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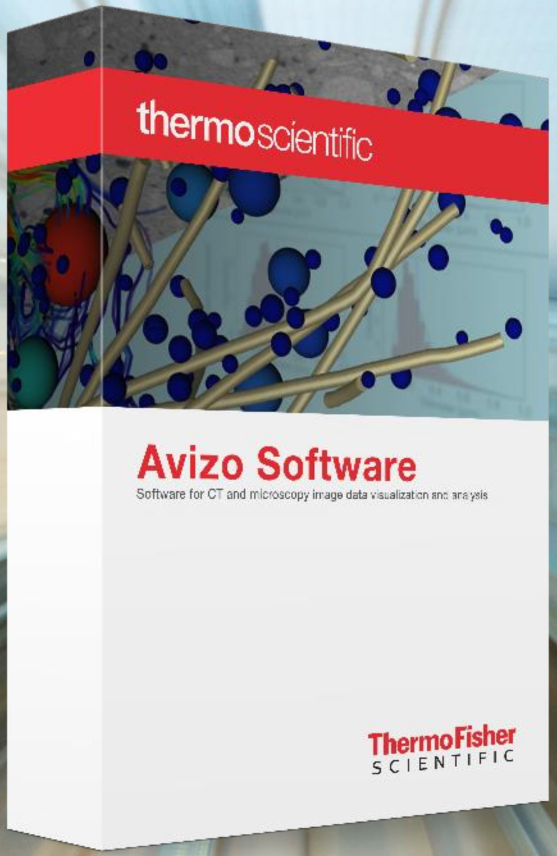
Avizo Software
Introductory Training



Sarawuth Wantha, PhD
Product Application Specialist
21-23 April, 2025

The world leader in serving science

A quick word about me

Sarawuth Wantha, Ph.D

Product Application Specialist,

Thermo Fisher Scientific

- Ph.D. in Biomedical Engineering: Focus on Biomedical Imaging and Image Processing
  Ludwig-Maximilians University Munich, 2013
- Joined Thermo Fisher Scientific in 2018

Agenda

Beginner: April 21 (Monday)

- Introduction to image/volume data and processing
- Software overview, general concepts and user interface
- Data visualization in 2D&3D and annotations
- Basic Data Manipulation: cropping, re-sampling, pixel type and conversion
- Image processing: denoising, filters, background correction
- Automatic Segmentation: binarization and thresholding
- Separation, classification, analysis & measurement
- Screen capture and animation
- Recipe and batch processing

Agenda

Intermediate: April 22 (Tuesday)

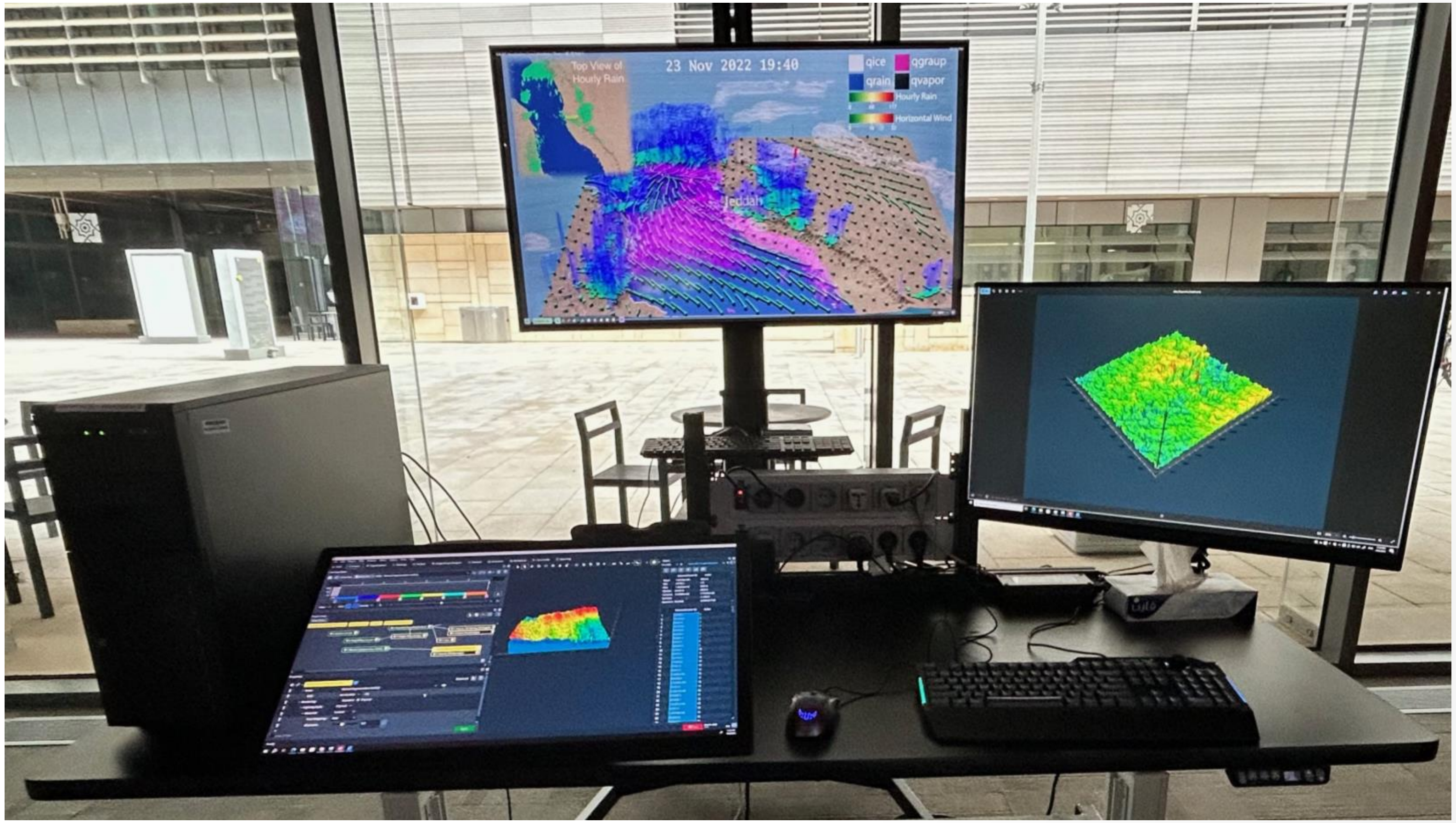
- Introduction to image segmentation
- New Segmentation+ workroom (requires Avizo 2024.2 and later)
- Multi-material segmentation
- Watershed segmentation
- Surface reconstruction and simplification
- Data registration and alignment
- AI-assisted segmentation tools

Agenda

Advanced: April 23 (Wednesday)

- Review of segmentation tools
- 2D & 3D Deep Learning Training module
- Deep Learning Prediction Module
- Deep Learning Denoising Module
- Core Profile (features from PerGeos)
- Overview of Avizo extensions
- Focus on specific extensions
- Avizo ToGo

KAUST Visualization Core Lab (KVL) Facilities



powerful workstations for Avizo processing and visualization

https://wiki.vis.kaust.edu.sa/booking

Book **AR/VR Hub**. Access is free but please acknowledge KVL in resulting publications!

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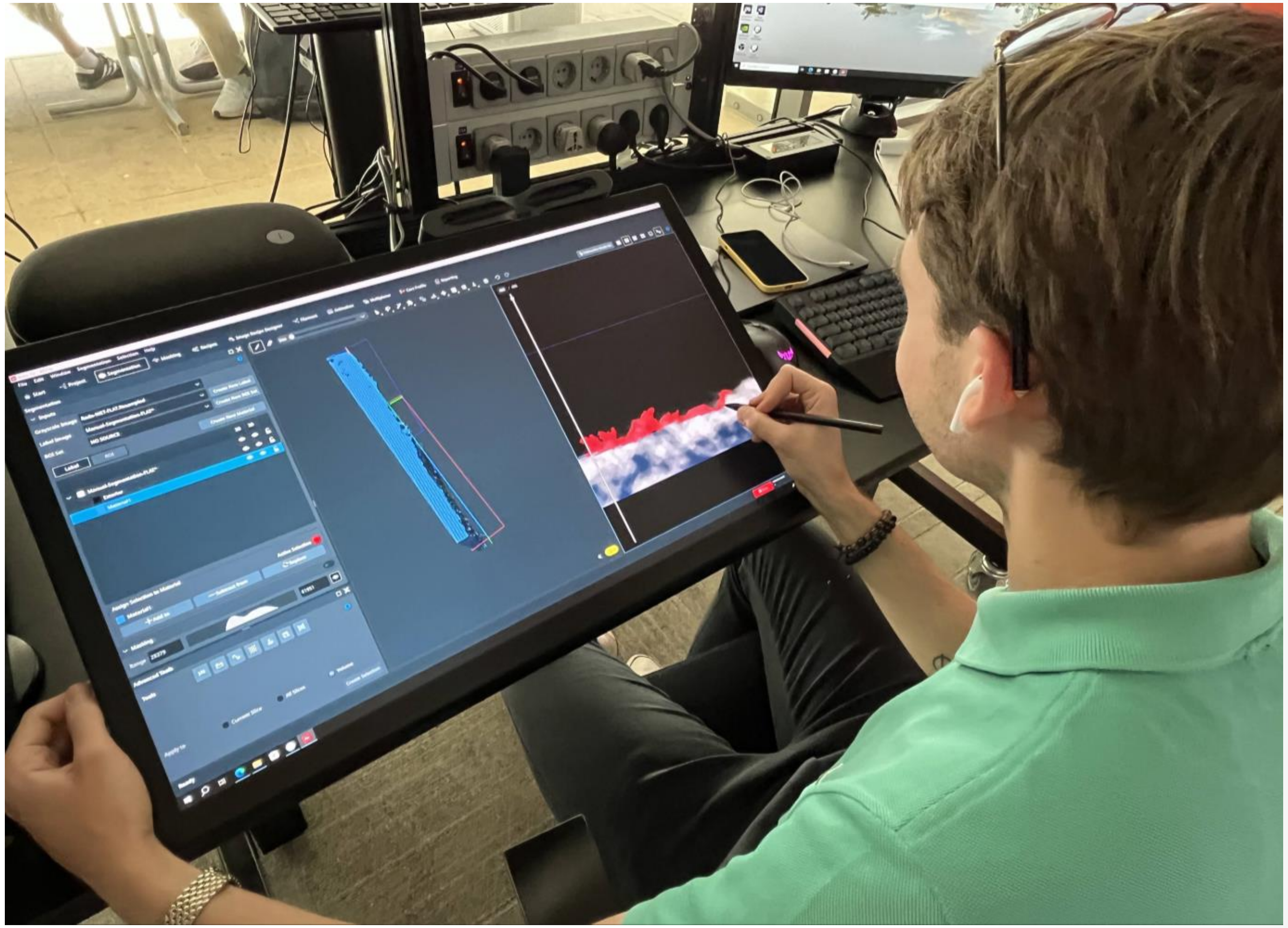
KAUST Visualization Core Lab (KVL) Facilities



pen + tablet interface
for manual segmentation

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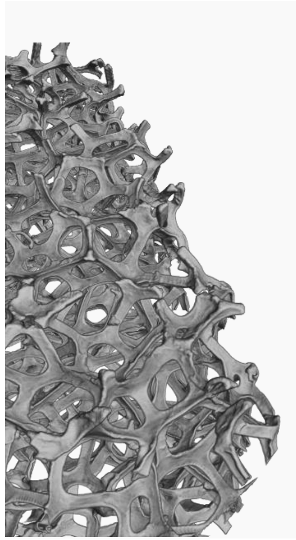
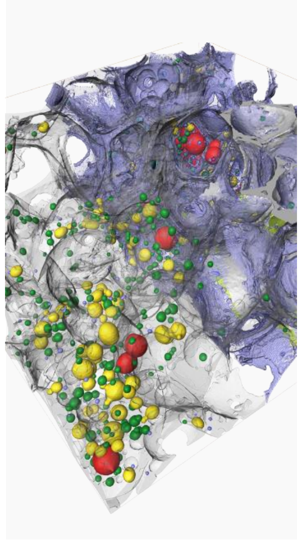
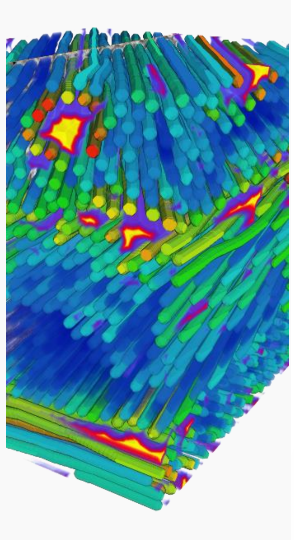
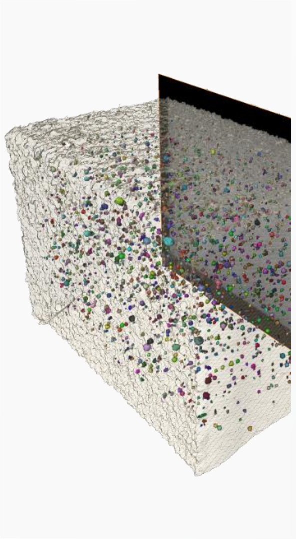
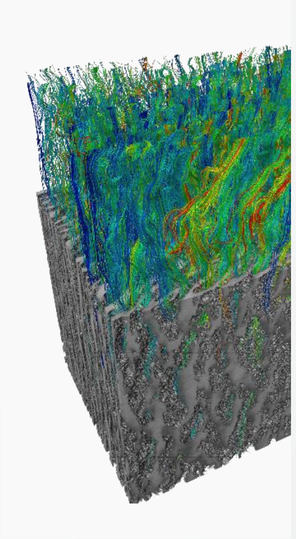
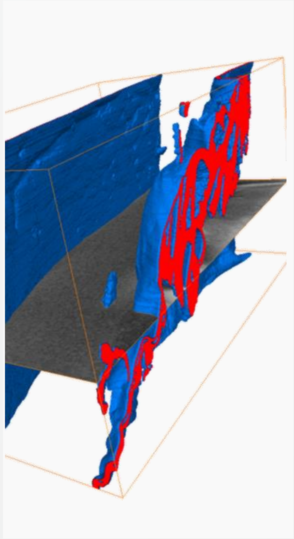
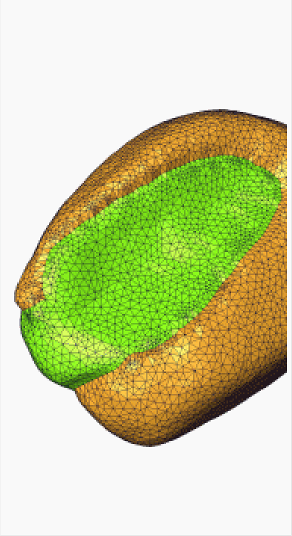
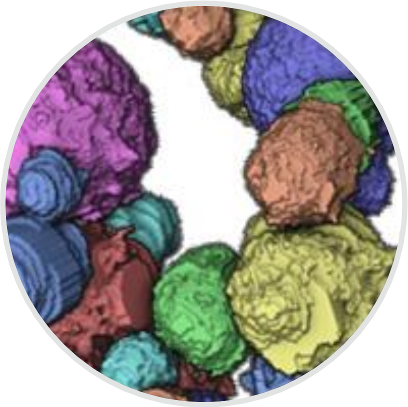
Avizo Software for Materials Research & Quality Control

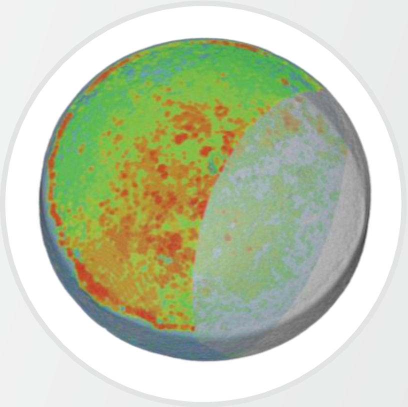
Quickly and accurately obtain properties from your imaging data

Metals and Alloy	Ceramics, Glasses and Porous	Composite, Polymer	Additive Manufacturing	Battery Energy Materials	Biomaterials	Food and Agriculture
						

Avizo Software provides unmatched imaging data analysis tools for numerous scientific and industrial applications from a single environment

Multi-Modality Imaging





Nanometer

Micrometer

Centimeter

Light microscopy
- Confocal
- Fluorescence
- Serial sections

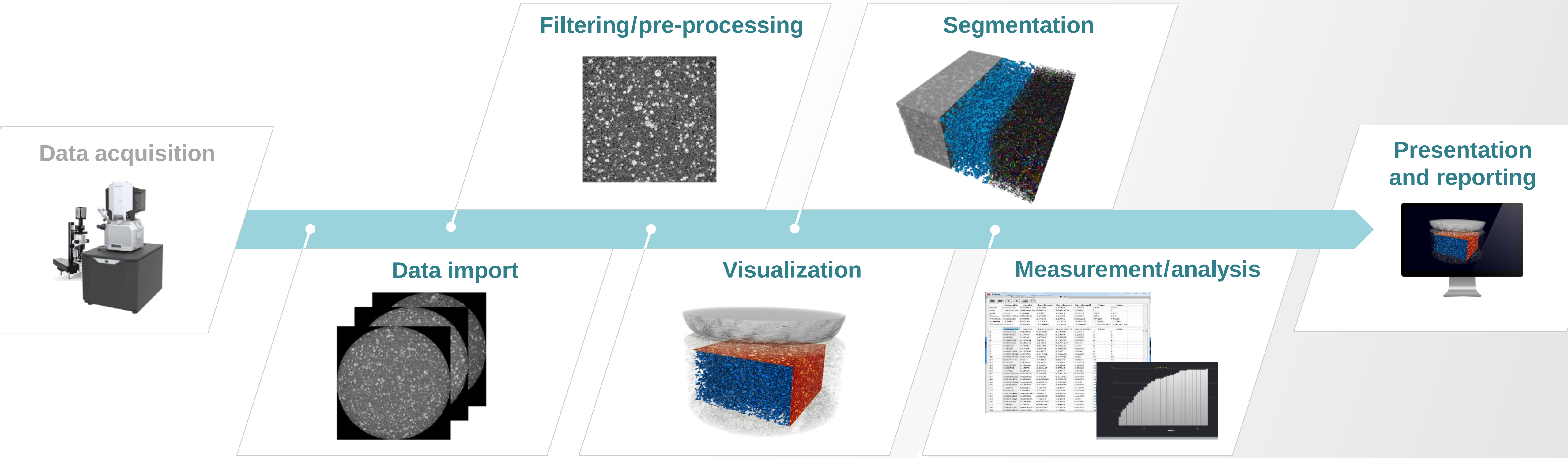
Electron microscopy
- SEM
- TEM-Tomography
- FIB/SEM

Computed tomography
- CT
- µ-CT
- Synchrotron-CT

Magnetic resonance imaging
- MRI
- DTI

Other
- PET
- 3D Ultrasound
- OTC

Complete workflows for visualization and analysis



Introduction to Image Analysis

Introduction to Image Analysis

What is Image Analysis ?

Image analysis involves **processing an image into fundamental components to extract meaningful information**.

Image analysis can include tasks such as finding shapes, detecting edges, removing noise, counting objects, and calculating statistics for texture analysis or image quality.

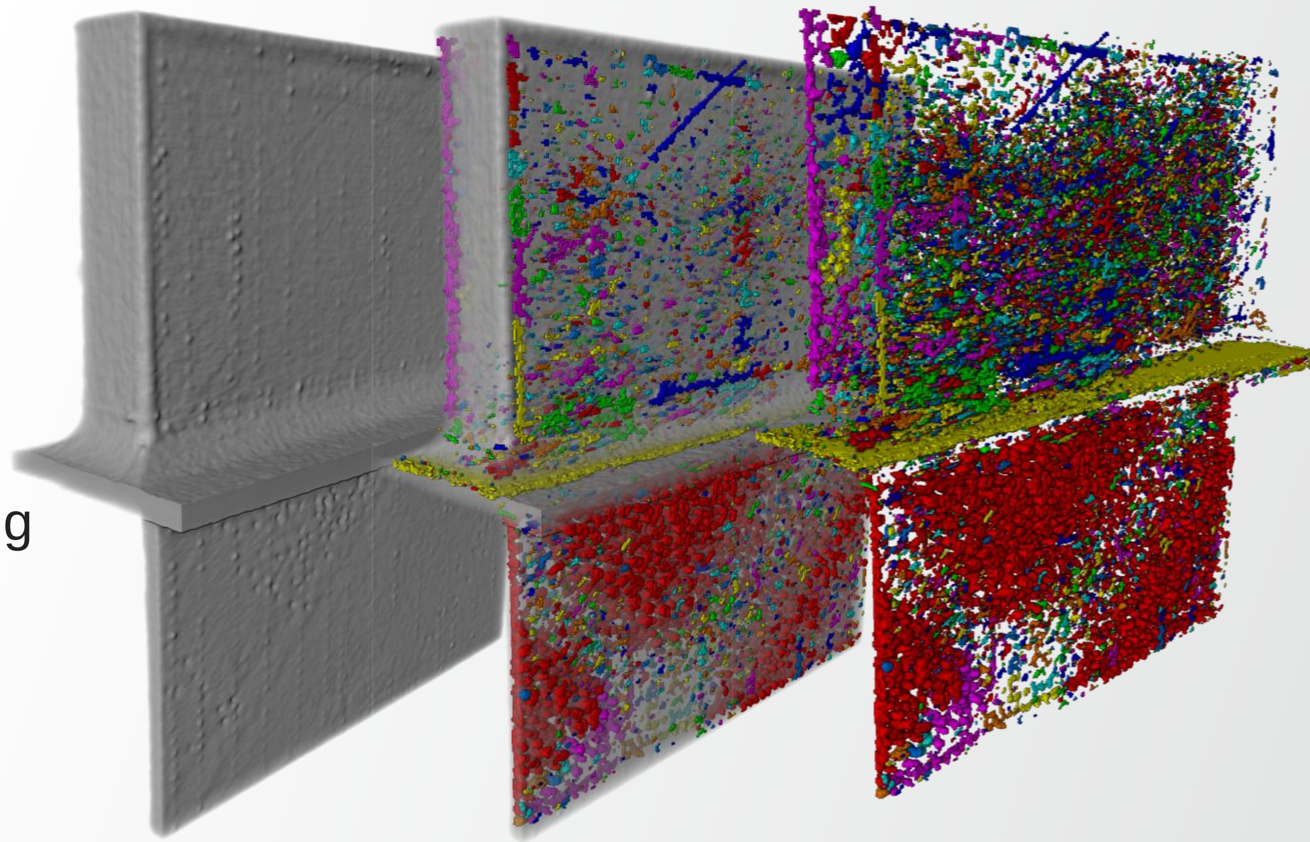
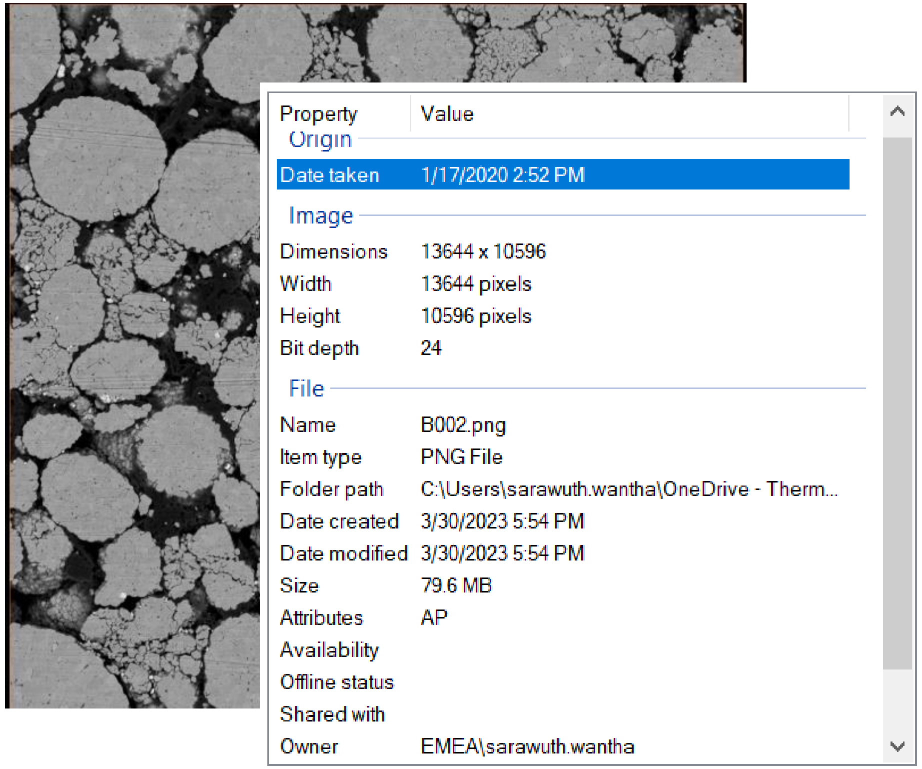


Image & Metadata

Background information of images

Image metadata is information about a visual file or resource.

This data describes what the file is, details about it, and how it should be used.



Technical metadata

- Image type
- Image size
- Microscope settings
- Date created
- Uploader credentials

Descriptive metadata

- Image descriptions
- Keywords
- Batch or collection
- Author's name

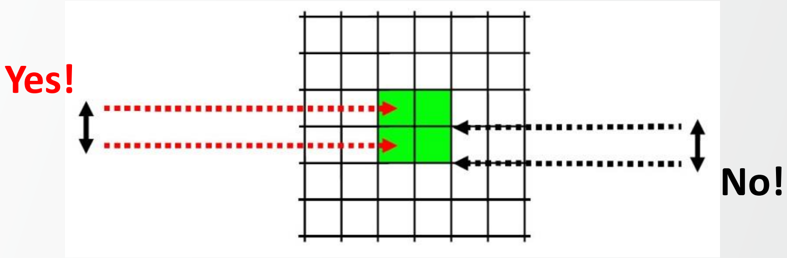
Administrative metadata

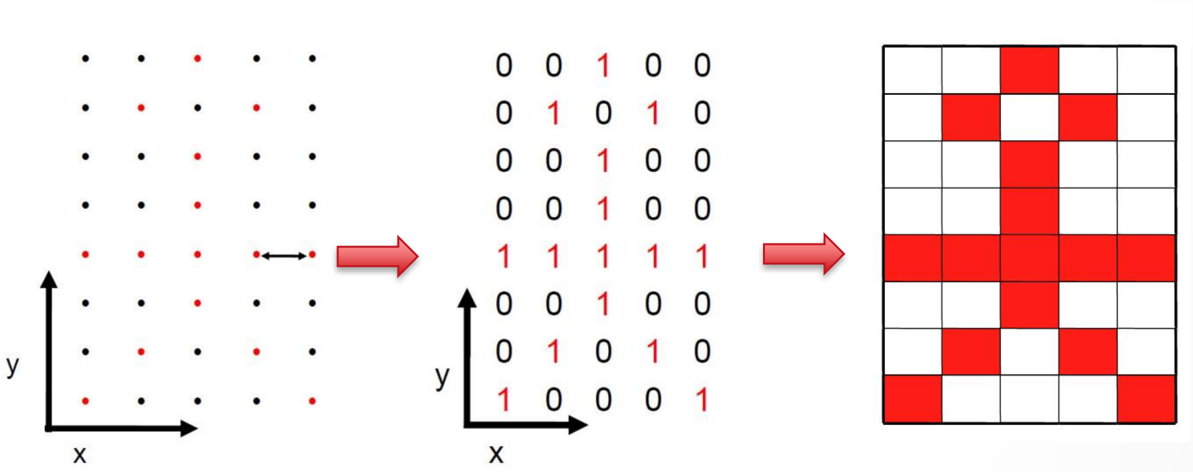
- Rights management
- Restrictions
- Licensing
- Expiration date

What is a digital image

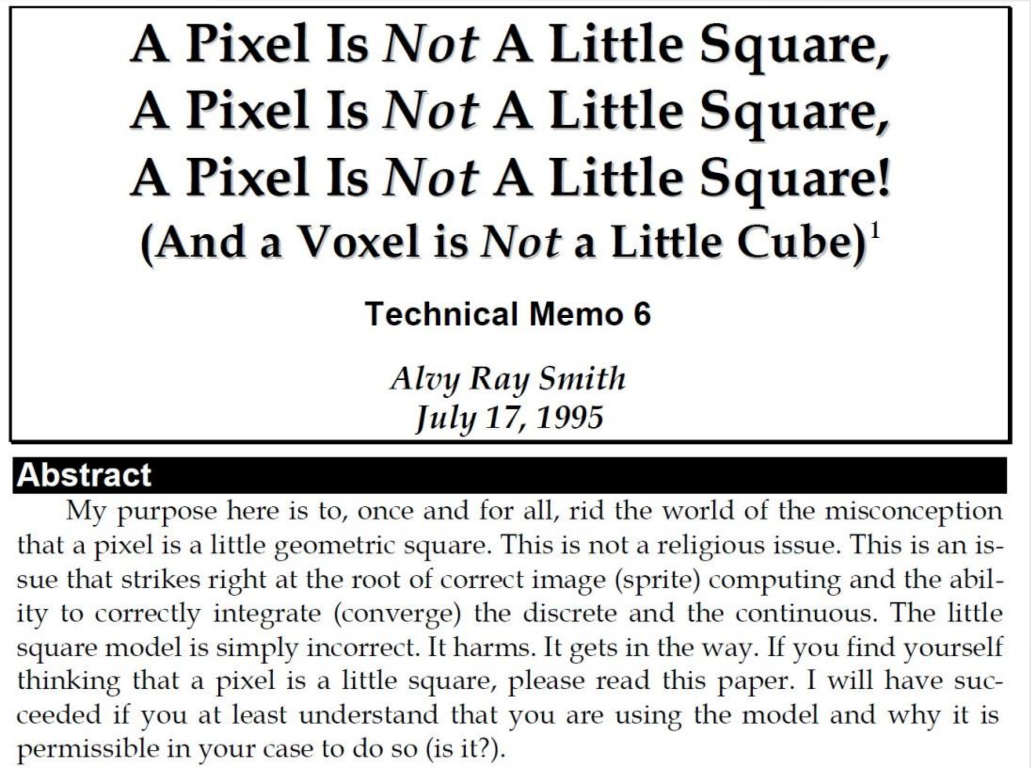
Definition of Pixel

- A pixel is a point sample of the intensity in space
- ≈ PSF (Point Spread Function)
- Pixel size = pixel spacing distance





Sampling **Data set** **Visualization**



A Pixel Is *Not* A Little Square,
A Pixel Is *Not* A Little Square,
A Pixel Is *Not* A Little Square!
(And a Voxel is *Not* a Little Cube)[1]

Technical Memo 6

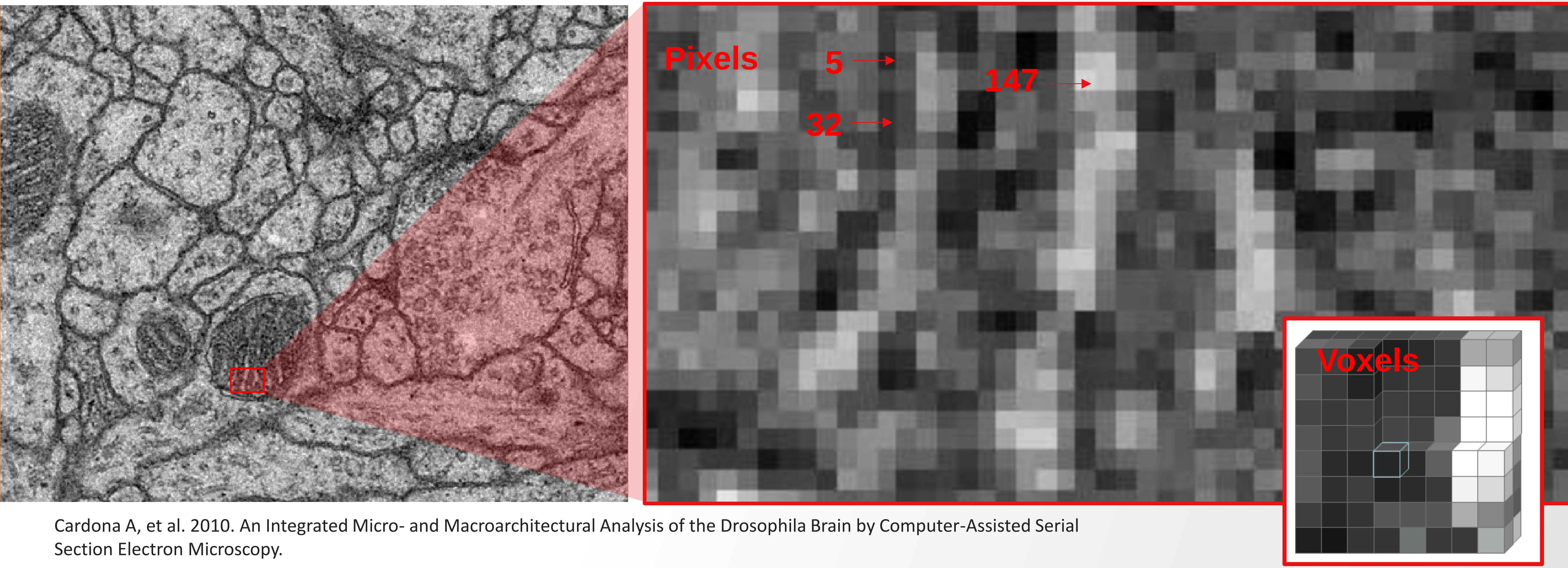
Alvy Ray Smith
July 17, 1995

Abstract

My purpose here is to, once and for all, rid the world of the misconception that a pixel is a little geometric square. This is not a religious issue. This is an issue that strikes right at the root of correct image (sprite) computing and the ability to correctly integrate (converge) the discrete and the continuous. The little square model is simply incorrect. It harms. It gets in the way. If you find yourself thinking that a pixel is a little square, please read this paper. I will have succeeded if you at least understand that you are using the model and why it is permissible in your case to do so (is it?).

What is a digital image

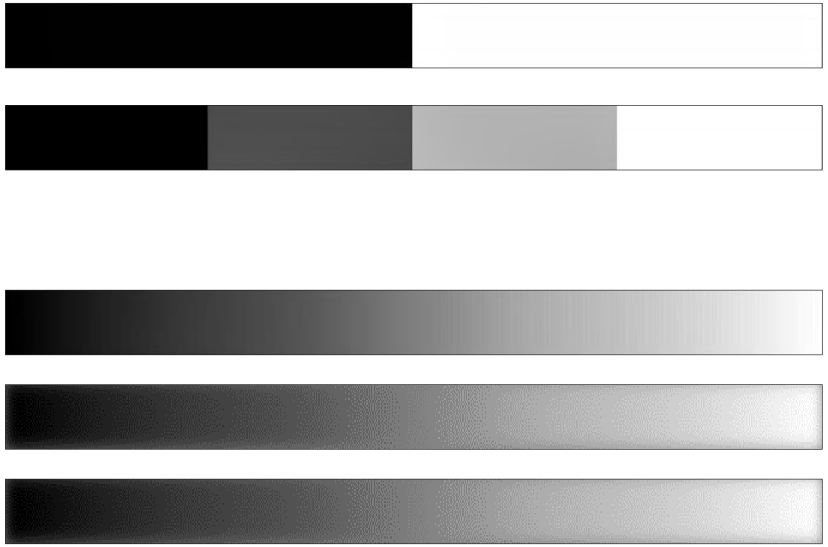
Grayscale image consists of a finite number of **pixels** (2D) or **voxels** (3D)



Cardona A, et al. 2010. An Integrated Micro- and Macroarchitectural Analysis of the Drosophila Brain by Computer-Assisted Serial Section Electron Microscopy.

What is a digital image

Bit depth of an image defines how many different grey values can be detected in the data



1-bit image (2^1) has 2 possible numerical intensity values

2-bit image (2^2) has 4 possible numerical intensity values

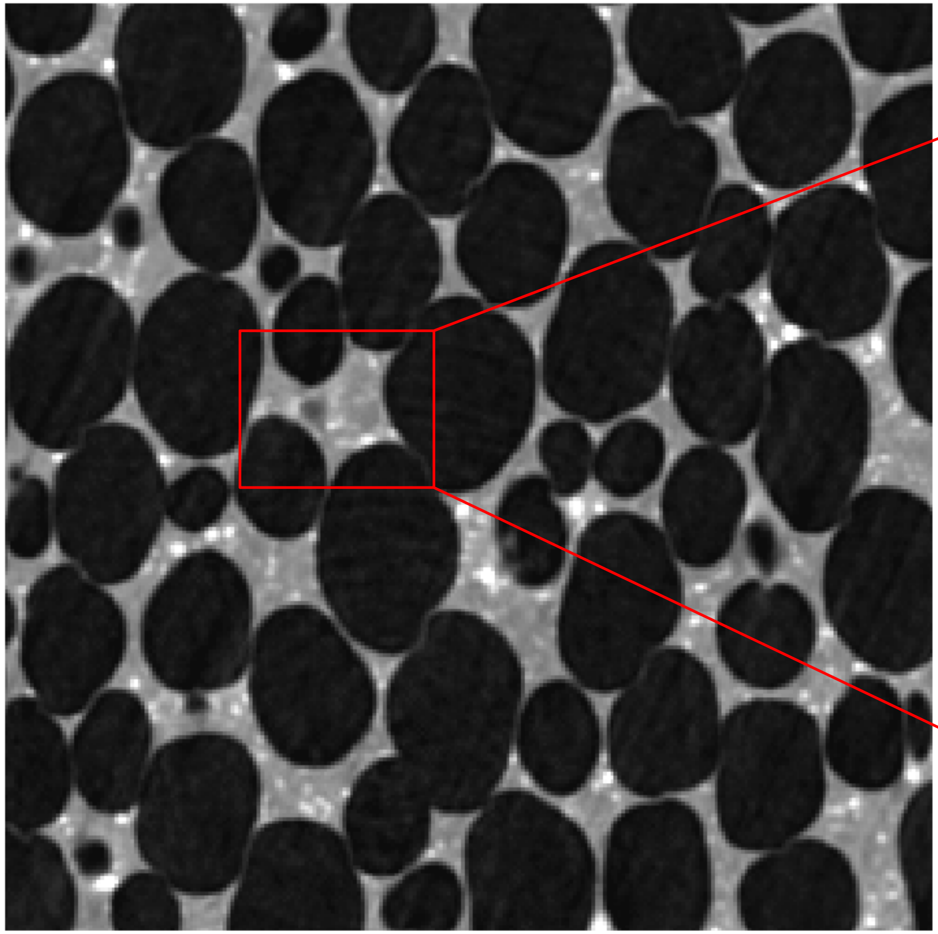
8-bit image (2^8) has 256 possible numerical intensity values

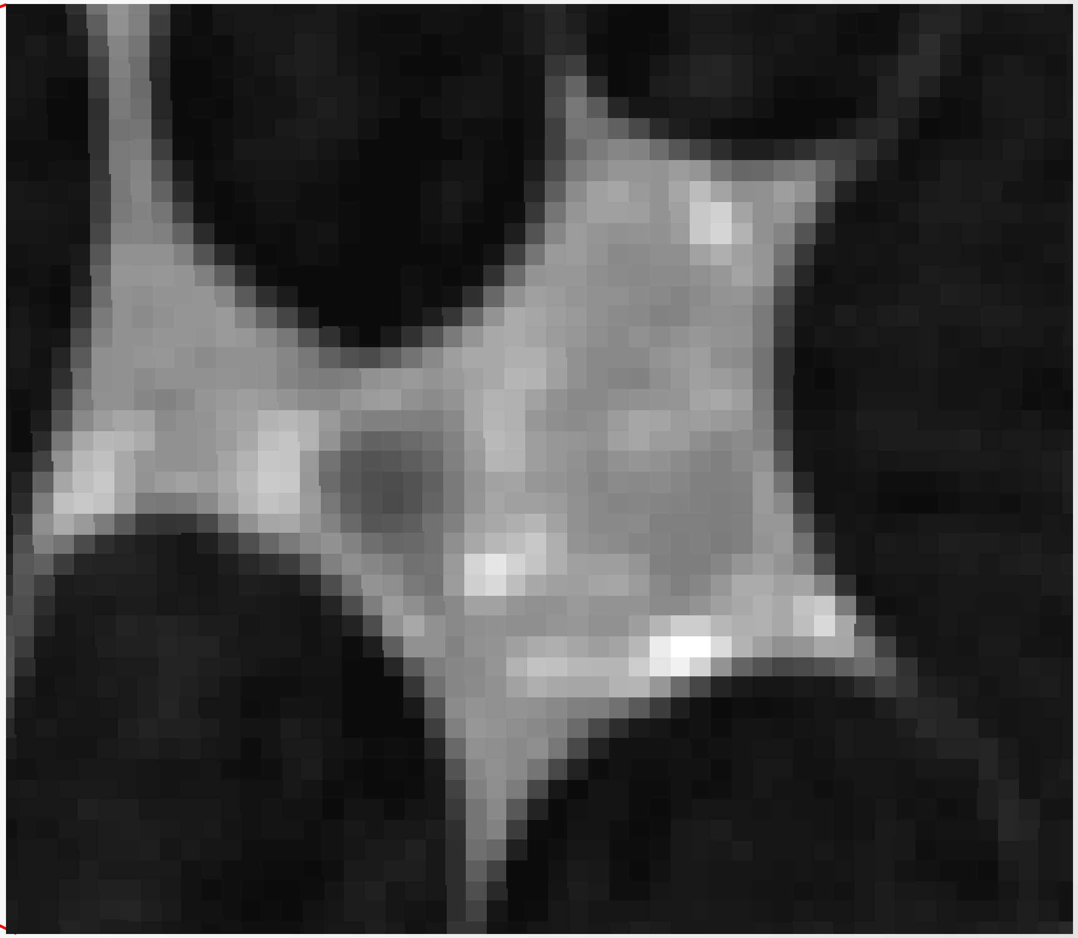
16-bit image (2^{16}) has 65,536 possible numerical intensity values

32-bit image (2^{32}) has 2,147,483,647 possible numerical intensity values

Intensity Image

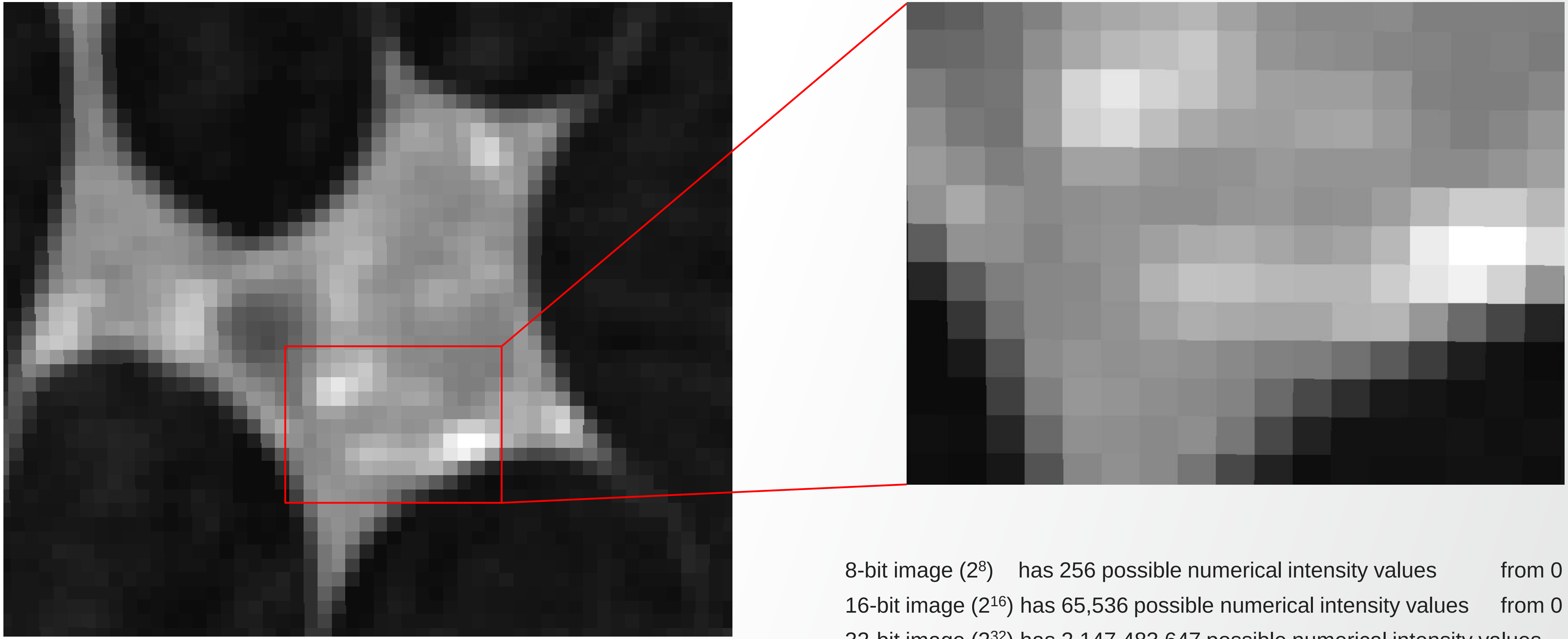
Grayscale Pixels





Intensity Image

Grayscale Pixels

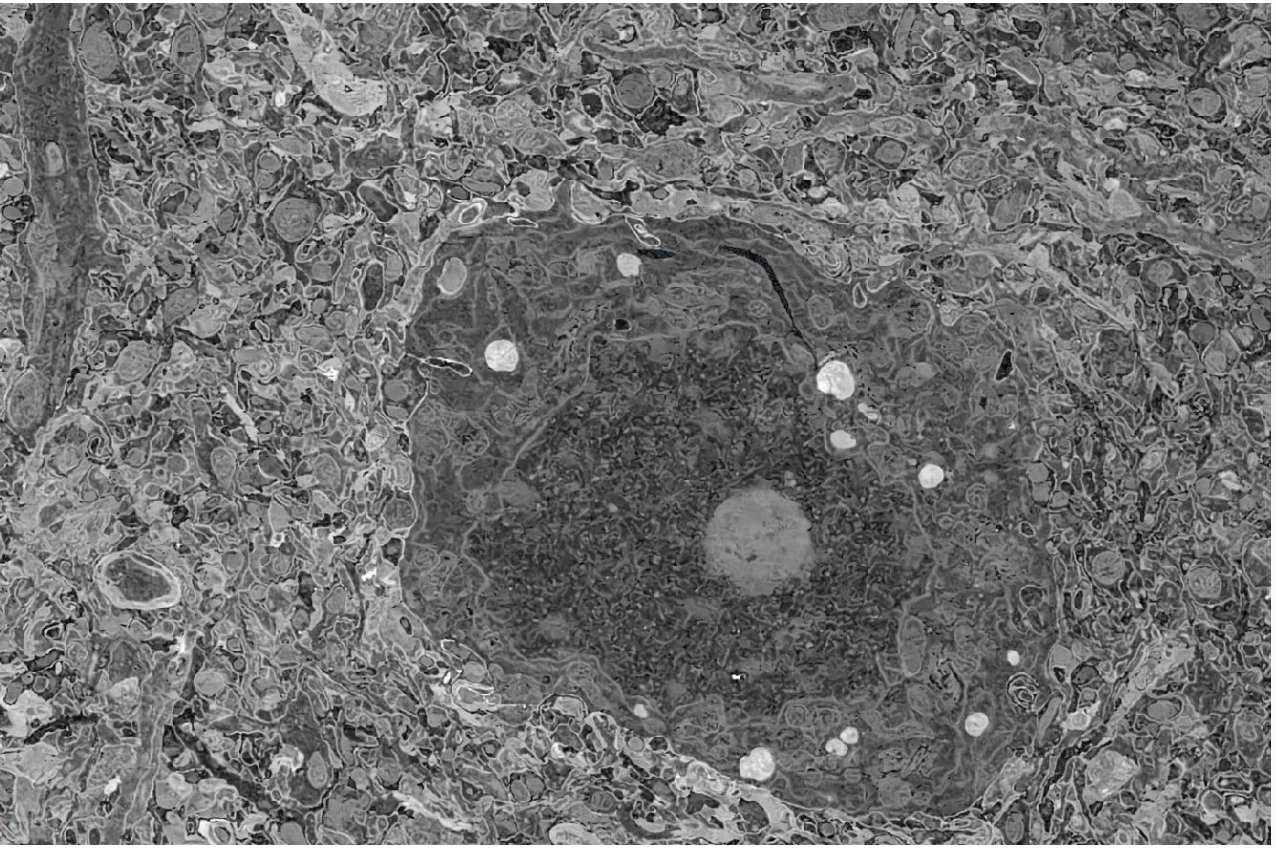


8-bit image (2^8) has 256 possible numerical intensity values from 0 to 255
16-bit image (2^{16}) has 65,536 possible numerical intensity values from 0 to 65,535
32-bit image (2^{32}) has 2,147,483,647 possible numerical intensity values

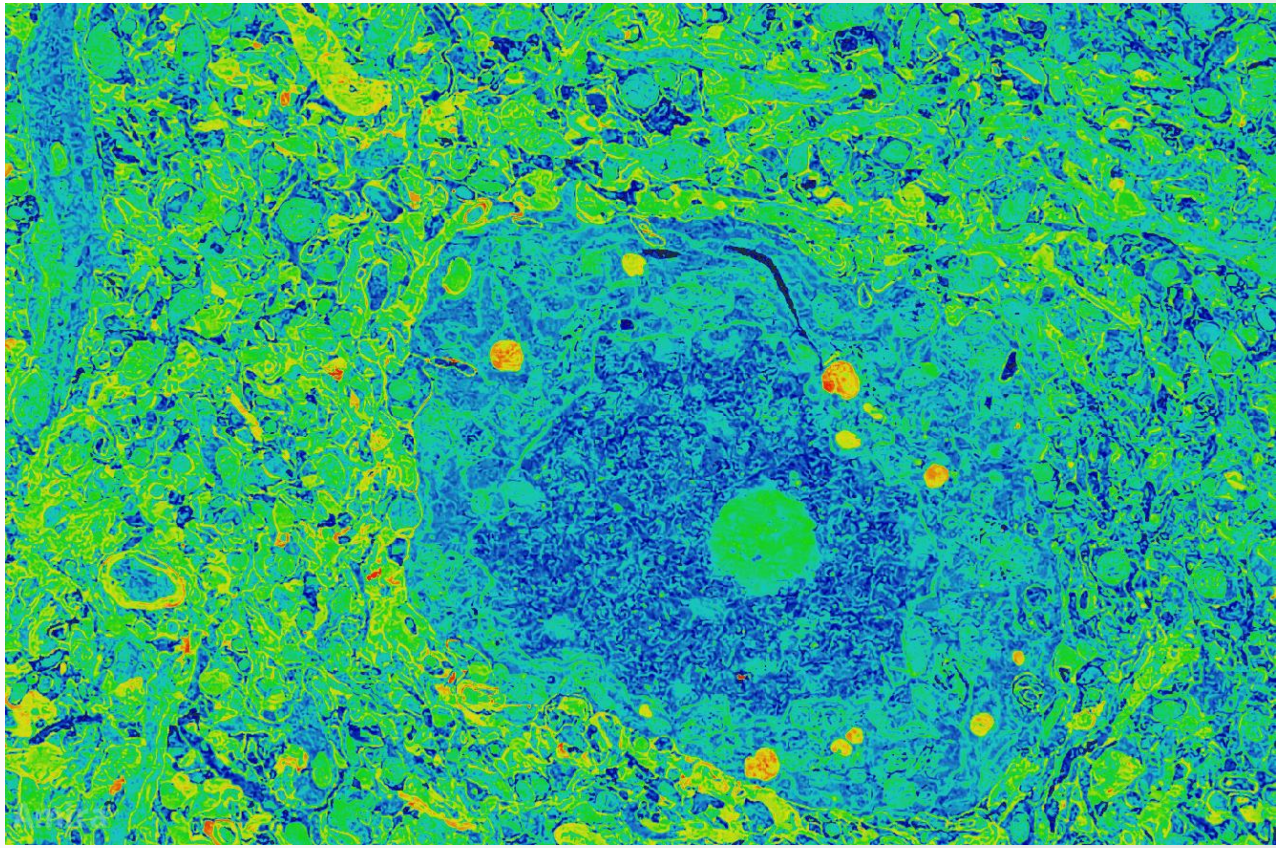
What is a digital image

Look-up tables assign color and transparency to each intensity value

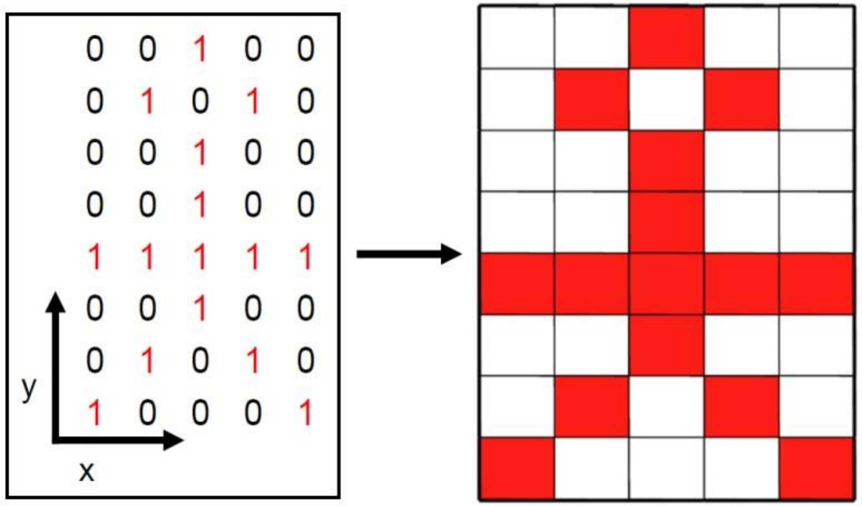
Grayscale



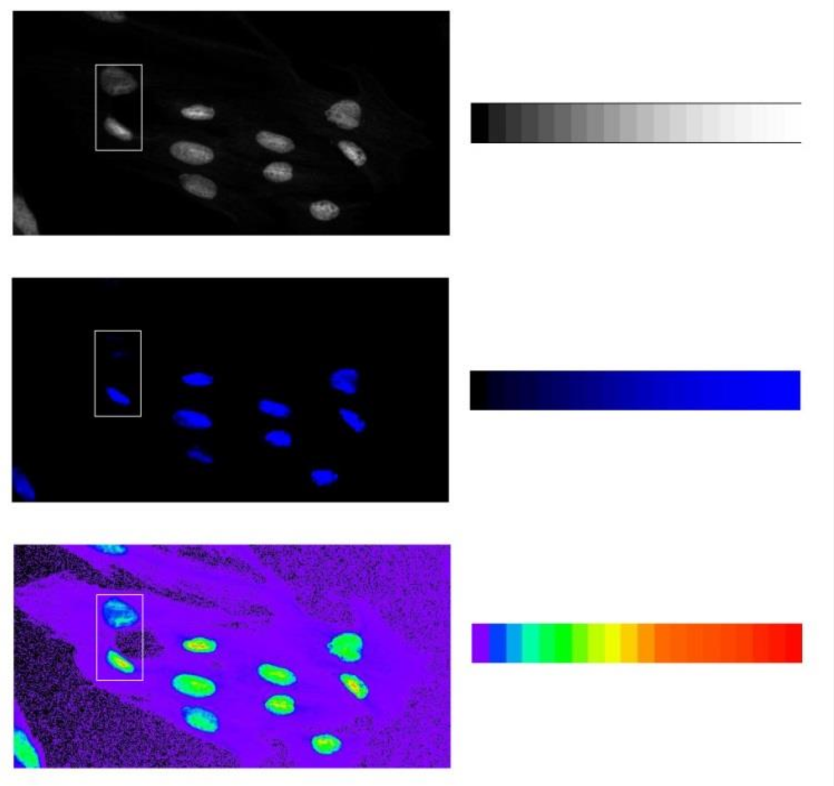
Physics



Data visualization: Colormap



Data **Visualization**



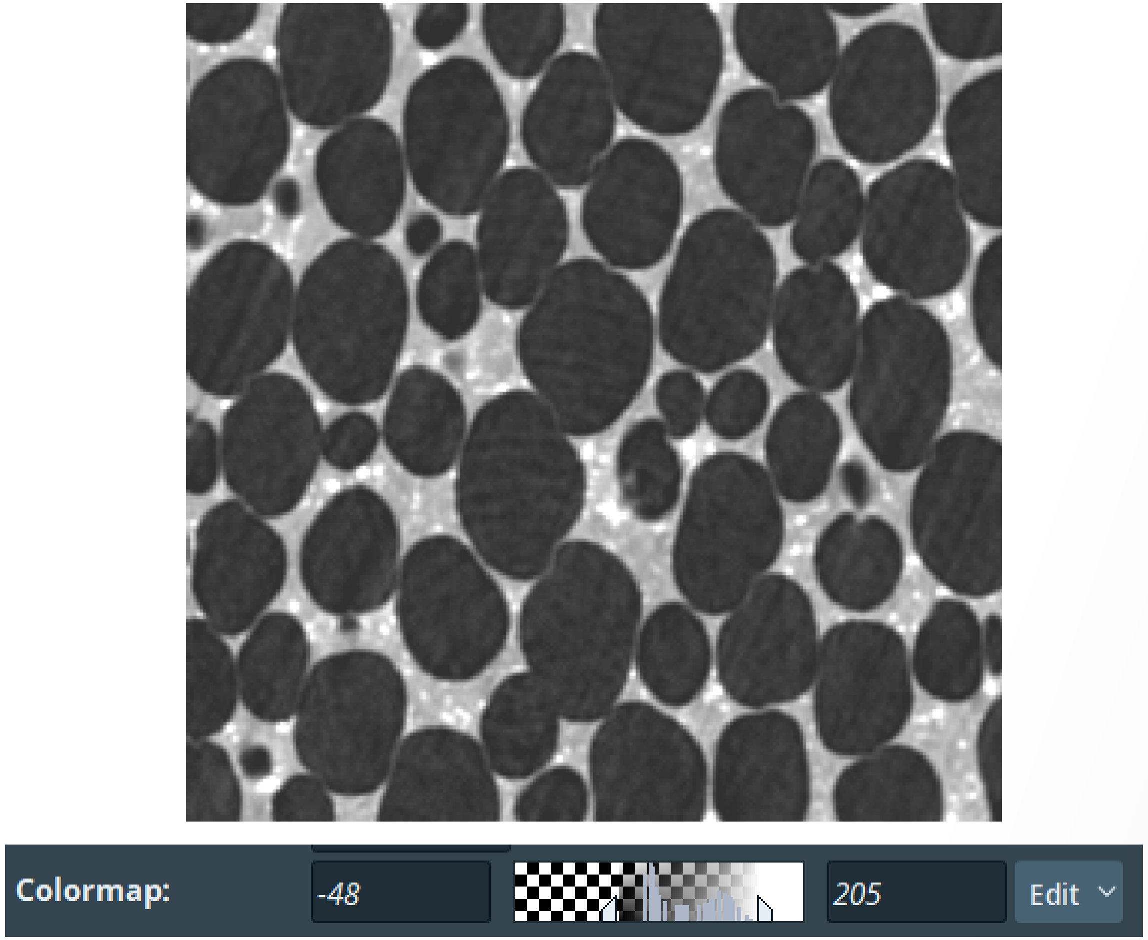
Gray

Blue

Physics

Color Mapping

Grayscale Color Mapping



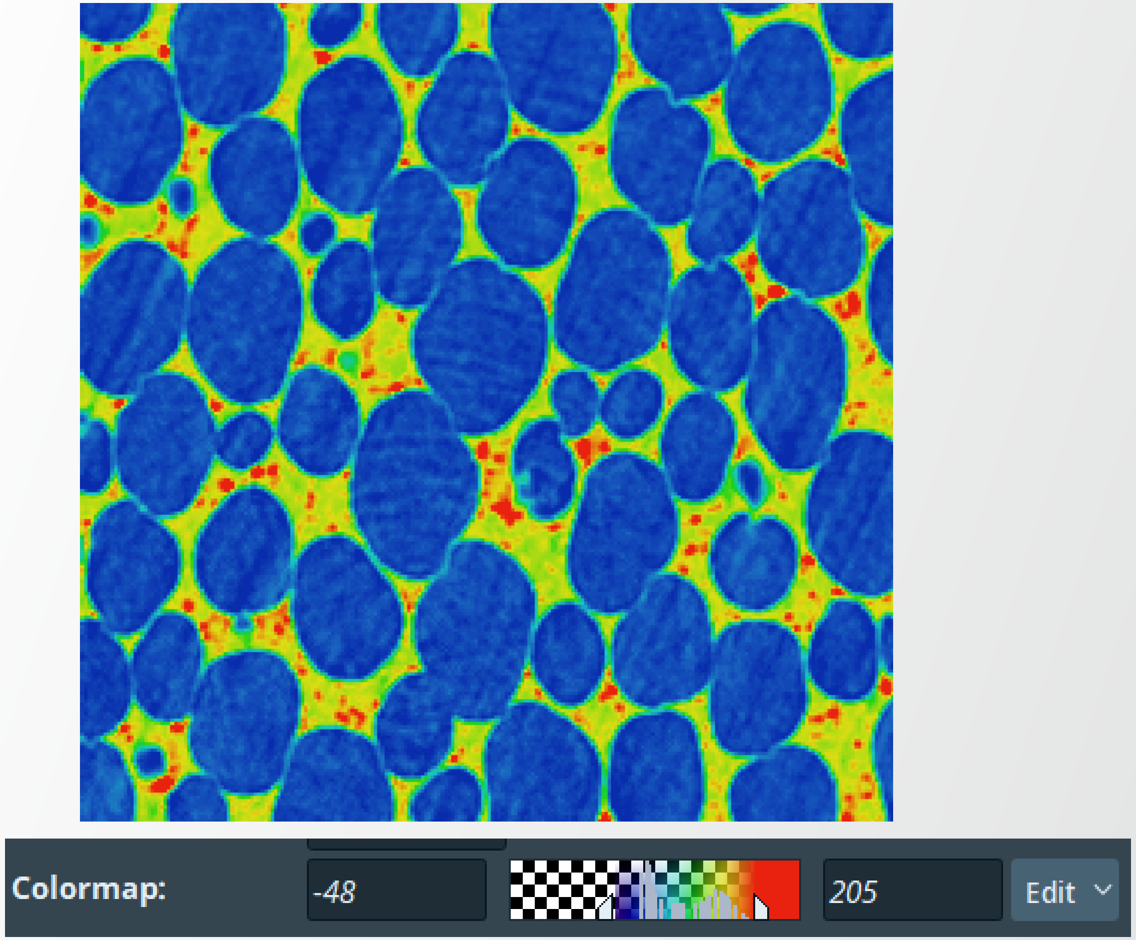
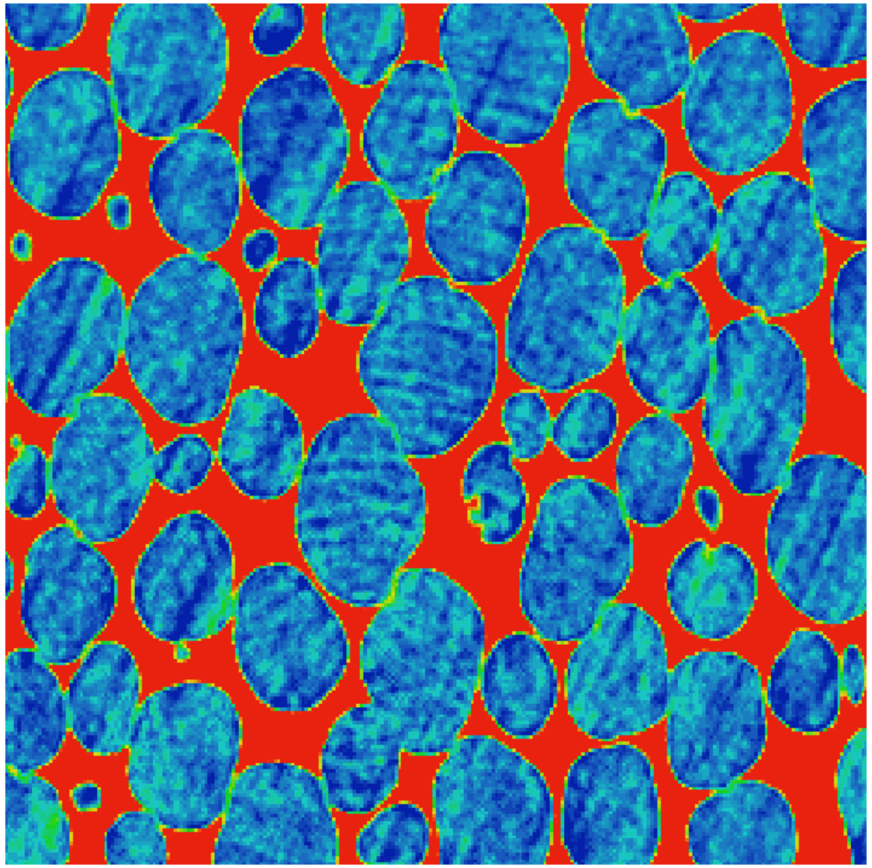


Image Visualization

2-Dimensional vs 3-Dimensional images (2D vs 3D)

2D Plane vs 3D Volume



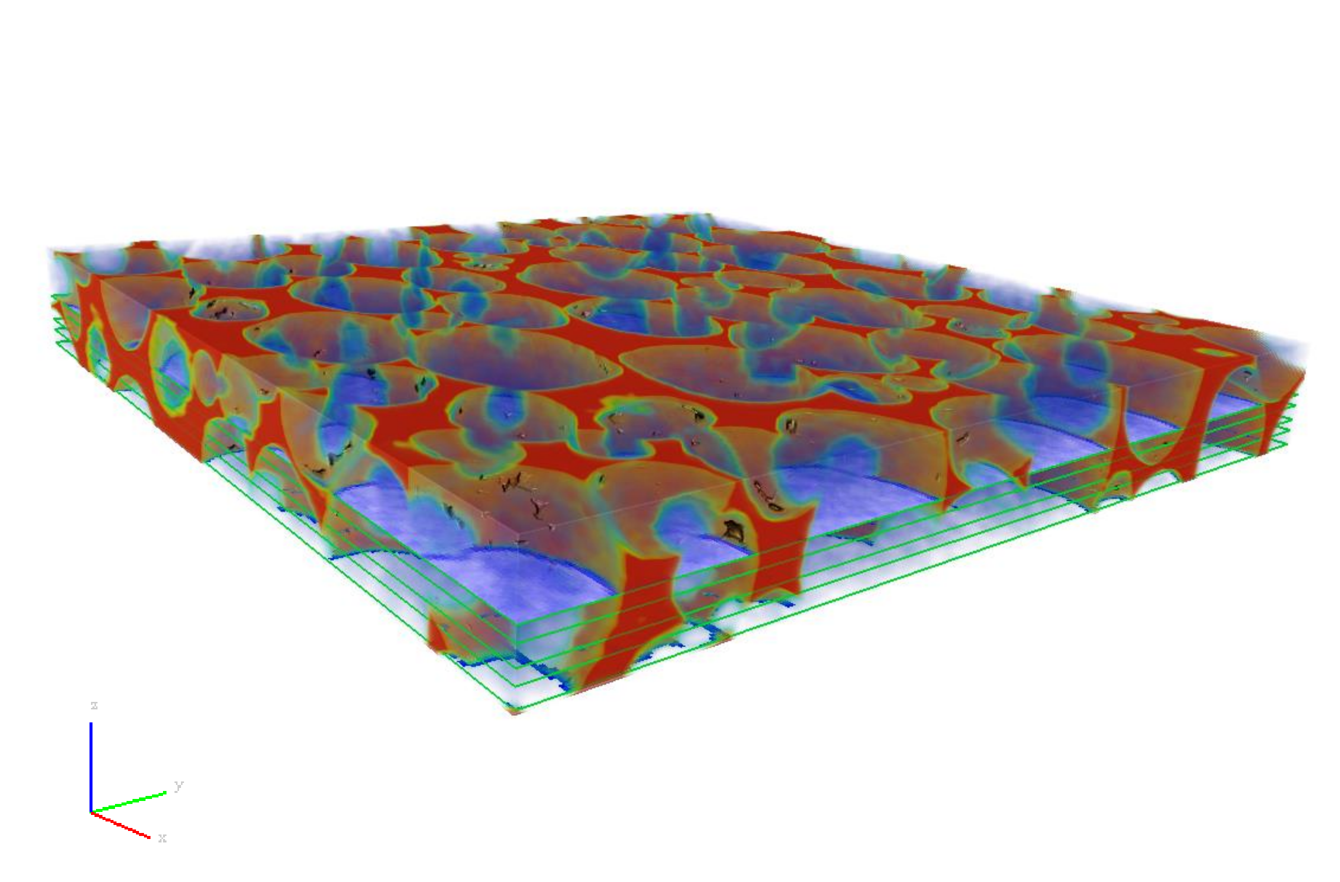


Image Visualization

Visualization of 2D & 3D Image Data

- **2D & 3D interactive scene**
- **Direct manipulation of objects**
- **Numerous visualization options:**
  - 2D Slices, with overlays
  - 3D Volume rendering
  - Surface rendering
  - Projections
  - Meshes, graphs
  - Isosurfaces
  - Histograms and curves

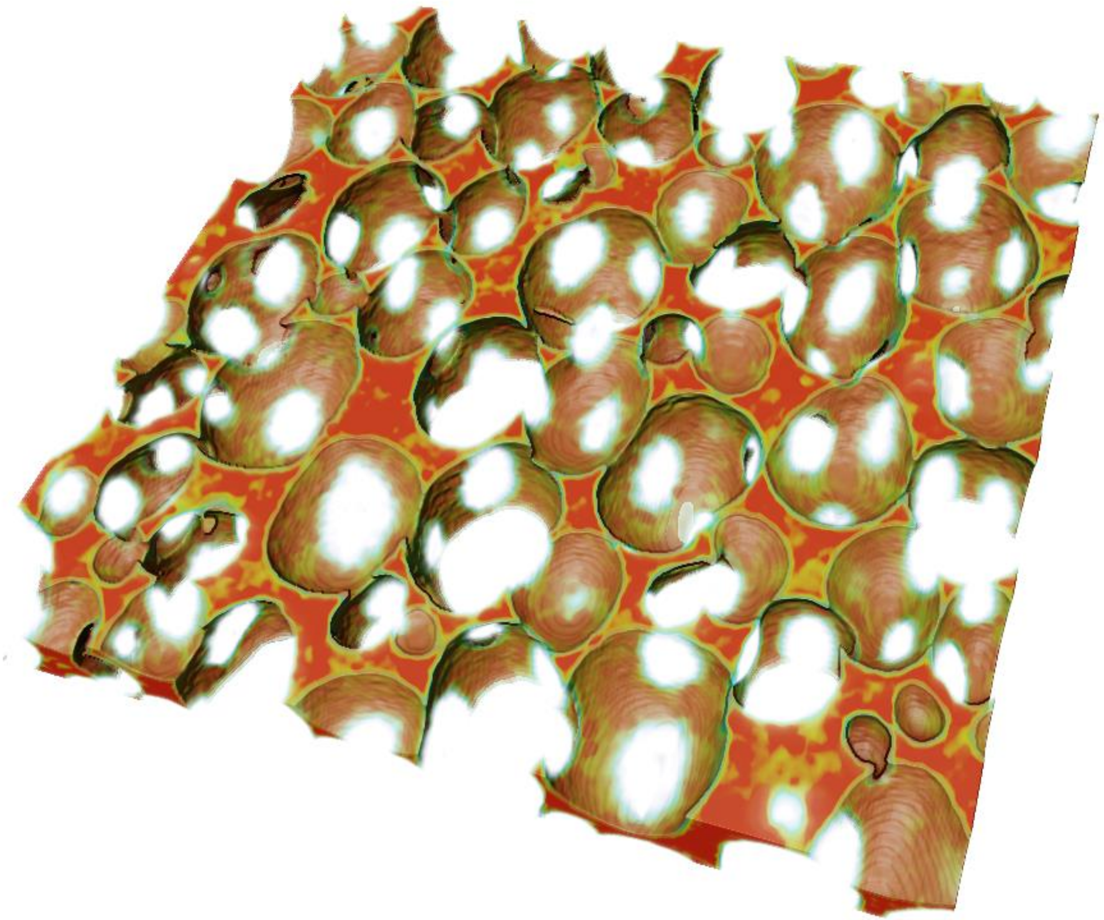


Image Visualization

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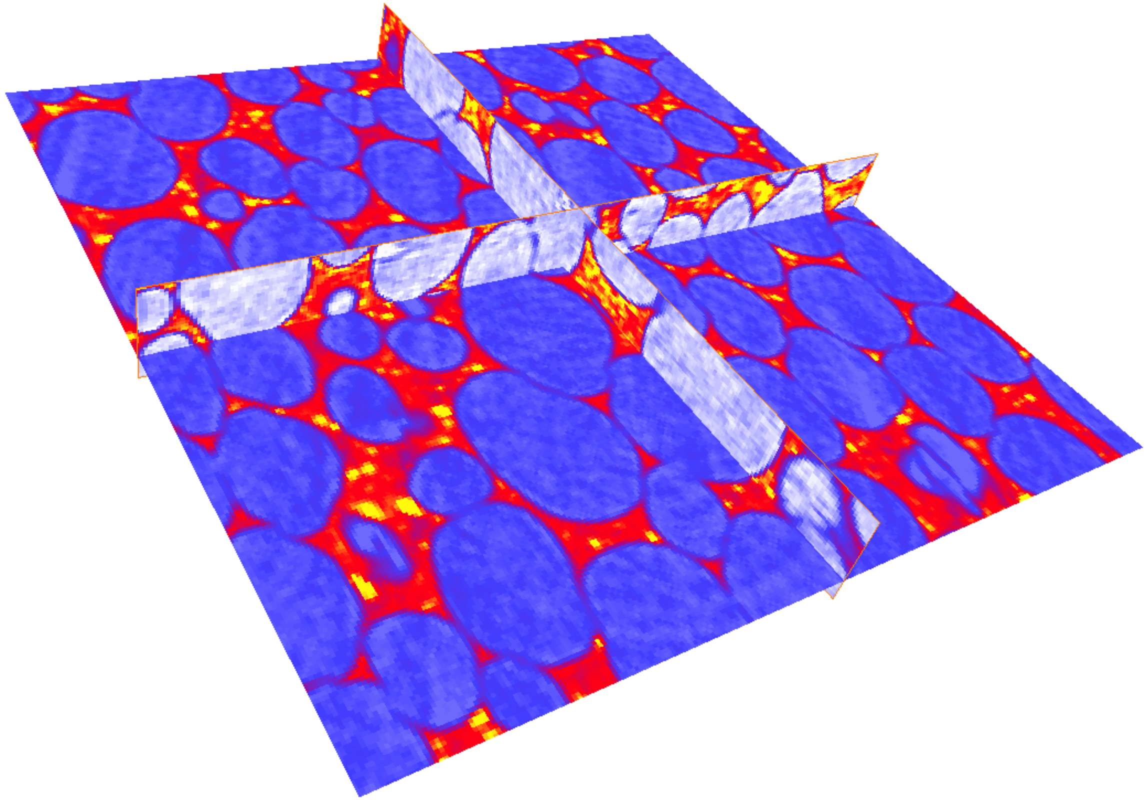
Visualization of 2D & 3D Image Data

- 2D & 3D interactive scene
- Direct manipulation of objects
- Numerous visualization options:
  - Ortho Slices



