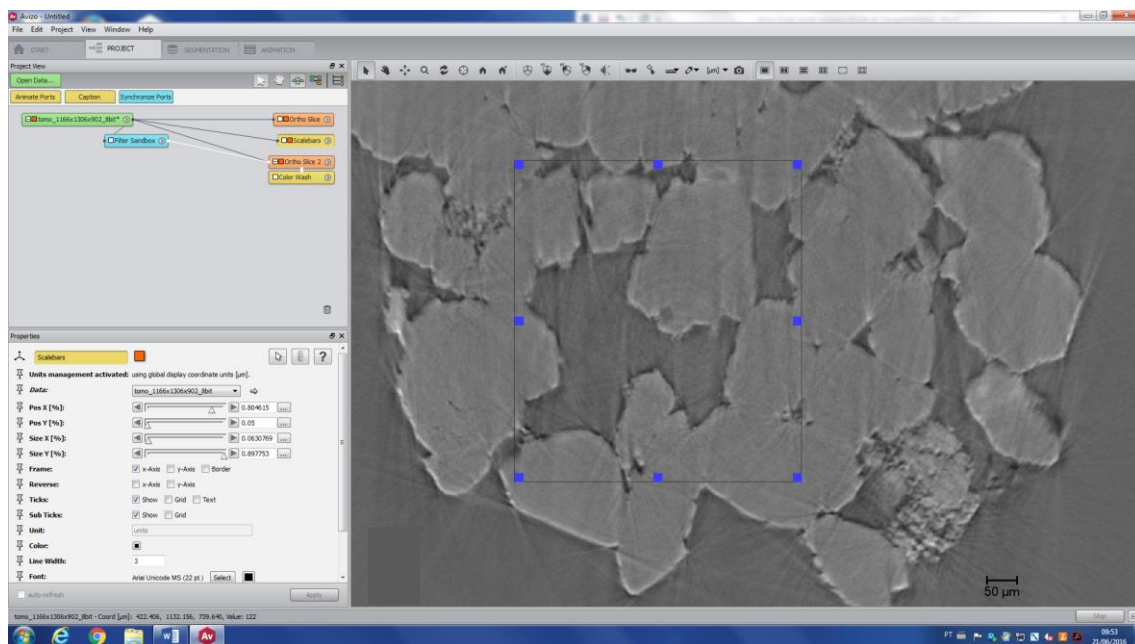
Load your image.



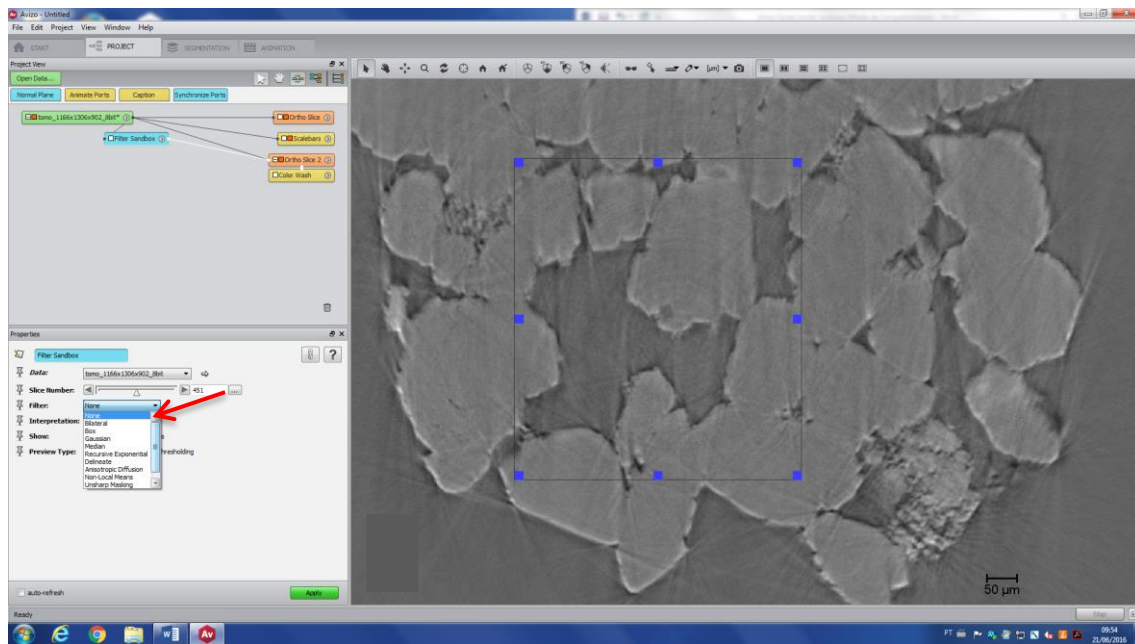
Using the *Filter sandbox* tool, you can test all the filters on a small piece of your image (little square in the image bellow). It is useful to help you to decide which filter is the best one for your case, in a very fast way.



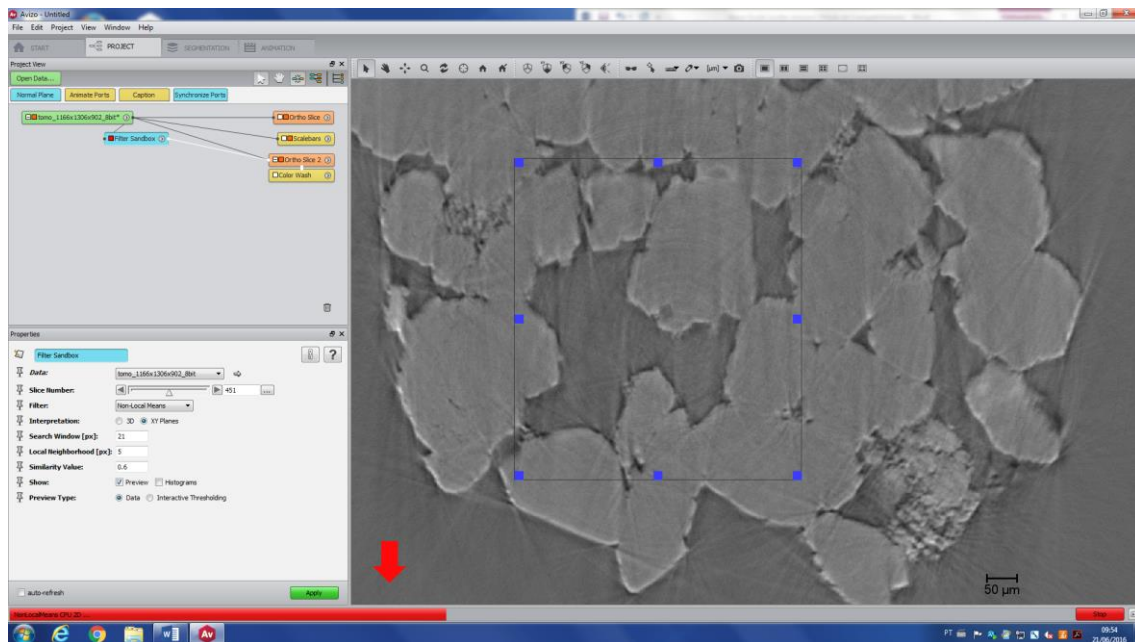
Zoom in the image and move the square to a desirable area.



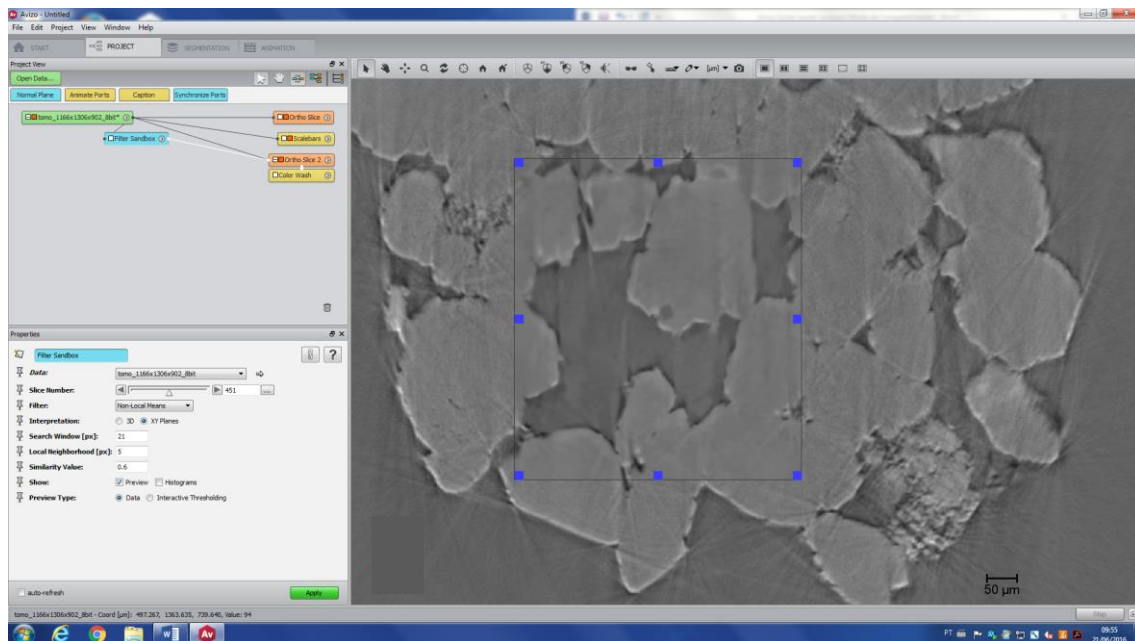
Click on *Filter Sandbox* (light blue) and its properties will be available. You can see that many different filters are available (red arrow).



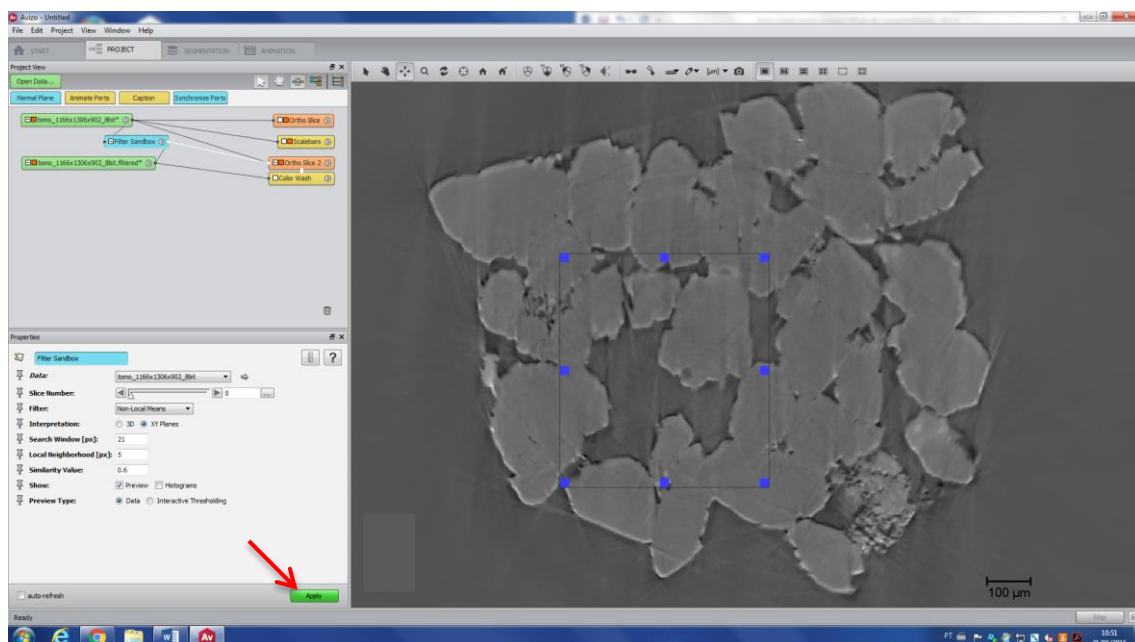
Choose the filter you want to test (in this case, it is Non Local Means). As this filter is very robust, it can take a while to apply to your image. You will see a red bar on the bottom of your screen, showing its progression.



The filtered image is inside the box.



You can make the same procedure to test the other filters. Once you have a decision, you can apply the filter to the whole image by pressing *Apply* (red arrow).



A brief description of every filter available is written bellow:

- **Bilateral:** It preserves and smooth the edge.
- **Box:** It is a lowpass filter that uses a box kernel.
- **Gaussian:** Smooths a image using a kernel (with Gaussian distribution). At general, the edge is not so preserved as the bilateral filter.
- **Median:** It set the voxels value to the median based on the neighborhood. The result image does not look so polished because it has a pixelated look.
- **Recursive exponential:** Smooths an image based on a variable scale. It doesn't preserve the edge as bilateral and also has a less intense smooth compared to gaussian.
- **Delineate:** It presents contrast enhancement.

- Anisotropic diffusion: Provides denoising of scalar volume data, preserving and enhancing the contrast of edges.
- Non-Local Means: Very useful to denoise scalar volume data. It makes the result image polished.
- Unsharp Masking: This filter enhances the edges. It's computed by a Gaussian filter of size kernel size.
- FFT magnitude: Used to remove periodical noises on image.

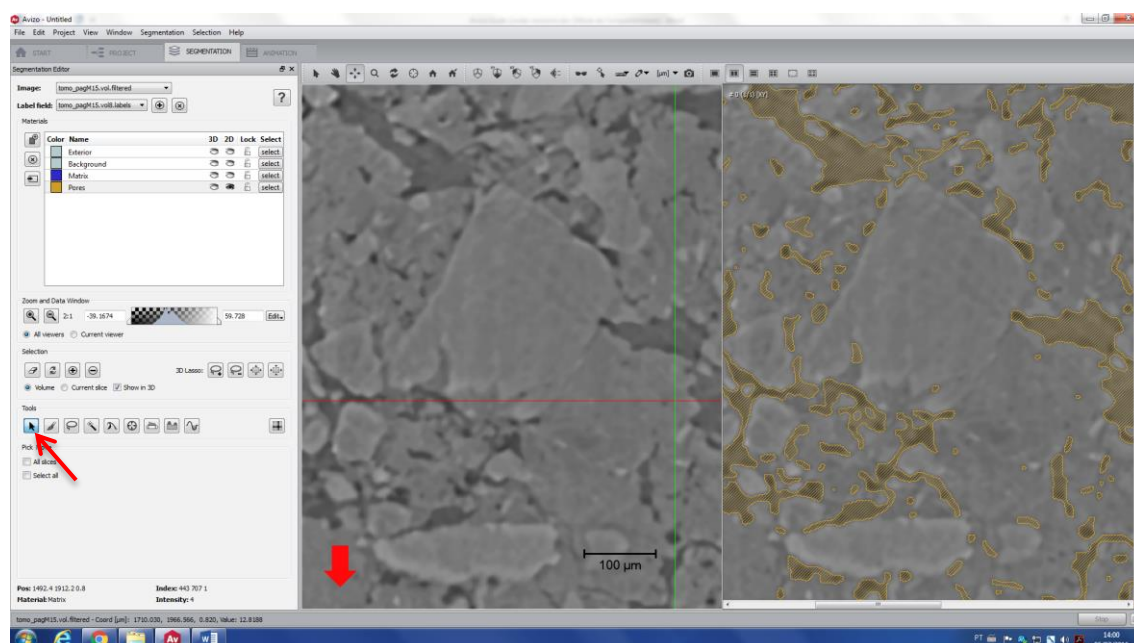
Tip: Sometimes using more than one filter can give you a better result. We suggest using Non Local Means to smooth and remove noise, and apply Unsharp Masking in the result image, in order to restore the edges.

4. Segmenting the image

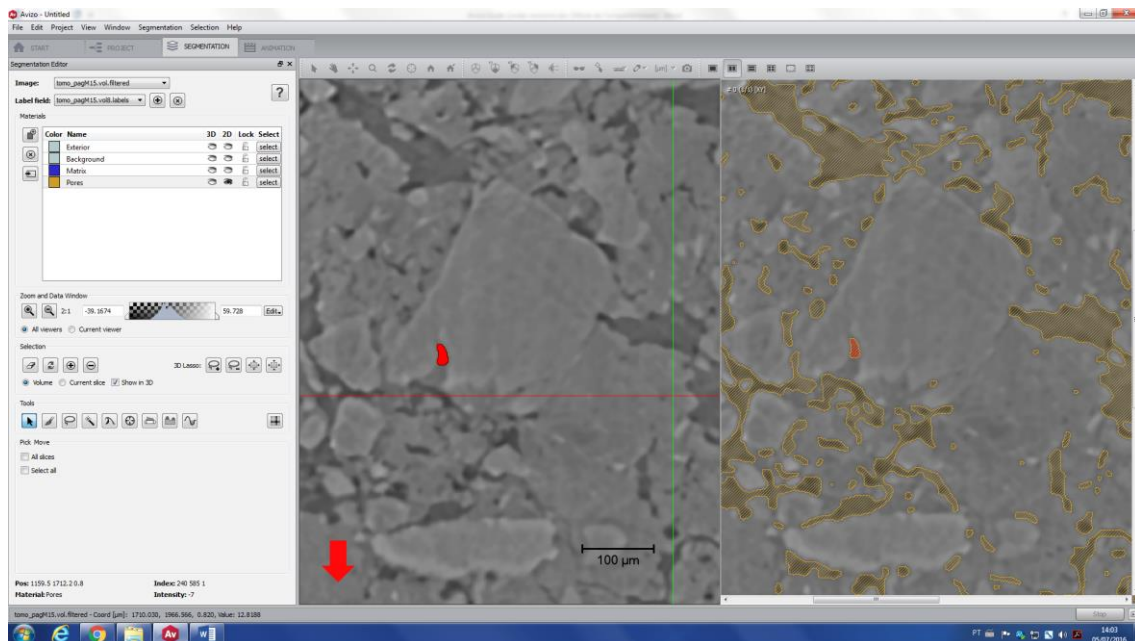
To access the Segmentation menu, right click on the image to be segmented (normally it is the filtered image) and type **Edit new label field**. On its properties menu, you will see many available tools for segmentation. All of them are described below.

a. Pick and Move

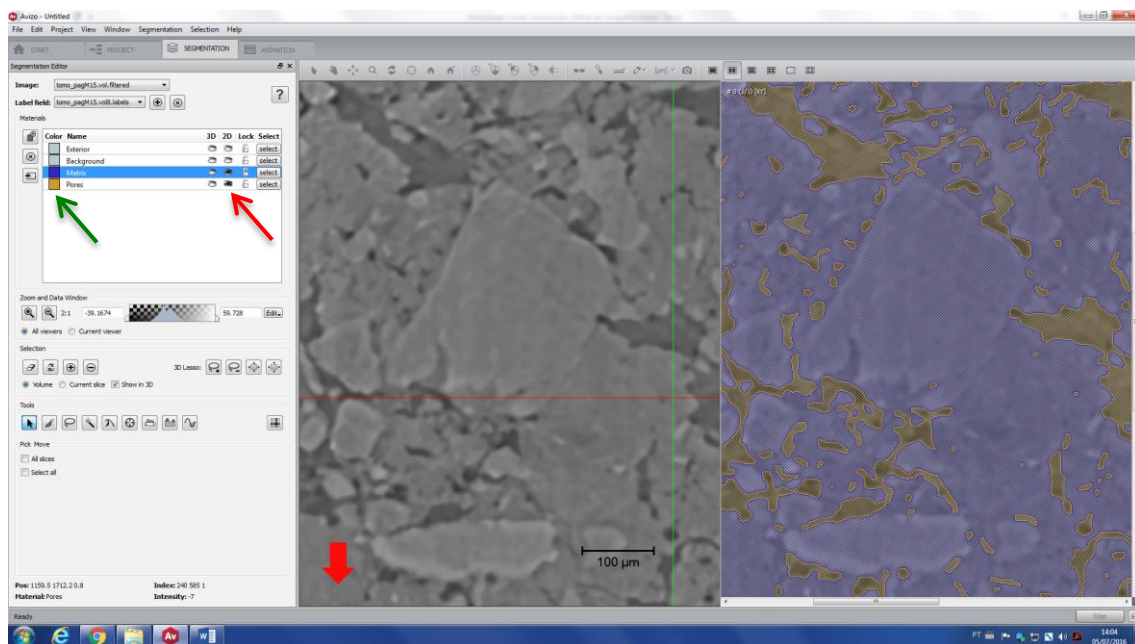
This tool (red arrow) is ideal to select an unconnected segmented area of your image and move it to another label. In the image below, the segmented area is shown in yellow. As an example, let's move one region that was wrongly assigned as pore space (black arrow), to the Matrix label.



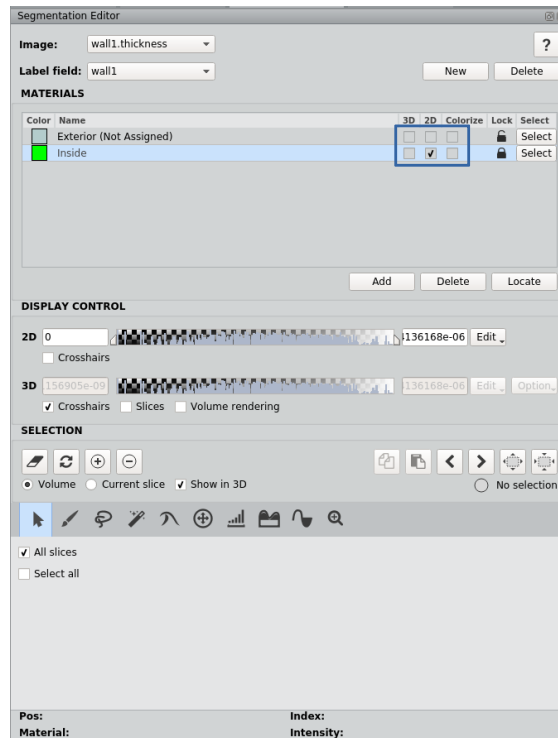
Using Pick and Move, click on the region you want to select. This is a 3D selection, so check the 3D volume to be sure that you really want to move the whole selection to another label.



Click on the label that you want this region to be moved (in this case, Matrix) and then click add (+). This region now is part of the Matrix label. See the final segmentation below, where the matrix is represented by the purple color and the pore space is in yellow. To show or hide a determined label, click on the **small eye*** (2D or 3D), next to the name of the label (red arrow). You can also check the label color and modify, by double clicking on the color box (green arrow).

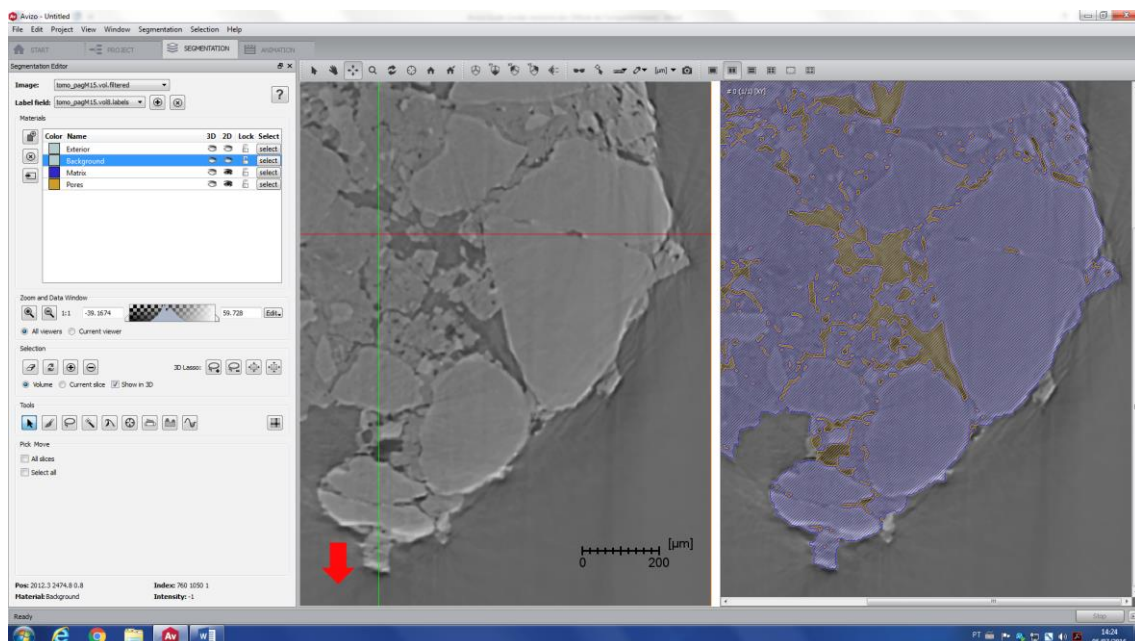


****Observation:** In the new interface, the eyes were replaced by squares (blue rectangle). This is the new interface:*

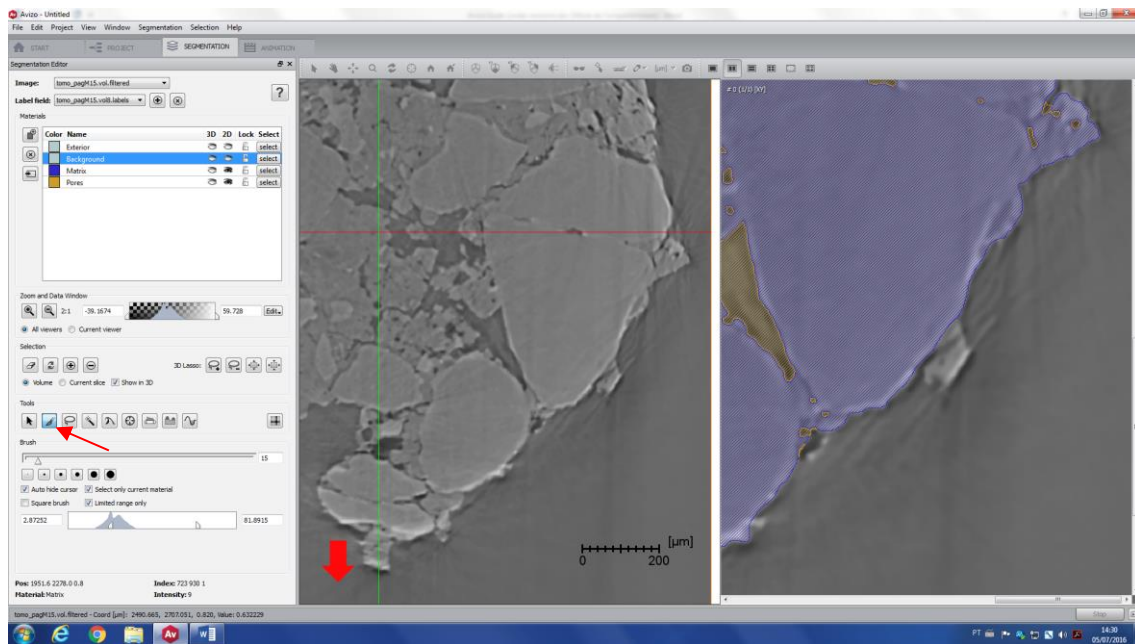


b. Brush tool

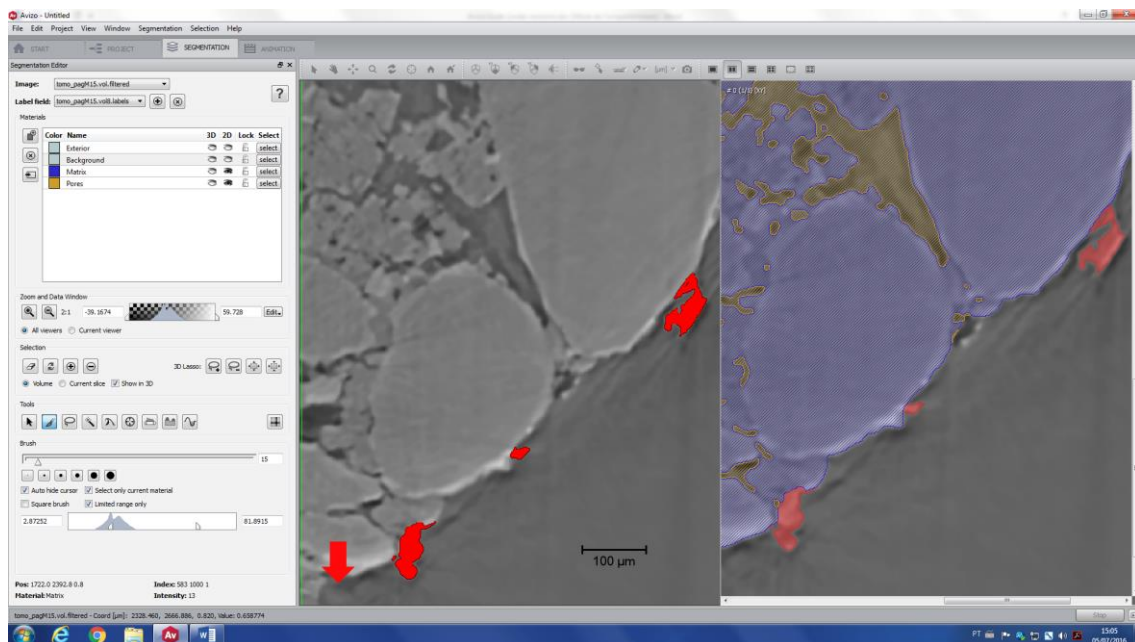
If you have a connected piece of a label that you want to move to another label, you have to use brush tool. For example, there are two regions (black arrows) that were wrongly assigned to Background label, but it should be Matrix.



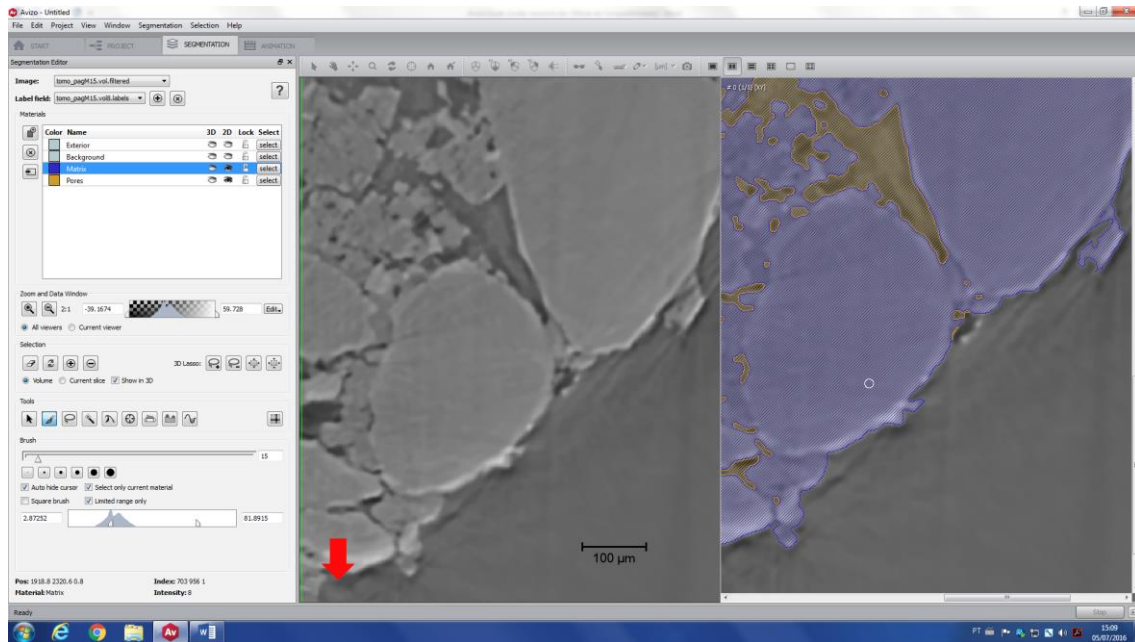
Go to Brush tool (red arrow), click on Background label (black arrow). Click on *Select only current material* and, if necessary, *Limited range only* (if you know the threshold value that contains the material you want to select) – if you do not select this, all the Background voxels will be available for selection.



Select the region of interest (this is a manual painting tool, so watch out for the edges). Attention, this is a 2D selection; you might change the slice number to select a 3D region.

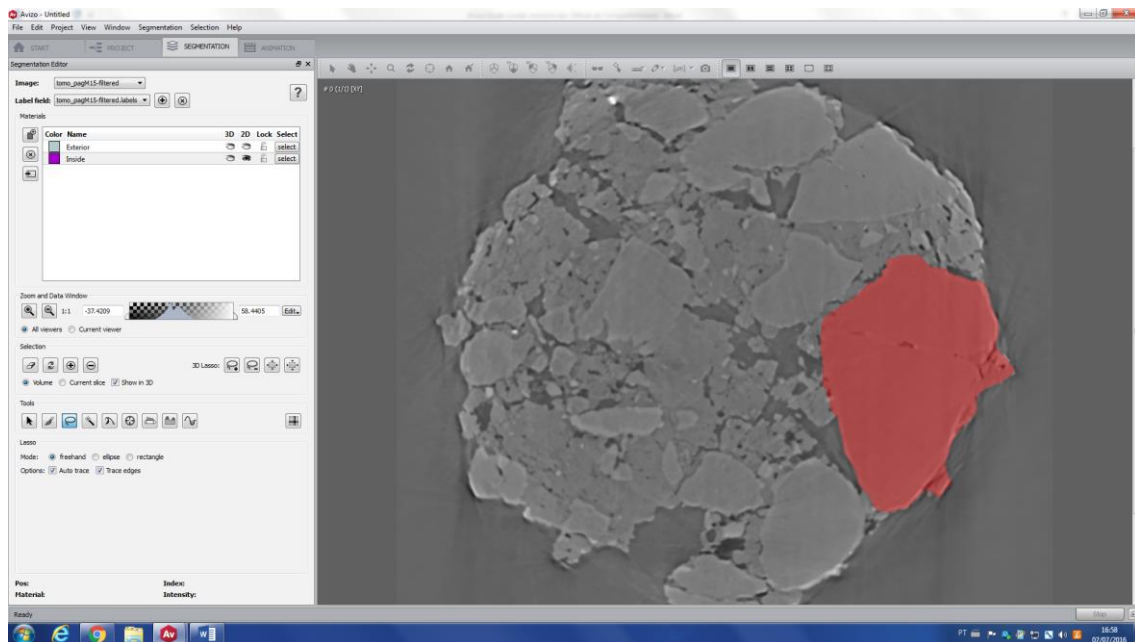


Add the selection to the correct label.