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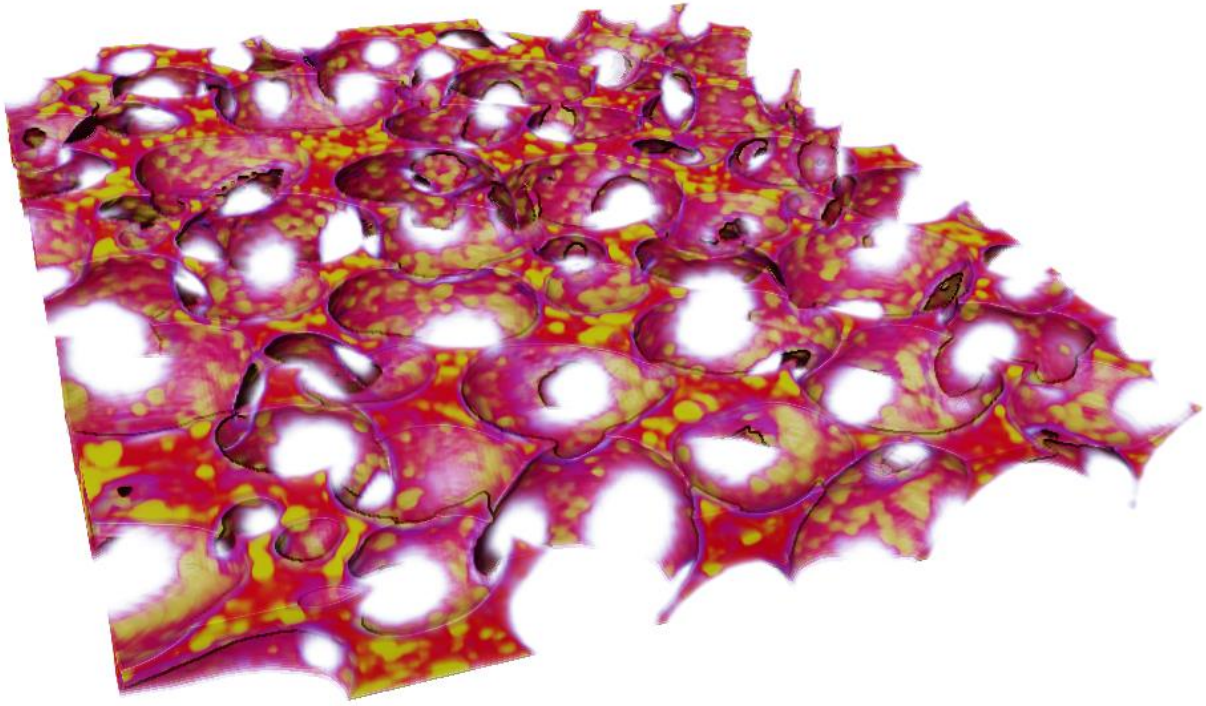

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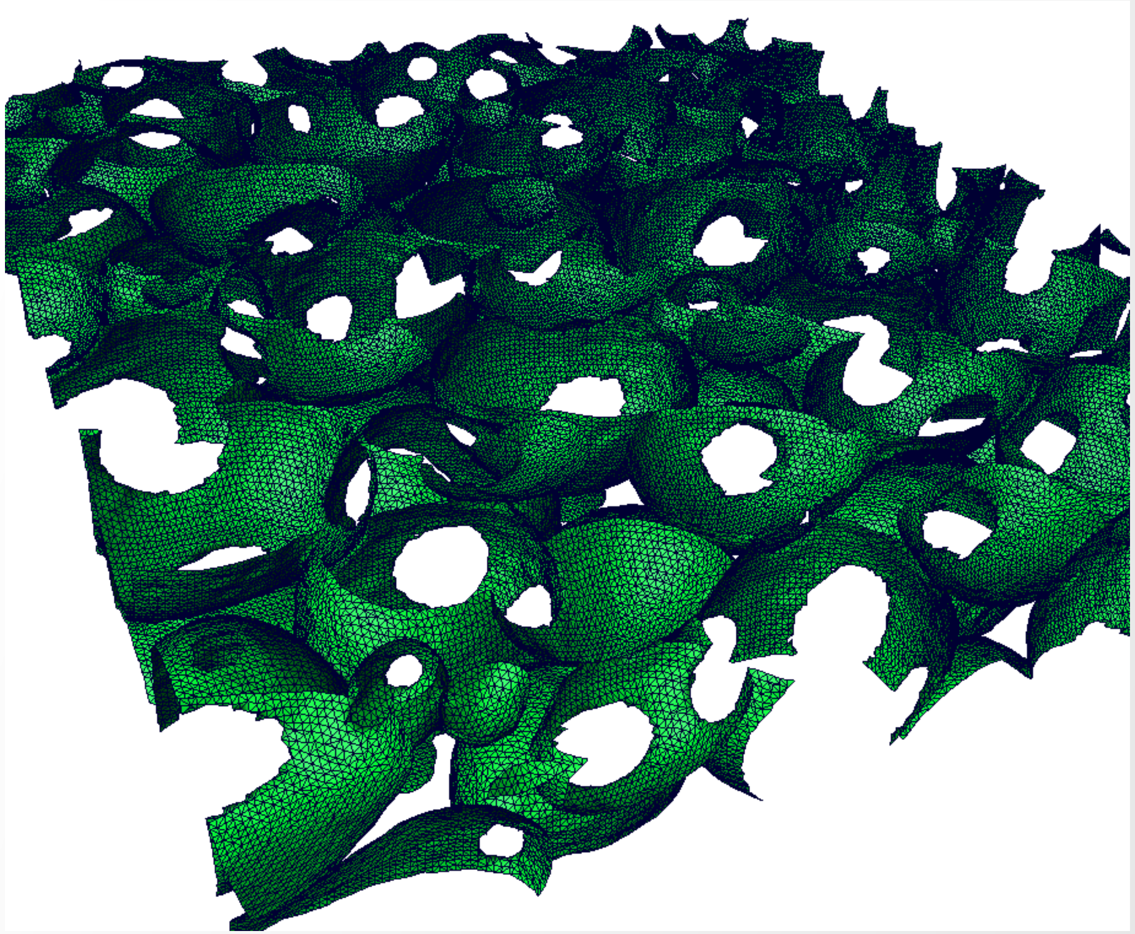

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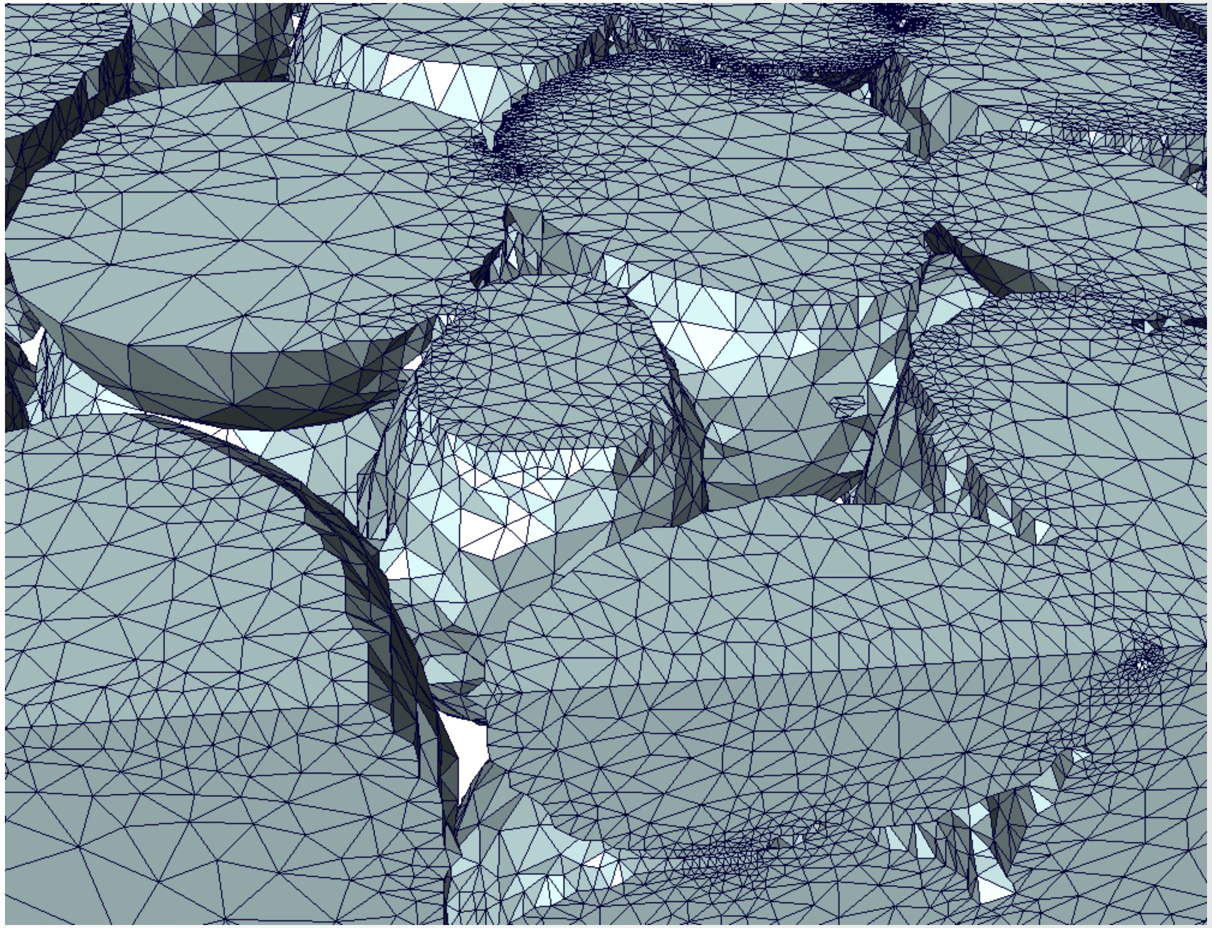
  - Meshes



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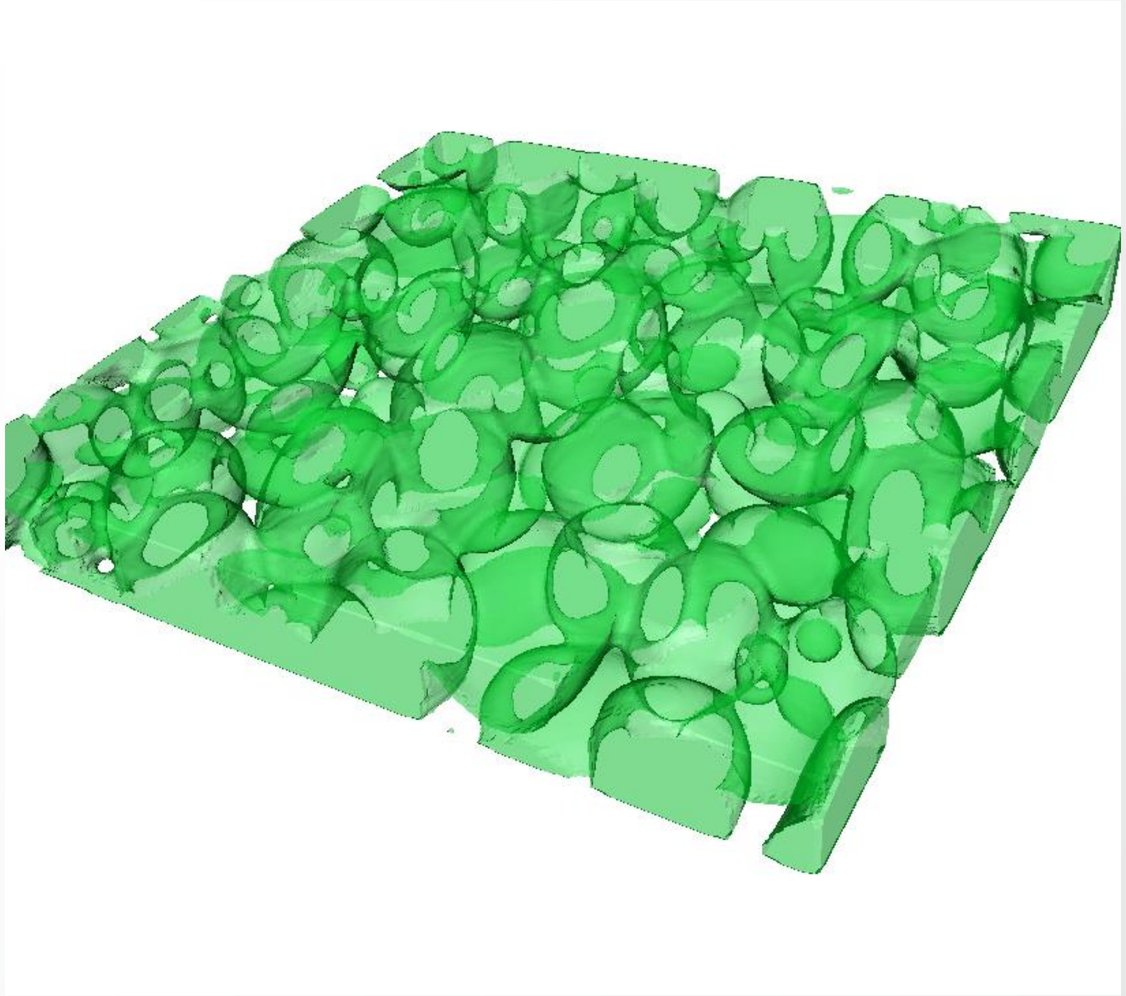

Image Pre-Processing

CT Reconstruction Artefacts

- Cone-beam
- Beam-hardening
- Phase-contrast
- Object motion
- Ring artefacts
- Scattering
- Metal-artefacts
- Inaccurate geometry
- Too few angles
- Missing angles

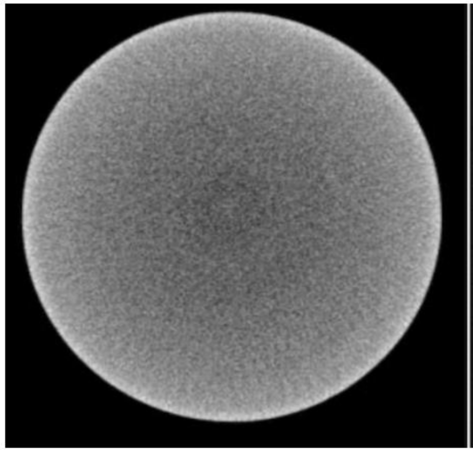
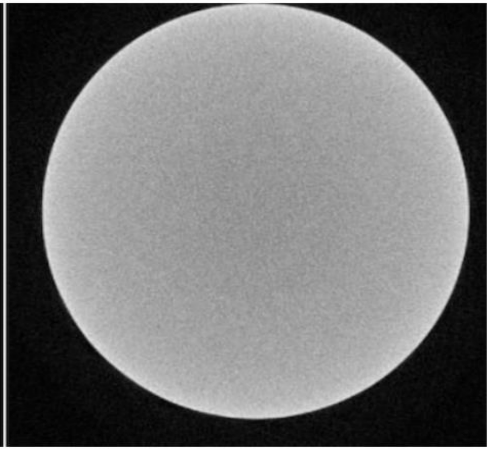
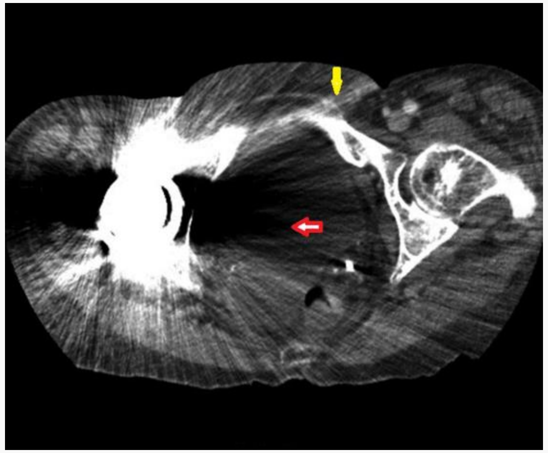
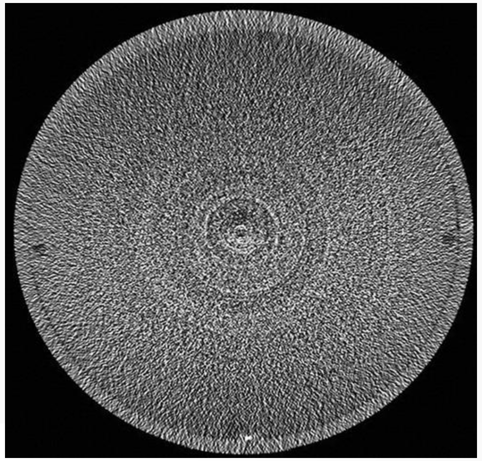
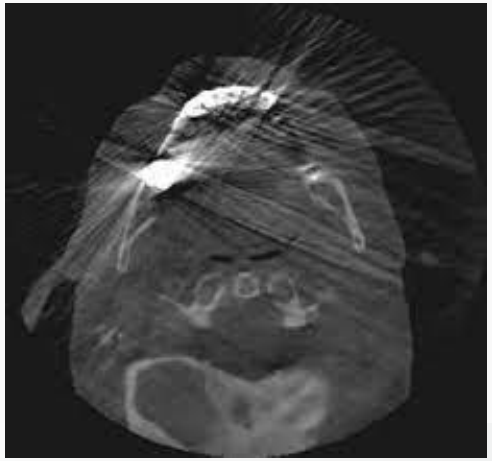
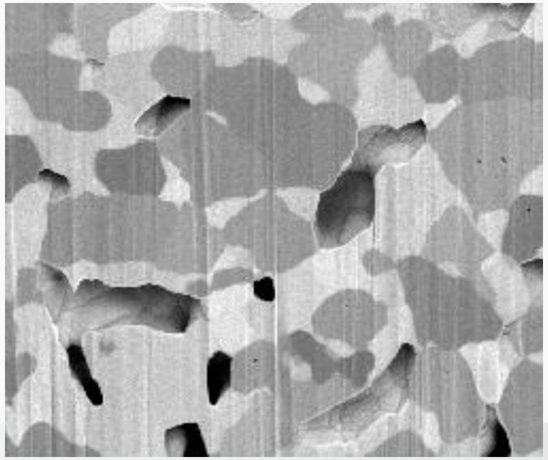

Image Pre-Processing

Denoising and Artifact Removing

- **Noise reduction**
  - Gaussian, median, Anisotropic diffusion
  - Non-local means
- **Image artefacts reduction**
  - Curtaining
  - Pore-back / shine-through
  - Slice alignment
- **Background correction**
  - Shading correction
- **Deconvolution**

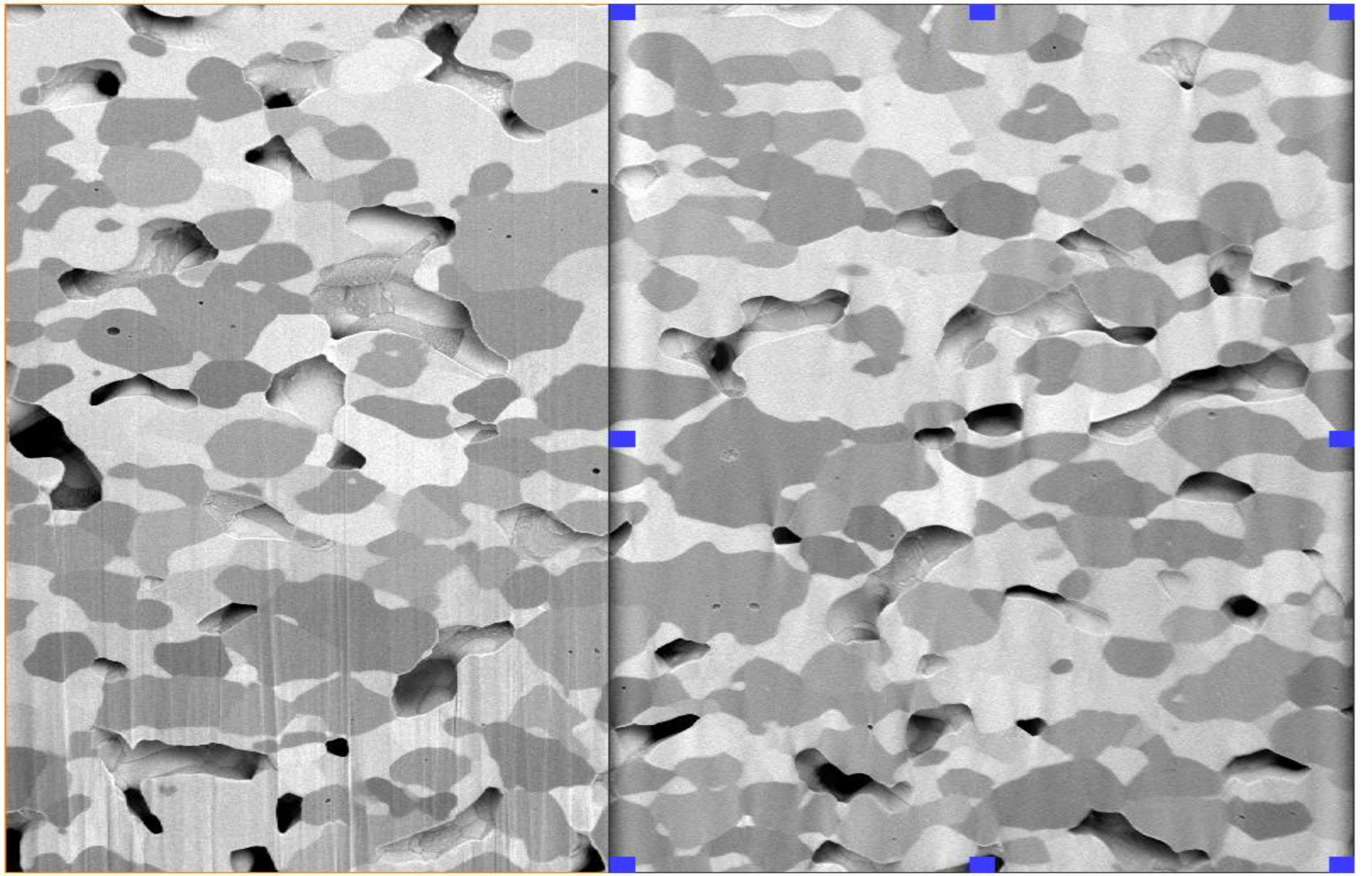

Image Pre-Processing

Denoising and Artifact Removing

- **Noise reduction**
  - Gaussian, median, Anisotropic diffusion
  - Delineate
  - Unsharp Masking, Non-local means

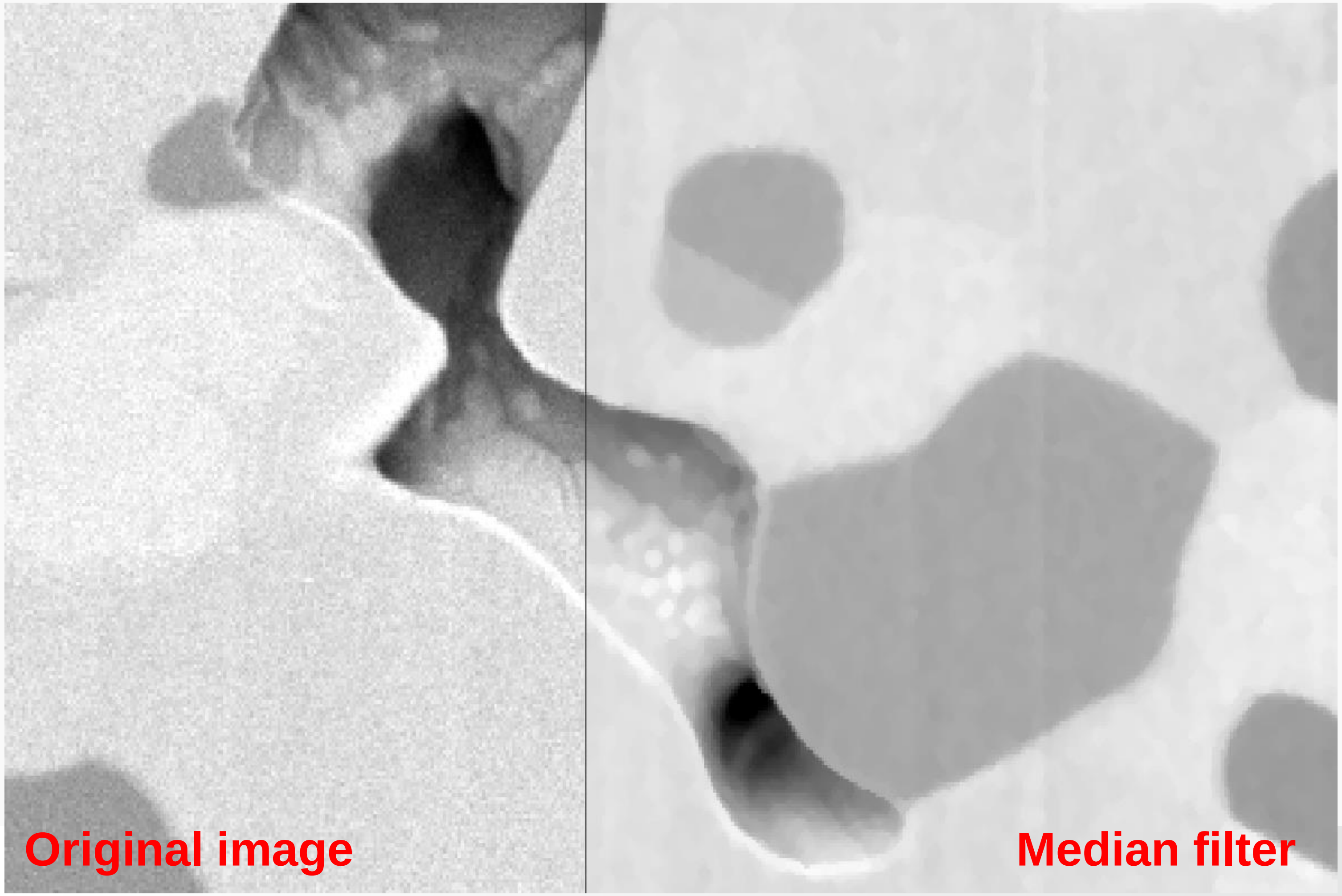

Image Pre-Processing

Denoising and Artifact Removing

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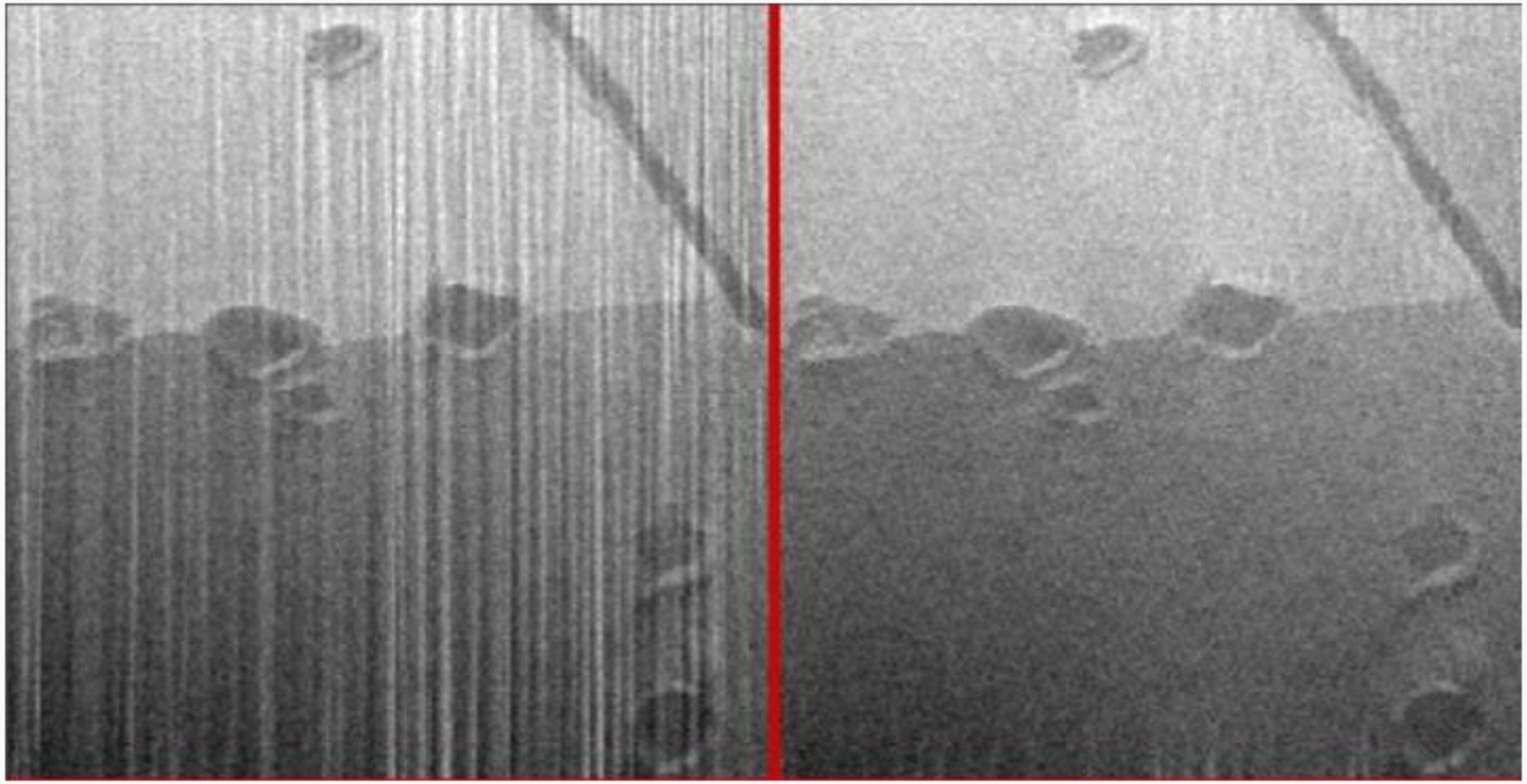

Image Pre-Processing

Denoising and Artifact Removing

- Noise reduction
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  - Slice alignment
- **Background correction**
  - Shading correction

Image Segmentation

Extracting meaningful information from images

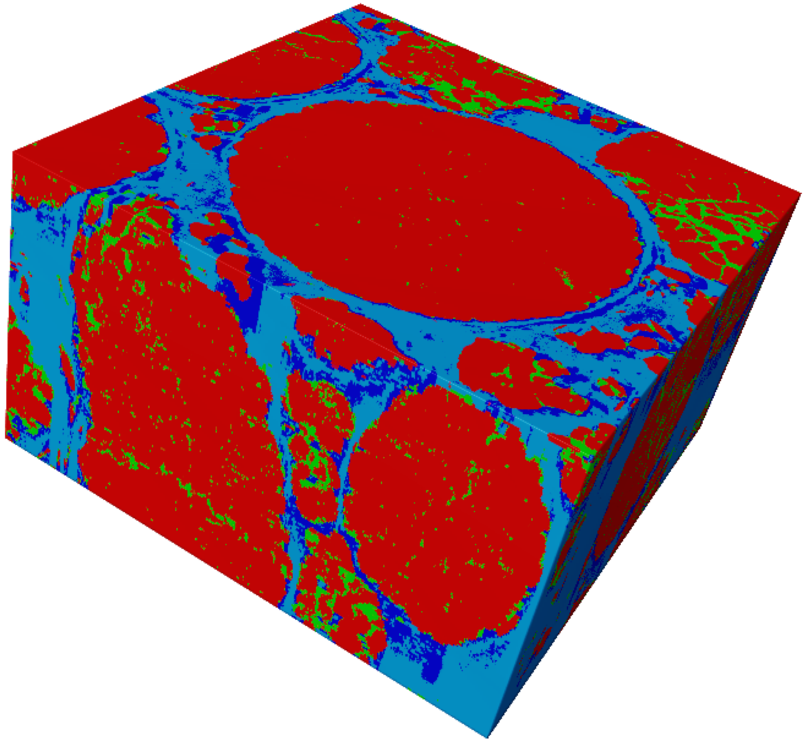


Image segmentation is a method of **dividing a digital image into subgroups called image segments**, reducing the complexity of the image and enabling further processing or analysis of each image segment.

Technically, segmentation is the assignment of labels to pixels to identify objects, cells, organelles or other important elements in the image.

Image Segmentation Principles

Thresholding: Binarization of graysale image into Label Image

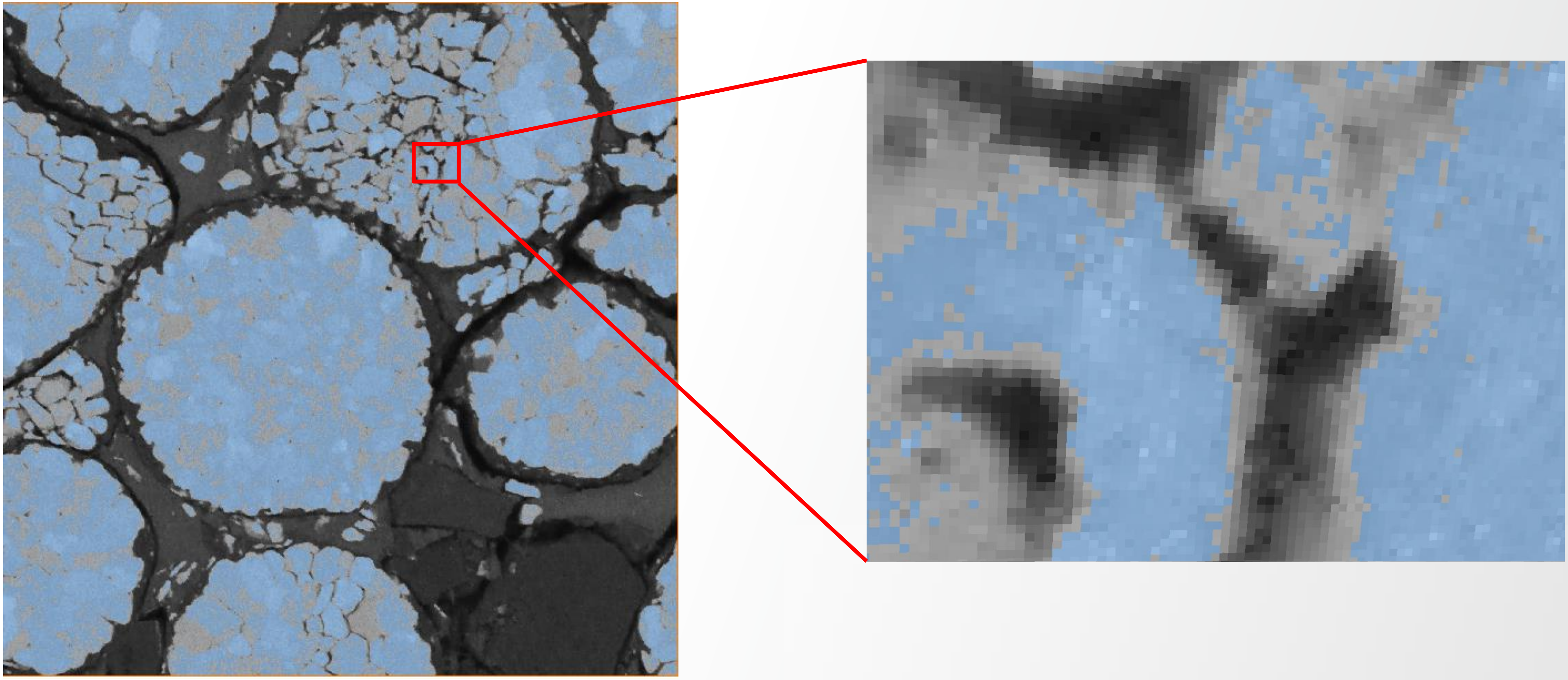
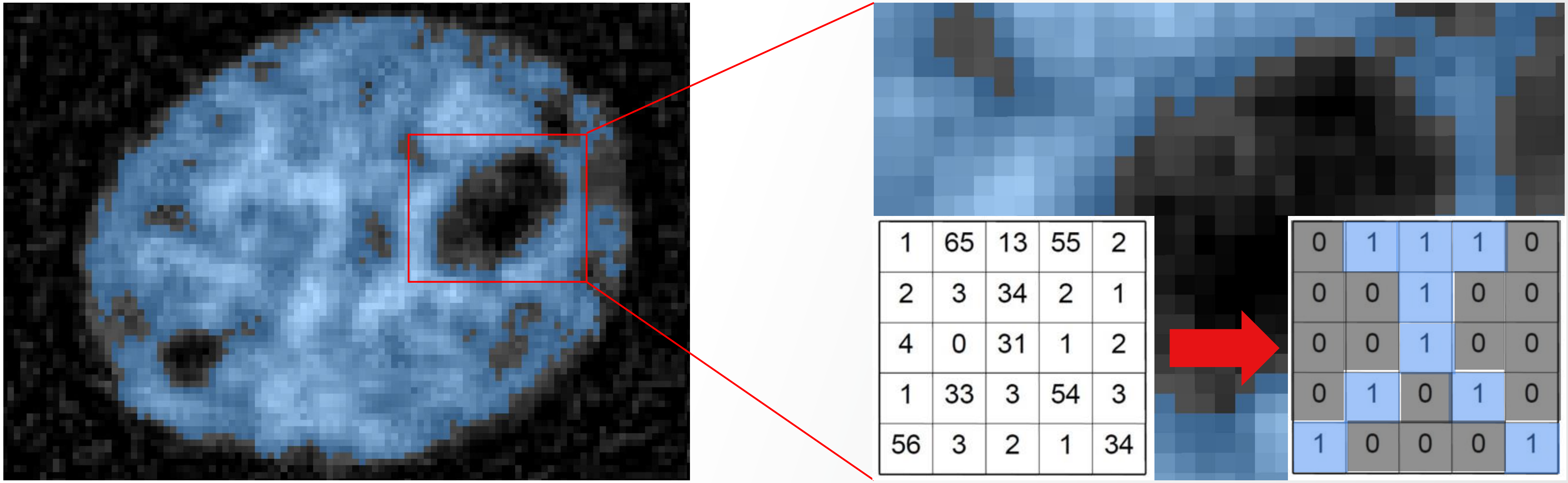


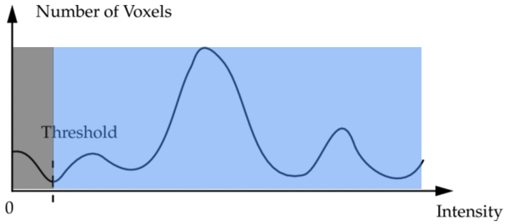
Image Segmentation Principles

Thresholding: Binarization of graysale image into Label Image



1	65	13	55	2
2	3	34	2	1
4	0	31	1	2
1	33	3	54	3
56	3	2	1	34

0	1	1	1	0
0	0	1	0	0
0	0	1	0	0
0	1	0	1	0
1	0	0	0	1



8-bit image (2^8) intensity values from 0 to 255

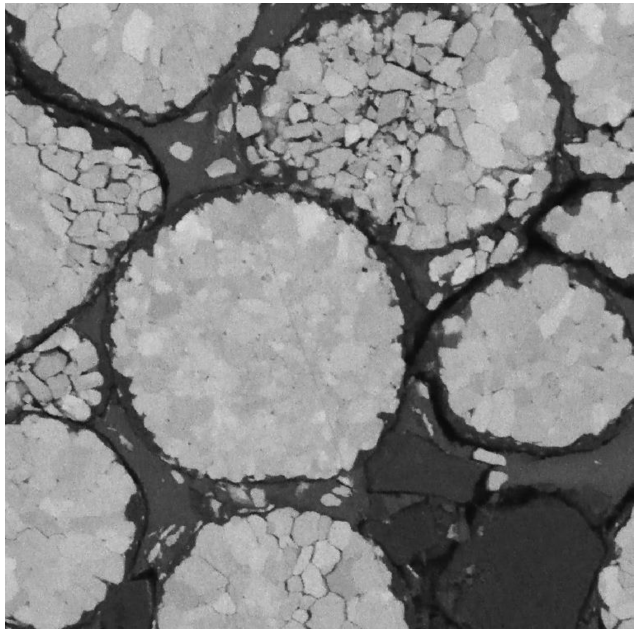
16-bit image (2^{16}) intensity values from 0 to 65,535

32-bit image (2^{32}) intensity values from 0 to 2,147,483,647

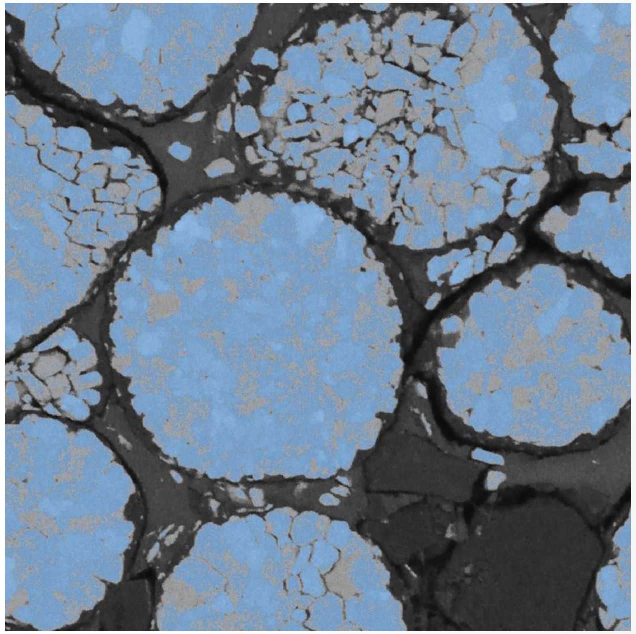
→ **Binary Image (2 possible) values 0 or 1**

Image Segmentation Principles

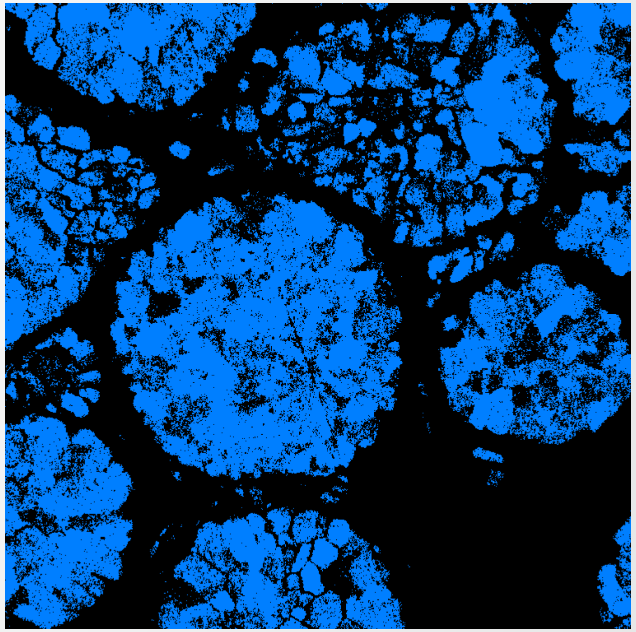
Thresholding: Binarization of graysale image into Label Image



Grayscale (intensity) Image



Thresholding (Binary mask)

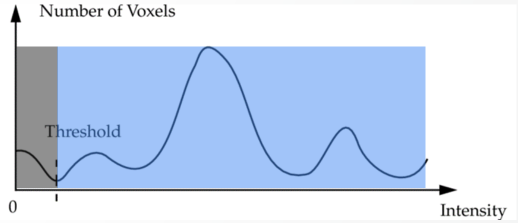


Binary (Label) Image

8-bit image (2^8) intensity values from 0 to 255

16-bit image (2^{16}) intensity values from 0 to 65,535

32-bit image (2^{32}) intensity values from 0 to 2,147,483,647



Binary Image (2 possible) values 0 or 1

Image Segmentation Principles

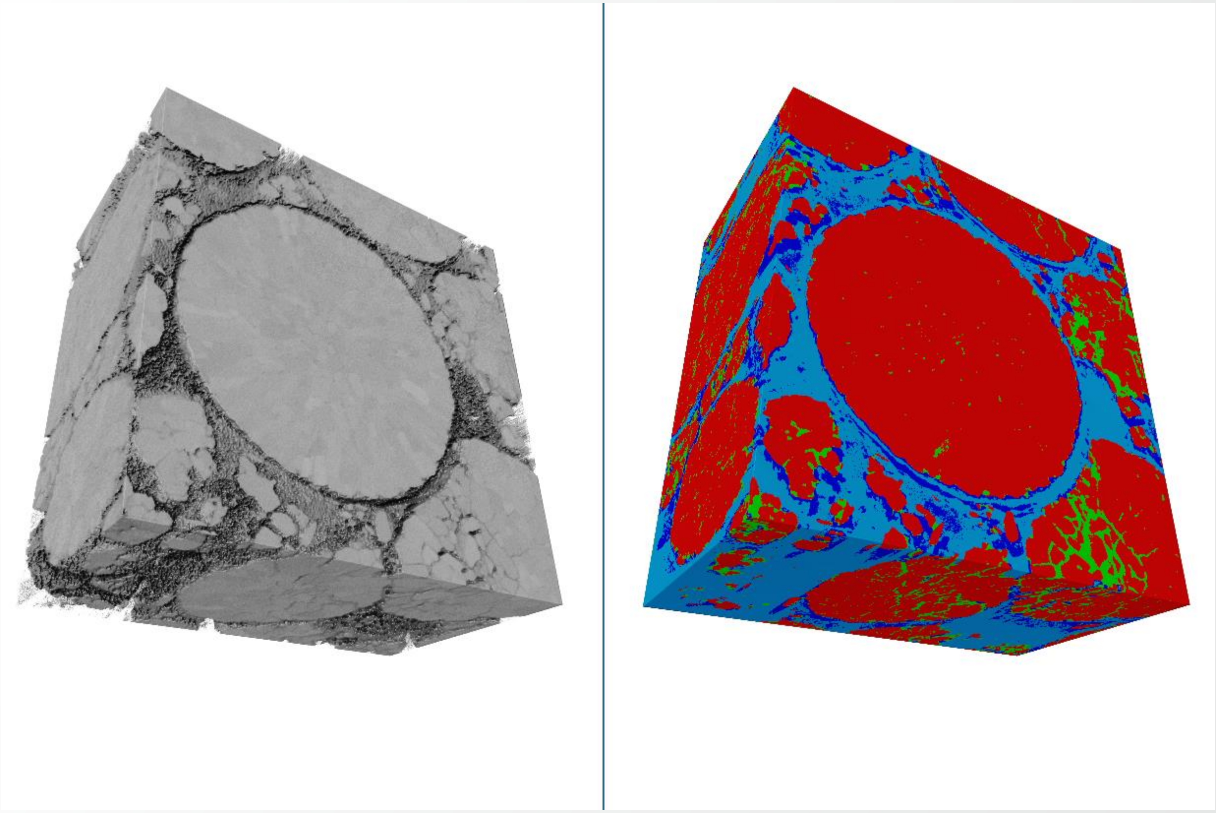
Binarization and Separation Methods

- **Thresholding**
  Global Thresholding, Local Thresholding, Multi-Thresholding, Auto-Thresholding

- **Watershed segmentation**
  Automatic edge detection
  Marker-based watershed with interactive / automatic markers

- **Mathematical morphology**
  Grayscale/binary morphology, variety of structuring elements
  Structure enhancement filter
  Membrane enhancement filter

- **Correlation-based Segmentation**
  Segmentation of regions that are co-localized in different channels of a multi-channel image

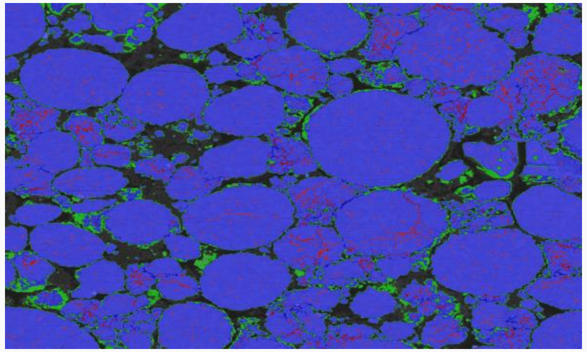


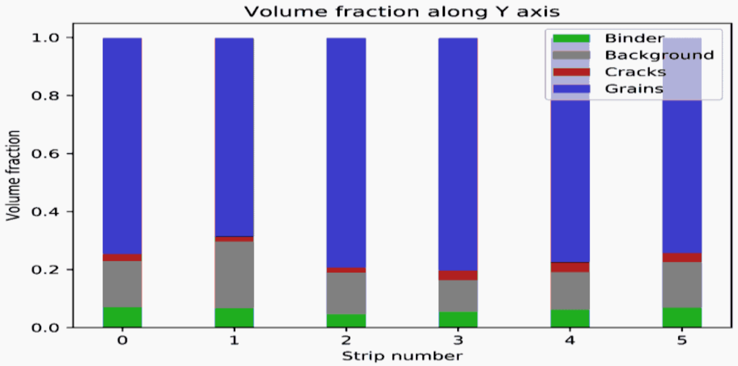
Data courtesy: Cell data: Mason Dean & Ronald Seidel (MPI Postdam-Golm), David Knoetel (Zuse Institute Berlin)
Brain data: Leslie Vosshall's Laboratory at HHMI-The Rockefeller University.
Membrane data: of Biophysical Fluorescence Laboratory Center For Bioimaging Sciences Departments of Biological Sciences & Chemistry National University Of Singapore

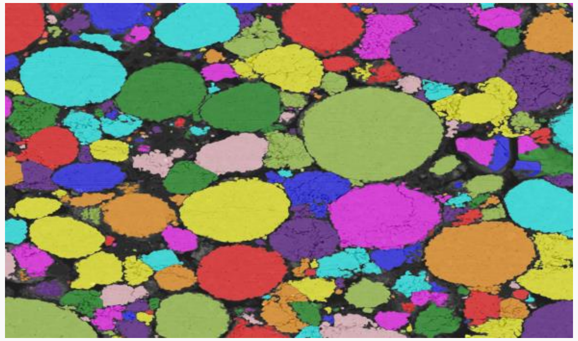
Analysis

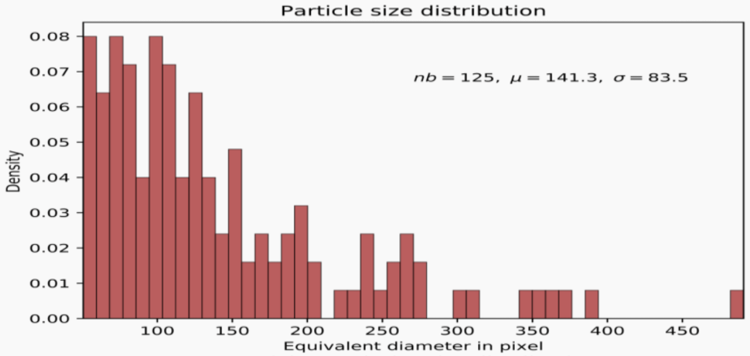
Shape and Size Distributions

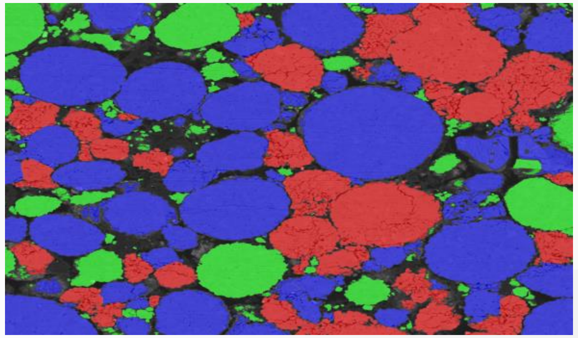
- Area, Volume, Surface
- Counting
- Intensity
- Distance, thickness
- Clustering
- Orientation, Length, Rugosity
- Co-localization
- Density

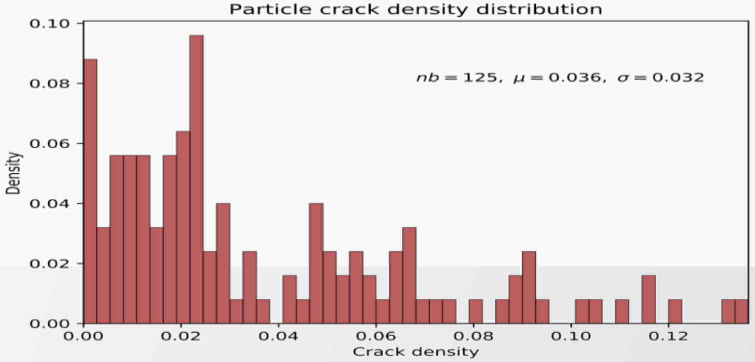












Complete Workflows

Summary: Step-by-step

- **Data import**

  - Reading image file formats & metadata
- **Visualization**

  - Display in 2D or 3D
- **Image Preprocessing**

  - Denoising
- **Segmentation**

  - Binarization (Thresholding)

  - Separation
- **Feature Extraction**

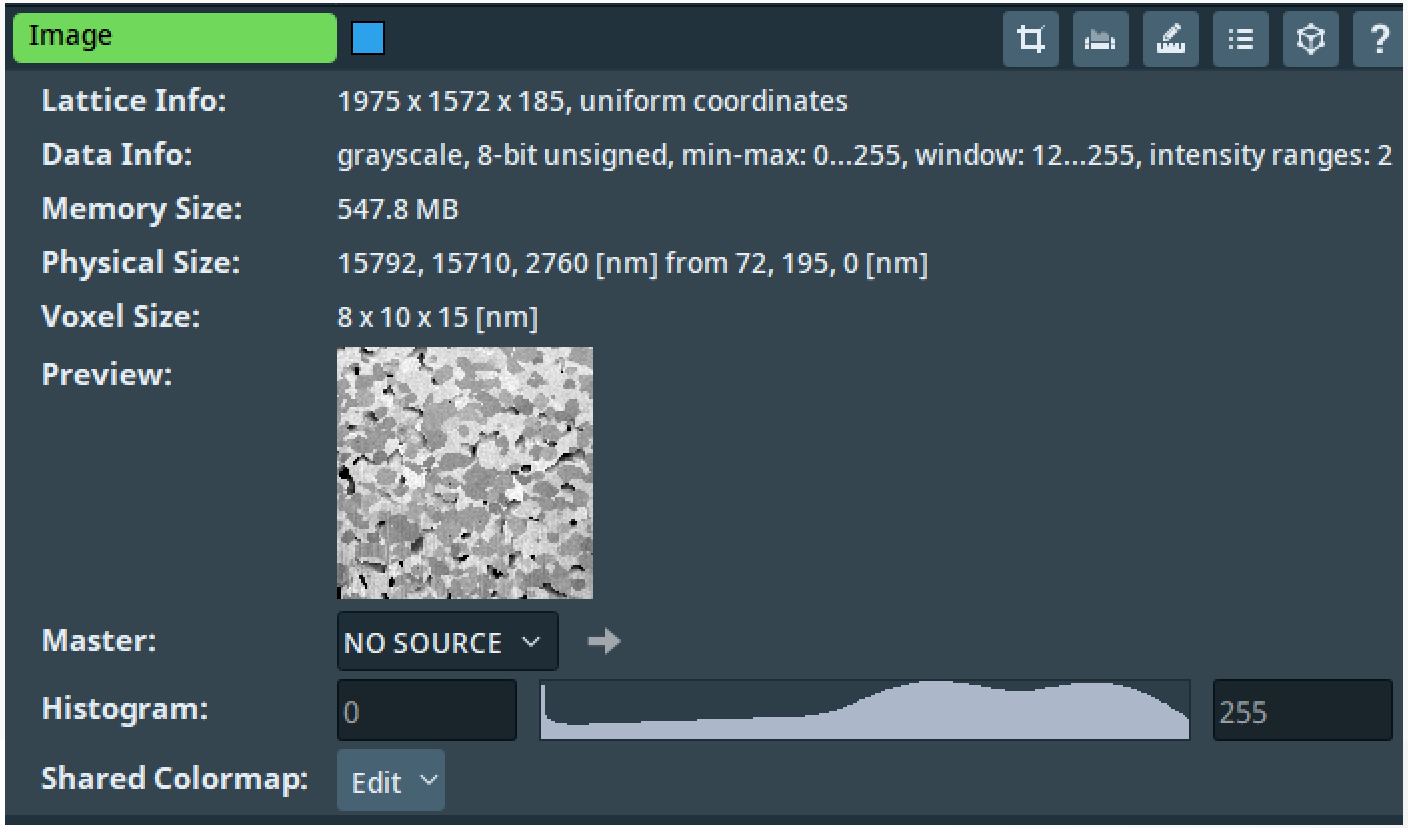
  - Statistic

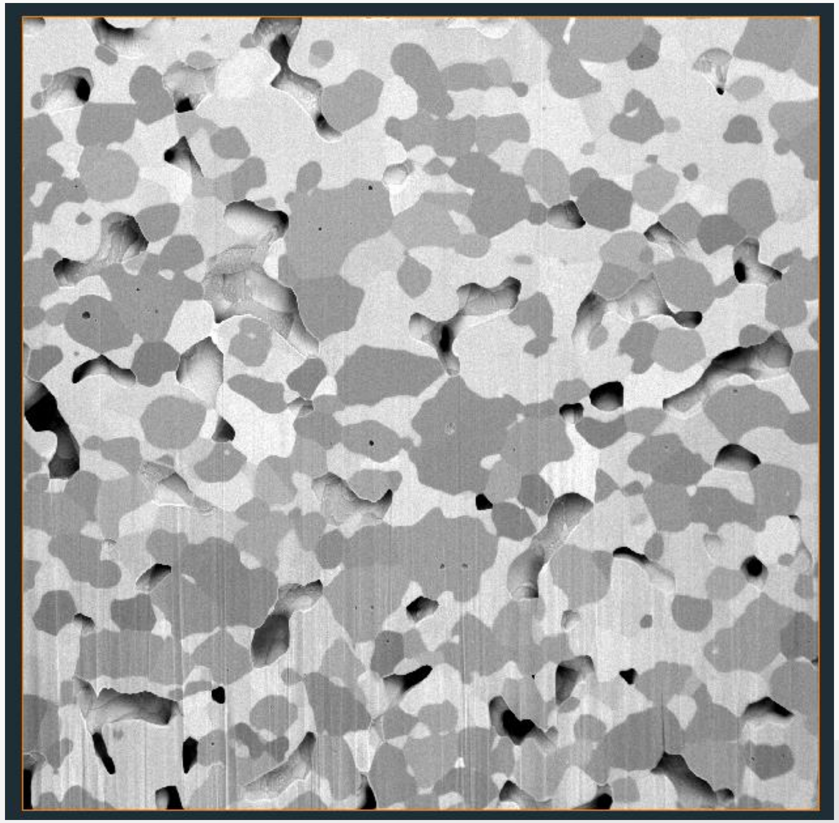
Complete Workflows

Step by step

- **Data import & Visualization**

  - Reading image file formats & metadata

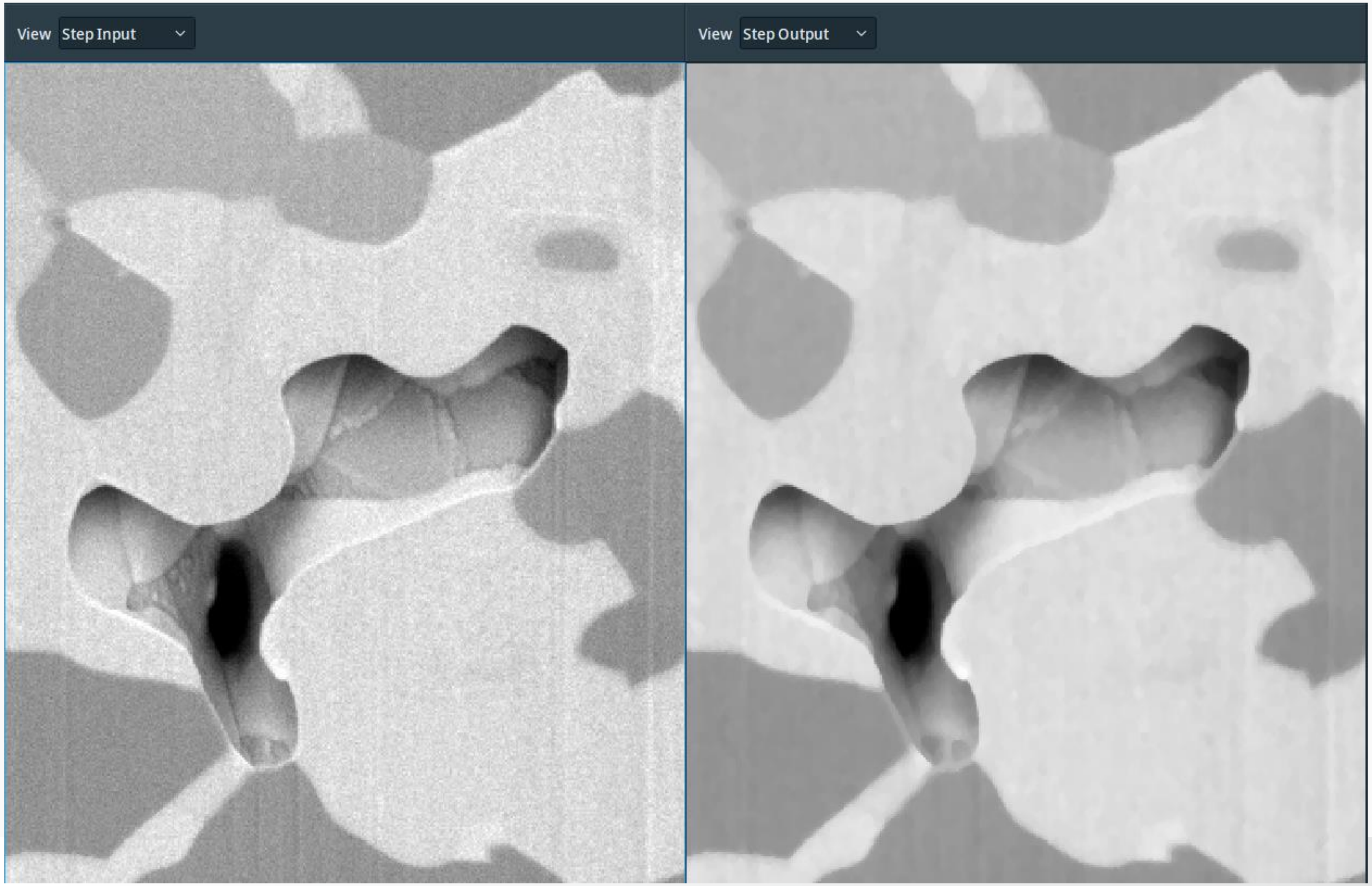




Complete Workflows

Step by step

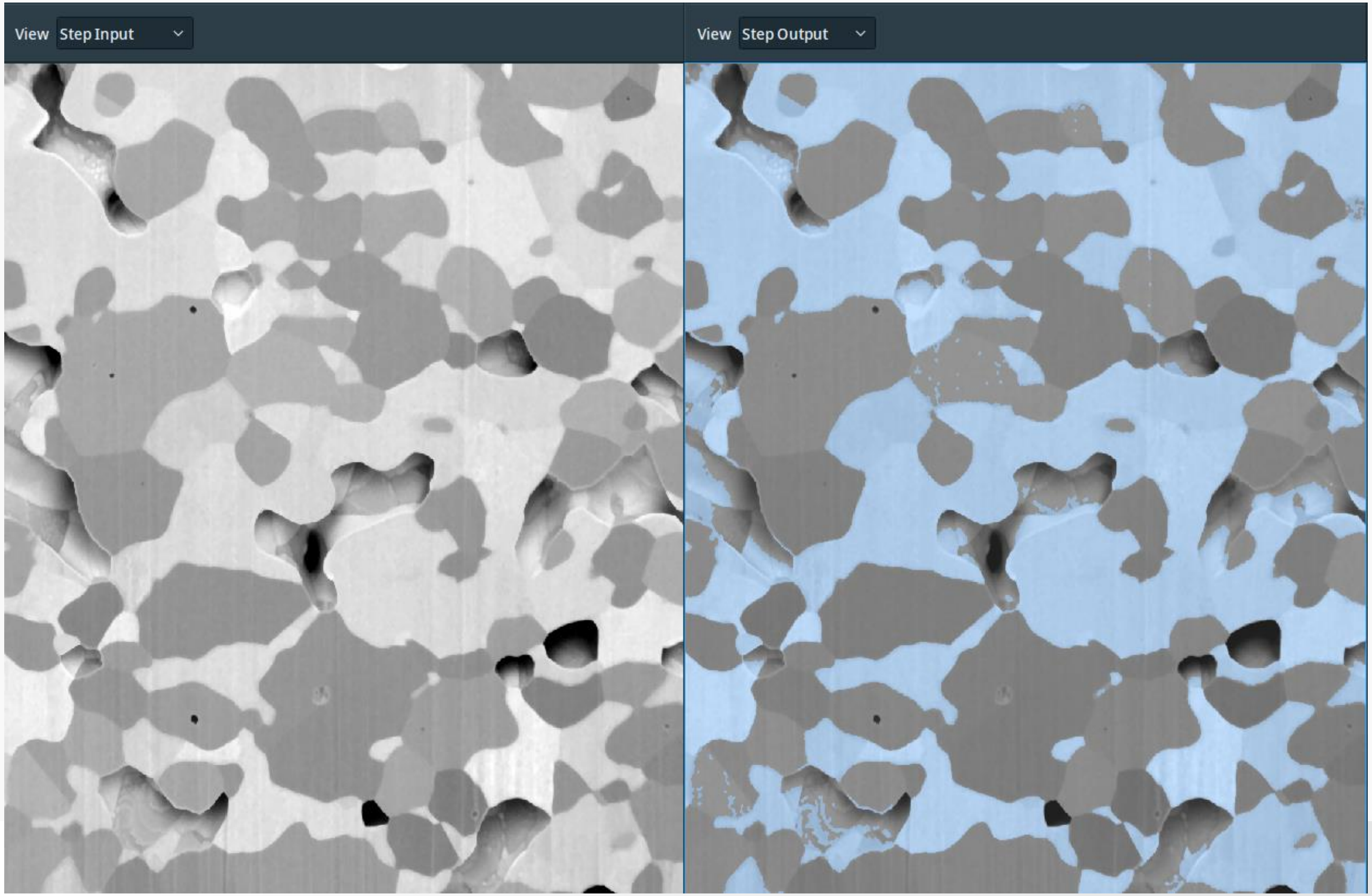
- Data import
  - Reading image file formats
- Visualization
  - Display in 2D & 3D
- **Image Preprocessing**
  - Smoothing & Denoising



Complete Workflows

Step by step

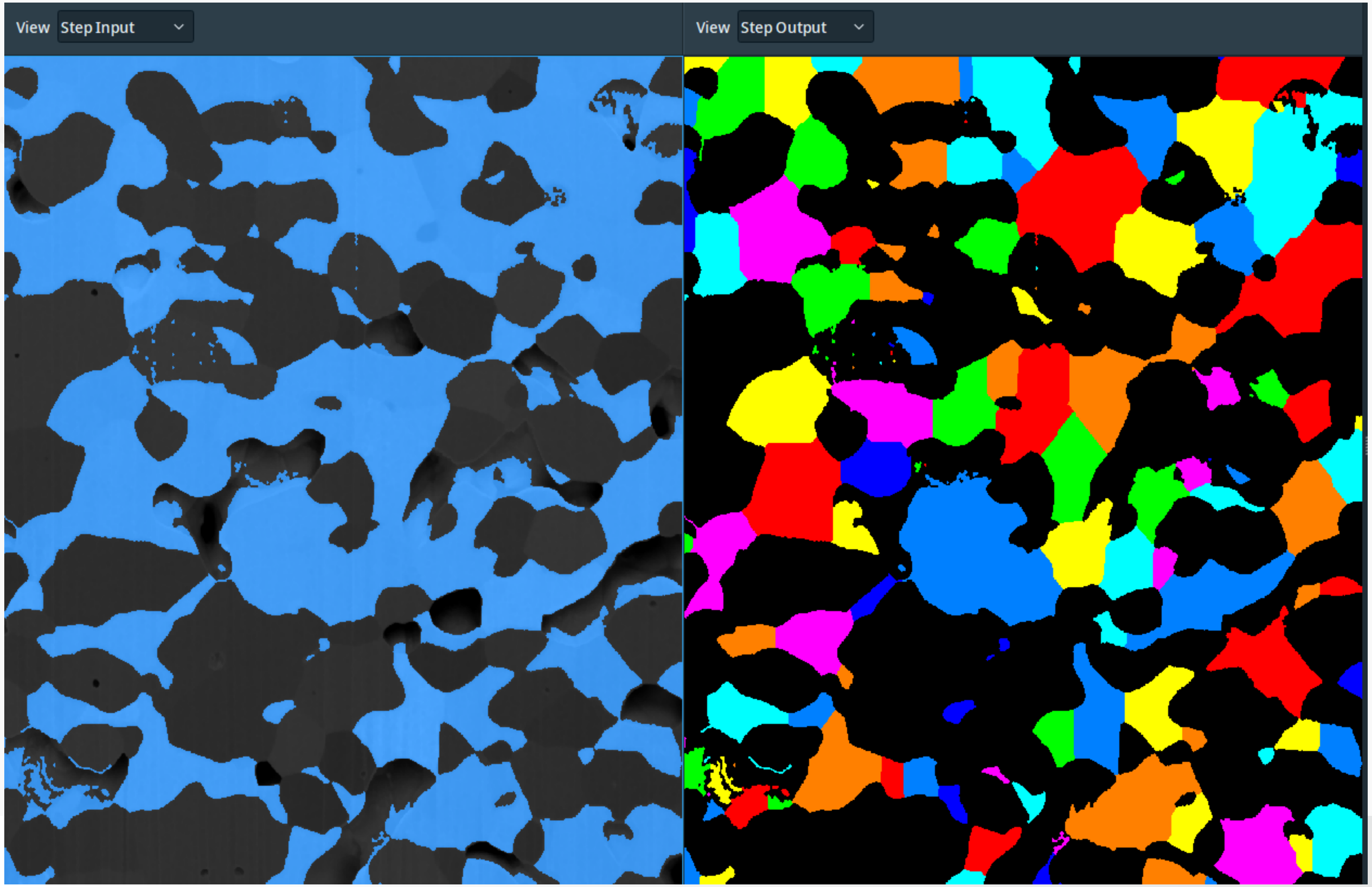
- Data import
  - Reading image file formats
- Visualization
  - Display in 2D & 3D
- Image Preprocessing
  - Denoising
- **Segmentation**
  - Binarization (Thresholding)



Complete Workflows

Step by step

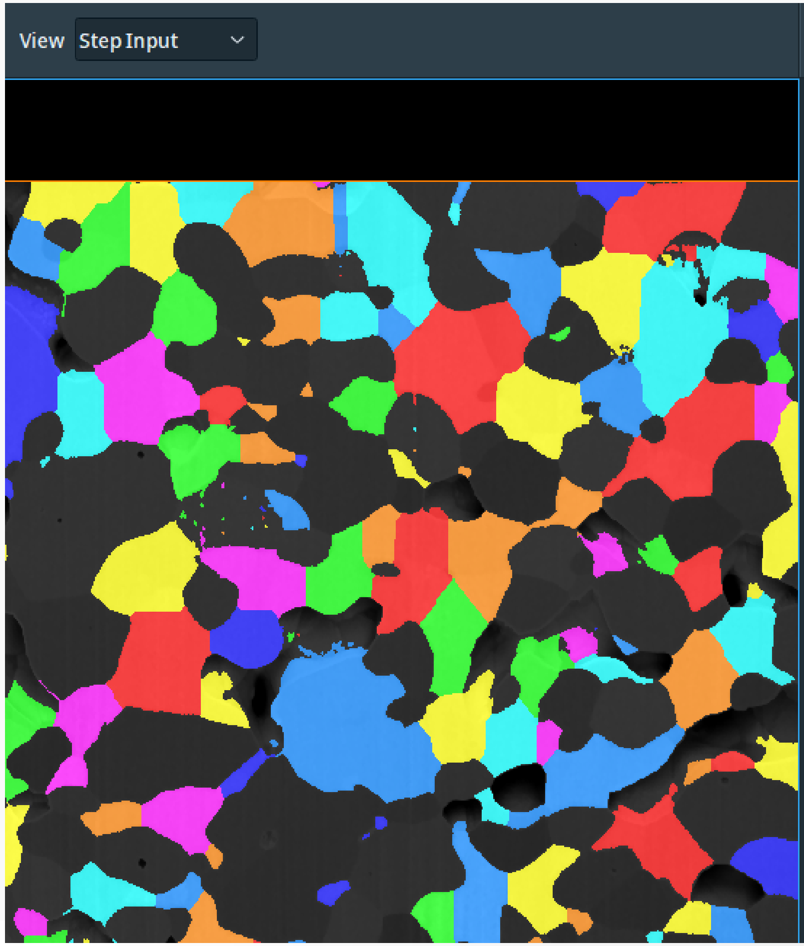
- Data import
  - Reading image file format
- Visualization
  - Display in 2D & 3D
- Image Preprocessing
  - Denoising
- **Segmentation**
  - Separation



Complete Workflows

Step by step

- Data import
  - Reading image file formats &
- Visualization
  - Display in 2D & 3D
- Image Preprocessing
  - Denoising
- Segmentation
  - Binarization (Thresholding)
  - Separation
- **Feature Extraction**
  - Statistics



View Step Output

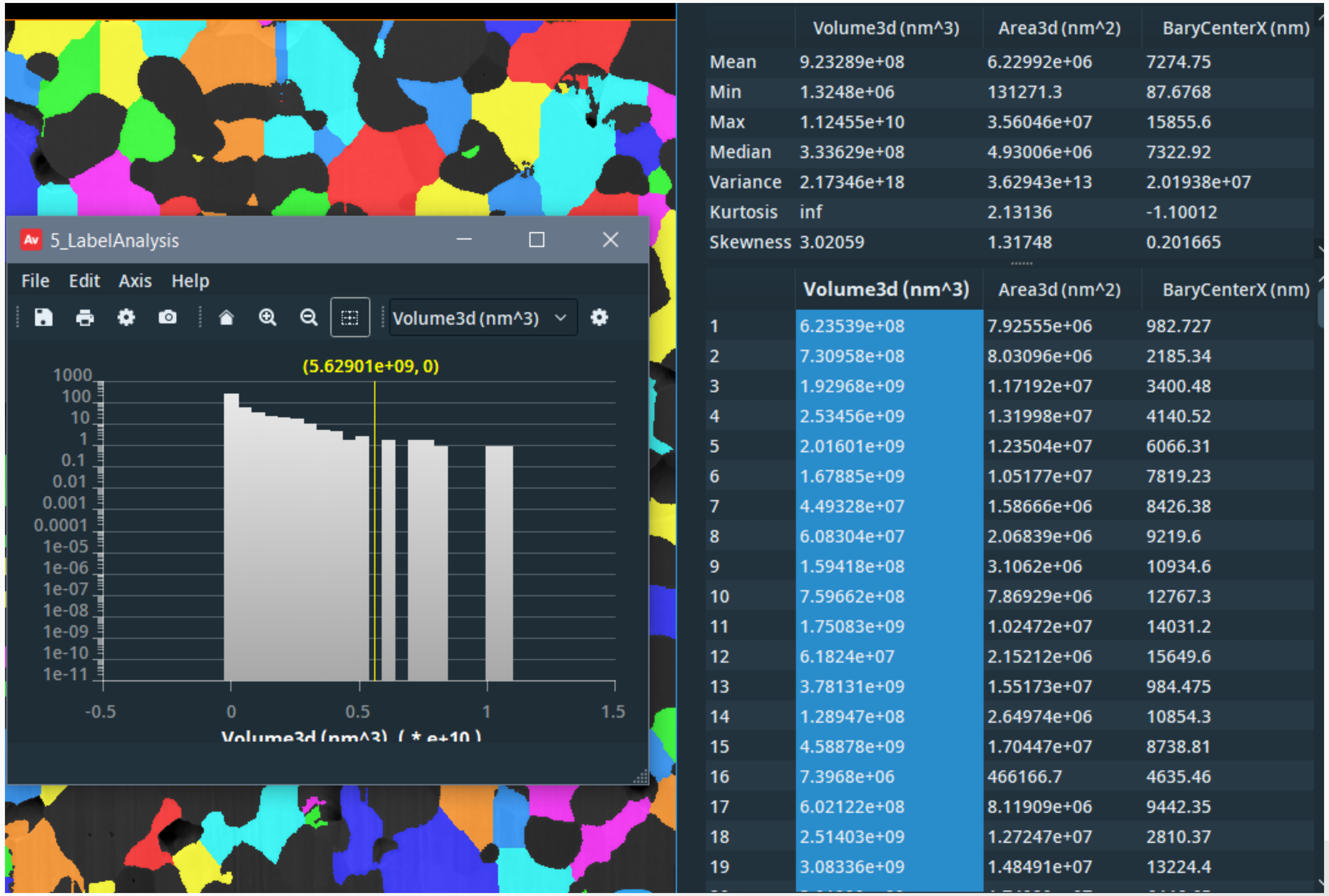
	Volume3d (nm^3)	Area3d (nm^2)	BaryCenterX (nm)
Mean	9.23289e+08	6.22992e+06	7274.75
Min	1.3248e+06	131271.3	87.6768
Max	1.12455e+10	3.56046e+07	15855.6
Median	3.33629e+08	4.93006e+06	7322.92
Variance	2.17346e+18	3.62943e+13	2.01938e+07
Kurtosis	inf	2.13136	-1.10012
Skewness	3.02059	1.31748	0.201665