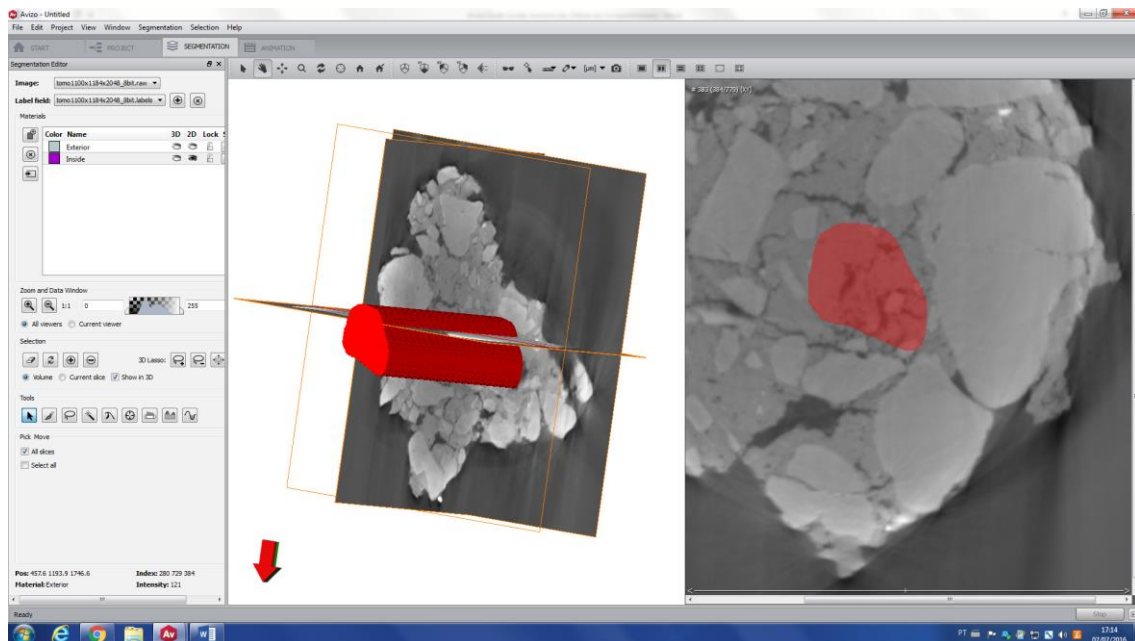


c. Lasso tool (2D and 3D)

This tool lets the user draw a closed contour, and selects all pixels within the contour. Some options are available for 2D: free hand, ellipse and rectangle. Using free hand, you can use auto trace, which will identify the edges of a certain area of the image.



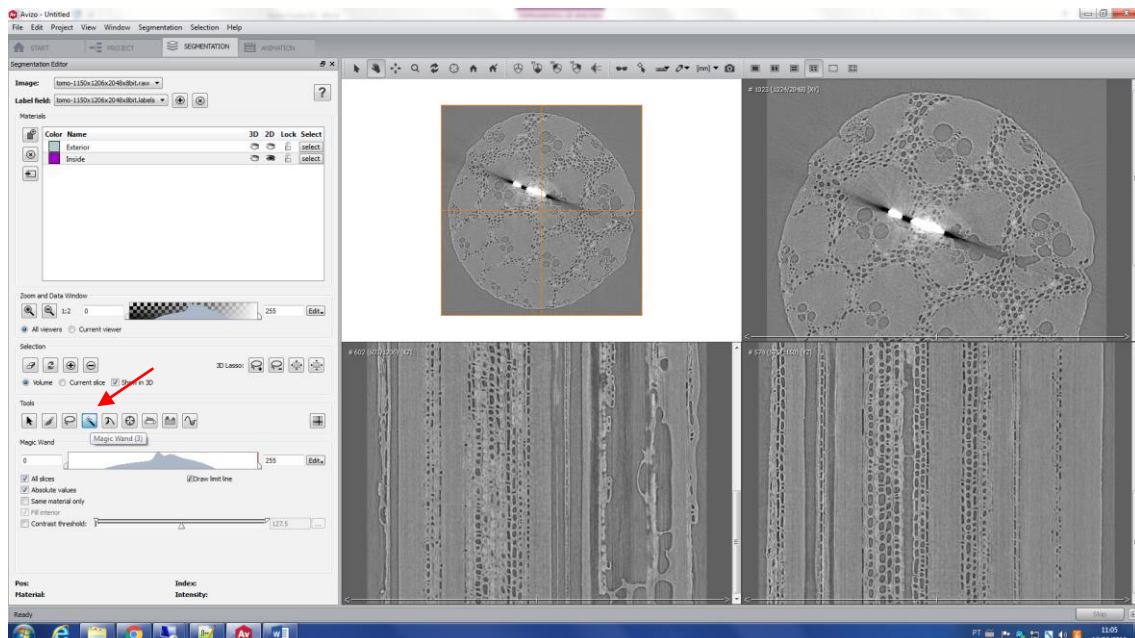
If you want to use the 3D lasso, you have first to change to **Two viewers** or **Four viewers** in order to see 3D and 2D simultaneously. Then select a 3D area (left part of the image below) and also check this in 2D (left part of the image). Note that you can only modify the selection shape in the 2D image and, in the third direction (in this case, Z direction), the selection is automatic.



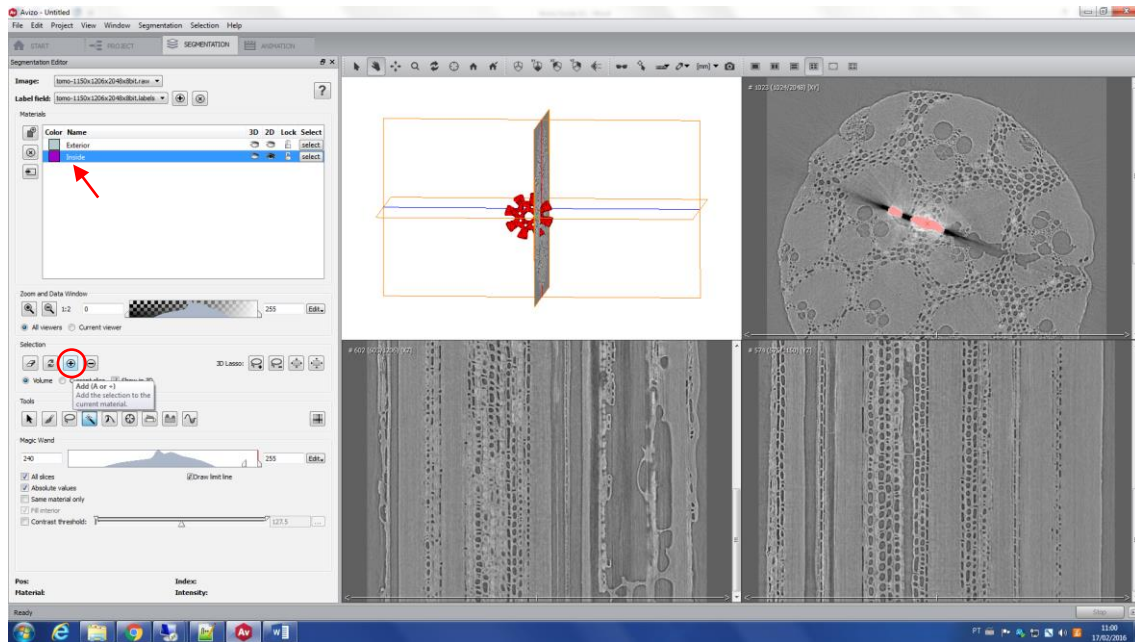
d. Magic Wand tool

Magic Wand is a region growing algorithm, which starts from a seed point and select all connected voxels with a gray value in a given tolerance interval. The advantage of magic wand over threshold tool is that it permits you to choose a single material/structure inside your image, i.e. if you have other materials/structures with the same gray level, but it is not connected to the region you want to segment, you will not select these voxels.

Click on magic wand tool (red arrow). In this example, the image is a toothpick with a micro gear inside (bright region).

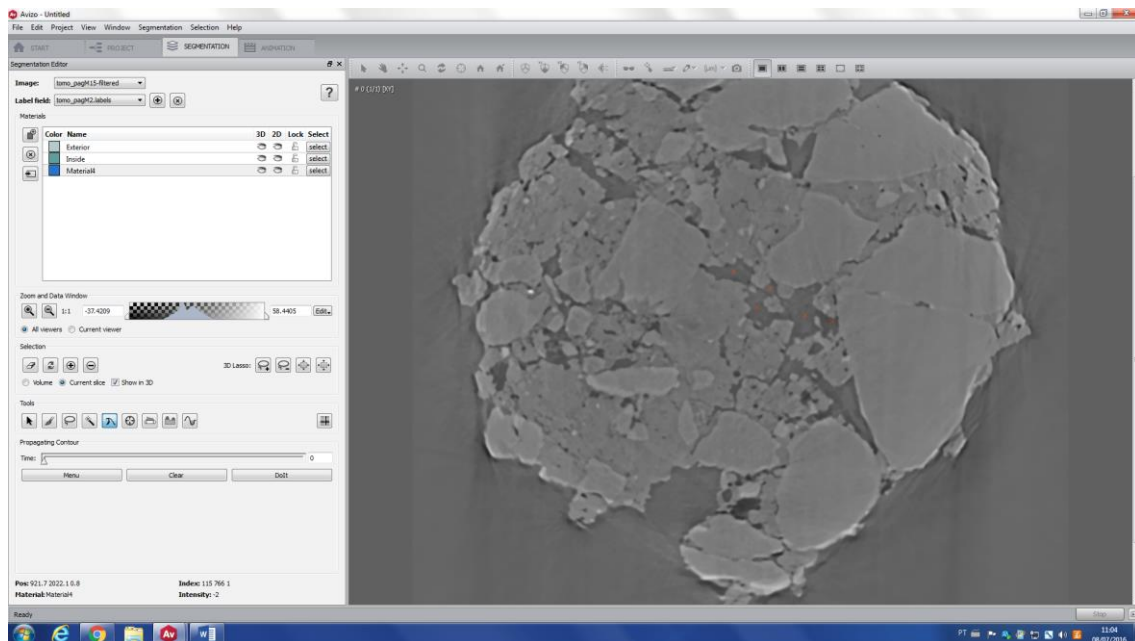


Click on the gear (red cross) and change the boundaries values (in this case, to 240 and 255).

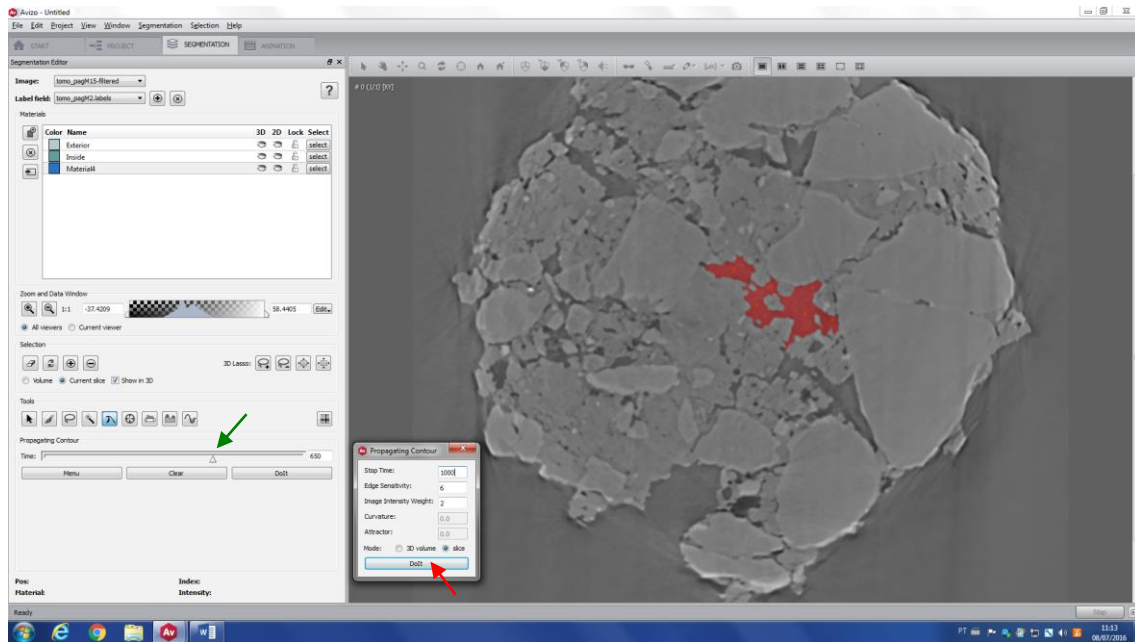


e. Propagation Contour

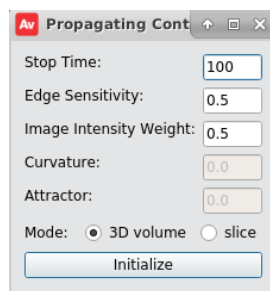
This tool is a fast active contour. From a seed, which is created manually, a boundary front evolves. Few parameters are available to be modified, as stop time and edge sensitivity. In this example, we will use propagation contour to segment a specific pore of this soil sample. First, you have to create the seeds manually - check the figure below (red crosses).



Then, you click on *Do it** (red arrow), and the front will start evolving. After it finishes, you can choose the best *Time* (green arrow) that represents the pore space.



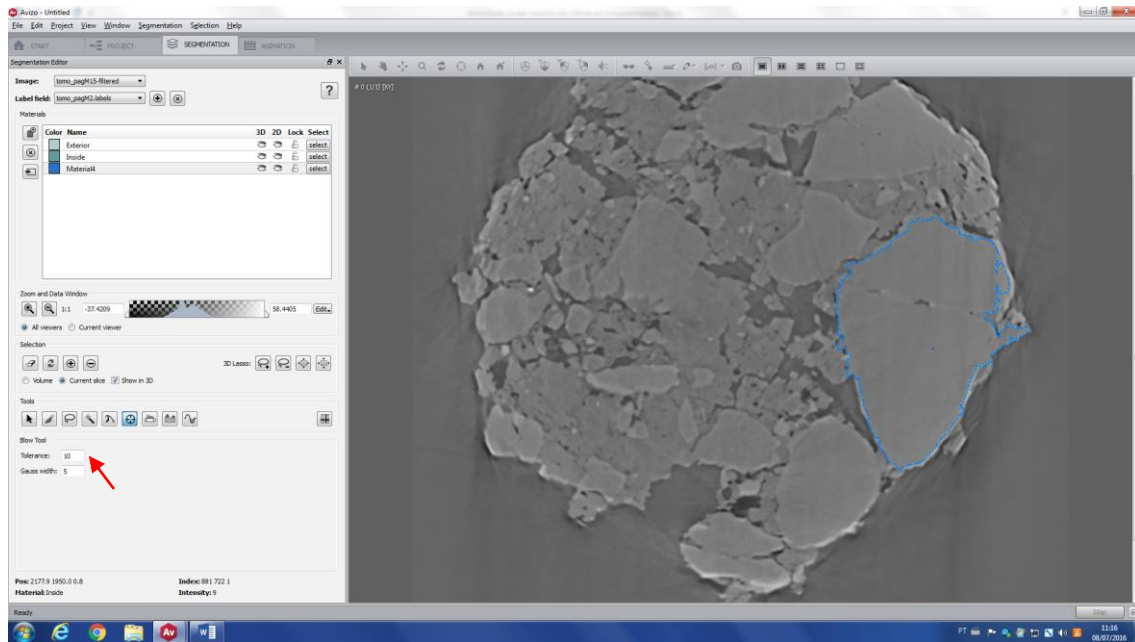
**Observation: In the new interface the phrase is “Initialize”.*



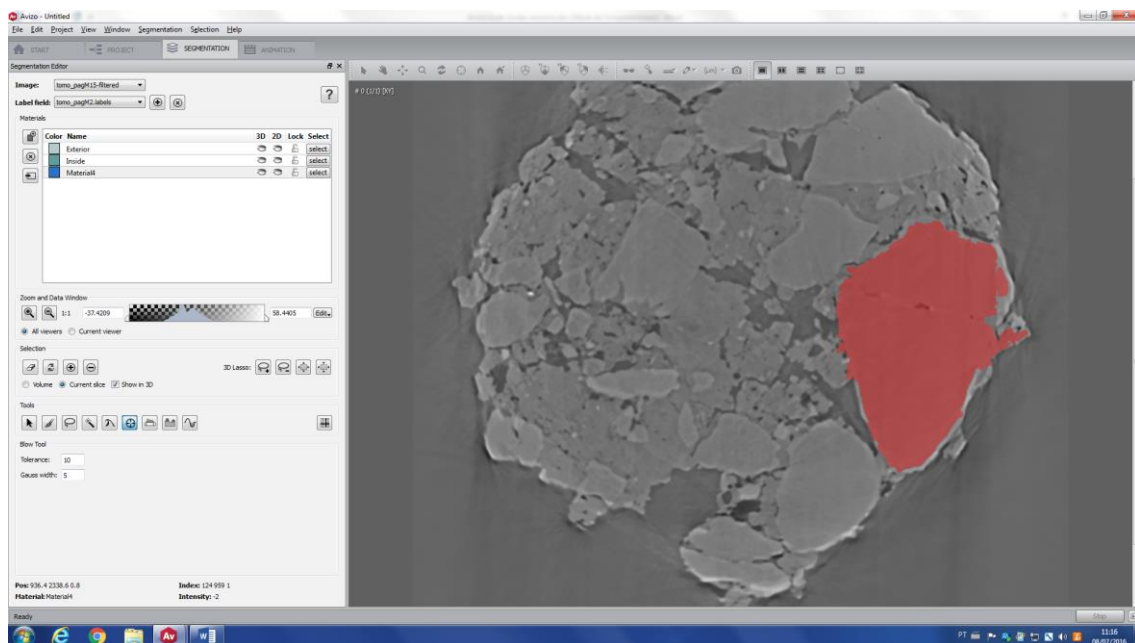
f. Blow Tool

This tool is useful to detect edges of a region and fill its interior, however it works only in 2D. Click (and hold the button) on the area of interest. Drag the mouse, and the greater the distance from the original point is, the more the contour grows. Release the button when the contour is in a satisfactory place, like shown in the image bellow. The contour will grow in areas with homogeneous gray values and will stop when it changes abruptly, i.e. when it finds an edge.

The **tolerance** (red arrow) controls how sharp the edge has to be in order to stop the contour growing - the smaller this number is, the sharper the edge has to be. If this number is too large, the contour will stop in weak edges.



After you release the mouse button, the area within the contour will be selected and you can add to the desirable label.



Tip: For 2D tools (Brush, Lasso, Blow) there is the possibility of **interpolating** the data in order to make manual segmentation faster. In this case, perform the segmentation on an x slide and advance some slides, let's assume an advance of 10 slides ($x + 10$) and segment on it as well. Click **ctrl + i** (attention, i must be lowercase) and note that the slides between x and $x + 10$ are segmented following the initial and final orientation.

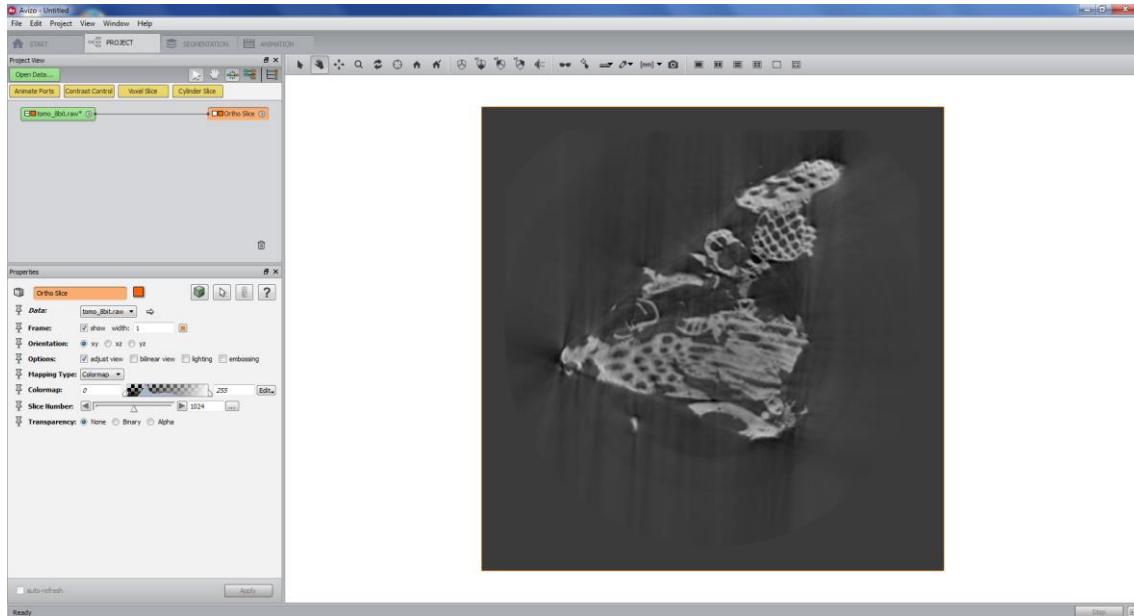
Tip 2: If you keep **shift** selected while doing blow or lasso, you can make more than one annotation per slice.

Tip 3: Use **shift + i** to invert the selection.

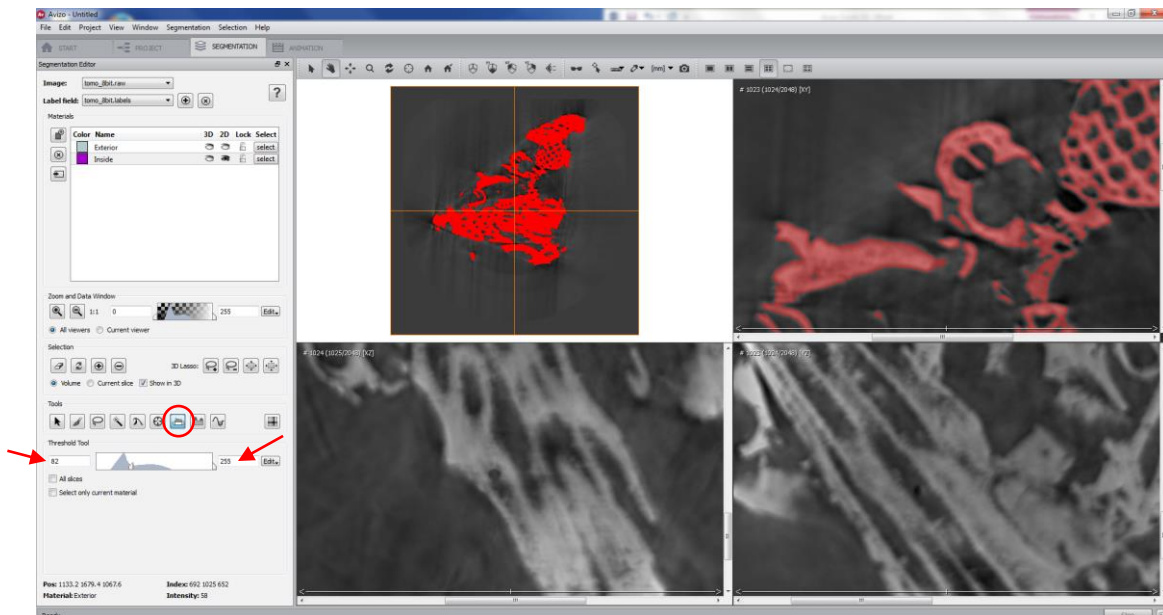
g. Threshold tool

The threshold tool separates the image into different regions according to the intensity values. To separate the image using this tool, you just need to choose one or more threshold points in the gray level histogram.

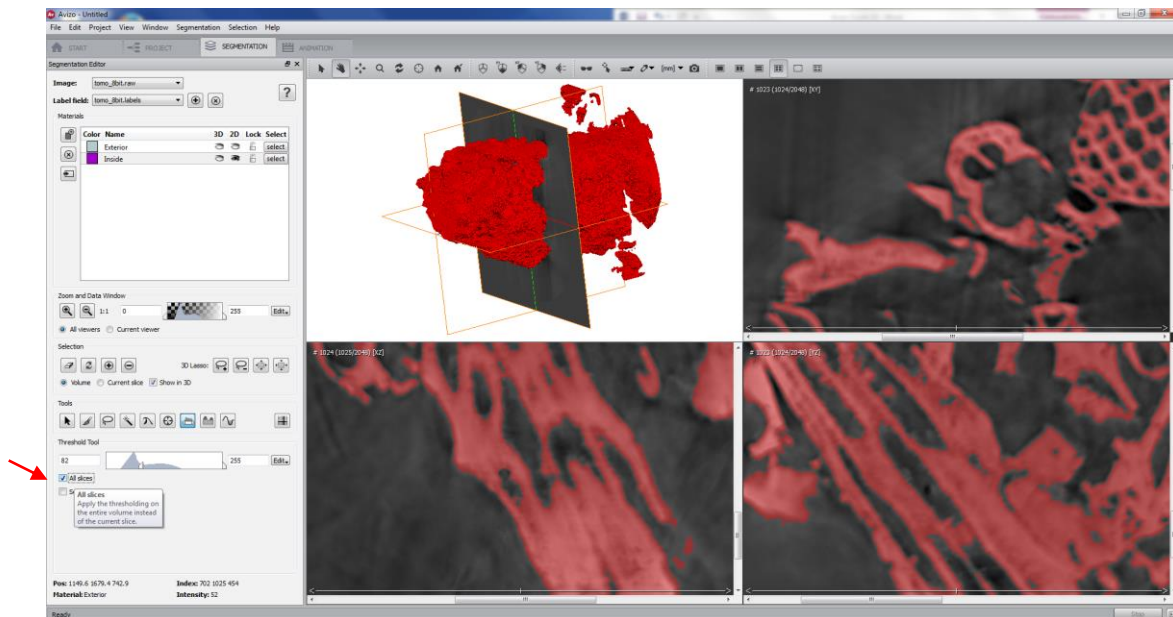
As an example, we have an image of a carbonate rock. Clearly, it is possible to see two different phases: matrix (light gray) and pores + background (dark gray).



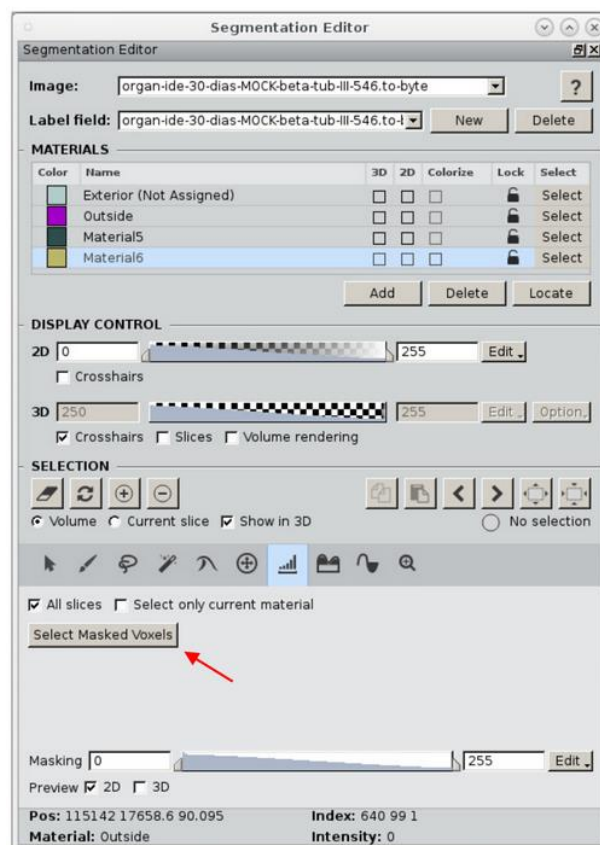
Click on **Threshold** tool (red circle) and change the gray level values (in this case, to 82 and 255) – this way, the rock matrix will be selected.



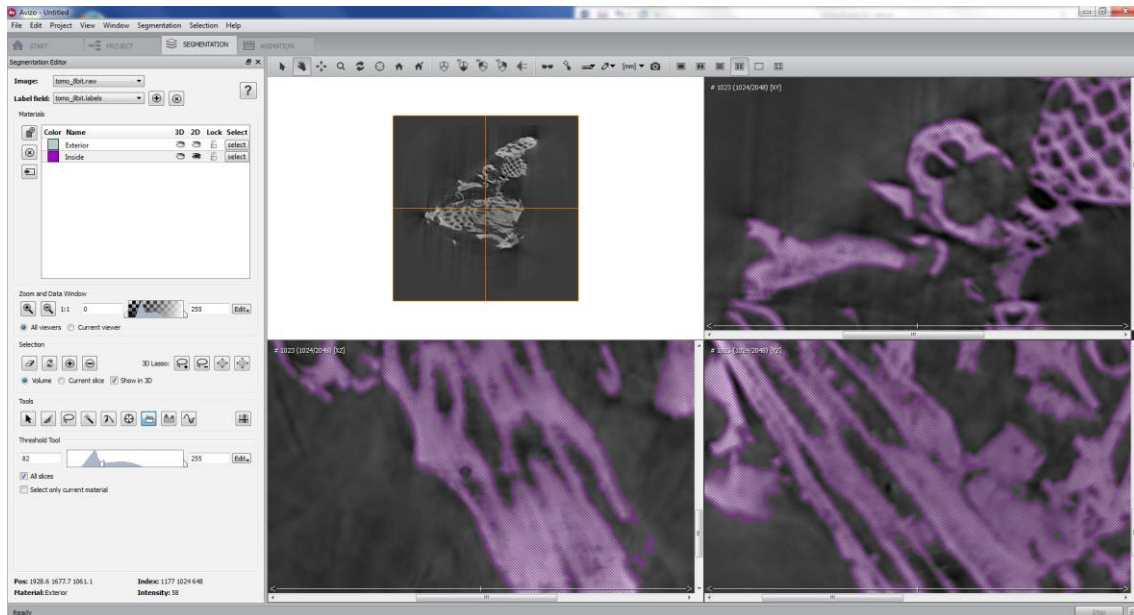
Each sample will require different numbers (threshold points). Once you have the best selection, you have to apply for all slices (red arrow).



Select **Masked Voxels** (red arrow), which only appears if you are using the threshold tool. Now, before adding a group of voxels to a specific label, you have to click on this button to select the voxels and, only after this, add them to the desirable label.

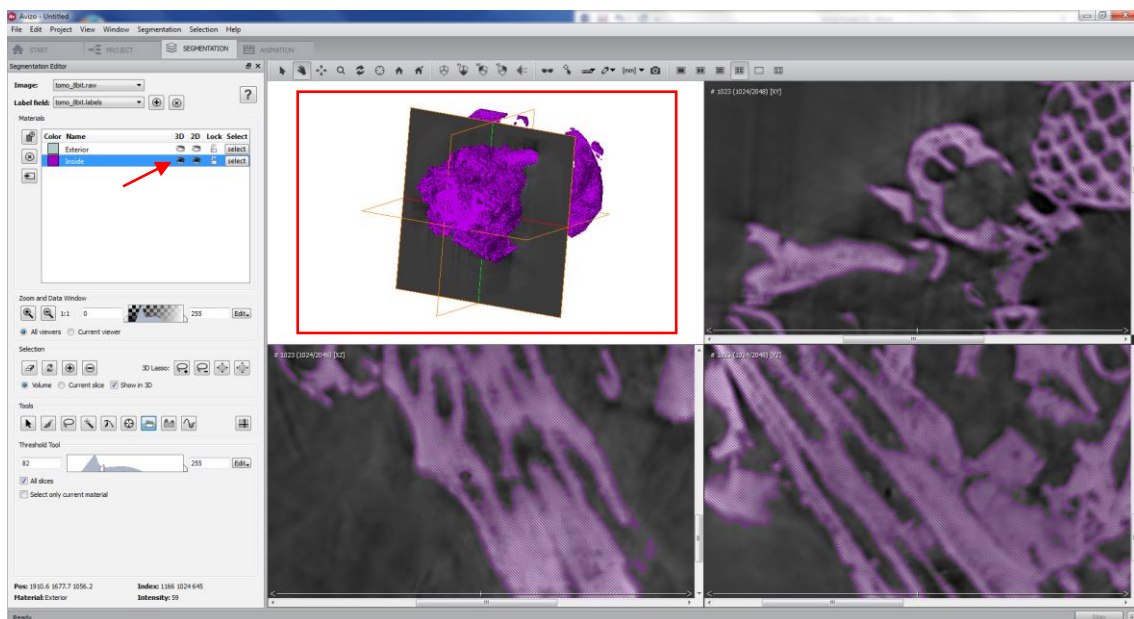


Add the selection to a label.