	Volume3d (nm^3)	Area3d (nm^2)	BaryCenterX (nm)
1	6.23539e+08	7.92555e+06	982.727
2	7.30958e+08	8.03096e+06	2185.34
3	1.92968e+09	1.17192e+07	3400.48
4	2.53456e+09	1.31998e+07	4140.52
5	2.01601e+09	1.23504e+07	6066.31
6	1.67885e+09	1.05177e+07	7819.23
7	4.49328e+07	1.58666e+06	8426.38
8	6.08304e+07	2.06839e+06	9219.6
9	1.59418e+08	3.1062e+06	10934.6
10	7.59662e+08	7.86929e+06	12767.3
11	1.75083e+09	1.02472e+07	14031.2
12	6.1824e+07	2.15212e+06	15649.6

Complete Workflows

Step by step

- Data import
  - Reading image file formats & metadata
- Visualization
  - Display in 2D & 3D
- Image Preprocessing
  - Denoising
- Segmentation
  - Binarization (Thresholding)
  - Separation
- **Feature Extraction**
  - Statistic



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10	7.59662e+08	7.86929e+06	12767.3
11	1.75083e+09	1.02472e+07	14031.2
12	6.1824e+07	2.15212e+06	15649.6
13	3.78131e+09	1.55173e+07	984.475
14	1.28947e+08	2.64974e+06	10854.3
15	4.58878e+09	1.70447e+07	8738.81
16	7.3968e+06	466166.7	4635.46
17	6.02122e+08	8.11909e+06	9442.35
18	2.51403e+09	1.27247e+07	2810.37
19	3.08336e+09	1.48491e+07	13224.4

General concepts and tools

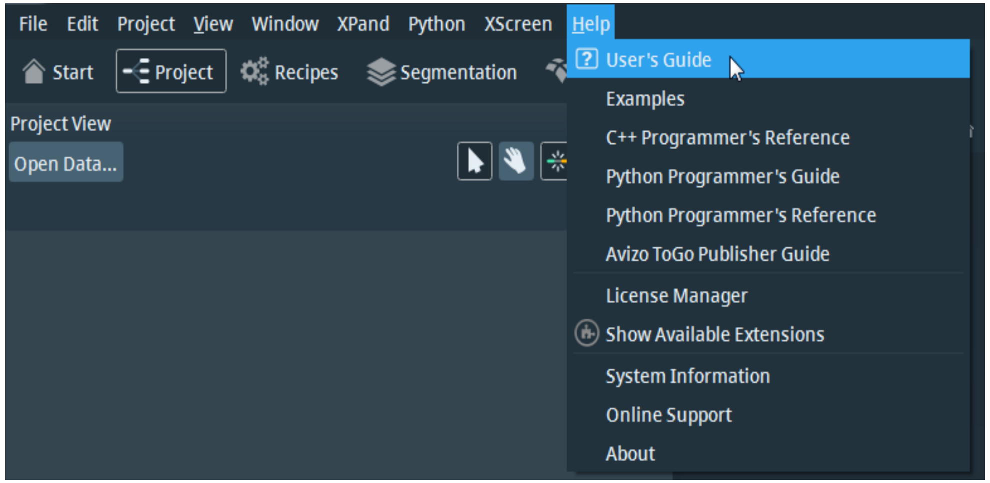
Getting started

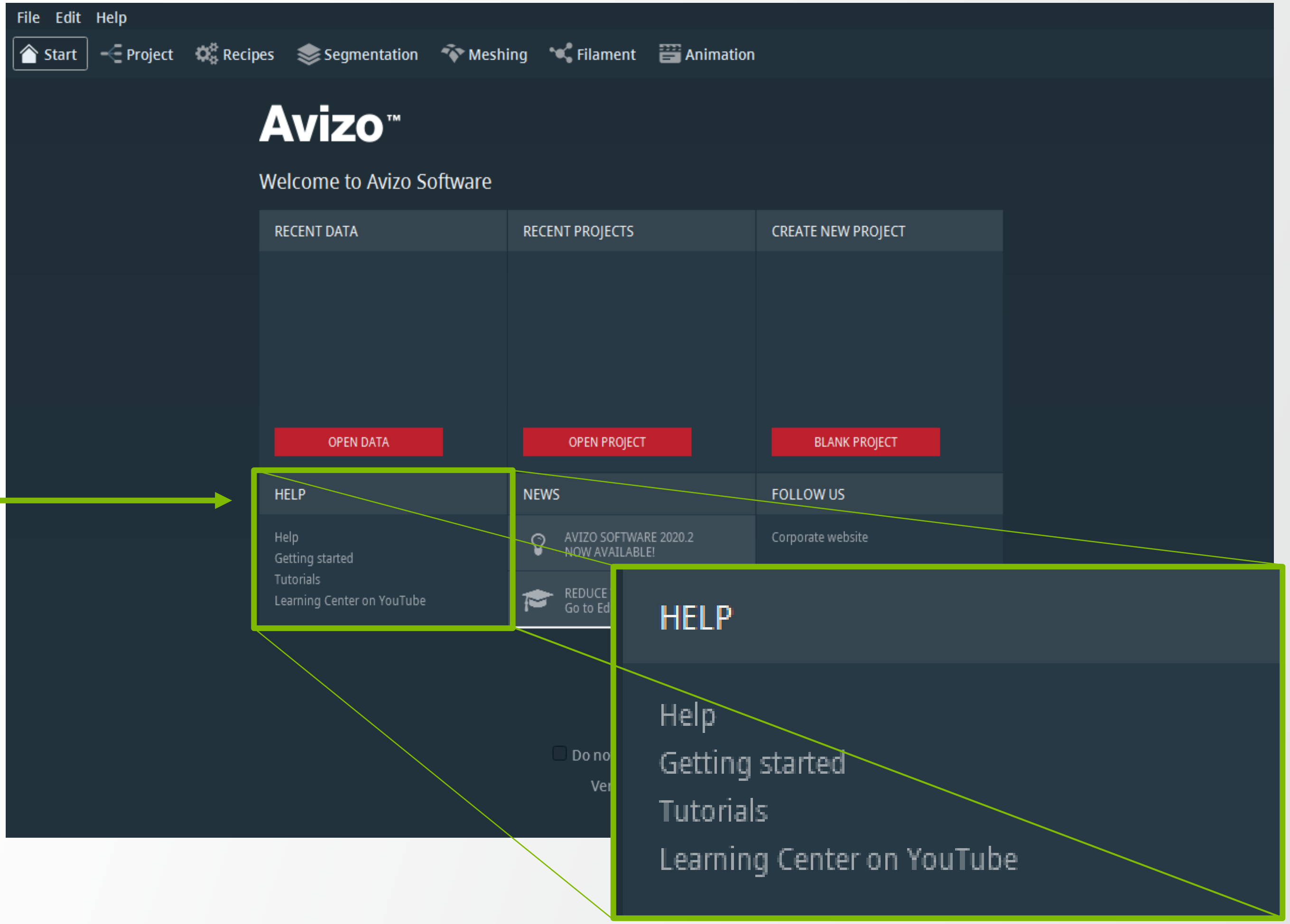
Help menu access

Avizo start page access

Learning resources:

- Tutorials
- YouTube Learning Center
- User's Guide





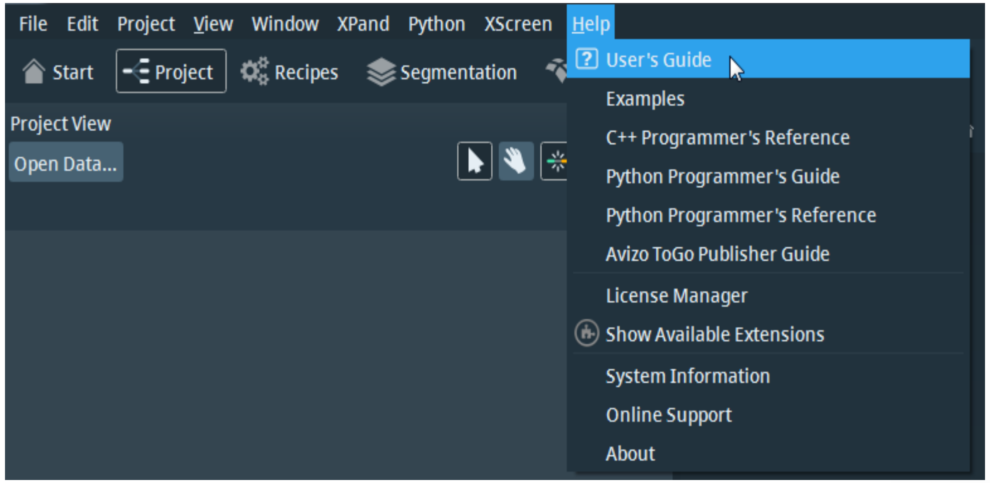
Getting started

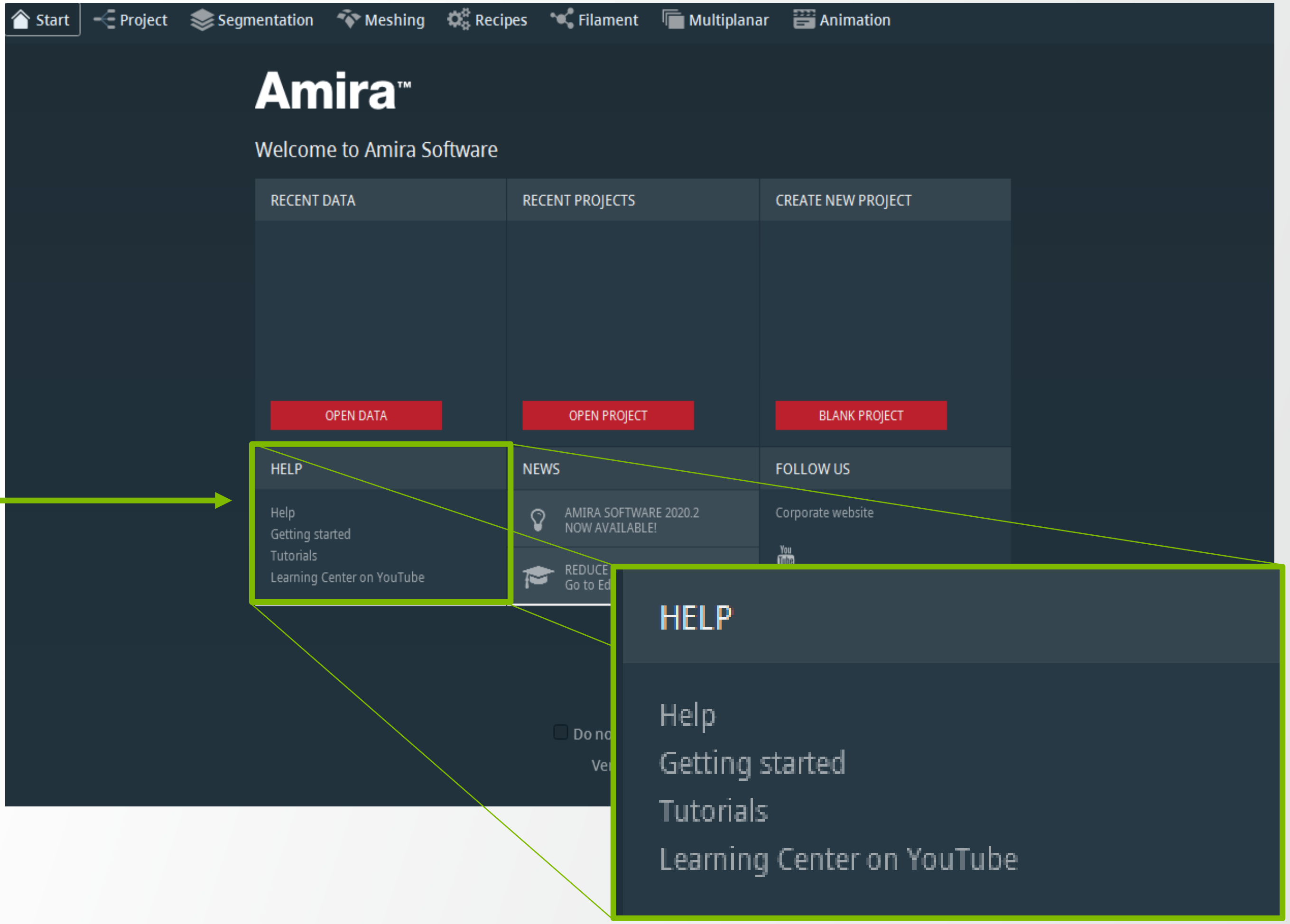
Help menu access

Amira start page access

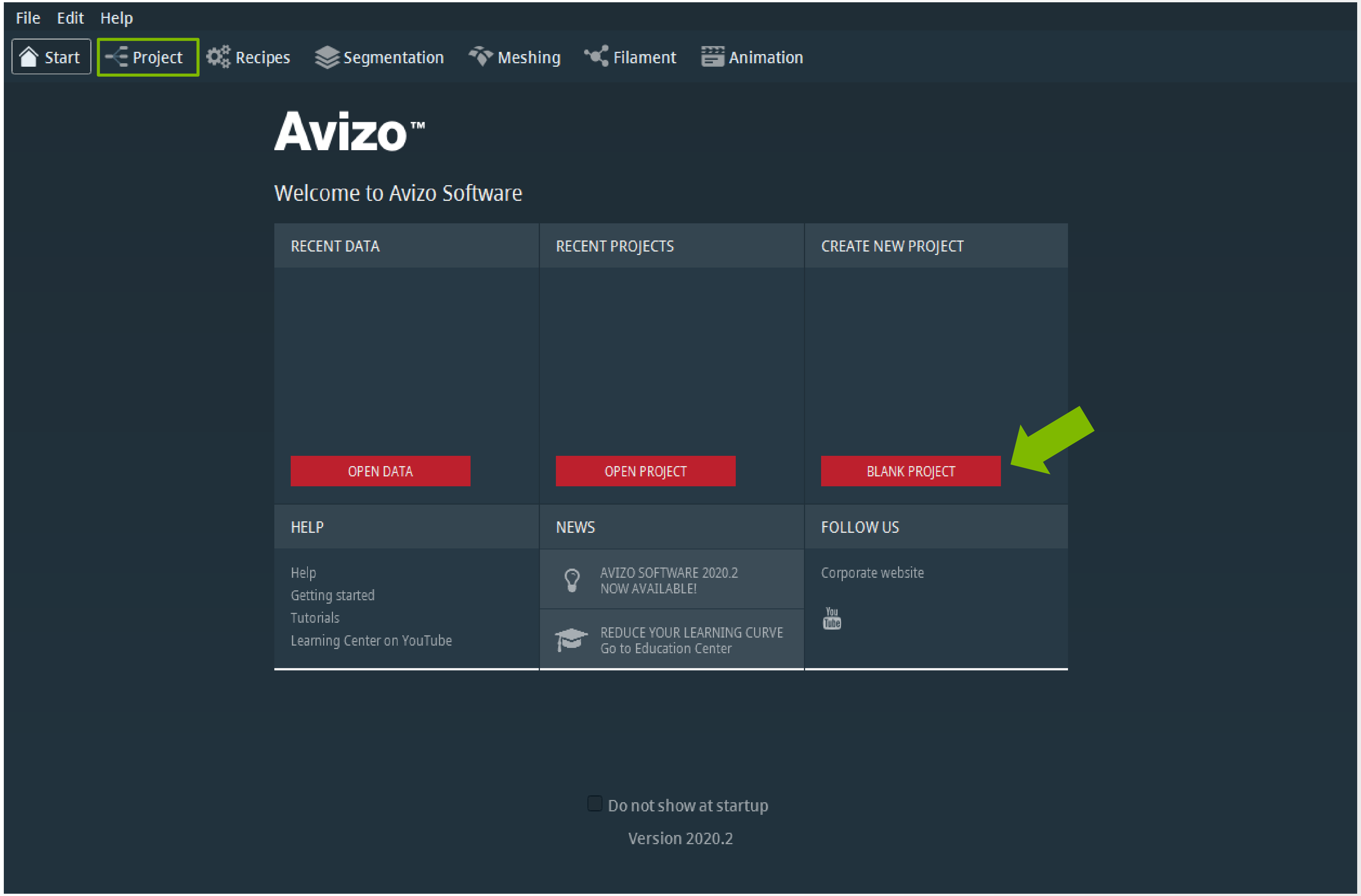
Learning resources:

- Tutorials
- YouTube Learning Center
- User's Guide

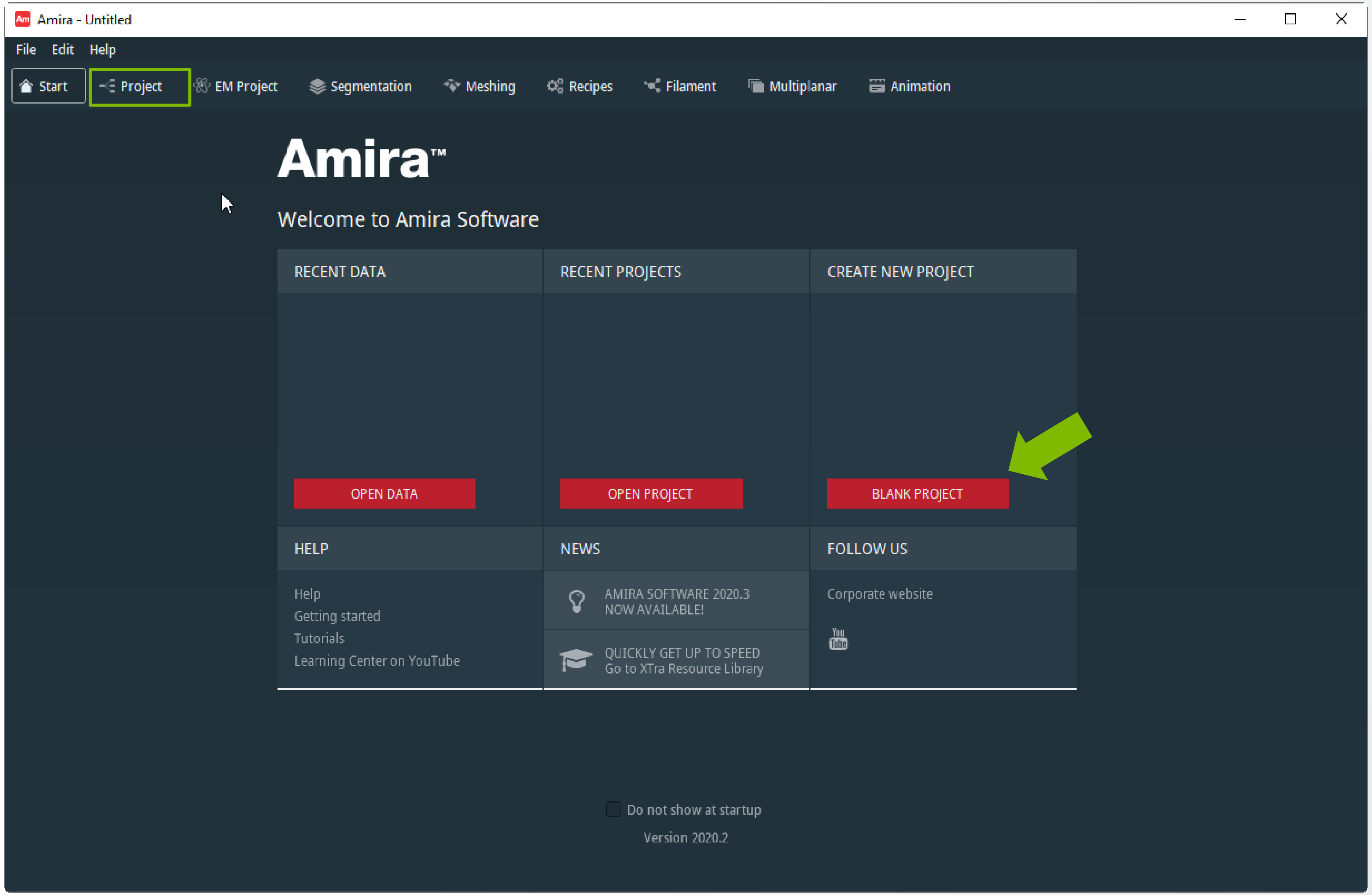




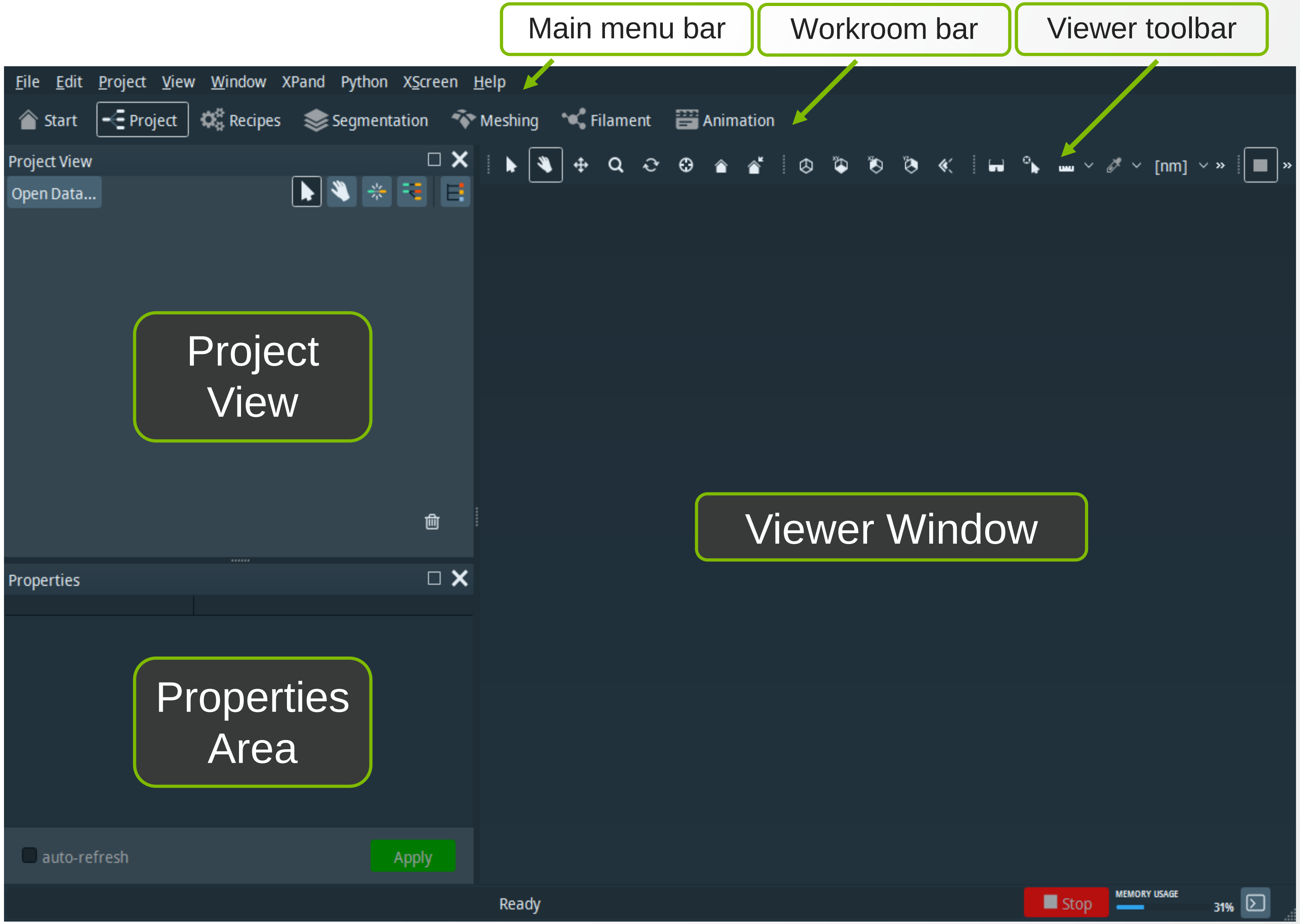
Avizo start-up page



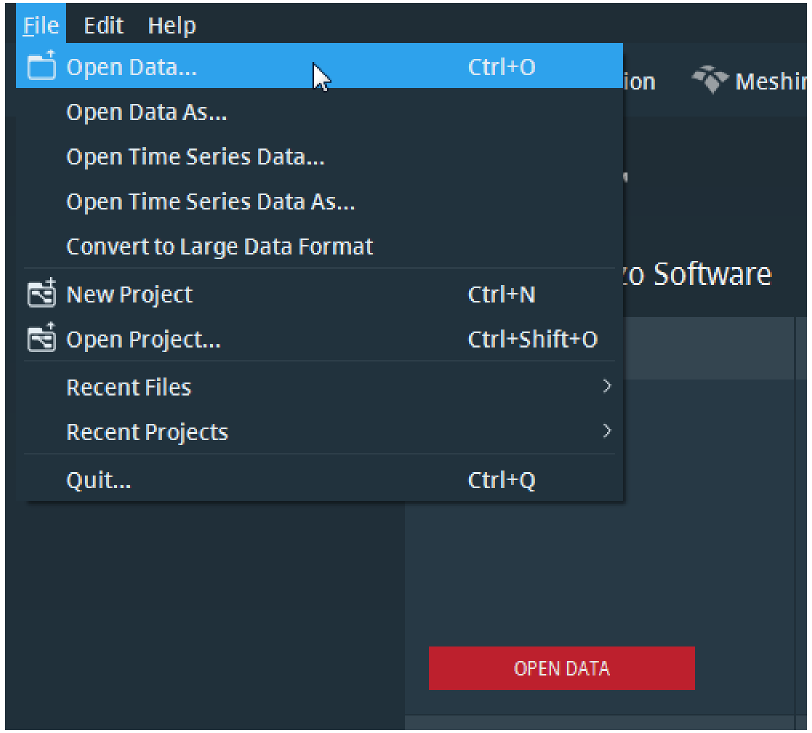
Amira start-up page

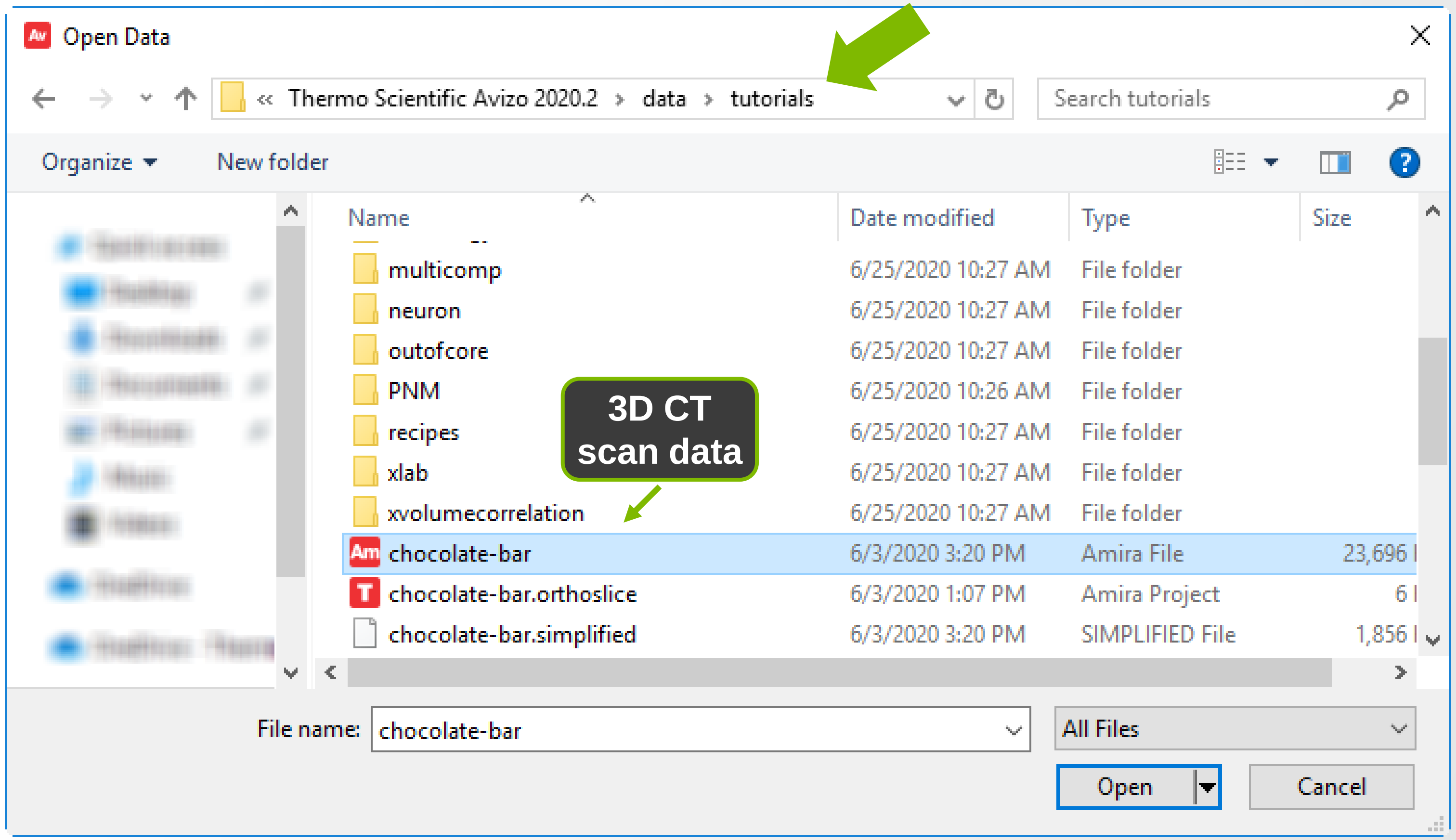


Workspace: user interface components

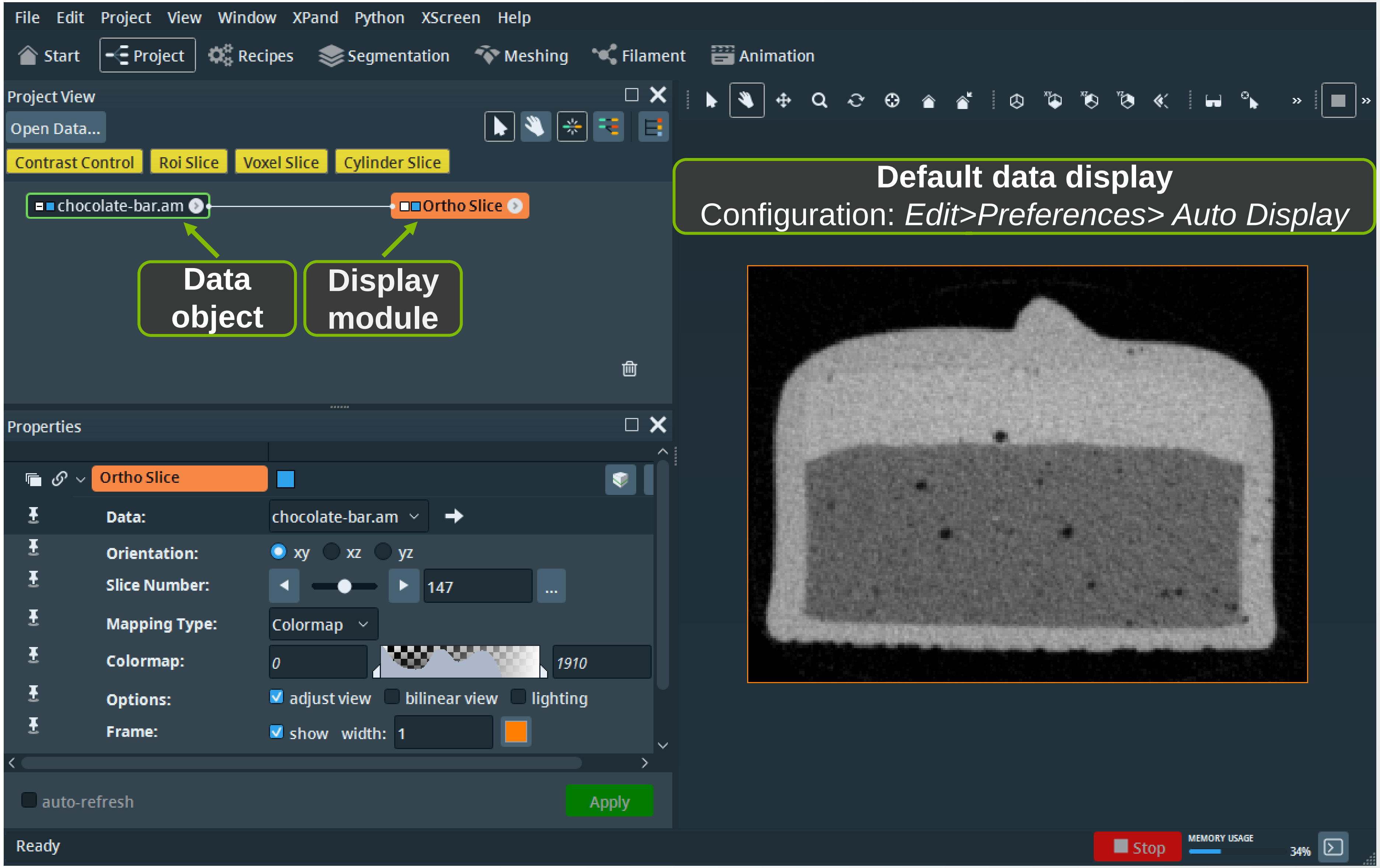


Loading a dataset into Project Workroom – part 1

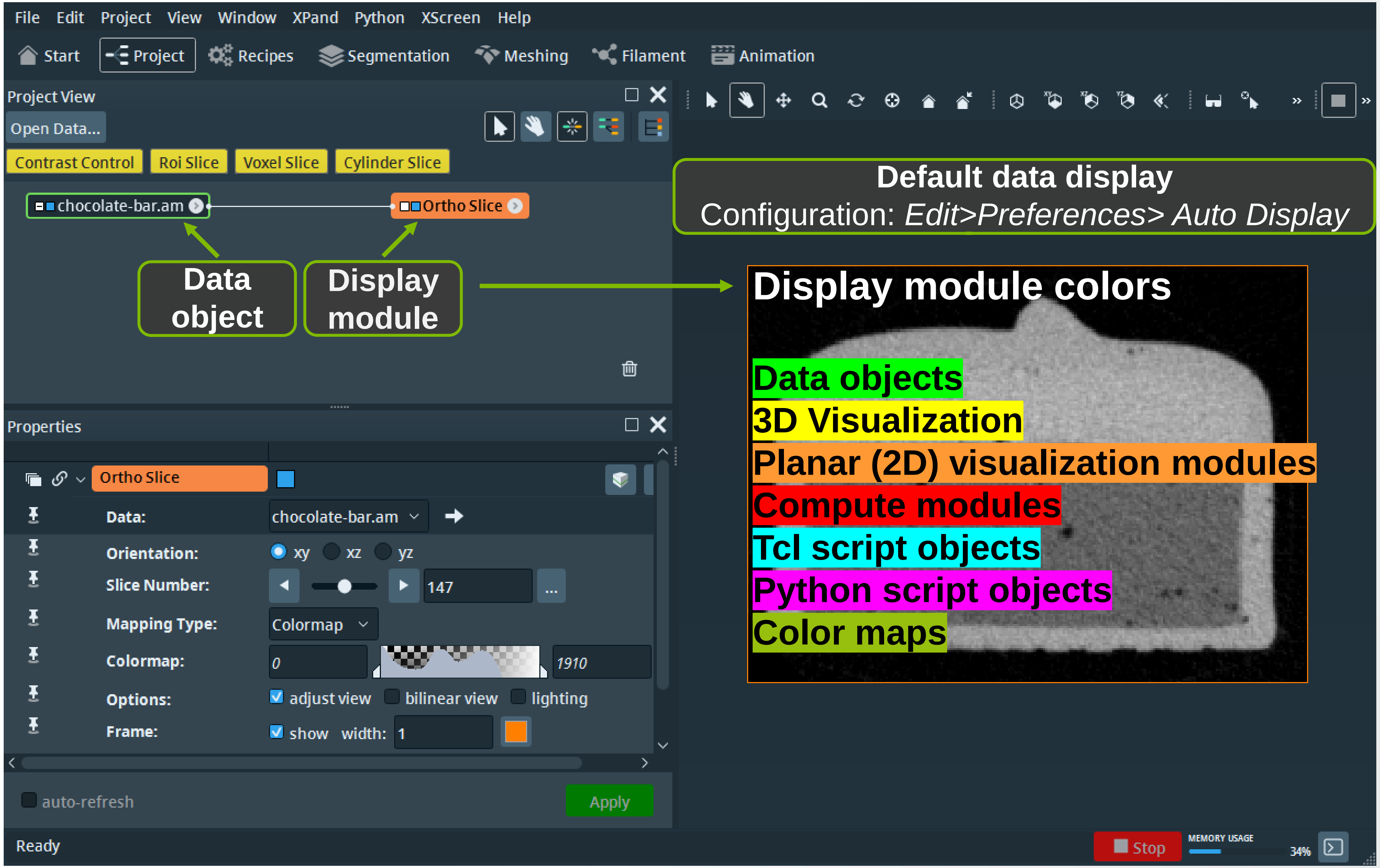




Loading a dataset into the Project Workroom – part 2

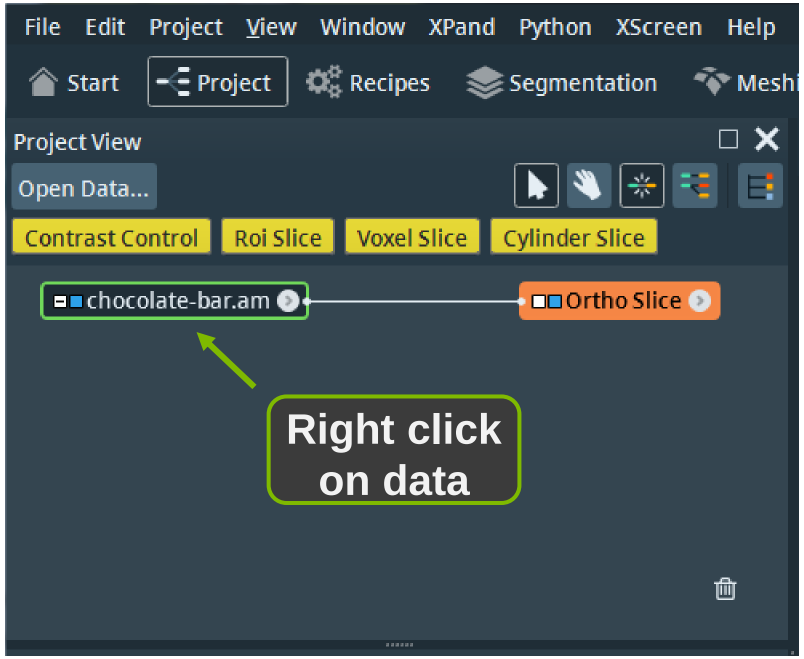


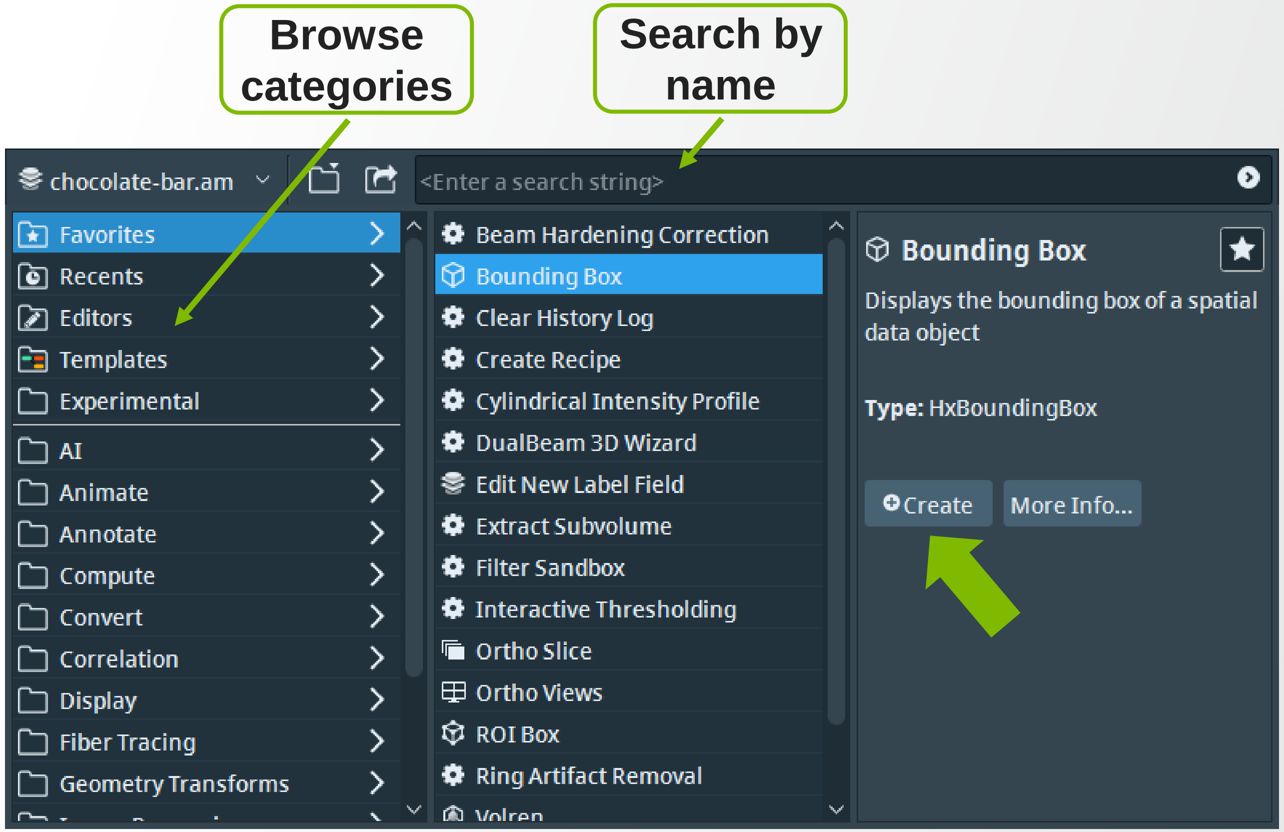
Loading a dataset into the Project Workroom – part 2



Attach a module to a dataset: e.g. Bounding Box

ThermoFisher SCIENTIFIC

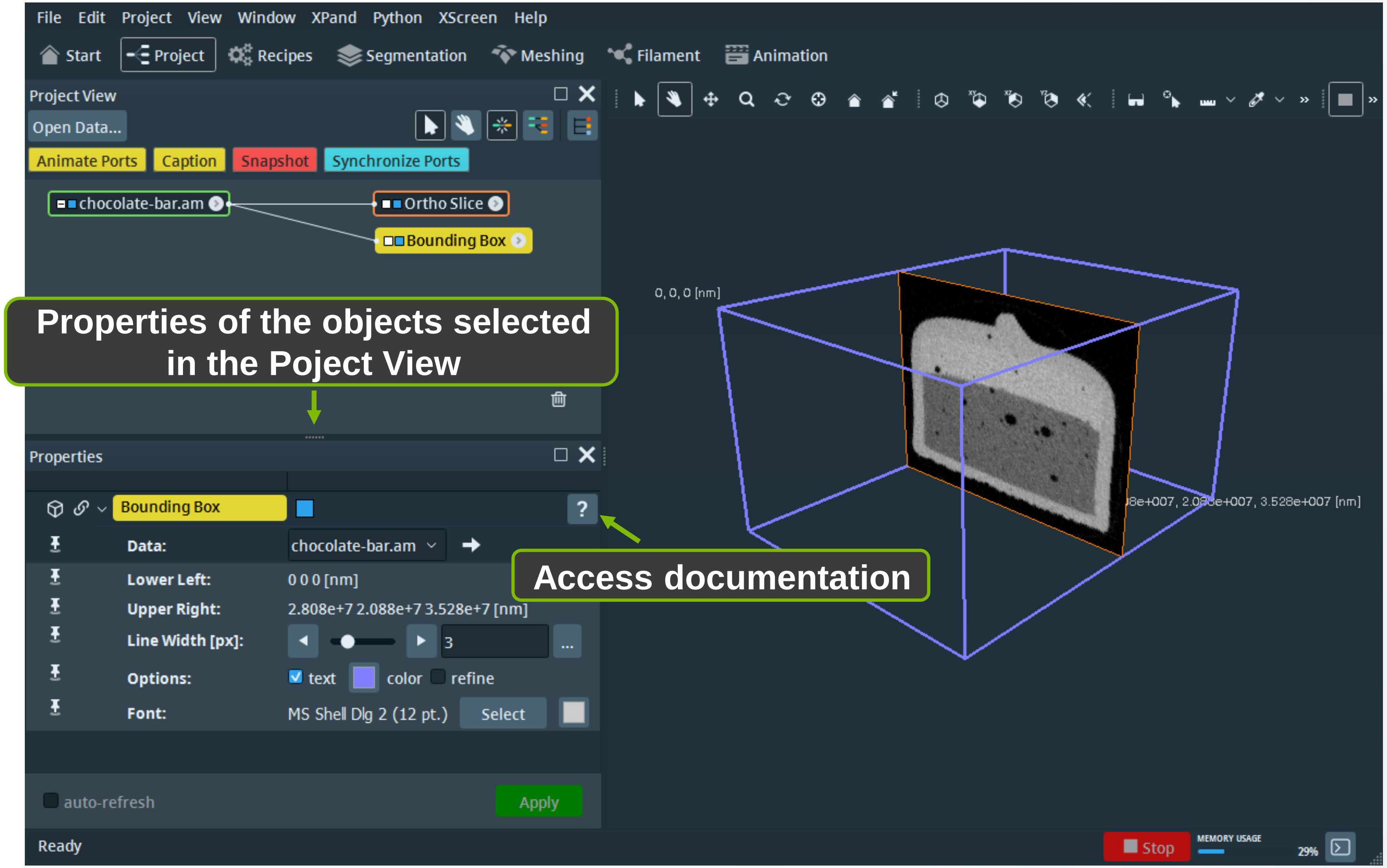




Ways to trigger module:

- Double-click
- Press Enter
- Press Create button

Module properties: e.g. Bounding Box

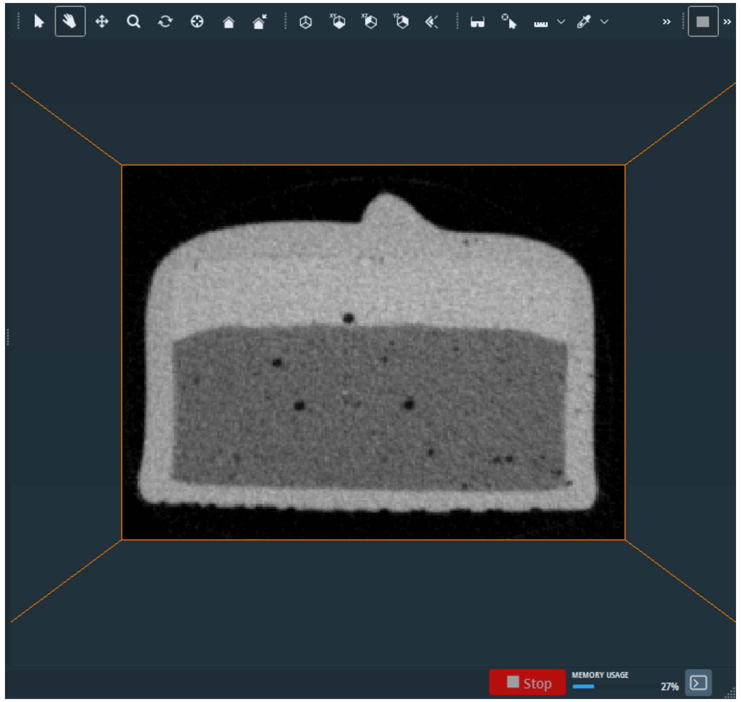


Properties:

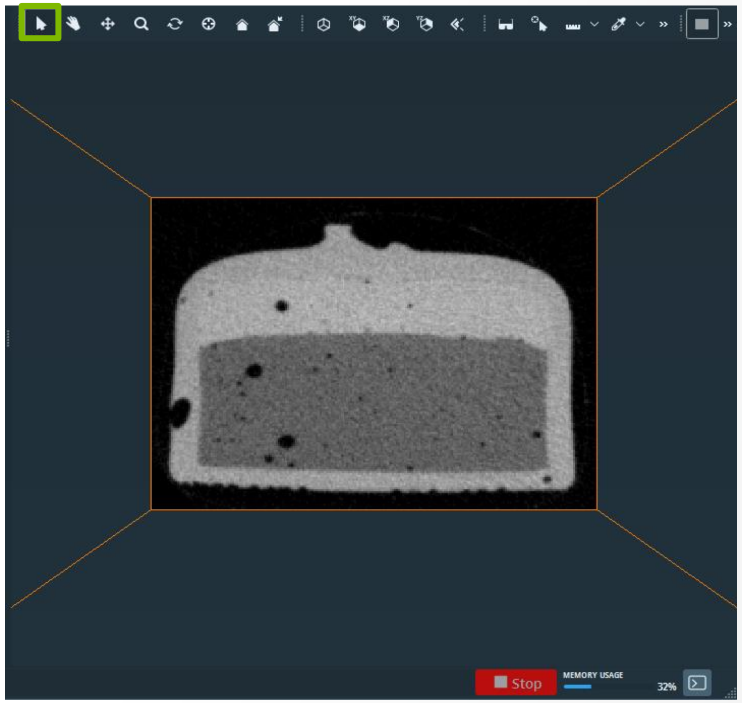
- Click on a module in the pool to display its properties (data module included)
- Click on “?” to access the module’s documentation
- Module properties are called *ports*

Navigate and interact in 3D – part 1

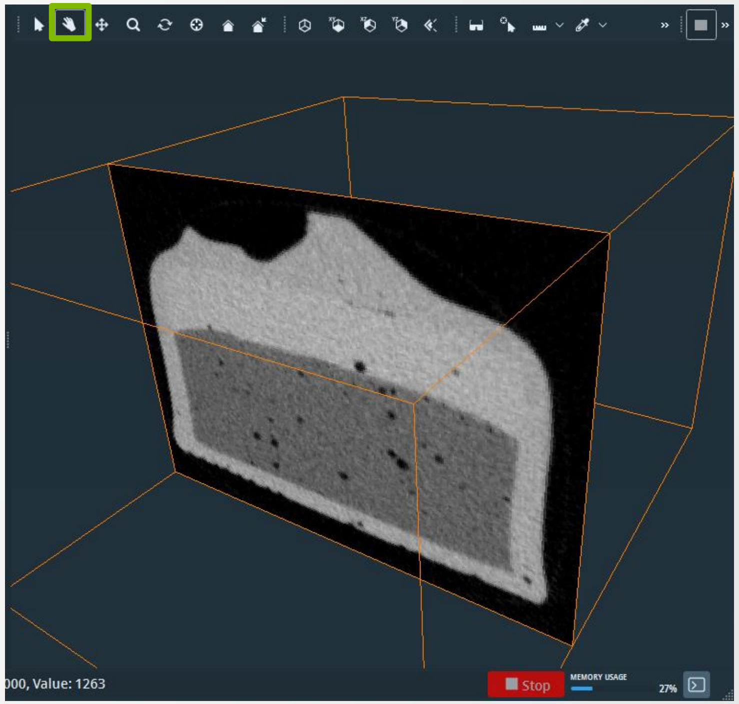
Bounding Box
Original View



Interact
[ESC]



Trackball
[ESC], [Alt]

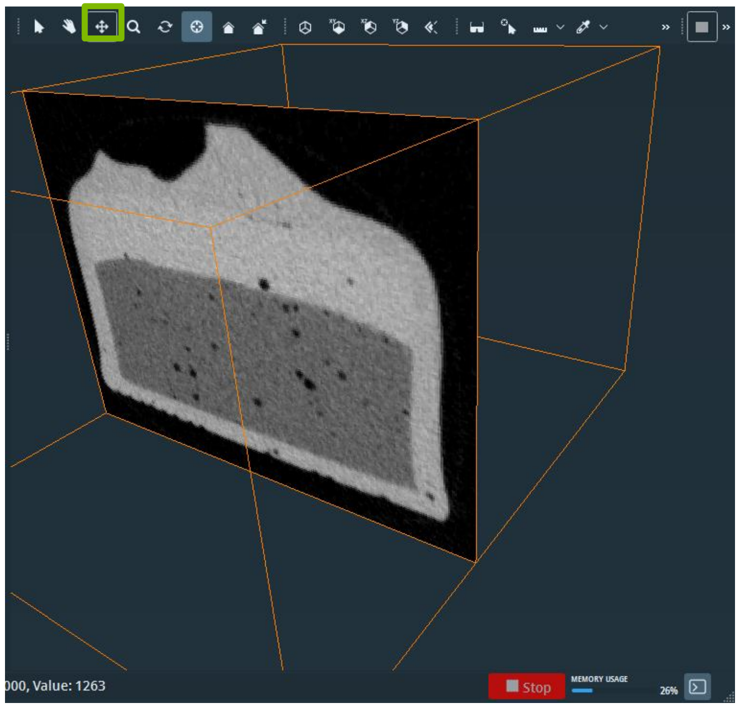


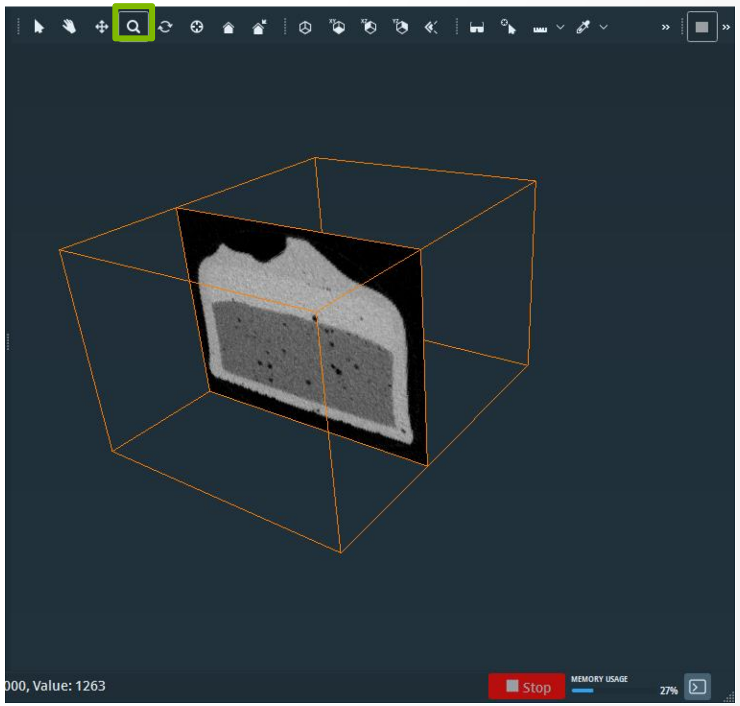
Navigate and interact in 3D – part 1

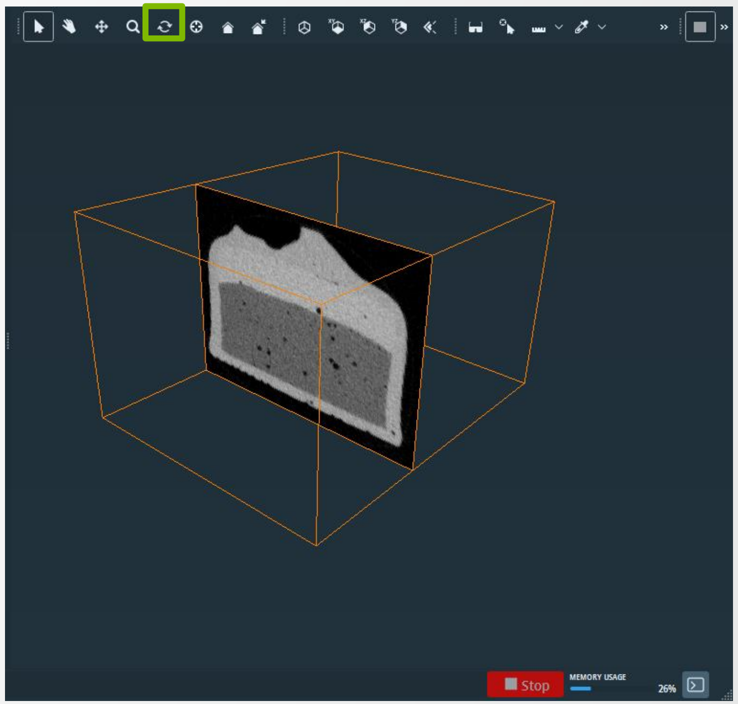
Translate
Left mouse button

Zoom
Mouse wheel/left button

Rotate
Click icon /+ [Shift]/[Ctrl]

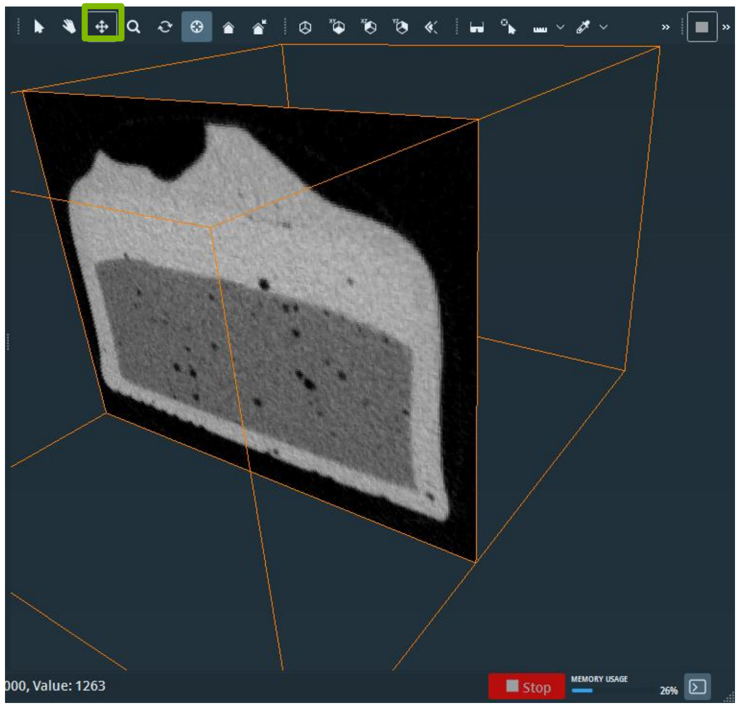




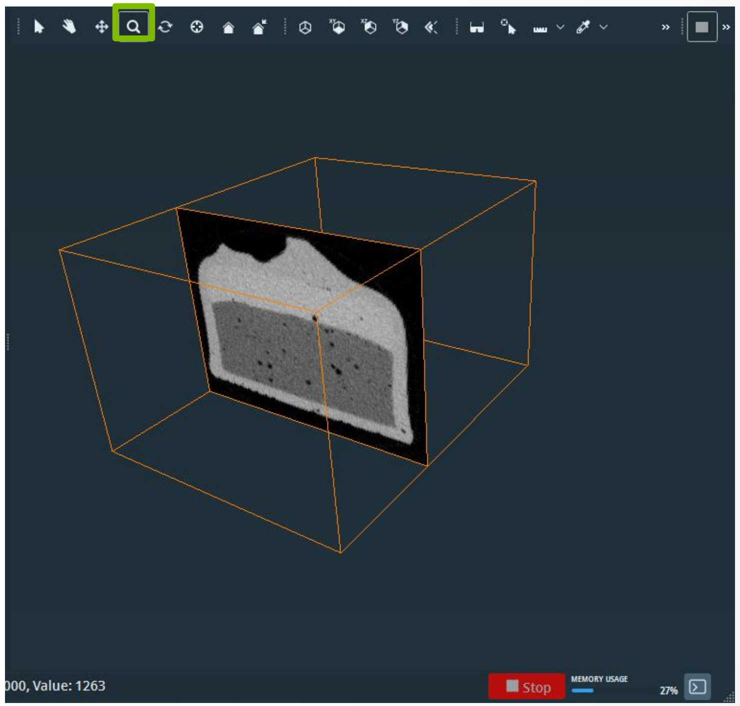


Navigate and interact in 3D – Part 2

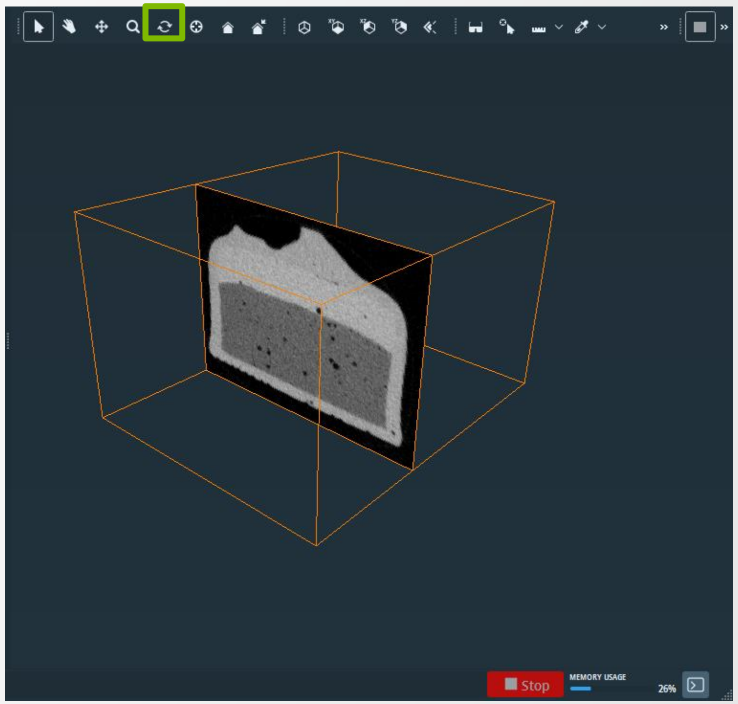
Translate
Left mouse button



Zoom
Mouse wheel/left button



Rotate
Click icon /+ [Shift]/[Ctrl]



Navigate and interact in 3D – good practice

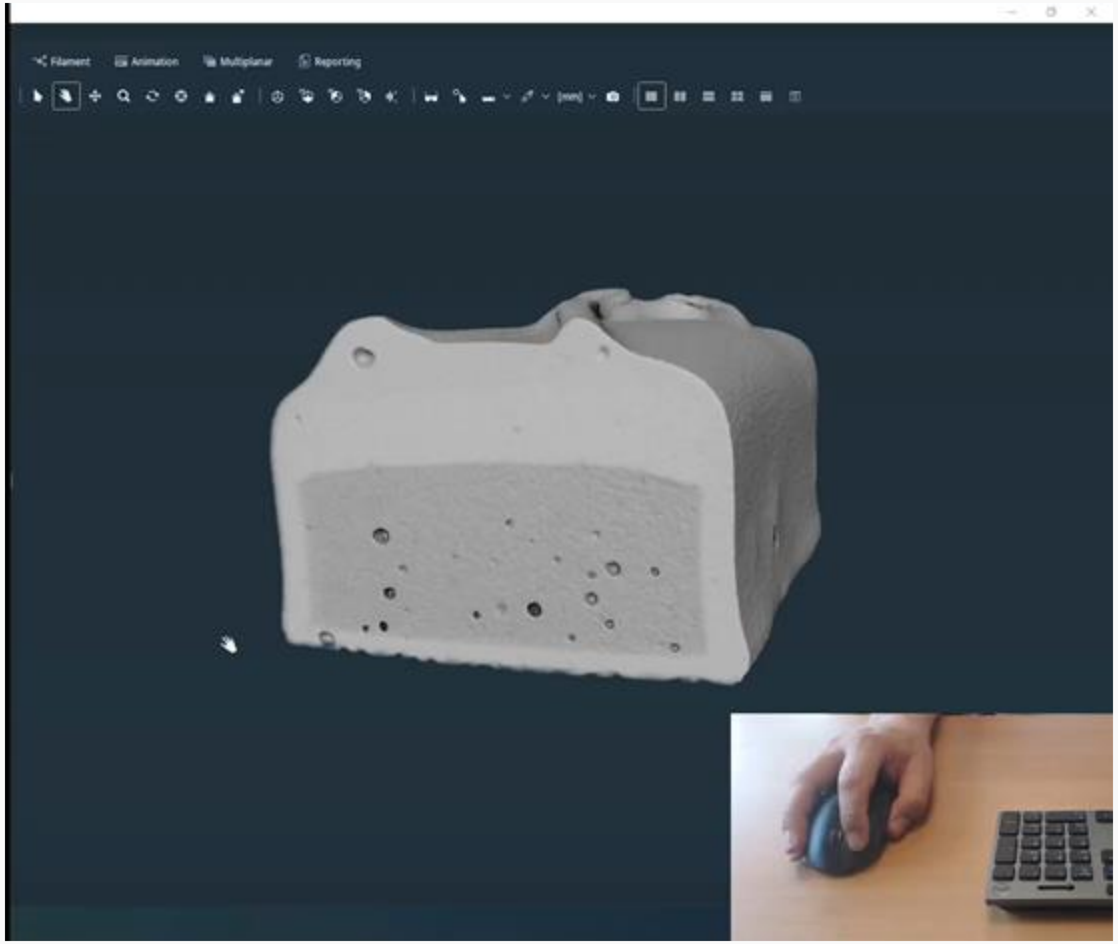
Most of the actions can be done in the trackball mode:

- Hold left mouse button for rotation
- Use the mouse wheel for fine zoom
- Hold left and middle mouse button for fast zoom
- Hold the middle mouse button for translation

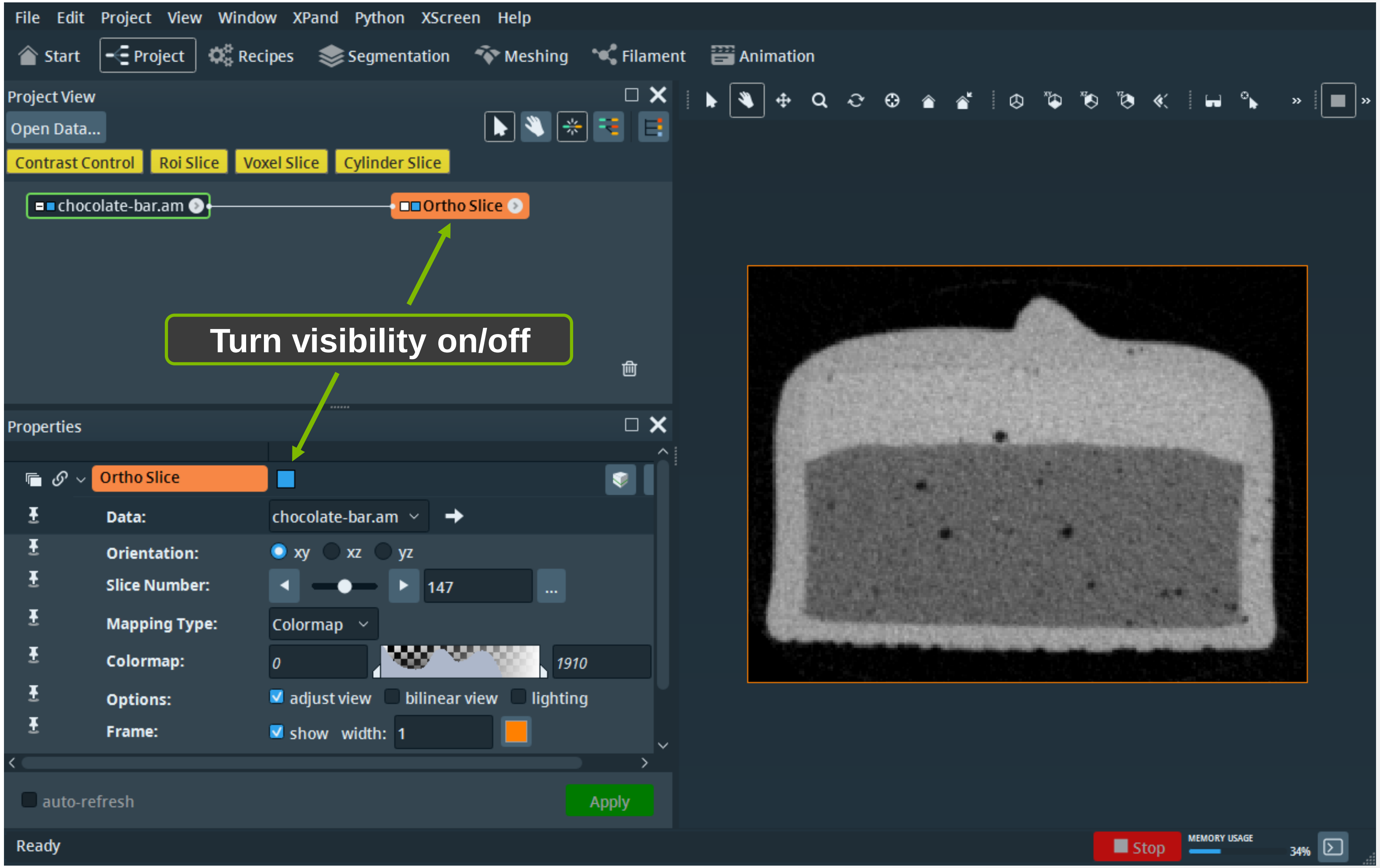
When in interact mode:

- Press and hold [Alt] to switch to trackball mode

Press [Esc] for switching between interact and trackball modes.