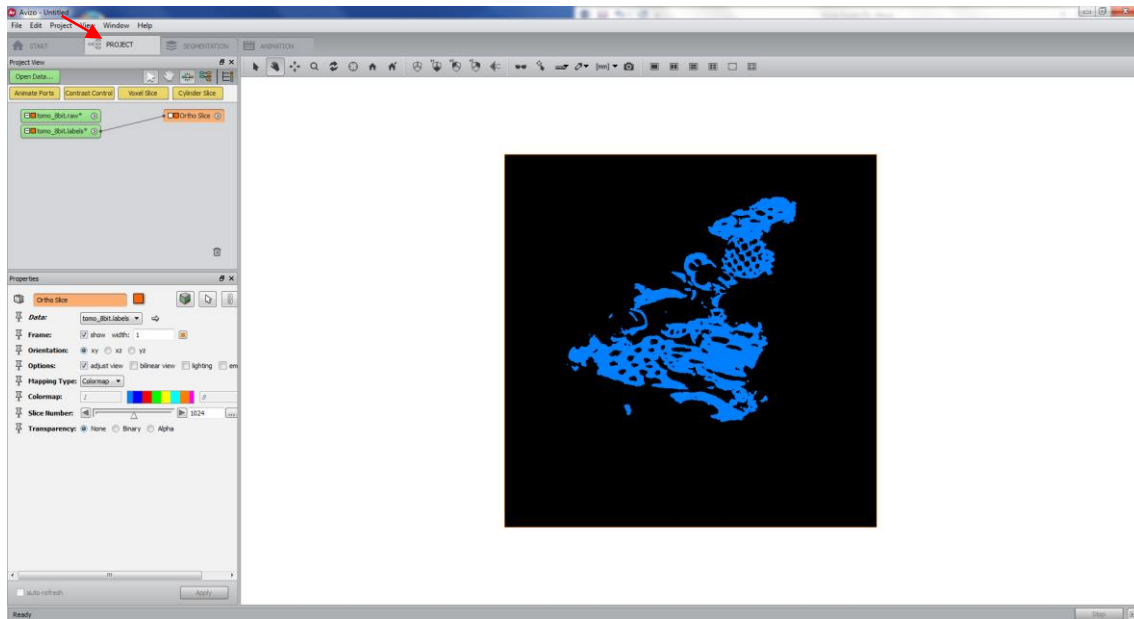


For a 3D view of your segmented image, click on the left small eye* (red arrow) and check the top left window.

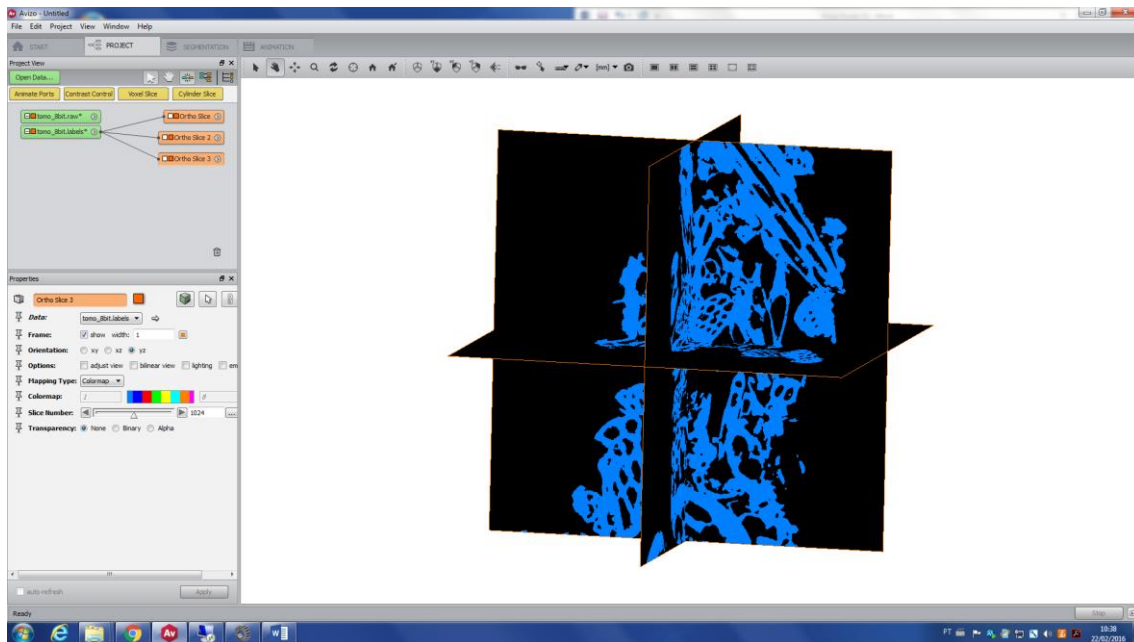
** **Observation:** In the new interface, the eyes were replaced by squares.*



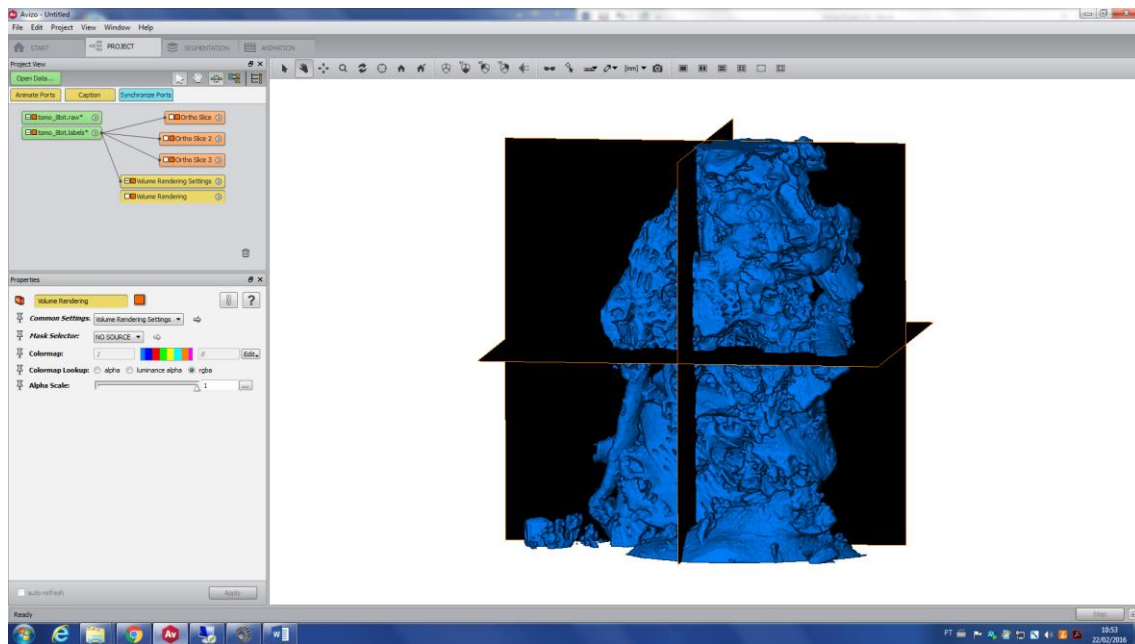
Once you finish the segmentation, come back to Project window (red arrow). Now you have a new image (.labels). This is your segmented image. Create an orthoslice and check the image.



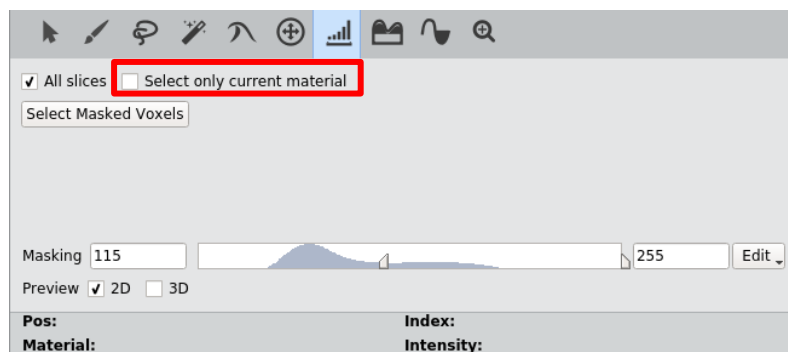
You can create two more orthoslices and check all views.



You can also create a Volume rendering of your segmented image.

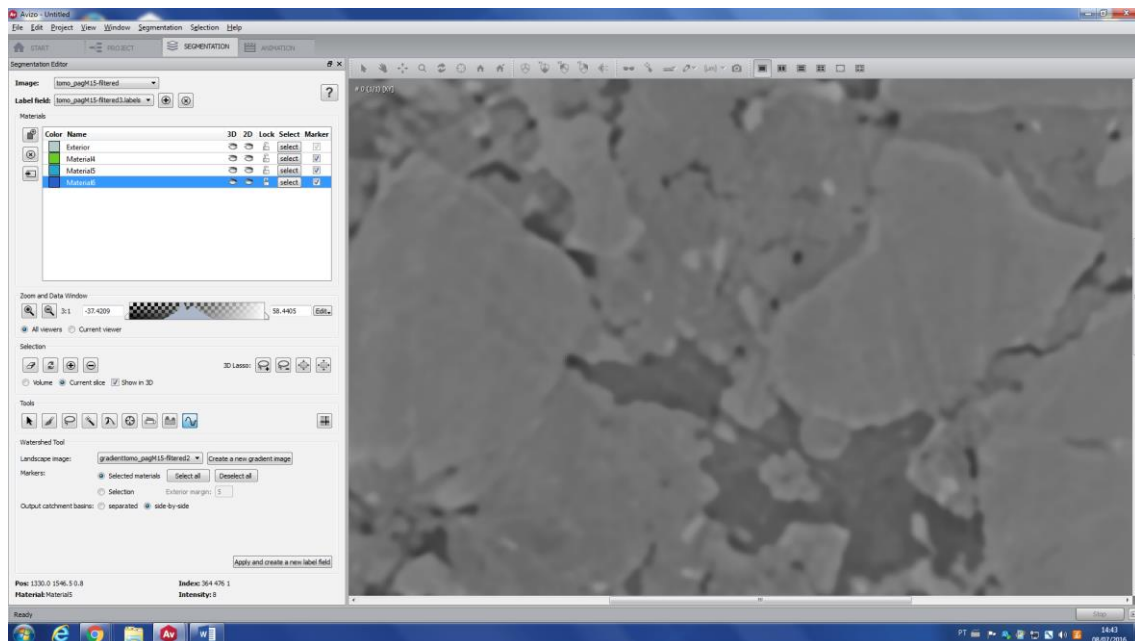


Tip: If the *Select only current material* option (red rectangle) is activated, only voxels assigned to the current material are selected. This can be useful, for example, to select pores inside the rock (avoiding the selection of background).

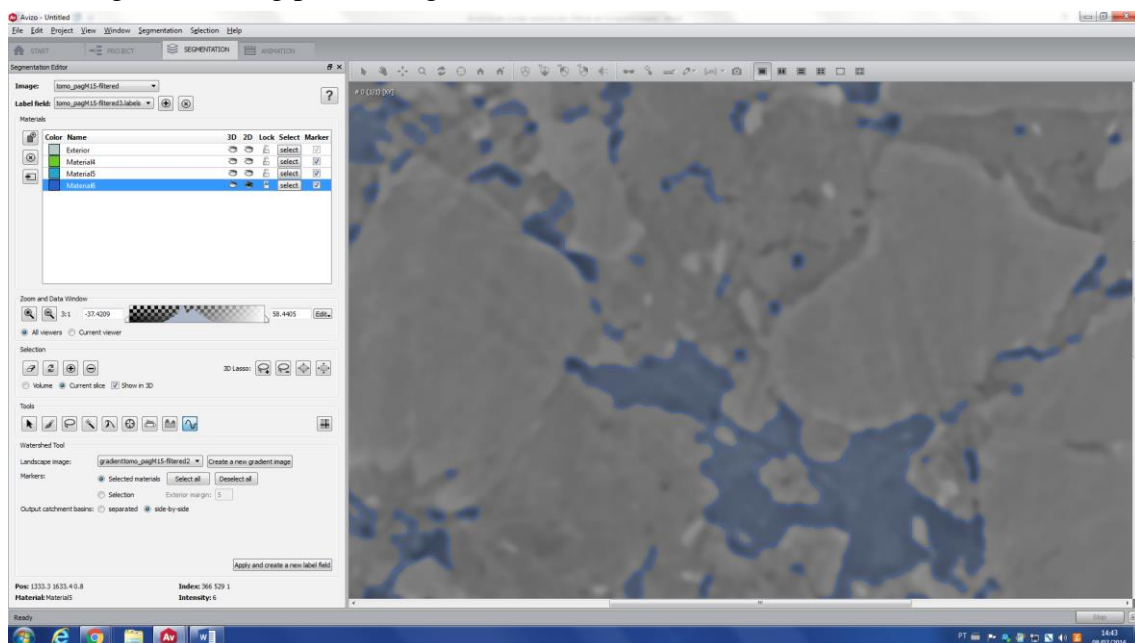


h. TopHat tool

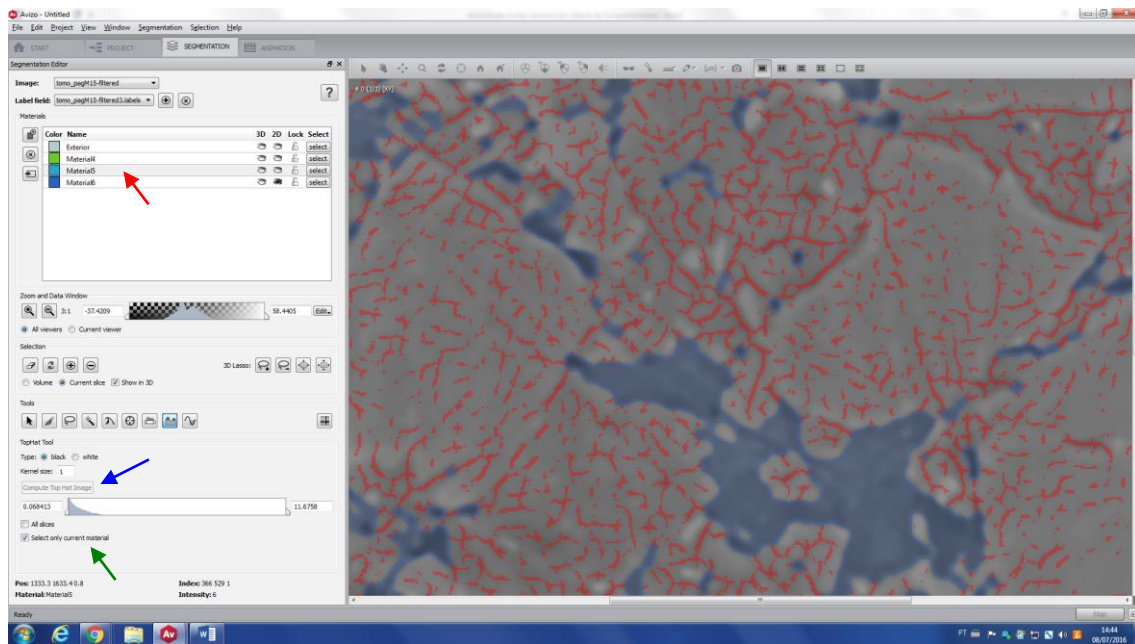
This tool allows the detection of dark or white areas of the image. This tool is very useful to pick small regions. As an example, this tool will be used to select the small pores of the following image.



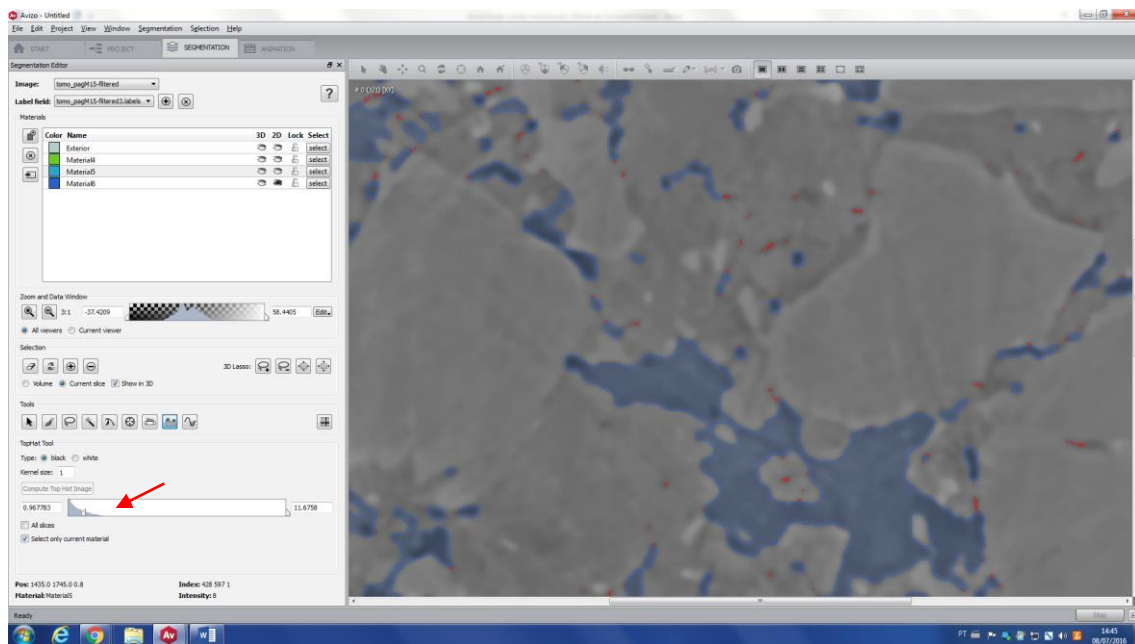
First, segment the big pores using **Threshold** or **Watershed**.



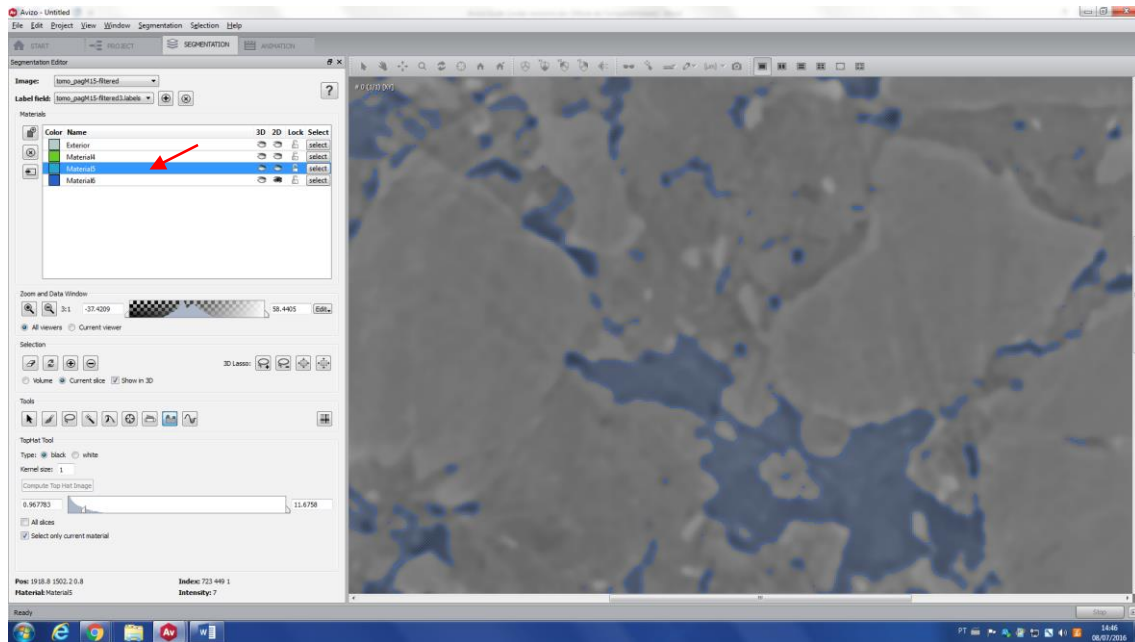
Then, the missing small pores will be segmented by **TopHat** tool. Click on *Material5* label (red arrow), which represents everything that is not a big pore in this image. Since all the unsegmented small pores are within this label, click on *Select only current material* (green arrow). Choose between black or white – in this case, as the goal is to segment pores, black is the best choice. Then, click on *Compute Top Hat Image* (blue arrow) – this will be an available option at this point of the segmentation process.



Change the range (red arrow) to adjust the selection.



And add this region to the pore label (Material6, in this case).



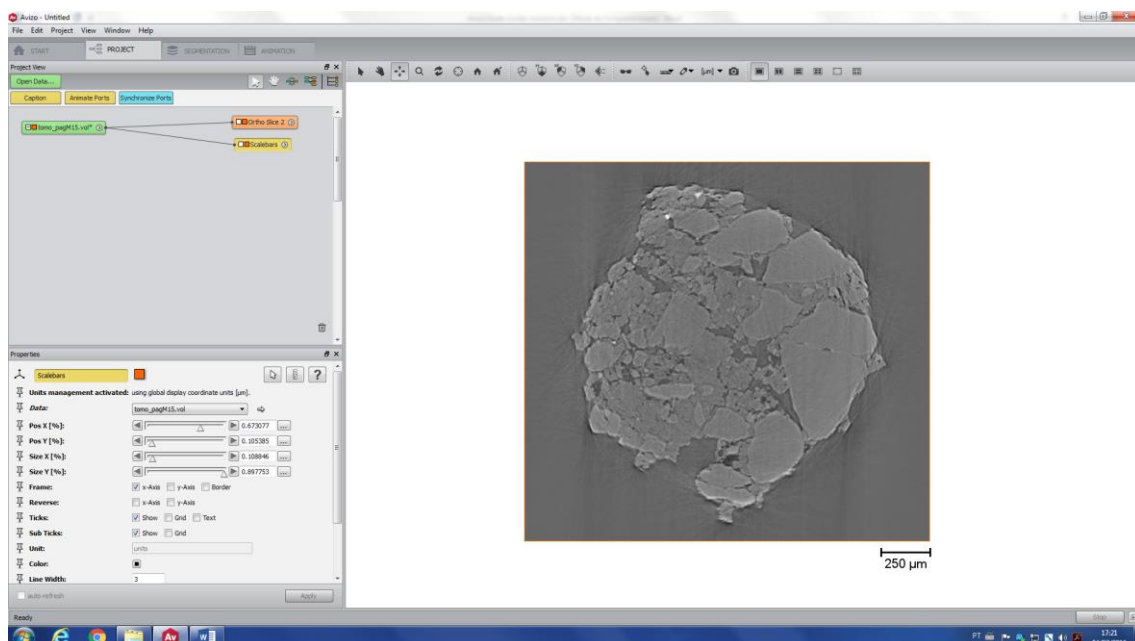
i. Watershed

Watershed is a robust segmentation tool and the idea consists of placing seeds in different regions of the image and start growing this until it touches a global maximum (barrier) of the image, which is determined by the image gradient.

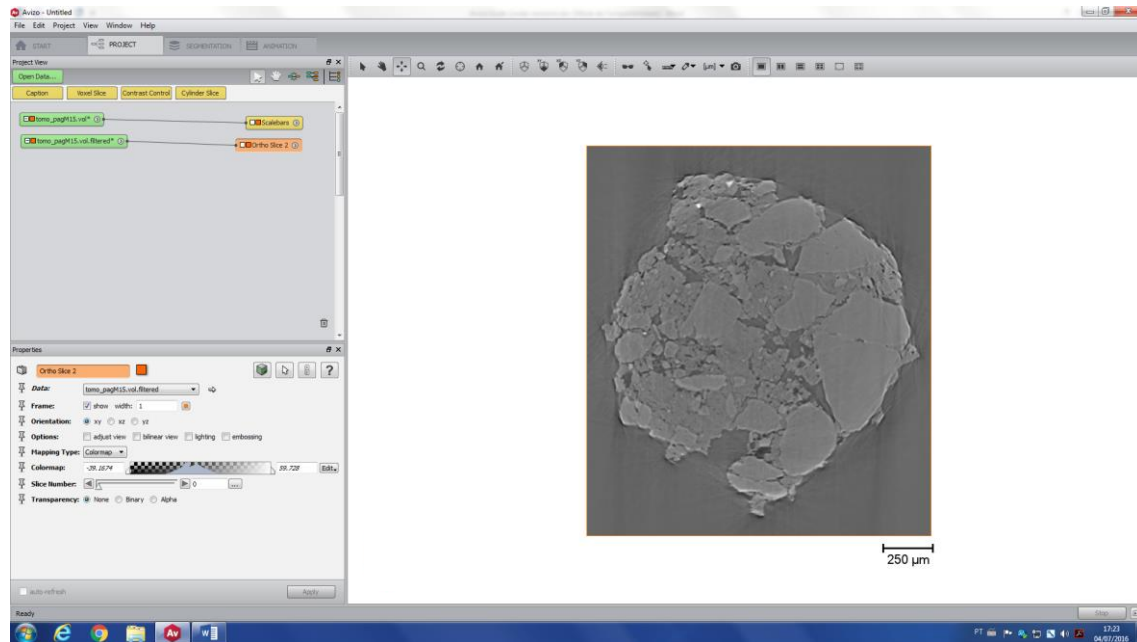
i. Special application: Removing the background

Remove the background of an image can be useful in many cases, e.g. you do not want to crop a region of interest, either due to its heterogeneity or because you want to study its surface, or you want to study the pore space of the sample - if the pore space is filled with air, there will be no difference in gray level between the internal pores and the background. To remove the background, follow the next steps.

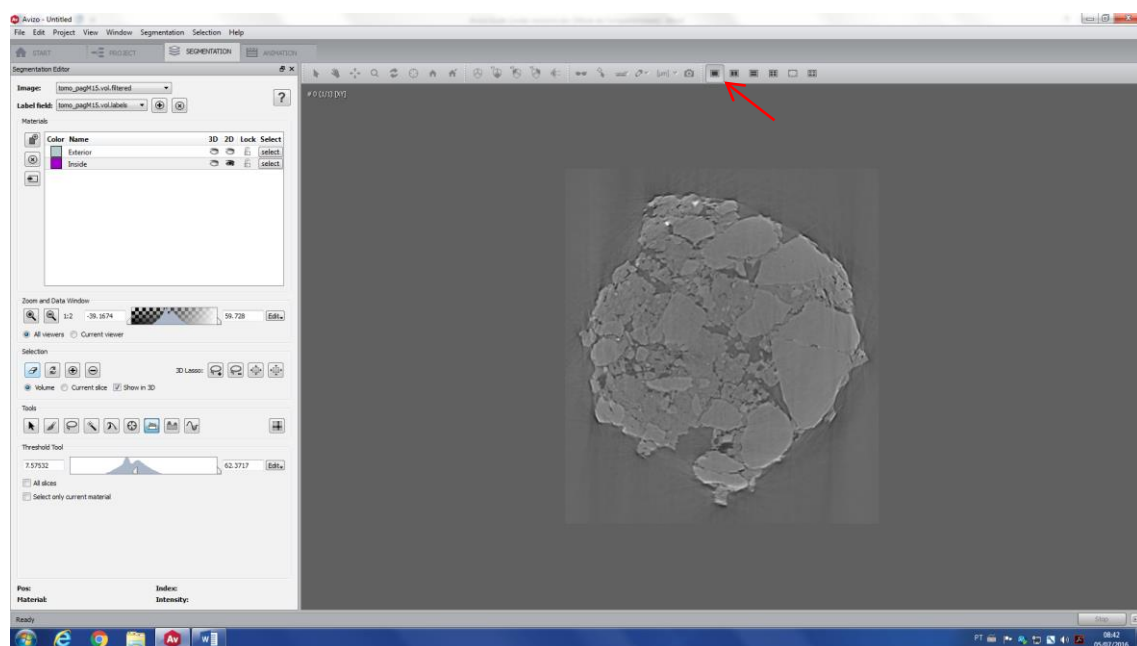
First, load the image.



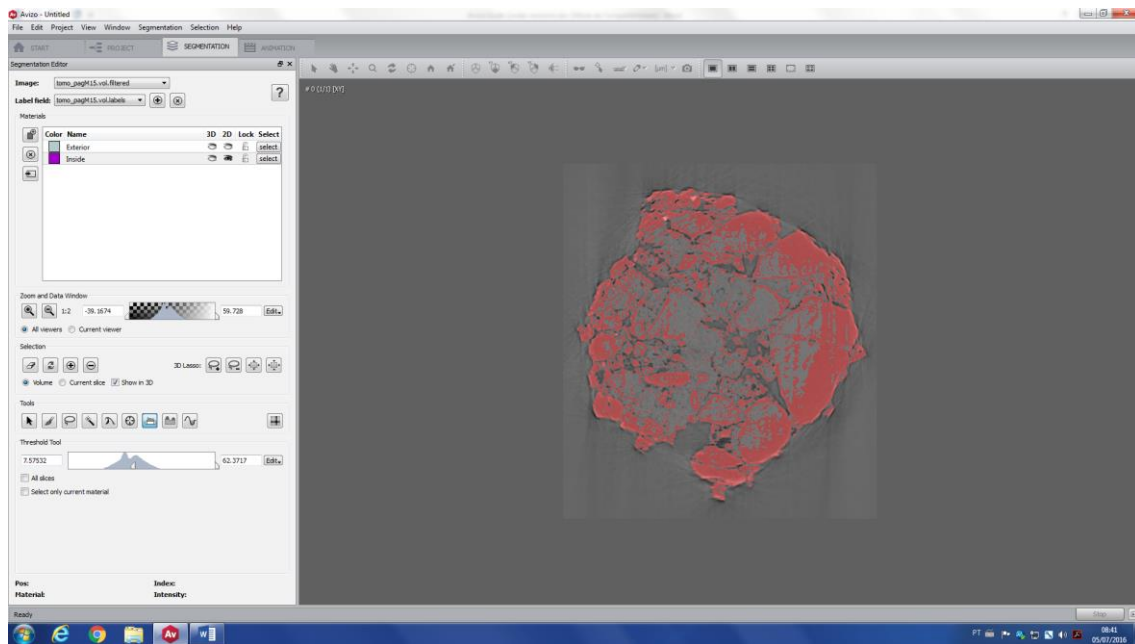
Apply a filter, if needed (in this case, NLM). Just be sure that the filter preserves the edges, which are necessary to create the gradient image.



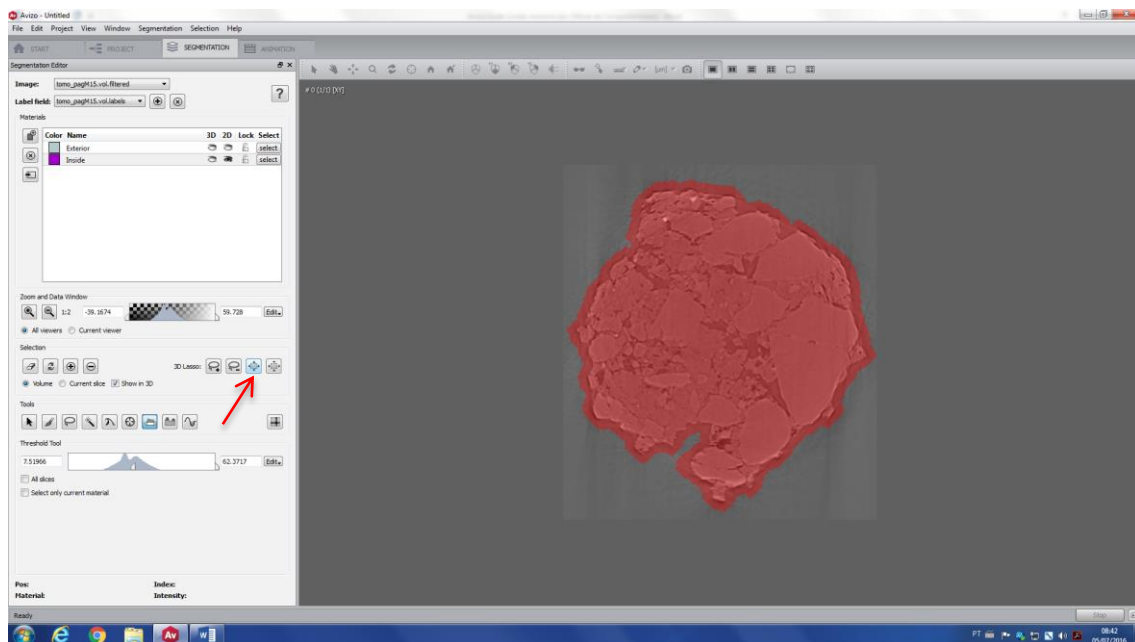
Click on **Edit new label field** and click on the full window (red arrow), for a better view.



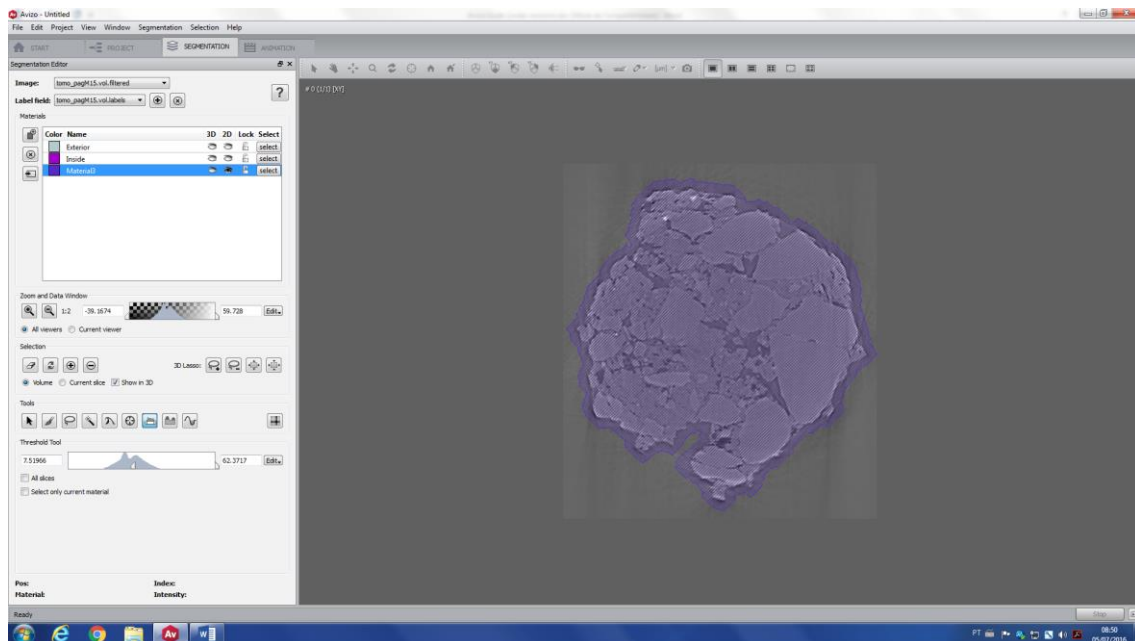
Using threshold tool, select as much as you can of your sample. Do not select the background, since this selection will be used as seeds.



Start growing (red arrow) the selection until you close all the internal pores (check all the slices, if necessary, grow a little bit more the selection).



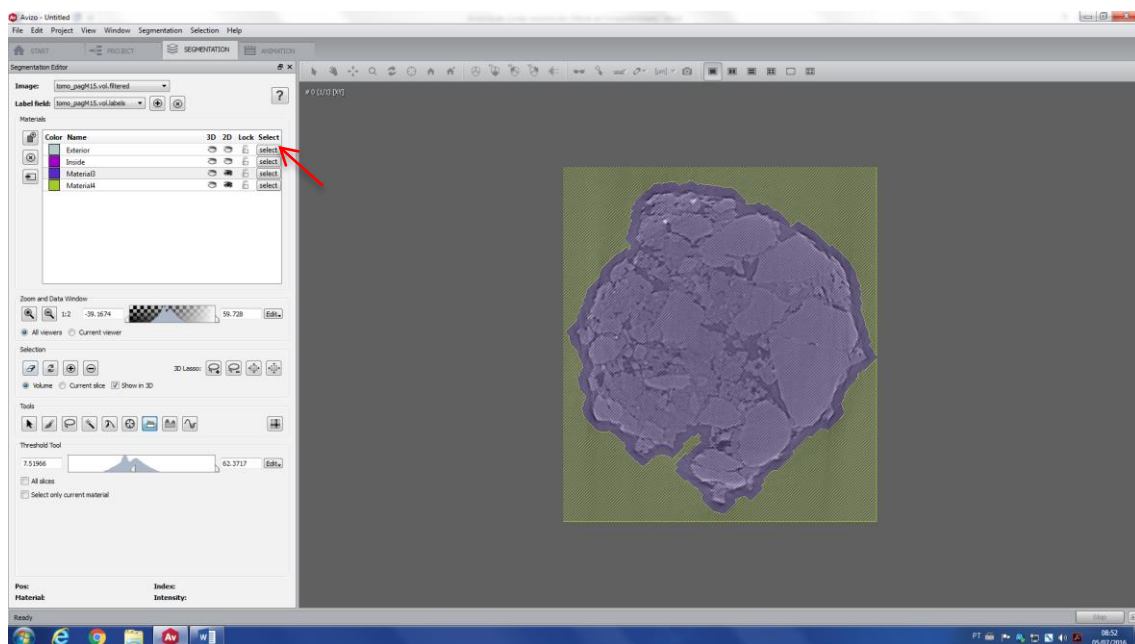
Add this selection to a new label (in this case, Material3).



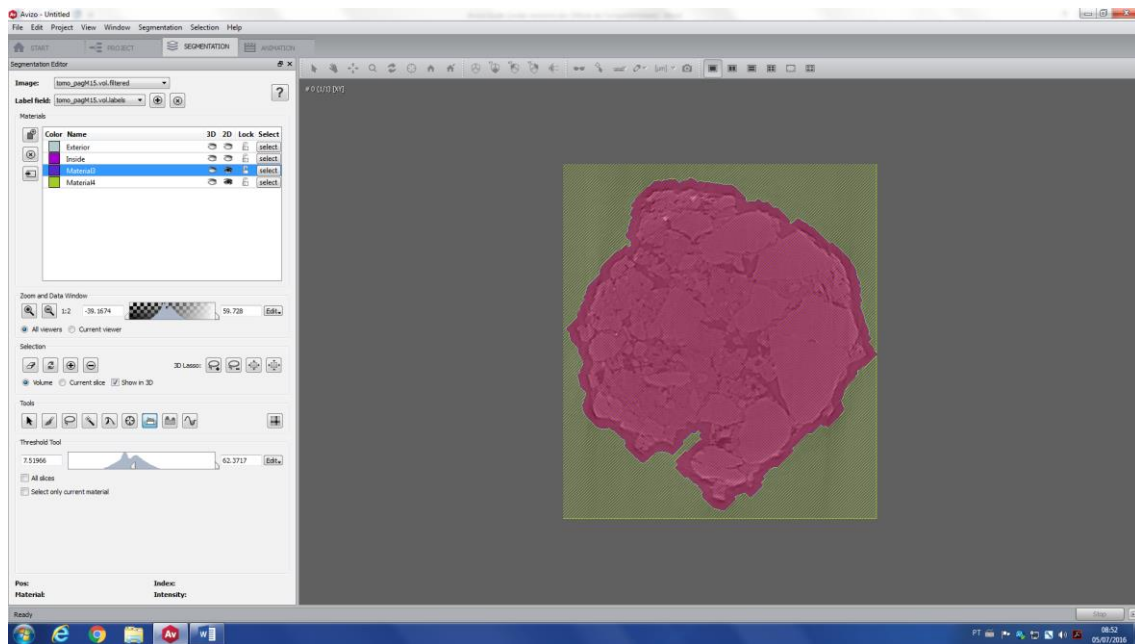
Tip: When the selection starts growing, small voxels can be selected at the background area. In that case it's useful to use **Remove Islands**. Select the Segmentation menu and click **Remove Islands**. This filter removes islands from the label image. In the case of multiple materials, check **Also merge n-neighbor islands** because if the length of that border is greater than a user-defined minimum length, the island is assigned to the material with the largest border, otherwise the island remains untouched.

Tip 2: With the purpose of filling the interior of the selection without having to greatly expand the border, go to the Segmentation menu and click **Fill holes**.

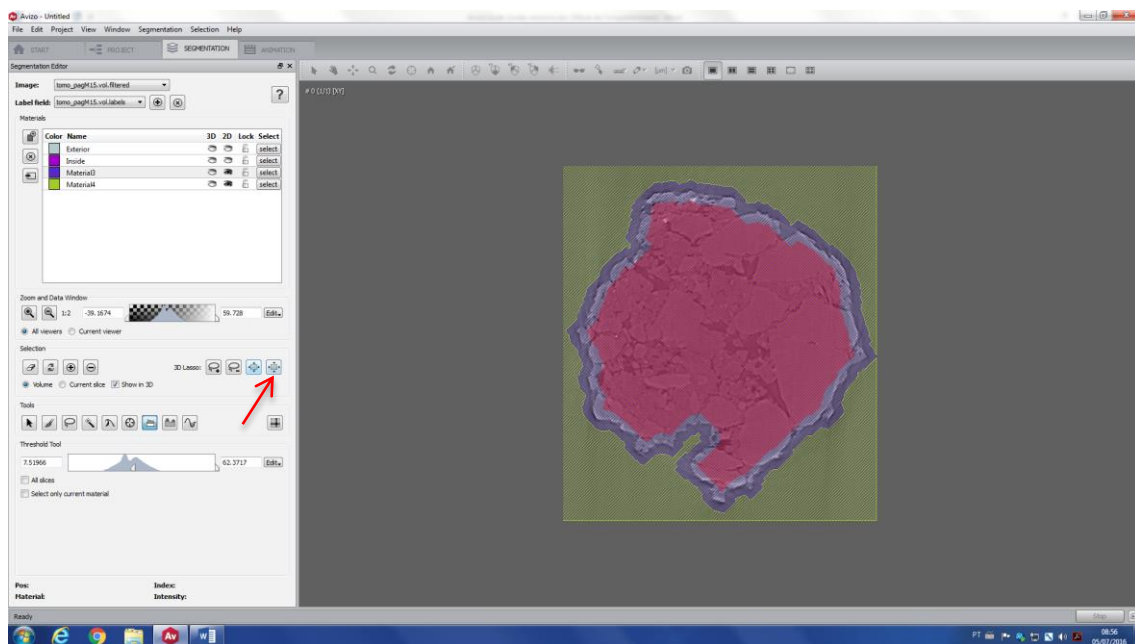
Select (red arrow) the Exterior label and add this to a new label field (in this case, Material4).



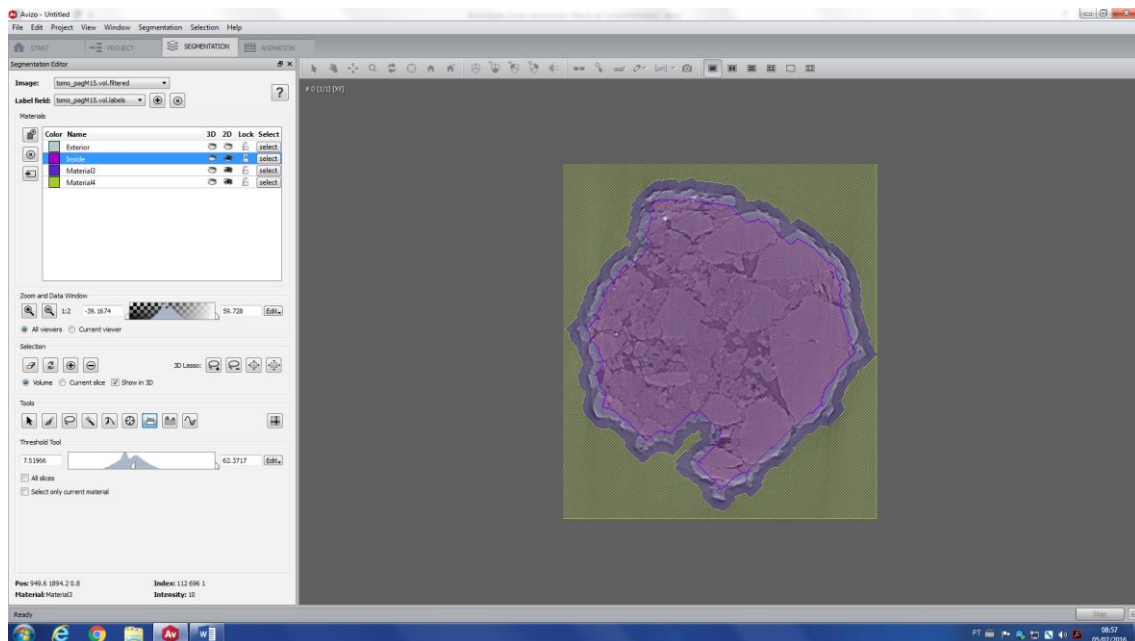
Select Material3 (the selected area will turn red).



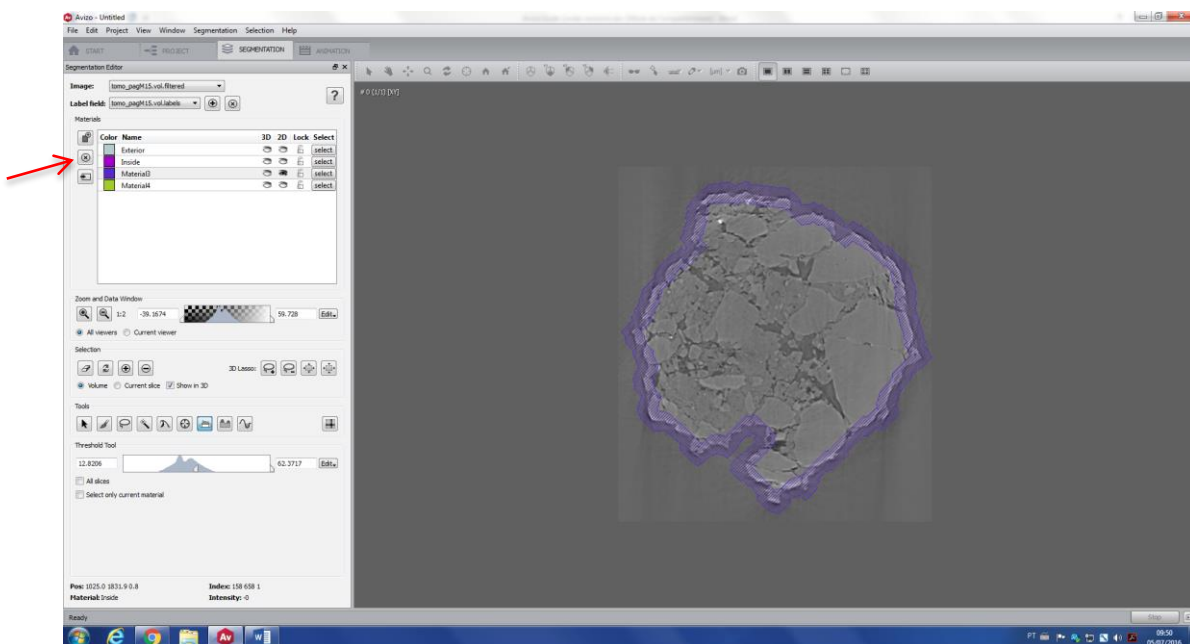
Start shrinking (red arrow) the region and stop when the red area is inside your sample.



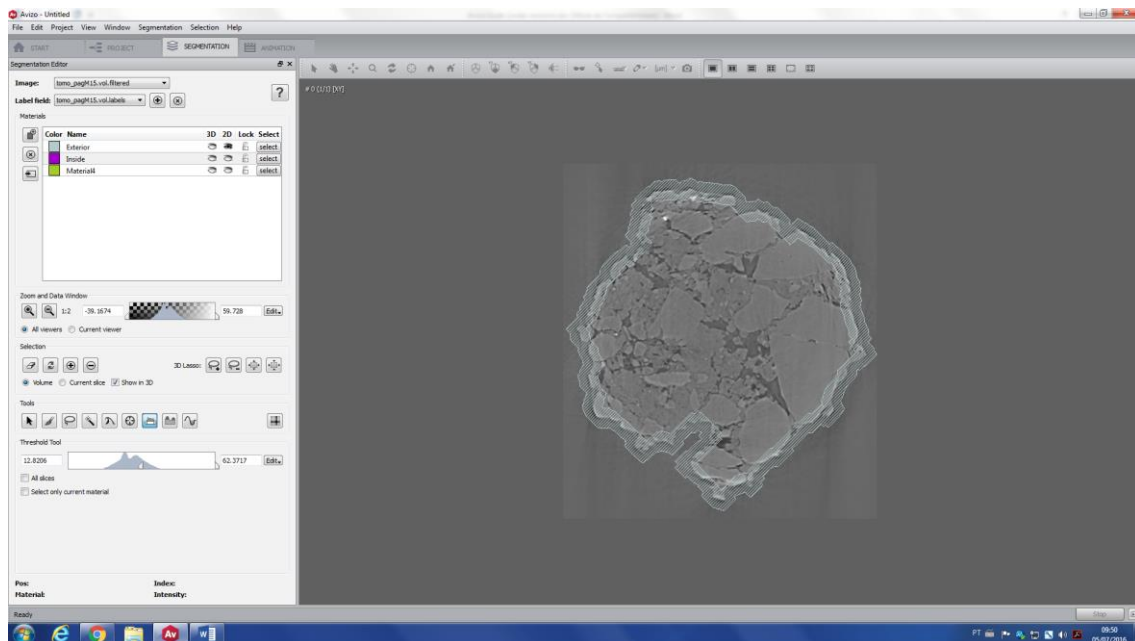
Add the selection to the Inside label. Now the whole image is divided into three different labels: *Inside label* has the seed of you sample (which also contains the internal pores); *Material3* contains the border of the sample and *Material4* has the seed for the background.



Delete Material3 (that represents the border) by pressing delete or clicking on the *Remove the selected material* (red arrow).

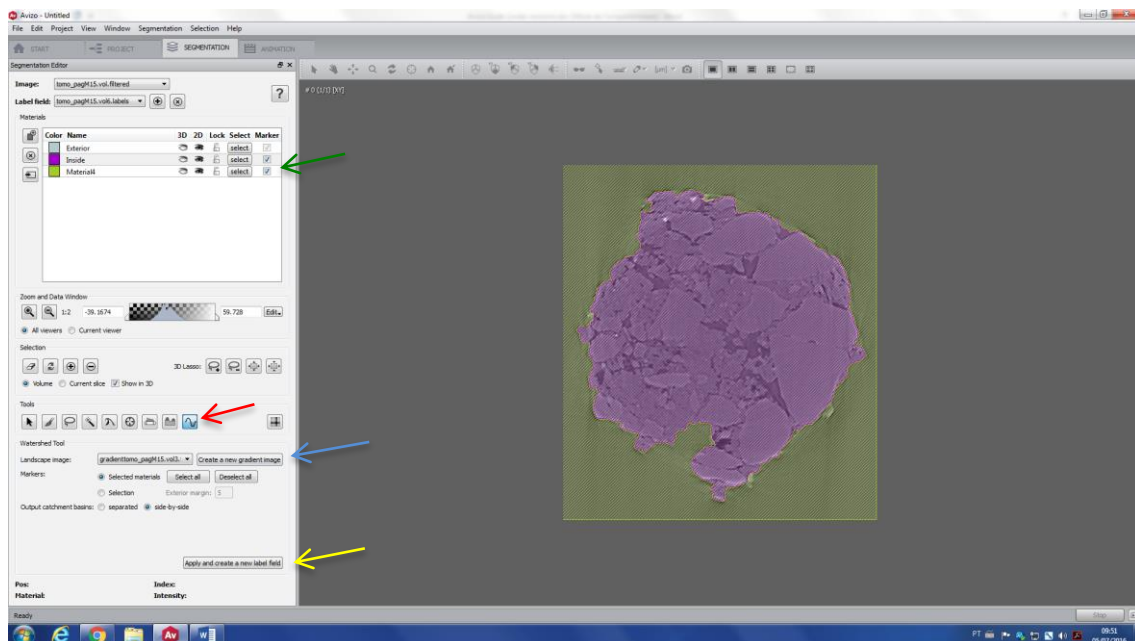


All the voxels of this material will be moved to the Exterior label.



Go to watershed tool (red arrow), be sure that *Inside* and *Material4* are marked (green arrow), create a *New gradient image* (blue arrow) and then click on *Apply and create a new label field* (yellow arrow).

The final result is showed bellow. The background is in yellow (*Material4* label) and the sample is in purple (*Inside* label). Check all slices and if you do not have a satisfactory result, you have to restart the process taking more care with the seeds selection, growing and shrinking processes.

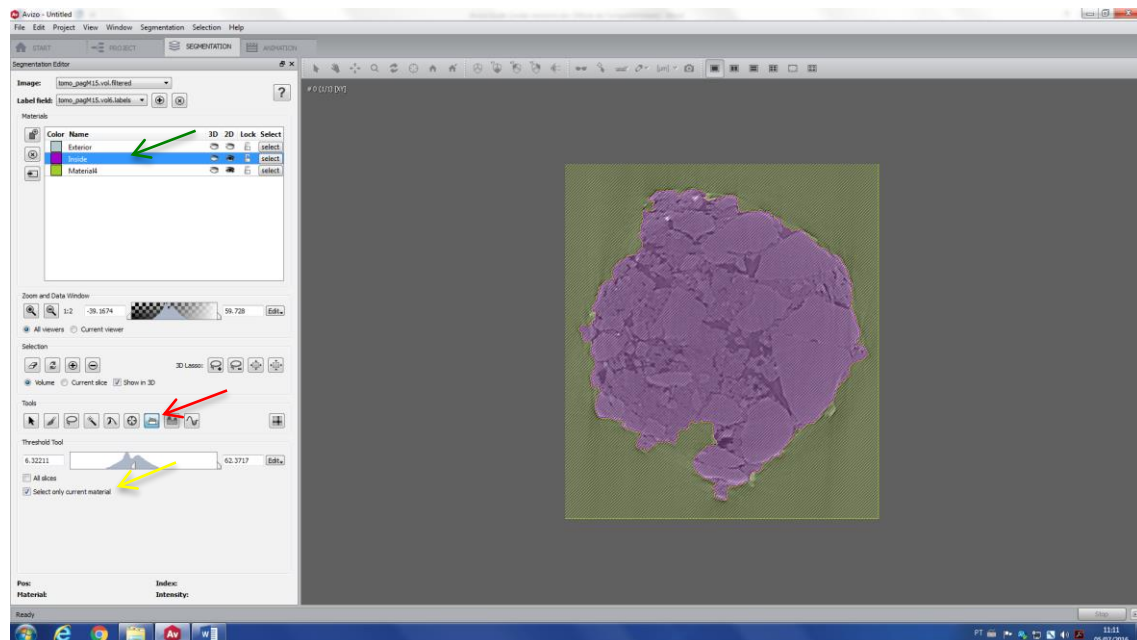


ii. Segmenting your image using a mask

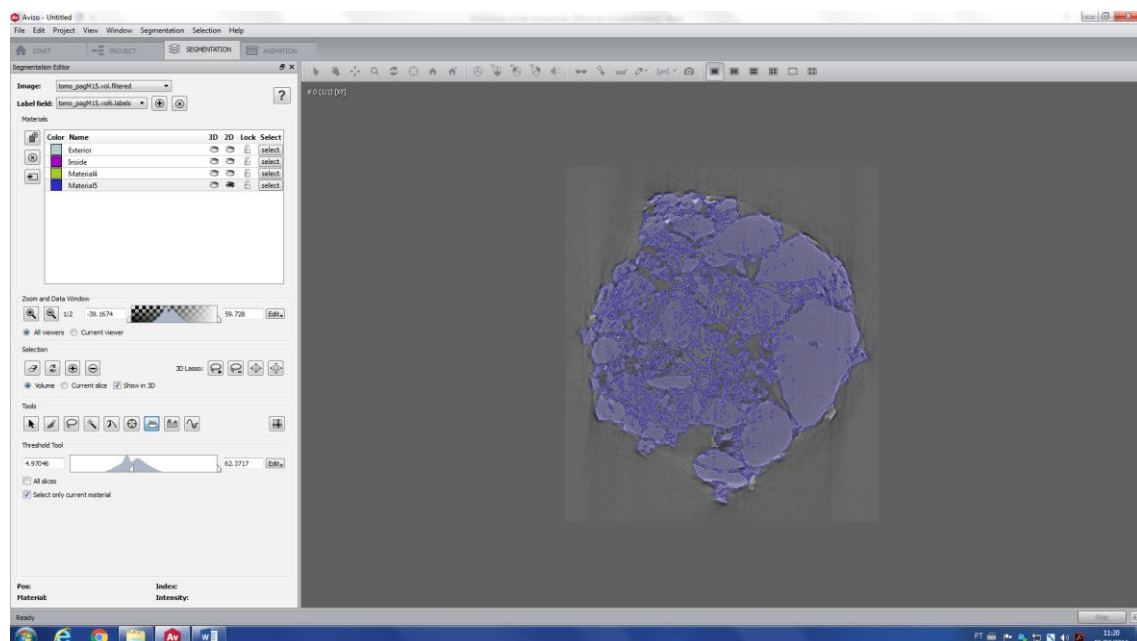
Section IX. i showed how to separate the image of your sample from the background, i.e. how to create a label (or mask) for each region. After this, it is possible to segment the sample using this mask. It is very useful, especially when you want to segment the pore space, which has the same gray level of the exterior (background).

For this example, let's use the image of the previews section. The idea is to separate the pore space from the matrix. For this, let's will use the *Watershed tool* again (but you can use any other segmentation tool).

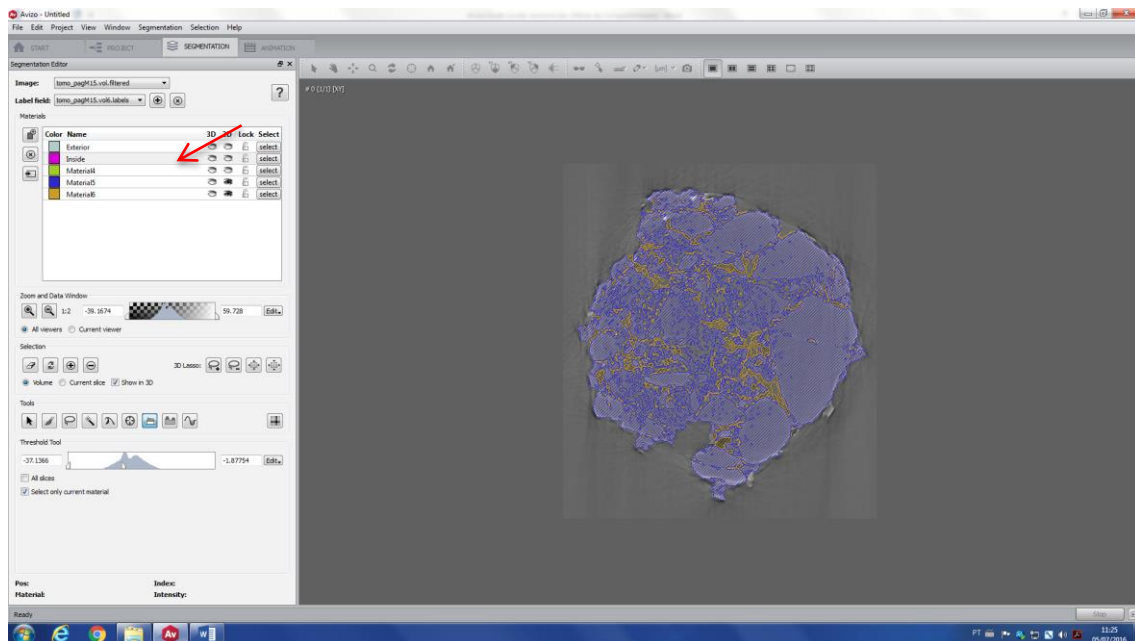
Go to threshold tool (red arrow), click on *Inside label* (green arrow) and then, *Select only current material* (yellow arrow). This way you are going to use the Threshold tool only on the selected material, in this case, the purple region.



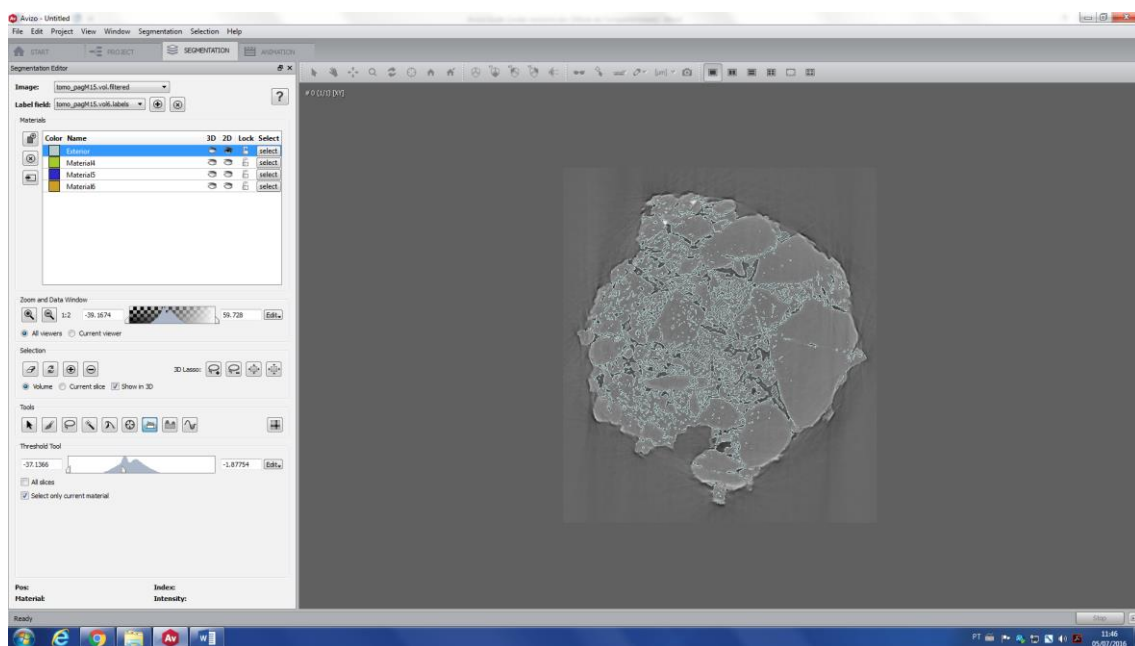
Select the seeds of the matrix. Make sure that there are no matrix seeds within the pore space. Add this selection to a new material (in this case, Material5).



Click on *Inside label* (red arrow) again and create the pore space seeds using the threshold tool.

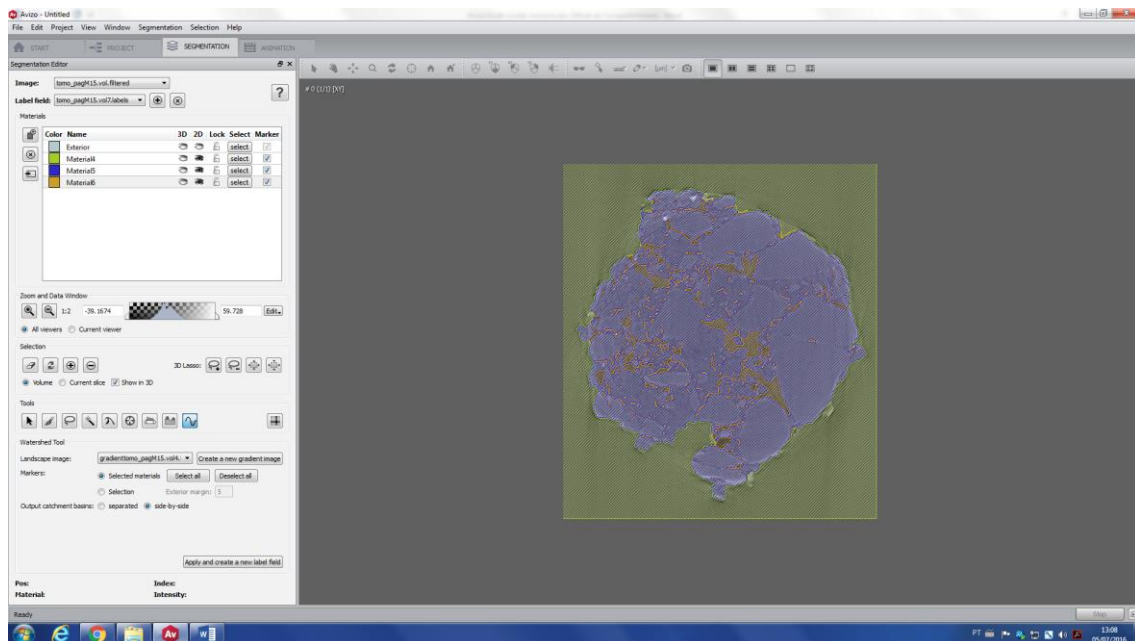


Move all the voxels within the *Inside* label to the *Exterior* label.

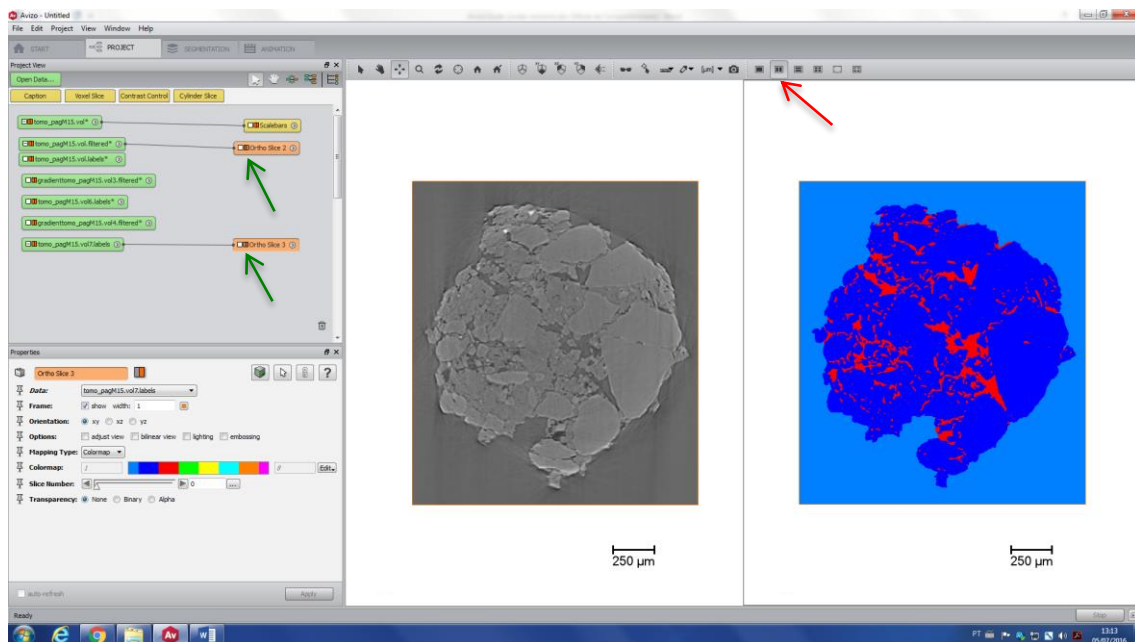


Go to *Watershed Tool*, be sure that Material4, Material5 and Material6 are selected, click *Create new gradient image* and then, click on *Apply and create a new label image*.