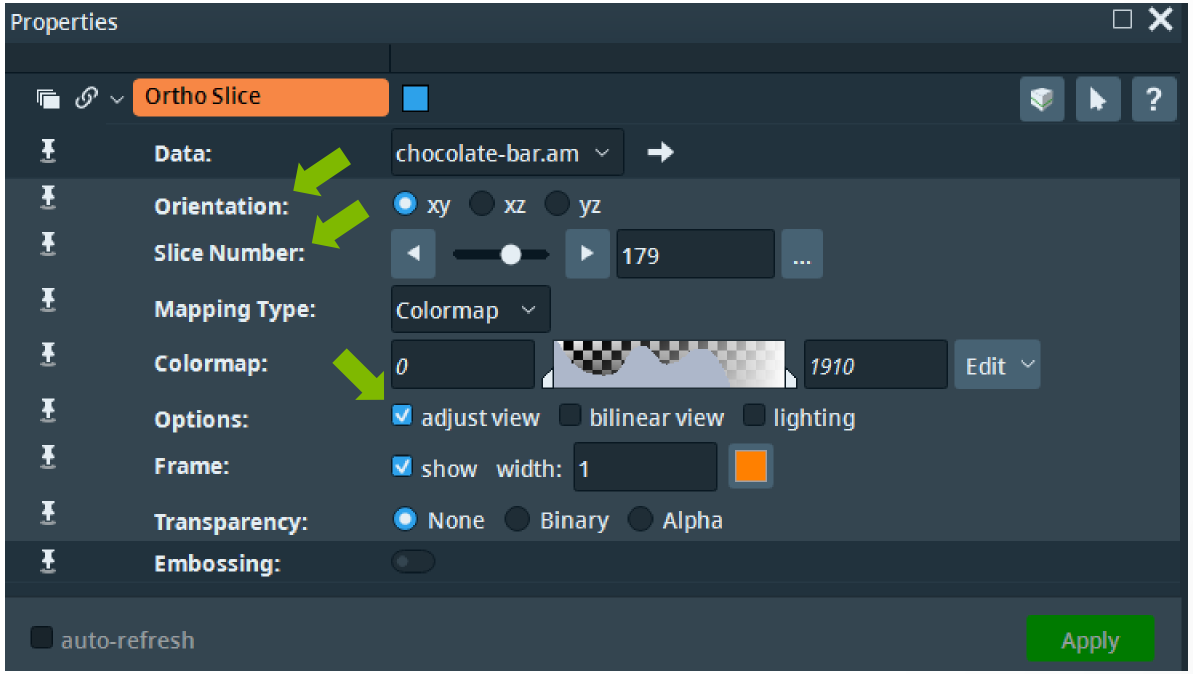


Visualize a dataset: e.g. Ortho Slice



- Ortho Slice connects Automatically if Auto Display is on
- Can otherwise be created like any other module
- A dataset is displayed in the viewer only if it has a visualization module attached
- Check that visibility is turned on (e.g. workflow with multiple data)

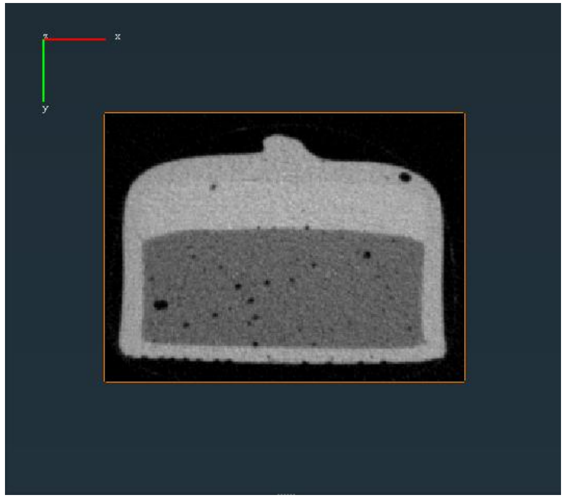
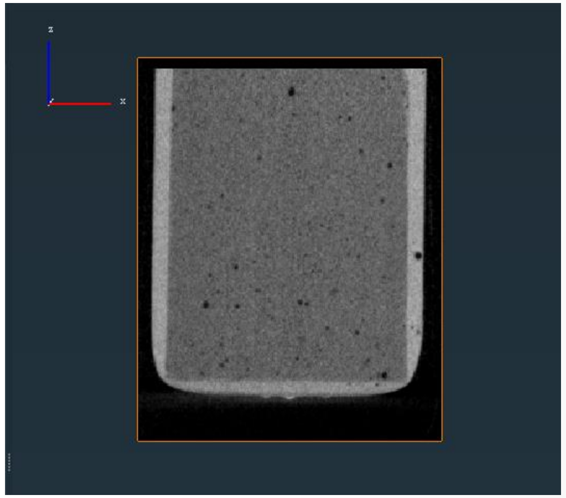
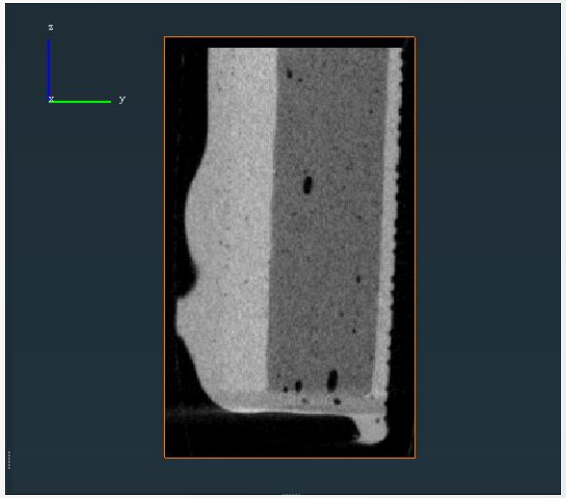
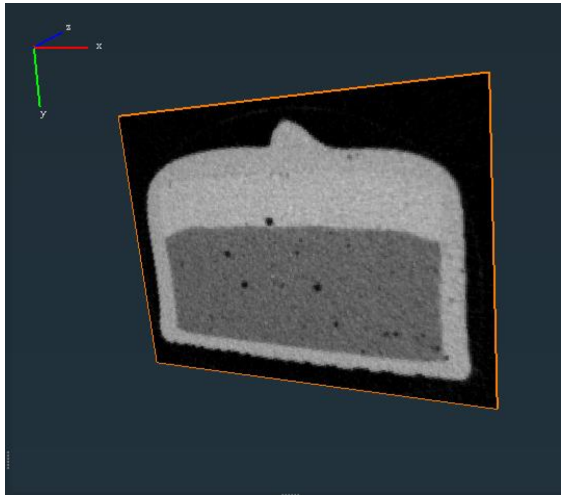
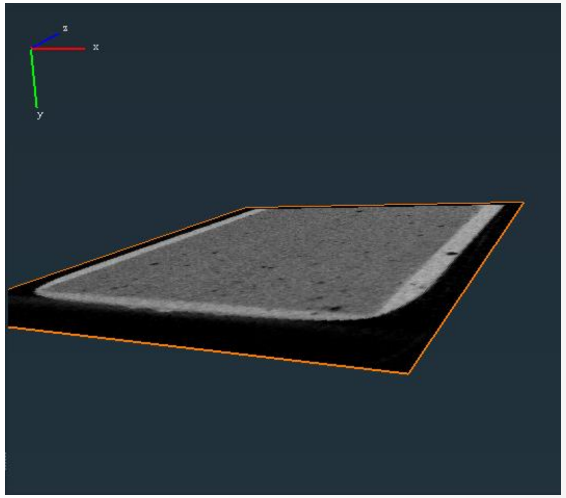
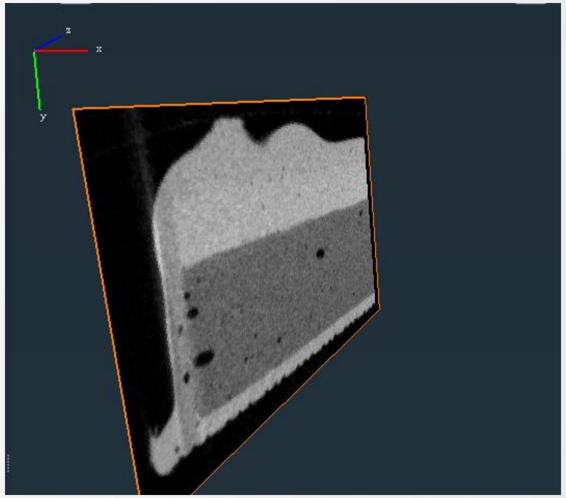
Visualize a Dataset: e.g. Ortho Slice properties



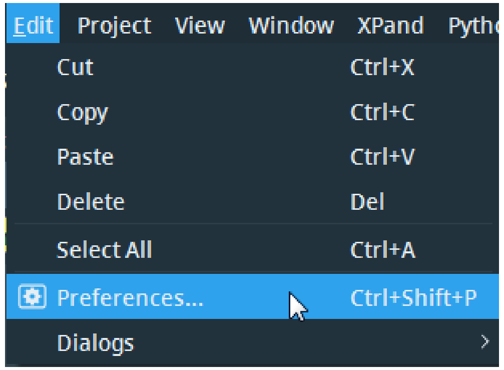
Some properties settings:

- Orientation port: choose the display plane of the slice
- Slice number: choose the slice to be displayed in the viewer - drag slider or use mouse wheel / insert value in the text box
- Adjust view: if on, the camera is reset each time a new slice orientation is selected

Visualize a Dataset: e.g. Ortho Slice properties examples

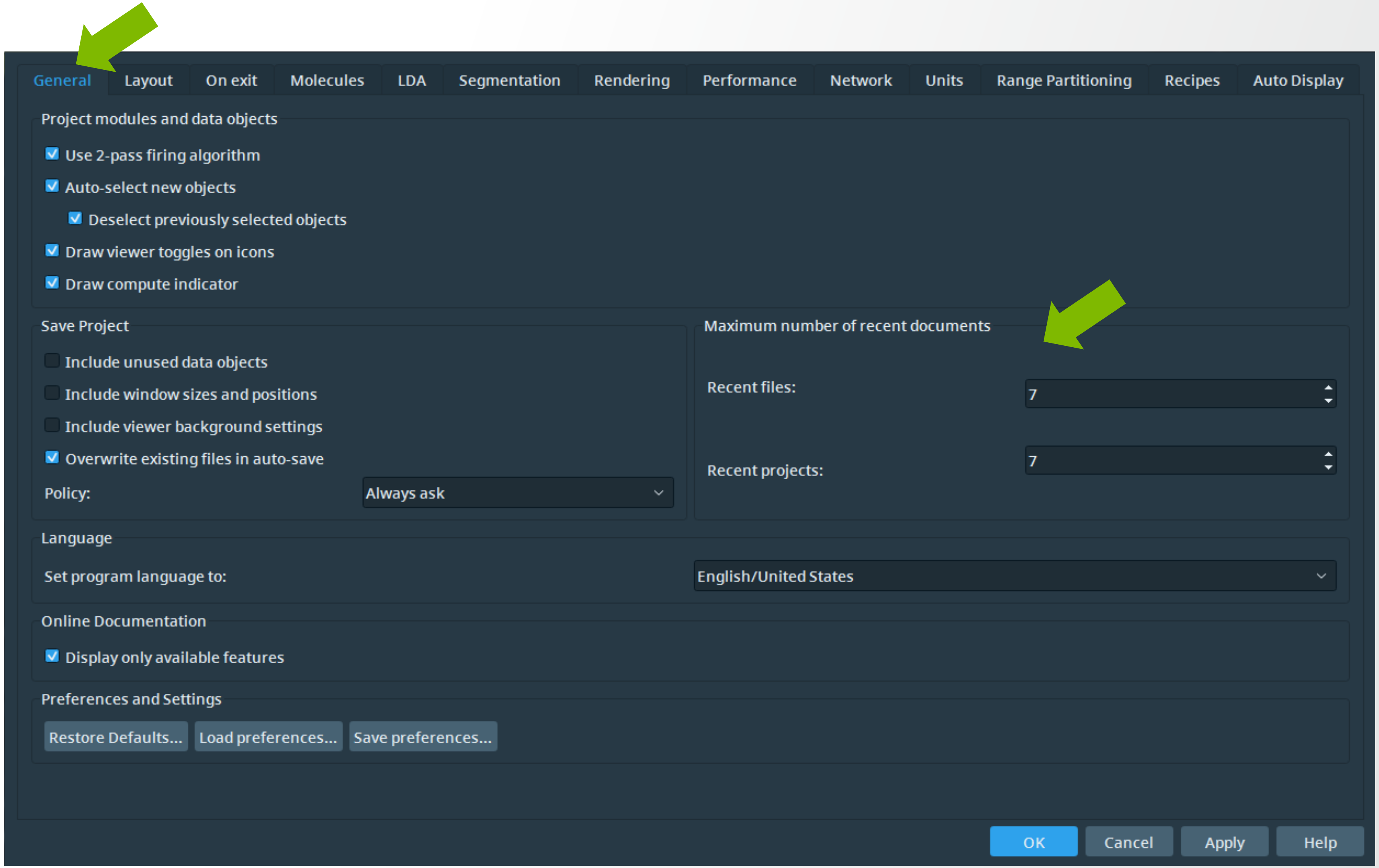
	Orientation xy	Orientation xz	Orientation yz
Adjust View on			
Adjust View off			

Setting preferences – part 1



Preference setting e.g.:

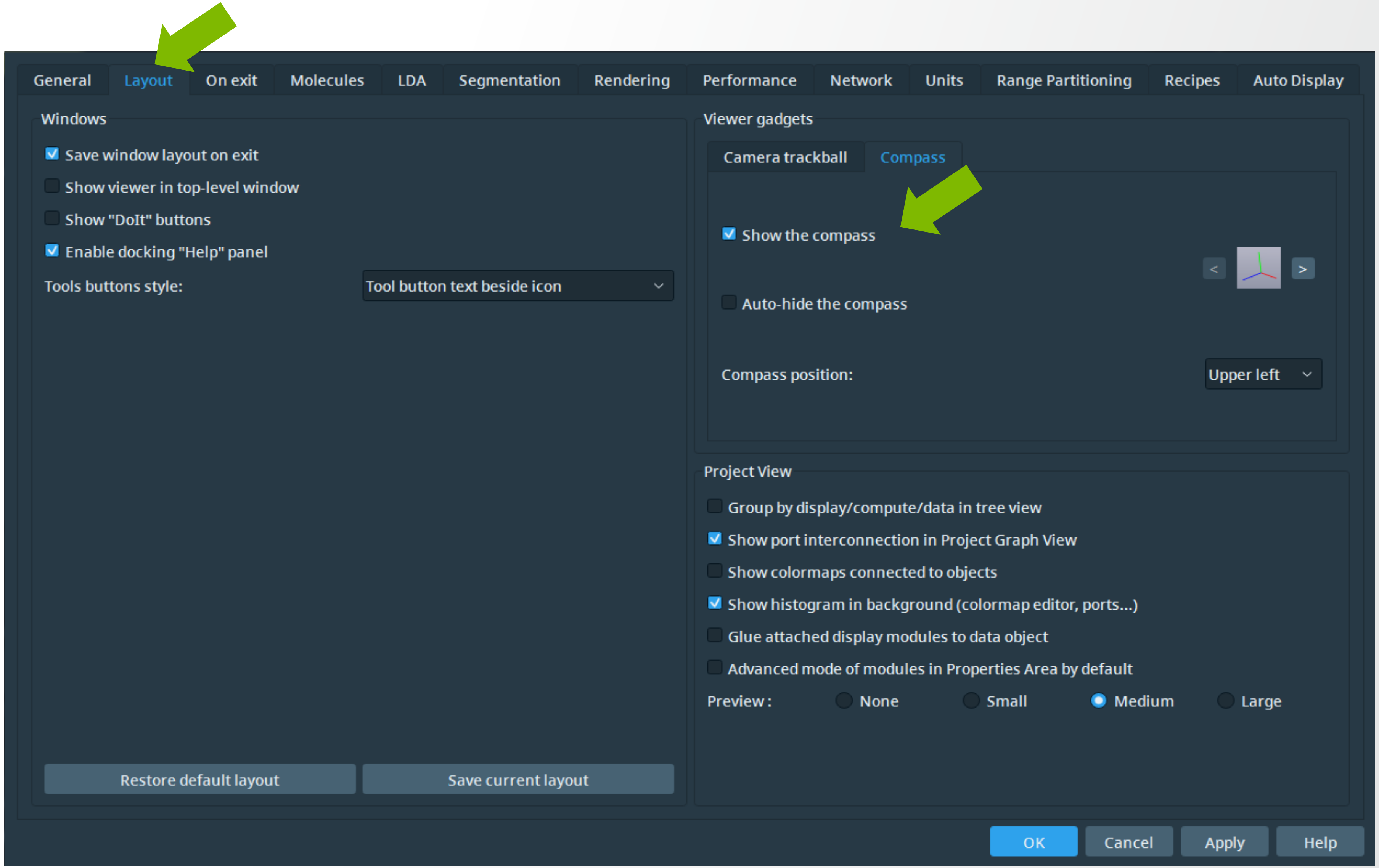
- Set the number of recent files and projects displayed on start page



Setting preferences – part 2

Preference setting e.g.:

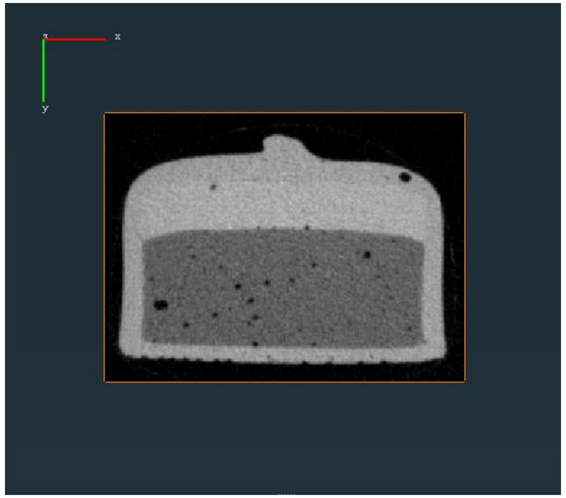
- Add compass in the 3D Viewer

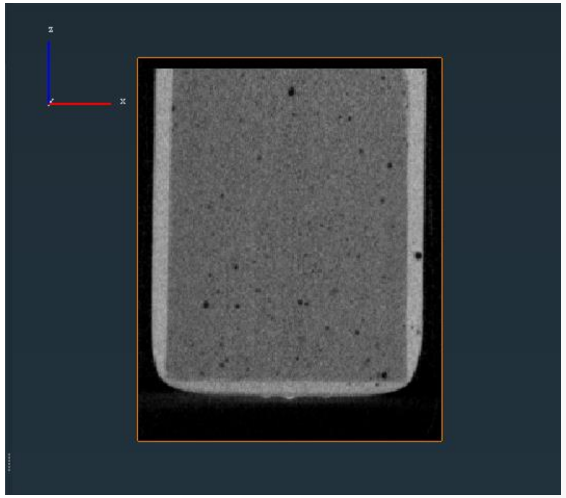


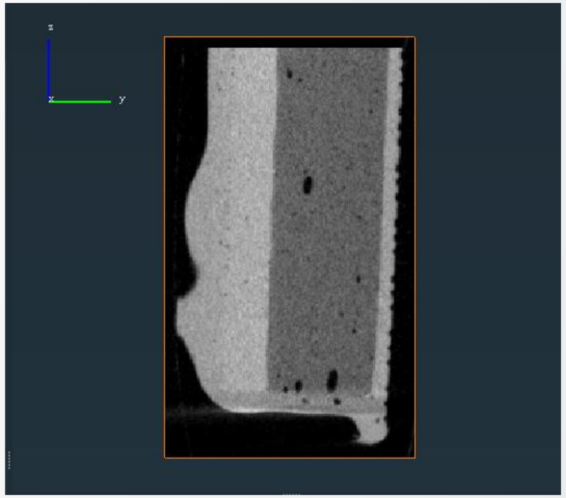
Visualize a Dataset: e.g. Ortho Slice properties examples

Orientation xy | Orientation xz | Orientation yz

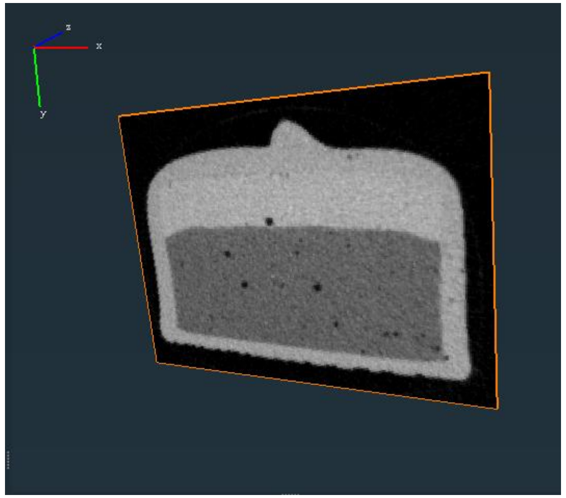
Adjust View on

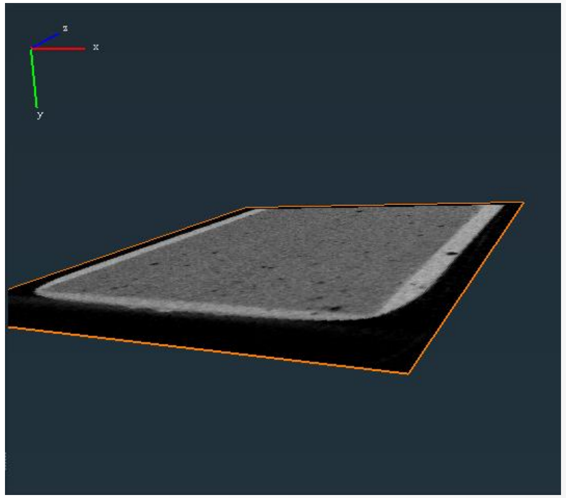


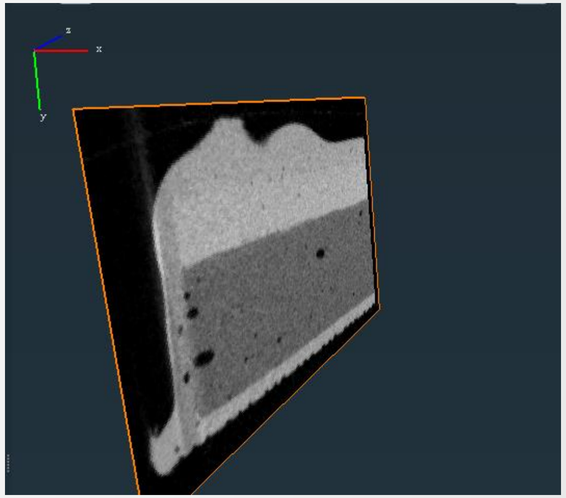




Adjust View off







Visualization of 2D and 3D data

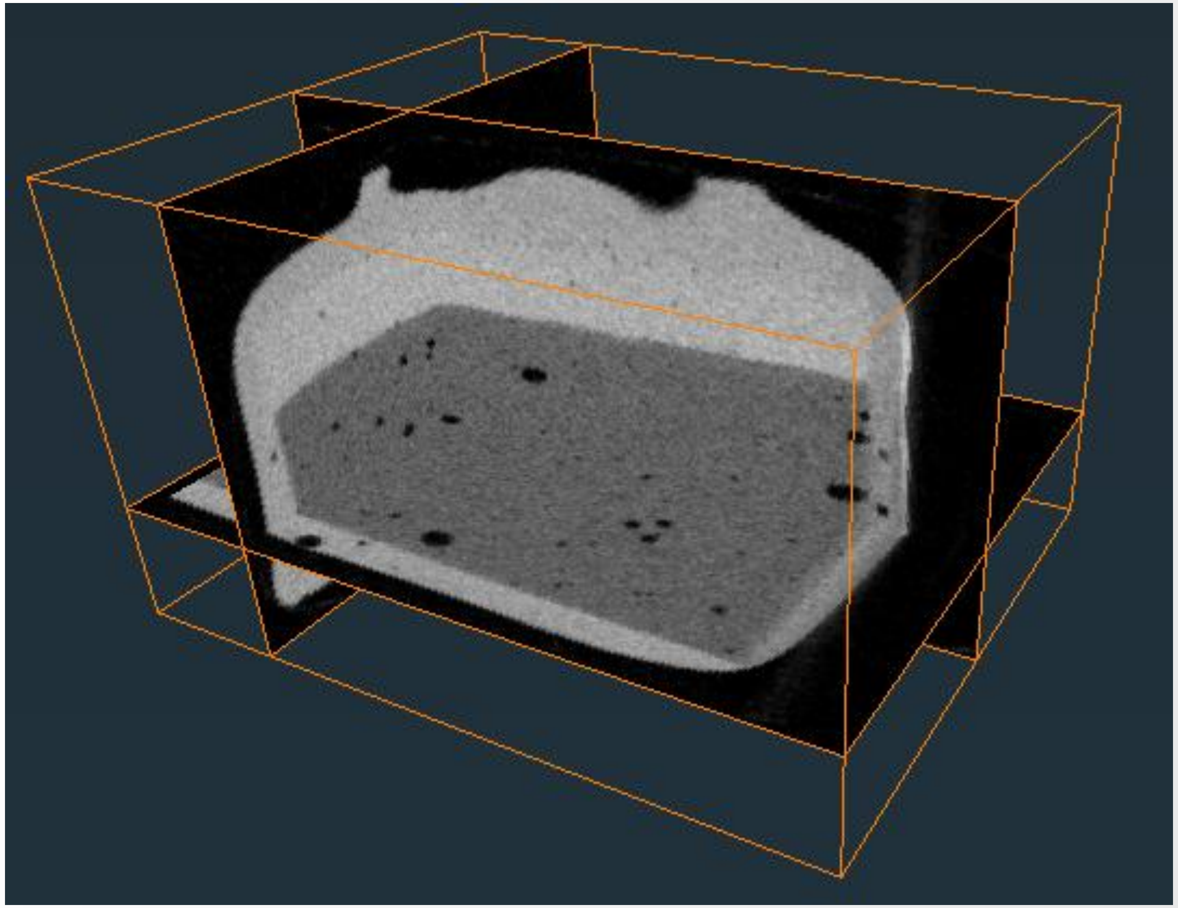
Data visualization: Exercise 1

Setting the orientation of the 2D view

Assemble the necessary modules to create a view like in the screenshot

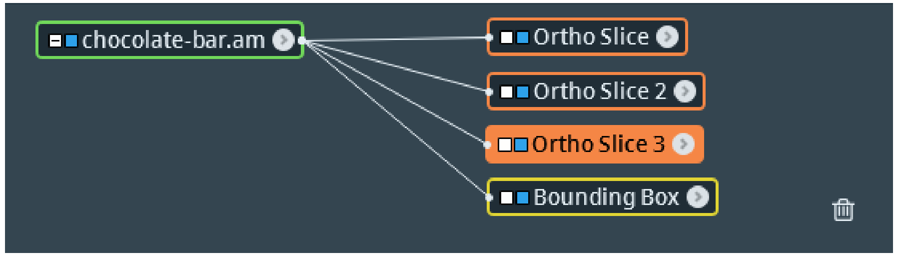
Dataset available at:

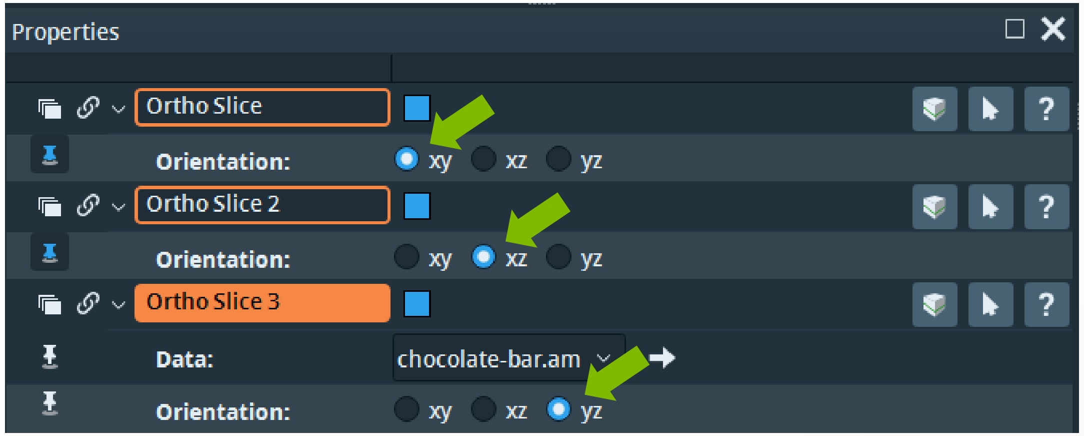
- $INSTALLDIR/data/tutorials/ chocolate-bar.am

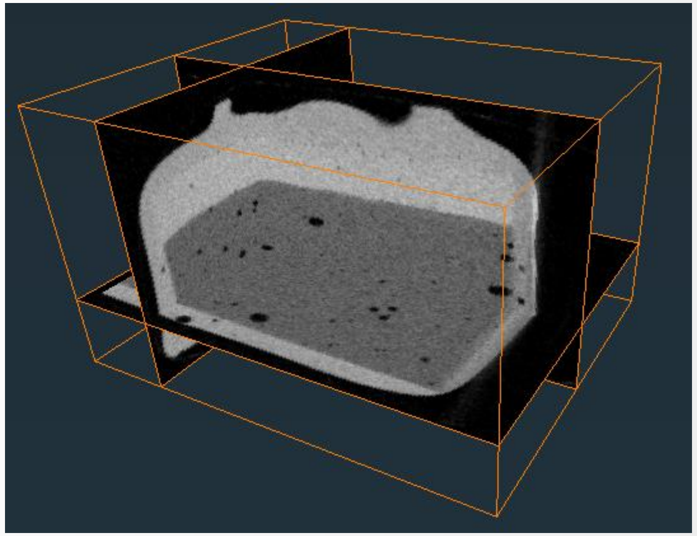


Data visualization: exercise 1

Solution





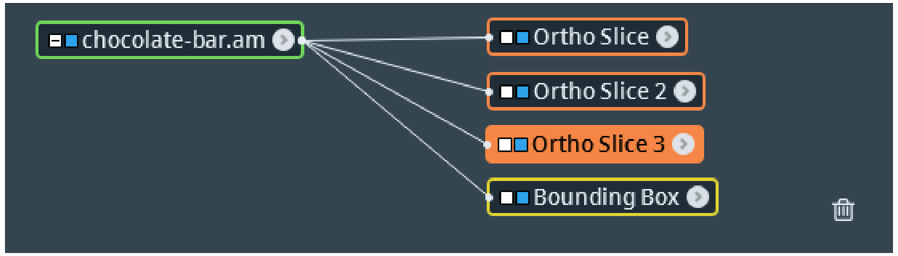


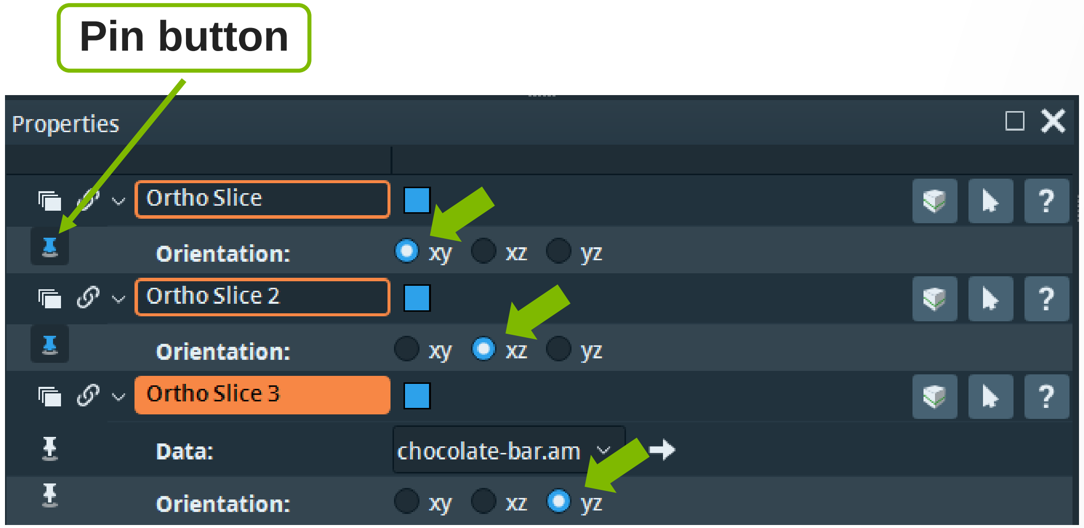
Solution:

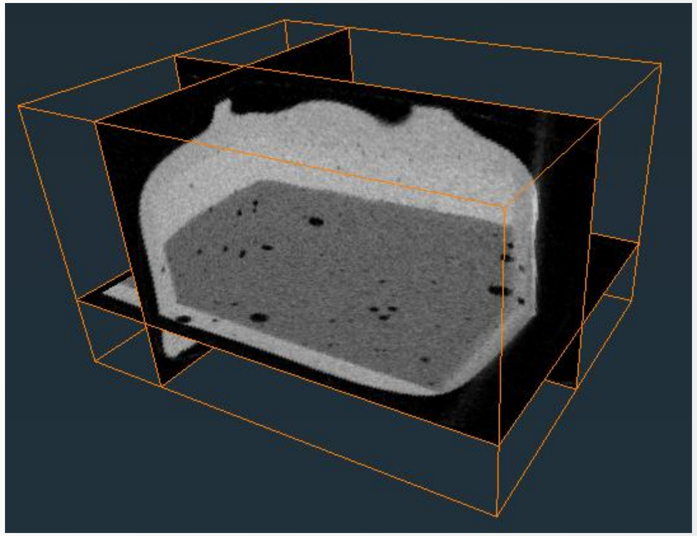
- Connect 3 Ortho Slice modules, each having a different orientation setting
- Note: Multiple modules with different ports settings can be connected to the same data

Data visualization: exercise 1

Tip



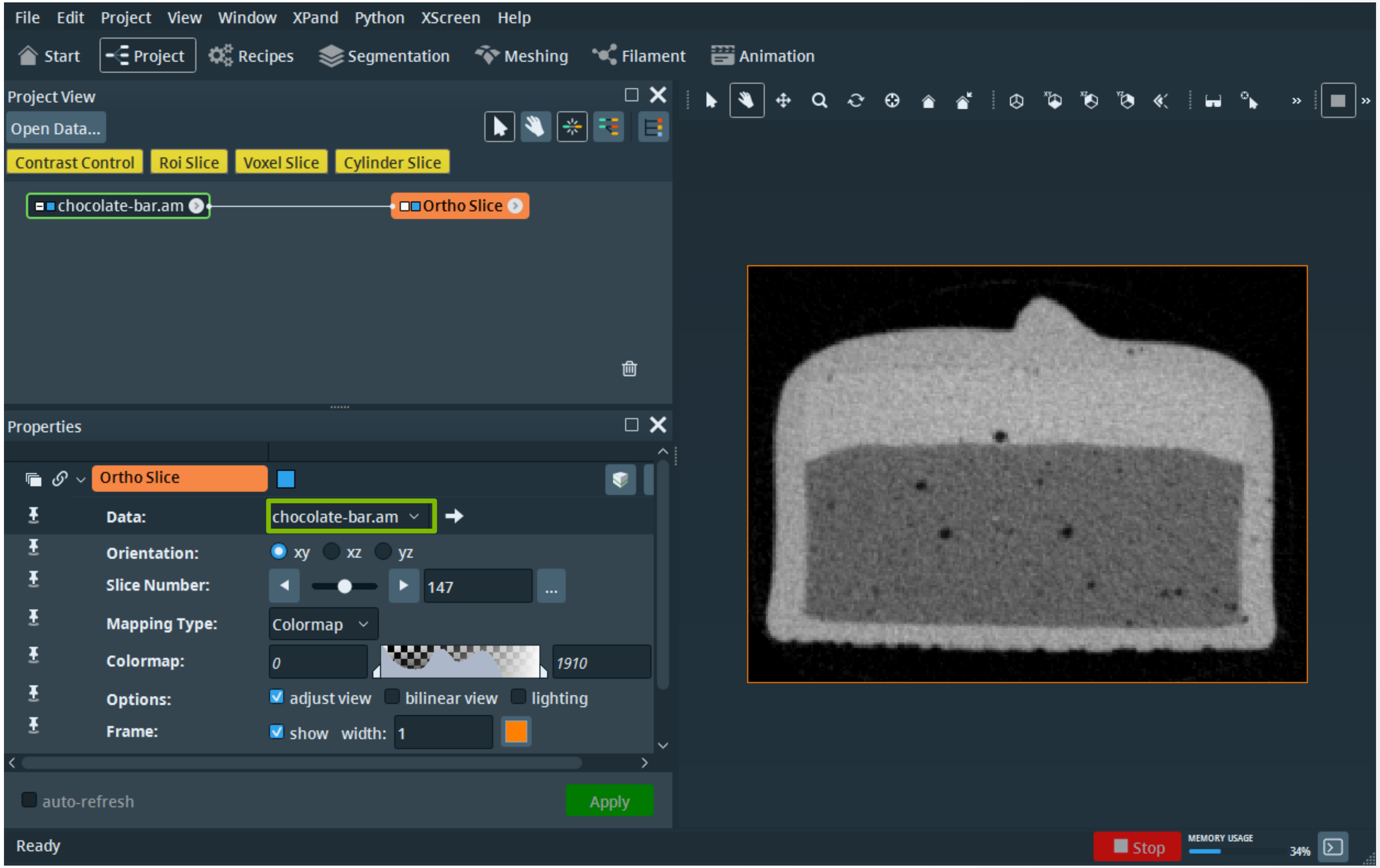




Tip:

- Module ports can be pinned by clicking the pin button: pinned ports are always displayed in the Properties window, even if the module is deselected

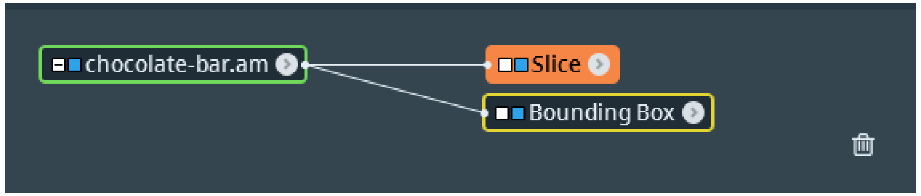
Visualize a dataset: e.g. Ortho Slice

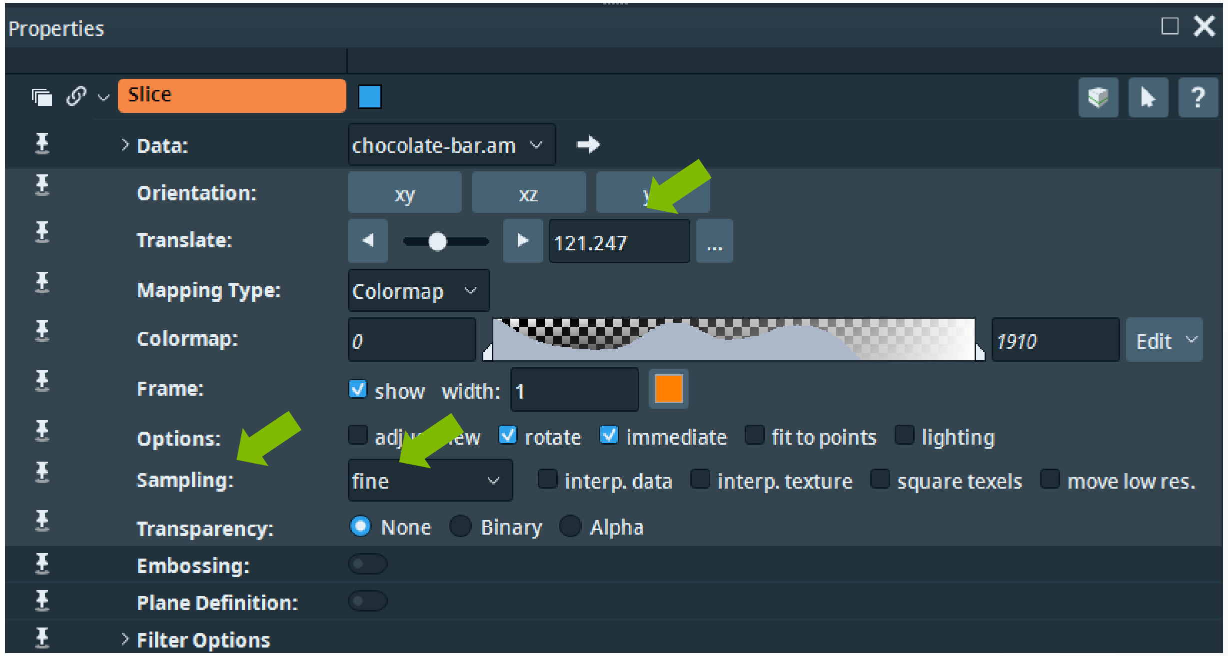


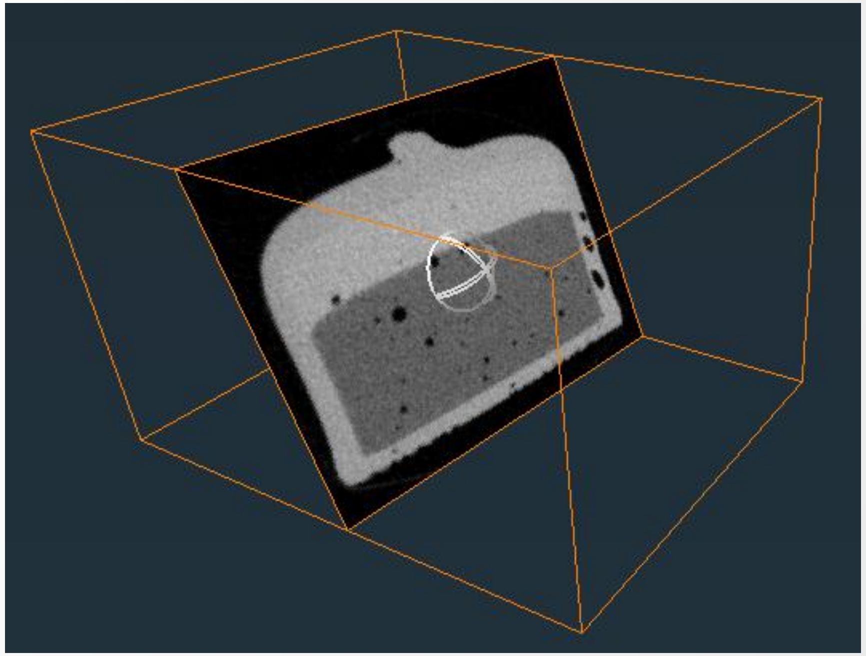
- Ortho Slice connects Automatically if Auto Display is on
- Can otherwise be created like any other module
- A dataset is displayed in the viewer only if it has a visualization module attached
- Check that visibility is turned on (e.g. workflow with multiple data)

2D visualization with Slice

Slice: visualize arbitrarily oriented slices in a volume





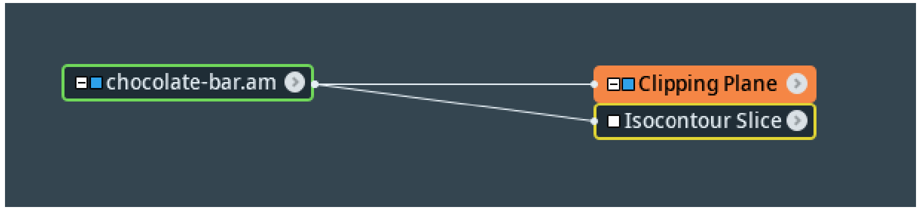


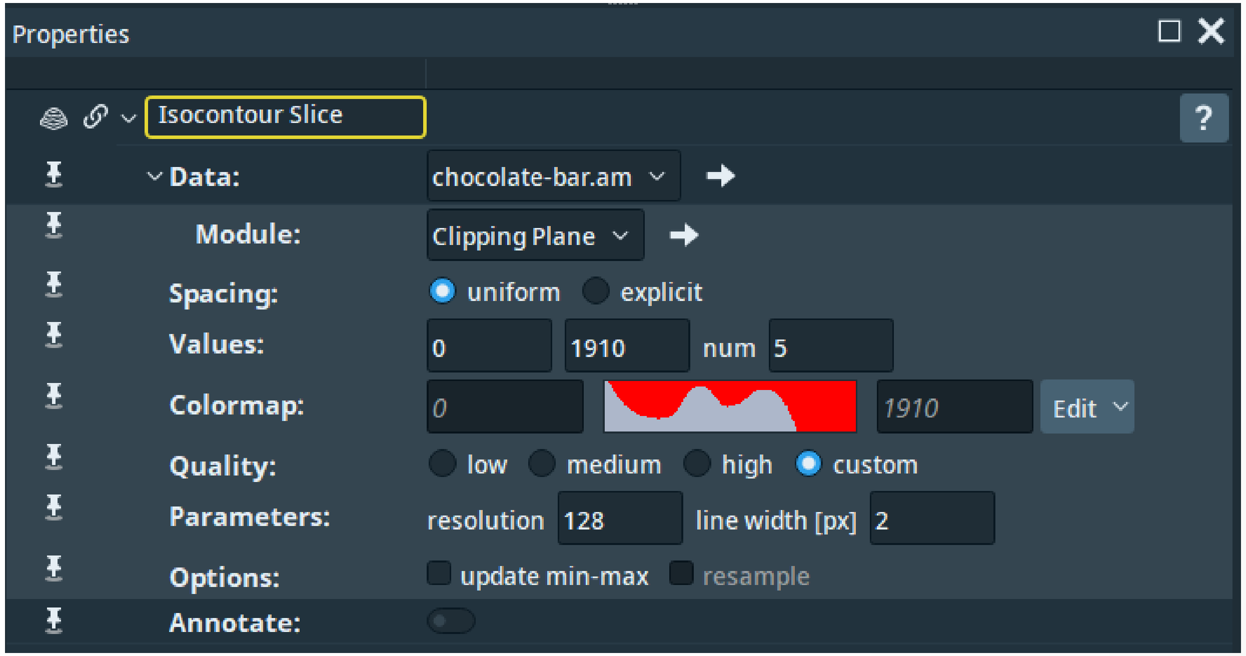
Slice – interpolation:

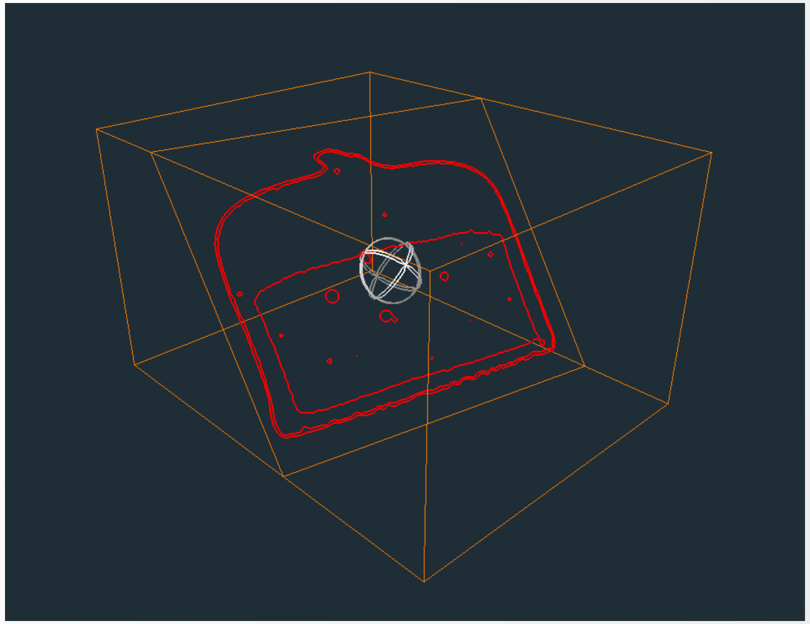
- Not necessarily axis-aligned, interpolation is necessary for reconstruction
- Interpolation can be tuned from Sample ports
  - Different sampling resolutions (fine, coarse, etc.) are available in the drop-down menu.
- Sample ports – no effect if the slice is axis-aligned.

2D visualization with Isocontour Slice

Isocontour Slice: computes isolines and their annotations for an arbitrary 3D scalar field on a 2D cutting plane.



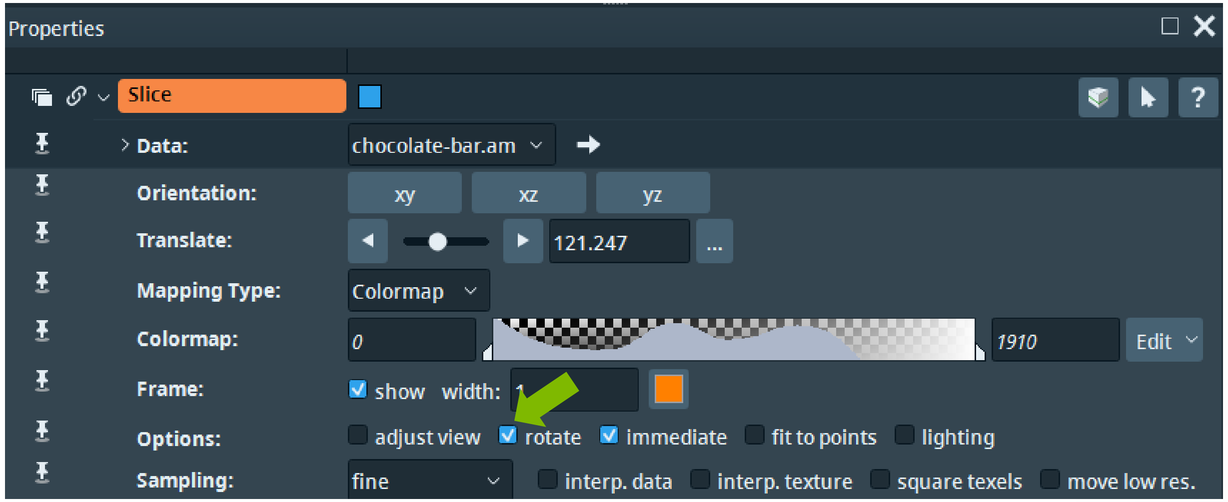


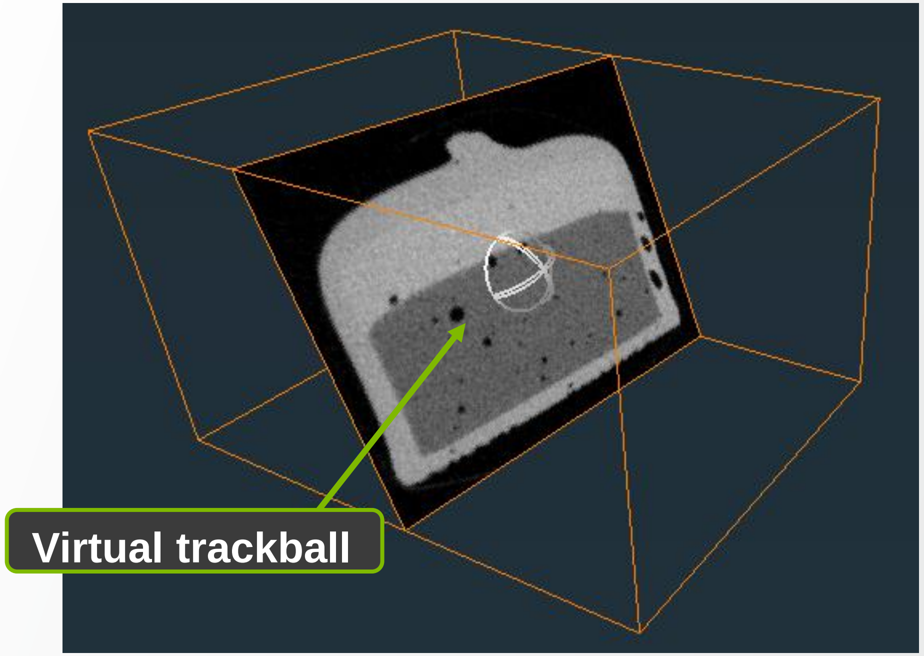


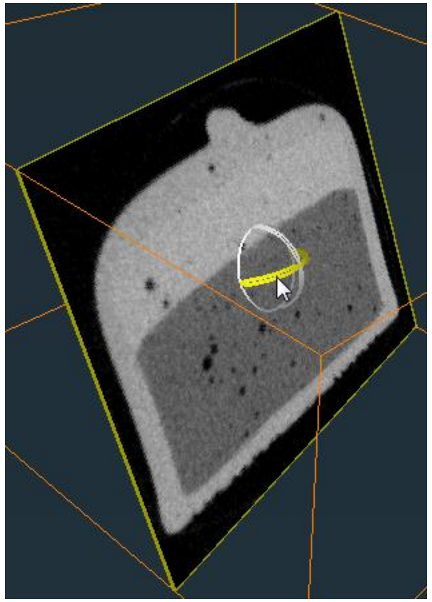
Isocontour Slice:

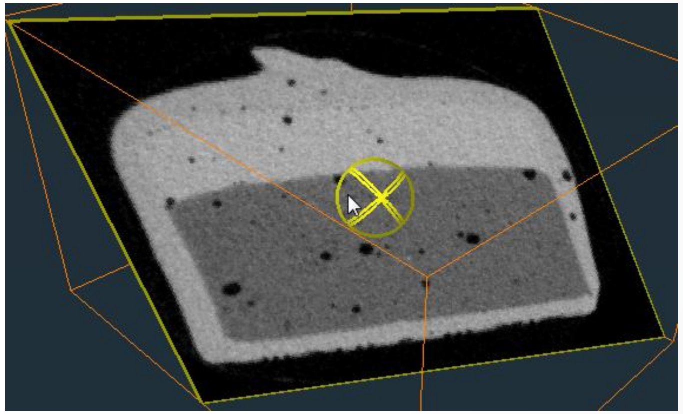
- Clipping Plane
- Isocontour
- Rotation
- Iso-Intensity values
- Colormap

2D visualization with Slice







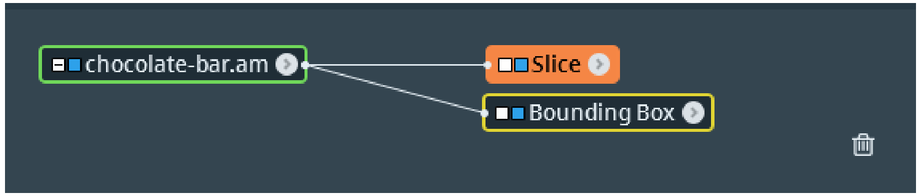


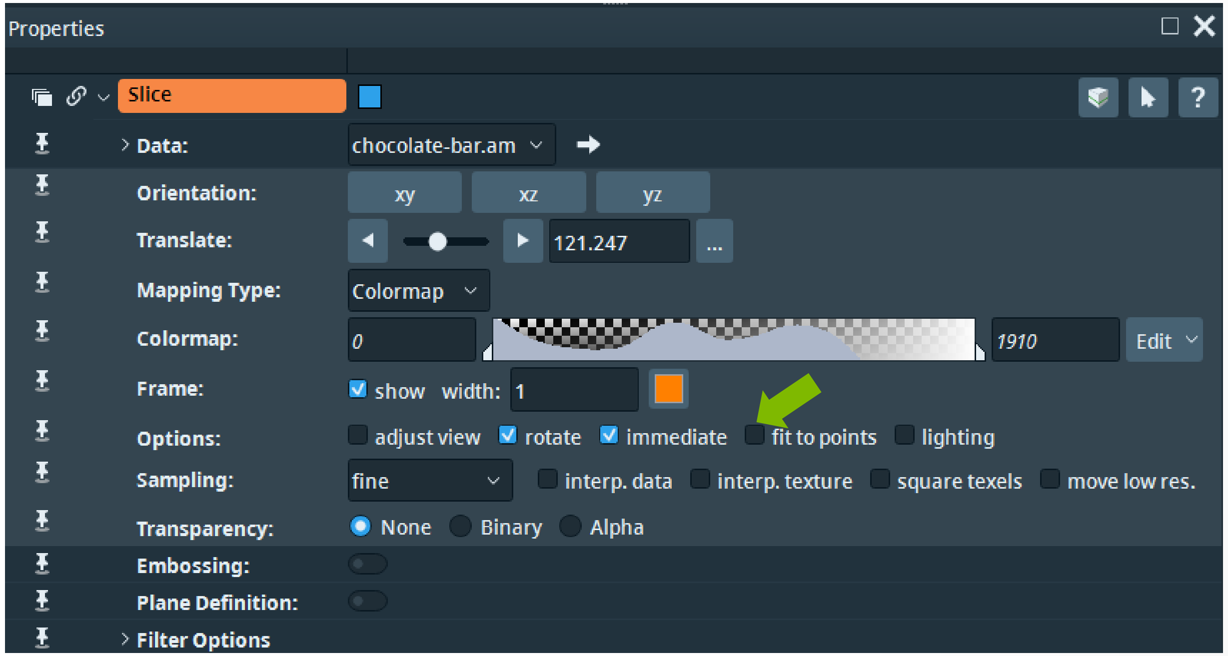
Slice – rotate:

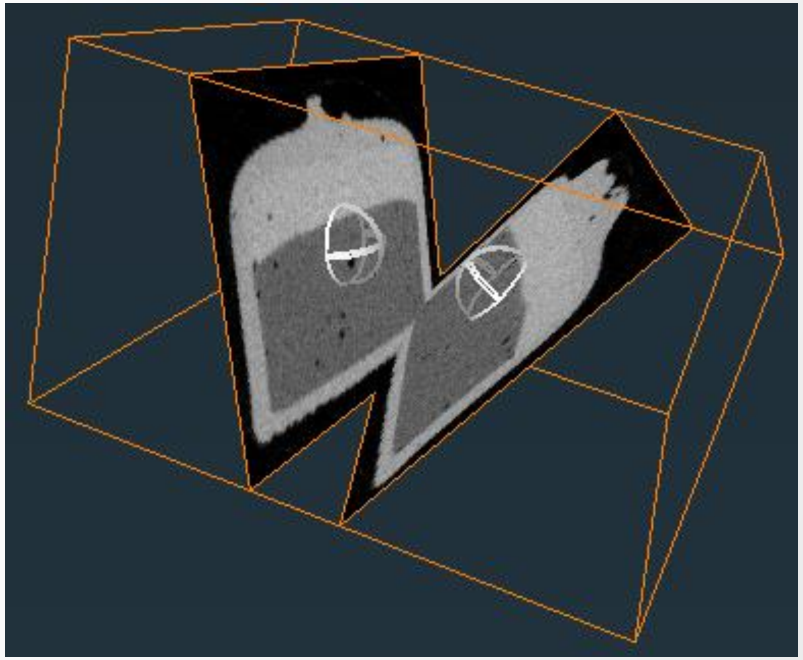
- Activate virtual trackball: Press [Tab] or select "rotate" (from Options)
- Hold the left mouse button in interactive mode:
  - Click inside the white lines of a trackball axis => rotate along the respective axis
  - Click outside the trackball axes => rotate in all directions

2D visualization with Slice

Slice: visualize arbitrarily oriented slices in a volume







Slice – defining a plane:

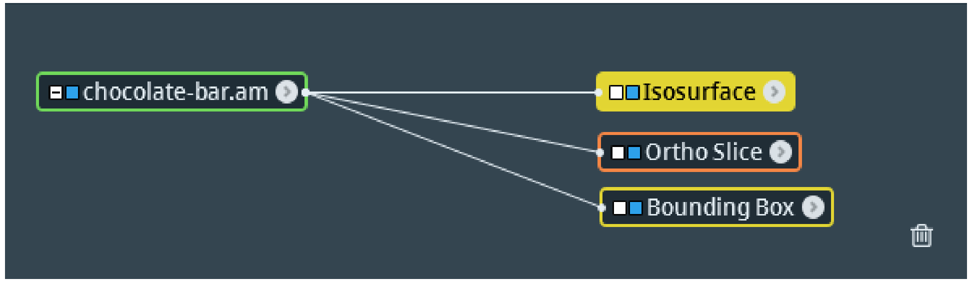
- Select "fit to points" (from Options)
- Click on 3 different points inside the object

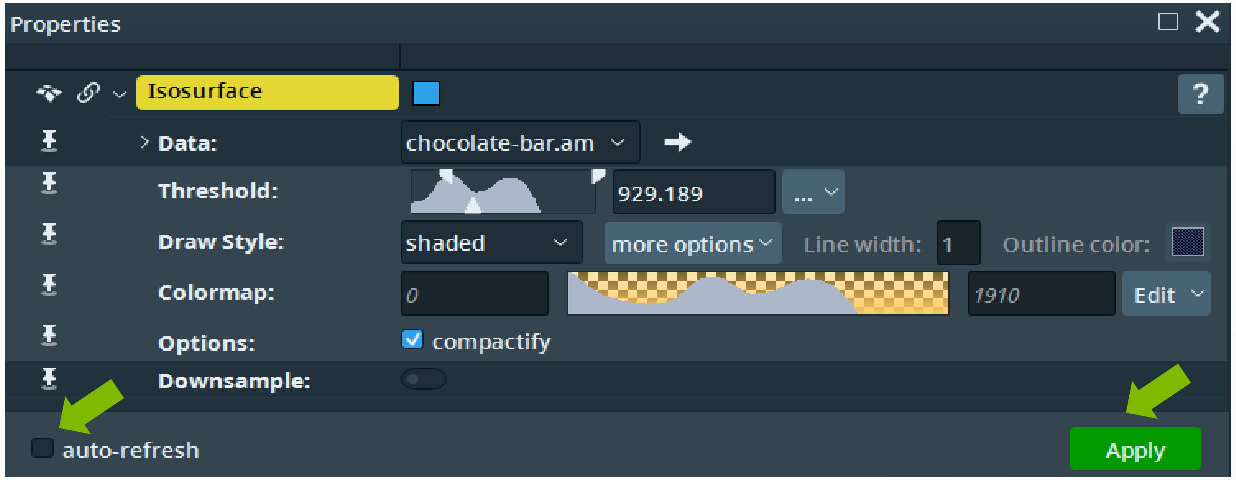
Press [Esc] to activate Interact mode.

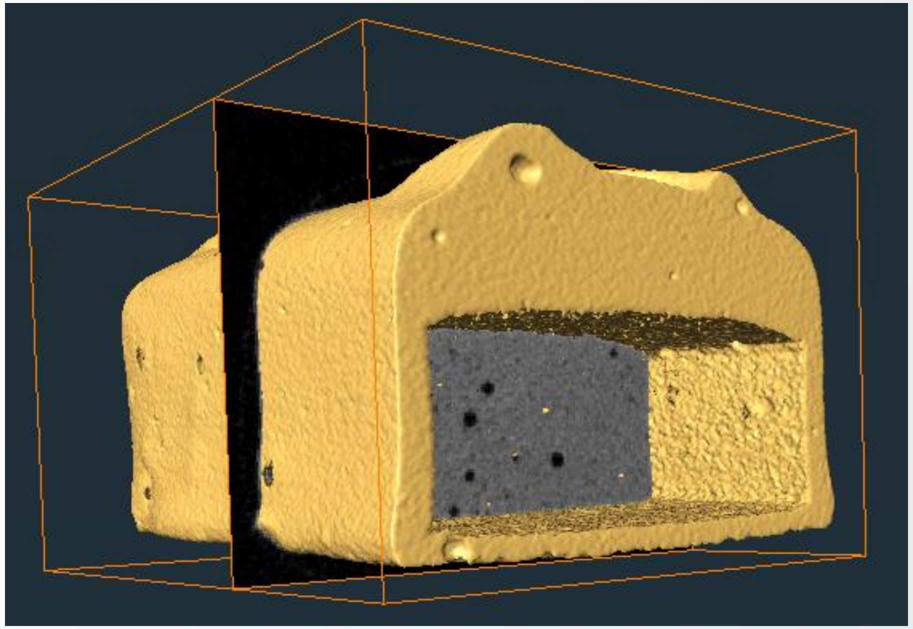
- After clicking 3 points, "fit to points" is automatically disabled.

3D visualization with Isosurface

Isosurface: visualization of surfaces of same value





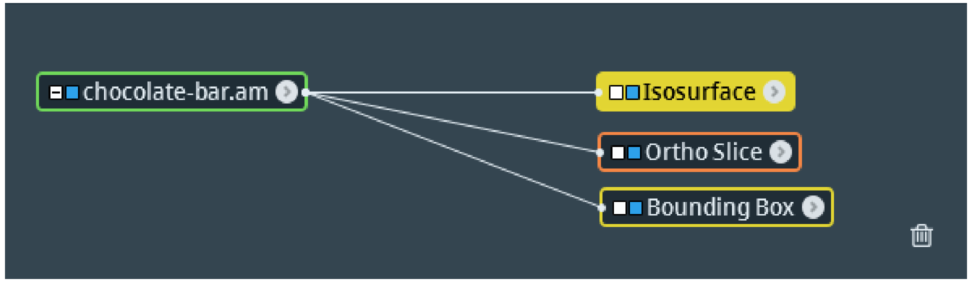


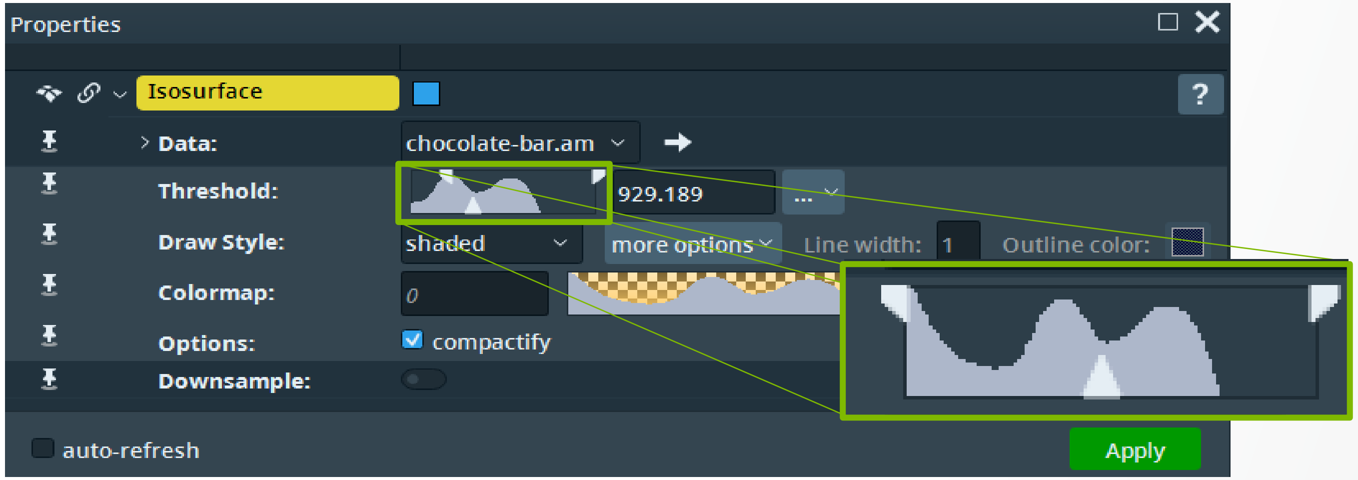
Isosurface – visualization module that requires processing. For launching the processing:

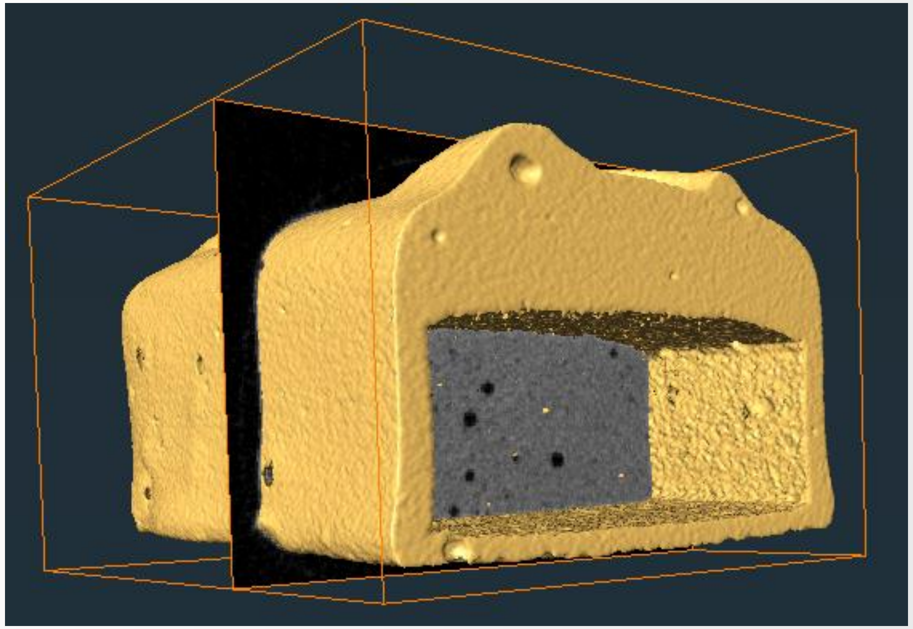
- Press the Apply button
- Check auto-refresh (use with caution)

3D visualization with Isosurface

Isosurface: visualization of constant value surfaces







A threshold value is necessary for computing the Iso-surface:

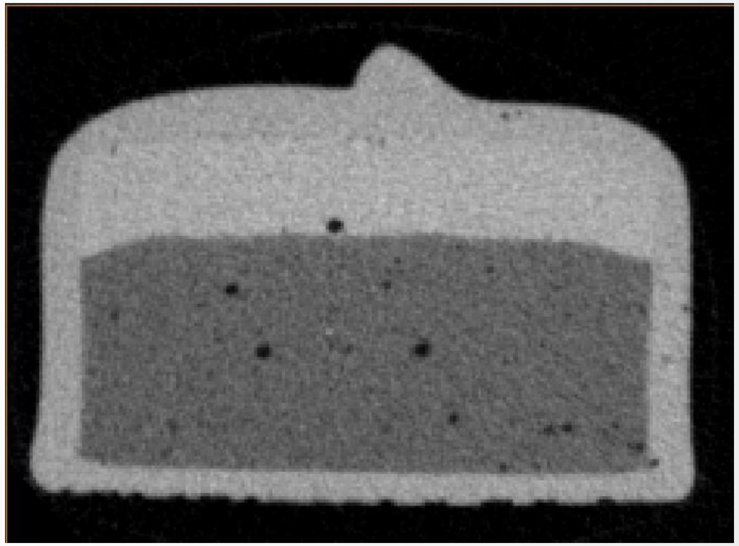
- set by default from data histogram
- can be manually adjusted (bottom slider)

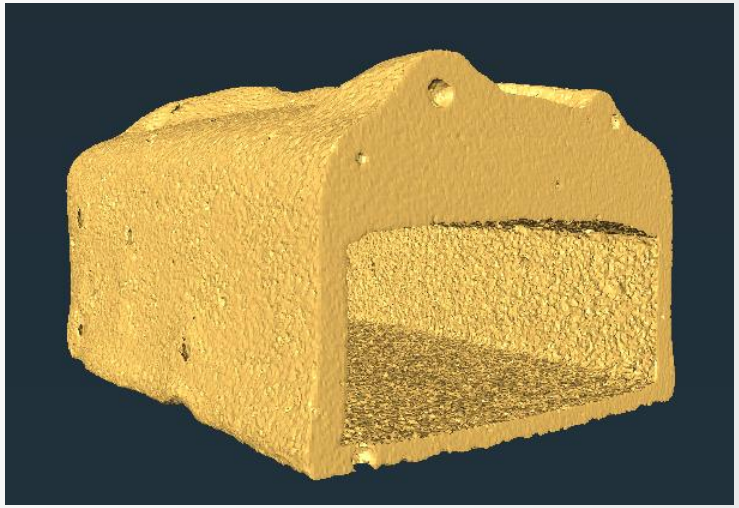
Top sliders – zoom on the histogram

3D visualization with Isosurface

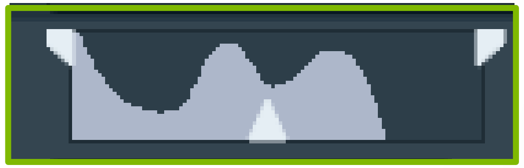
Histogram Thresholding

- Histogram – the distribution of voxel intensity values
- Can be computed by "Histogram" module
- Its shape is informative for thresholding a dataset
- In the case of chocolate bar data, the histogram has 3 lobes for:
  - background and porosities
  - for the "mousse" (inside)
  - for the chocolate and caramel (outer layers)





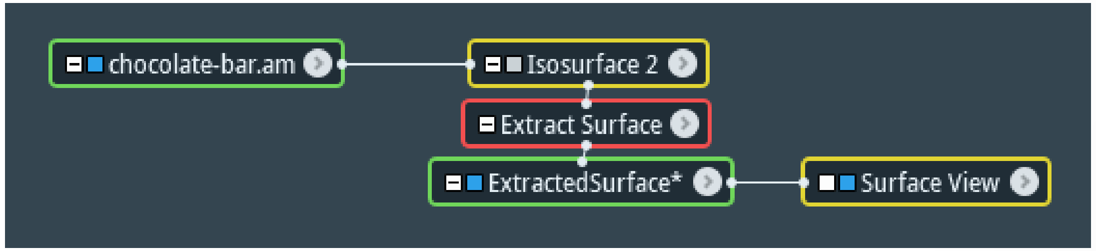
By setting a histogram threshold value as indicated below, The outer chocolate bar layers will be selected, as they correspond to the third histogram lobe (highest intensity values).

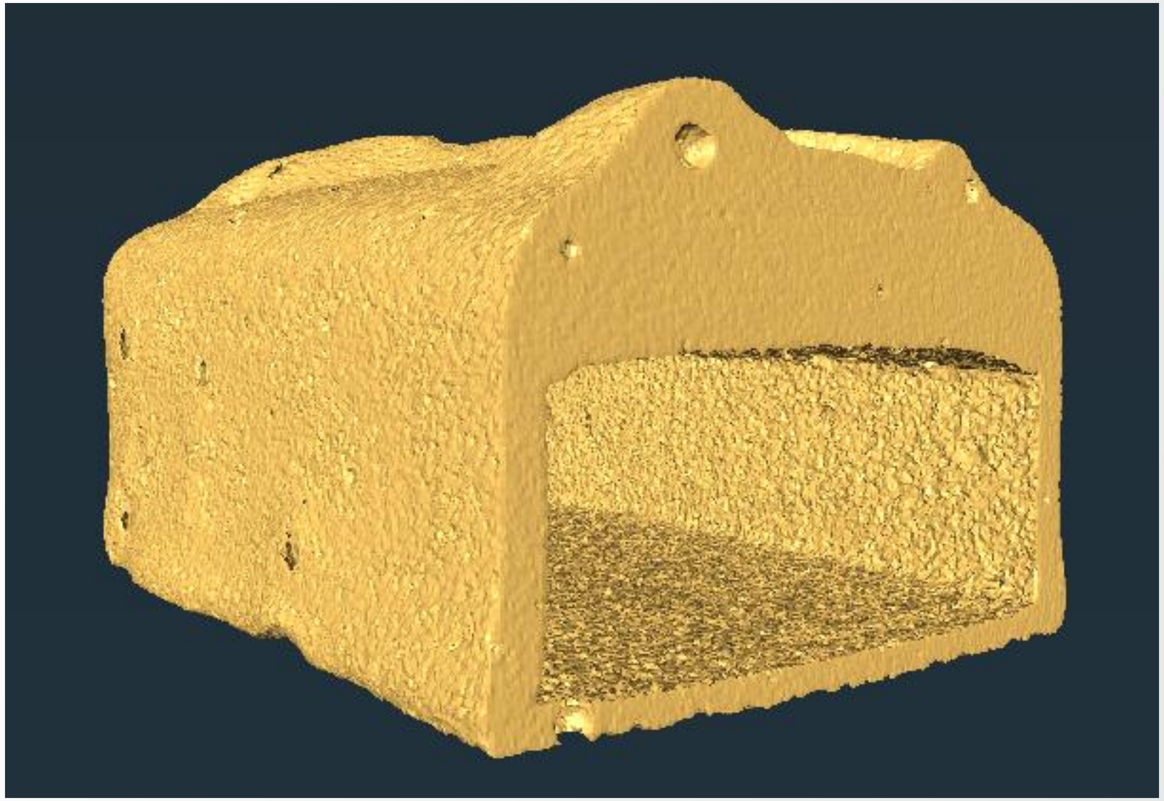


3D visualization with Isosurface

Isosurface:

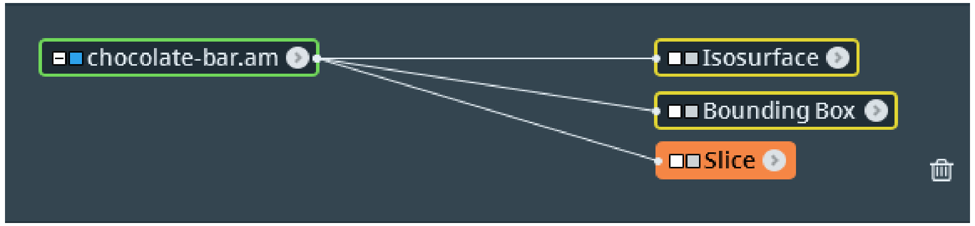
- For generate the surface computed by Isosurface, use “Extract Surface” module.
- For visualizing the generated surface, use “Surface View” module.



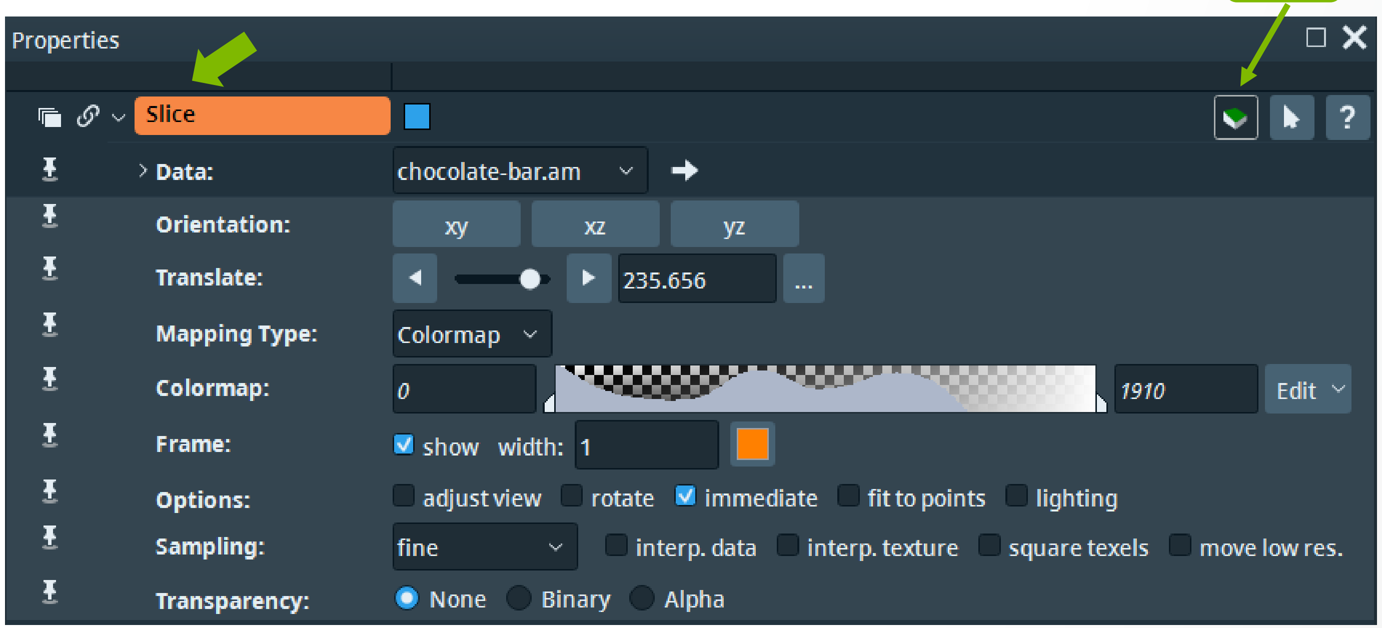


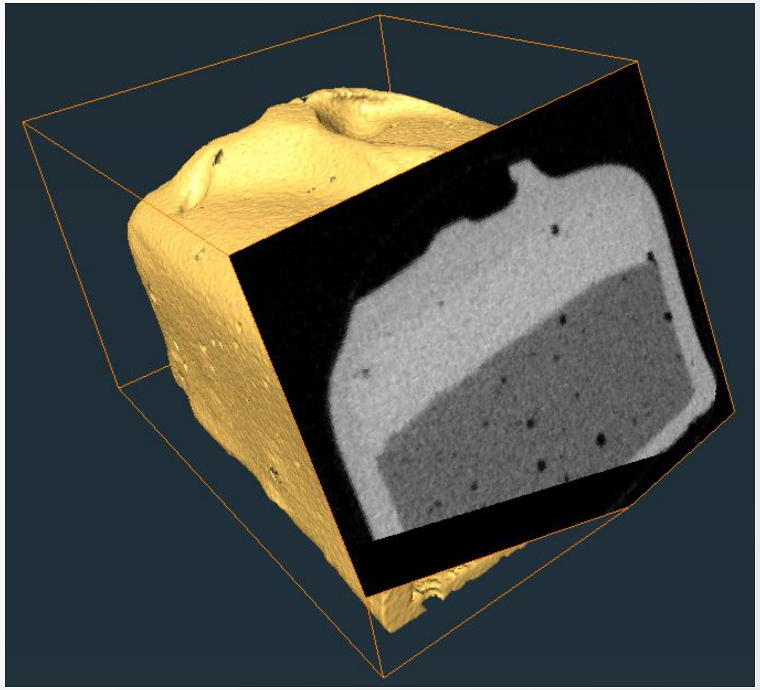
Planar visualization modules - clipping

Planar visualization (orange) modules can be used for clipping.
Clipping applies to all the objects that have the visibility on.