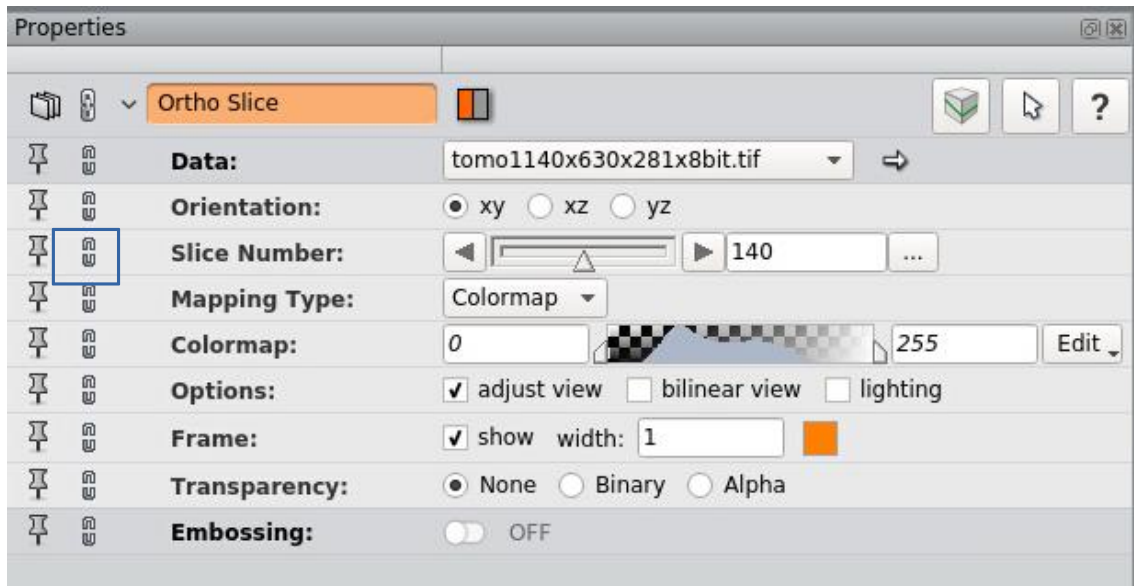
The final result is shown below.



To check if the segmentation is good, you can compare the original and segmented image, side by side. Go to *Project*, you will see that there are three new *.labels files. The last one is your final segmented image – create an orthoslice for this. Click on *Two Viewers* (red arrow), and choose the image you want to show by hiding/showing the orthoslice (green arrow).



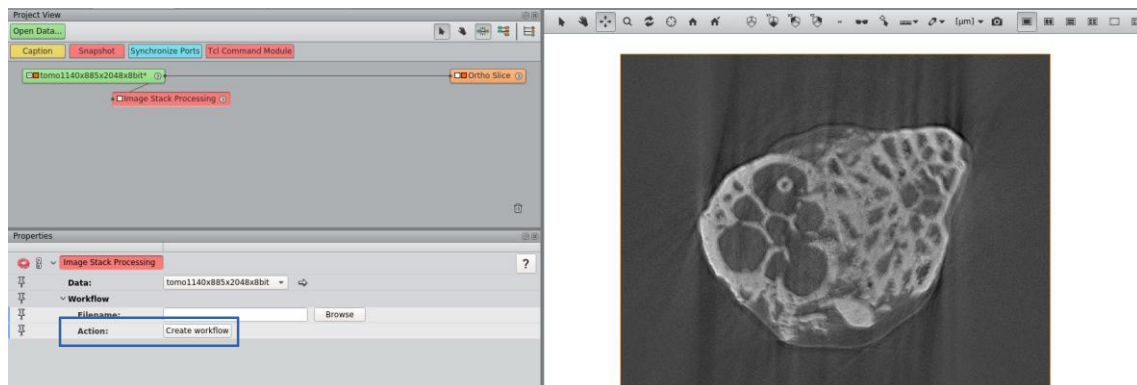
To make the two orthoslices move together, click on the orthoslice and then on the clip at slice number section (blue rectangle). Drag this clip to the other orthoslice.



5. Image Stack Processing (2D Recipes)

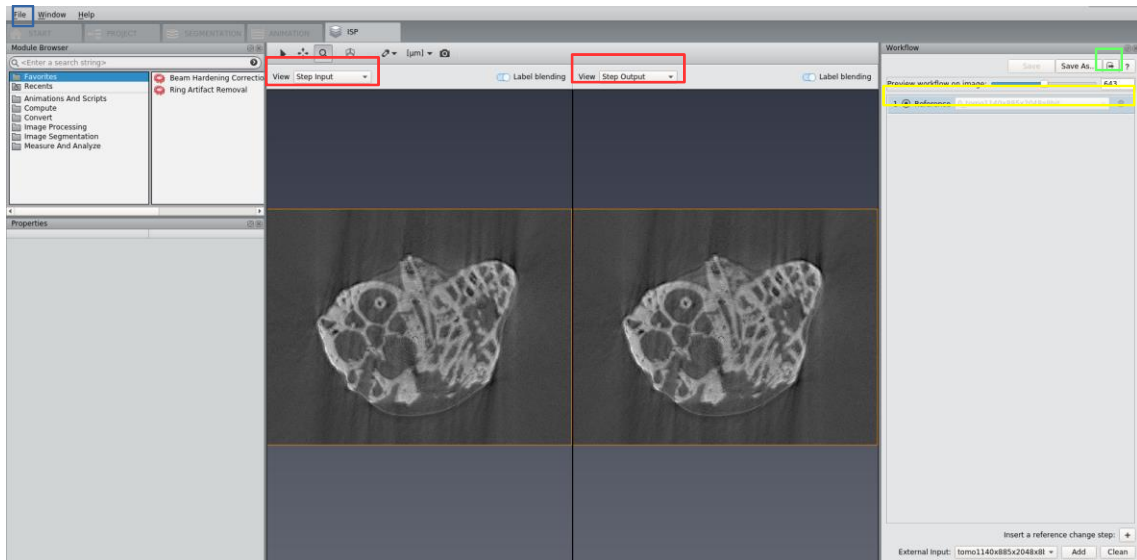
a. Workroom

This is a new feature that allows an image processing workflow for 2D data. It gives you tools available for **preprocessing** and **segmentation**. The creation of a workflow is good to automatize image processing if your samples need the same applications of features. Right click at your data and search for **Image Stack Processing**. Then, select creation workflow (blue rectangle).



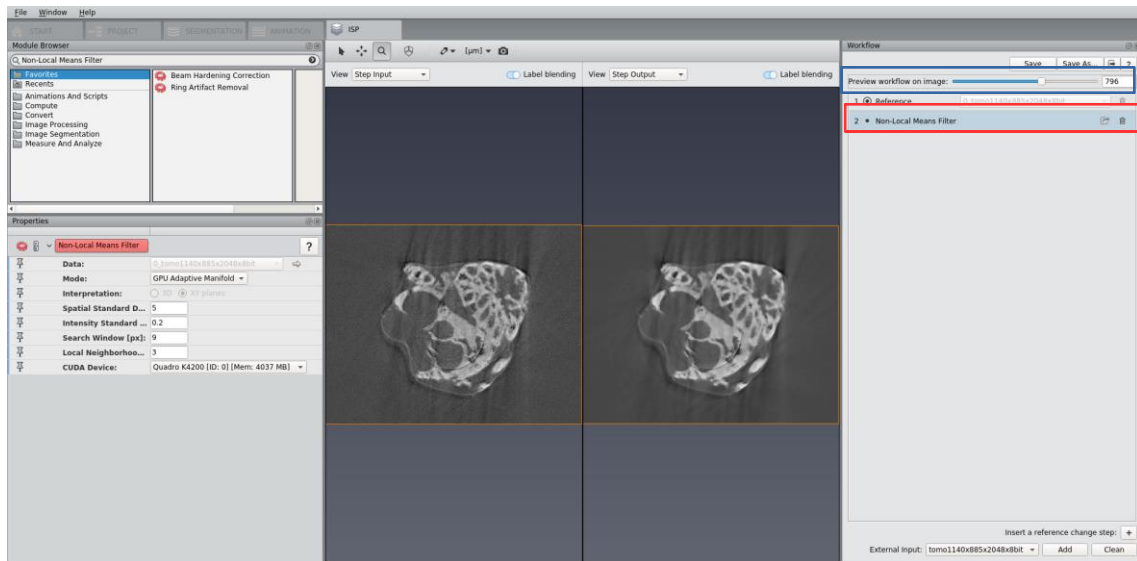
At the workspace you'll see that the "reference" is the input data. There is **two views** (red rectangle) and you can adjust to see **step input/step output/workflow input/workflow output**. There is a search box at left where you can find the tools and at right is the linear workflow. If you want to leave the Image Stack Processing, press **Quit** (green rectangle). If you press Quit in File (top left, blue rectangle) you'll leave the program.

At **reference** (yellow rectangle) there is the main data, which means that every tool selected will automatically connect to the previous output. If you want to connect with a different data or output, then it's necessary to change the reference (this will be explained later).

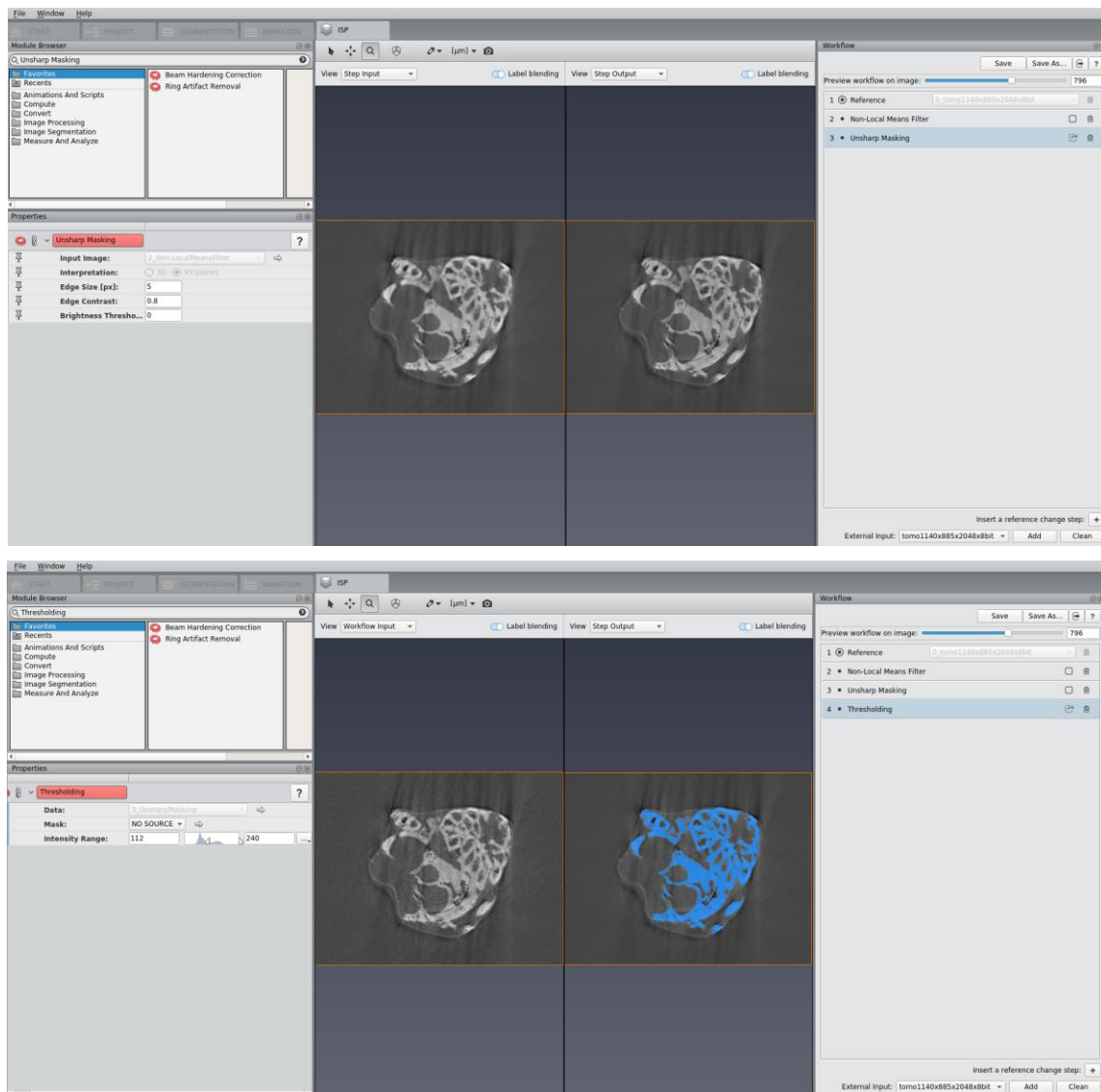


b. Adding and removing tools

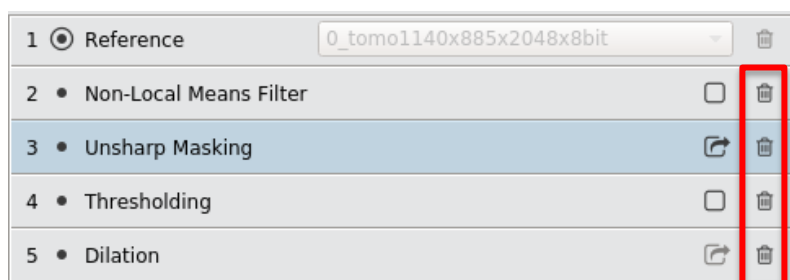
Type in the module browser window the tool that you need (e.g. non-local means filter) and select it. The result image will appear at the right side (step output) and the previous will appear at the left (step input). Notice that this step appears as 2 at the workflow section (red rectangle). You can move through the slices at Preview workflow on image (blue rectangle).



Now, apply **Unsharp Masking** and then **Thresholding**. As you can see, every tool added is inserted below the selected step.

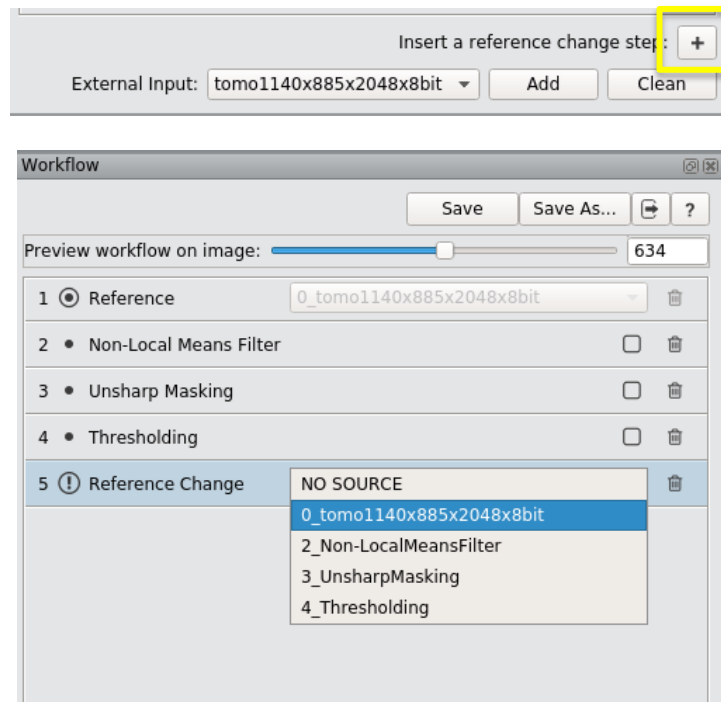


To remove a step, simply press the **Trash** button.

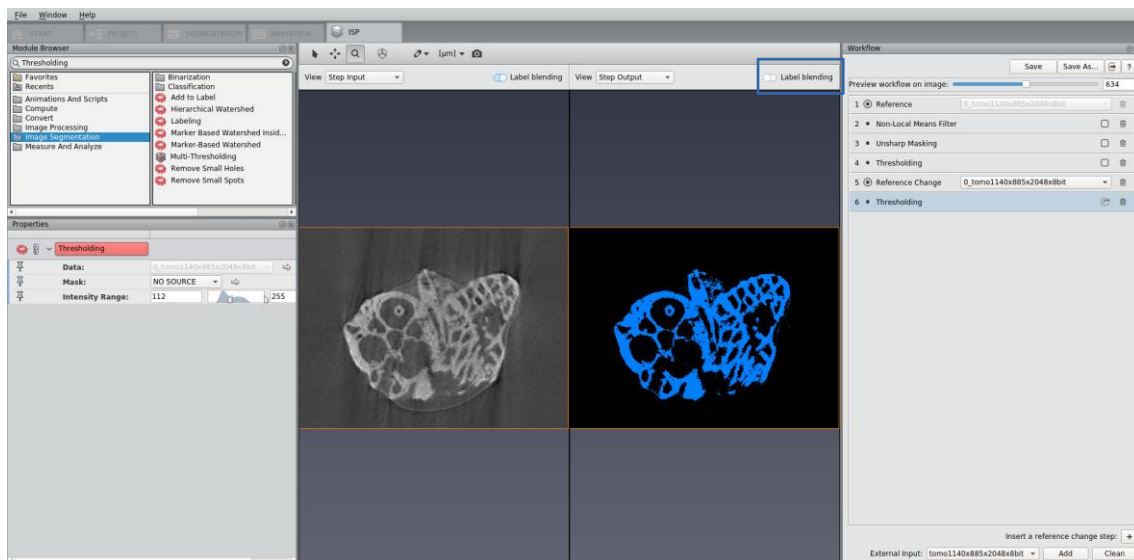


c. Changing the reference

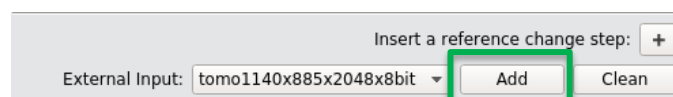
The selected tool automatically connects to the preceding output, to change that you need to click “Insert a reference change step” at the end of the Workflow (yellow rectangle). For example, we’ll apply Threshold at the original data.



If you disable **Label Blending** (Blue rectangle), the background becomes black instead of the input data.

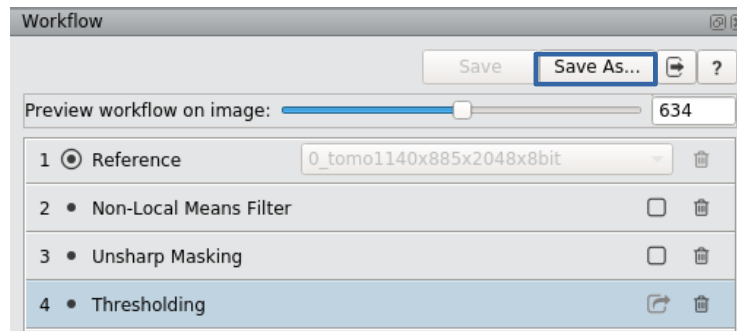


To add an exterior data (green rectangle), it needs to have the same dimension in X, Y and Z axis.

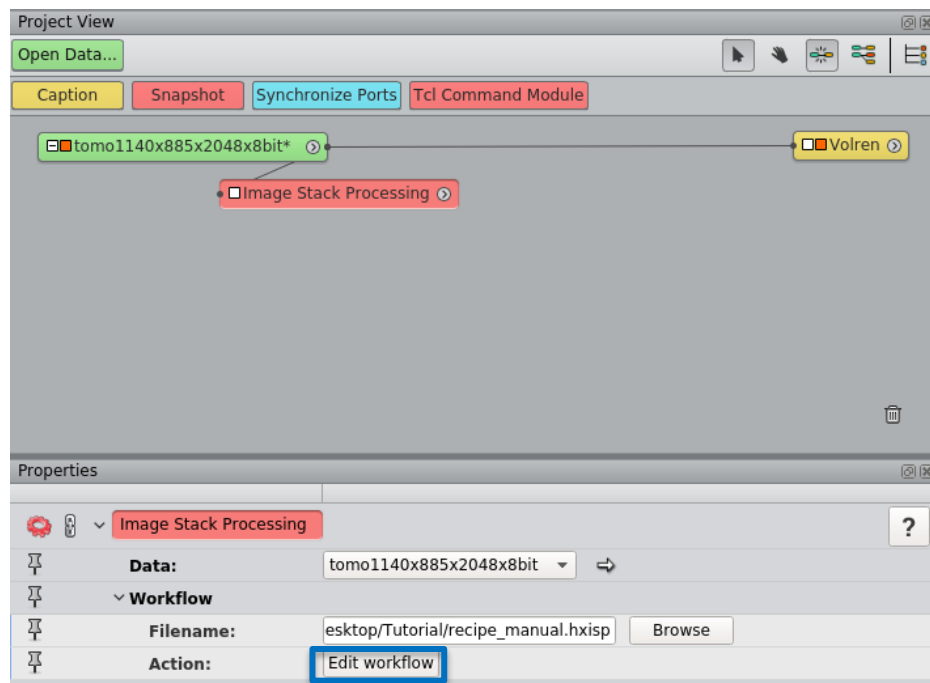


d. How to save and apply to other data

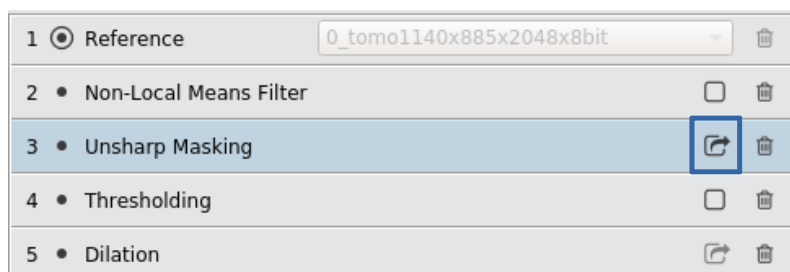
In the Workflow window, select **Save As** (blue rectangle) and then save the Workflow on disk. The file extension is .hxisp.

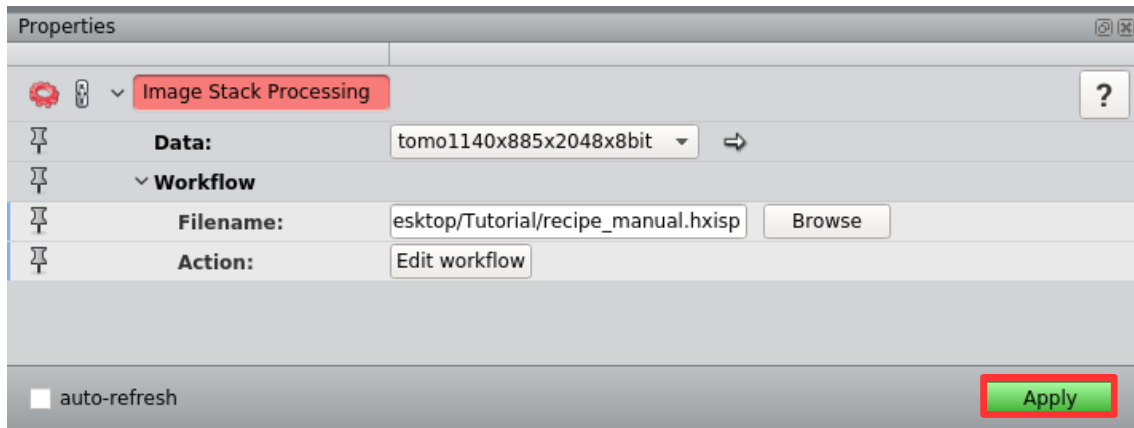


To edit the recipe later, after attaching the Image Stack Processing module, browse the name of the recipe and click **edit workflow** (blue rectangle).



By default, only the last step of the workflow is exported. To export additional data of the select output icon. After you select, **Quit** and select **Apply**.
















6. Didactic Images

a. Setting the space

At the section 2.c is the description of how to apply scalebar and alter the color of background. Now here are other options to improve your working space.

Click on Edit > Preferences > Layout dialog. In there you can control the visibility, auto-hide option, and position of the **trackball** and the **compass**. There are four options of compass available.

In order to decide the position, the toolbar of the main viewer windows provides several buttons and controls. The main ones are:

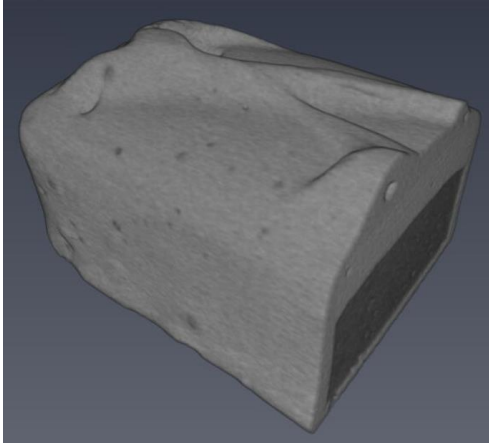
-  Interact: Changes to interaction mode.
-  Trackball: Changes to viewing mode. Click at the left mouse button to alter the view direction.
-  Translate: Use the left mouse button to activate the translation.
-  Zoom: The vertical motion of the left mouse button controls the zoom.
-  Rotate: Rotates the camera following a clockwise rotation of one degree. [SHIFT]+clicking - 90 degree rotation. [CTRL]+clicking - counterclockwise rotation.
-  Seek: Seek button+clicking on an arbitrary object - It moves the object into the center of the viewer window.
-  Home: Resets the camera to the home position.
-  Set Home: Sets the current position as the new home position.
-  View All: Makes all objects visible.
-  XY-, XZ- and YZ-Views: Moves the camera perpendicular to the specified coordinate plane.[SHIFT] – Changes to the opposite view direction.
-  Perspective/Ortho: Toggles between a perspective and an orthographic camera.

b. 3D Rendering

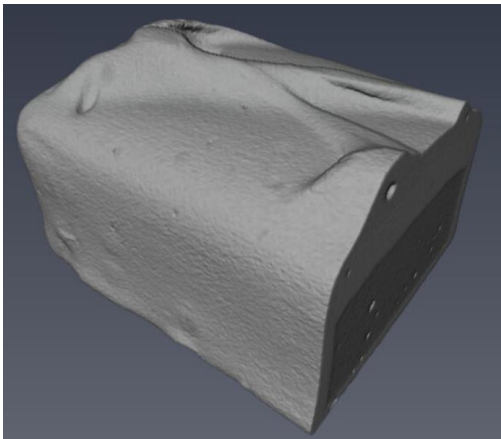
Once your space is settled, it's necessary to give dimension to your data. The next option are 3D rendering that are applied by right clicking on the data (e.g., .raw) and searching

their names at the search box. The advantage of this tools are that they don't need segmentation to generate the surface.

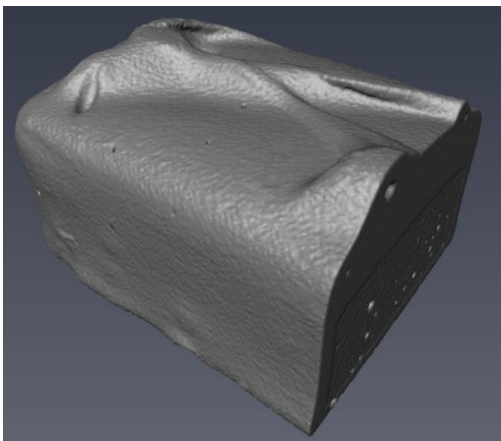
Volren: It has less features available but allows a faster manipulation (if a slowdown occur, select the option LowRes only).



Volume Rendering: This is a richer renderization. It provides different lighting and shade effects options. The Help at Avizo provides a Table that compares the features available in Voltex, Volren and Volume Rendering.

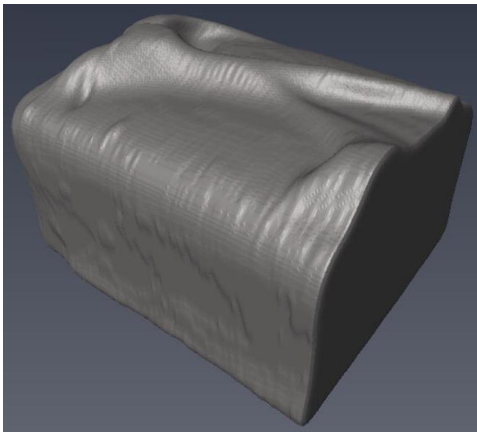
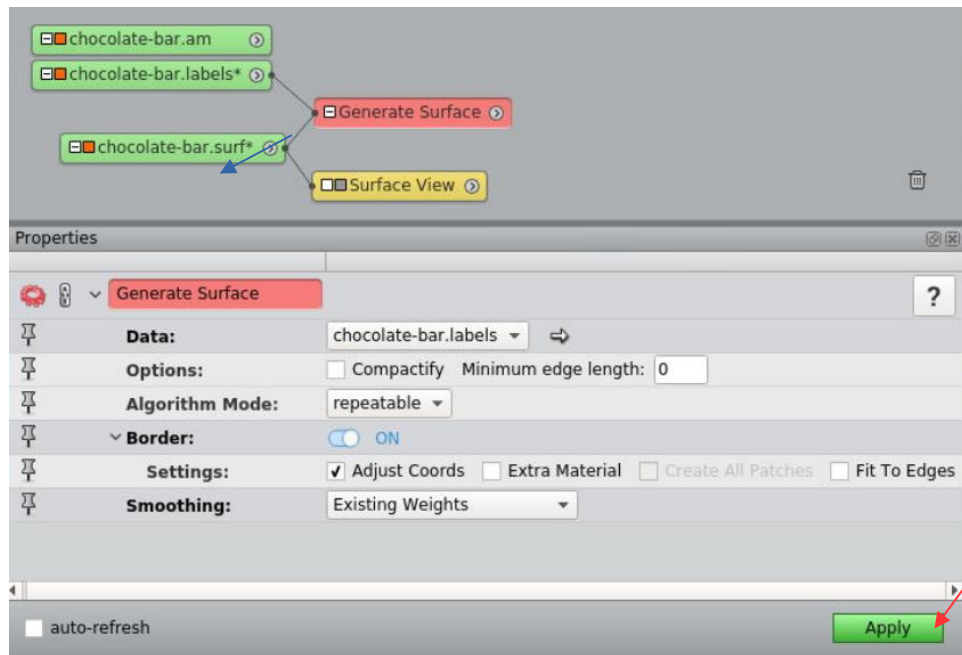


Isosurface: The main difference between this one and volren or volume rendering is that isosurface renders just the surface pixels (not whole volume). It creates a less pixelated image, but it also means that it demands much more graphic resources (significant slowdown can happen).

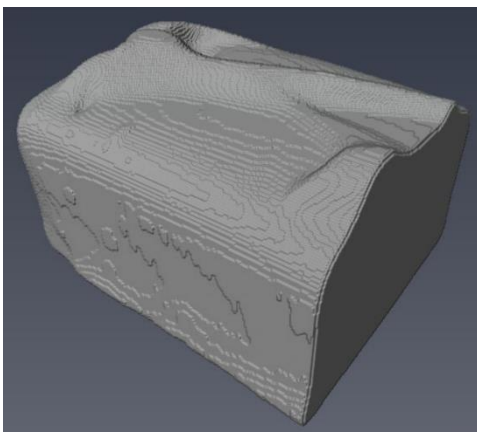


c. Label Rendering

Generate surface and Surface view: In this case, right click at the label, type **Generate Surface** at the search box and click on it. After, apply (red arrow), That will create a .surf file (blue arrow). In order to visualize it, right click and select **Surface View** .



Voltex: As this module is deprecated, it's better to use Volume Rendering instead.

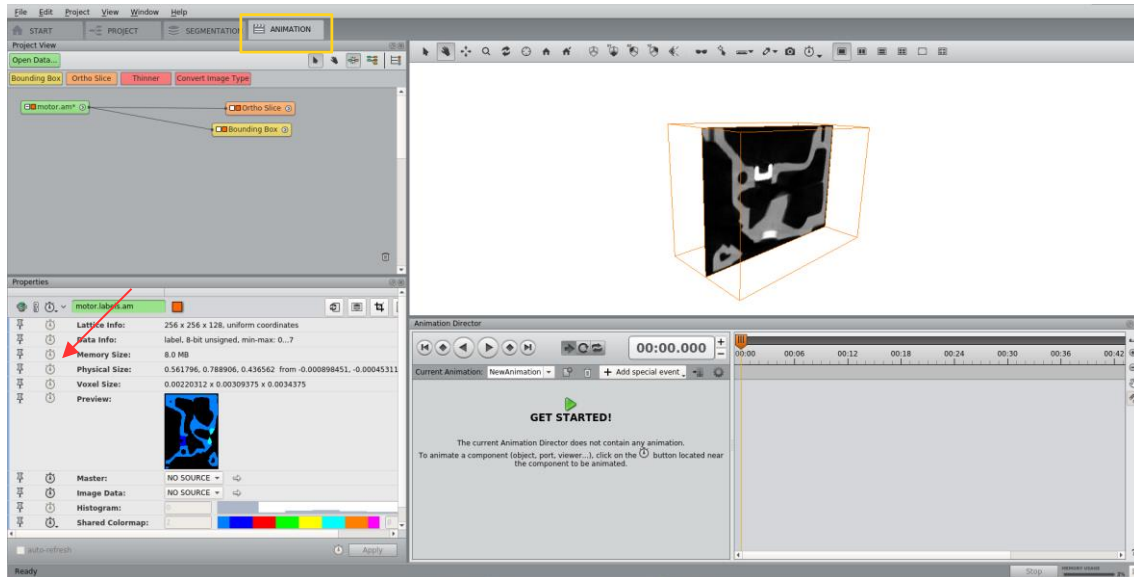


7. Animations

a. Orthoslice

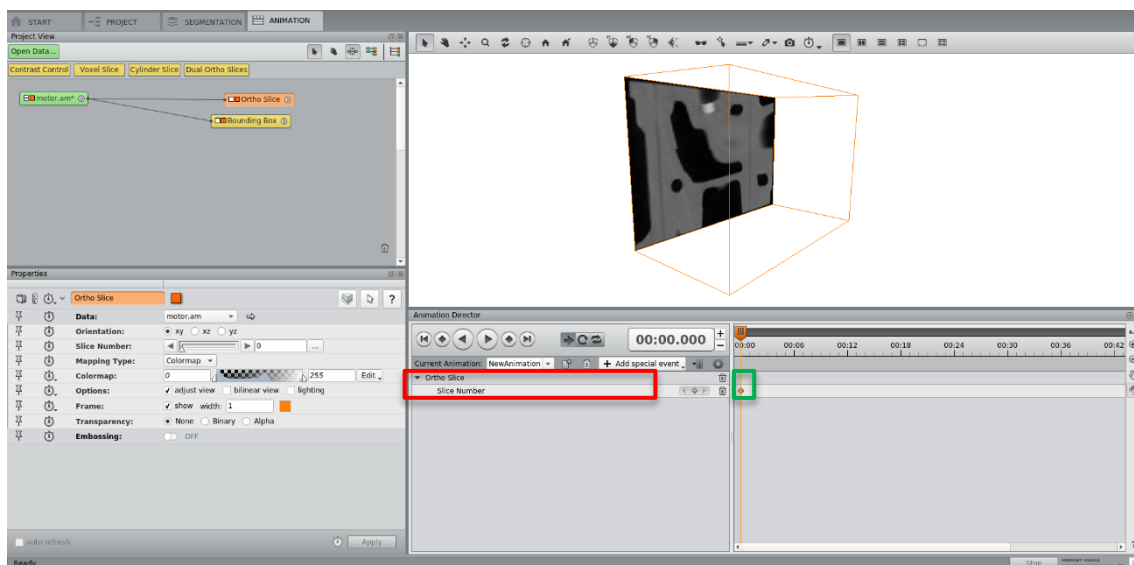
To help the visualization, attach a **bounding box** and **orthoslice** module to the data (right click and then type in the search area).