Clip

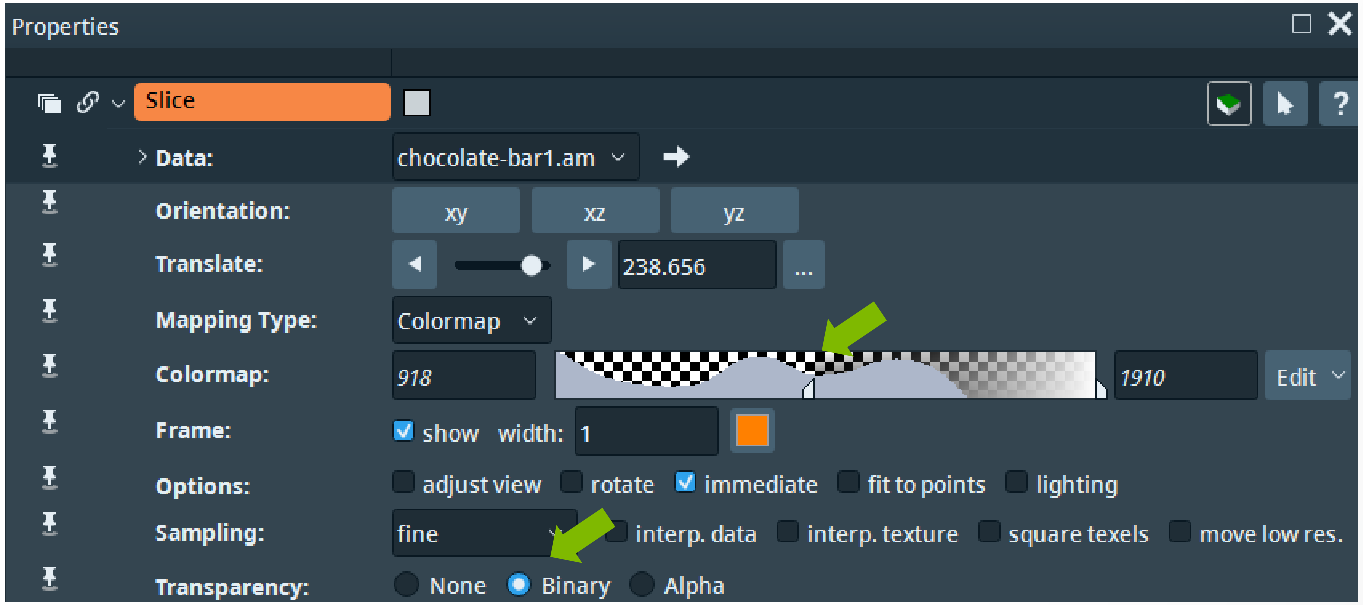


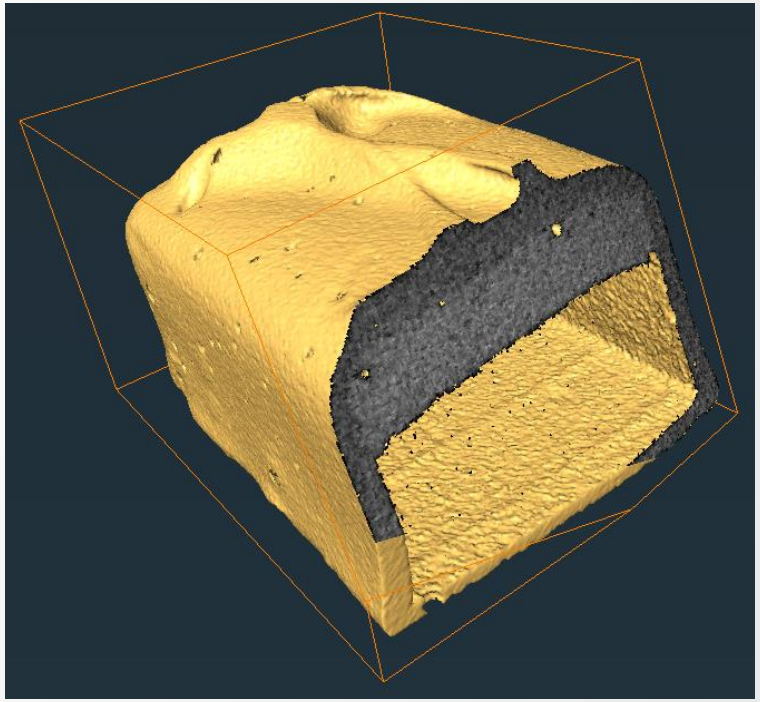


Clipping:

- Define the clipping plane by e.g. Slice
- Click on the “Clip” icon – clip on one side of the object
- Click again – disable clipping
- Click again – clip on the other side

Planar visualization modules - clipping





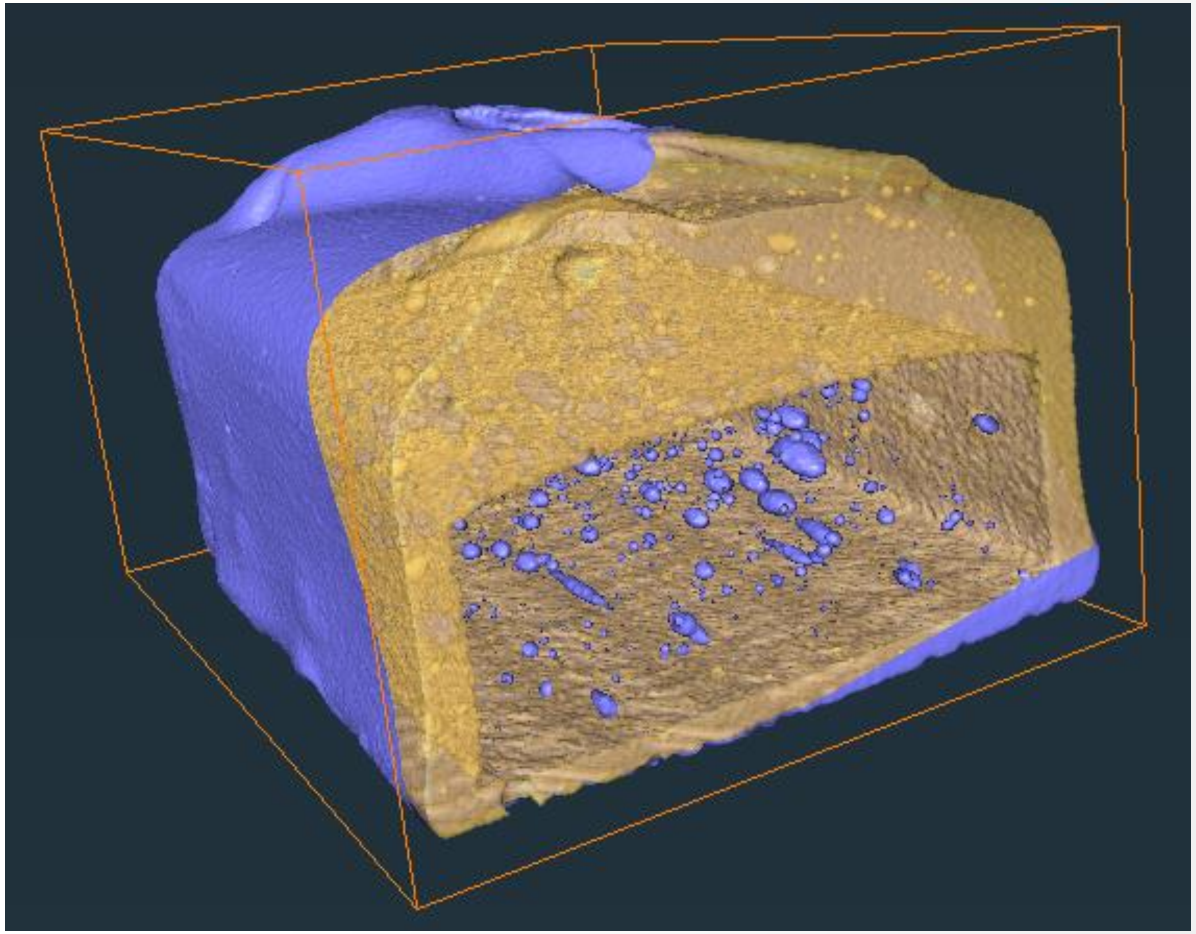
Setting Slice transparency:

- Choose Binary mode (from Transparency) – regions of voxel intensity values outside the colormap range are fully transparent while others are fully opaque.
- Adjust the colormap range to obtain the view in the example above.

Data visualization: exercise 2

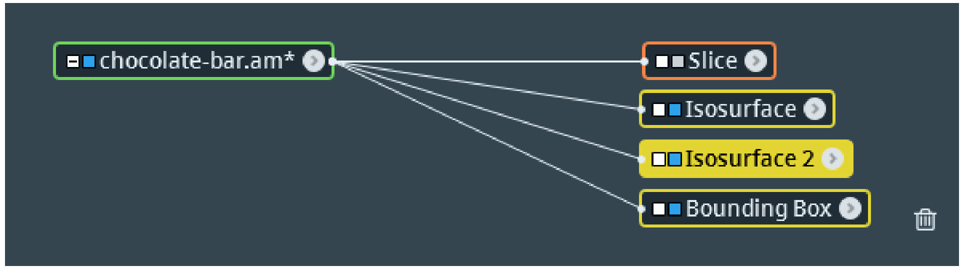
Clipping volumes and setting transparency

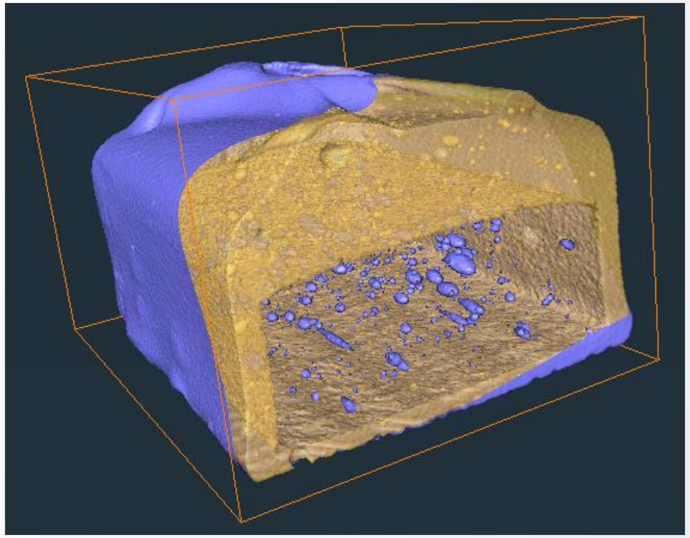
Assemble the necessary modules to create a similar view:

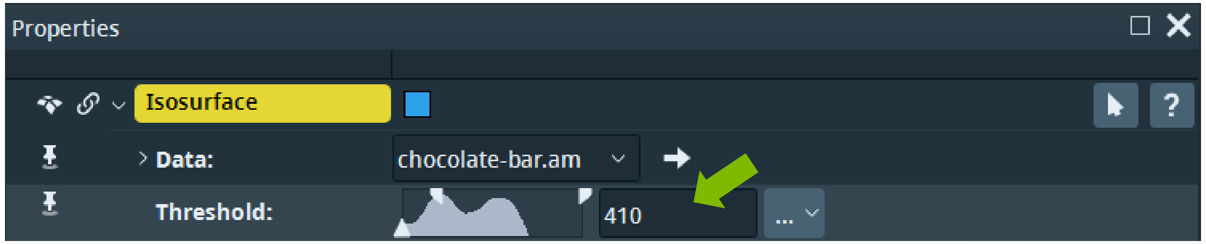


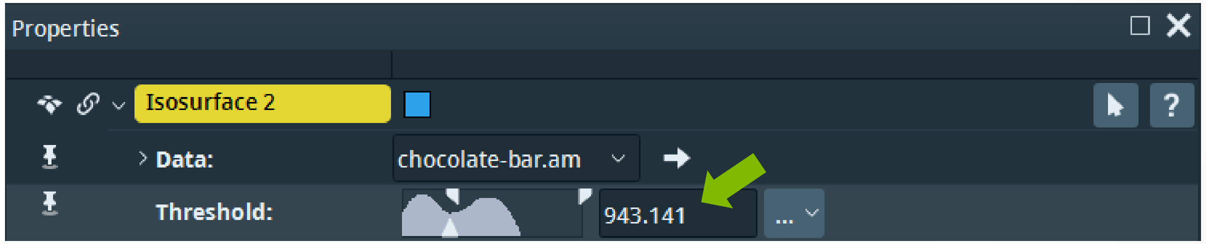
Data visualization: exercise 2

Solution – Step 1







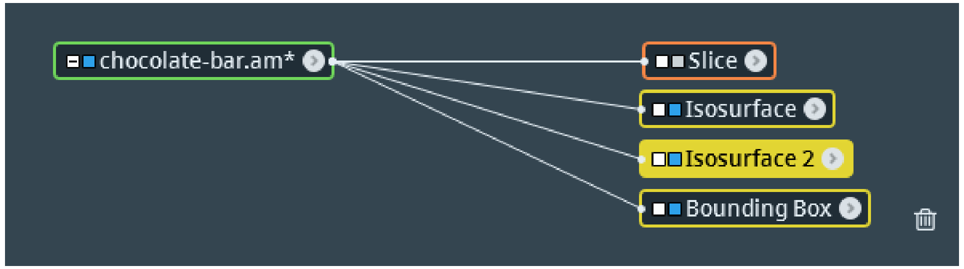


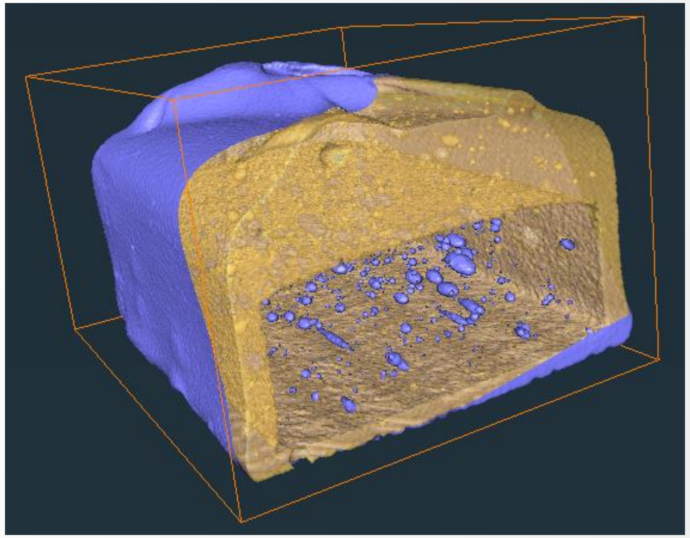
Generate 2 isosurfaces for two thresholds:

- Low threshold: ~ 410
- High threshold: ~ 940

Data visualization: exercice 2

Solution – Step 2





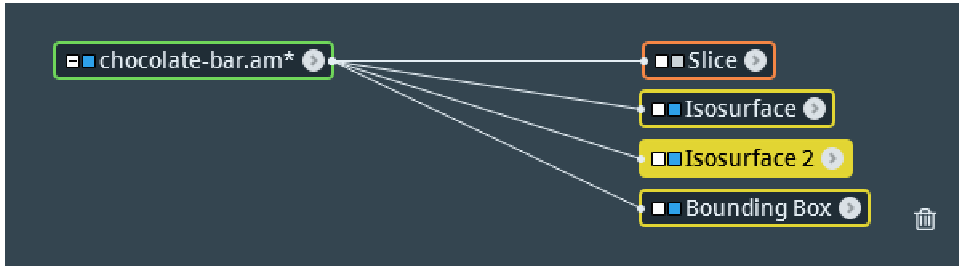
Clip the 1st Isosurface with Slice:

- All visible modules are clipped => the visibility of all other modules except the 1st Isosurface should be turned off.
  Tip: Select 1st Isosurface and press [h] => only he selected module has the visibility switched on.
- Select the clipping plane with Slice and then clip.

Data visualization: exercise 2

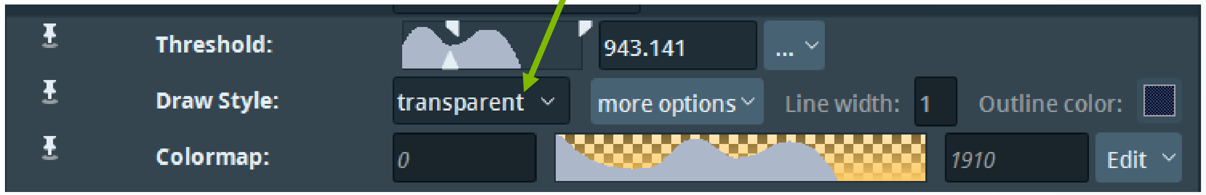
Solution – Step 3

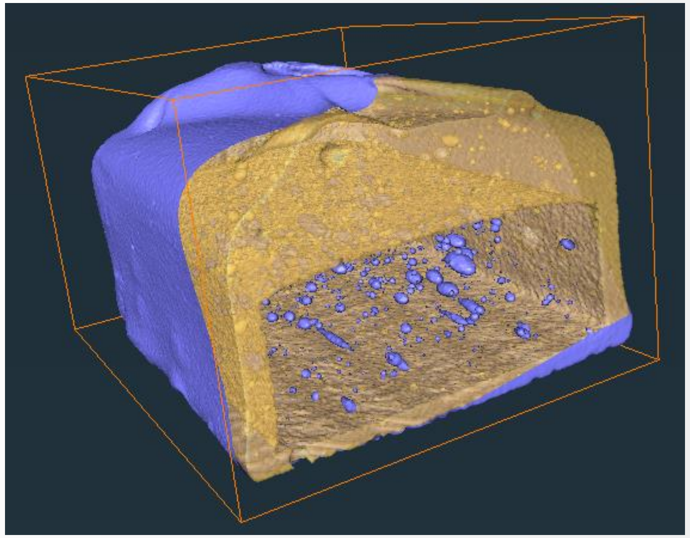
Full solution available at:



Double click to change color

Set transparency





Setting visibility, colors and transparency:

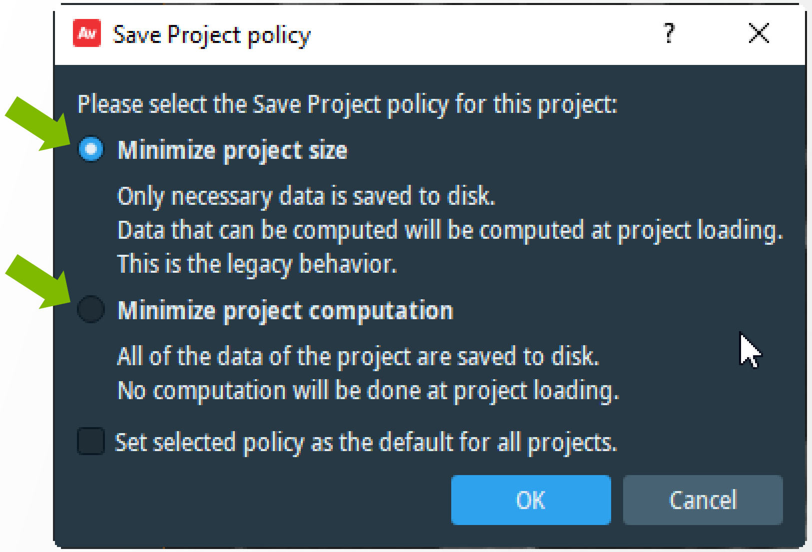
- Turn off the visibility of Slice
- Change the color of the 1st Isosurface: double click on the colormap to pick color
- Set a transparency to the 2nd Isosurface – select “transparent” (“Draw style” port)

Project save

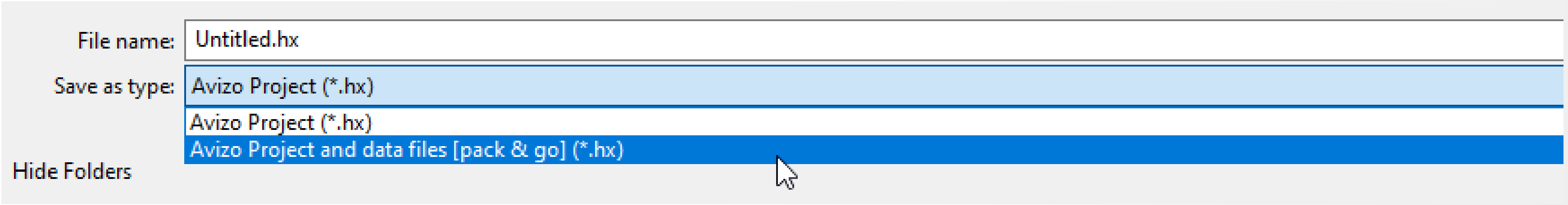
Saving a project: File > Save Project (As) …

- Use "Minimize project computation" when a module takes a long computation time

- Use "Minimize project size" otherwise

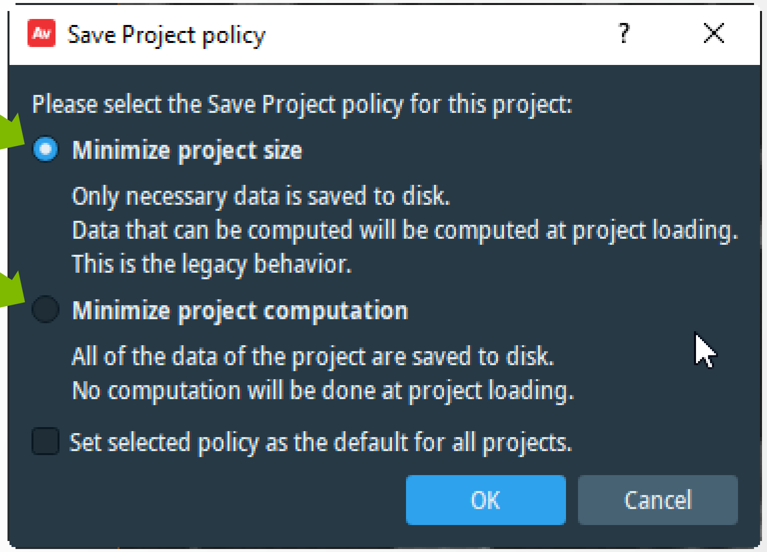


- Use '[pack & go]' option if you need to archive or transfer the project to a different computer.
  - This will copy the input dataset(s) inside the project folder. Otherwise, these files are only referenced via their path on the disk, and the project will not load if this path is no longer valid.



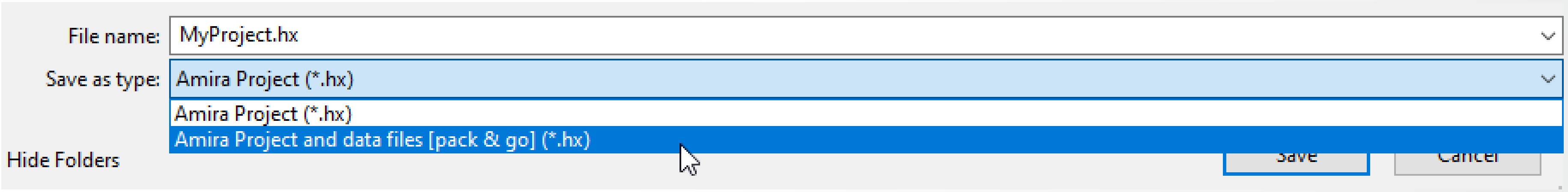
Project save

Saving a project: File > Save Project (As) …



- Use “Minimize project computation” when a module takes a long computation time
- Use “Minimize project size” otherwise

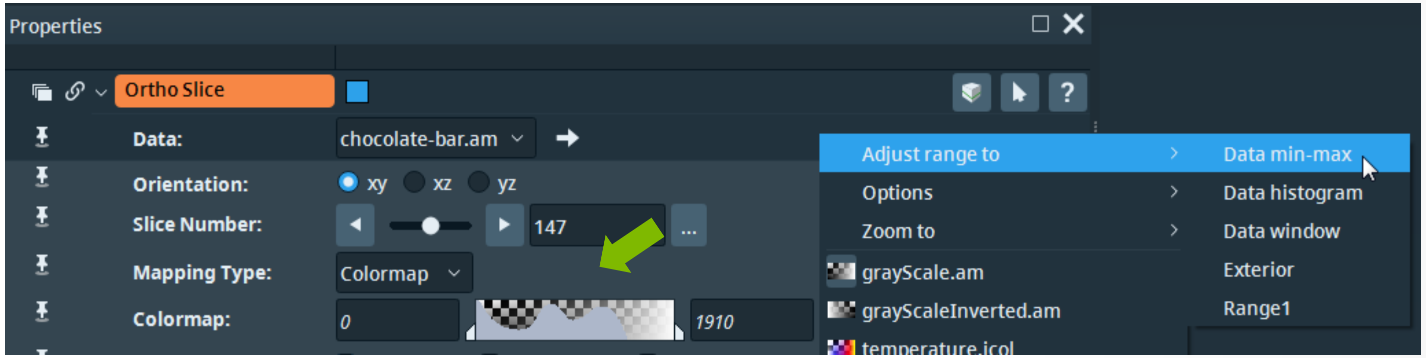
- Use ‘[pack & go]’ option if you need to archive or transfer the project to a different computer.
  - This will copy the input dataset(s) inside the project folder. Otherwise, these files are only referenced via their path on the disk, and the project will not load if this path is no longer valid.

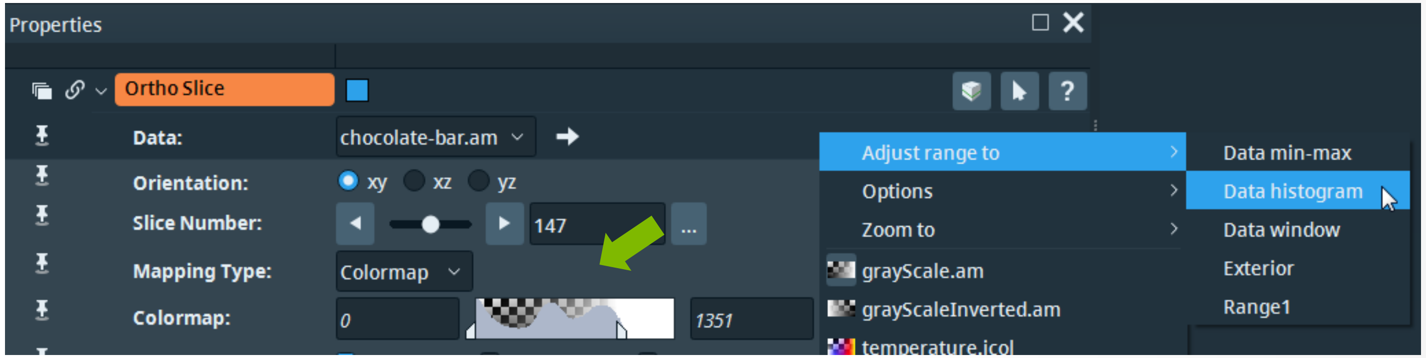


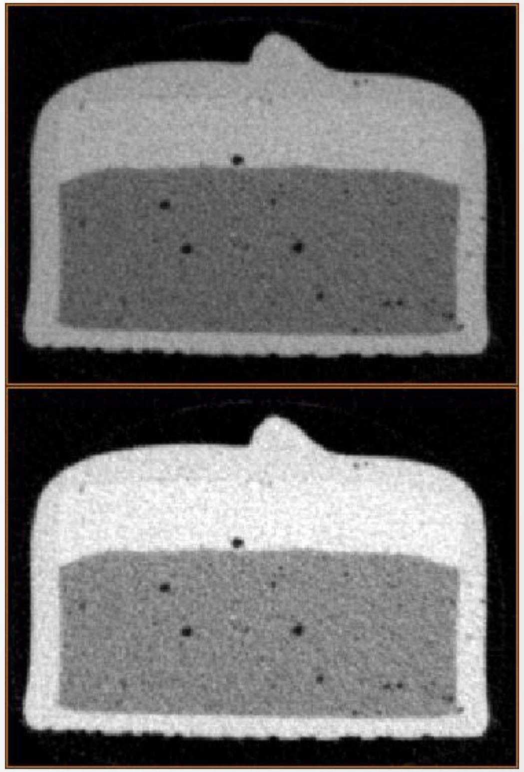
Setting a colormap: e.g. Ortho Slice

- A colormap is used to map scalar values to intensity levels or colors
- The colormap range can be modified manually in order to adjust brightness, darkness or contrast
- Predefined colormap settings are also proposed.

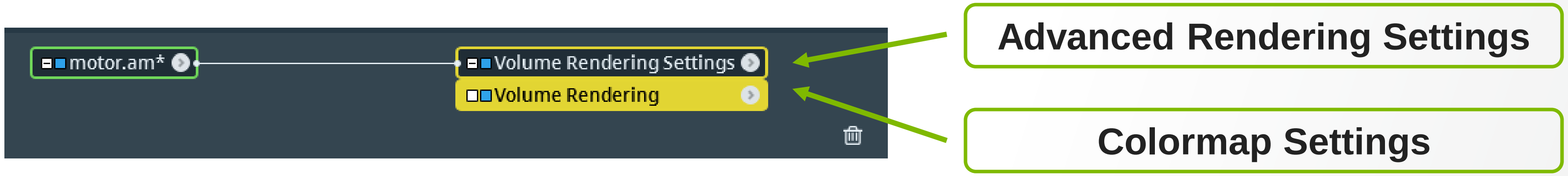
Some examples of grayscale colormap setting for Ortho Slice module (click on the ”Edit” button of “Colormap” port):





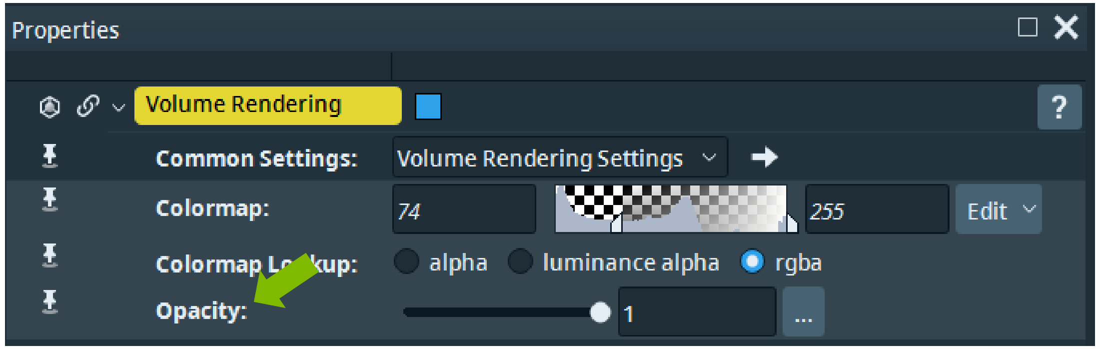


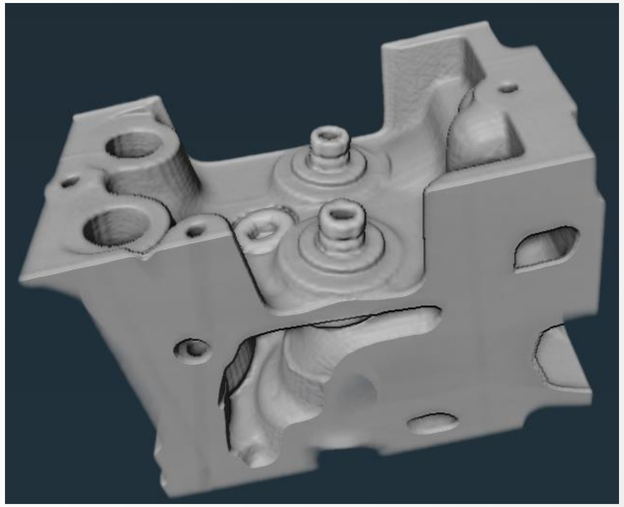
3D visualization with Volume Rendering

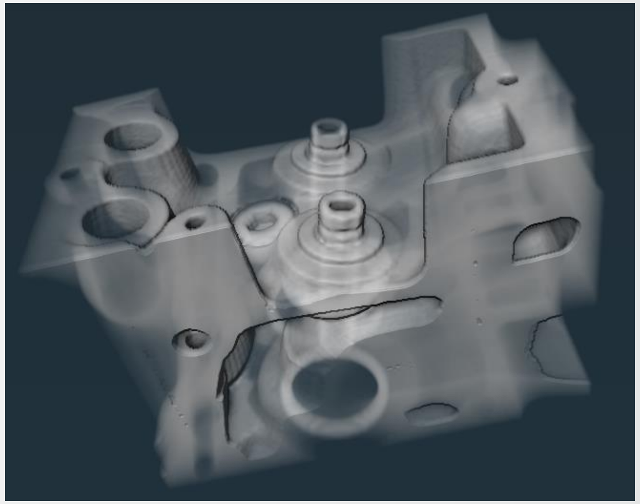


Volume rendering – colormap settings for assigning color and transparency to each voxel value

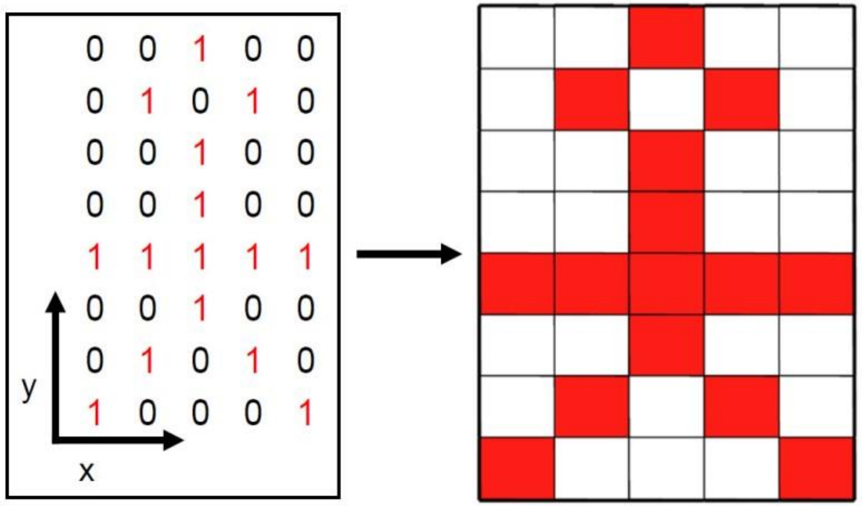
"Opacity" port – for tuning the transparency





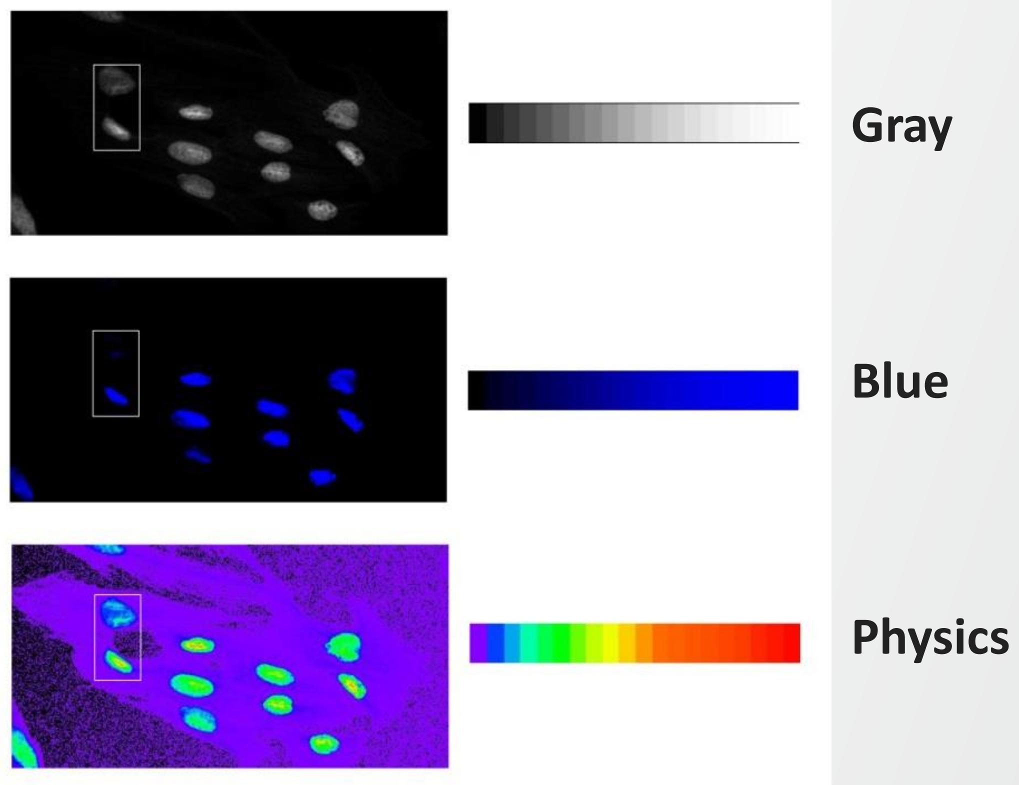


Data visualization: Colormap



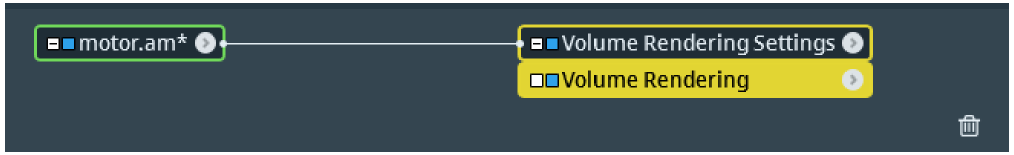
Data

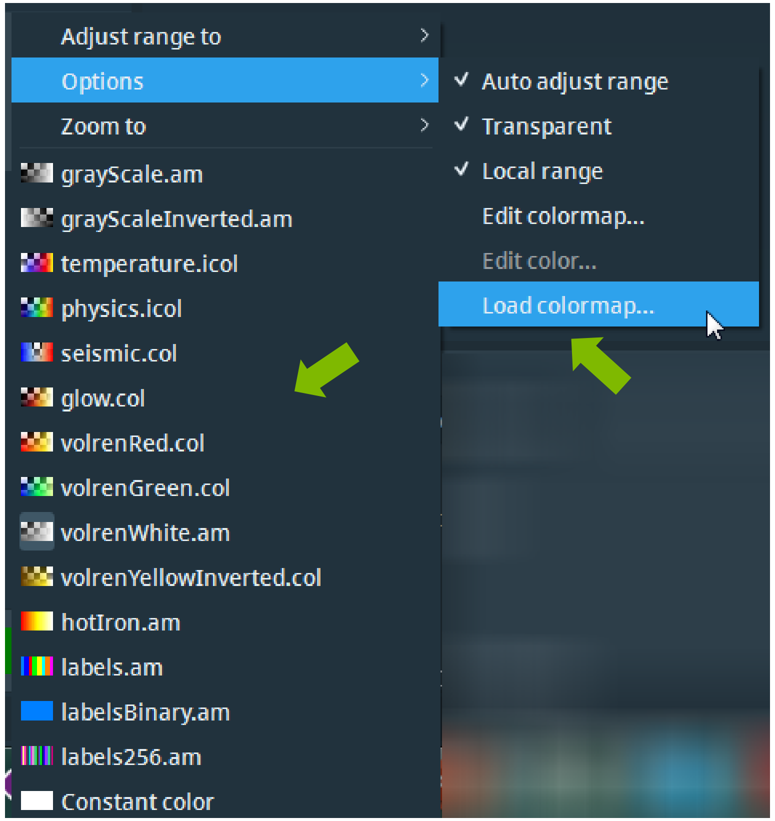
Visualization



3D visualization with Volume Rendering

Volume rendering: Colormap Settings





For changing the default colormap:

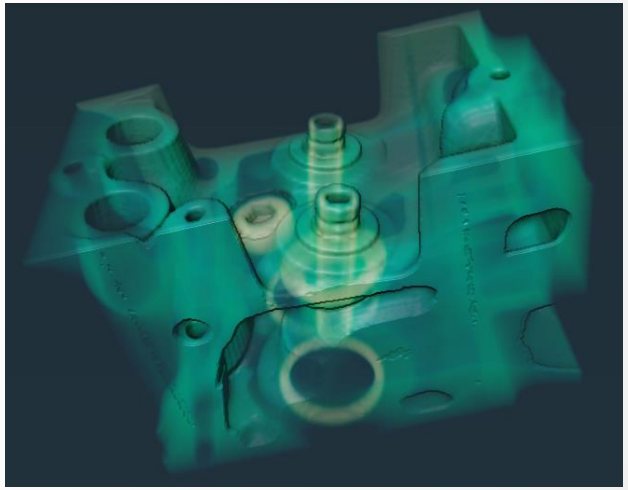
- Click on the “Edit” button of “Colormap” port
- Pick a colormap from the drop-down list

OR

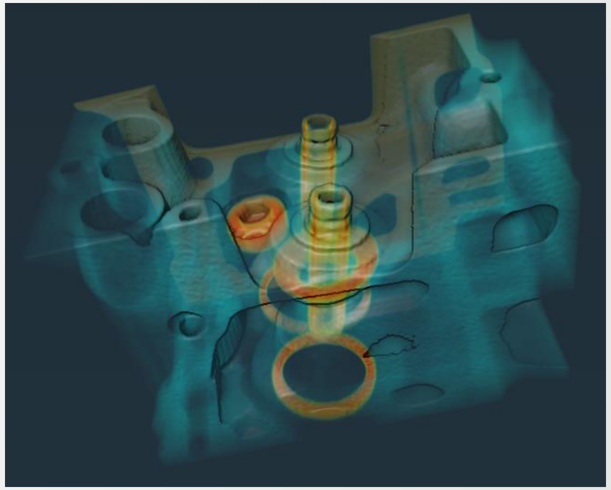
- Load a colormap from the disk

Once loaded, a colormap will be added to the drop-down list

volrenGreen Colormap (default)

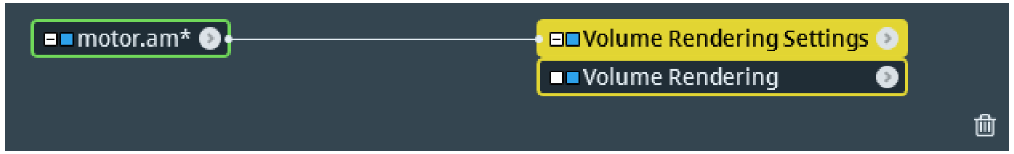


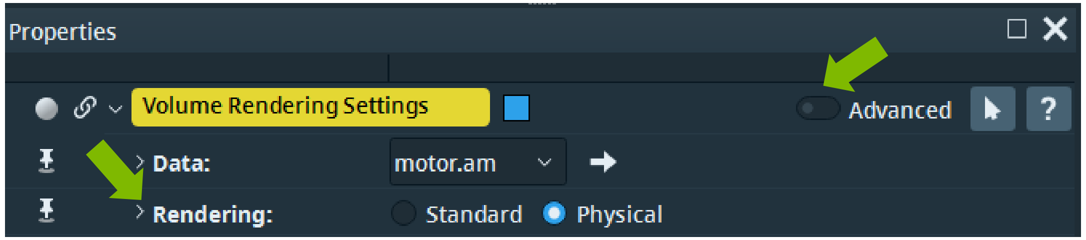
volrenPhysics Colormap (loaded from disk)



3D visualization with Volume Rendering

Volume rendering Settings



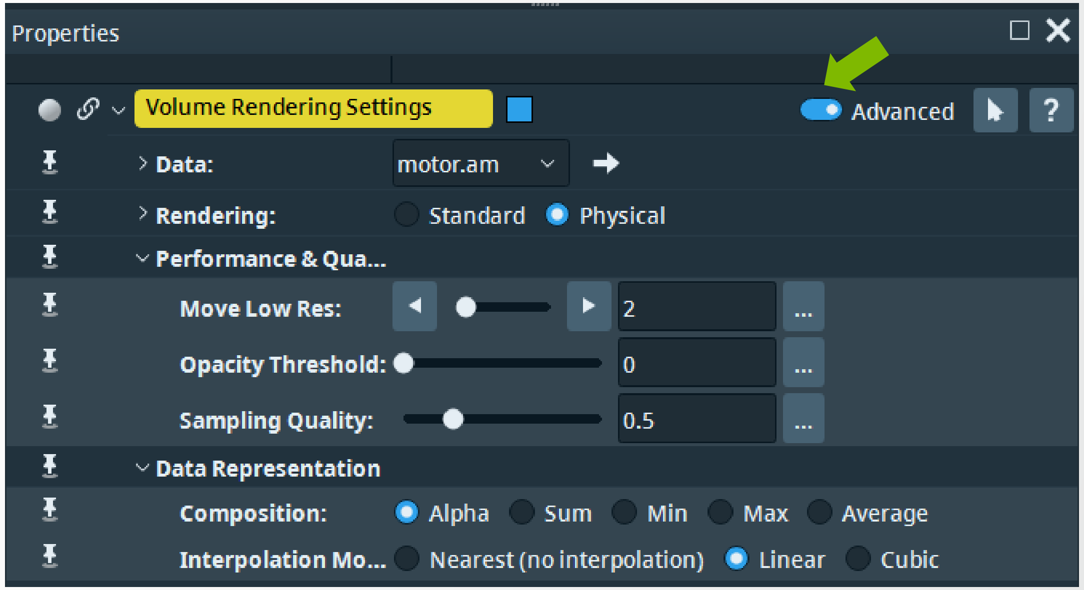


Rendering type:

- Standard
- Physical – mostly used

According to the selected rendering type, different rendering options are proposed.

Click on the arrow on the left of the “Rendering” port in order to show more settings.

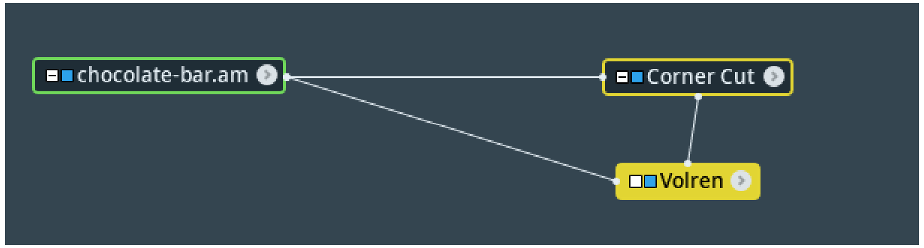


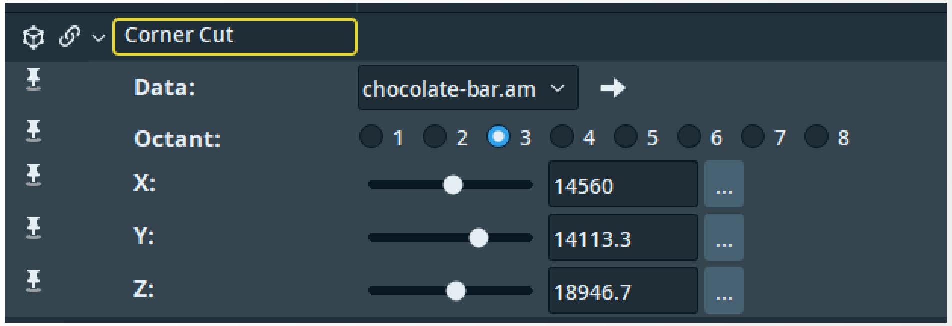
Switch on the “Advanced” ribbon for advanced Volume Rendering settings

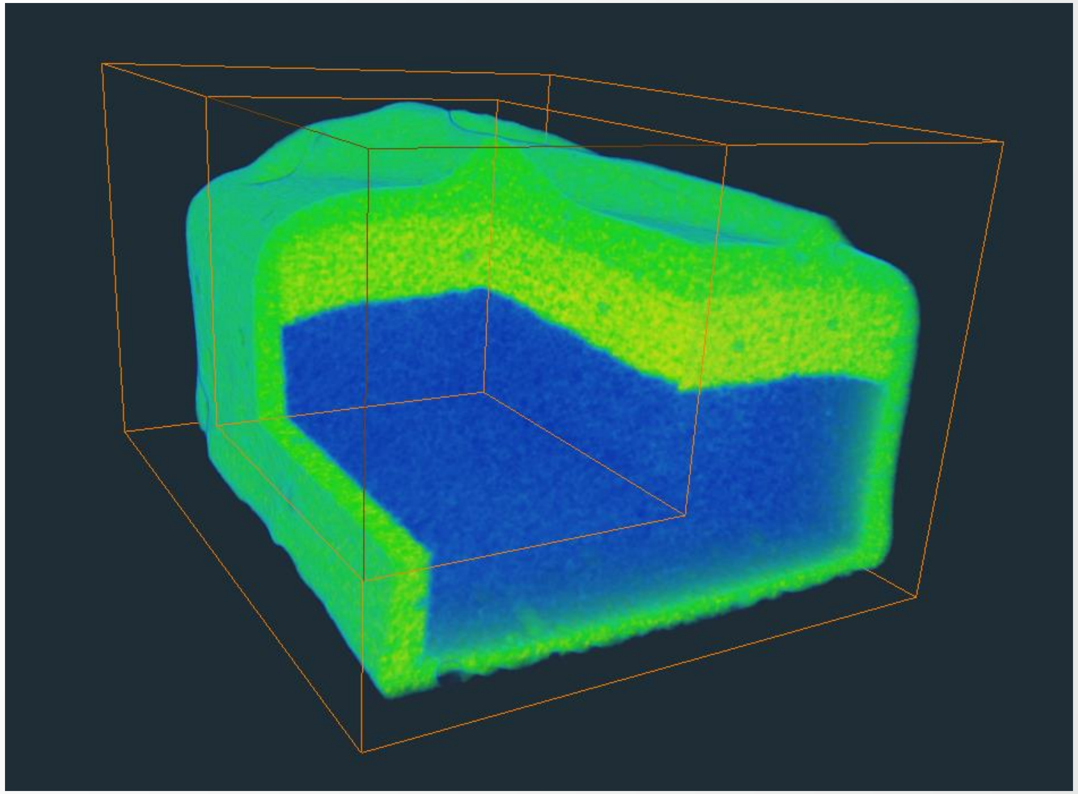
- “Move Low Res”– low resolution mode when moving the camera, for real time rendering

3D visualization with Volren

Volren: Support Corner Cut module







Volren:

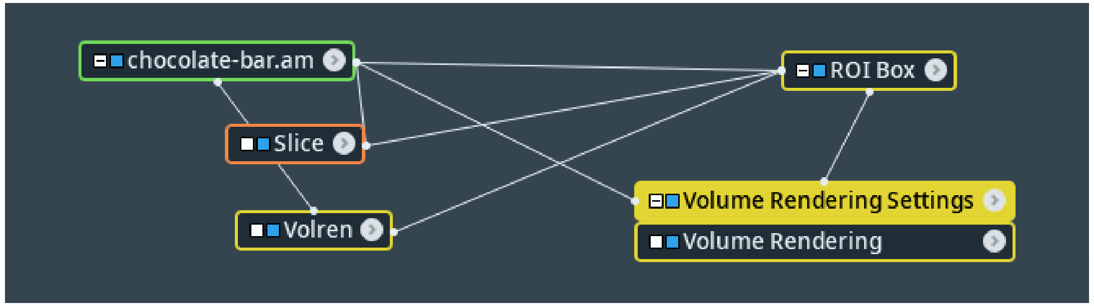
- Fast preview
- Limited by RAM memory
- Corner Cut possible

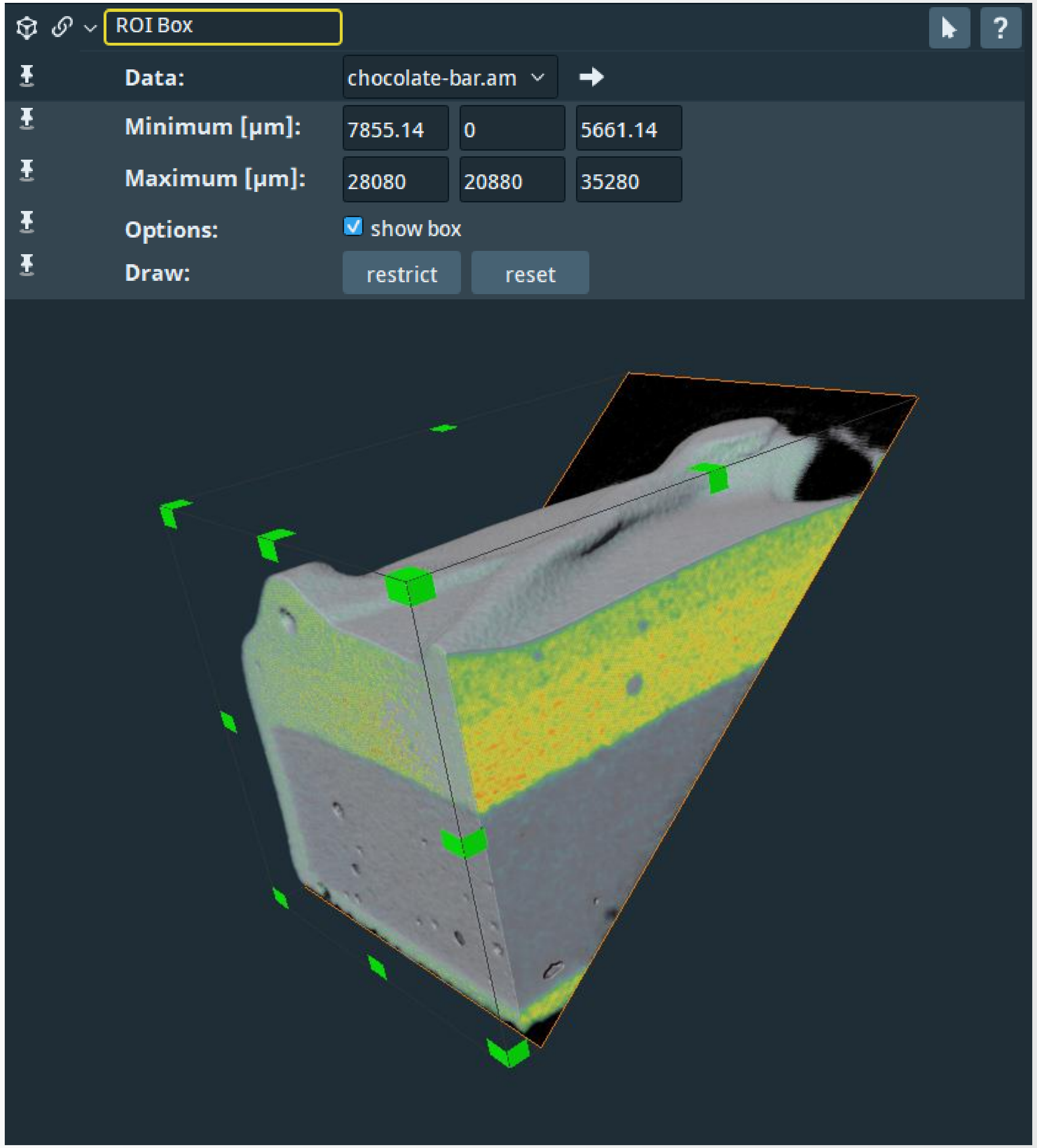
Volume Rendering:

- Realistic visualization
- Limited by VRAM memory
- Corner Cut not possible

3D visualization with ROI Box

ROI Box: Region of Interest





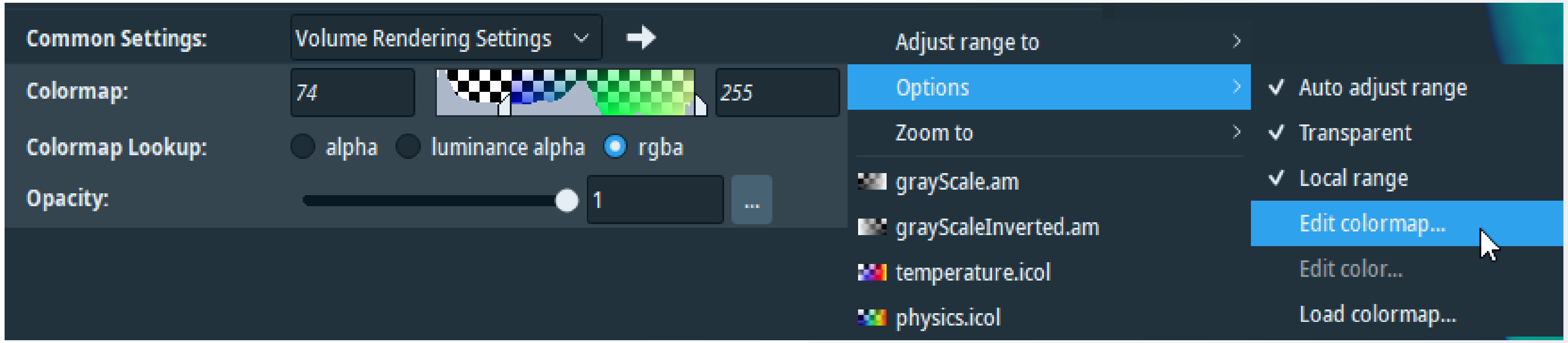
Connected to:

- Slice
- Volren
- Volume Rendering
- Iso-Surface
- Triangulated Surface

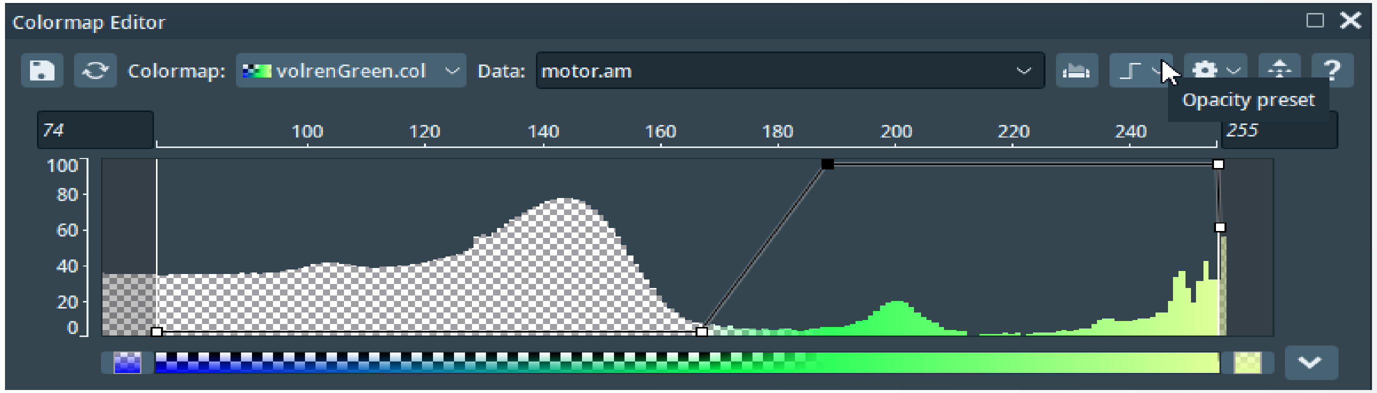
Setting a colormap: Colormap Editor

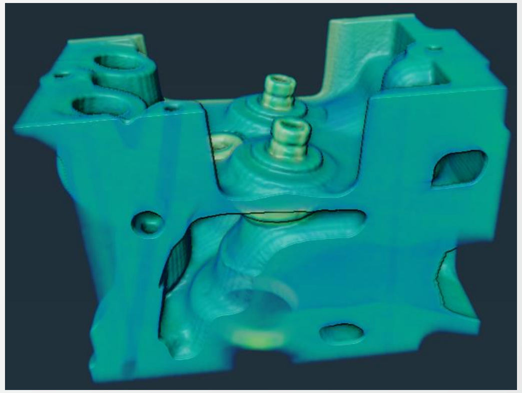
In addition to choosing a default colormap or loading one from the disk, one can also edit a colormap by means of Colormap Editor. To access Colormap Editor:

- Click the “Edit” button of the “Colormap” port of any visualization module and select Options -> Edit Colormap



Colormap Editor window will be displayed on top of the Project View window:

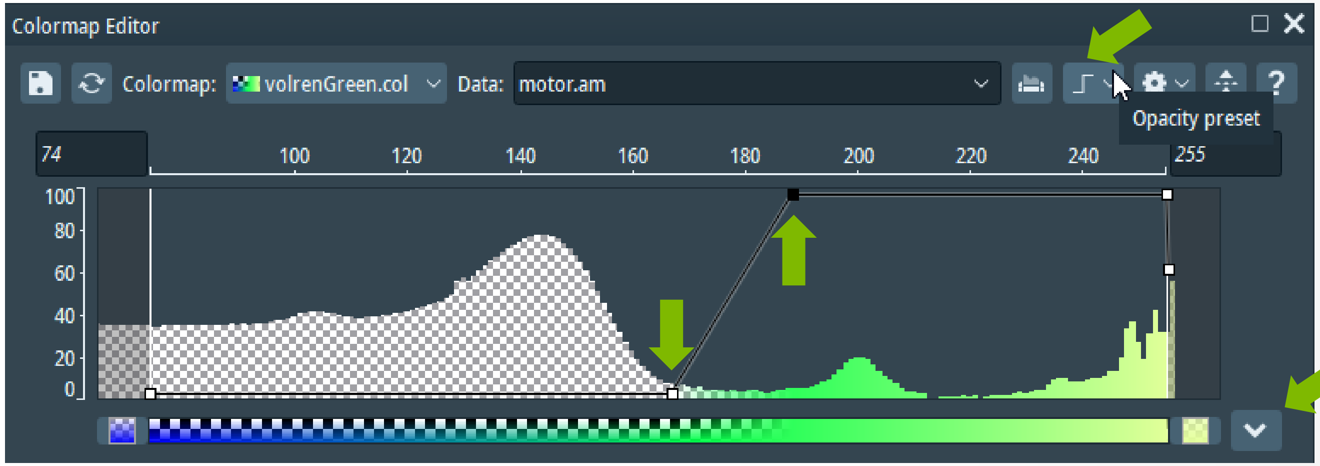


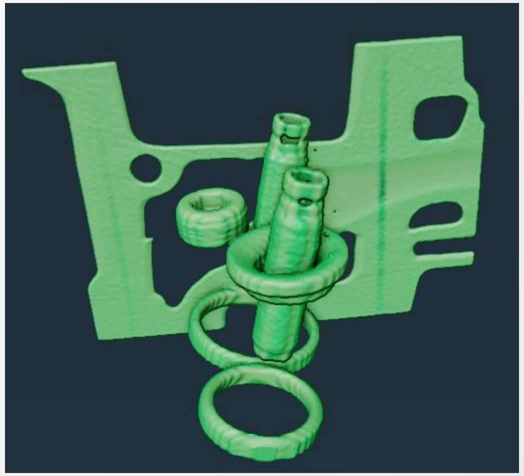


Setting a colormap: Colormap Editor

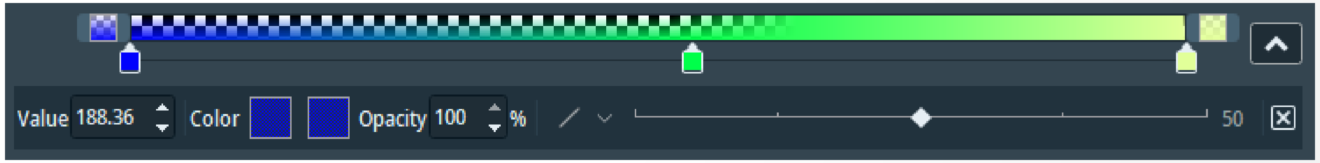
Opacity curve – allows controlling the transparency of the colormap:

- Pick a default preset from the “Opacity preset” menu
- Manually adjust the curve:
    - Left-click on the curve to add a point at the respective location
    - Click and hold on a point in order to move it
    - Right click on a point in order to remove it





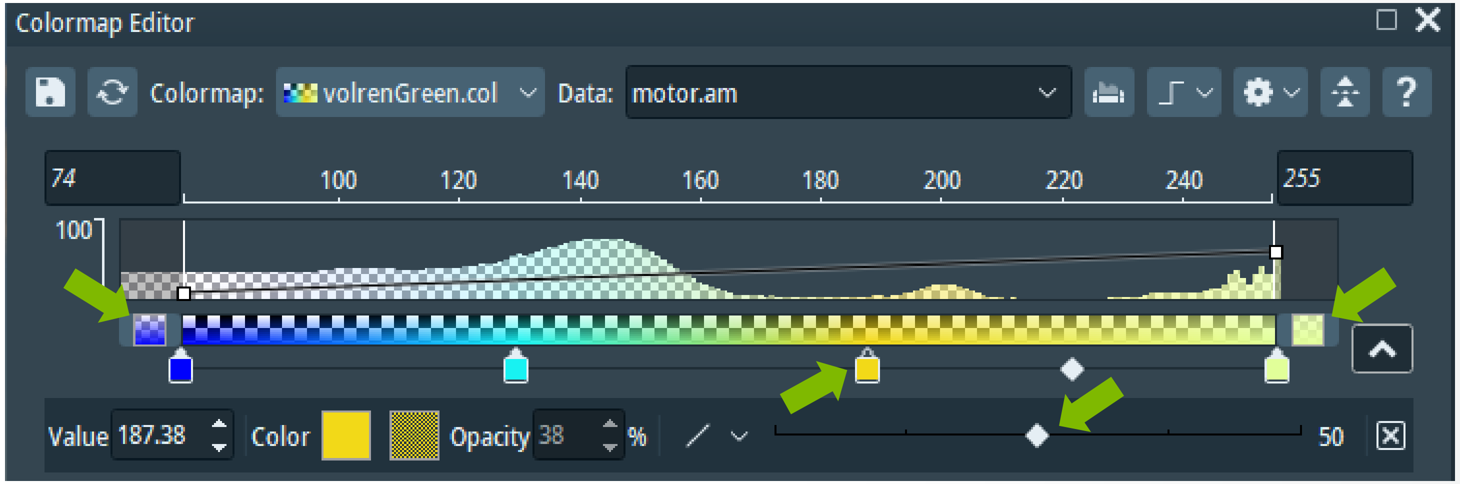
Click on the arrow on the Editor’s bottom-right corner for opening the Color Editor (below the histogram):

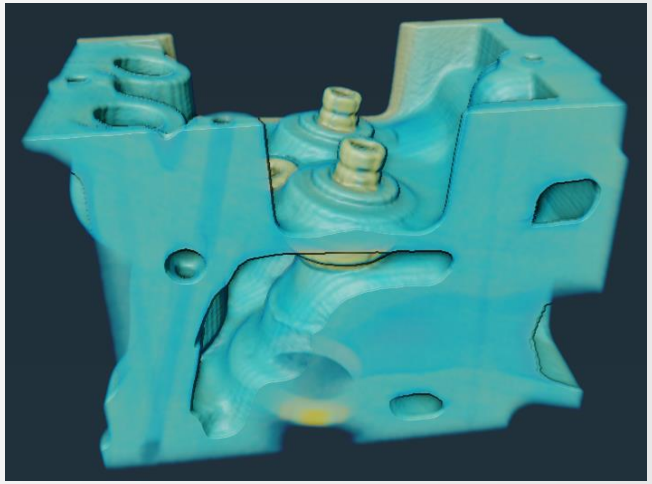


Setting a colormap: Colormap Editor

Colormap gradient – allows modifying the colormap using color markers

- To modify the color gradient:
  - Left-click on the markers line to add a marker at the respective location
  - Click and hold a marker in order to move it
  - Right click on a marker in order to remove it
  - Double click on a marker for color settings
  - Drag the diamond shaped button to adjust the location of the inflection point
- The data points outside the colormap range will be mapped to the color defines by the extreme left and right boxes.

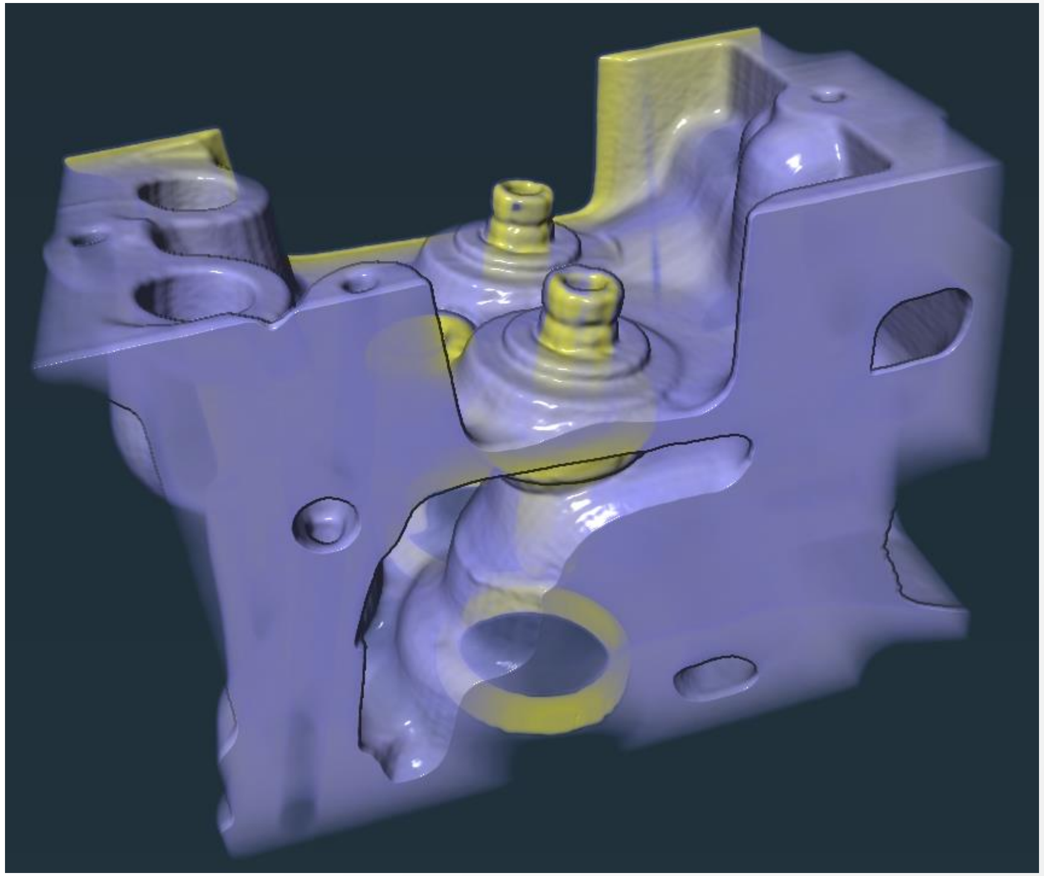




Data visualization: exercise 3

Setting colormaps

Use the Volume Rendering module and tune the colormap to obtain a similar view:

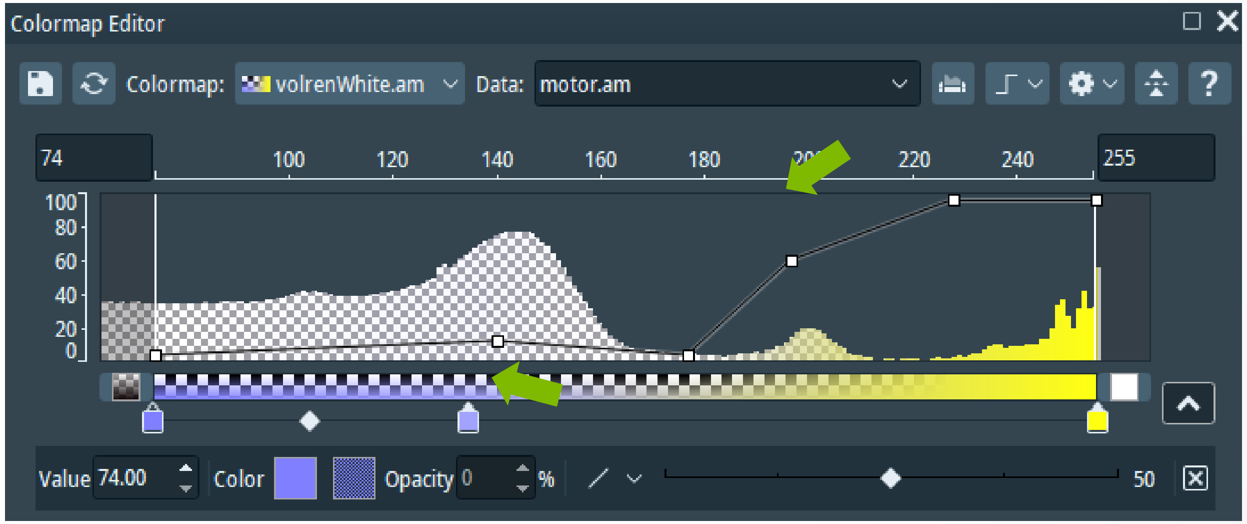


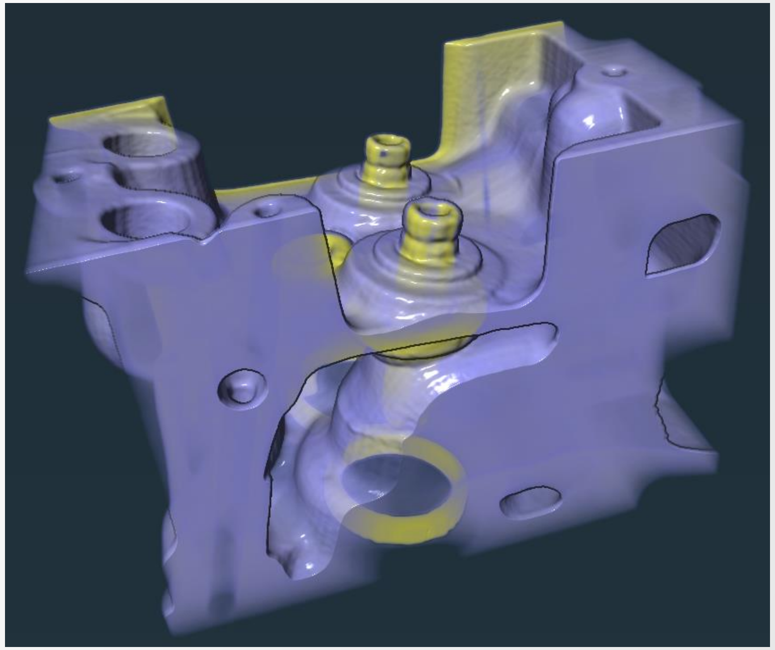
Data visualization: exercise 3

Solution

Colormap Editor Settings:

- Add and move points on the opacity curve for setting the transparency of the colormap
- Add and tune color markers for setting the color gradient of the colormap



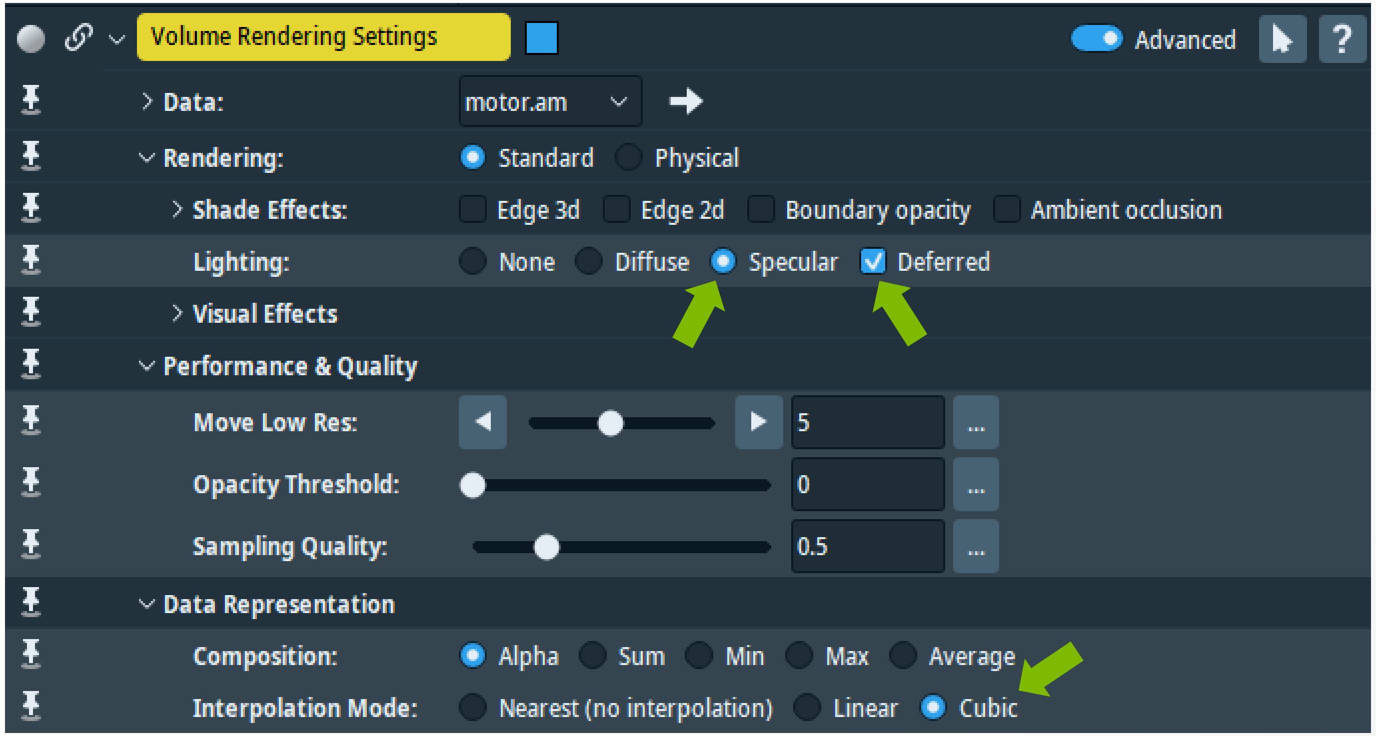


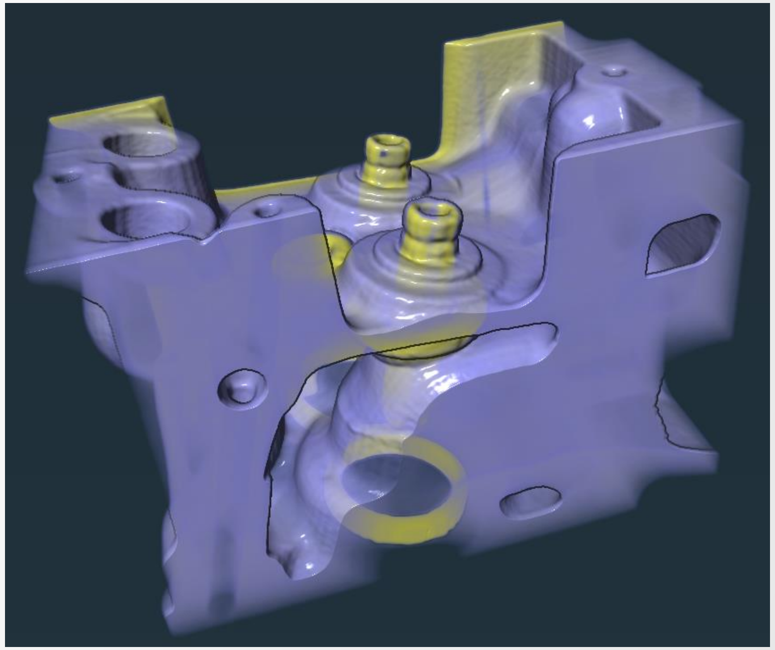
Data visualization: exercise 3

Solution

Volume Rendering Settings:

- Adjust the lighting effects: specular and deferred for highlighting reflections and shadows
- Choose interpolation mode: cubic for smoother result

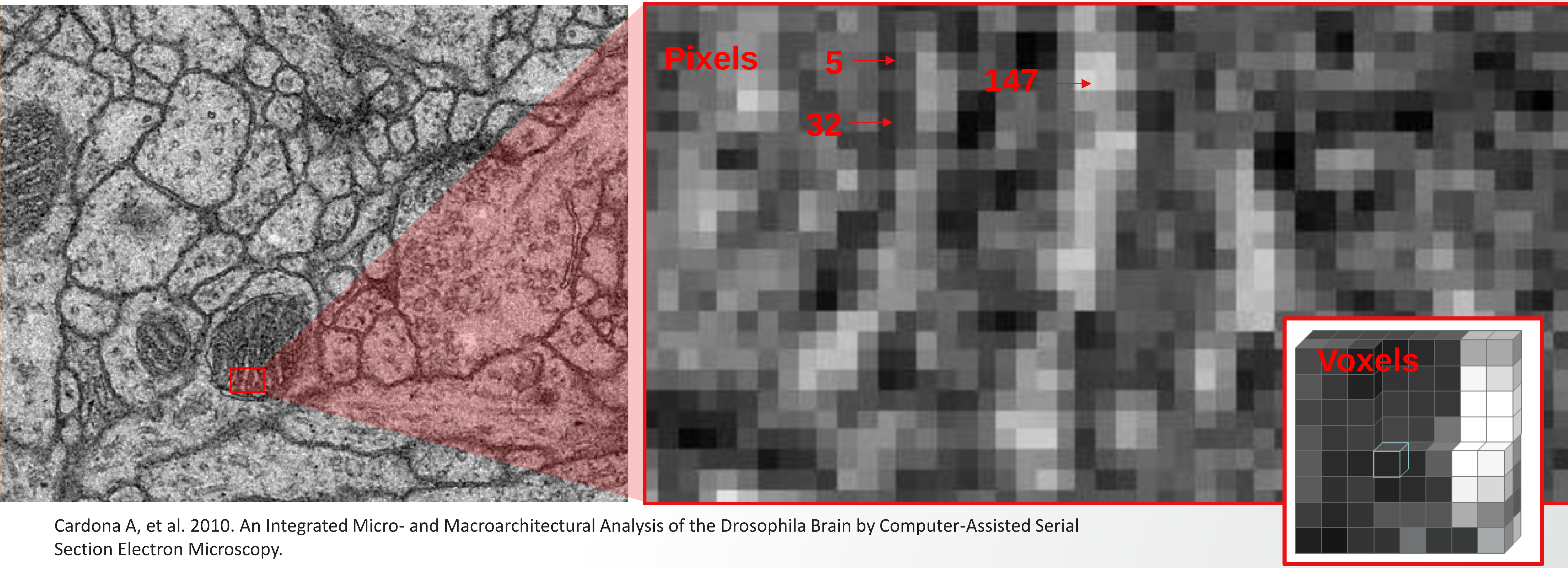




Basic data manipulation

What is a digital image

Grayscale image consists of a finite number of **pixels** (2D) or **voxels** (3D)

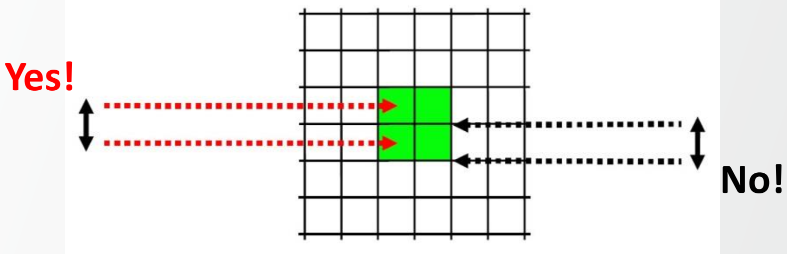


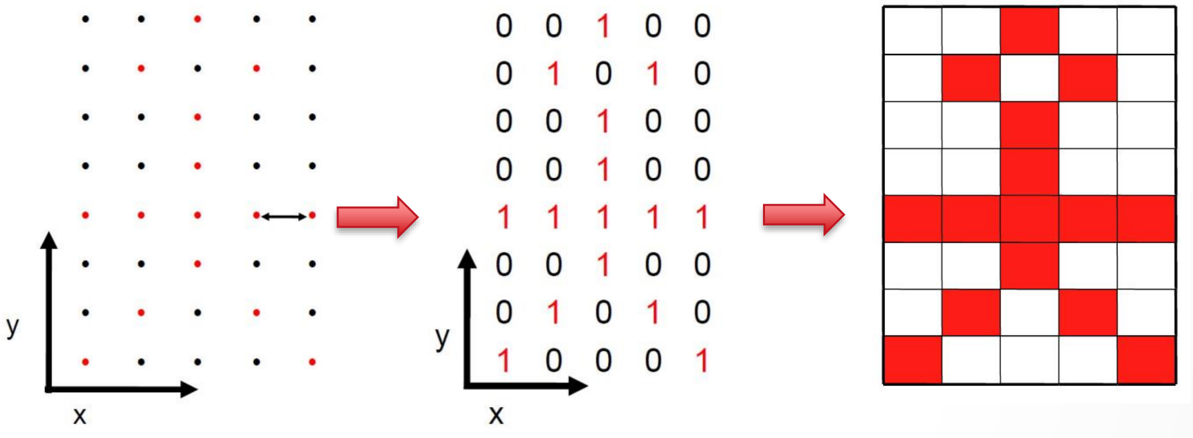
Cardona A, et al. 2010. An Integrated Micro- and Macroarchitectural Analysis of the Drosophila Brain by Computer-Assisted Serial Section Electron Microscopy.

What is a digital image

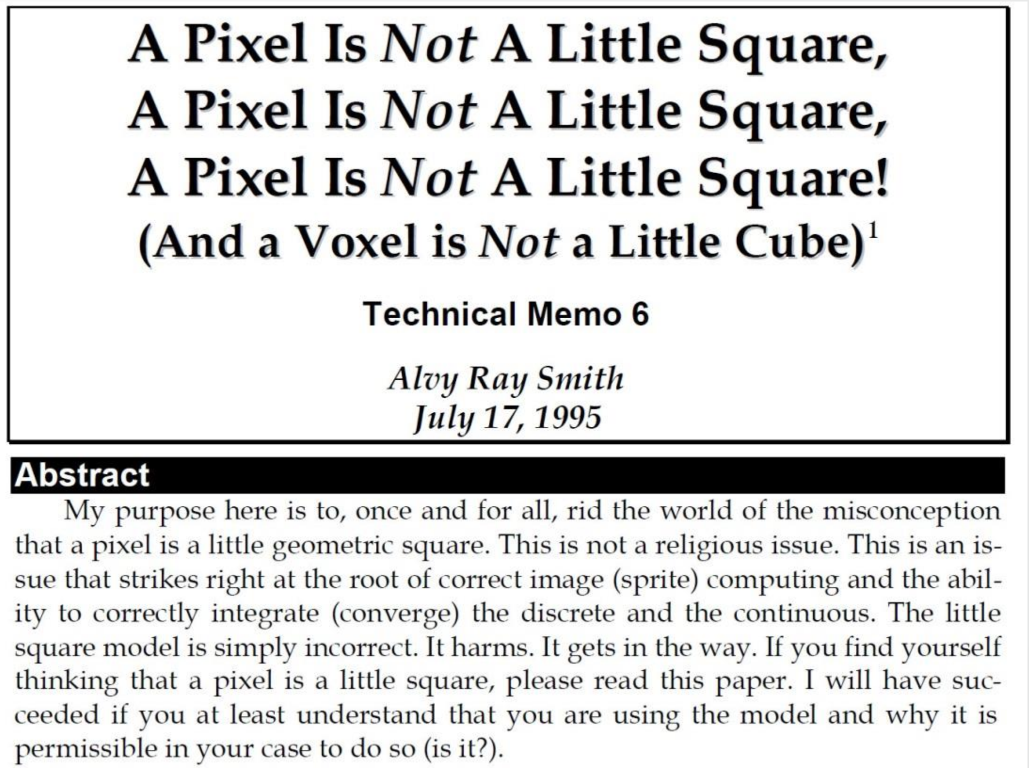
Definition of Pixel

- A pixel is a point sample of the intensity in space
- ≈ PSF (Point Spread Function)
- Pixel size = pixel spacing distance





Sampling Data set Visualization



A Pixel Is *Not* A Little Square,
A Pixel Is *Not* A Little Square,
A Pixel Is *Not* A Little Square!
(And a Voxel is *Not* a Little Cube)[1]

Technical Memo 6

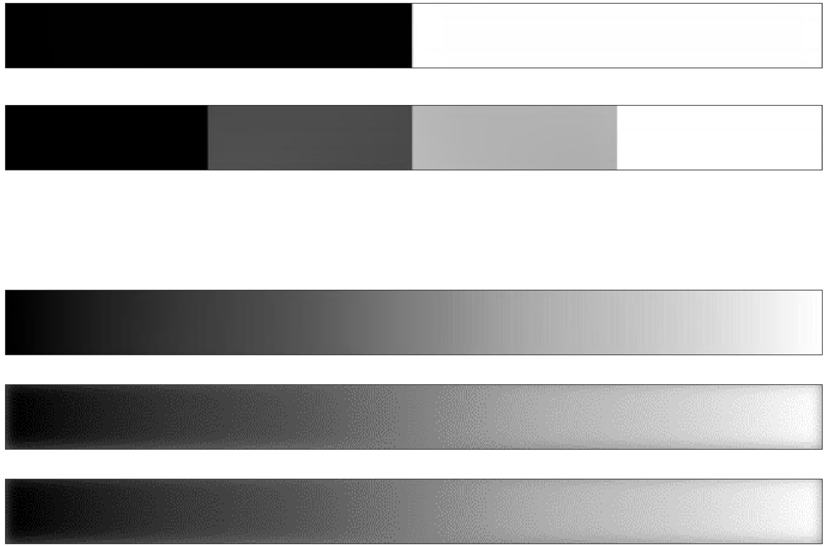
Alvy Ray Smith
July 17, 1995

Abstract

My purpose here is to, once and for all, rid the world of the misconception that a pixel is a little geometric square. This is not a religious issue. This is an issue that strikes right at the root of correct image (sprite) computing and the ability to correctly integrate (converge) the discrete and the continuous. The little square model is simply incorrect. It harms. It gets in the way. If you find yourself thinking that a pixel is a little square, please read this paper. I will have succeeded if you at least understand that you are using the model and why it is permissible in your case to do so (is it?).

What is a digital image

Bit depth of an image defines how many different grey values can be detected in the data



1-bit image (2^1) has 2 possible numerical intensity values

2-bit image (2^2) has 4 possible numerical intensity values

8-bit image (2^8) has 256 possible numerical intensity values

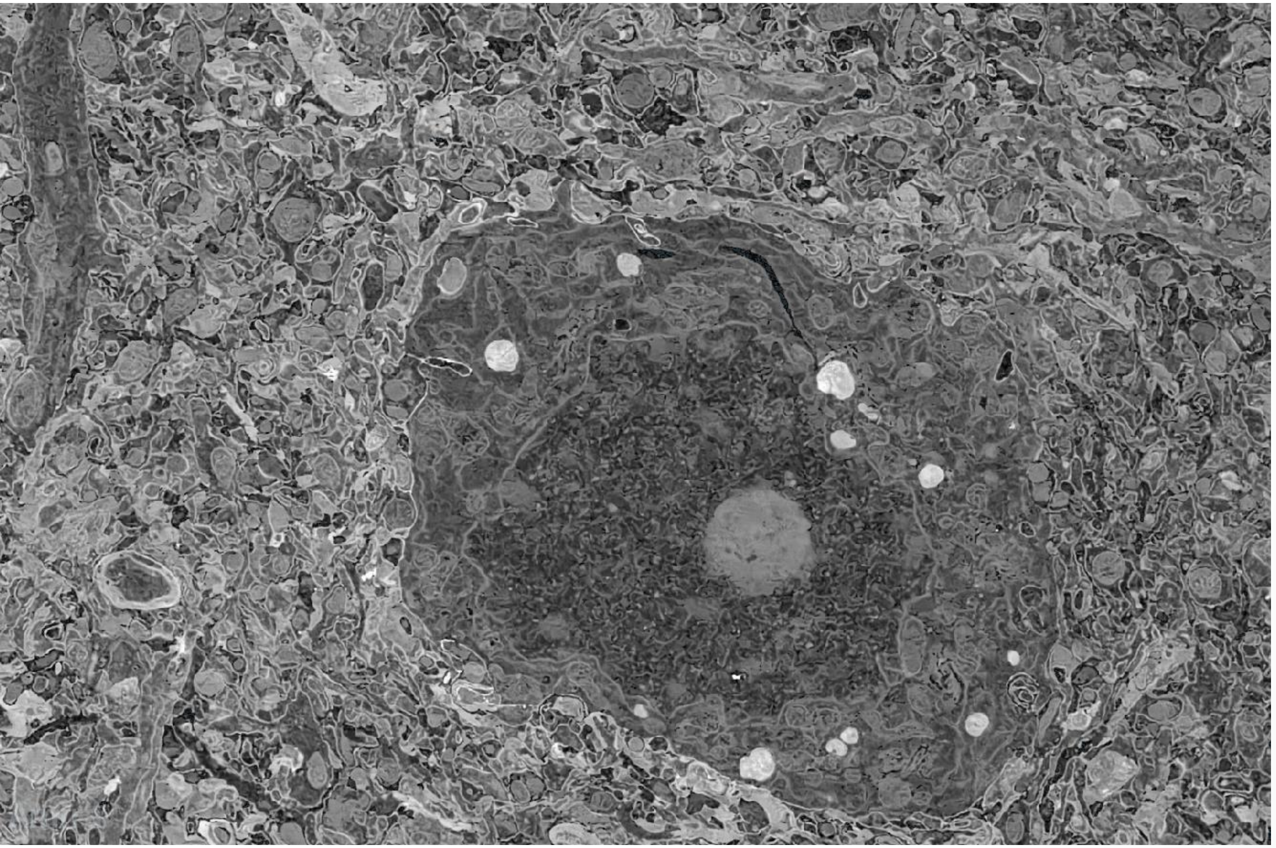
16-bit image (2^{16}) has 65,536 possible numerical intensity values

32-bit image (2^{32}) has 2,147,483,647 possible numerical intensity values

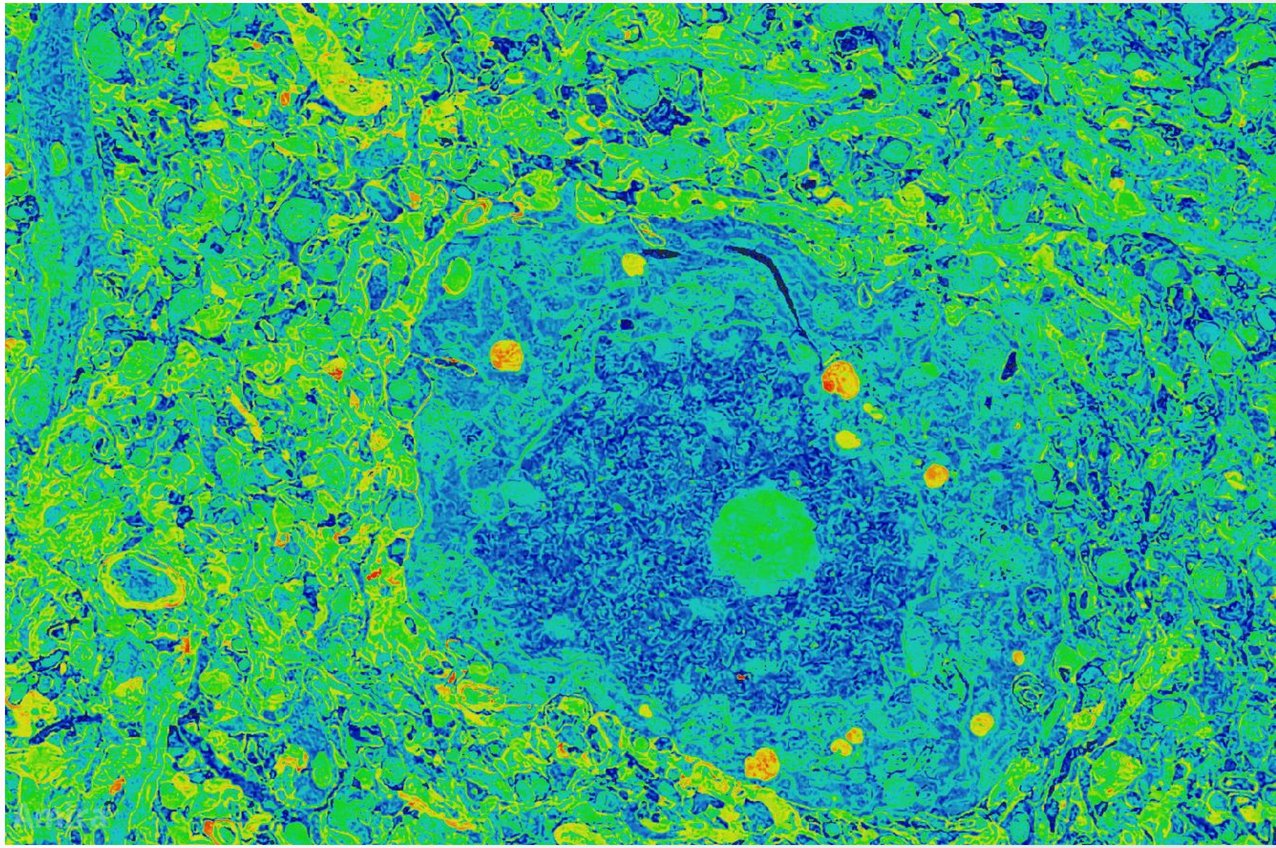
What is a digital image

Look-up tables assign color and transparency to each intensity value

Grayscale

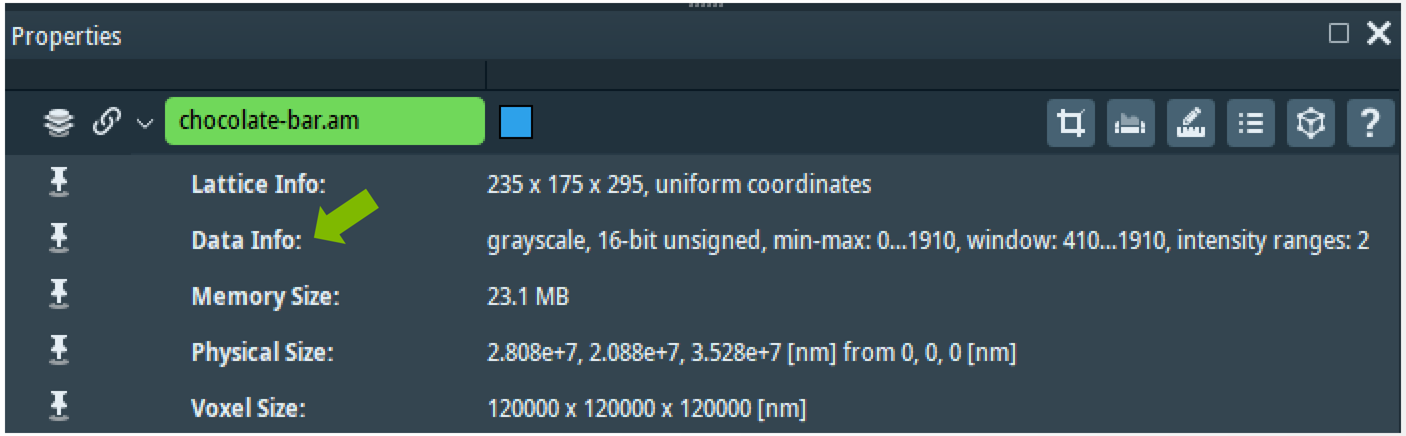


Physics



Data and voxel properties

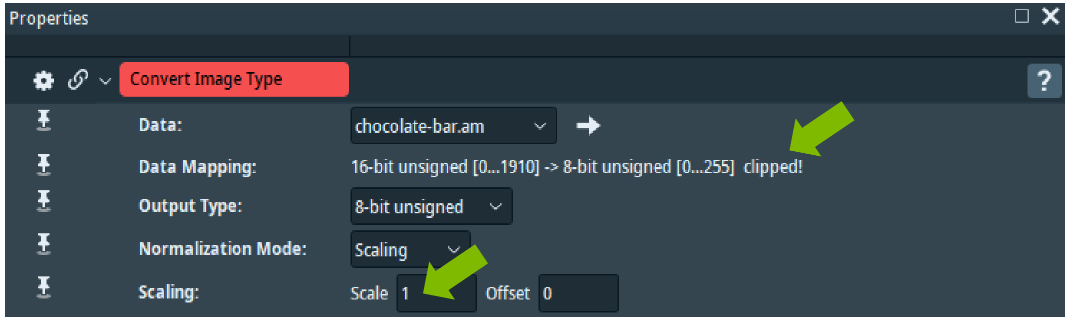
Click on a data in the pool in order to have data and voxel properties displayed in the properties window: e.g. chocolate bar:

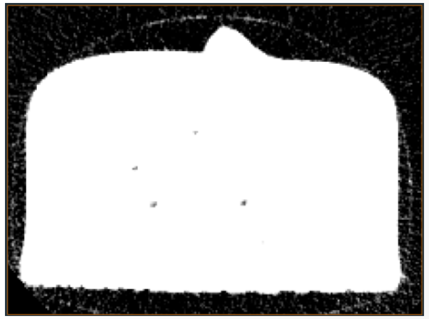


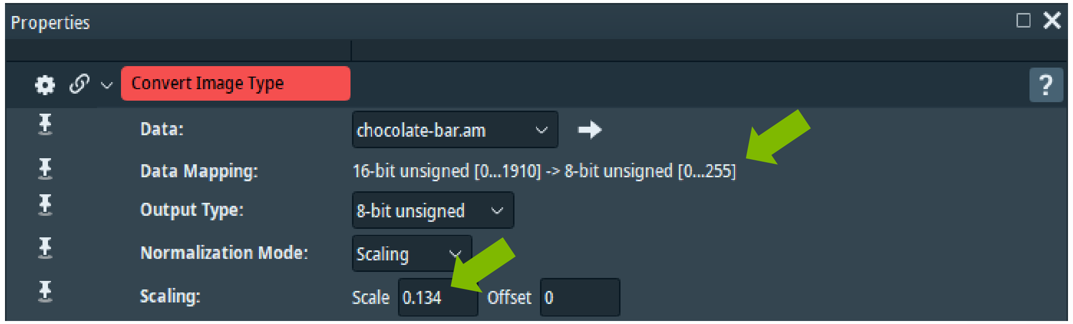
- Type: grayscale, label, RGBA, etc.
- Precision: number of bits used for coding the value stored by a voxel. E.g.:
  - 8-bits <-> values in [0-255]
  - 32-bits float <-> values in ~ [-1e38, 1e38], finer precision but 4x more memory required
- Minimum and Maximum voxel values – give the intensity range (do not confuse with colormap range)
- Window: voxel values range outside the background

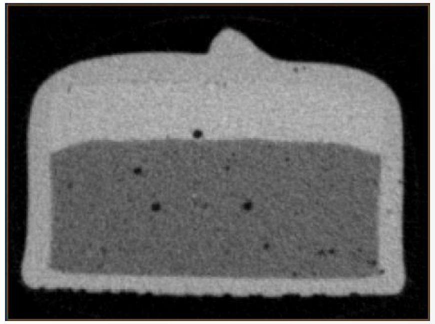
Converting types and re-mapping intensities

Changing type: Convert Image Type module







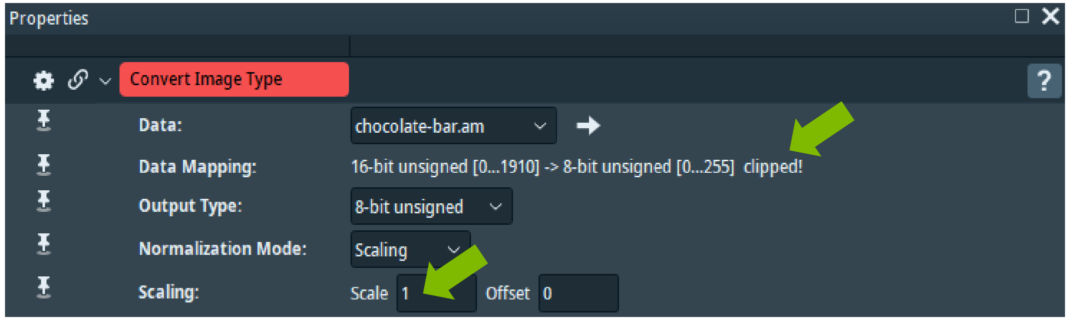


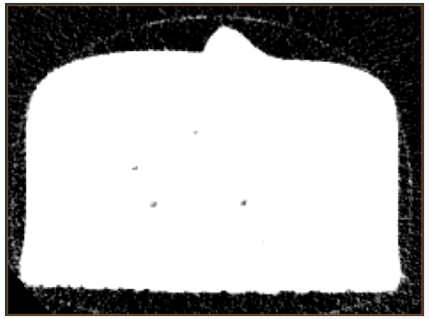
Scaling is necessary to avoid clipping. For scale tuning check:

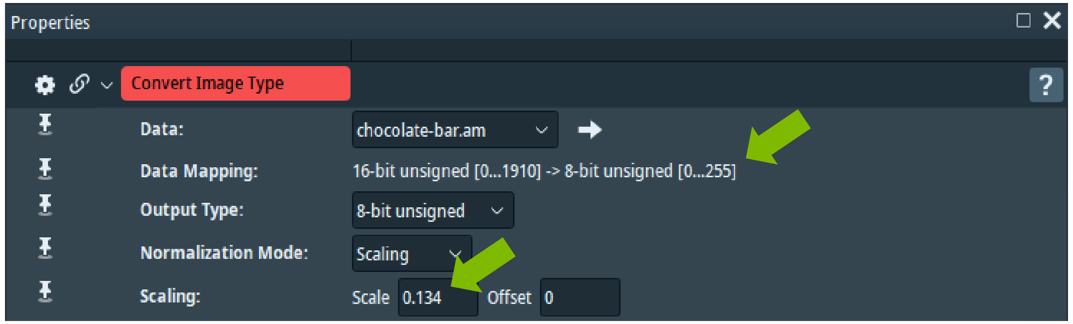
- The intensity range of the input data
- The intensity range of the converted data

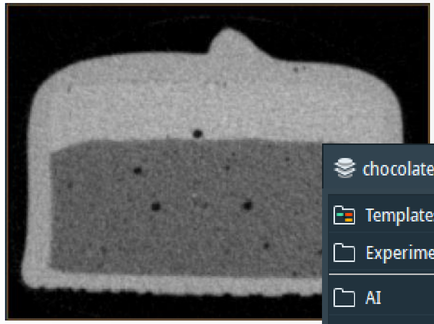
Converting types and re-mapping intensities

Changing type: Convert Image Type module





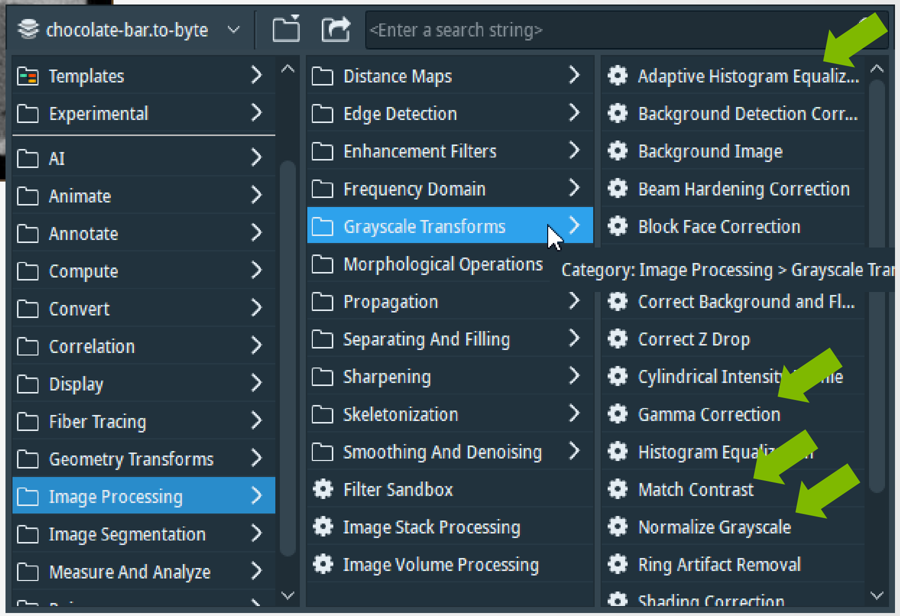




Scaling is necessary to avoid clipping. For scale tuning check:

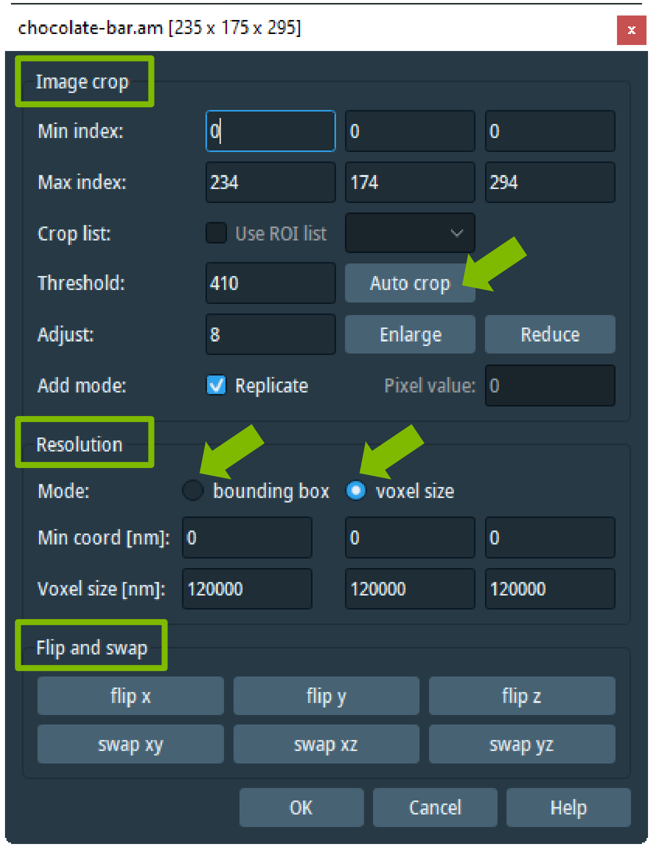
- The intensity range of the input data
- The intensity range of the converted data

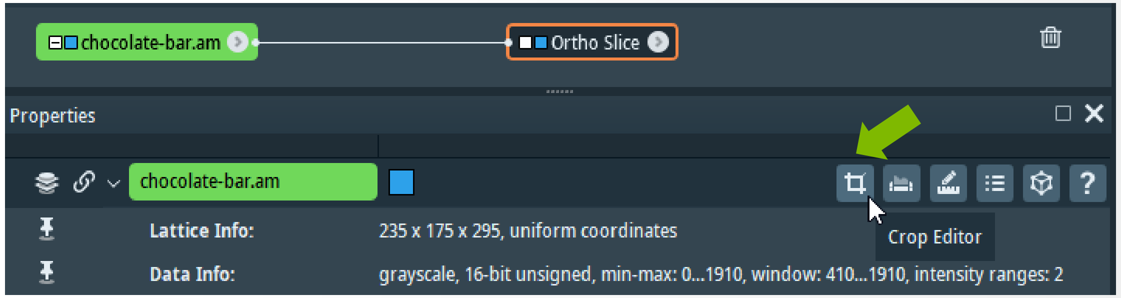
Re-mapping intensities: Different modules are available in the category: Image Processing -> Grayscale Transform



Crop Editor: main functionalities

Access Crop Editor: select a dataset in the pool and click on the Crop Editor icon in the data properties window





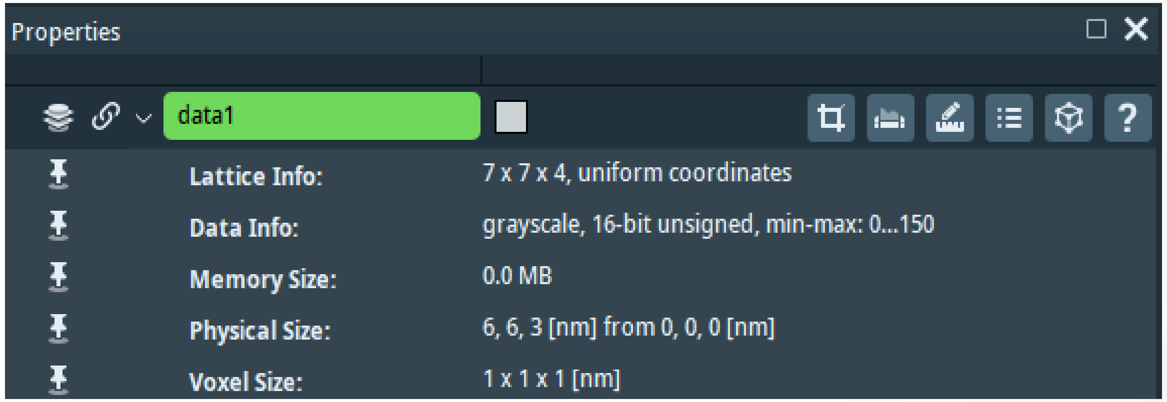
Crop Editor – main functionalities:

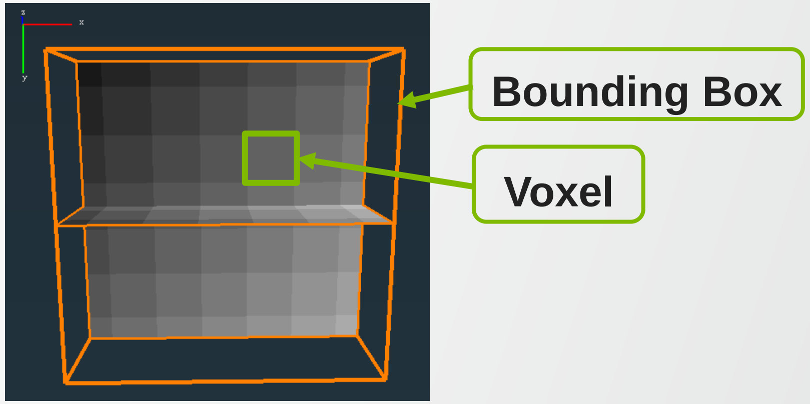
- Crop: reduce/enlarge image frame
  - Manually
  - Automatically (by an automatically set gray-level threshold for separating the data into background and object)
- Change resolution
  - Change voxel size
  - Change bounding box size (the new voxel size is automatically computed

  The number of voxels is preserved
- Modify axes:
  - Flip an axis' orientation – the slices along the respective orientation will have their order reversed
  - Swap – allows interchanging two axis

Crop Editor: good practices

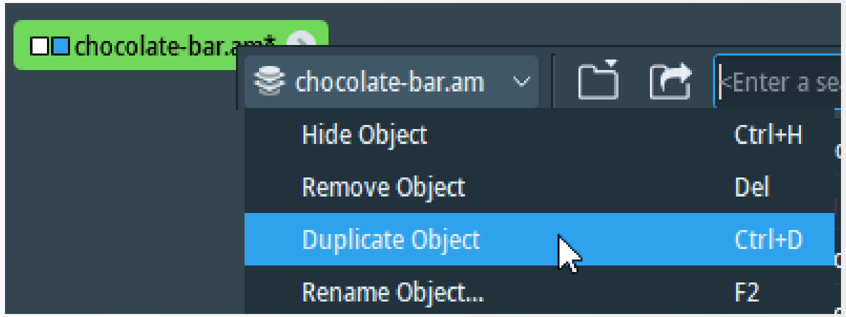
Note: Bounding Box is defined from voxel centers, i.e. :
bbox_size = voxel_size * (#voxels – 1) for each dimension (x, y or z)
A slice is defined from voxels centers too.





Warning: editors directly modify the data
no undo available (the only way to get the original data back is by reloading/re-generating it)
Good practice: duplicate data before editing:

- Keyboard shortcut: [Ctrl] + [d]
- Data object menu



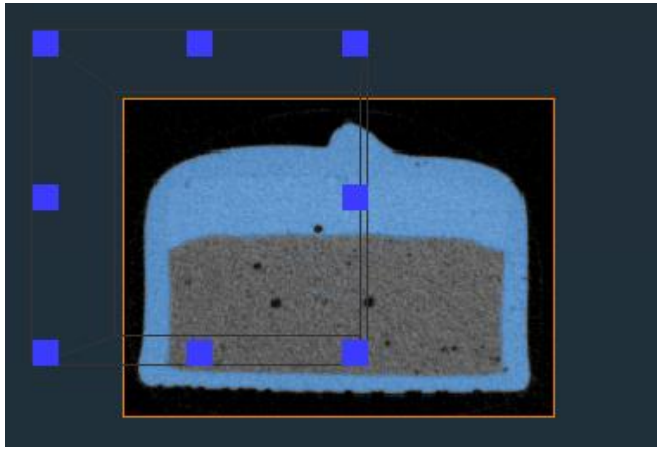
Crop Editor: alternatives

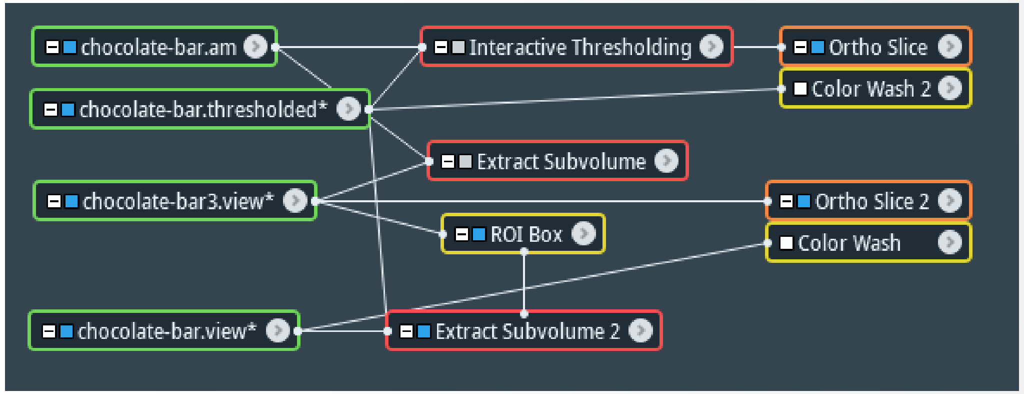
Extract Subvolume module can be used as an alternative to Crop Editor (for cropping only).

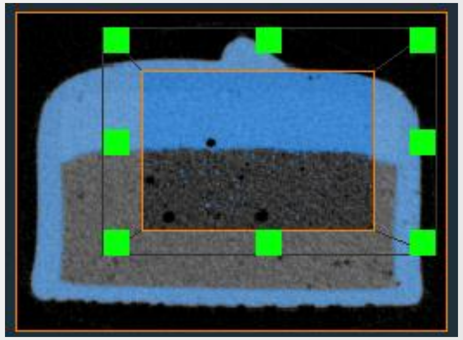
Extract Subvolume + ROI Box – allow cropping multiple datasets the same way:
e.g. input dataset and its segmentation result

- Extract the desired sub-volume of the input dataset (by means of Extract Subvolume)
- Connect a ROI Box to the extracted sub-volume
- Connect an Extract Subvolume module to the second dataset. Connect the ROI input of the Extract Subvolume module to the ROI Box module and then press Apply

=> The second dataset will be cropped as the first one.







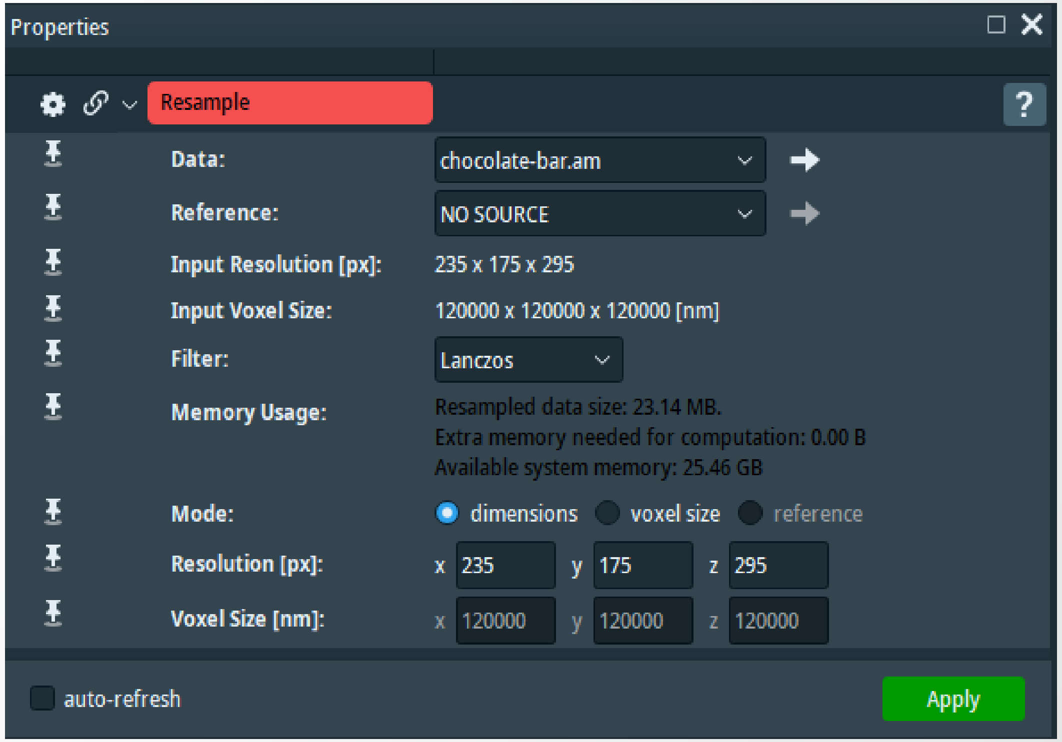
Data resampling

Resampling

Allows enlarging/shrinking the regular grid on which an image is defined (interpolation is necessary):

- Change the number of voxels
  e.g. reduce => reduced data size but lowered quality
- Change voxel size
  e.g. adjust size in order to make the voxels isotropic

This can be done in Avizo via the Resample module.



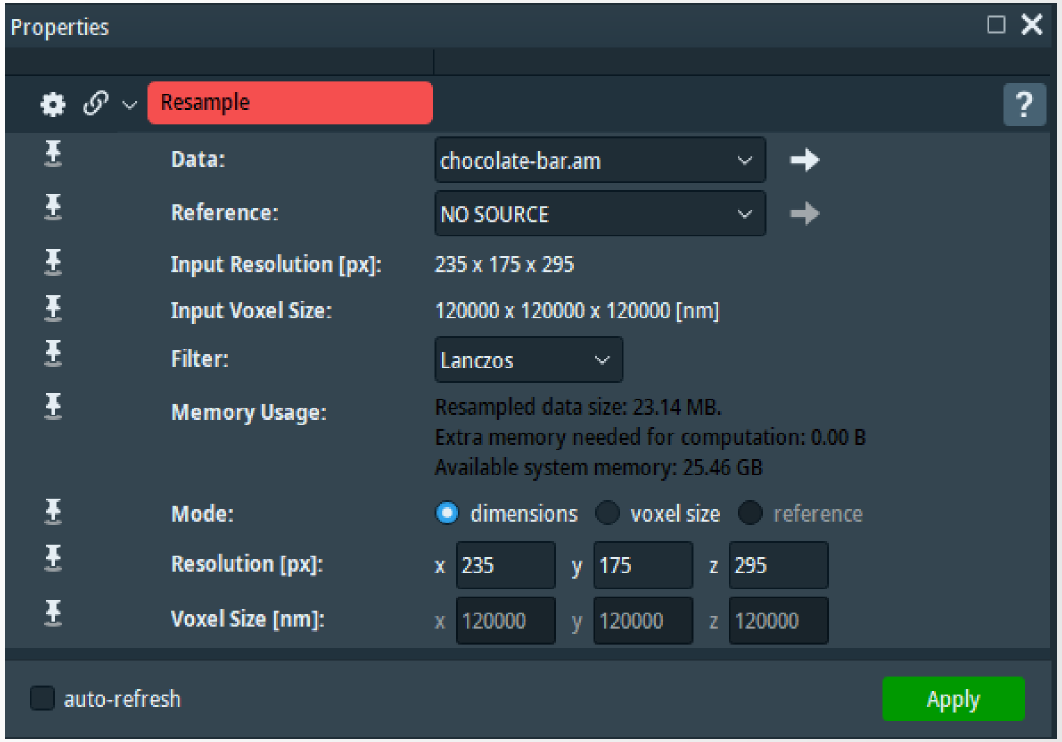
Data resampling

Resampling

Allows enlarging/shrinking the regular grid on which an image is defined (interpolation is necessary):

- Change the number of voxels
  e.g. reduce => reduced data size but lowered quality
- Change voxel size
  e.g. adjust size in order to make the voxels isotropic

This can be done in Amira via the Resample module.



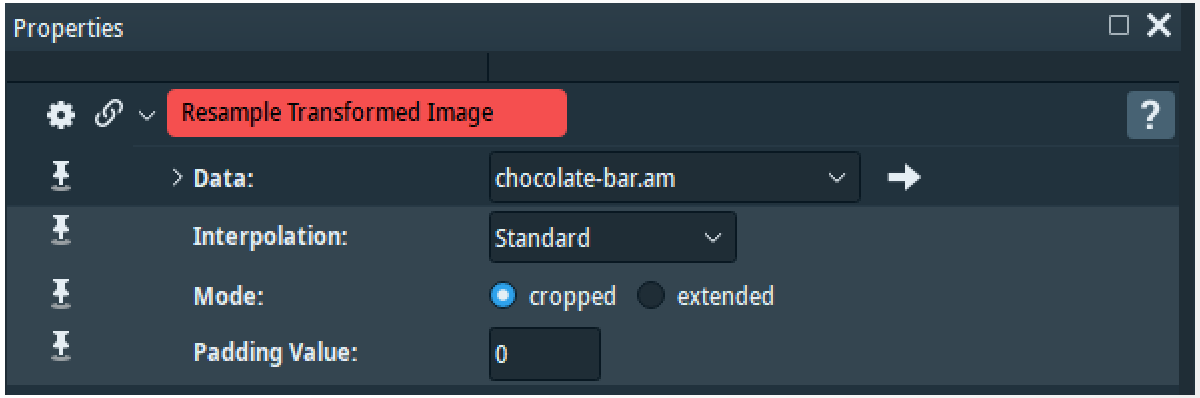
Data resampling – apply transformation

Resample Transformed Image module

Oftentimes, when a data is transformed in Avizo, only the visualization of the data is changed and not its representation in memory. Apply this module to implement the transformation carried by a dataset and to change the dataset representation in memory.

Use cases examples:

- Generate the data resulted after applying a rotation, scaling or other transformation
- Generate the resampled image after registration with a reference
- Apply the rotation necessary to a dataset after aligning the data bounding box with the object's axes.



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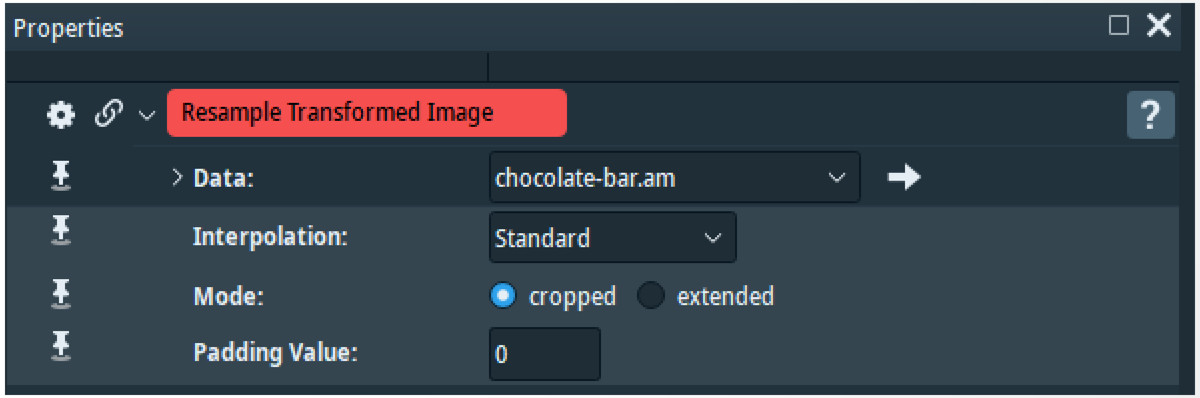

Data resampling example

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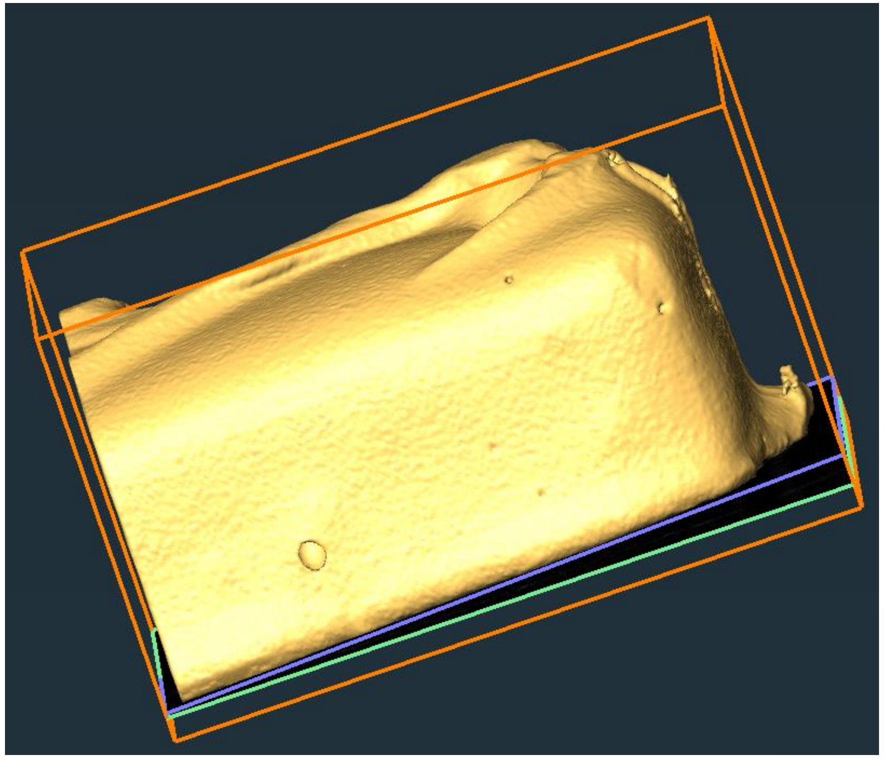
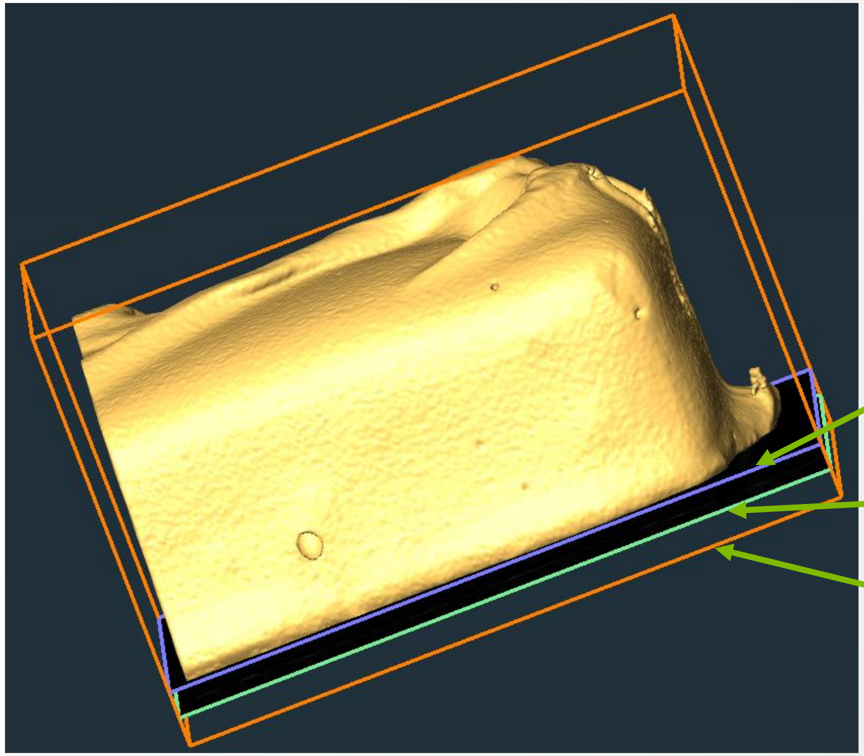
Resampling to an oblique plane

Issue: the object's axes and the bounding box's axes are not aligned
Goal: align the bounding box's axes to the object's axes

Before alignment



After alignment



Slice (aligned to the object's bottom plane)

Ortho Slice

Bounding Box

Data resampling example

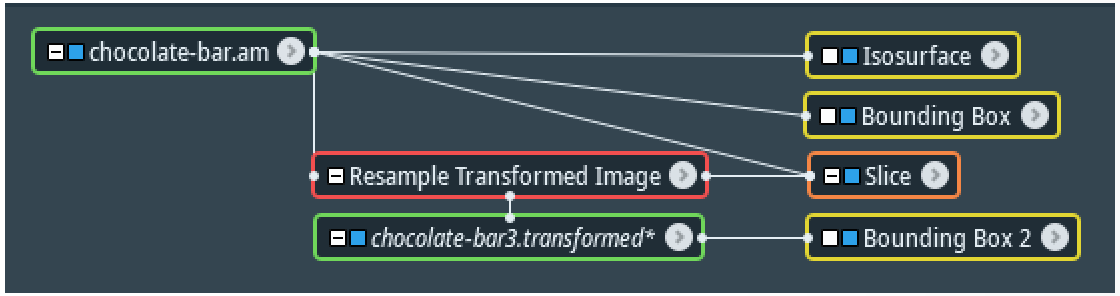
Resampling to an oblique plane

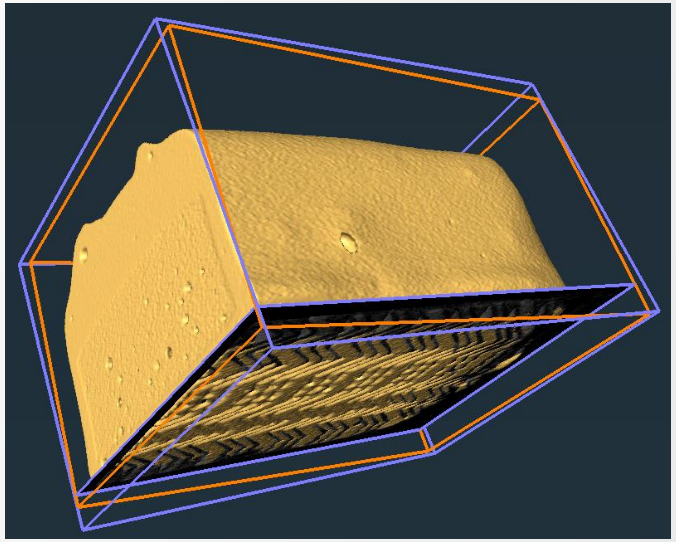
Solution:

- Use “fit to points” option of Slice on an Isosurface or Volume Rendering and fit the Slice to the object’s xz plane (the bottom of the chocolate bar).
- Apply a Resample Transformed Image module and connect:
  - “Data” input to the data object
  - “Reference” input to the Slice

  Select “extended“ option and press Apply

A new data is generated with the bounding box’s axes aligned to the object’s axes.



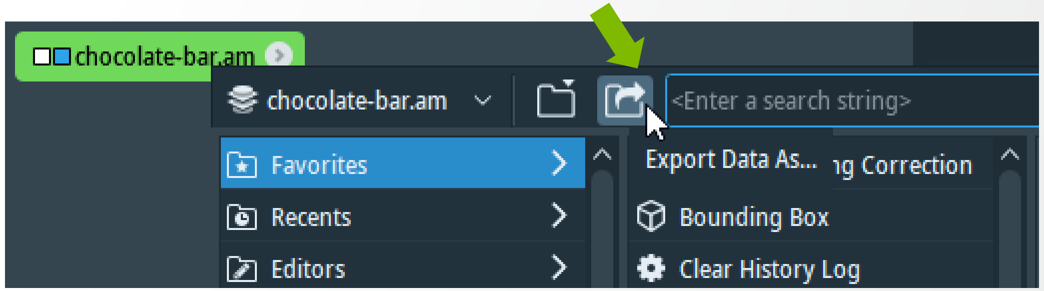


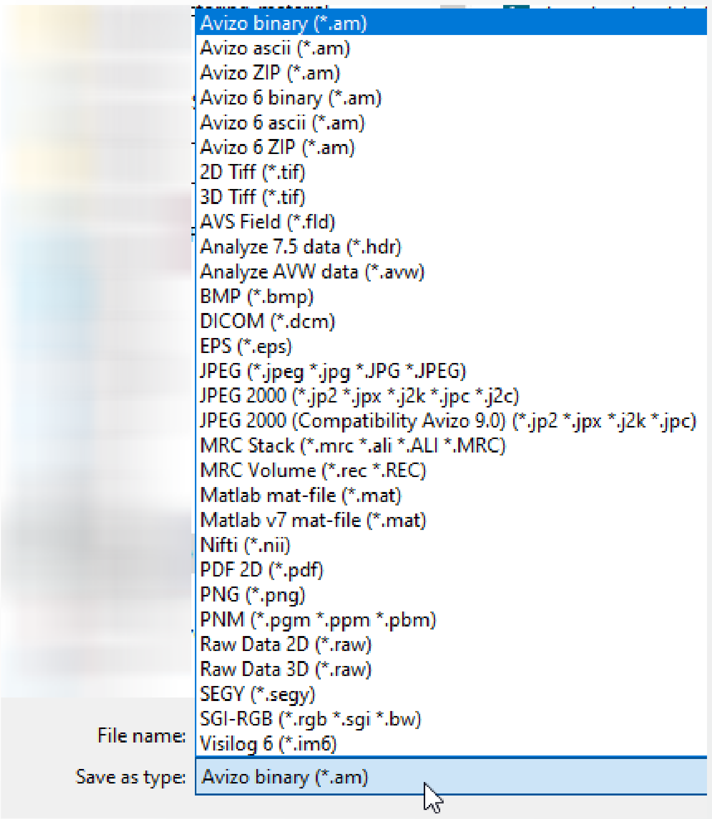
Export data

The results of the different processing modules can be exported from the Avizo pool.

Exporting data on the disk:

- Right-click on the dataset you want to save
- Click on the “Export data as” icon
- Select the relevant format





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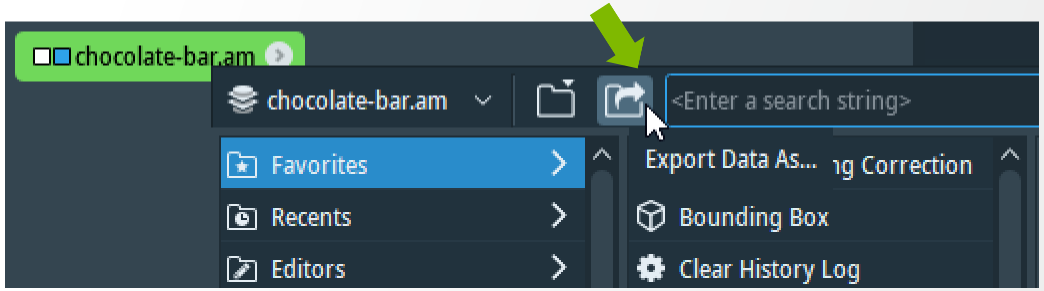


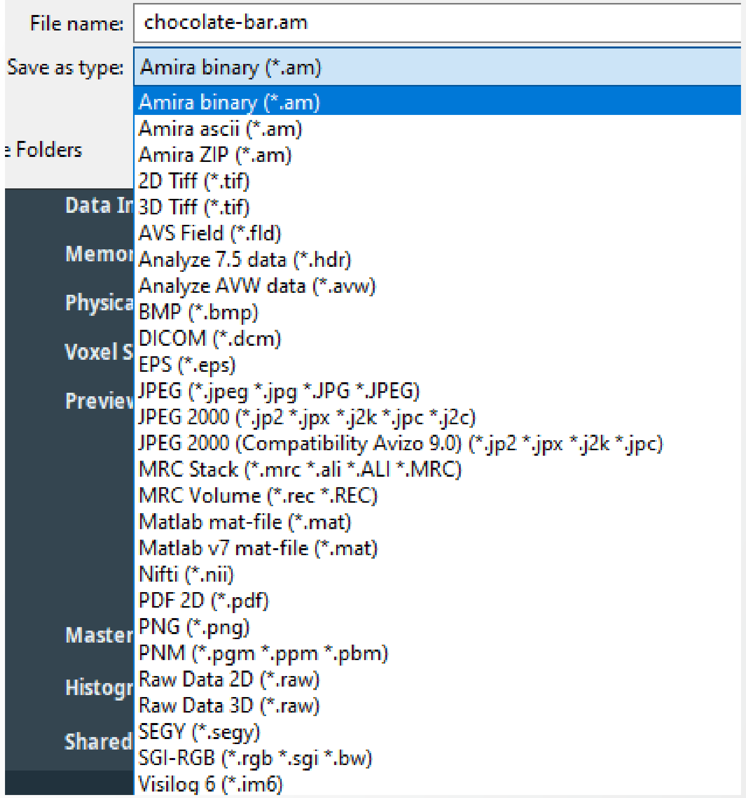


Image pre-processing: adjustments

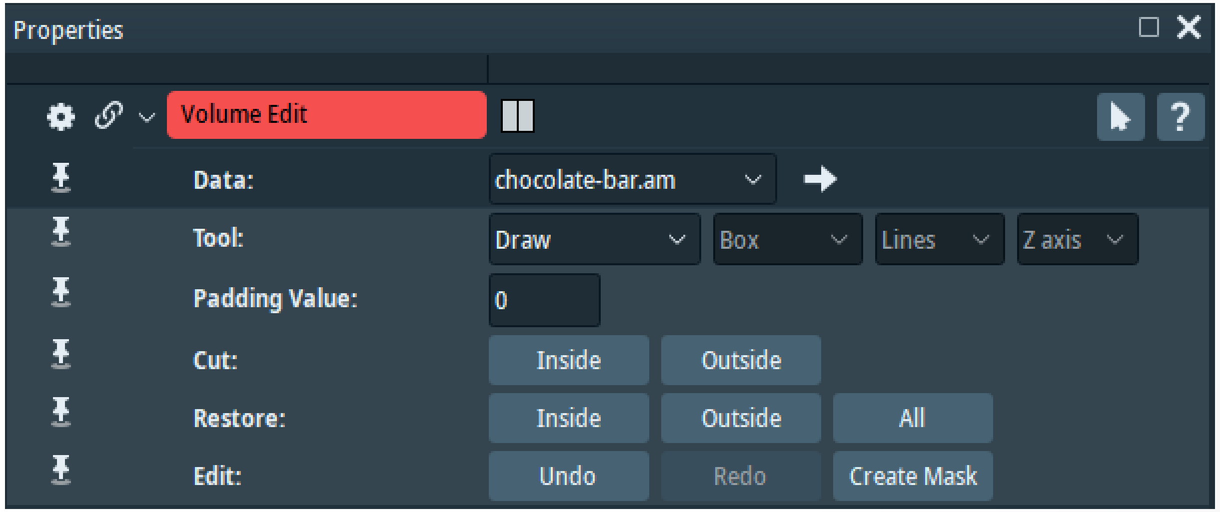
Basic image data editing – Volume Edit

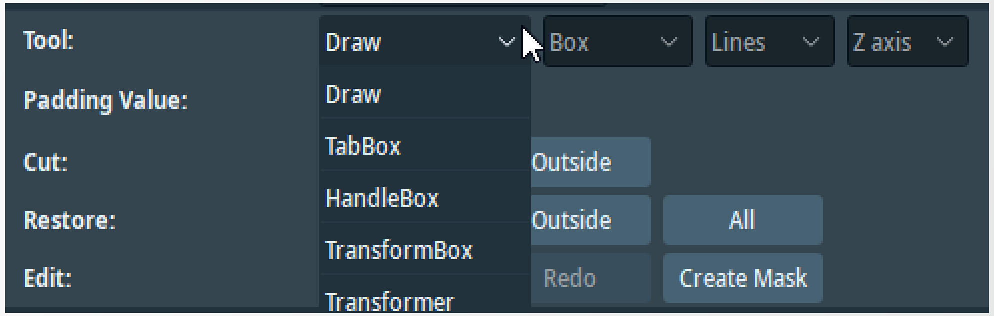
Volume Edit: interactive editing with various tools

Two types of tools are available:

- A draw tool:
  allows selecting the 3D projection behind a contour drawn in the viewer

- Dragger tools:
  allow selecting a region by dragging, rotating, resizing a 3D shape (box, ellipsoid, cone, etc.).

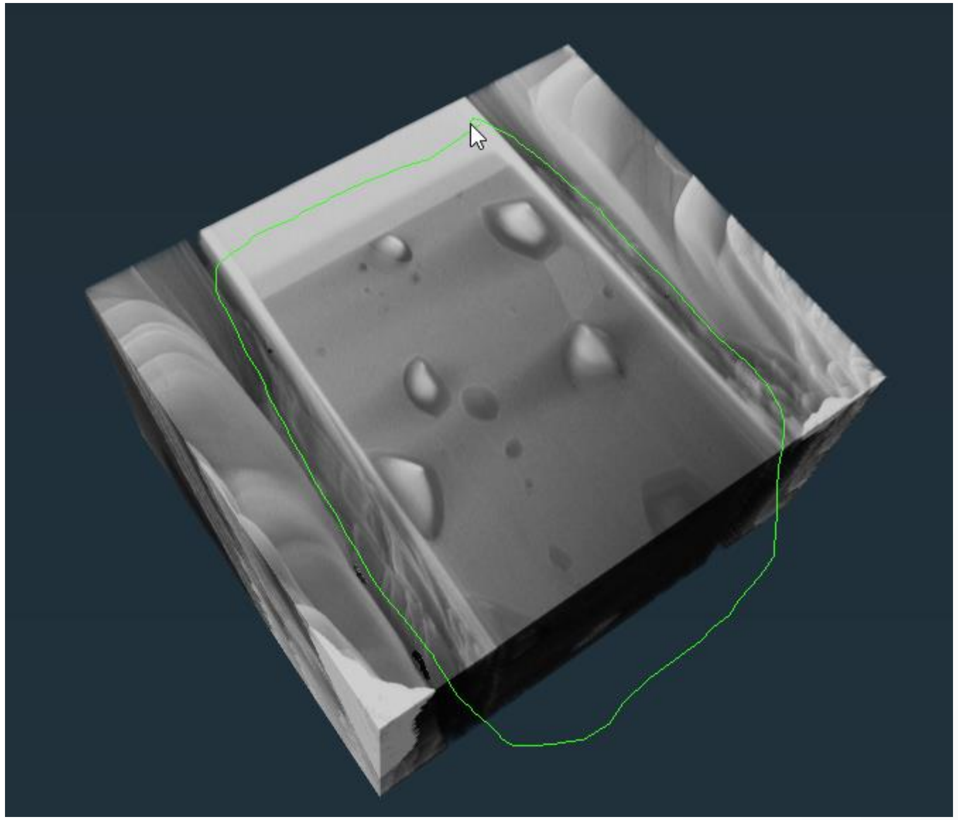




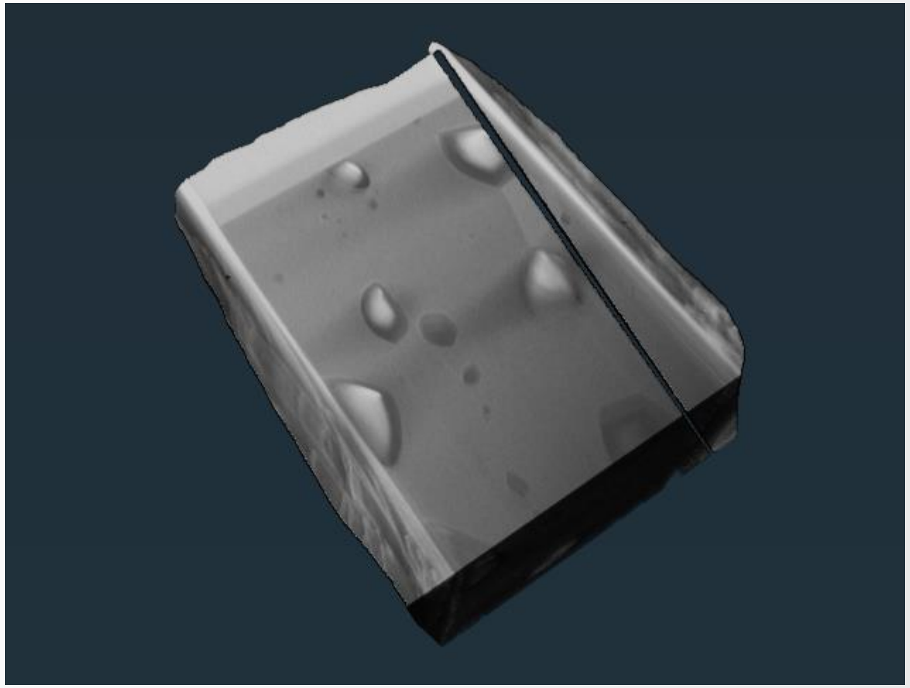
Basic image data editing – Volume Edit

Volume Edit – example of creating a sub-volume with the draw tool:

Draw tool selection



“Cut outside” result

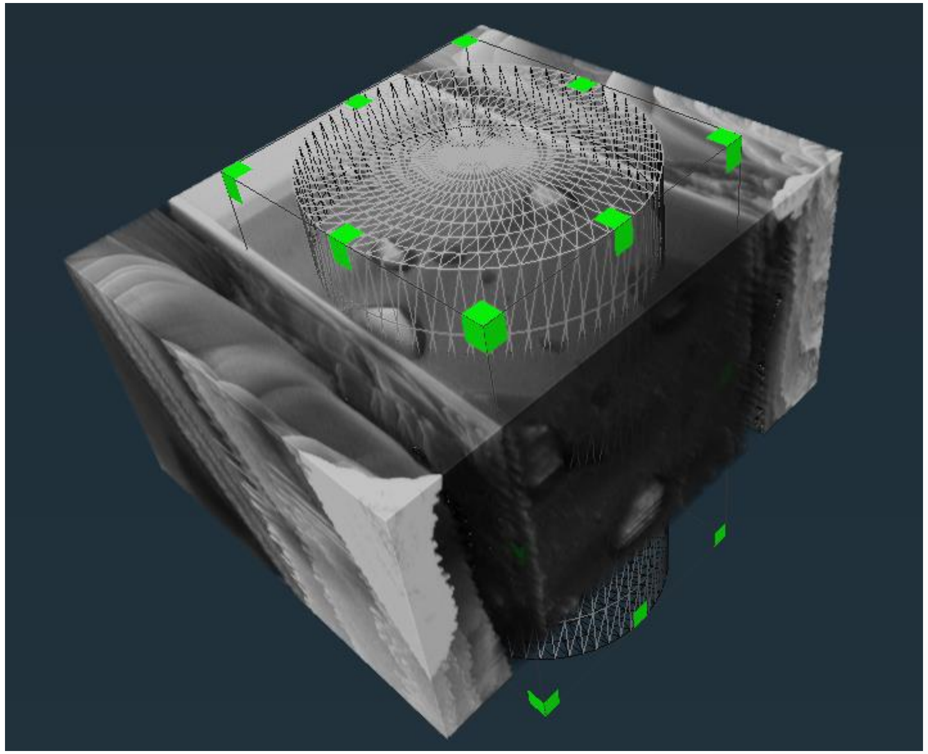


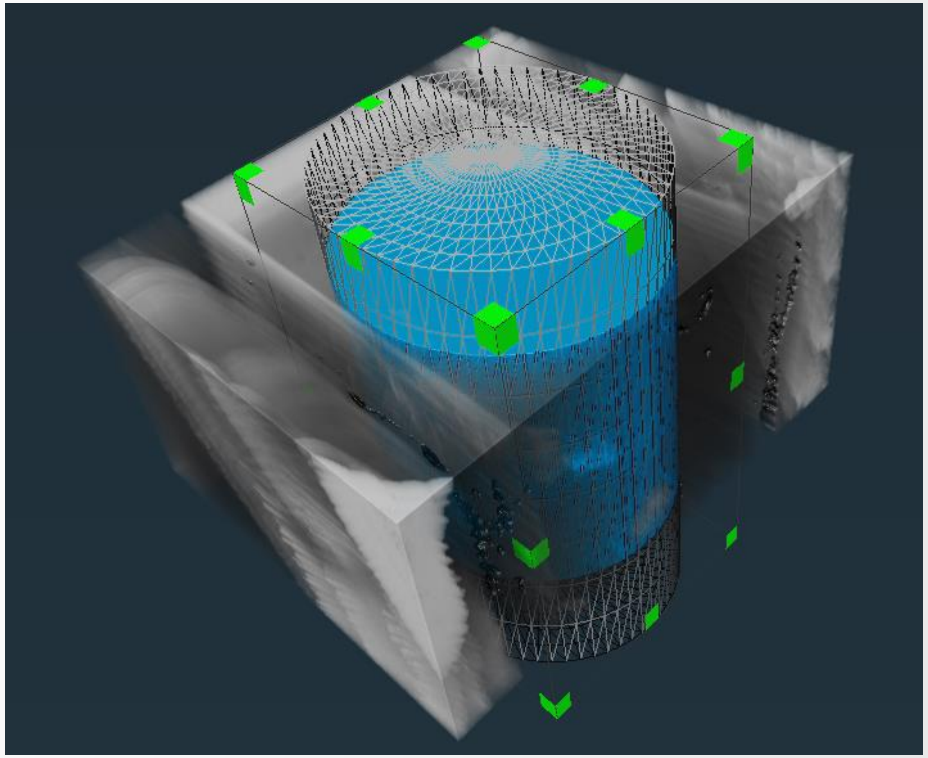
Data: MoSi2-shear-corrected.am

Basic image data editing – Volume Edit

Volume Edit - example of creating a Cylindrical Mask:

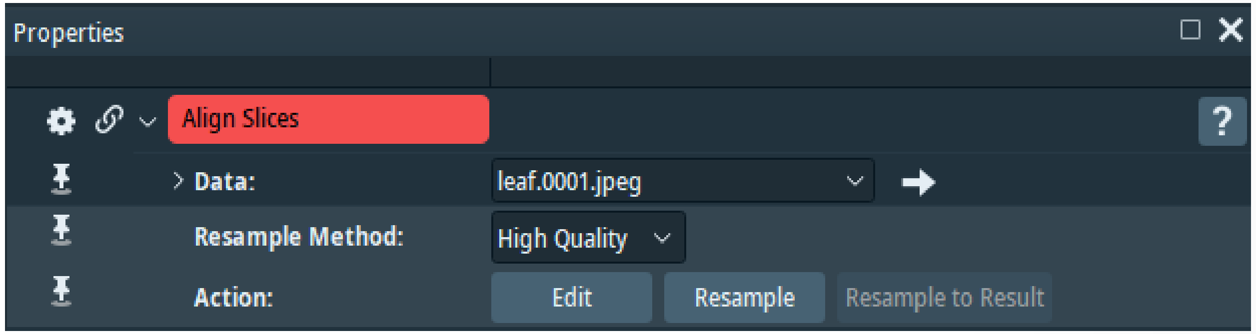
- Tool port settings: TabBox, Cylinder, Z Axis
- Adjust cylinder using Orthographic Camera mode
- Set Padding value to the value of the voxels of the “exterior” (e.g. 0)
- Push buttons: Cut – Outside and Edit – Create Mask

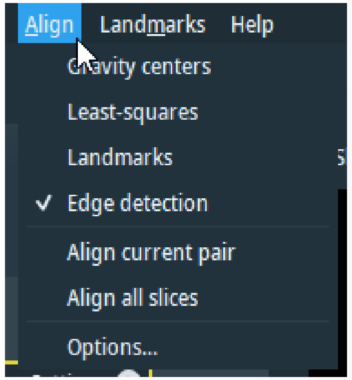


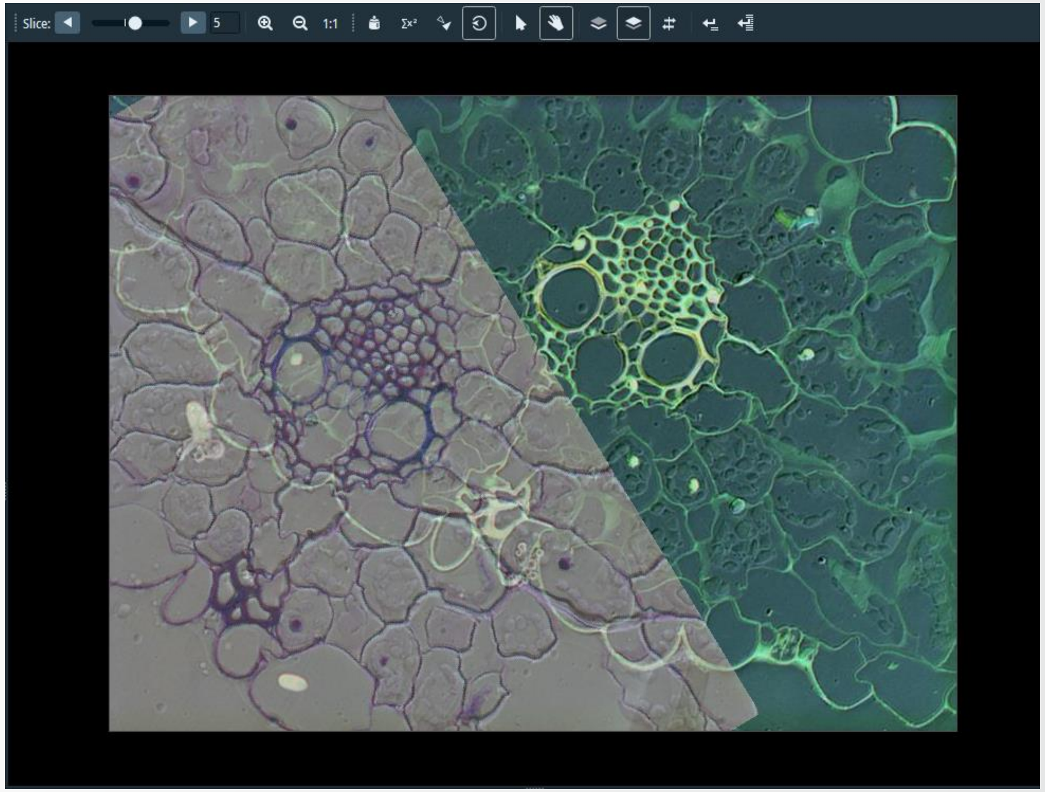


Align Slices

- For serial sections (e.g. Light Microscopy)
- Pushing the Edit button activates the Alignment viewer and tools and the Align and Landmarks menus
- Visualization of two consecutive slices in overlay







Data: leaf image stack

Align Slices

- Available operations:
  - Translate
  - Rotate
  - Mirror

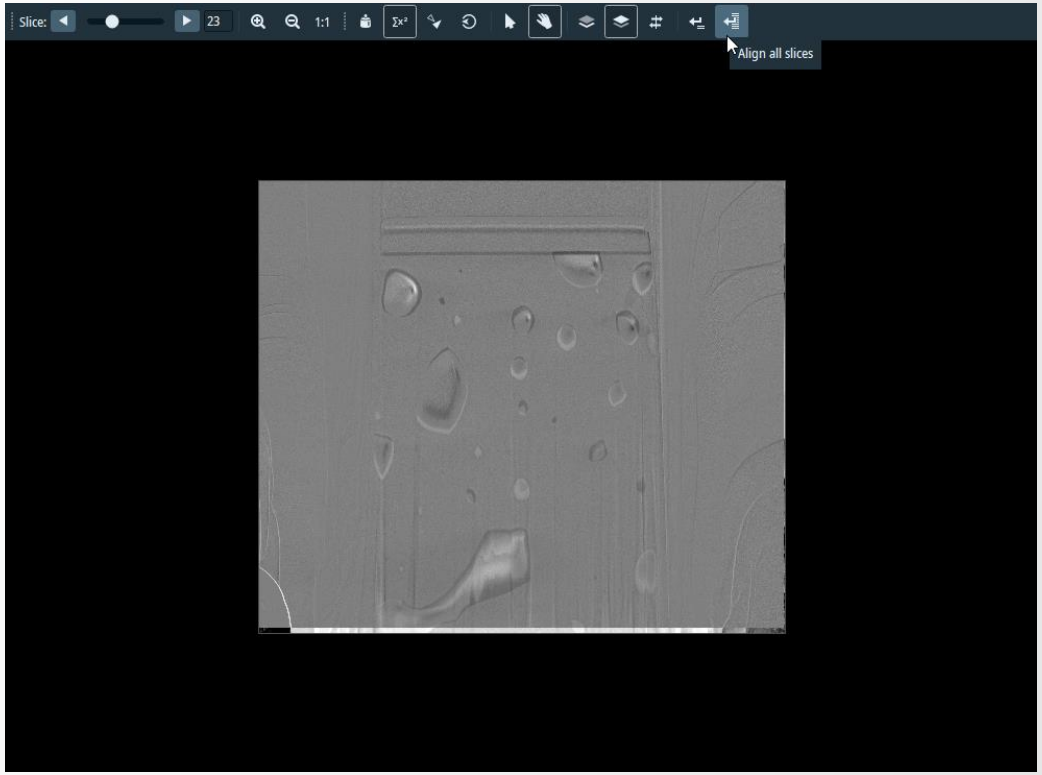
  each slice with respect to the next one.