To start an animation, enter on the **Animation Director** (yellow rectangle). Notice that all properties have a stopwatch button on the left side (red arrow).

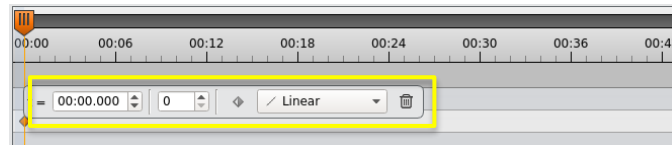


The first step is the animation of the orthoslice position. Click on the **stopwatch button** of the Slice Number section (blue rectangle) with a slice of your choice to start (this image has 127 slices, in the example we'll start with 0).

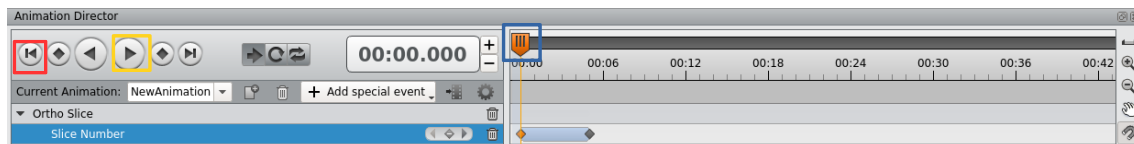
It will create a new event in the left side of the Animation interface (red rectangle) and a new **orange diamond** on the right side (green rectangle).



If you need to adjust the time, it's possible to just drag the diamond to the new position or put the mouse on this button and type the desired time (yellow rectangle).

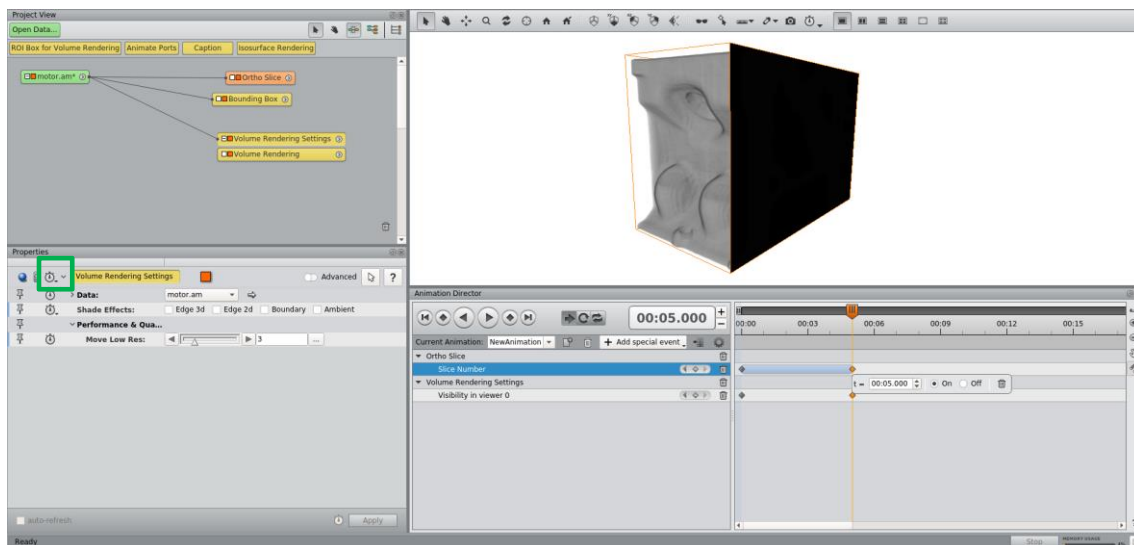


To finish the animation, drag the **master time slider** (blue rectangle) to proper time (in this case, 00:05:00), adjust the orthoslice to a new position (e.g. 127) and click the stopwatch button again. Click on the **Jump to start** button (red rectangle) and start the animation (click on **Play Forward** button, yellow rectangle). If you want to stop the animation, click on the **Stop** button



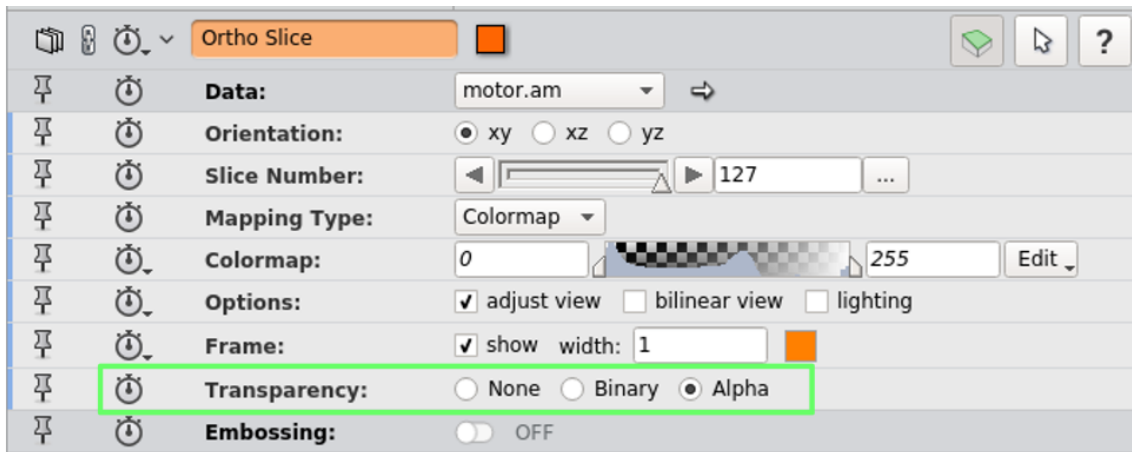
b. 3D rendering and Clipping

Attach the desirable 3D rendering (e.g. volume rendering or surface view) at your data. Move the master time slider to 00:05:00 and then click on the stopwatch button (green rectangle), select **Visibility in Viewer 0**. This option will create a new event, making this new one invisible until the time selected. Make sure the first diamond is off and the second is on.

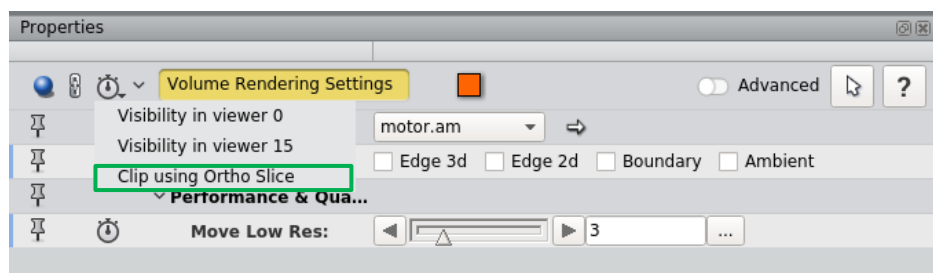


Sometimes can be interesting to show the orthoslice in some transparency and the volume gradually as the orthoslice is moving.

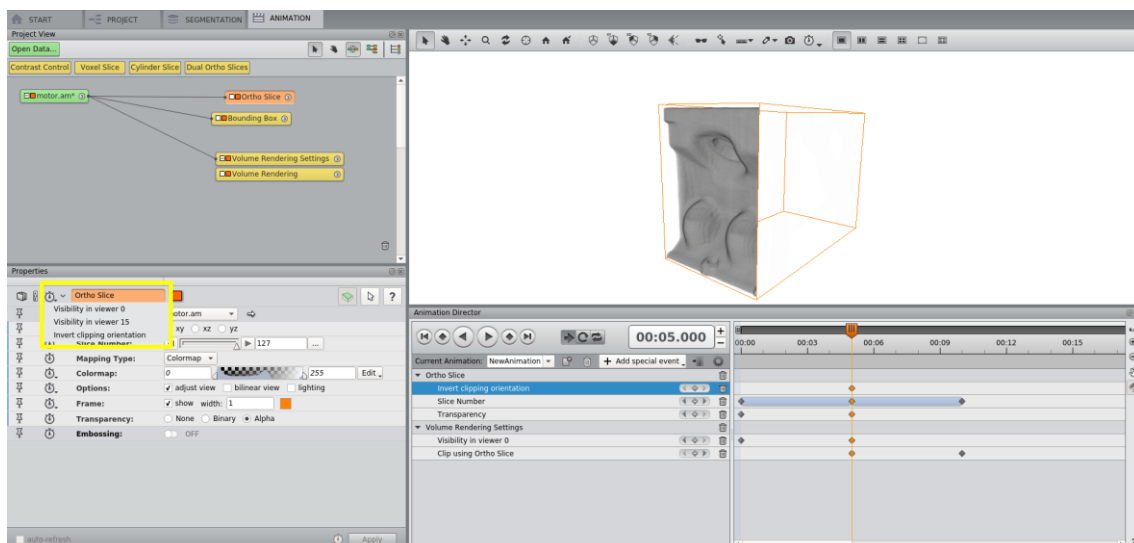
To change the **transparency of your orthoslice**, keep the master time slider to 00:05:00, change the transparency None to Alpha and then click on the stopwatch button next to it (green rectangle). This option will create a new event, make sure the first diamond is None and the second is Alpha.



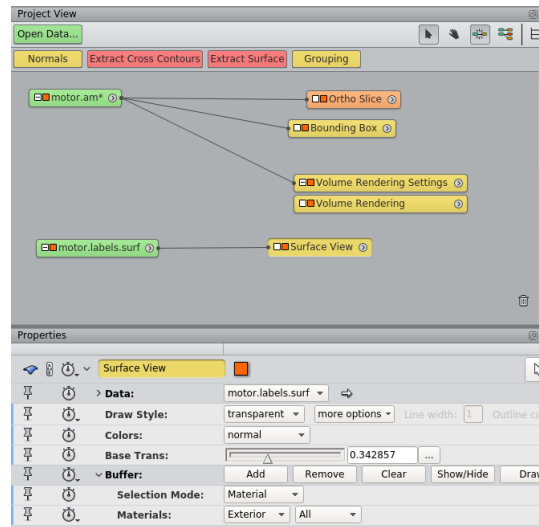
To clip with an orthoslice, first drag the **master time slider** to 00:10:00, adjust the **orthoslice** to a new position (e.g. 0) and click the stopwatch button. After, select the **Volume Rendering** module, and at the same stopwatch button on the top left, select **Clip using orthoslice** (green rectangle). This option will create a new event, make sure the first diamond is at position 00:05:00 and on and the second is at position 00:10:00 and also on.



If you play the animation, you will realize that the orientation of clipping is not desirable. If you are in this situation, drag **master time slider** to 00:10:00 (the time when the clipping starts). Then click at the orthoslice module and select the stopwatch button at the top left (yellow rectangle). Click on **Invert clipping orientation**. Start the animation again and check the results.

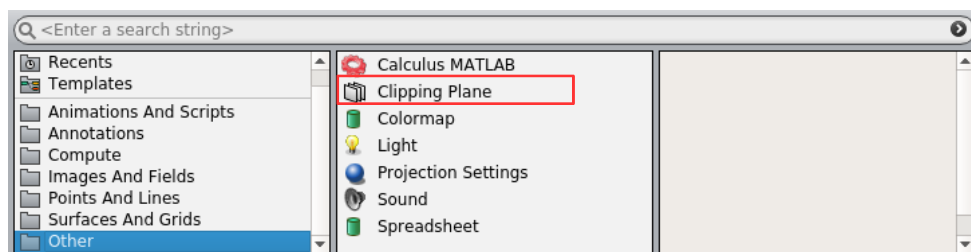


Observation: If you want to clip again at another circumstance, create another Volume Rendering and orthoslice and perform the clipping in the same way as described before.



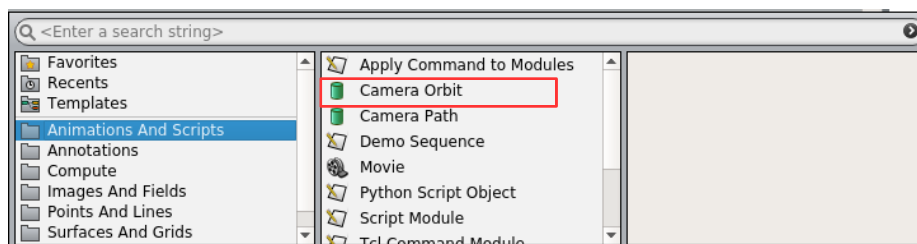
Tip: If you have a label data, right click on it, type **Generate surface** and that will result in a .surf file. Attach a **Surface View** module. Instead of leaving all the material, you can show only the interior materials and show them gradually as explained. For that, click at **Transparent** at Draw Style. In the buffer, select the materials you want to delete (e.g. exterior) and all (red rectangle); after select remove. Then proceed with the clipping.

Tip 2: If you want to clip without an orthoslice, right click in the project view and create object. Select **Other** and then **Clipping plane**. The clip mechanism is the same as the orthoslice, but instead you change the position at the translate section.

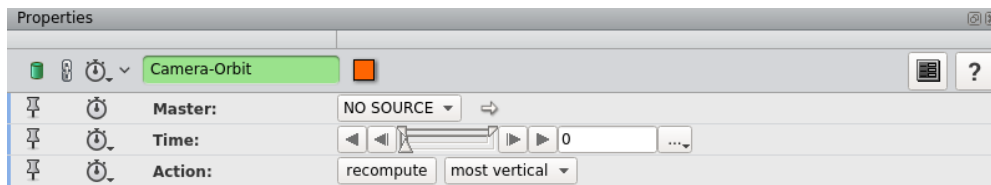


c. Camera Rotation

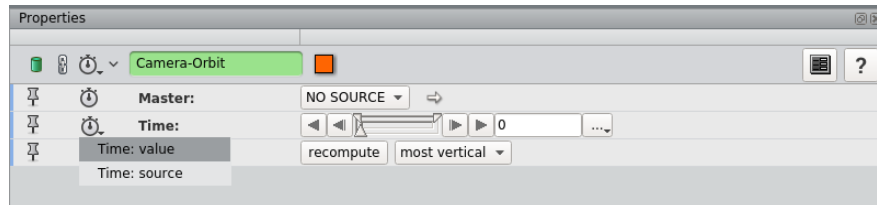
Right click in the project view and create object. Select **Animations and Scripts** and then **Camera orbit**.



The time slider determinates the angle (0 to 360) and action allows the modification of the axis of rotation. You select the axis on the right and then select **recompute**.



Set the master time slider to the time you want (e.g. 00:10:00) and then adjust the angle and the axis. After, select the stopwatch button at time section and click Time: value.

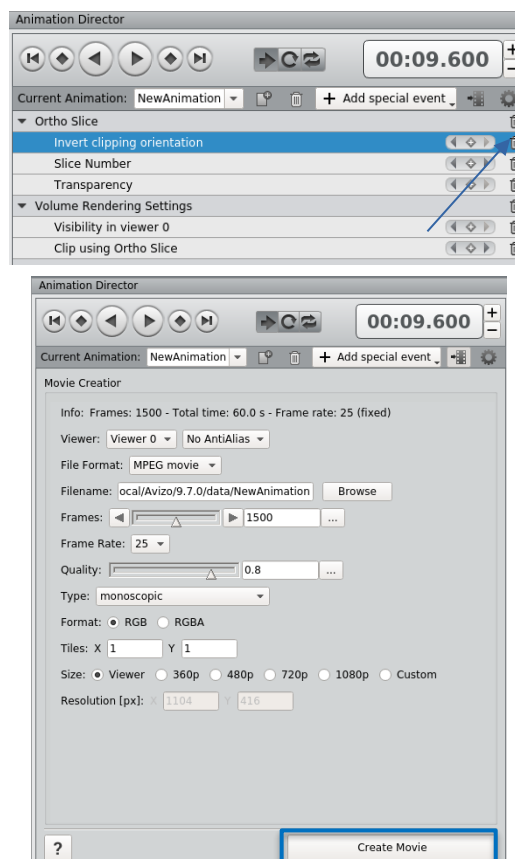


Adjust the master time slider to the ending point, modify the angle and click at the stopwatch button again.

d. Saving and exporting

The animation is automatically saved when the Avizo project is saved. Select **file > Save Project** in the top right of the menu.

To create a movie you need to click on the **Movie Creation Button** (blue arrow). A panel will appear and it permits the adjust of some parameters. Click **create** to export your movie.



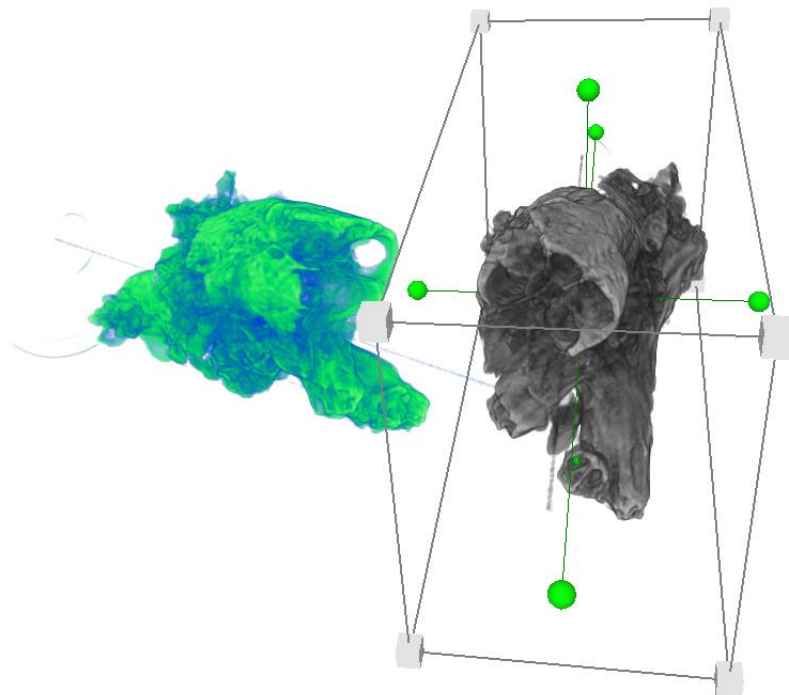
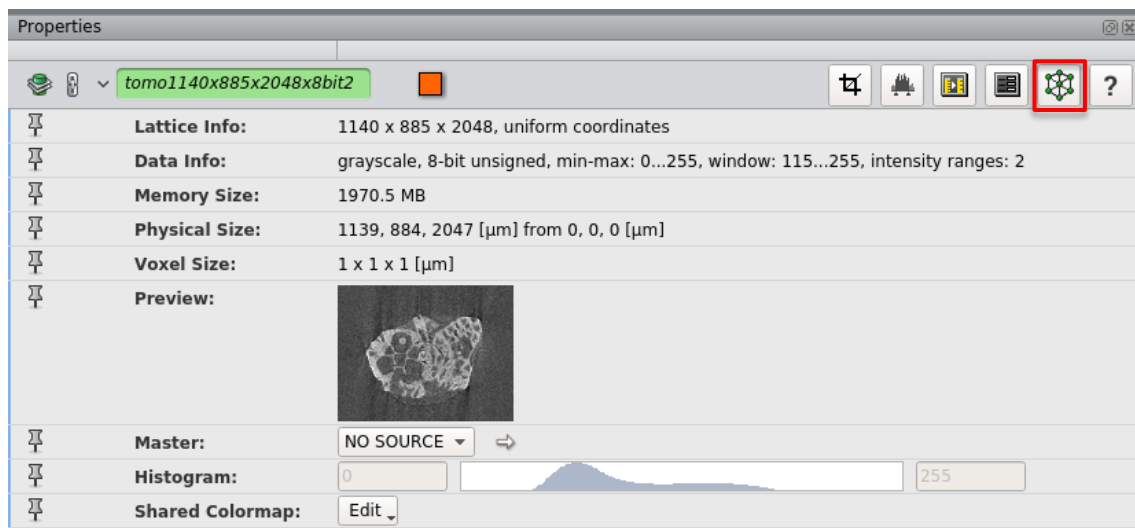
8. Registration of images

The registration of images becomes necessary when you have 2 equal images (or very similar) and you need to adjust one of them to be in the *same coordinate, rotation, translation and/or scale factor* as the other. You can do it manually (Transform Editor) or automatically (Register image).

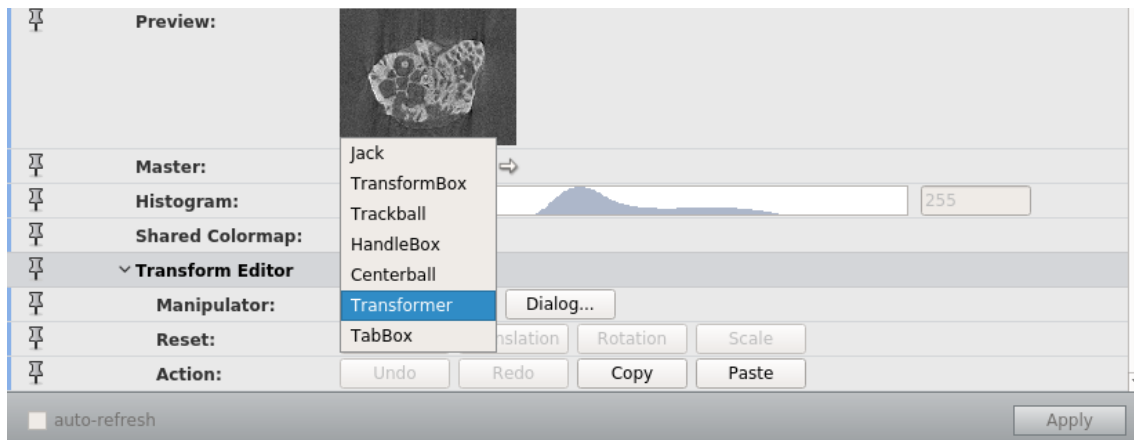
a. Transform Editor

Once you have the two datas at your project view, apply a 3D rendering to make the visualization easier. In this example there is the carbonate rock used in the threshold section with volren attached. When you click in the green module of the data, a properties panel will appear at the left bottom.

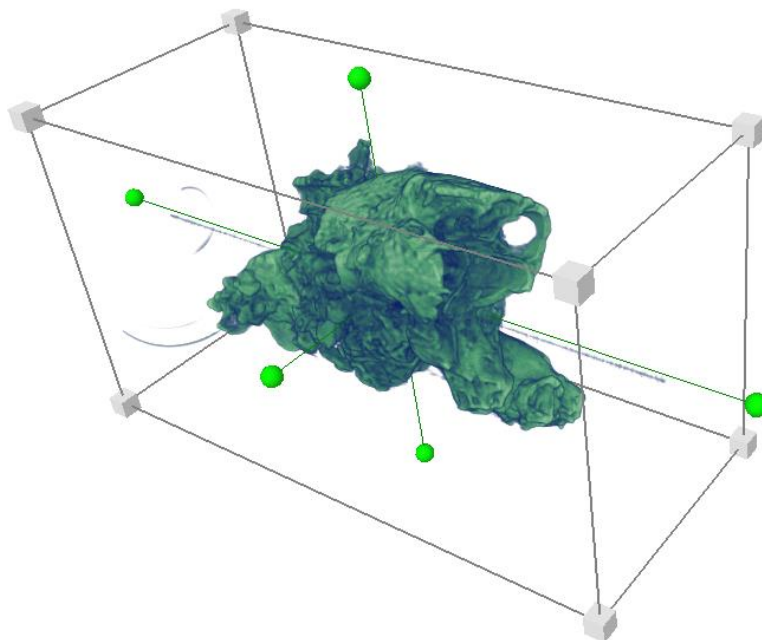
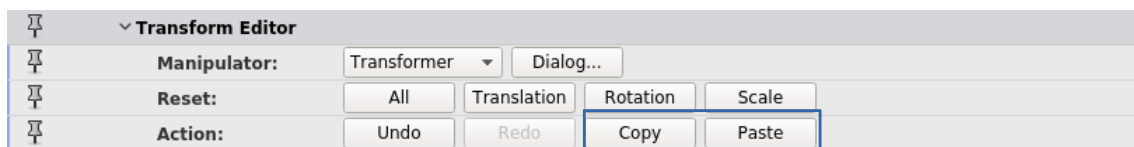
Click on the **Transform Editor Button** (red rectangle). You'll notice a box with white vertex and six green spheres automatically appear.



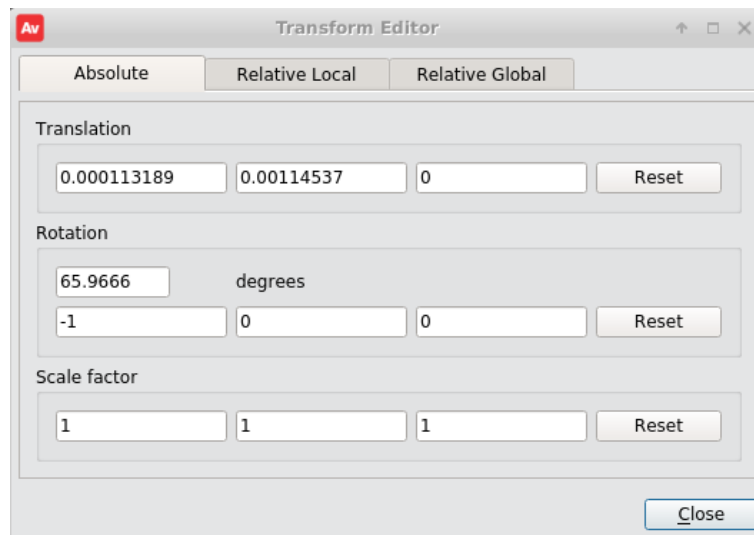
At the Transform Editor section, the manipulator lets you choose between seven types of interactive manipulations (type “**transform manipulators**” at the Avizo User’s guide to see their description). The reset lets you restore transformation, rotation, scale or all. The undo/redo at action allows you to restore one action at time.



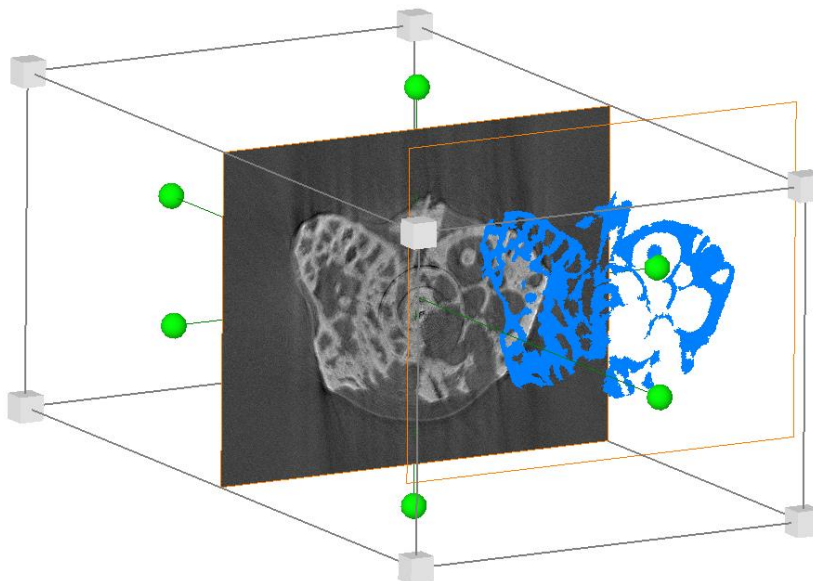
In this example, we altered the green rock and we want to copy that to the gray rock. To copy/paste a transformation/rotation/scale factor you need to click on the **Copy** button (action port, blue rectangle) in the altered data. Activate the transform editor of the other data and press **Paste** button (action port). **Deactivate the Transform Editor** by clicking on its button again.



In this situation we had the transformation to copy. If you don’t have, you can use the option of manipulation to manually adjust your data. In some situations, it’s better to type specific alterations, for that, select **Dialog** (manipulation port) and that will open this table.



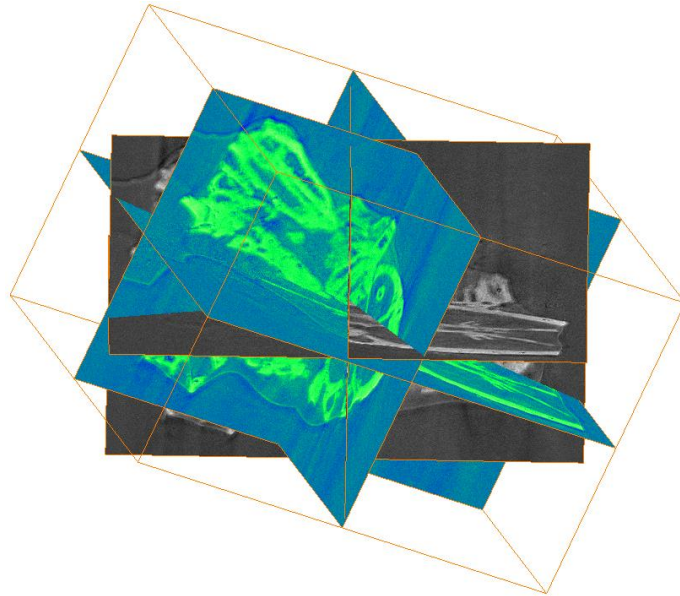
Tip: When you have many measures of the same data, sometimes there will be little changes in the position of the objects from one to another. If you have the segmentation done for one of them, you can use the label as your guide to the alterations. For that, change the transparency of the orthoslice attached to the label from none to alpha and then align manually or from the dialog box the data orthoslice.



b. Register Image

This is the automatic option, but a pre-alignment is necessary if the two object are too far away from each other. In that case, use the Transform Editor to make them as close as possible.

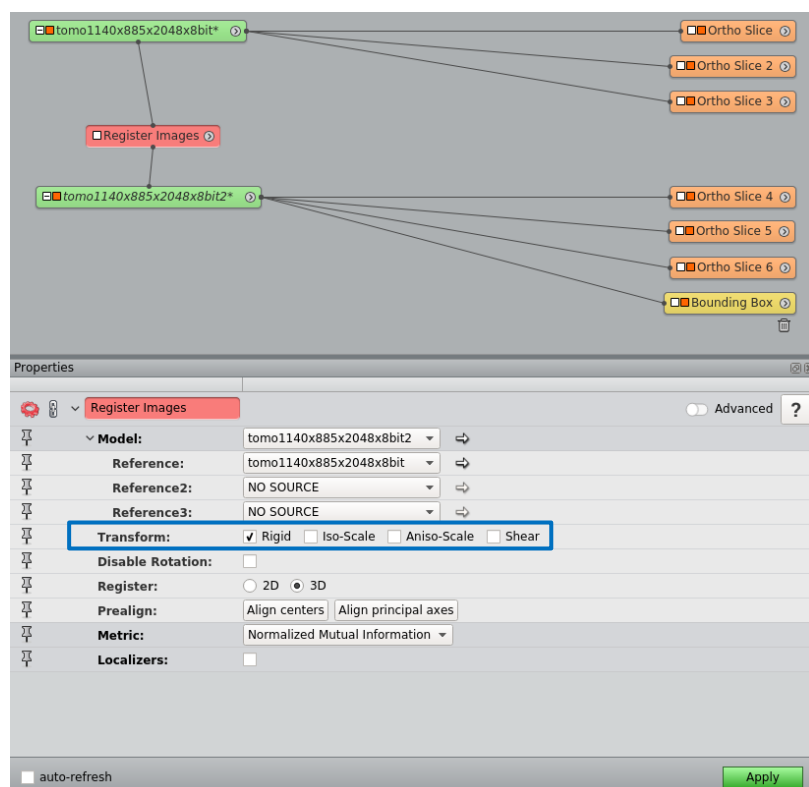
This example uses the same data, but the green data has a rotation different from the gray one.



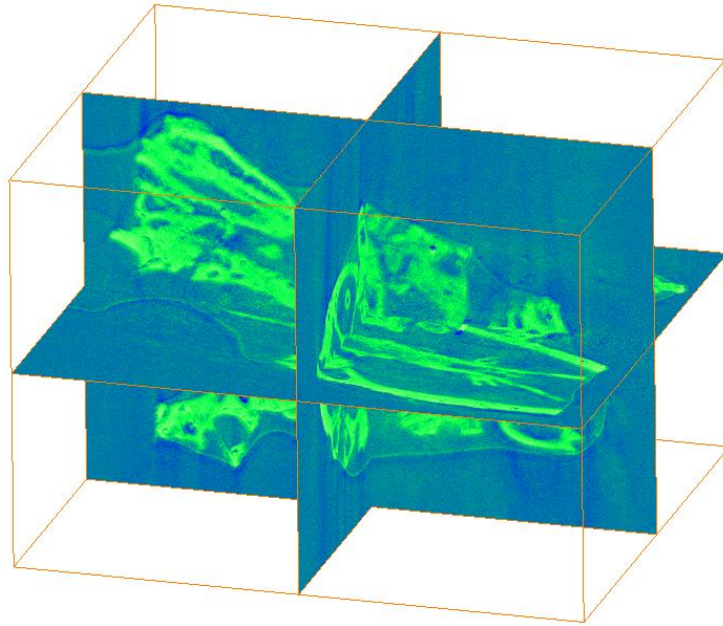
Right click at the data you want to move (e.g. green data) and search for Geometry Transformations > Register Imagens. The Model port is related to the data that will change and the Reference is the data that will remain static. After that, you have to make some decisions about the **Transform port** (blue rectangle).