- Modes and options (some examples):
  - Manual
  - Land-Mark
  - Intensity-based

- Possibility to use a mask

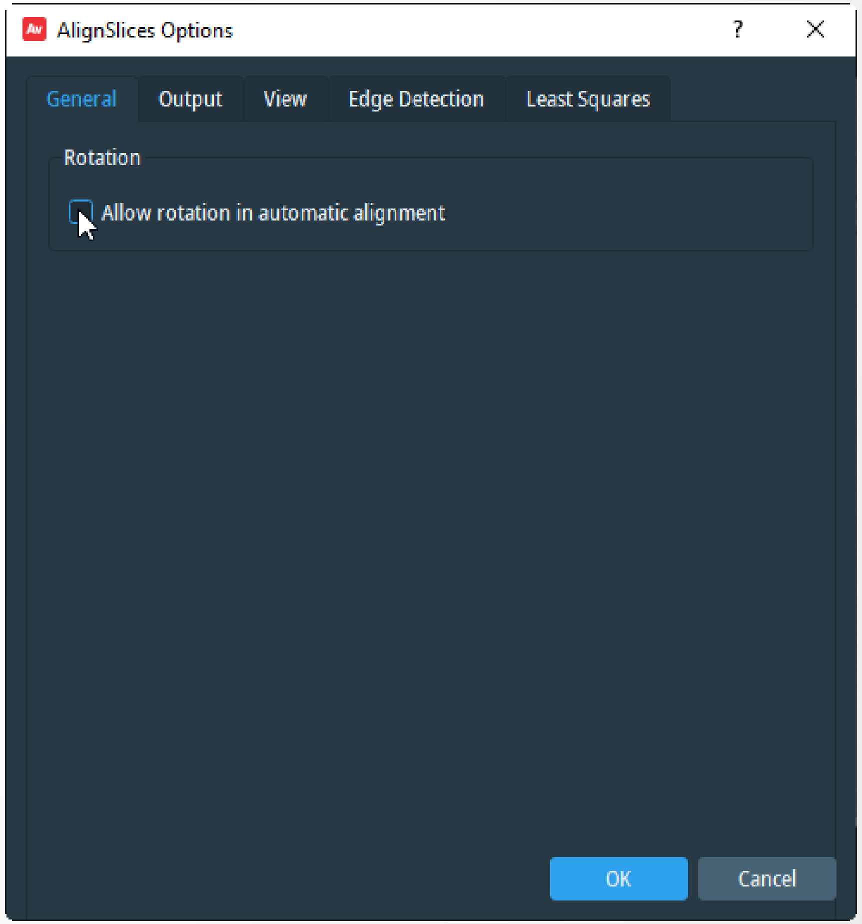
- Resample aligned stack (by pressing the Resample button of the module).

Automatic alignement modes



Align Slices

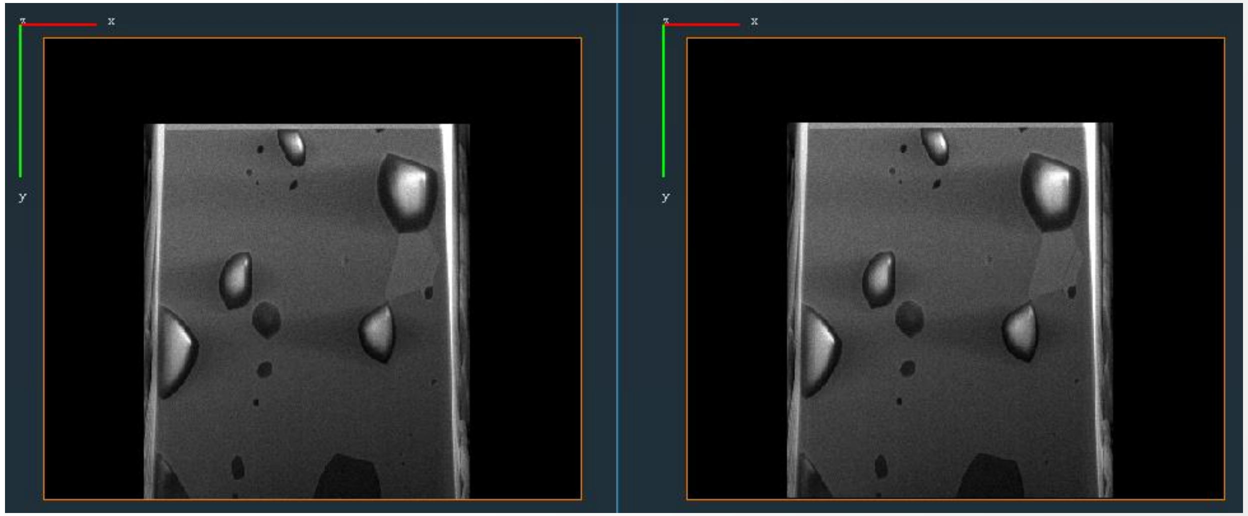
- Rotation alignment should be disabled for FIB-SEM (Align / Options)
- No rotation while FIB-SEM data collection!
- For disabling rotation go to Align menu – Options in order to access the AlignSlices Options pop-up.



Align Slices

Alignment example – comparison of raw data set (left-side) with aligned data set (right-side)

First xy slices of the volume



Last xy slices of the volume

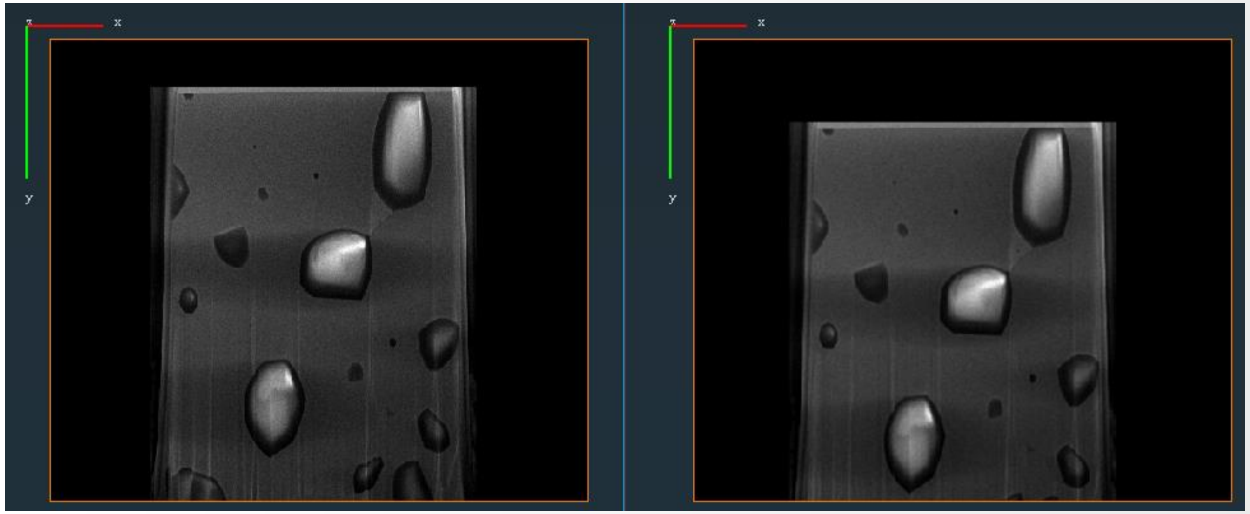


Image pre-processing: filtering

Introduction to spatial domain filtering

Filtering – enhance the data quality

Most filters operate in the spatial domain – each pixel/voxel is evaluated and its filtered valued is given by applying a formula to the values of its neighbors in the input data:

- The neighborhood type needs to be defined
- The formula is filter specific.

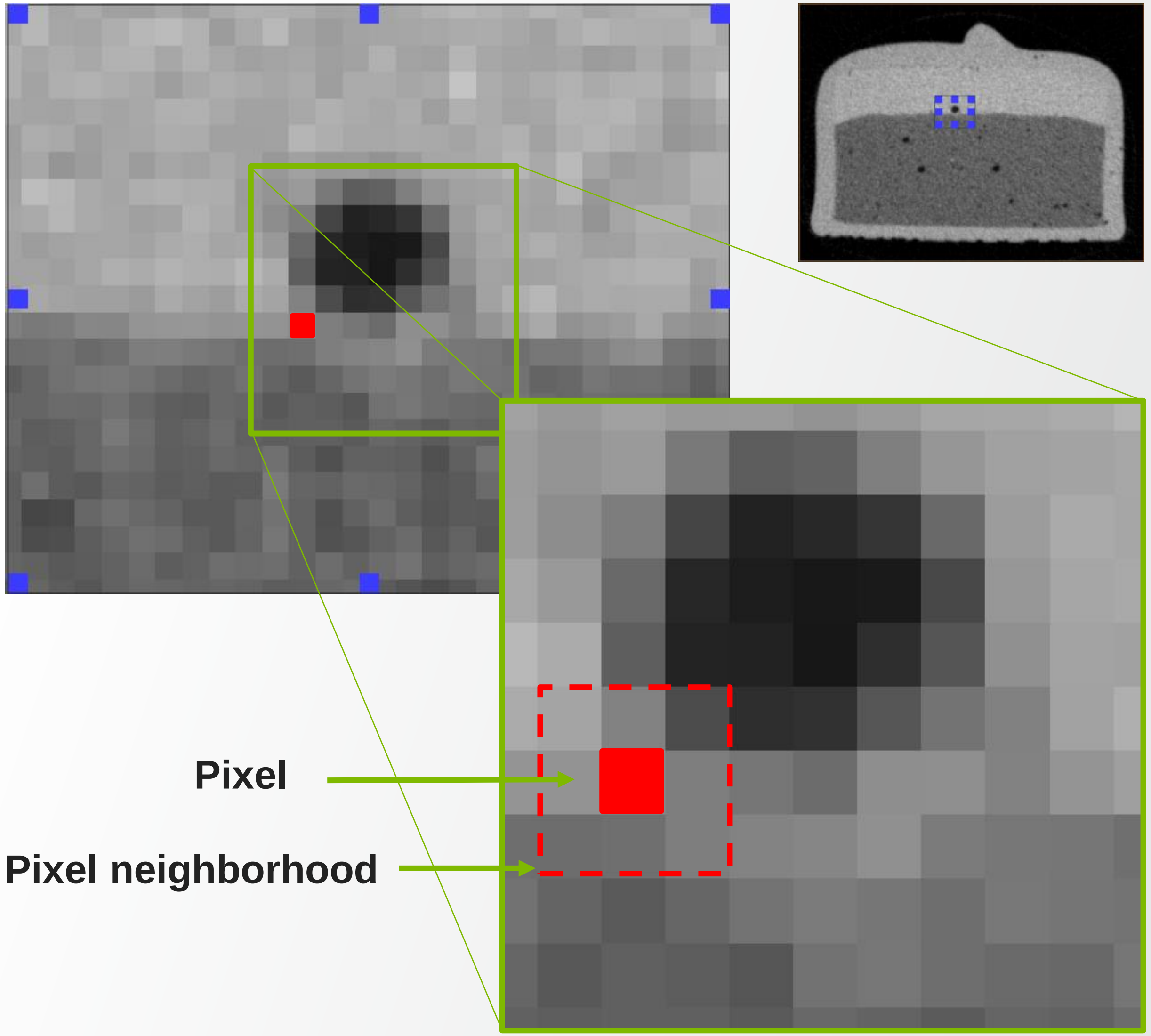


Image filtering: how to apply a filter in Avizo

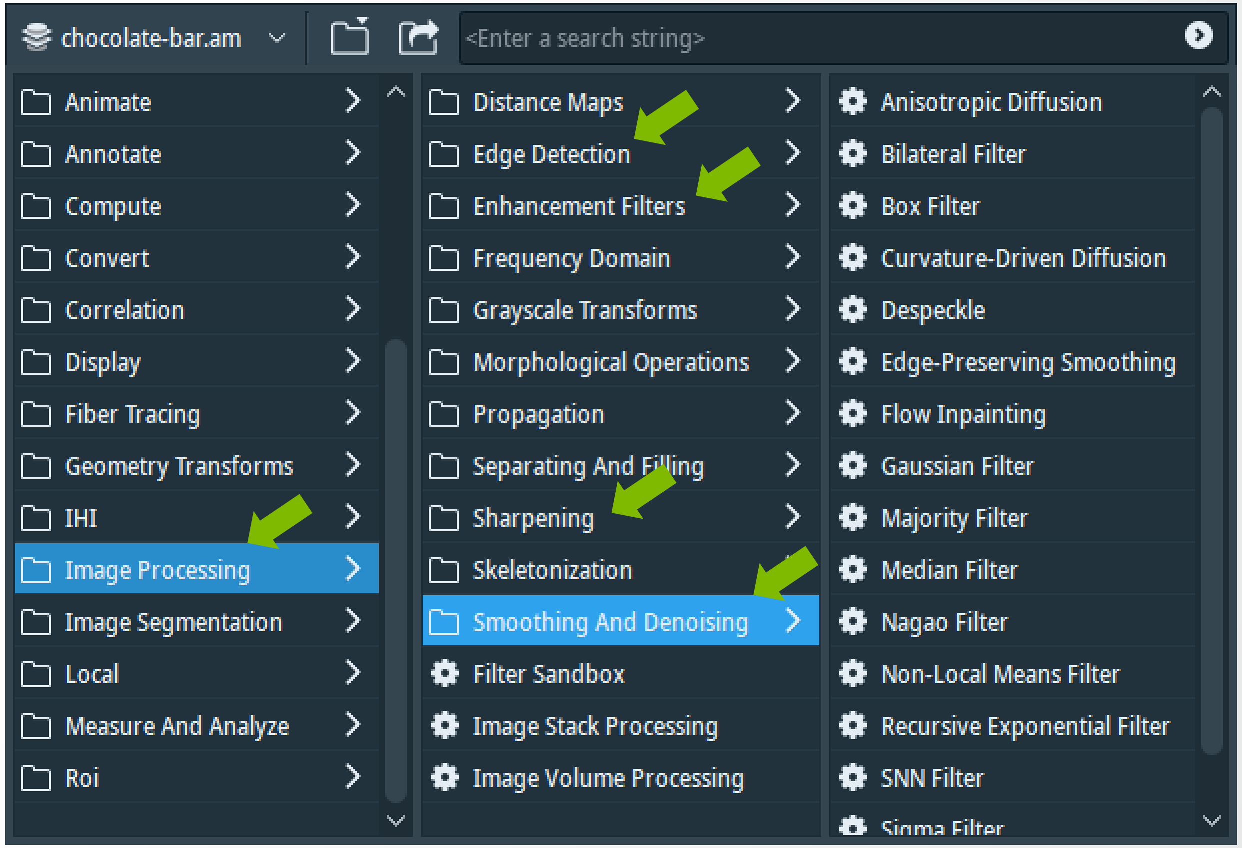
Thermo Fisher SCIENTIFIC

Various filtering modules are available in Avizo.

They are mainly grouped according to their types into different sub-categories of the Image Processing category :

- Edge Detection
- Enhancement Filters
- Sharpening
- Smoothing and Denoising
- …

Right click on the data set in order to Access the module category menu.



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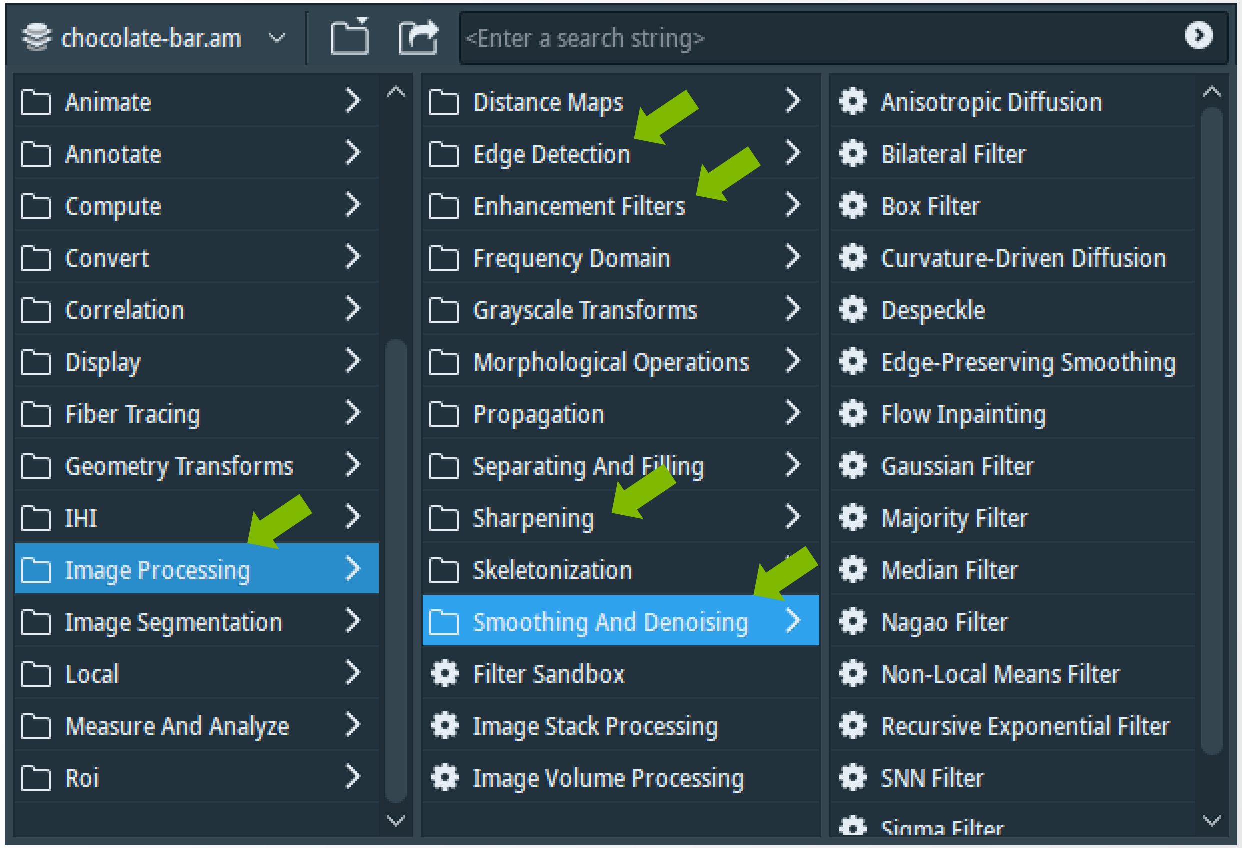
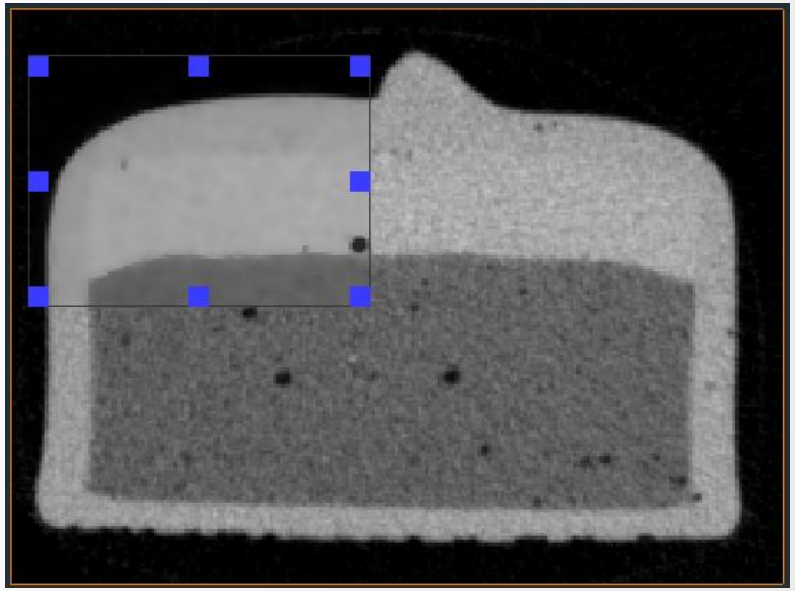


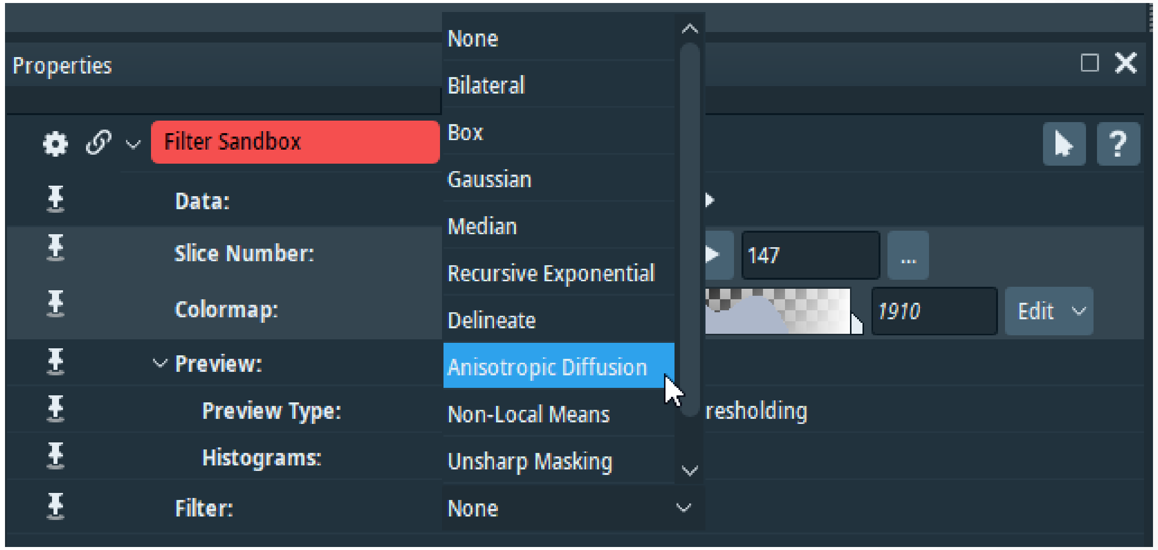
Image filtering: how to apply a filter in Avizo

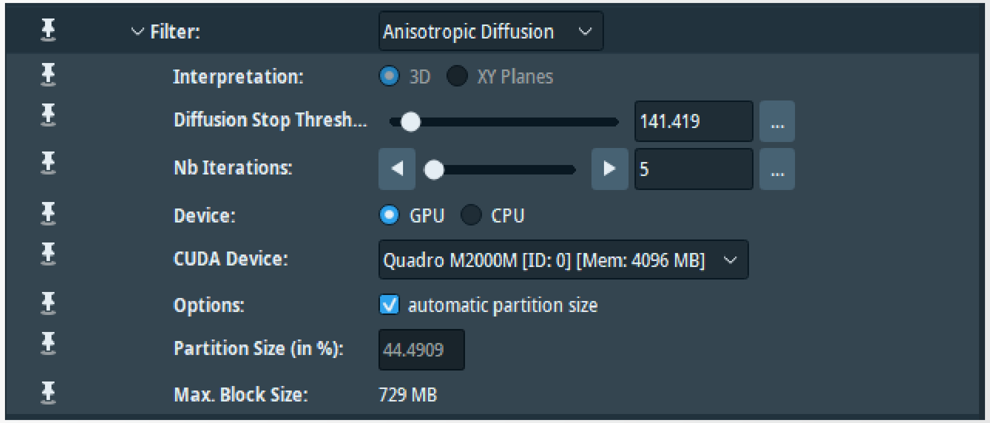
Filter Sandbox module allows testing different filters in order to make the best choice for a given dataset:

- Select a filter
- Tune its parameters
- Appy result on a sub-volume of the dataset (useful for large datasets)

E.g. Anisotropic Diffusion







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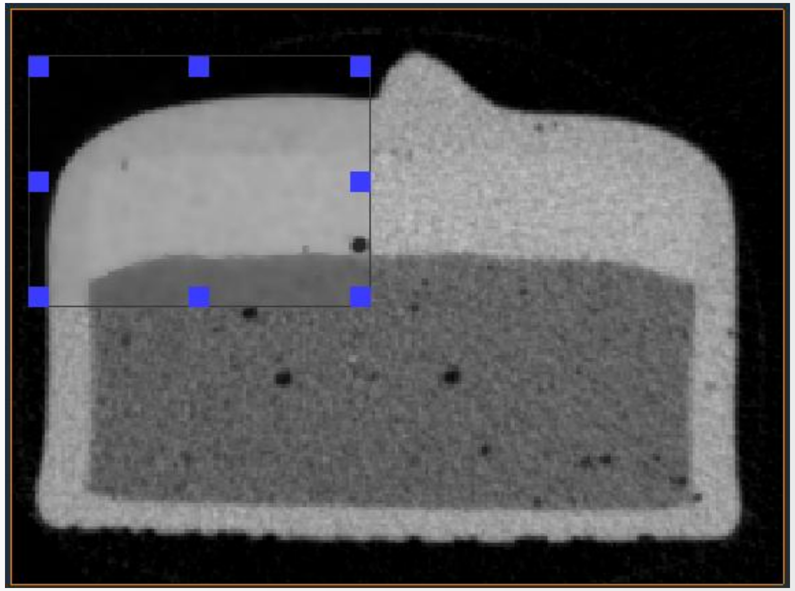
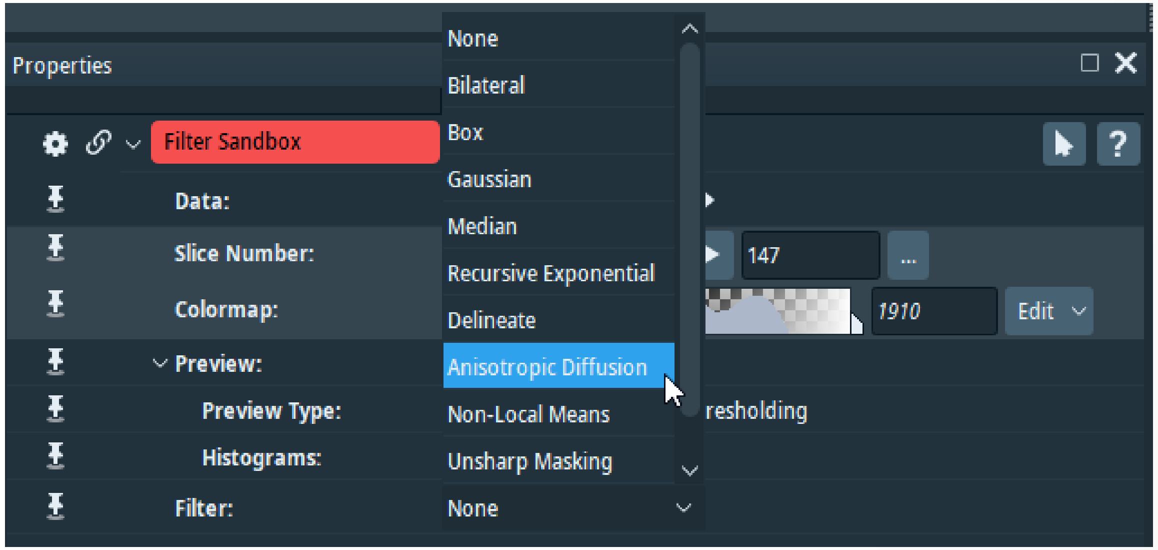




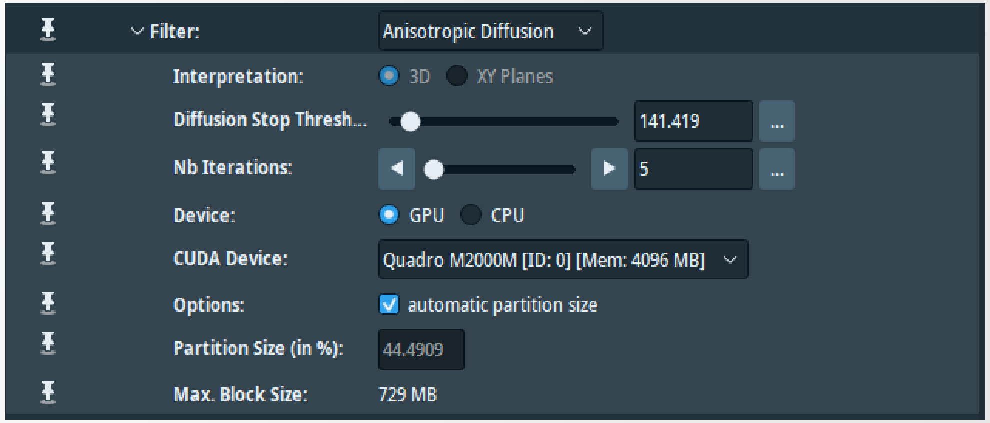


Image filtering: Box

Box Filter performs the arithmetic mean of the pixel/voxel values in the neighborhood window

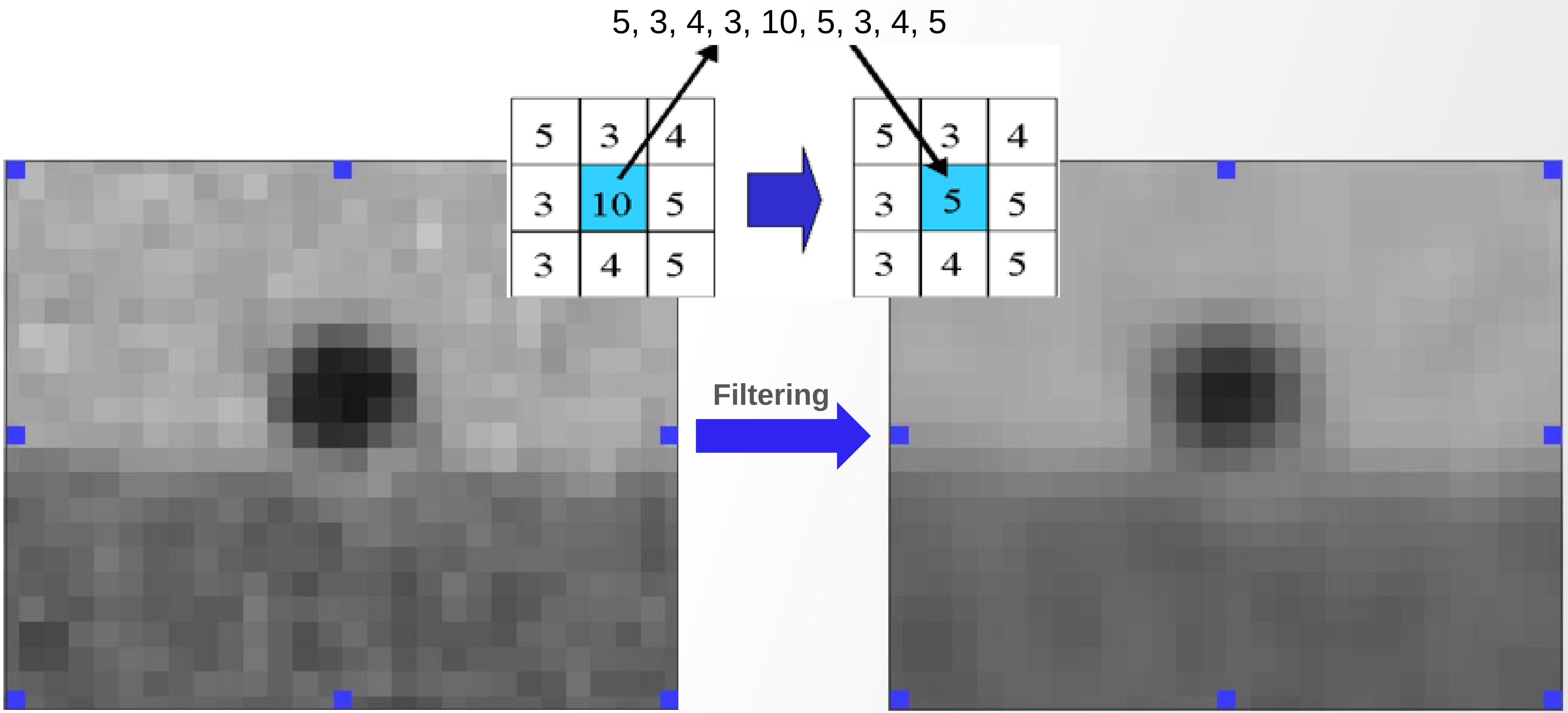


Image filtering: Median

Median Filter outputs the median value of the pixel/voxel values in the neighborhood window

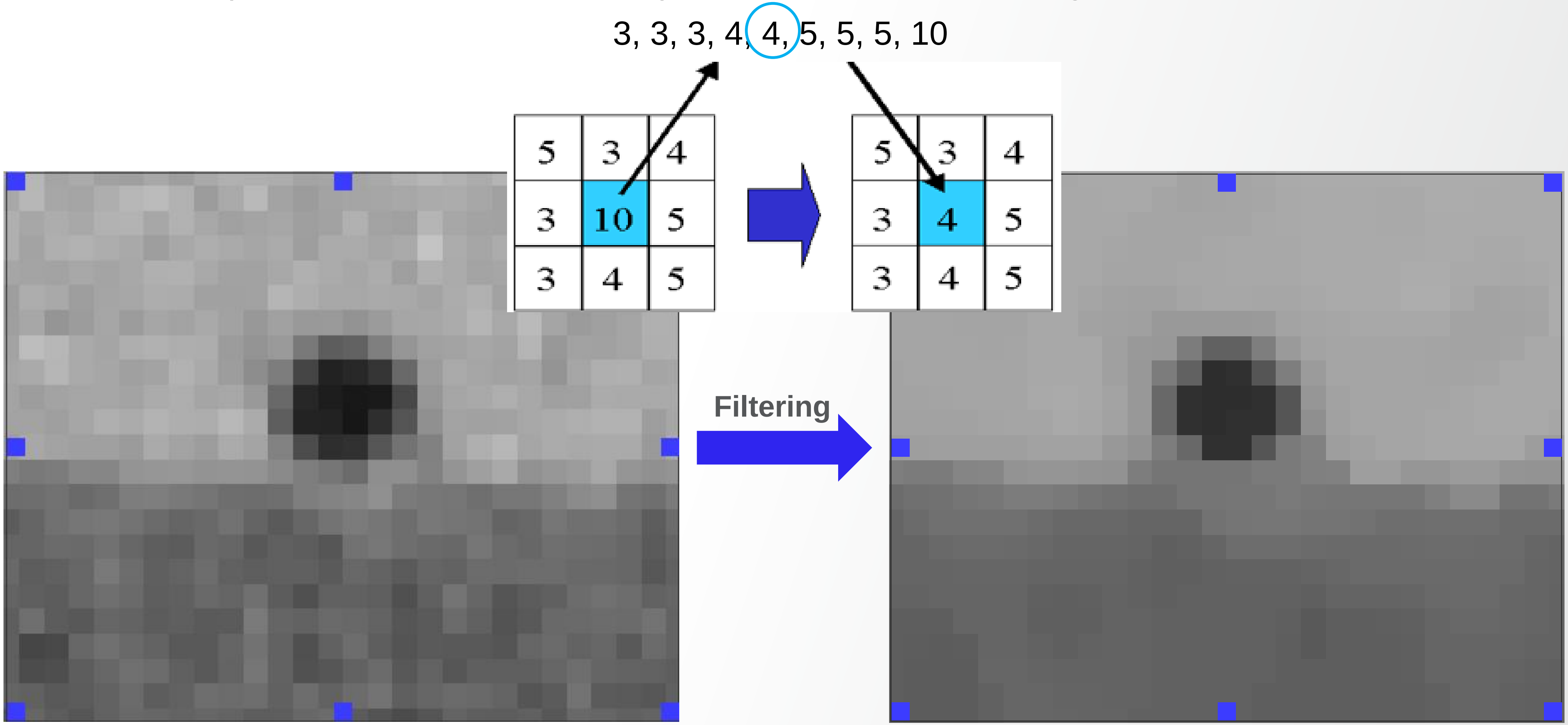


Image filtering: Non-Local Means

Non-Local Means outputs the weighted average of the values in the local neighborhood. The weight of each pixel/voxel is given by how similar its neighborhood (non-local) is to the local neighborhood.

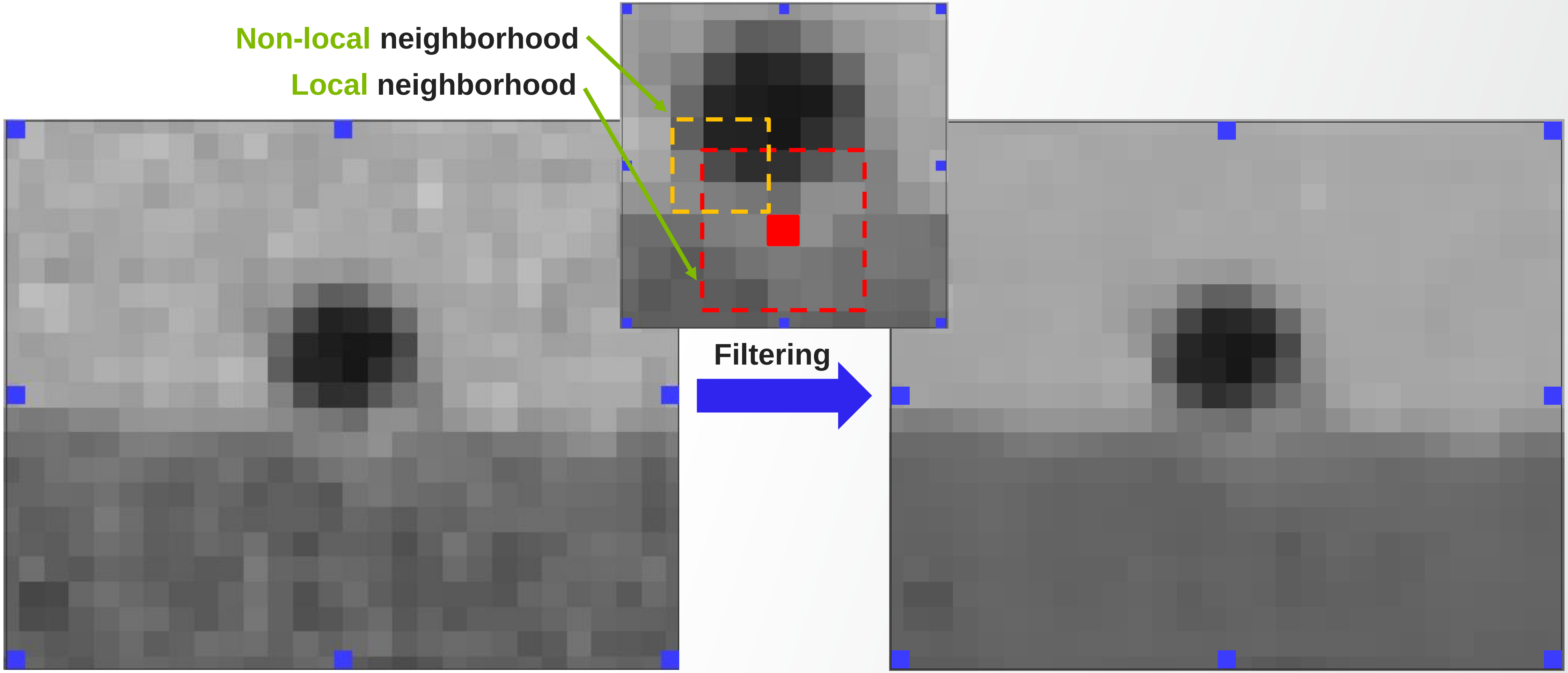
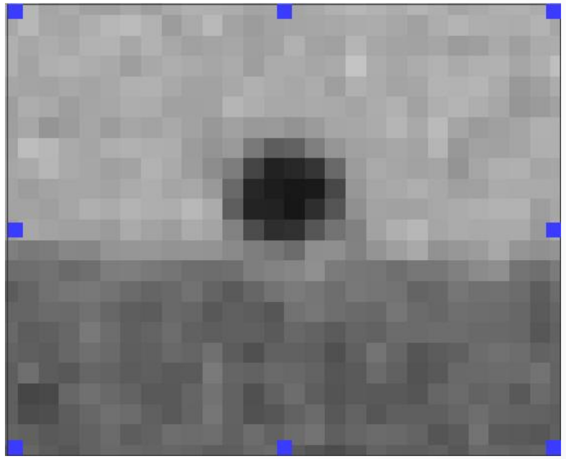
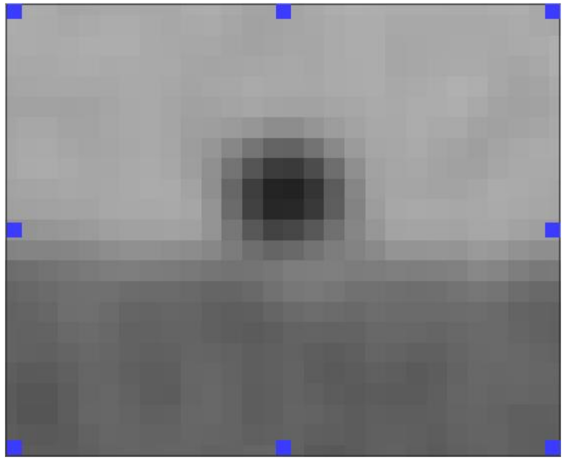


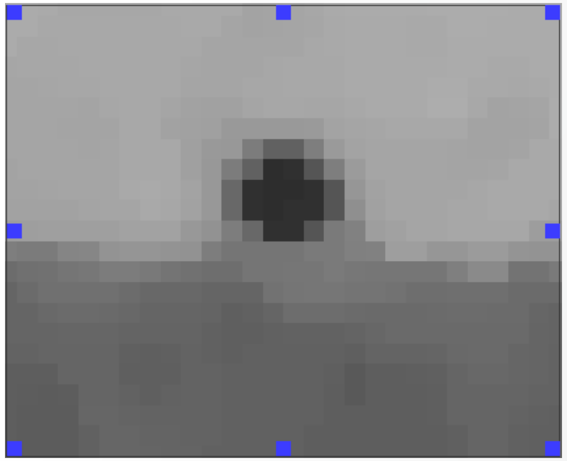
Image filtering: adapt filter choice to dataset and problem



Box
Smoothening
Not edge preserving



Median
Edge preserving
but some details are lost



NLM
Edge preserving
Better preservation of details

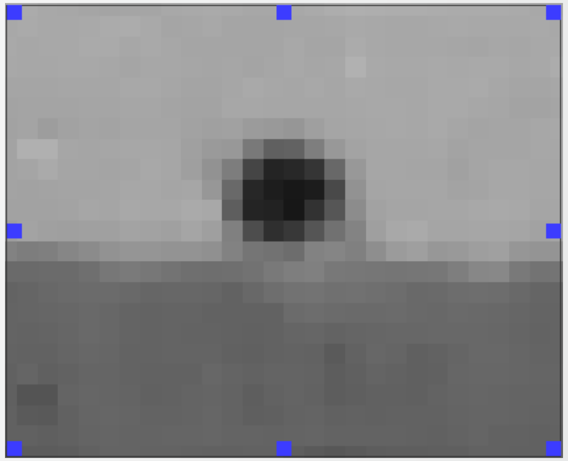
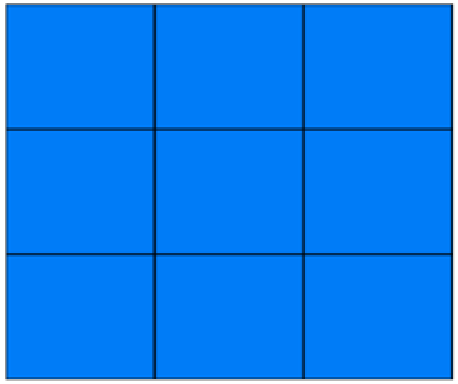


Image filtering: kernel type and connectivity

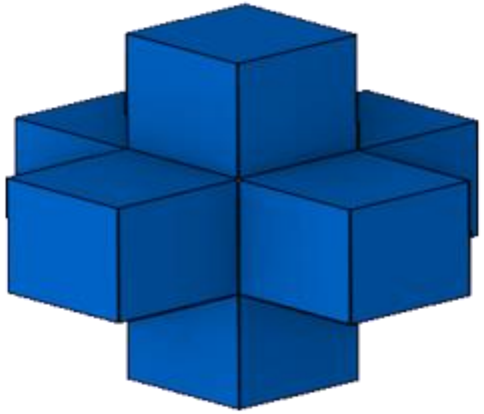
Kernel type – defines the neighborhood configuration, e.g: cube

2D

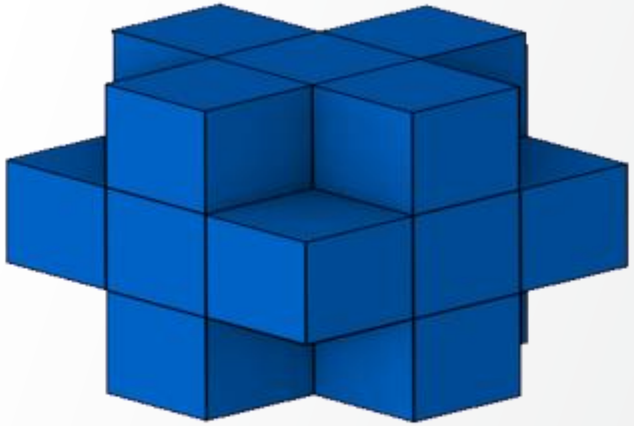


Kernel connectivity in 3D (for a cube kernel)

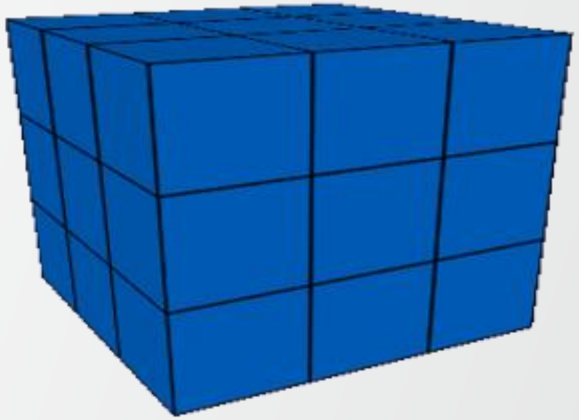
3D



6 voxels



18 voxels



26 voxels

Image filtering: kernel size

Kernel size – refers to the half kernel. Example for a cube type kernel:

Size 1 Size 3 Size 6

2D

3D

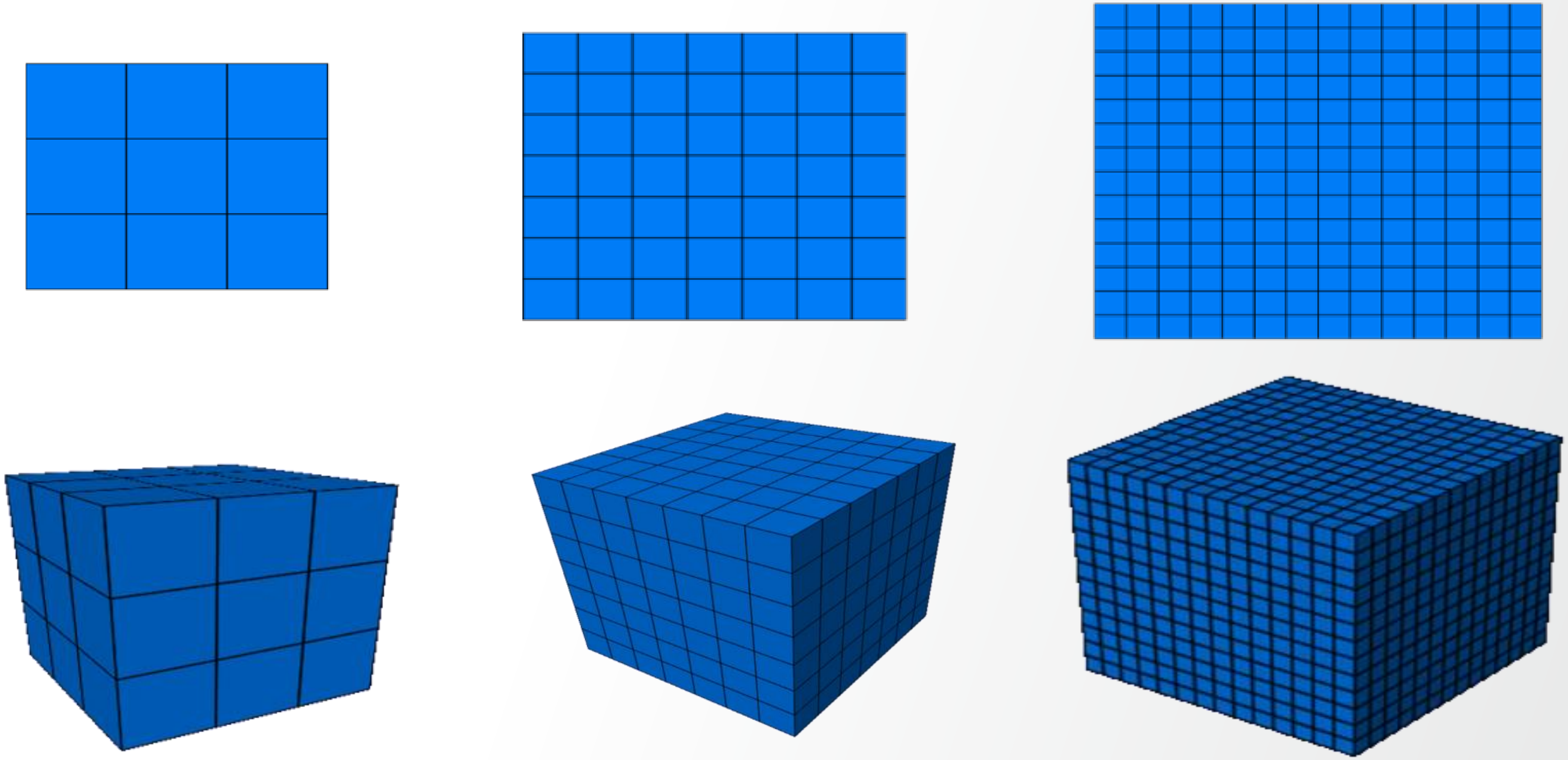
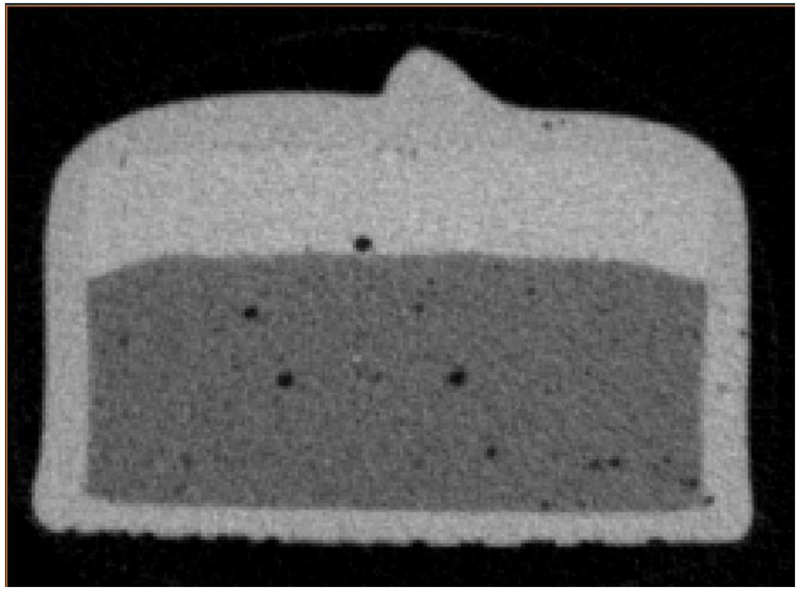


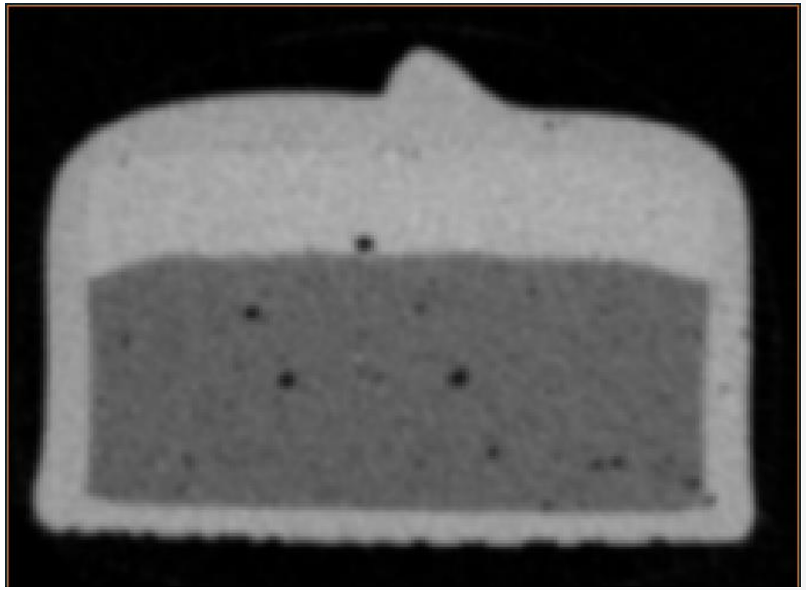
Image filtering: denoising filters

Examples of filters for removing “salt and pepper noise” (white and black dots on the image):

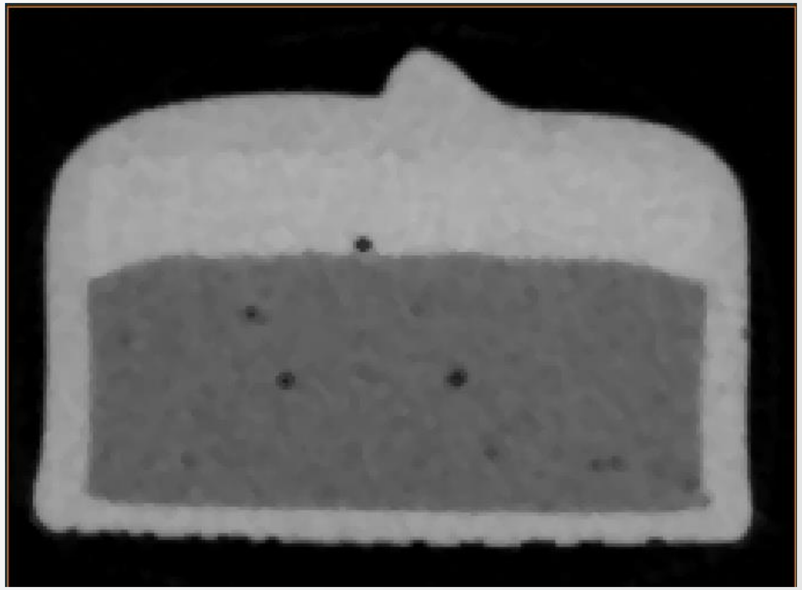
- Gaussian – smoothening, not effective for removing high contrast local noise
- Median – fast and efficient but tends to remove small details and to blur the result



Input



Gaussian

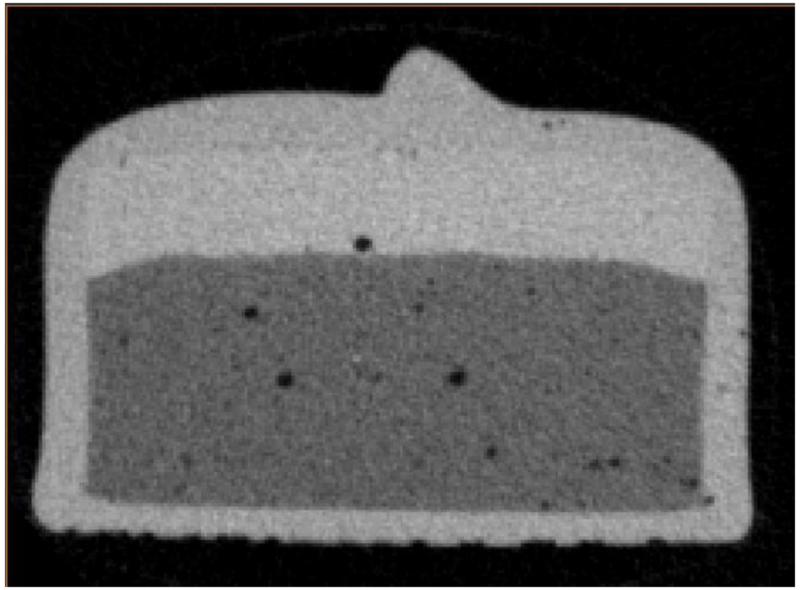


Median

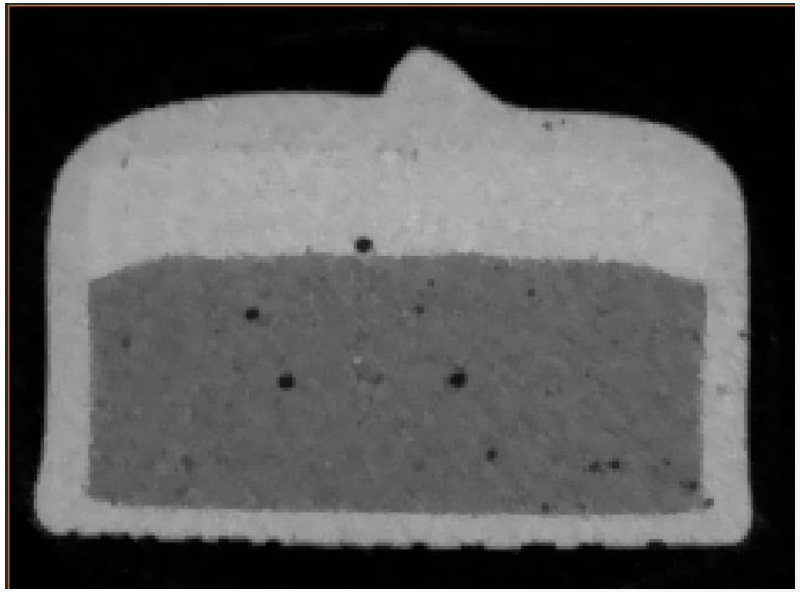
Image filtering: denoising filters

Examples of filters for removing “salt and pepper noise” (white and black dots on the image):

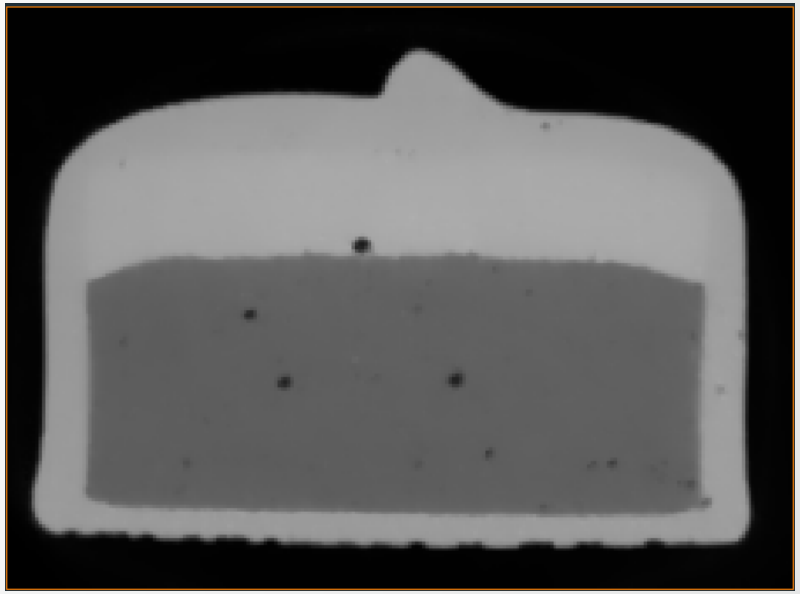
- Bilateral – performance in between median filter and NLM
- Non-Local Means – very effective at removing noise while preserving the edges but slow



Input



Bilateral

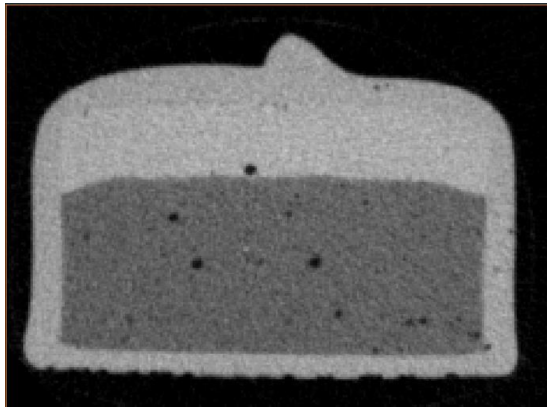


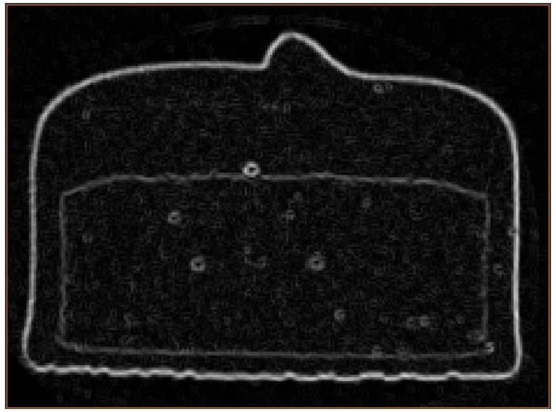
NLM

Image filtering: contour detection and enhancement

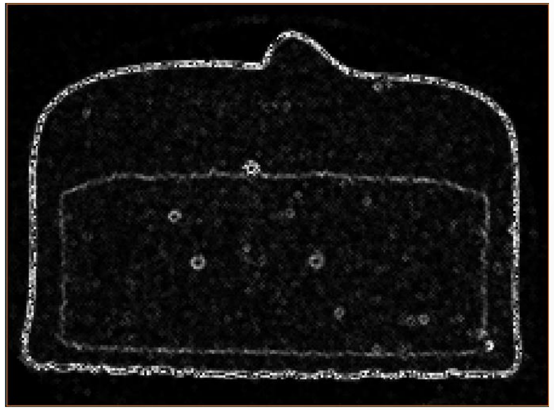
- Sobel and Moments (e.g. variance, kurtosis) – edge detection filters
- Unsharp Masking – edge enhancement filter

  Best practice: employ unsharp masking after denoising

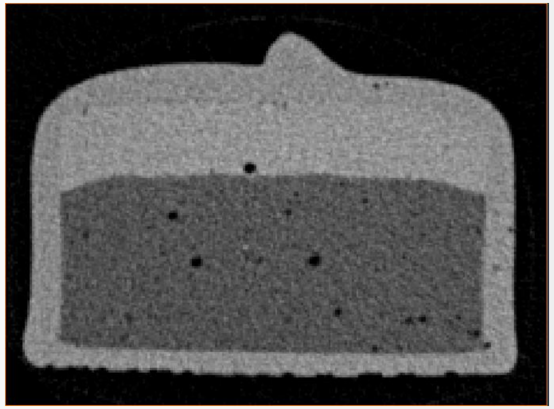




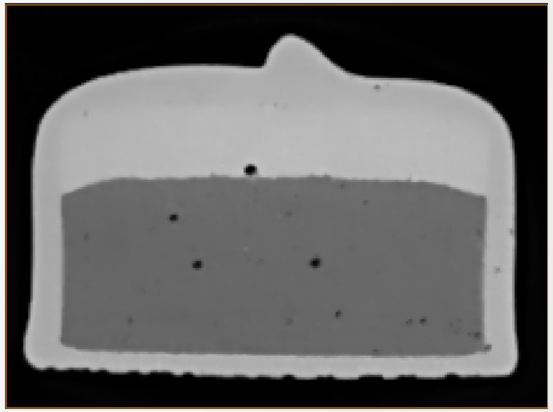
Sobel



Moments



Unsharp Masking



NLM + Unsharp Masking

Background correction

Basic idea: remove low frequencies in image

Can be done in Avizo by means of different modules:

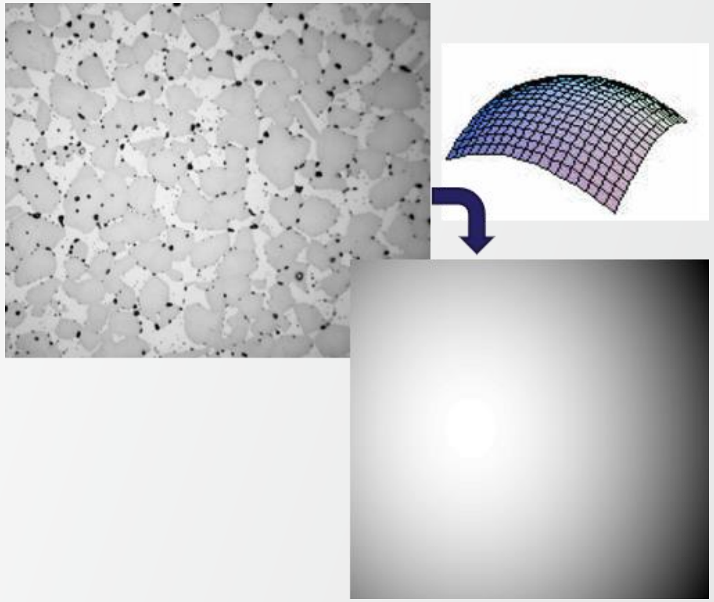
- Correct Z Drop:
  fits an arbitrary function of z to the average intensity in each slice

- Block Face Correction:
  matches masked-slice average intensity to volume average intensity

- Background Image:
  estimates background image, slice by slice, by fitting a 2nd order polynomial (to the masked region)

- Shading correction wizard:
  removes image low frequency by dividing the input image by a background image

- Background Detection Correction:
  estimates the background from a B-spline model (for example) with specified grid and removes it.



Background correction

Basic idea: remove low frequencies in image

Can be done in Amira by means of different modules:

- Correct Z Drop:
  fits an arbitrary function of z to the average intensity in each slice

- Block Face Correction:
  matches masked-slice average intensity to volume average intensity

- Background Image:
  estimates background image, slice by slice, by fitting a 2nd order polynomial (to the masked region)

- Shading correction wizard:
  removes image low frequency by dividing the input image by a background image

- Background Detection Correction:
  estimates the background from a B-spline model (for example) with specified grid and removes it.