- *Rigid*: No distortion. Only translation and rotation.
- *Shear*: They are not at the same direction.

After that, you have to use the **Prealign port**. First, press **Align centers** and then **Align Principal Axes**. That will make an optimized registration.



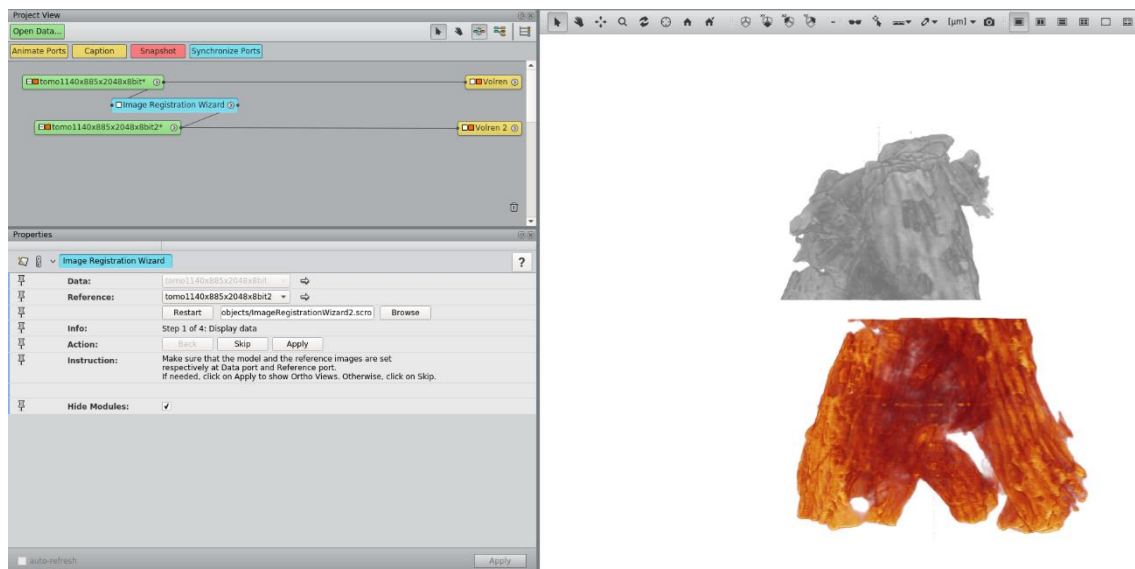
After that, click on the **apply** button. The result image is perfectly aligned.



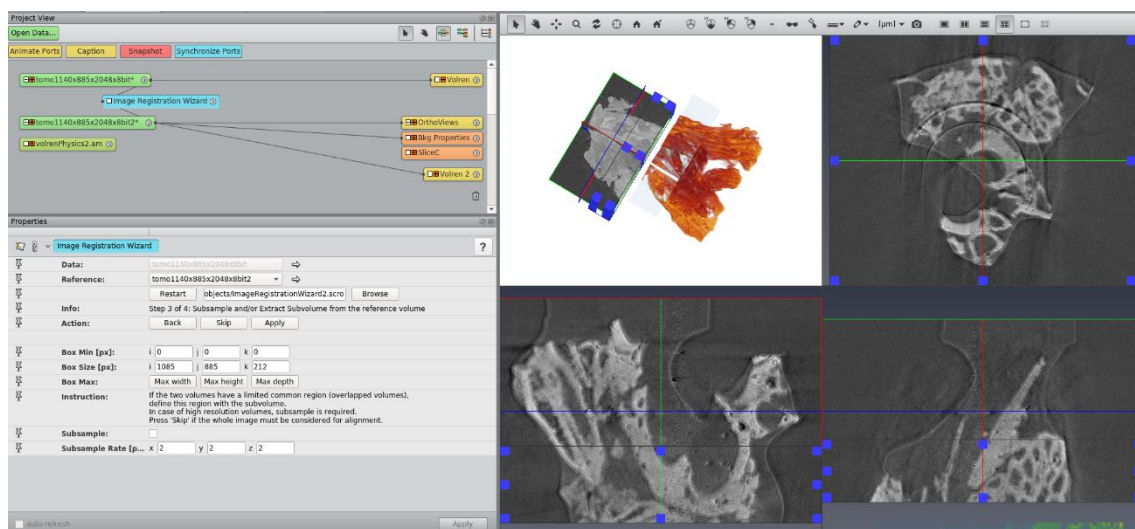
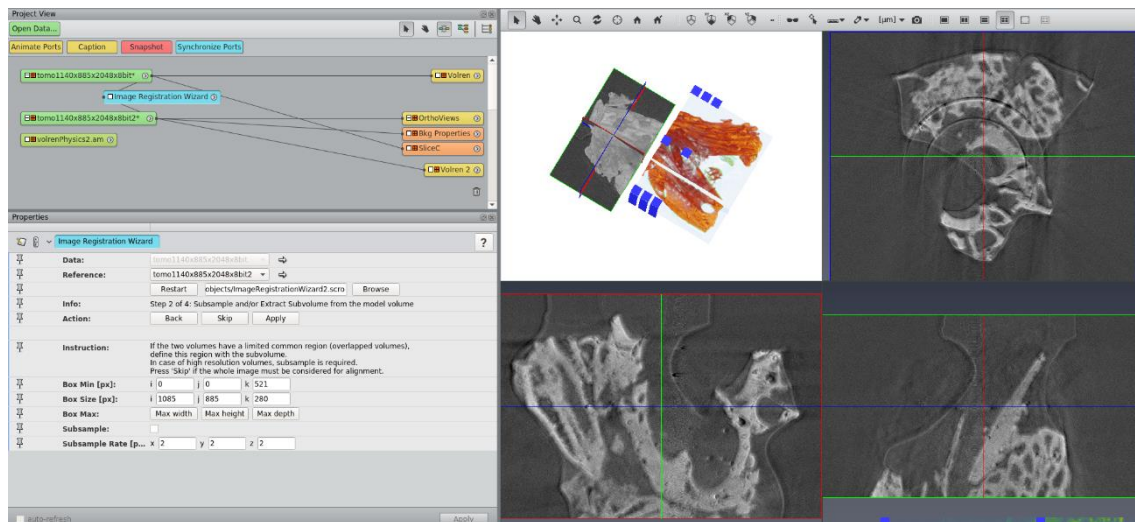
Attention: This is a good tool only to **EQUAL** objects. If your sample is deformed from one to another or have different characteristics, the tool will work poorly. In those situations, use a manually registration with Transform Editor.

c. Image Registration Wizard

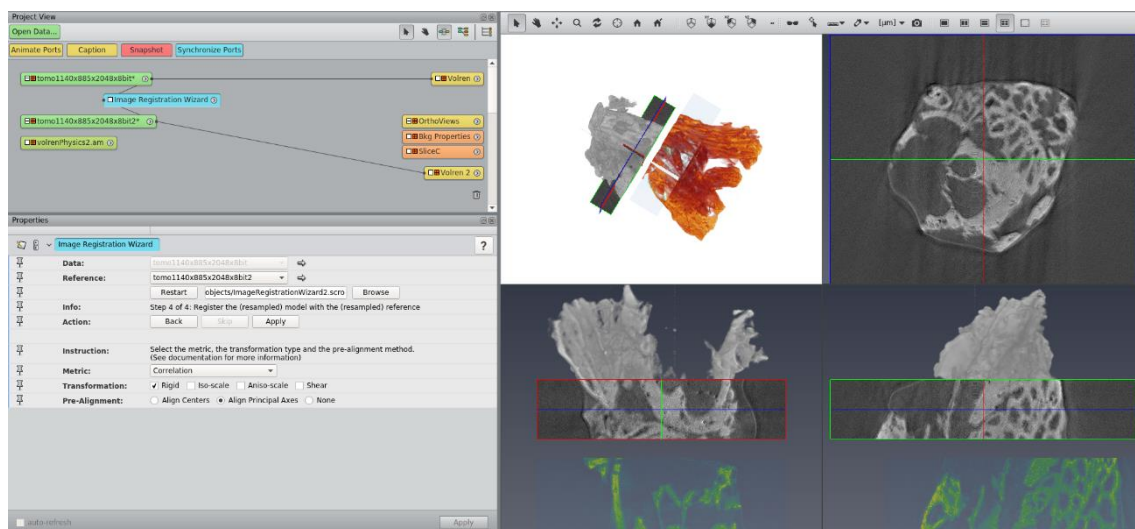
This is a tool to connect regions that are overlapping each other. Load the two parts and attach a 3D volume (e.g. volren). It's easier to see if you change the colors of the surfaces. Then right click on one of the data (part 1 - orange) and search **Geometry Transform > Image Registration Wizard**. Set the reference to the other part (2 - gray). Select Apply.



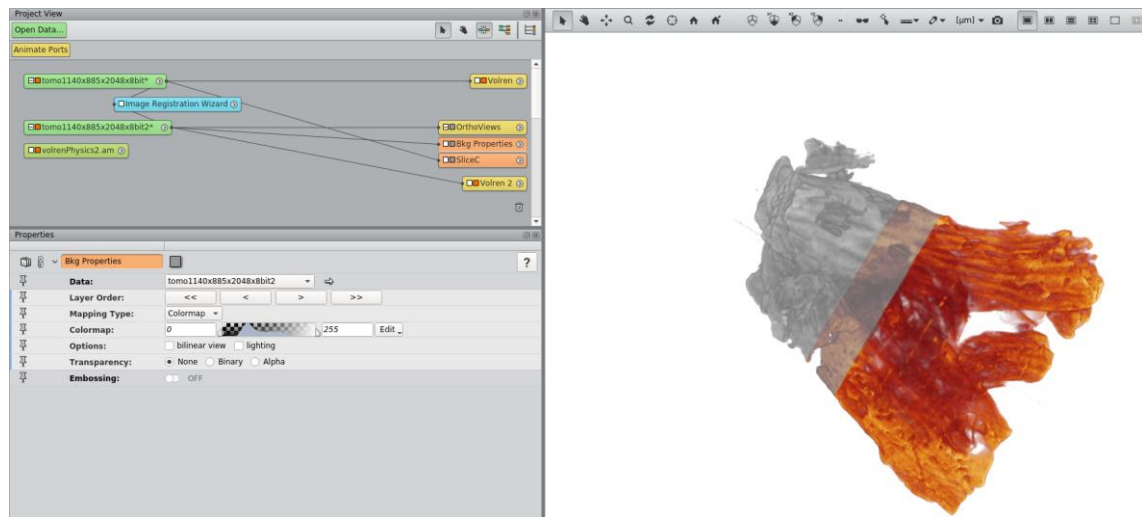
After that, the wizard will set up Ortho Views and there will be a box around one of the data. At this part it's necessary to specify overlapping subvolumes. Drag the box in the part 1 of the data (it should be the common region between the model and the reference) and then select apply. Do the same with the part 2.



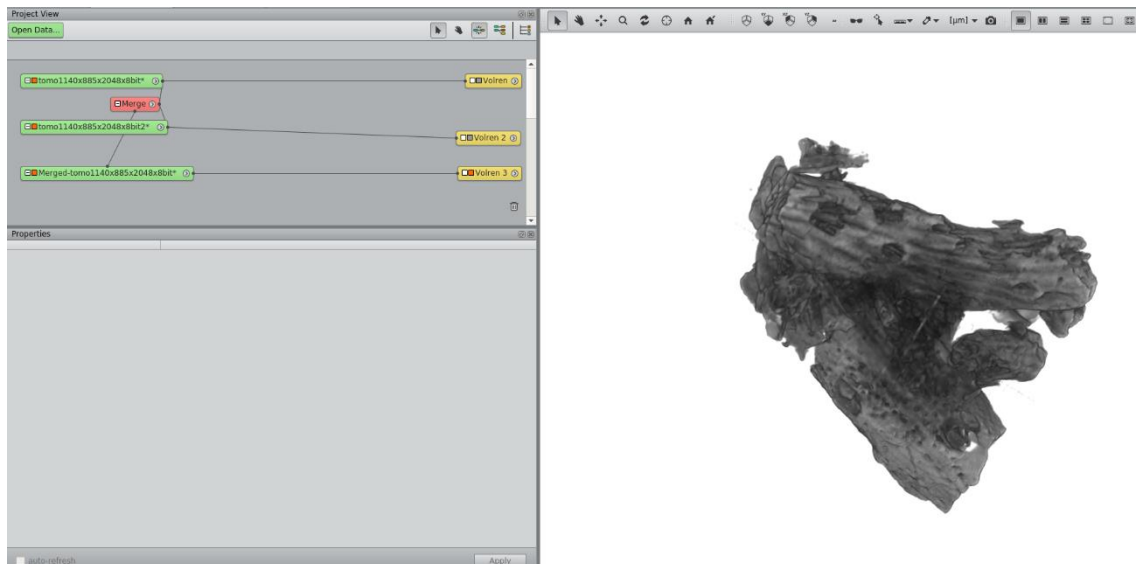
At this part you can adjust to start the registration. Since this model is not aligned with the reference, select Align principal axes and then select apply.



The registration is done. You can go back to previous steps or remove the Image Registration Wizard module. To finish the work, you need to merge the part 1 and 2.



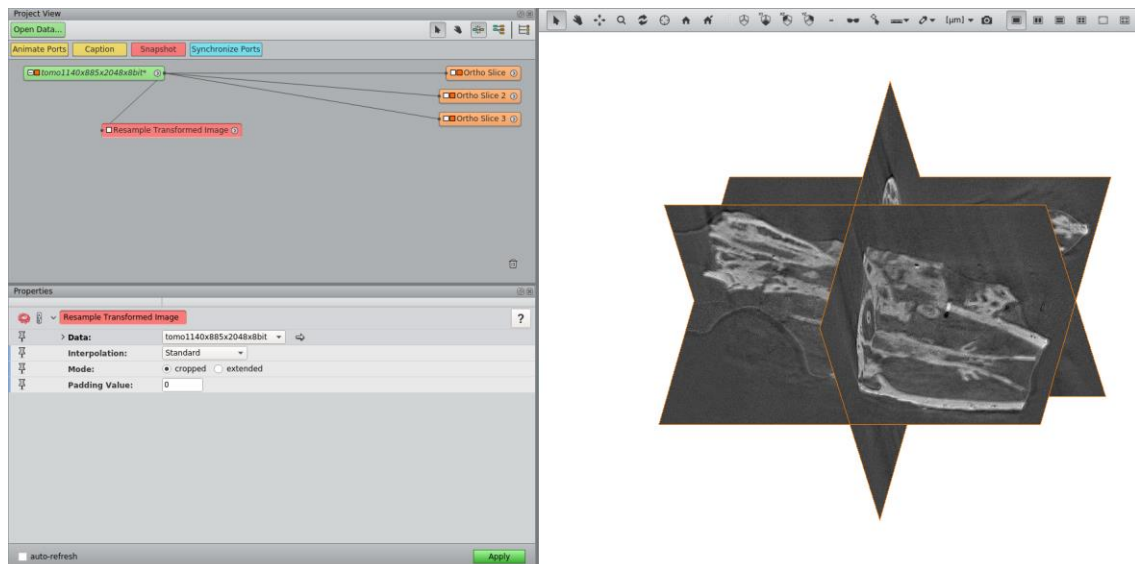
Attach a **Compute > Volume Operations > Merge** module to part 1 and then attach to part 2. Select apply and then generate a Volren to see the results.



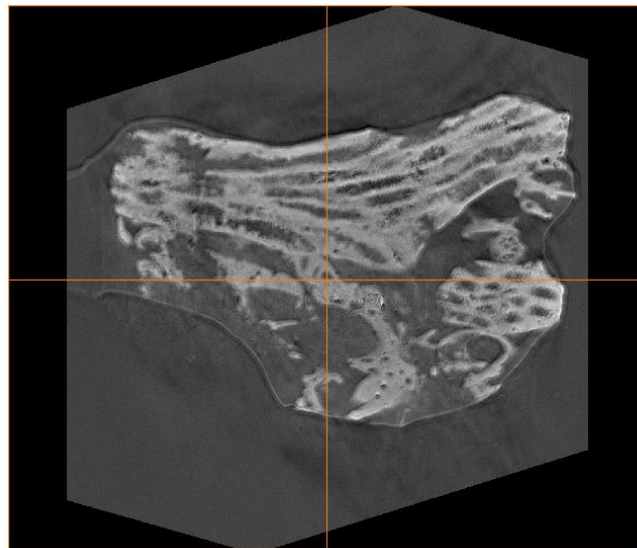
d. Resample Transformed Image

This is another way of registration, but it will move the data to the same location without any transformation set. It means that this tool recalculates x, y, and z axis considering the new position of the axis (it's basically "sampling a volume data onto a reference lattice connected as input"). Attach a **Resample Transformed Image** (right click at the data and type it in the search box).

If you select **Extended at the Mode port**, the result will have all the source data. Than, click on apply.



The next figure is the image resampled as XY view.



It's important to point out that your **data will lose resolution** in that process! That can be bad if you need to quantify the information in your data.

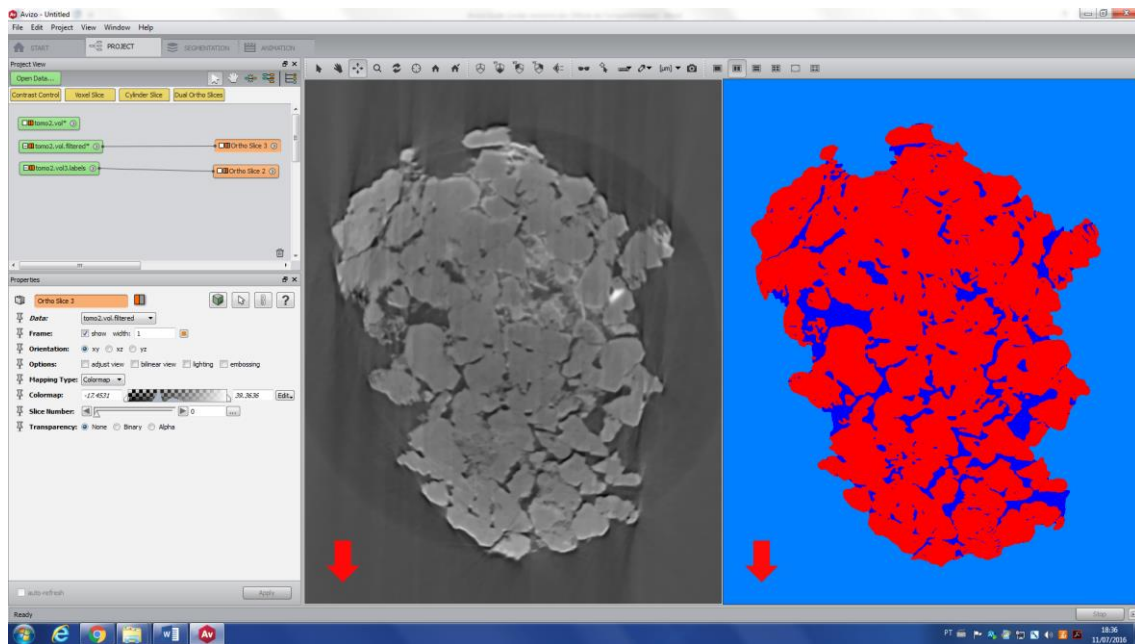
Tip: Use *Subtract Image* as a way to see what is moving or changing in your samples.

9. Quantification

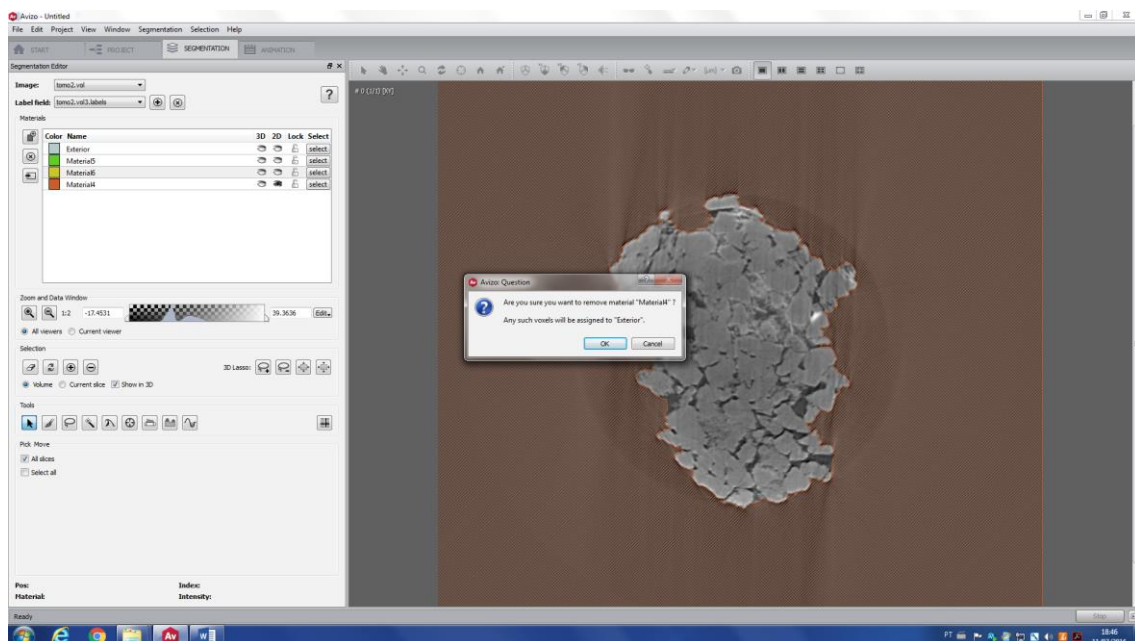
In this chapter, some possibilities of quantification of a segmented image will be shown. So, at this point, it is very important to have a good segmentation of your image, otherwise, you won't have a trustworthy result.

a. Volume of a label

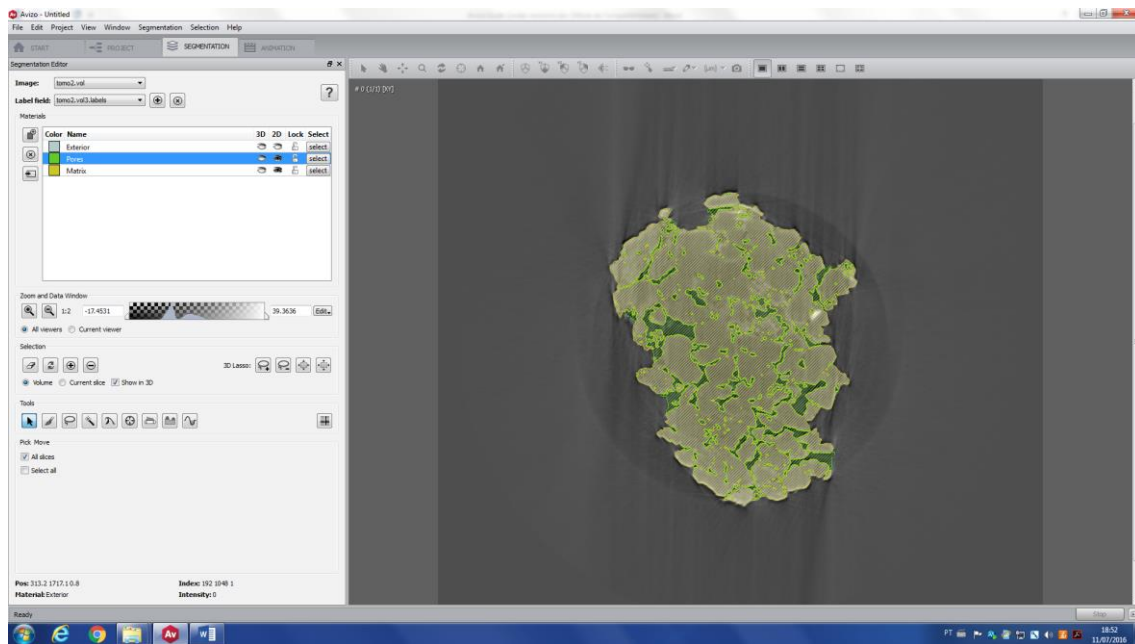
The image below shows a sandstone rock, filtered with the non-local means and segmented with a combination of threshold and watershed.



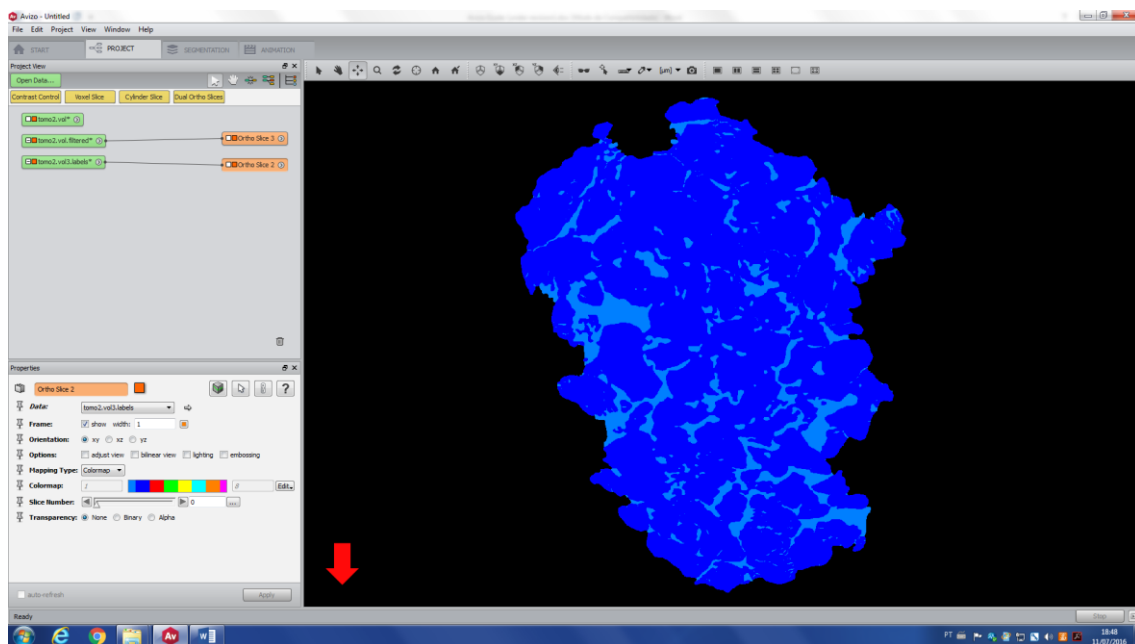
First, move the background to the *Exterior* label, since this is not a region of interest, in this example.



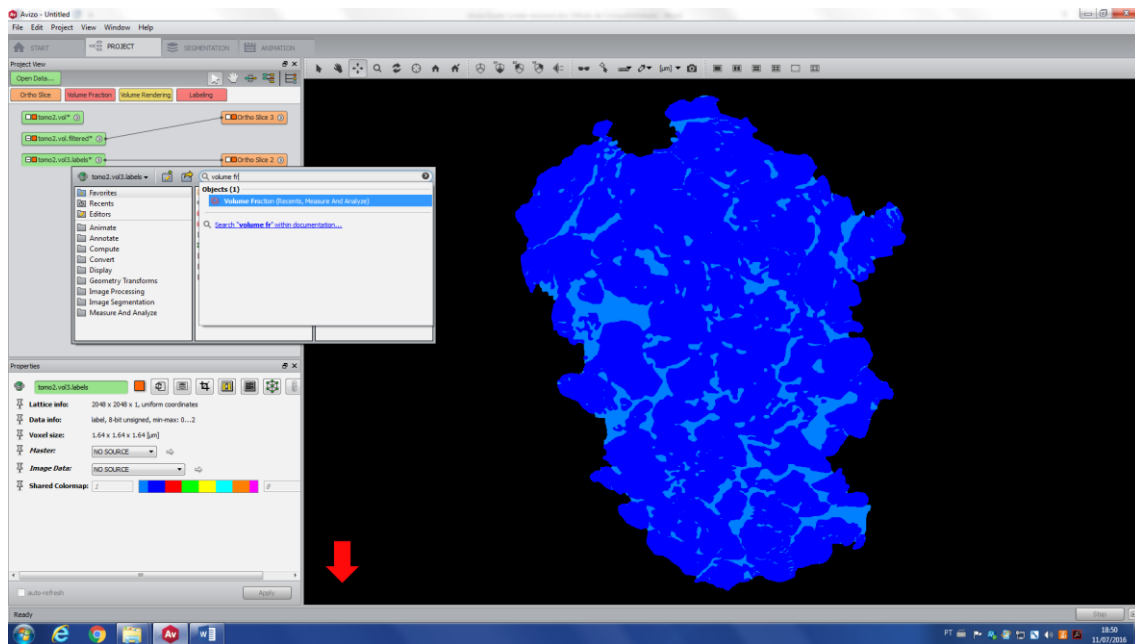
It is possible to rename the label, by double clicking on it. This way is easier to understand the final results.



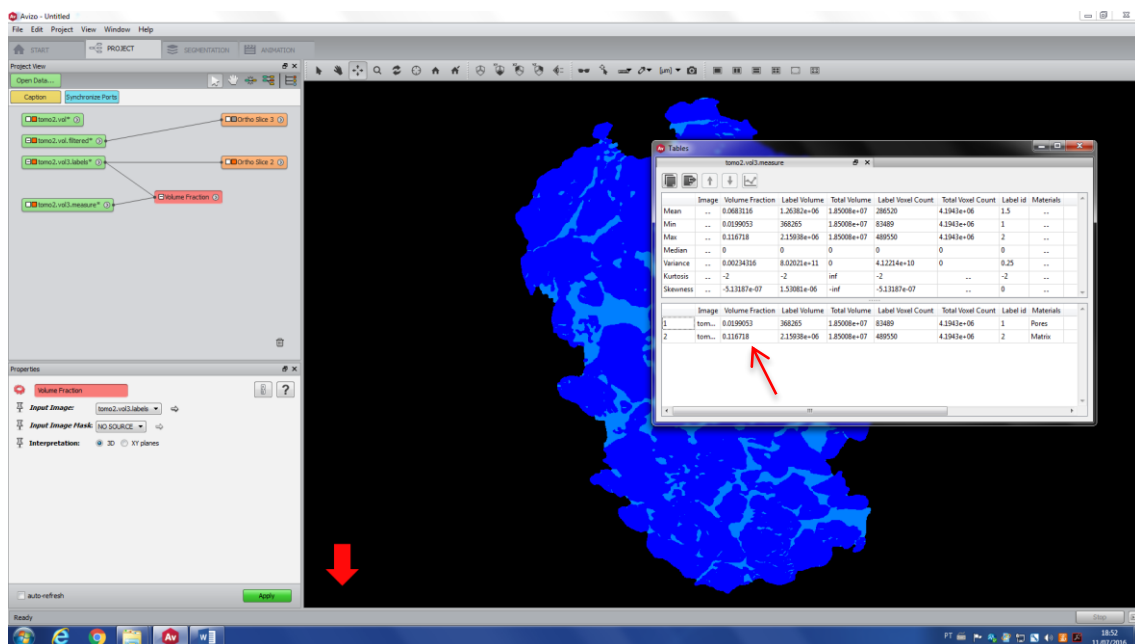
Go to *Project* and check the labeled image (*.labels), the black region represents the background and the sample (with its pore space) is represented by both dark and light blue.



Use the *Volume Fraction* tool to obtain information of the volume of pores and matrix.

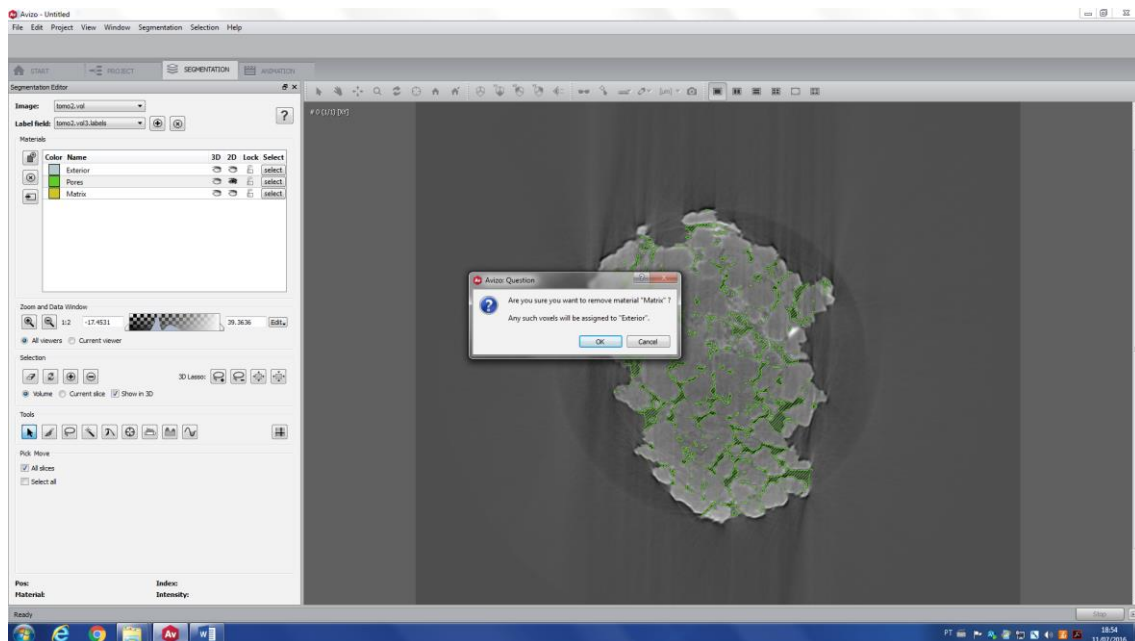


A table will appear with the volume fraction values (red arrow). Adding both of the values, you see that it is not equal to 1 (i.e. 100%), just because the software also considers the background as part of your image, but a simple rule of three can give you the proper fractions.

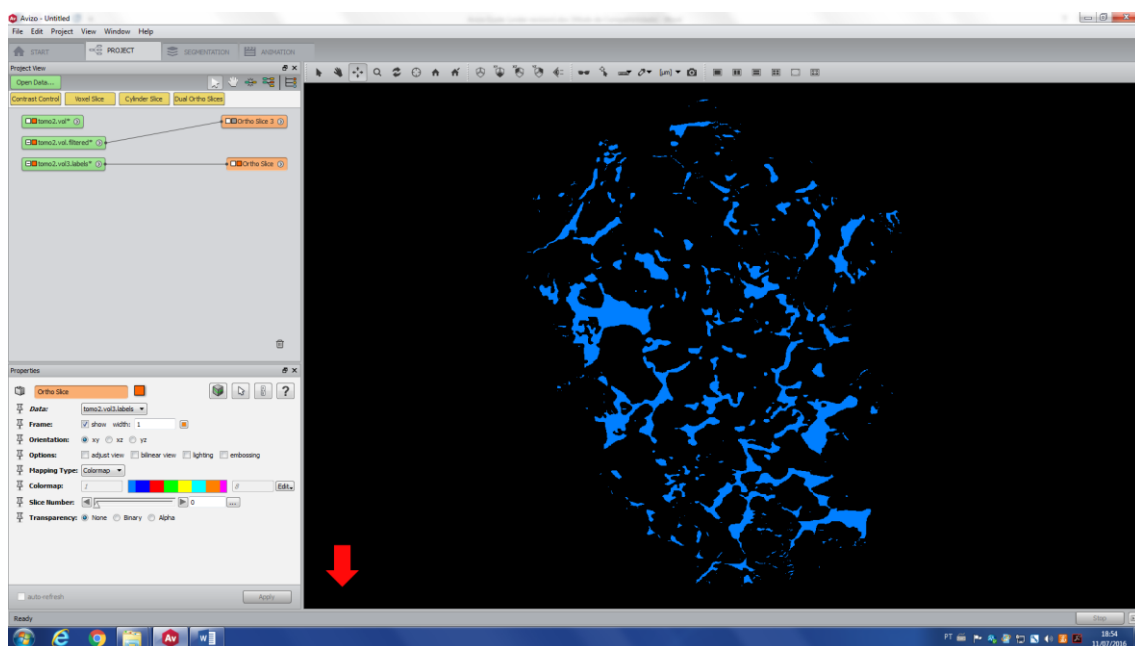


b. Separate tool

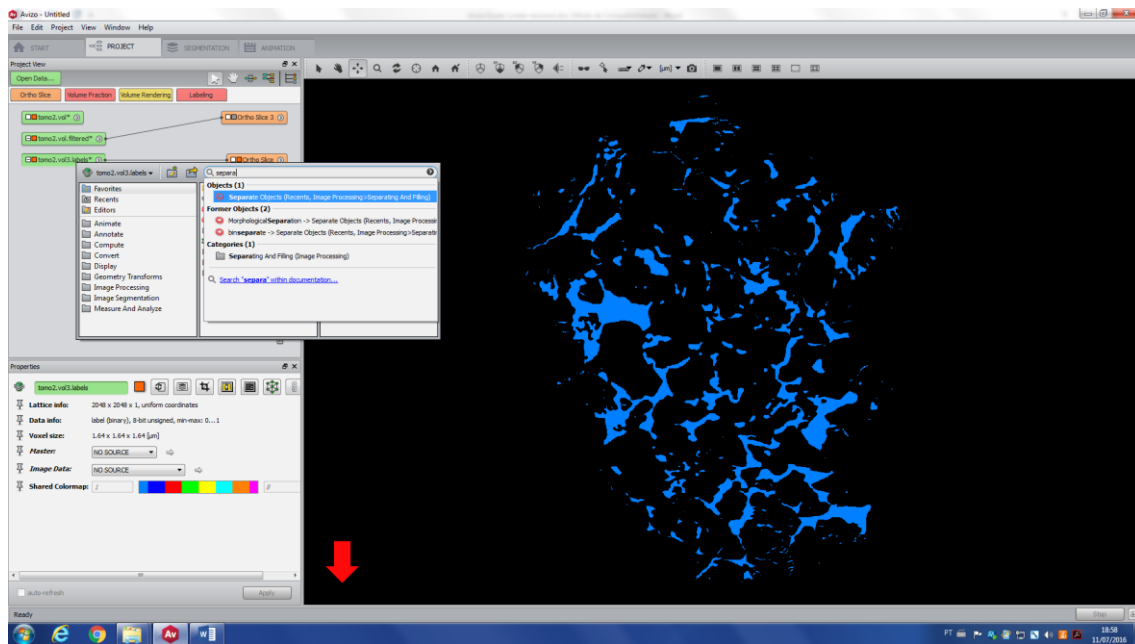
Separation is a tool to individualize regions of an image, which can be, for example, pores or grains. Let's keep the same image used in the previous example, but now, the idea is to transform the pore space into many individual pores (the same can be done with the matrix). Move all the labels to the exterior, except the one you want to individualize. In this case, Matrix label will be moved to the exterior.



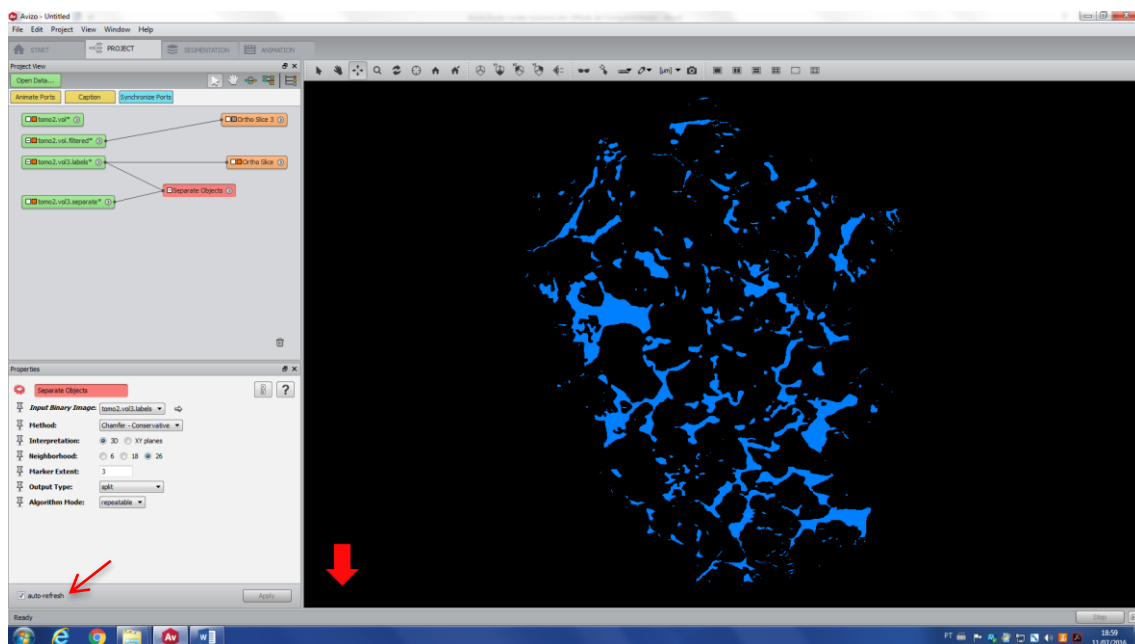
And only the pore space will be left.



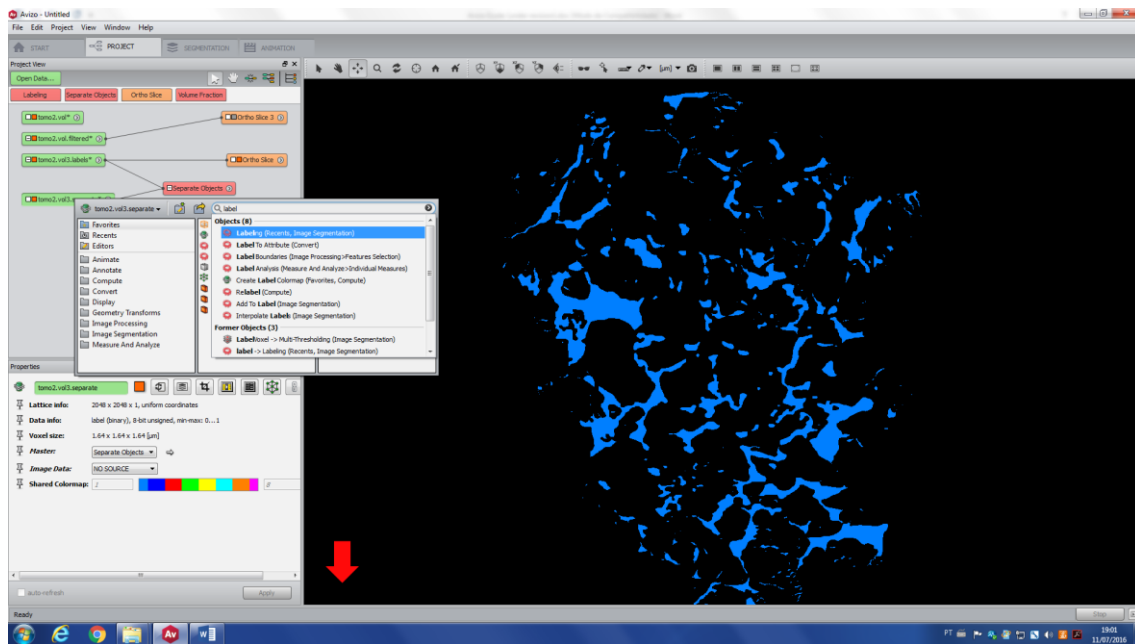
Right click on the image and type *Separate Objects*.



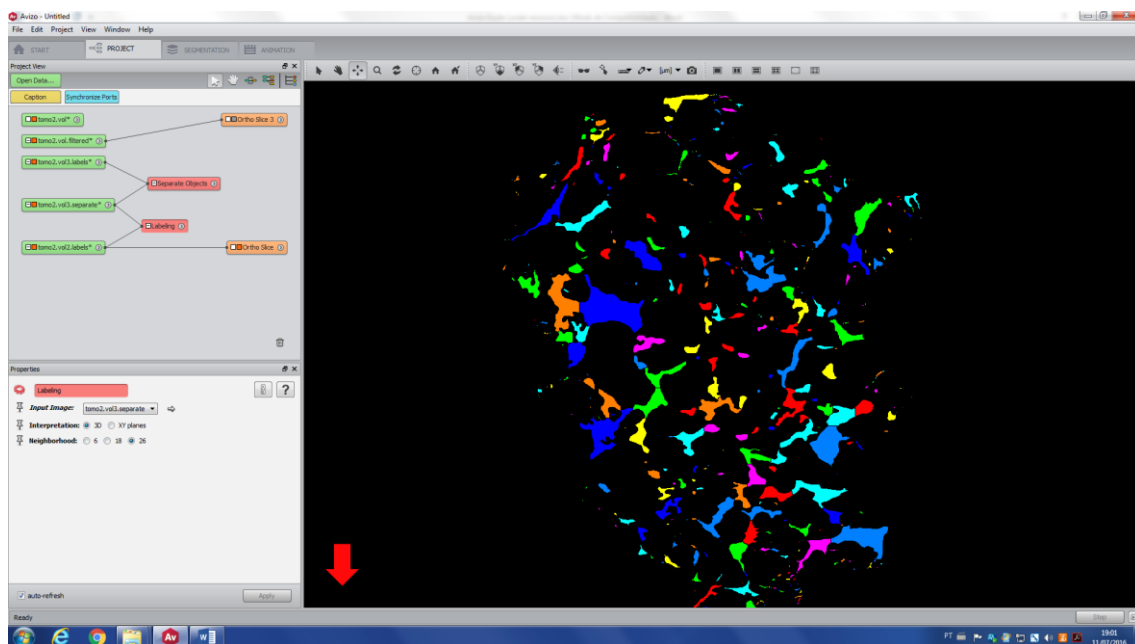
The *Properties* window will open. This tool is very sensitive to the *Marker* value (which works like a seed), so, it might be a good idea to vary the marker and check the final result, to make the best decision. For that, click on *Auto-refresh* (red arrow) and it will create a new file (*.separate).



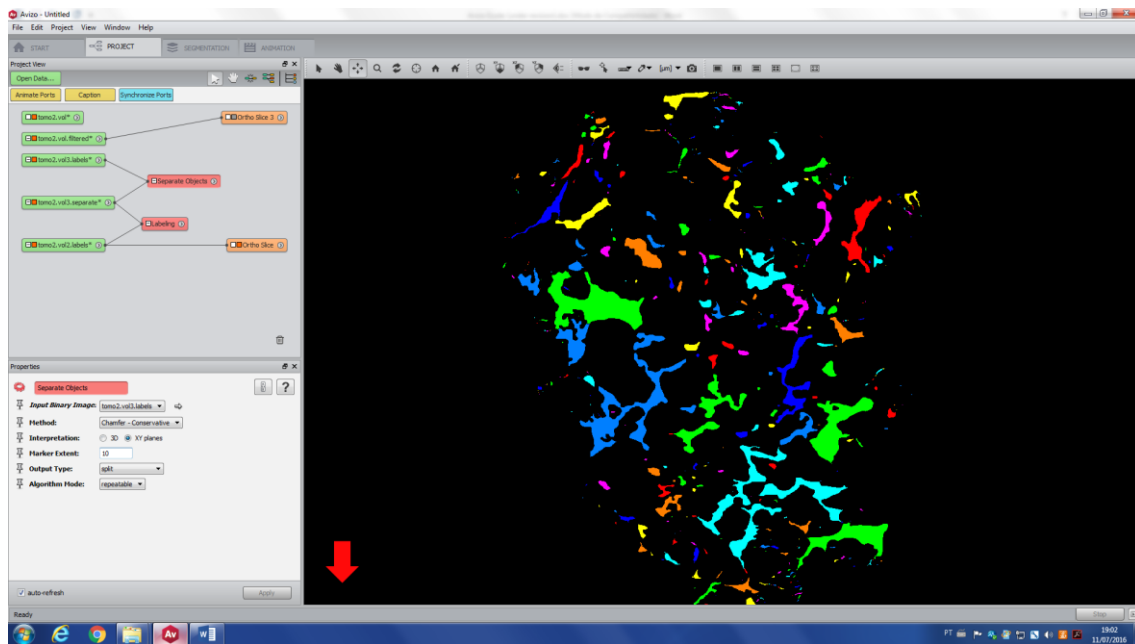
To make it easier to check the final result, click on the *.separate image and type *Labeling*. Now, every pore will receive a different color.



Create an orthoslice for this new image and check the result. There is a repetition of colors, but it does not mean necessarily that they are the same pore (somehow connected in 3D), it is more likely due to the high amount of pores and the lack of enough different colors.

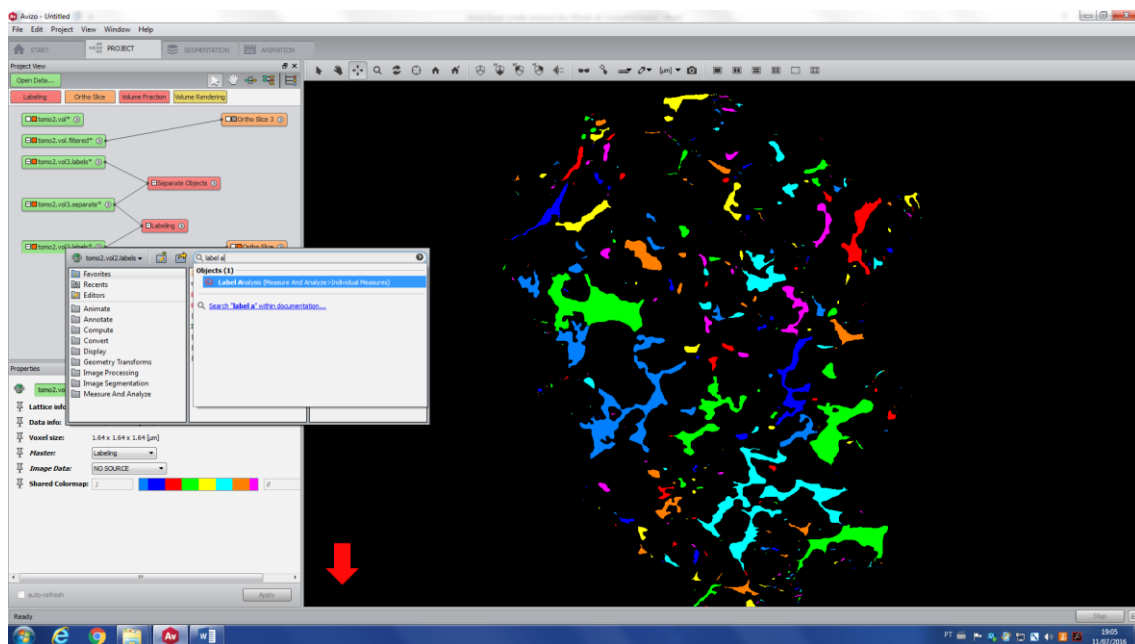


If you go back to the *Separate Object* and change the *Marker Extend*, you will see that the separation changes. The above image was with Marker = 3 and the following image, Marker = 10. Choose the best marker for your case.

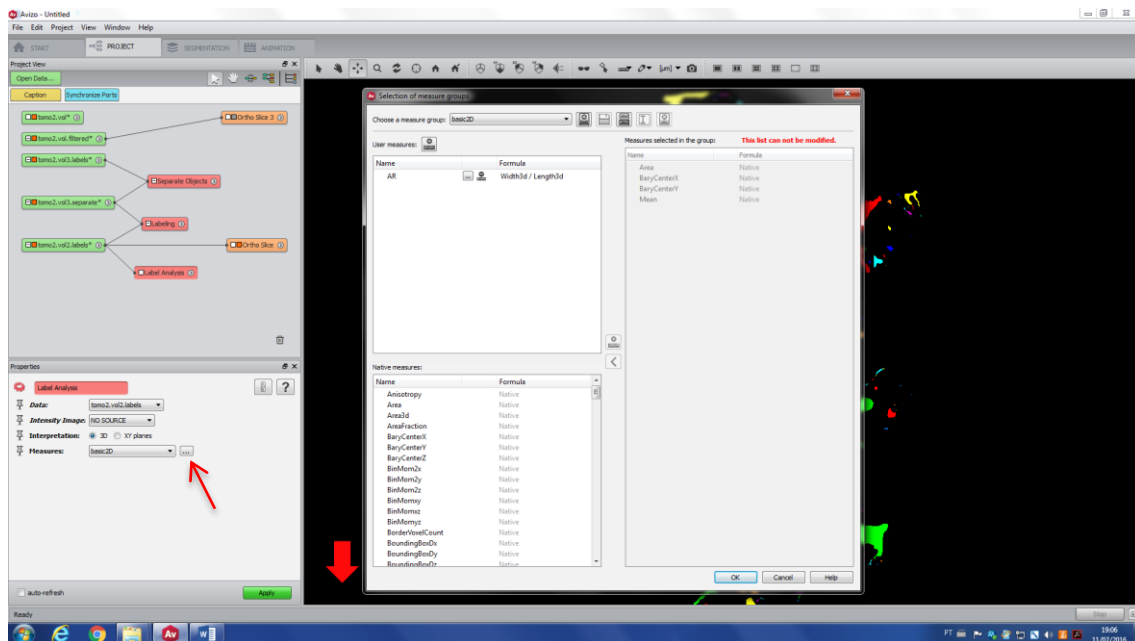


c. Label analysis

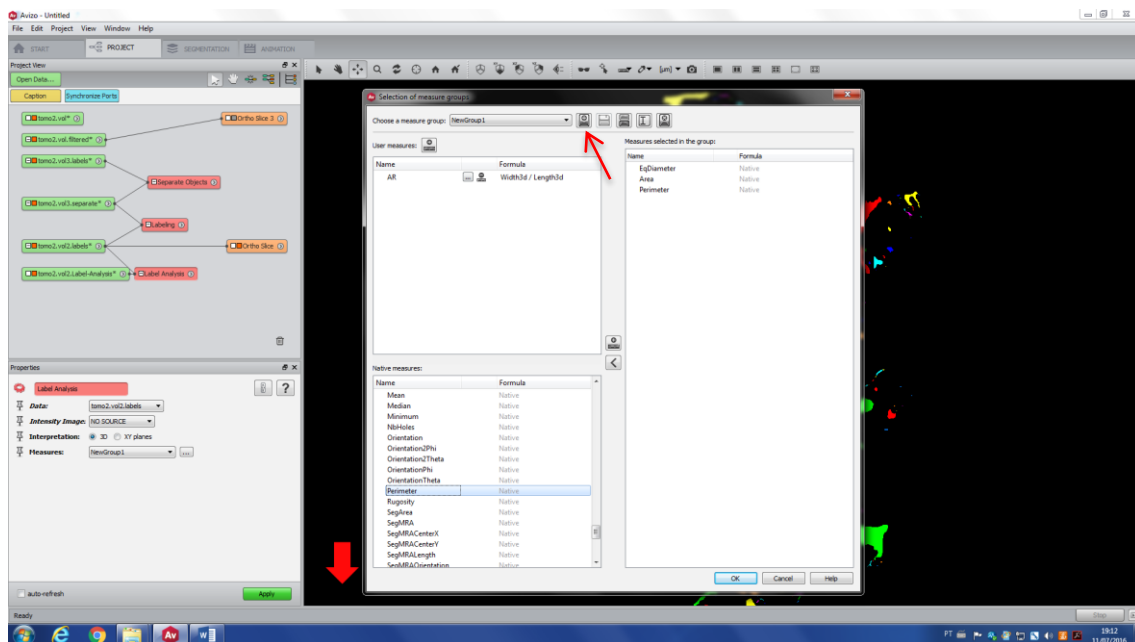
Label analysis is a tool that permits to quantify your image. In this example, we will use the last image, showed below, to obtain few parameters from each pore. For that, right click on the image and type *Label Analysis*.



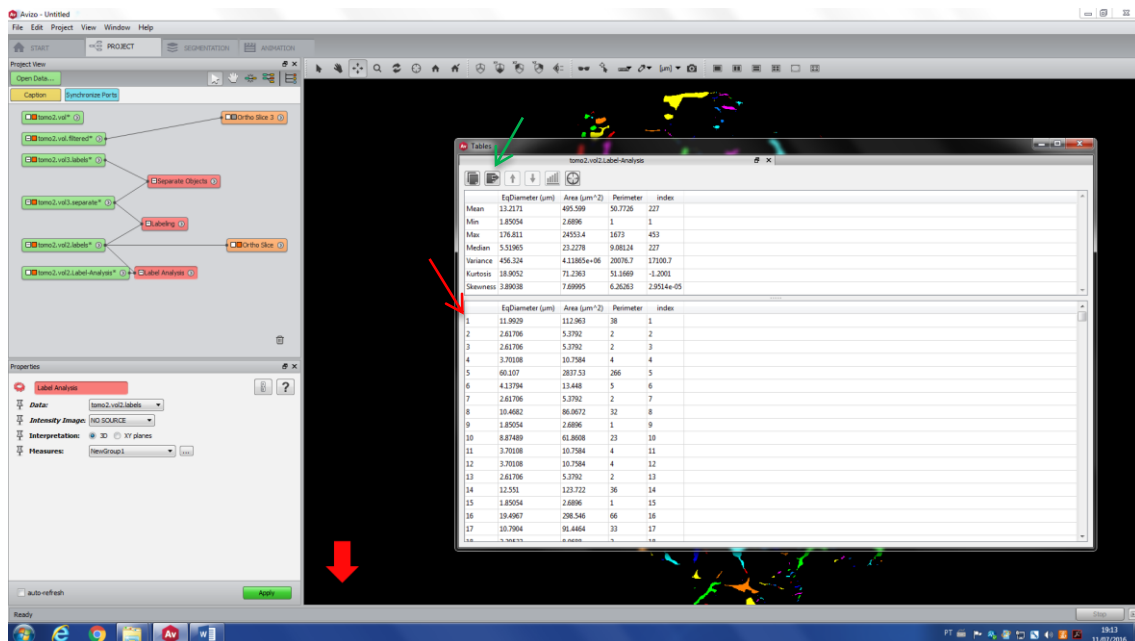
Clicking on '...' (red arrow), you will open all the possibilities of measurements and you will also be able to create your own list of measurements.



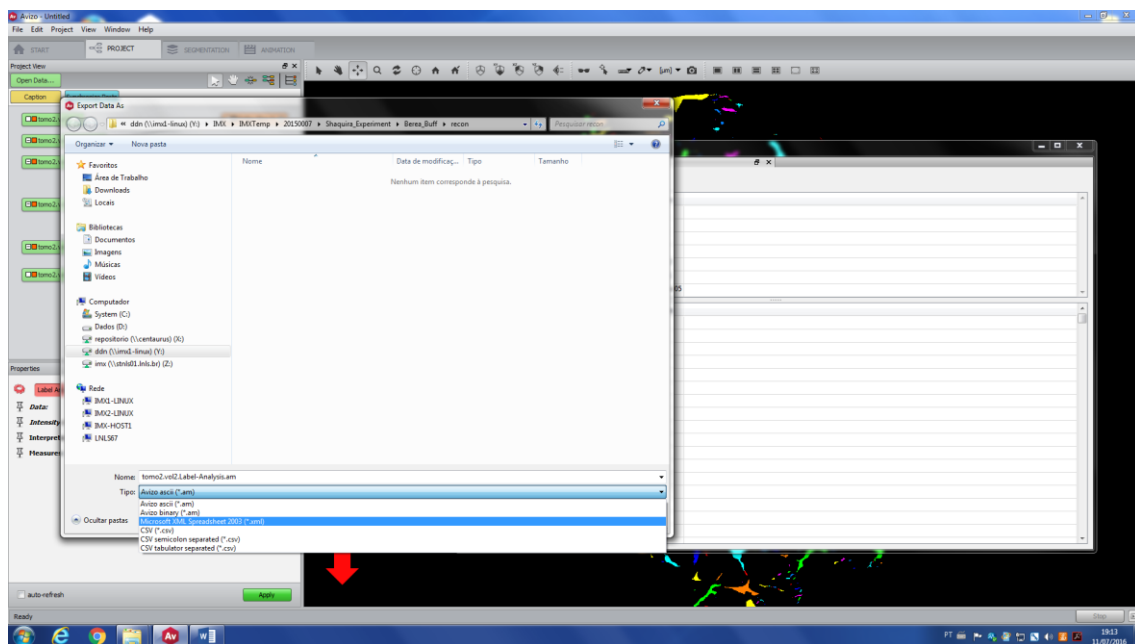
Create your measure group (red arrow) and choose all the properties (from the list) you want to measure.



Apply to your image and check the table with the results. Each pore is represented by a different number (red arrow), and you have the selected measures from each one of them. You can also export your table (green arrow).



And save this in different formats and work with your data in any other desirable software.

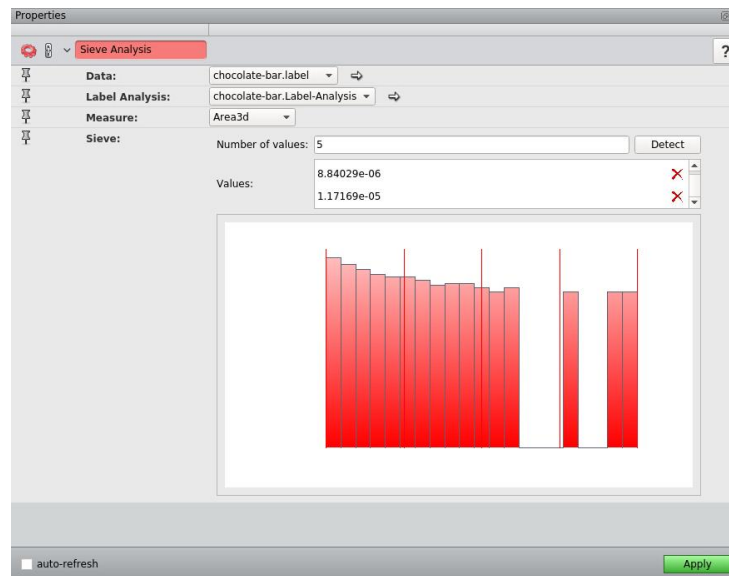


OBS: Click Window > Restore Default Layout if you need to bring things back to their original place.

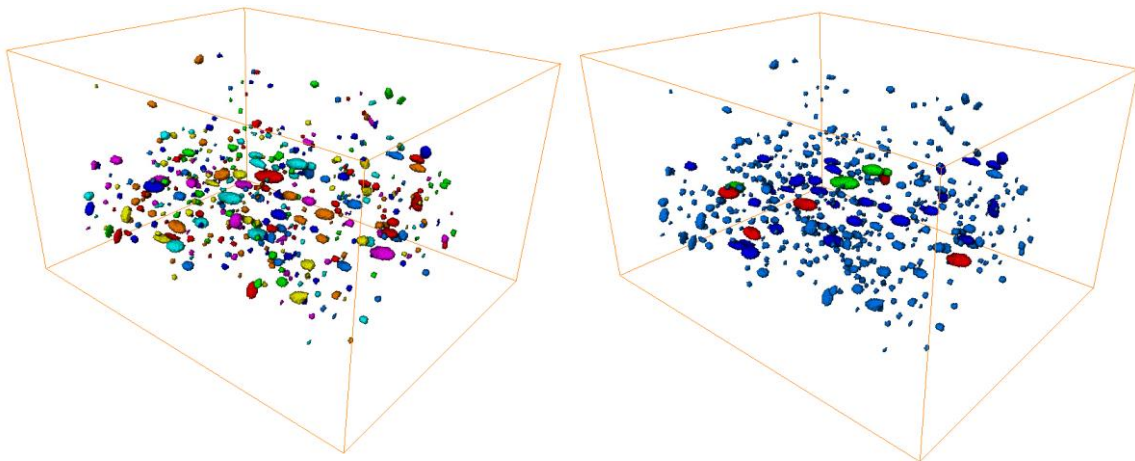
d. Sieve Analysis

After you applied the label analysis, you can classify you data based on the measure. Attach the Sieve analysis module to your .label image (this will be the Data). Select the .Label-Analysis generated. Define one of the physical measure of the input analysis spreadsheet.

After that, the respective histogram will appear. You can define how many categories you want to divide (the range for each bin) at the Sieve portion > Number of Values. The limit of range is represented by vertical red lines in the histogram and the value limits are presented above the histogram.

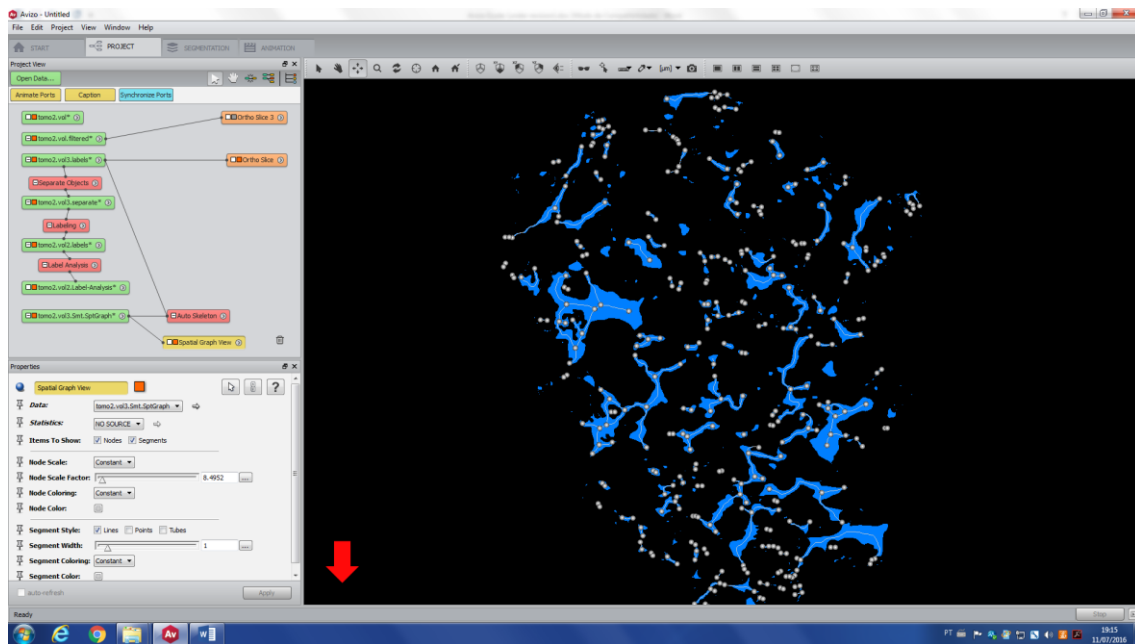


The result is a new label image by binning components of the input label image. At left is an label image generated by labeling; at right is a label image with sieve analysis based on Area3d with 5 values.



e. Connectivity

The final example of this guide is the connectivity tool, which will give you information of the number of neighbors of each pore (i.e. coordination number of the pore). For that, you have to use the first segmentation of your pore space (before the separation) and use the *Auto Skeleton* tool. It will create a new file (*.SptGraph) and to visualize the result, right click on this and type *Spatial Graph View*. The final result is shown below. Each single pore is represented by one segment and two dots. The segment represents the pore body and the dots, either an end point (border) or a point of connection to another pore.



If you click on the *.*SptGraph* file, you will open its *Properties* window. Go to the Table option (red arrow) and click on *Show* and it will open the table with some information regarding the skeletonization. There you can find information regarding the coordination number of each pore and the size of the segment (pore body) in the three directions. Note: *Z coord* is always zero here because there is just one slice loaded, but in a real 3D image, this number is always bigger than this.