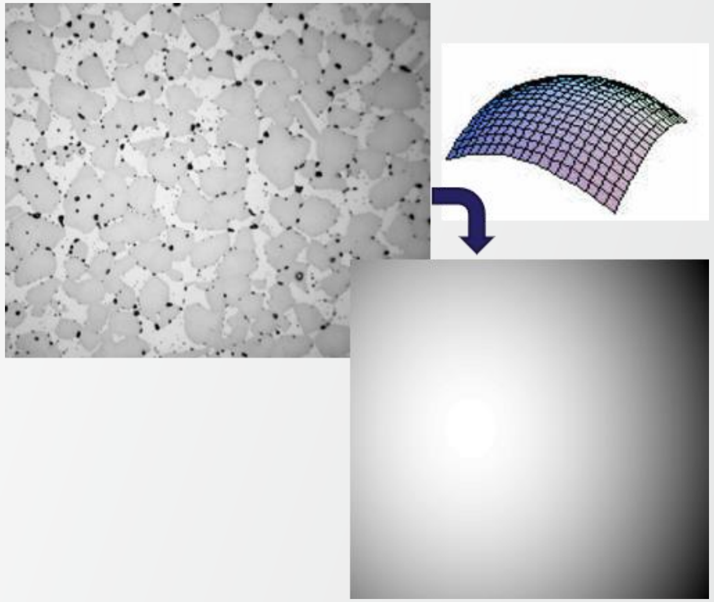
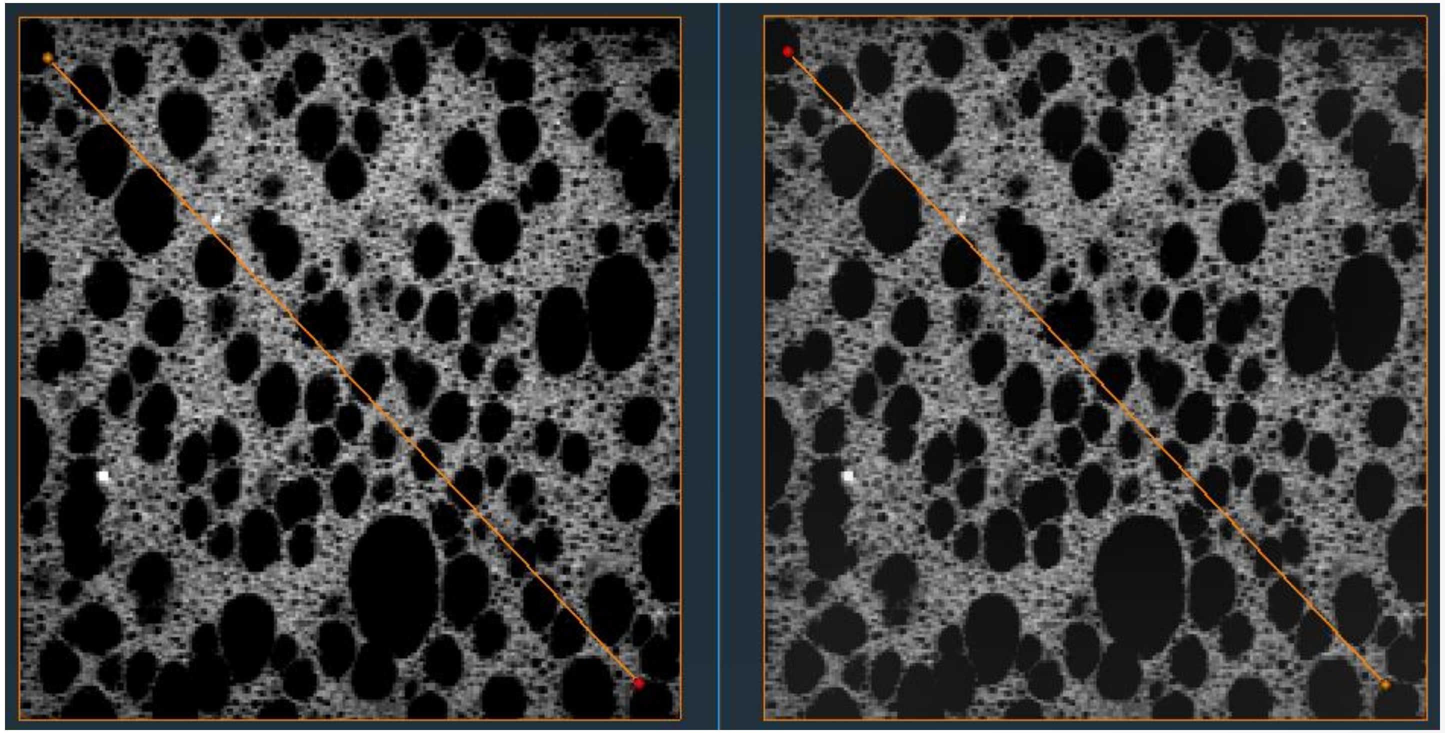


Image pre-processing: exercise 1

Background correction

Apply a background correction method in order to obtain a similar result:

Original **Corrected**



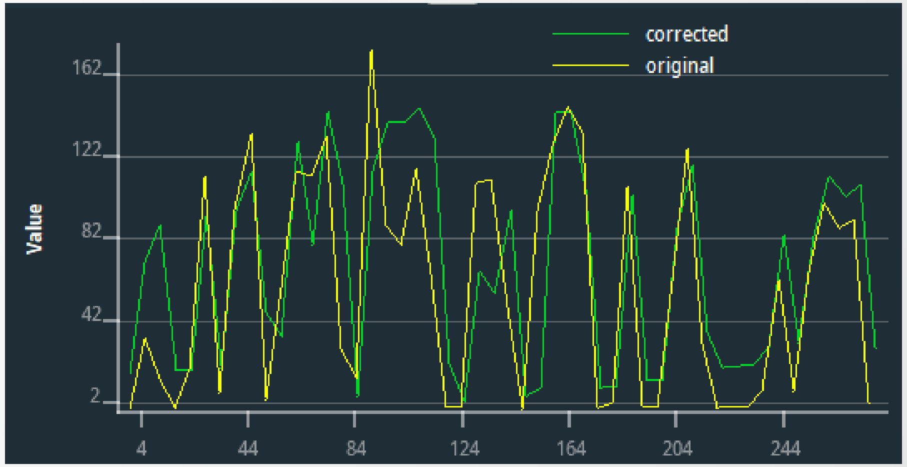


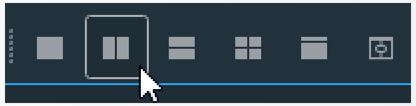
Image pre-processing: exercise 1

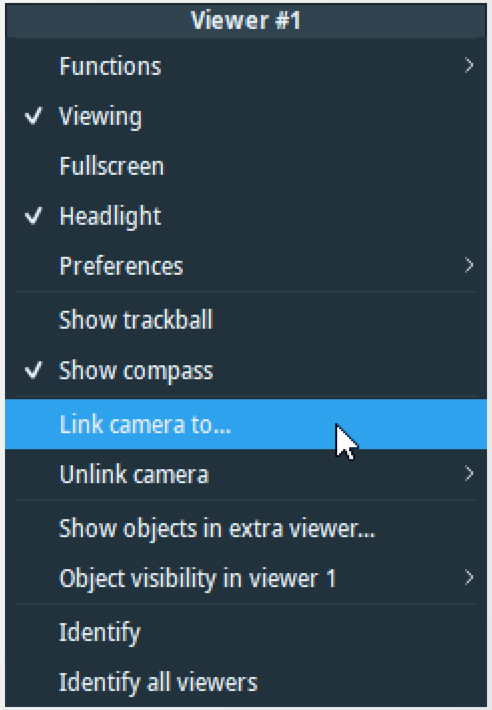
Solution

- Example 1: Shading Correction Wizard
  - Threshold 75-255
  - Normalization 130
- Example 2: Background Detection Correction
  - Type: B-spline
  - Size: 5, 5, 5

Tips:

- Visualization setup for comparing images:
  - Use multiple viewers with linked camera
    - right-click on one of the images in the two viewers
    - select “Link camera to…”
    - then click on the image in the second viewer.





- Assess background
  - Use Line Probe module and increase number of samples if necessary « take average » (with increased radius / long. Width)

Introduction to frequency domain filtering

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Fourier Transform

- Filters out low frequency (small intensity variations) or high frequency (strong intensity variations e.g. edges) components in images

Steps

- Compute the Fourier Transform of the image
- Multiply the images Fourier Transform by a filter function (low-pass filter, high pass-filter, etc.)
- Compute the inverse Fourier Transform of the result (the result is mapped back to the spatial domain)

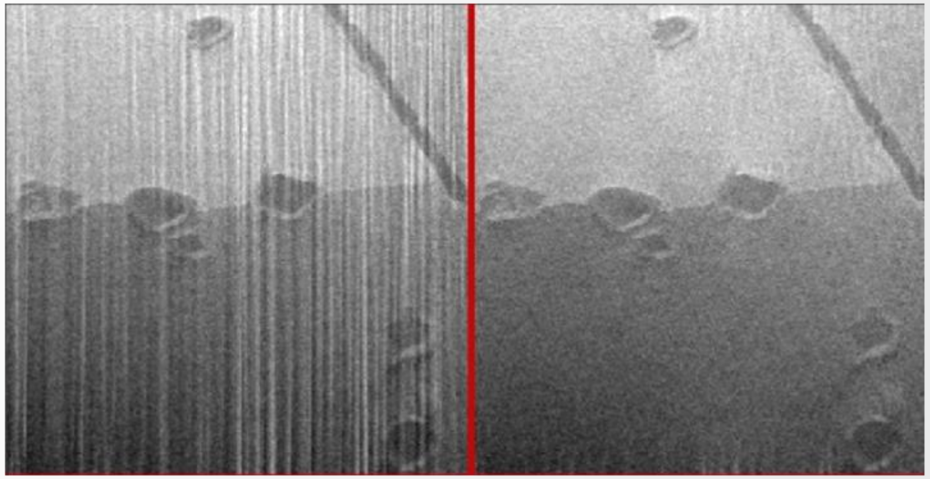
Why filter in the frequency domain ?

- Can be much faster than the spatial domain filtering
  (a convolution in the spatial domain is replaced by a multiplication in the frequency domain).

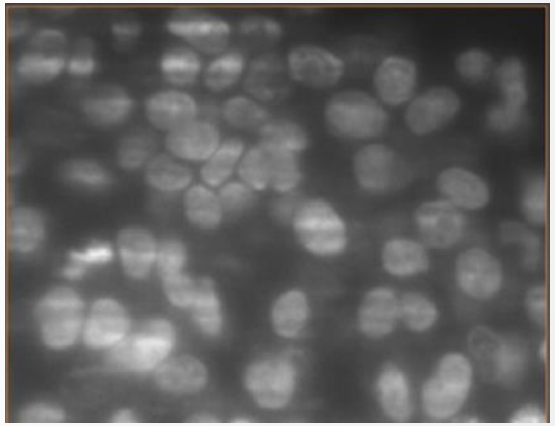
Image filtering: FFT

Frequency domain filtering can be achieved in Avizo by means of FFT Filter module

FFT Filter has two main functioning modes:
- Spatial: removal of periodic structures or stripes
- Frequency: removal of periodic/directional structures and spots from the FFT magnitude (advanced-user mode)



Principal use-cases:
- Curtaining artefacts in FIB-SEM
  (the module's parameters are set by default for filtering vertical stripes)
- Horizontal stripes in light-sheet microscopy images



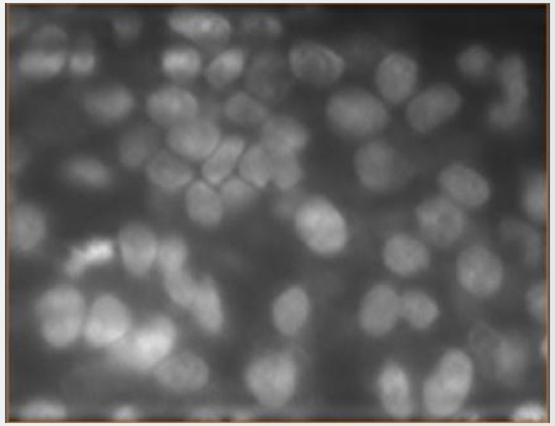


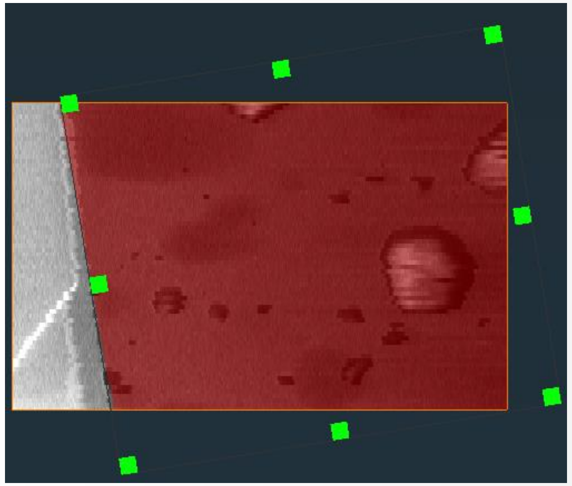
Image pre-processing: exercise 2

Slice Alignment

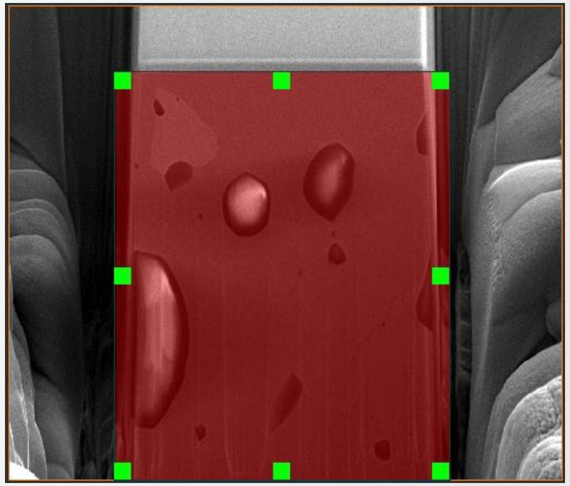
- Load fib/MoSi2-shear-corrected.am
- Use Volume Edit to create a mask
  - Use the different transformers to rotate and position a box
  - Exclude the trench and upper surface
  - Use “Cut Outside”
- Use Align Slices on masked image
  - Translation only
  - Automatic Least Squares Mode

Exercise solution:
https://youtu.be/HKh4rCr_bIg

xy slice



yz slice



yz slice before and after alignment

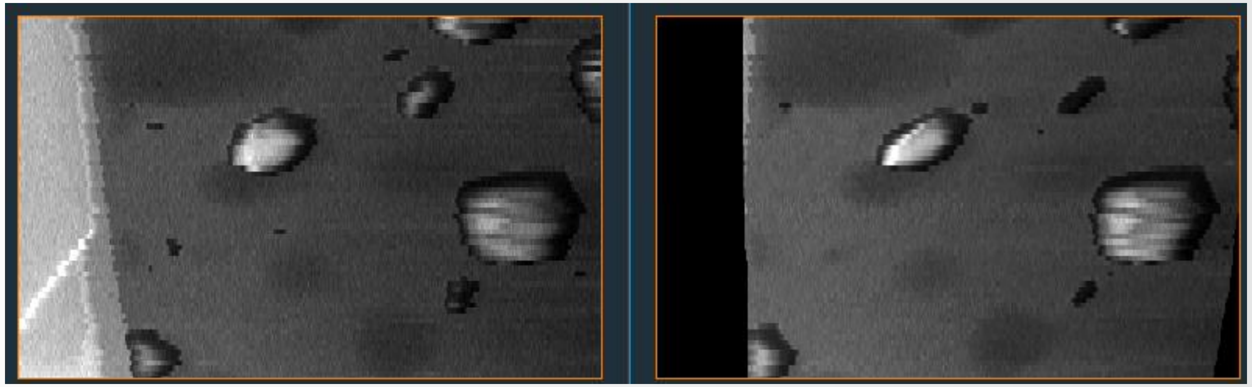
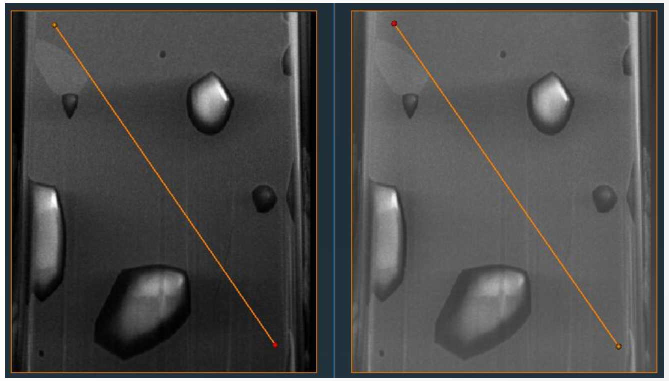


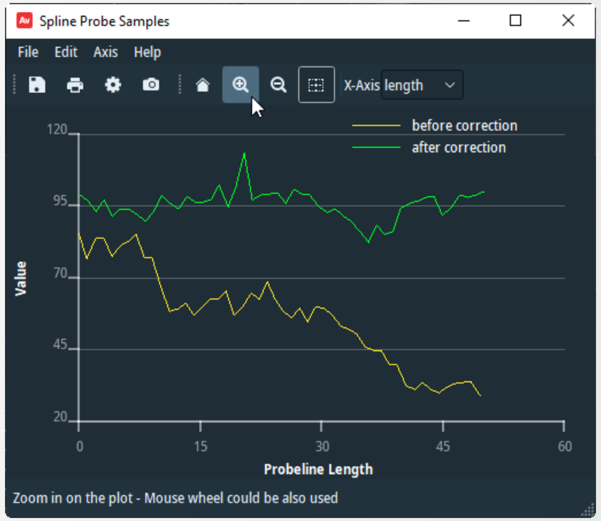
Image pre-processing: exercise 3

Background Correction, Frequency Domain Filtering, Denoising

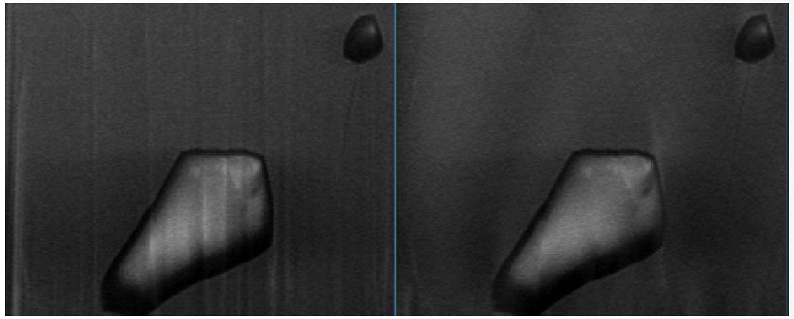
- Background correction
  Try:
  - Shading Correction Wizard
  - Background Detection Correction

Original   Corrected

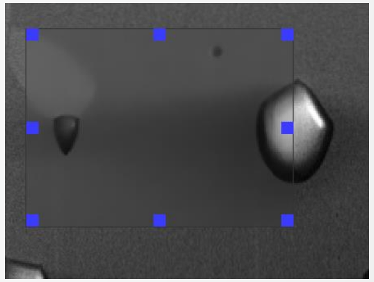




- Reduce curtaining artefacts
  - FFT Filter



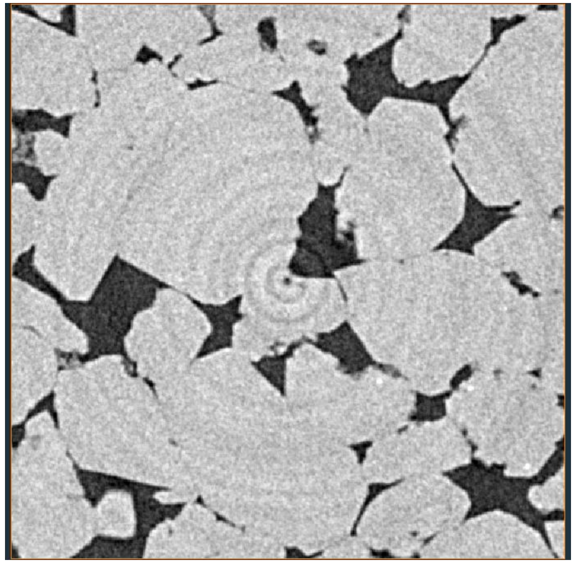
- Denoise
  - Filter Sandbox: e.g. NLM, median, bilateral



Exercise solution: https://youtu.be/HKh4rCr_bIg

See also

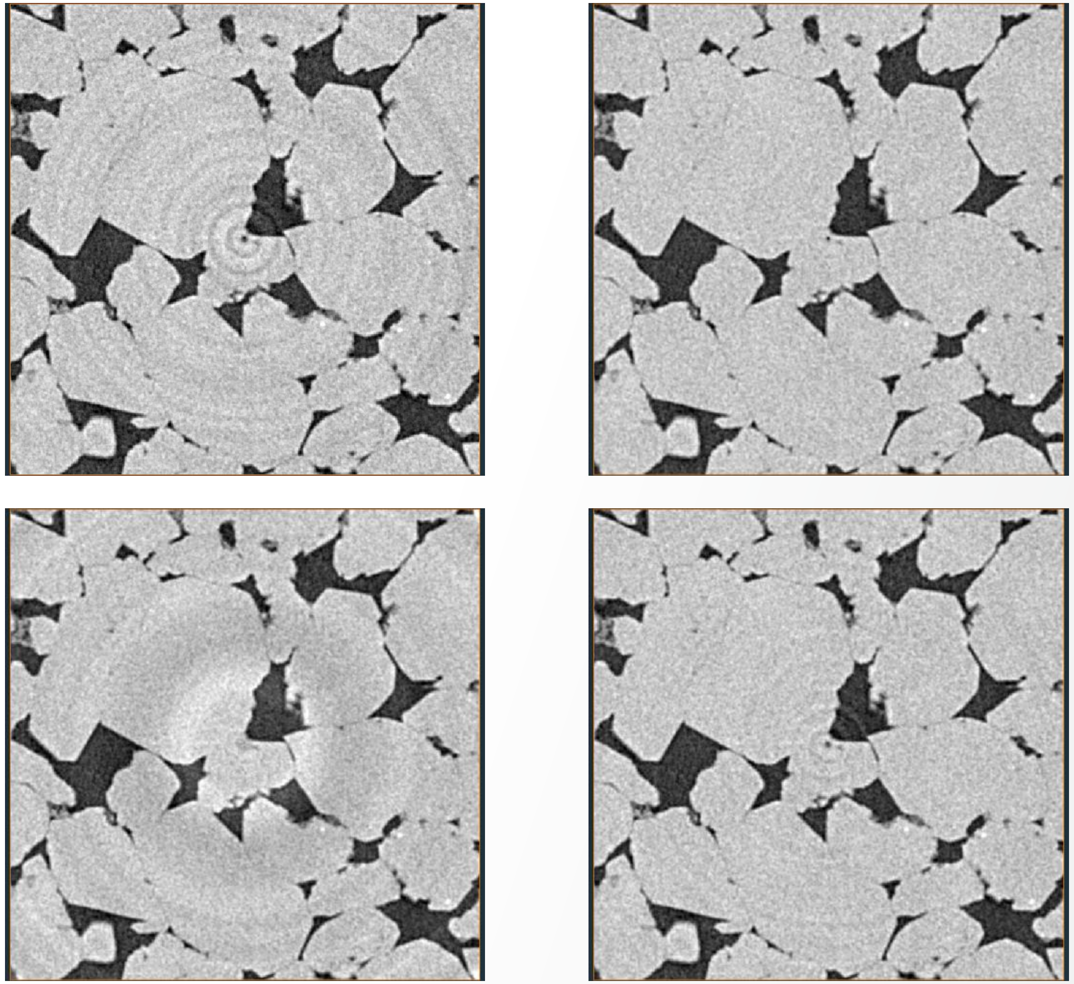
Ring Artefact Removal



- Adjusts the mean intensity of the pixels of concentric rings to the mean intensity of the whole image
- The rotation axis is assumed to be aligned to the Z-axis of the data-set's local coordinate system
- The center of the rings needs to be adjusted manually if:
  the center of the image ≠ the center of rotation during CT acquisition
- Using the settings "Lower Threshold" and "Upper Threshold", the calculation of the mean values can be restricted to a certain intensity interval. This might be necessary:
  - For objects with inhomogeneous density (large pores, multi-material, etc.)
  - For a geometry deviating from a cylinder
  - If a cylindrically shaped object was measured de-centered.
- The input data-type must be 16-bit unsigned.
  Use "Convert Image Type" if necessary.

See also

Ring Artefact Removal examples:



original

best correction

no intensity range adjustment

slightly wrong detection axis

Image pre-processing: deconvolution

Image deconvolution

Iterative Maximum Likelihood Image restoration algorithm. Types of **Deconvolution**:

- Non-blind (a measured or computed PSF – *Point Spread Function* – is used)
- Blind (the PSF is estimated along with the data)

Theoretical PSF generation via **Generate Point Spread Function** module (Project View → Create Object)
PSF estimation via **Extract Point Spread Function** module (bead extraction).

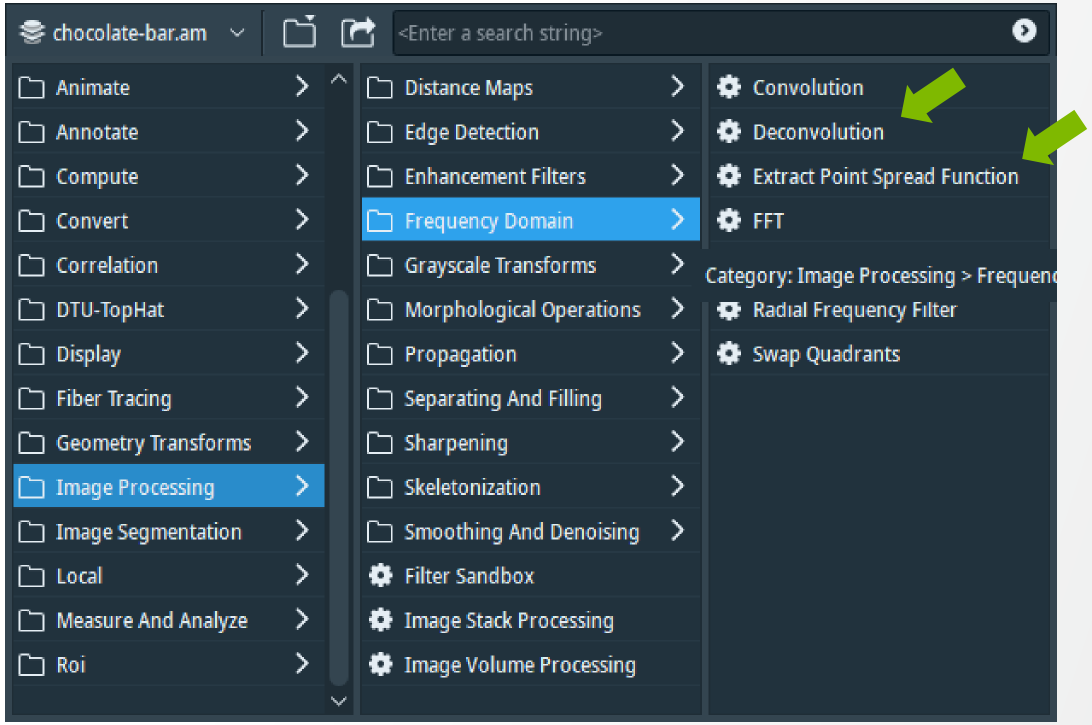
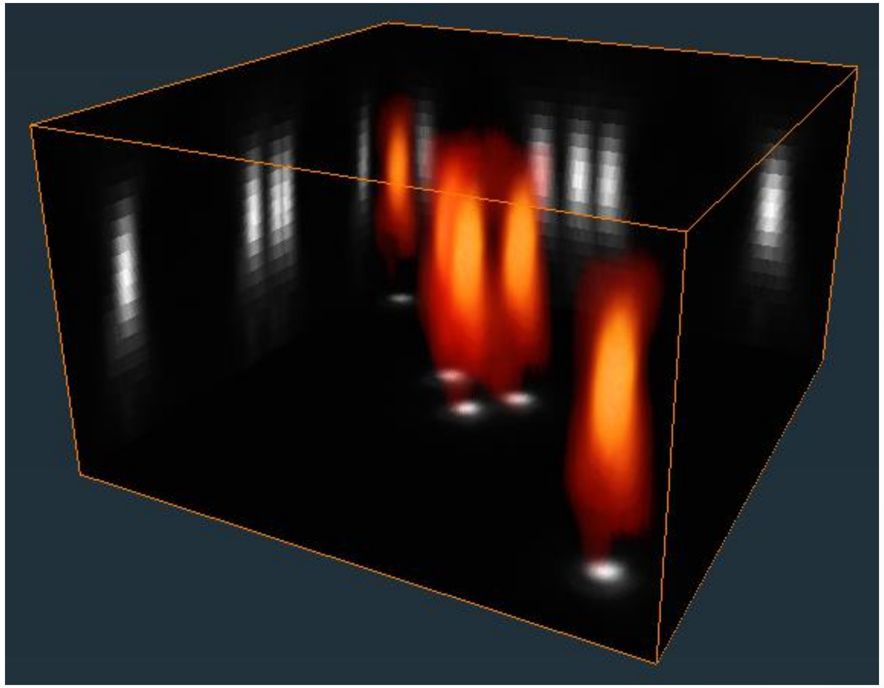


Image deconvolution: measuring the PSF

Imaging beads with desired acquisition settings (dataset available at …data/deconv/beads.am)
Beads image visualization: **Image Ortho Projections** + **Volren**
PSF Estimation:

- **Projection Cursor** (for creating Landmarks)
- **Extract Point Spread Function** (Adjust centers + Estimate size)

Data before deconvolution



Data after estimating the PSF and applying standard deconvolution

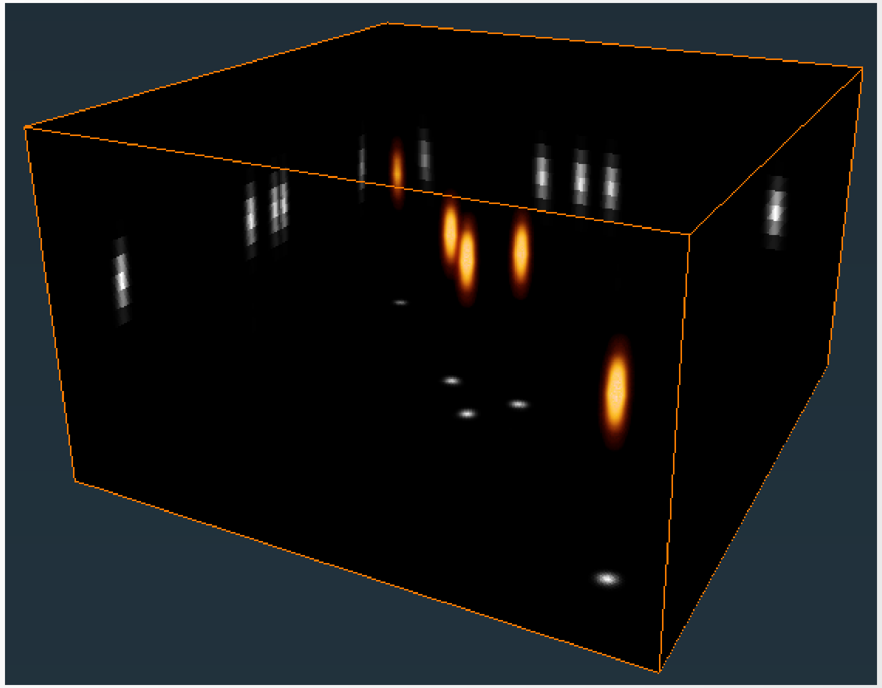
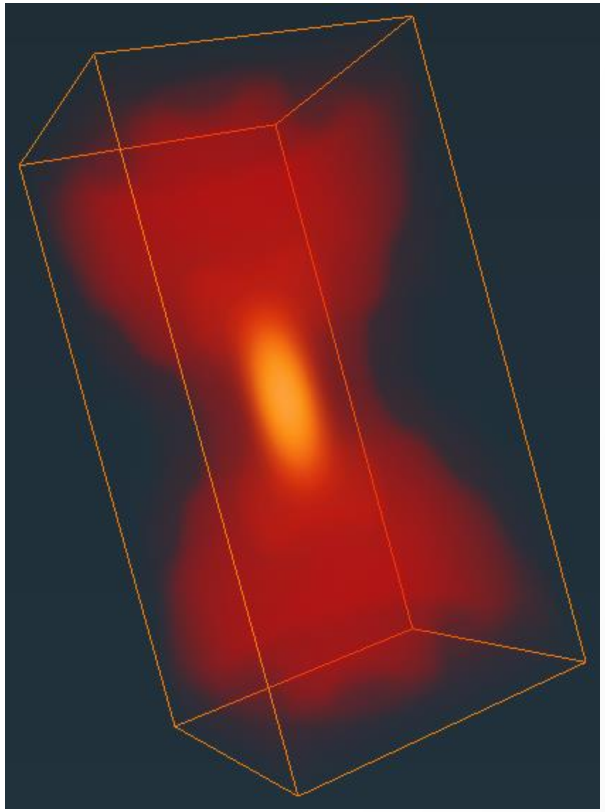


Image deconvolution: implemented mathematical PSF models

For theoretical PSF computation: **Generate Point Spread Function** module

- Choose type of microscopy: widefield or confocal
- Set microscope parameters: numerical aperture, wavelength, refractive index

Widefield



Confocal

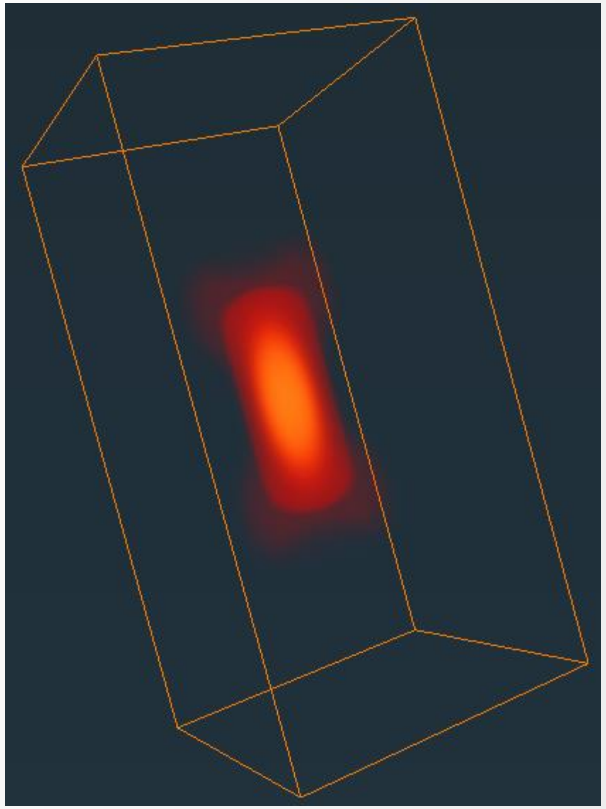


Image deconvolution: Blind method

Simultaneous data restoration and PSF estimation

- Can be initialized with a theoretical or measures PSF (that will only be used for the first iteration of the algorithm)

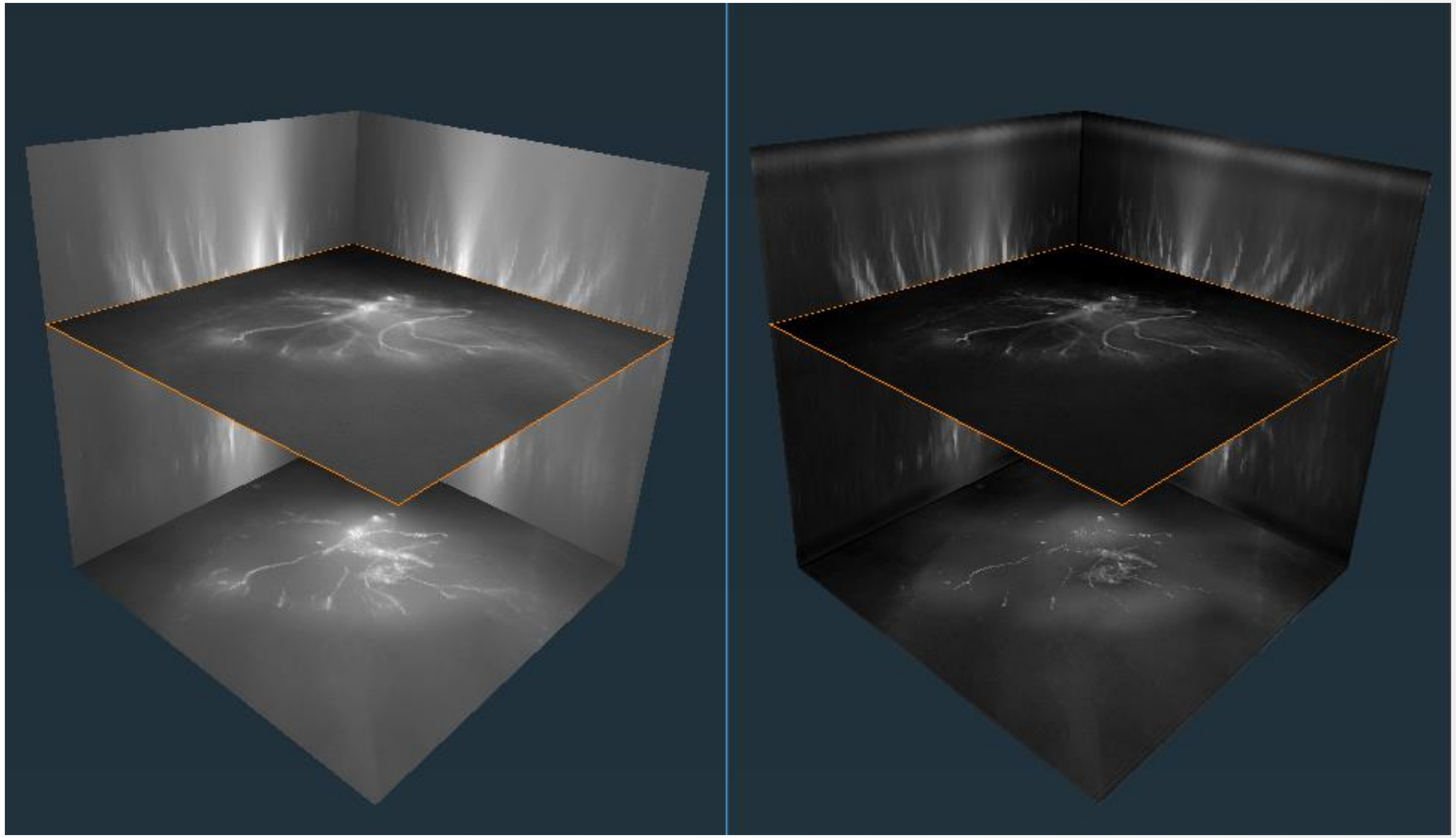
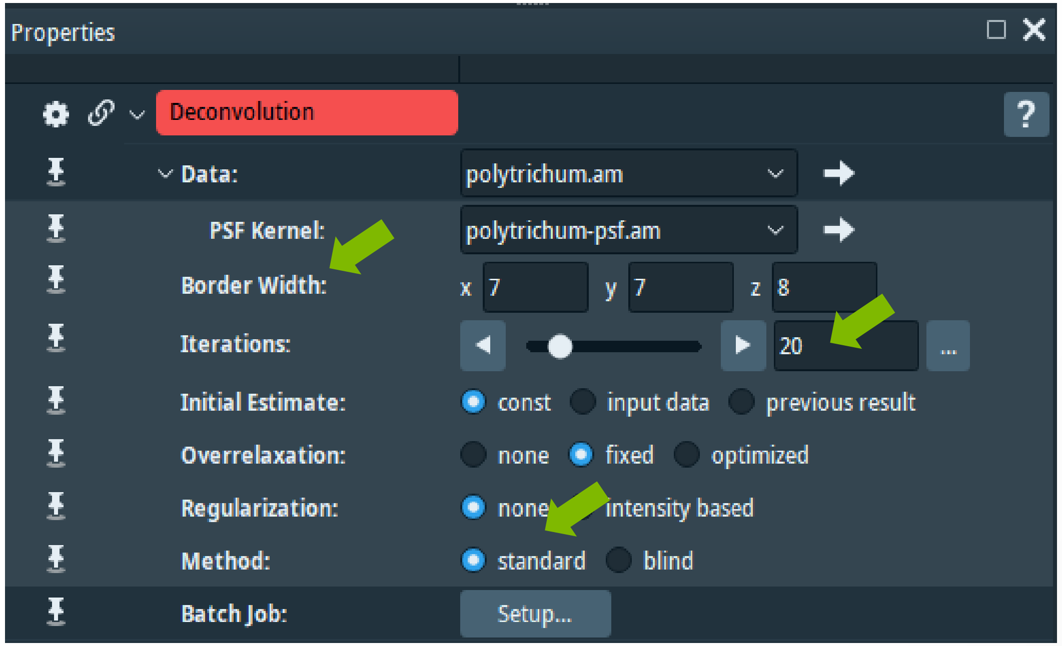


Image deconvolution: Standard method

Standard deconvolution example (dataset available at: data/deconv/polytrichum.am & polytrichum-psf.am):

- Resample PSF (optional)
- Apply Deconvolution module in standard mode



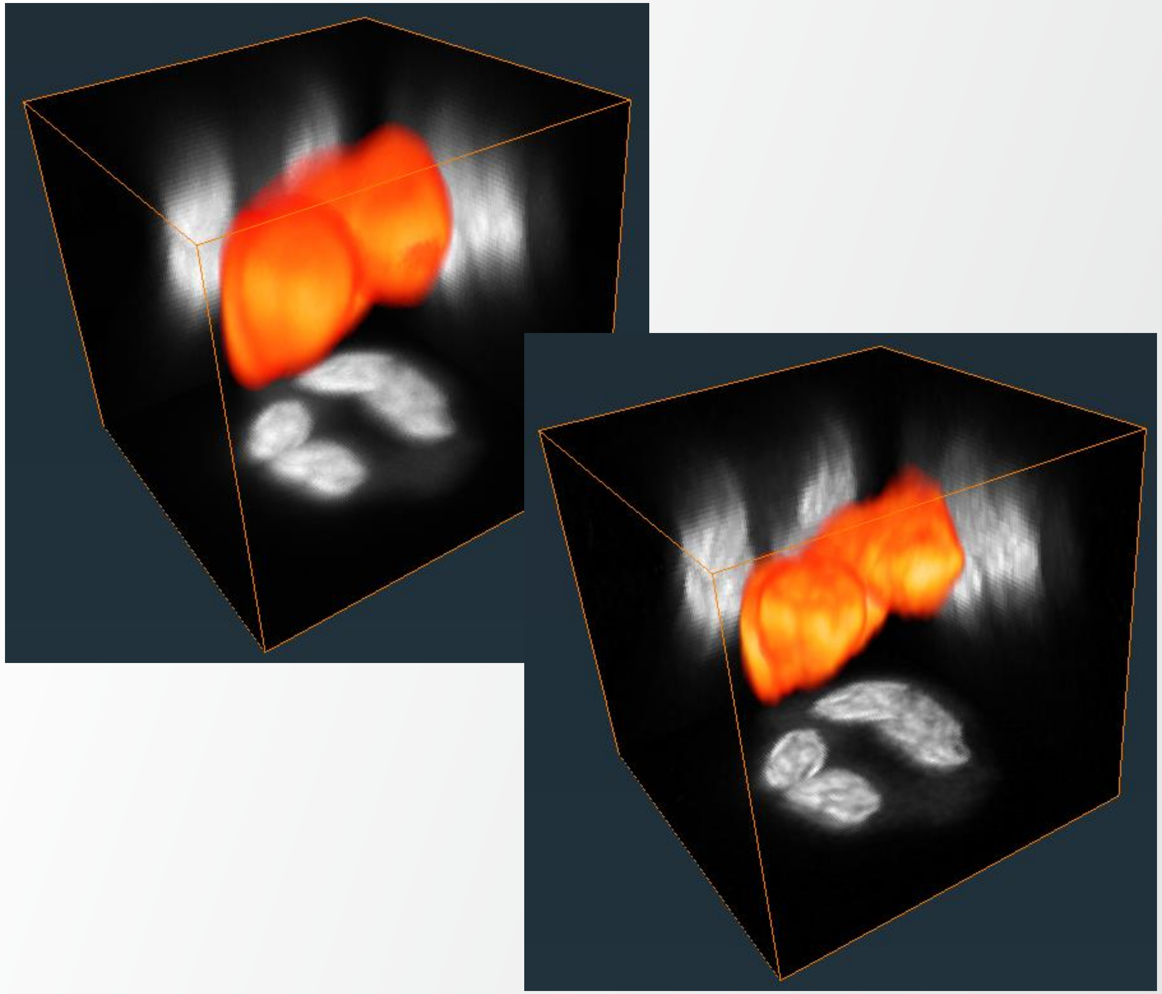


Image segmentation: Segmentation Editor

Segmentation Editor: workroom

Dedicated workroom for interactive segmentation

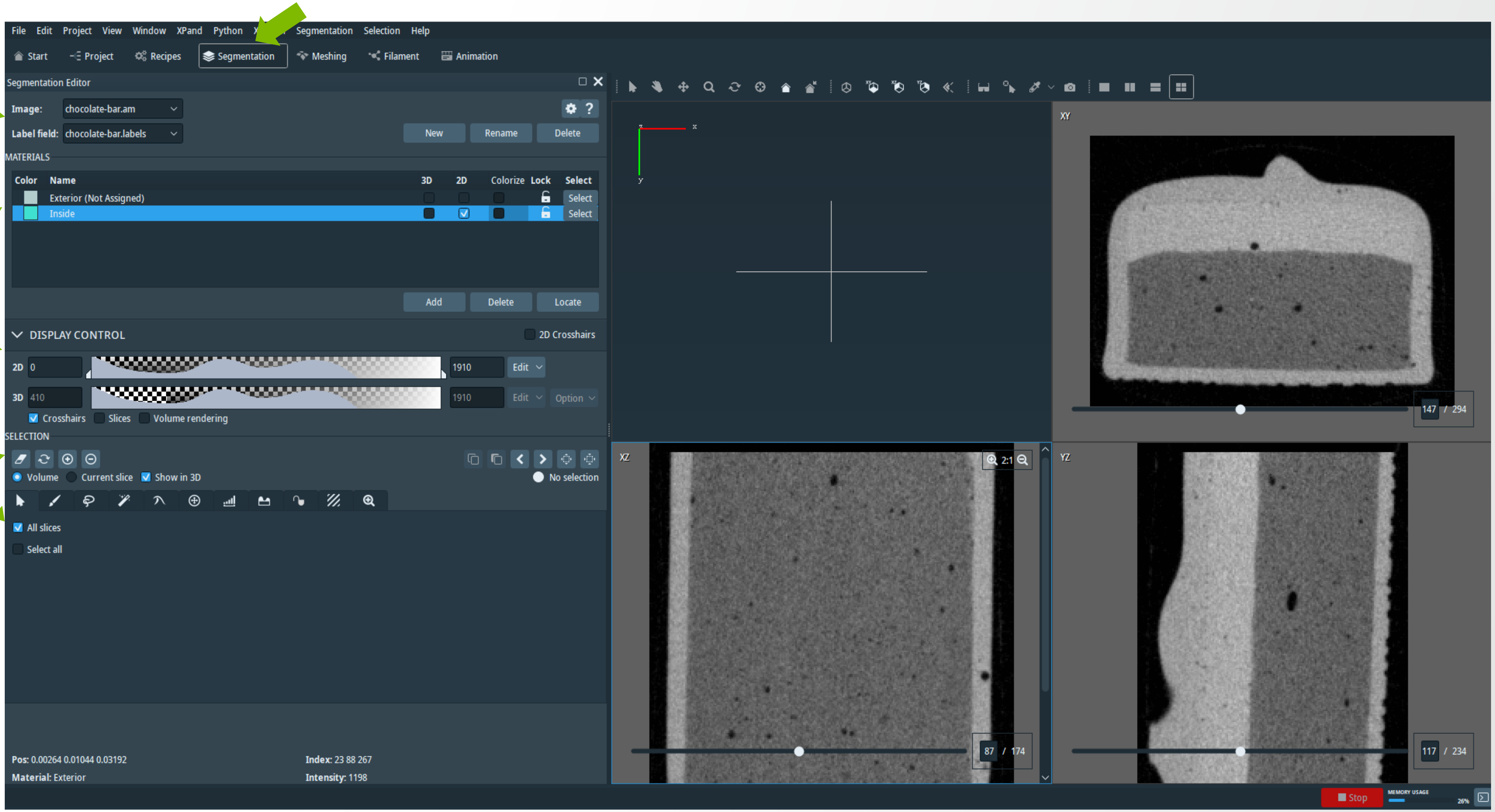
Image and Label Field

List of materials (labels)

Configuration of display

Selection manipulation

Tools for generating selections



Segmentation Editor: workroom

Dedicated workroom for interactive segmentation

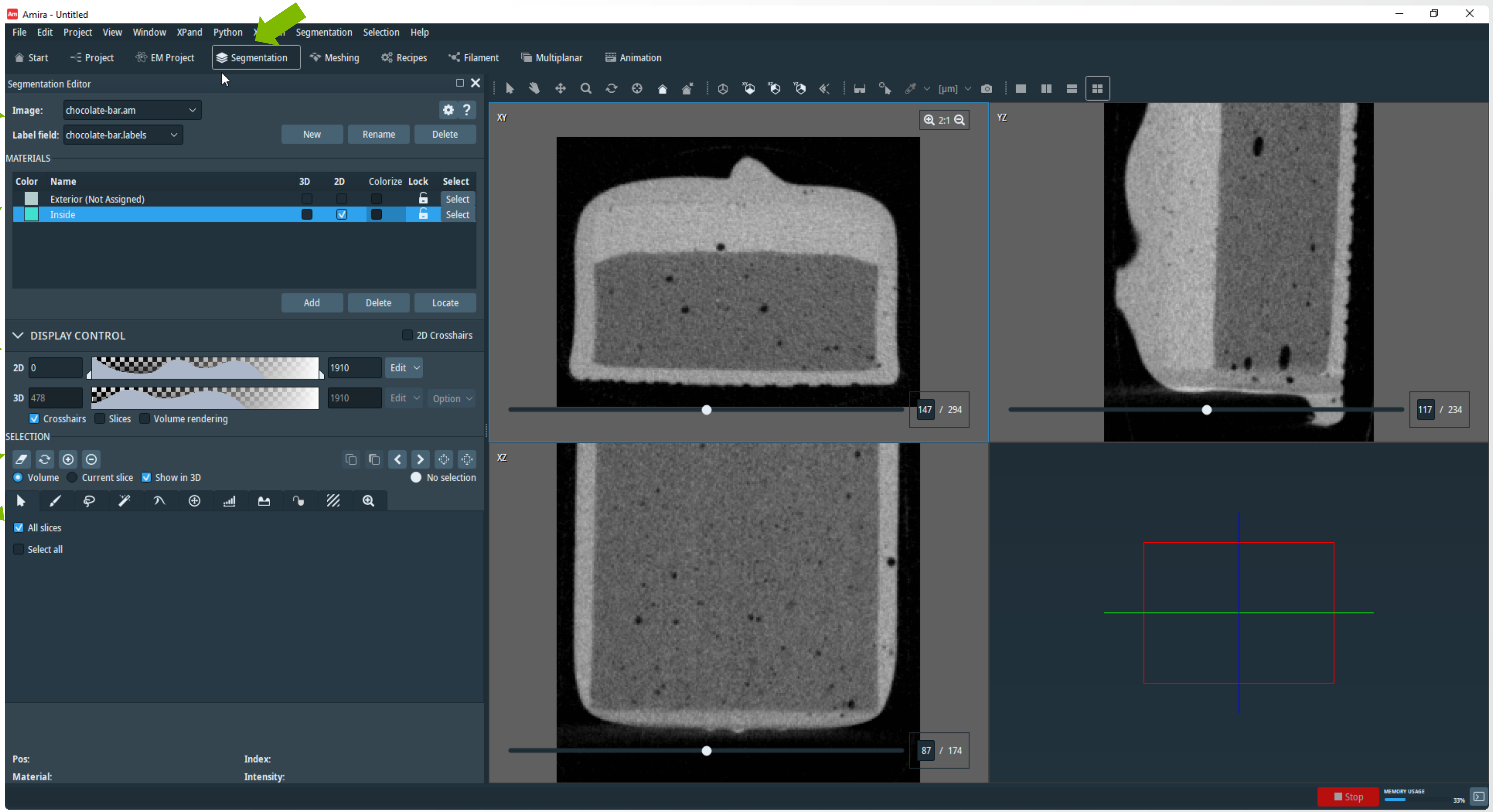
Image and Label Field

List of materials (labels)

Configuration of display

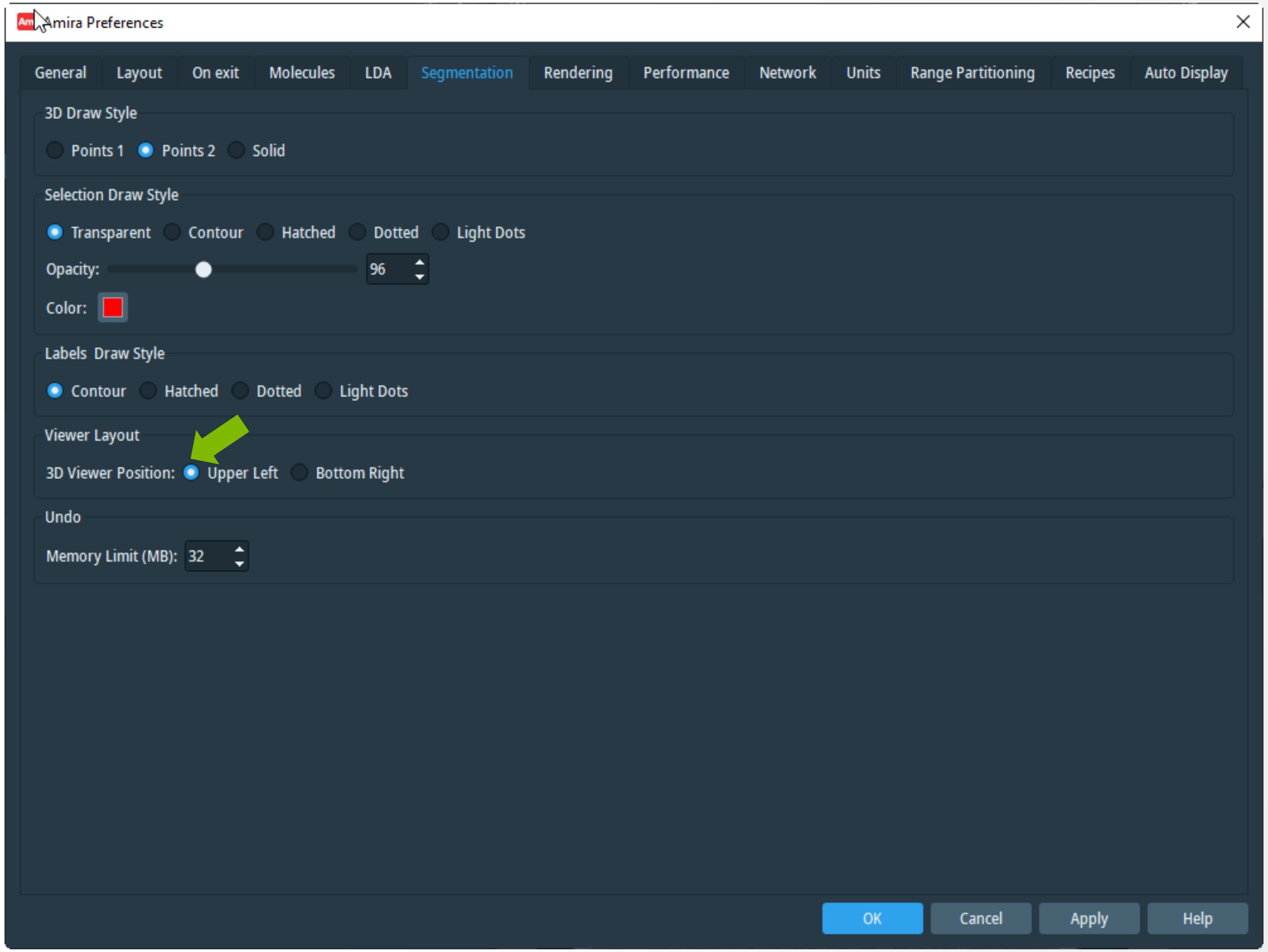
Selection manipulation

Tools for generating selections



Segmentation Editor: 3D Viewer Position

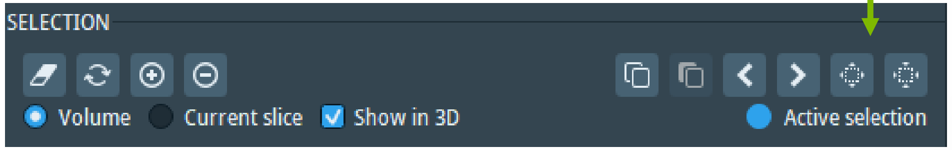
The default **3D Viewer Position** in Amira is “Bottom Right”. You can switch it to “Upper Left” from: Edit -> Preferences ->Segmentation

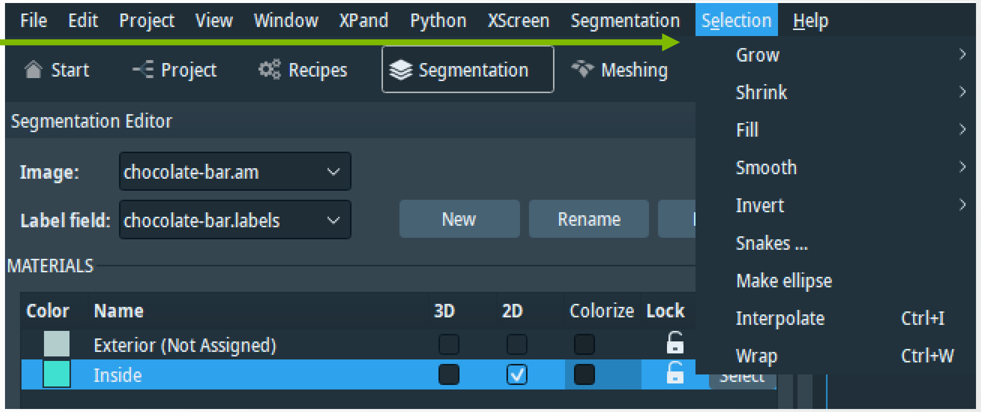


Segmentation Editor: general principle

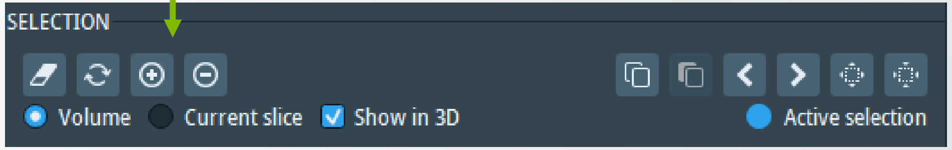
- **Generate selection** using the tools available in the selection generation toolbar
  e.g. Brush, Lasso, Magic Wound (region growing), Threshold, Blow

- **Modify selection** using the tools available in the:
  - Selection menu
  - With keys (shift/ctrl)
  - Selection modification toolbar





- **Assign selection** to material using the tools available in the selection assignment toolbar



Rules of thumb:

- Only one label per voxel
- Always keep the “Exterior” material

Segmentation Editor: selection tools

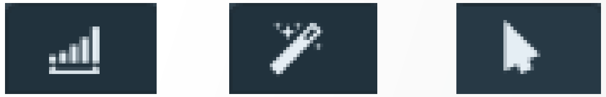
- **Brush: 2D painting**
  - Right click inside close contour: flood fill in 2D
  - CTRL: erase
- **Lasso: 2D & 3D closed contours**
  - Generate selections in 2D and 3D by defining closed contours
  - “Auto-trace” option: snap to gradient (in 2D)
- **Pick & Move: 2D & 3D**
  - Pick and move (translate/rotate) selection
  - Can be applied to all slices or current slice only
- **Threshold: 2D & 3D masking**
  - Select all voxels in intensity range
- **Magic Wand:**
  - Region growing within intensity range
  - CTRL: add new seeds
  - Draw 2D barriers (“**Draw limit line**” option)
- **Blow:**
  - Blow a 2D balloon that stick to edges

Segmentation Editor: selection tools

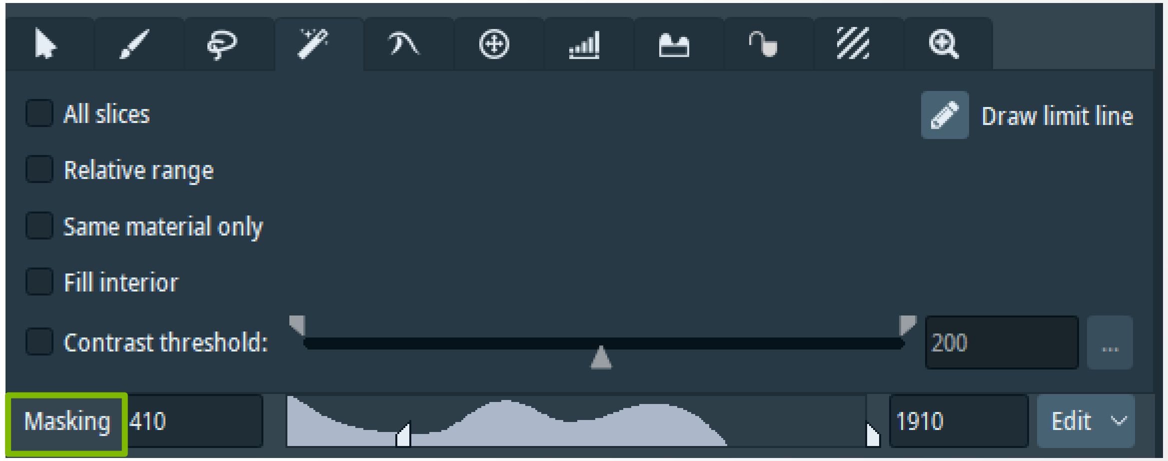
Tips:

- "**Same Material Only**" option available for: Brush and Magic Wand tools

- "**All Slices**" mode available for: Threshold, Magic Wand and Pick & Move tools



- "**Masking**" can be enabled: selection only within specified intensity range



Segmentation Editor: selection modification

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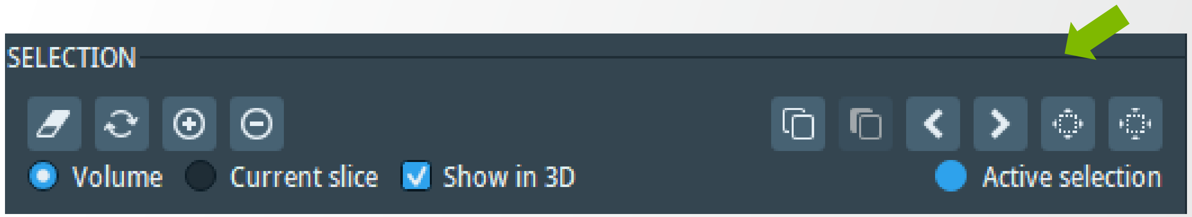
Selection modification

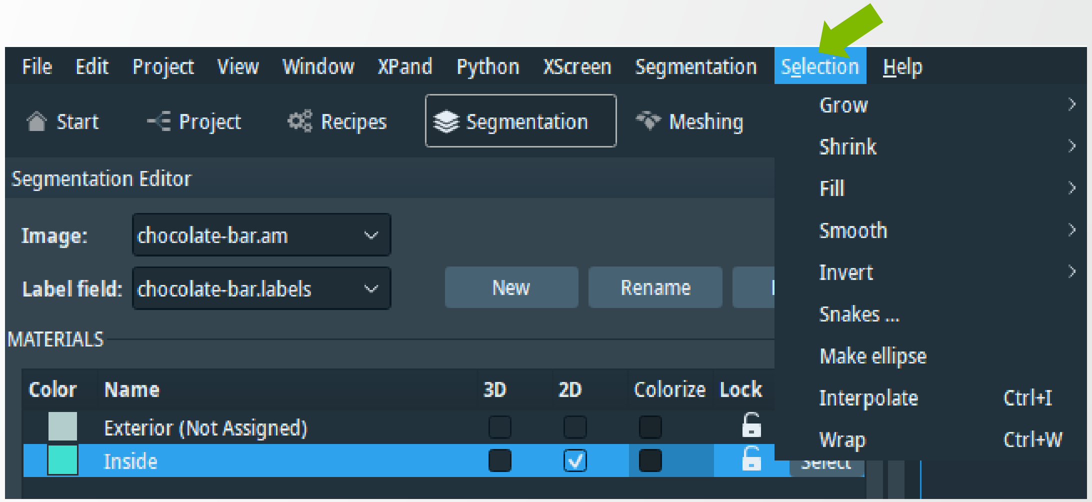
- **Grow / Shrink** (2D or 3D)
- **Fill Holes** (2D or 3D)
- **Smooth** (2D only)
- **Invert** Selection

- **Snake**: propagate a 2D selection to the next/previous slice, following grayscale intensities

- **Interpolate**: between selections from parallel slices
  - Shape-based interpolation

- **Wrap**: between selections from orthogonal slices
  - Shape-based RBF interpolation

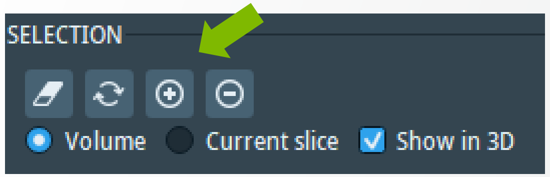


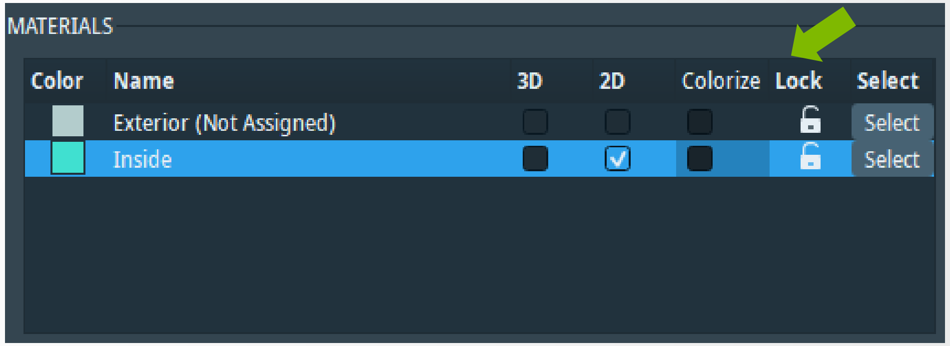


Segmentation Editor: materials assignment and modification tools

Materials assignment:

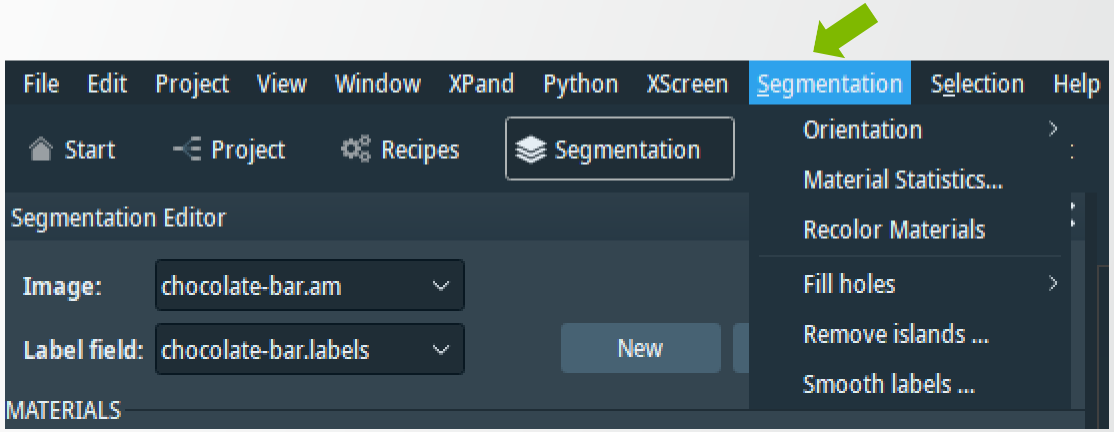
- Add selection to material (or replace / substract)
- Materials can be locked





Materials Menu:

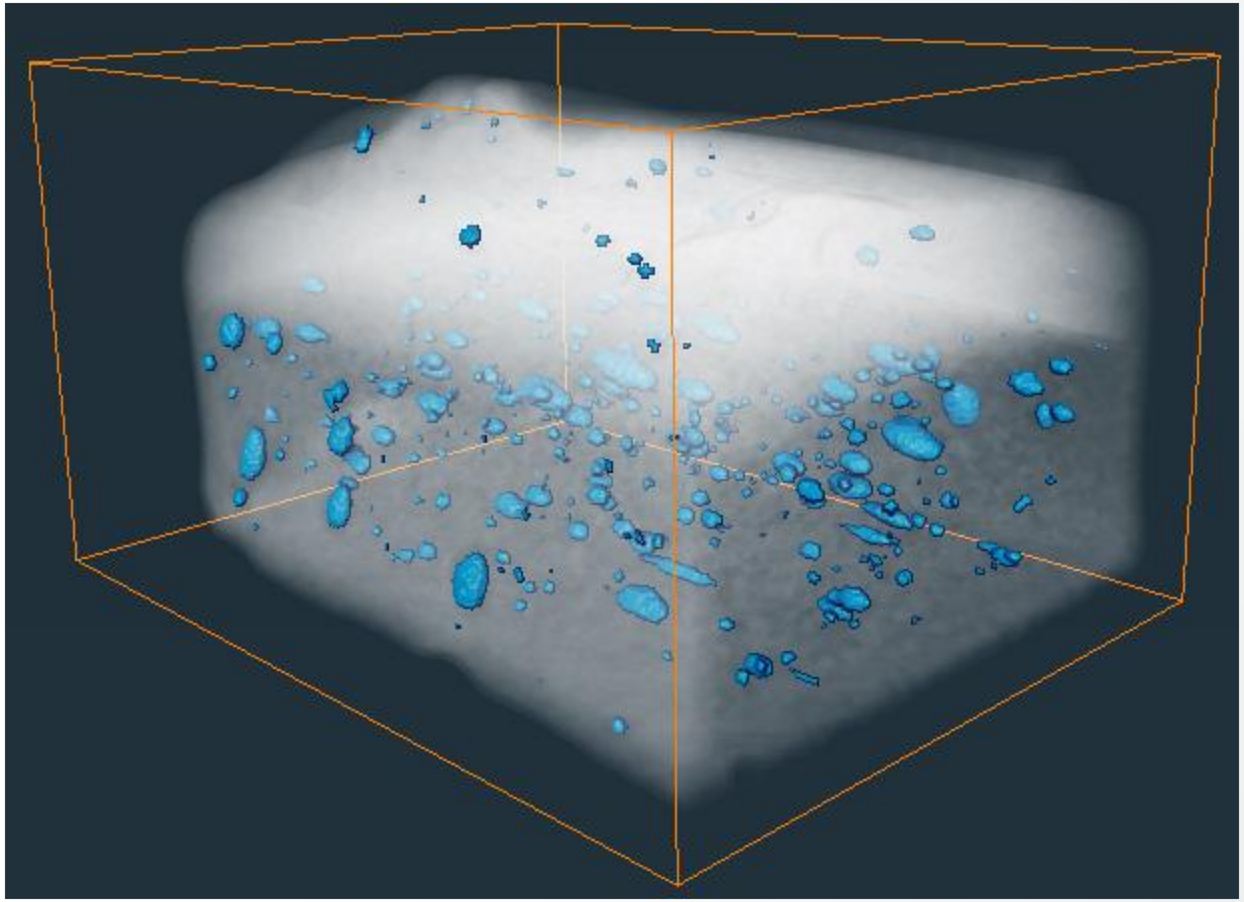
- Fill holes (slices only)
  - For 3D rather use Selection > Fill Holes
- Smooth Label:
  - 2D or 3D smoothing of the label map (shape only)
- Remove Islands:
  - Select small connected components
  - Relabel them according to the dominant neighboring label



Segmentation Editor: exercise 1

Bubble segmentation

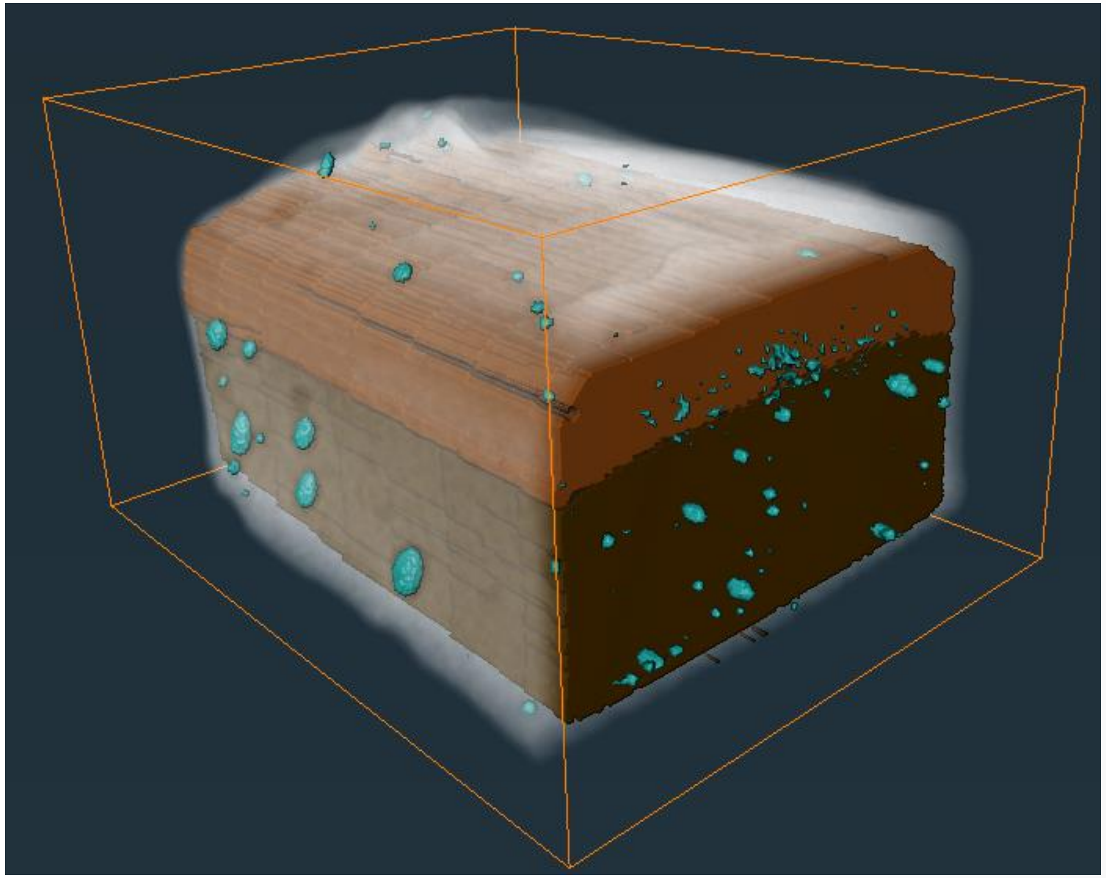
Use “**Thresholding**” and “**Fill Selection**” to segment chocolate bar and bubbles:



Segmentation Editor: exercise 2

Caramel and biscuit segmentation

- Use “**Brush**” or “**Blow**” tool and “**Interpolation**” to segment the chocolate mousse.
- Use “**Lasso**” tool and “**Interpolation**” to segment the caramel.

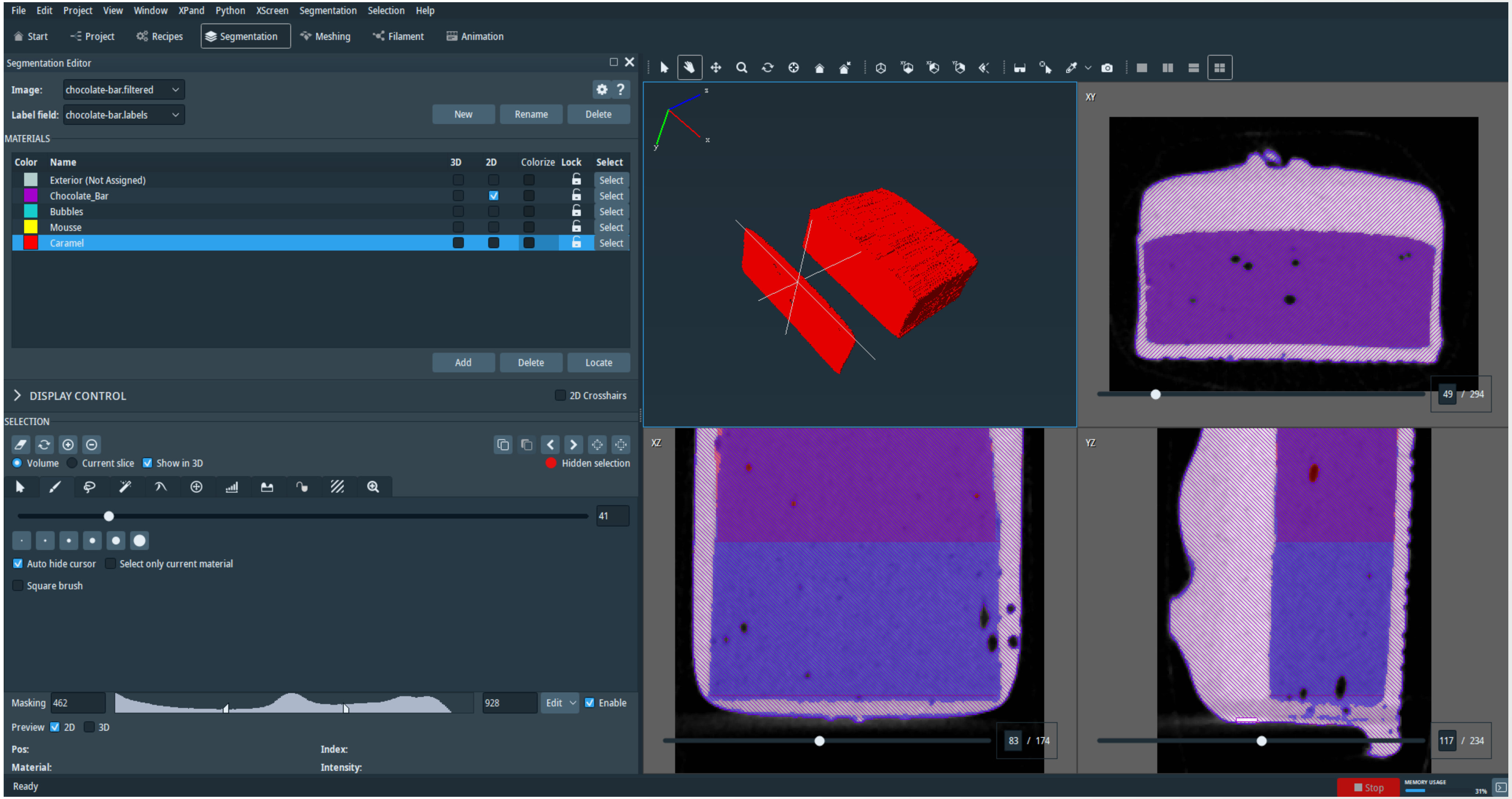


Tips:

- Segment the **denoised** image rather than the noisy one
- "**Blow**” tool and “**Lasso**” (Auto trace) are sensitive to the visualization range. Adjust the contrast accordingly.

Segmentation Editor: solution to exercises

Tutorial: https://youtu.be/lQsKXRr9Njs

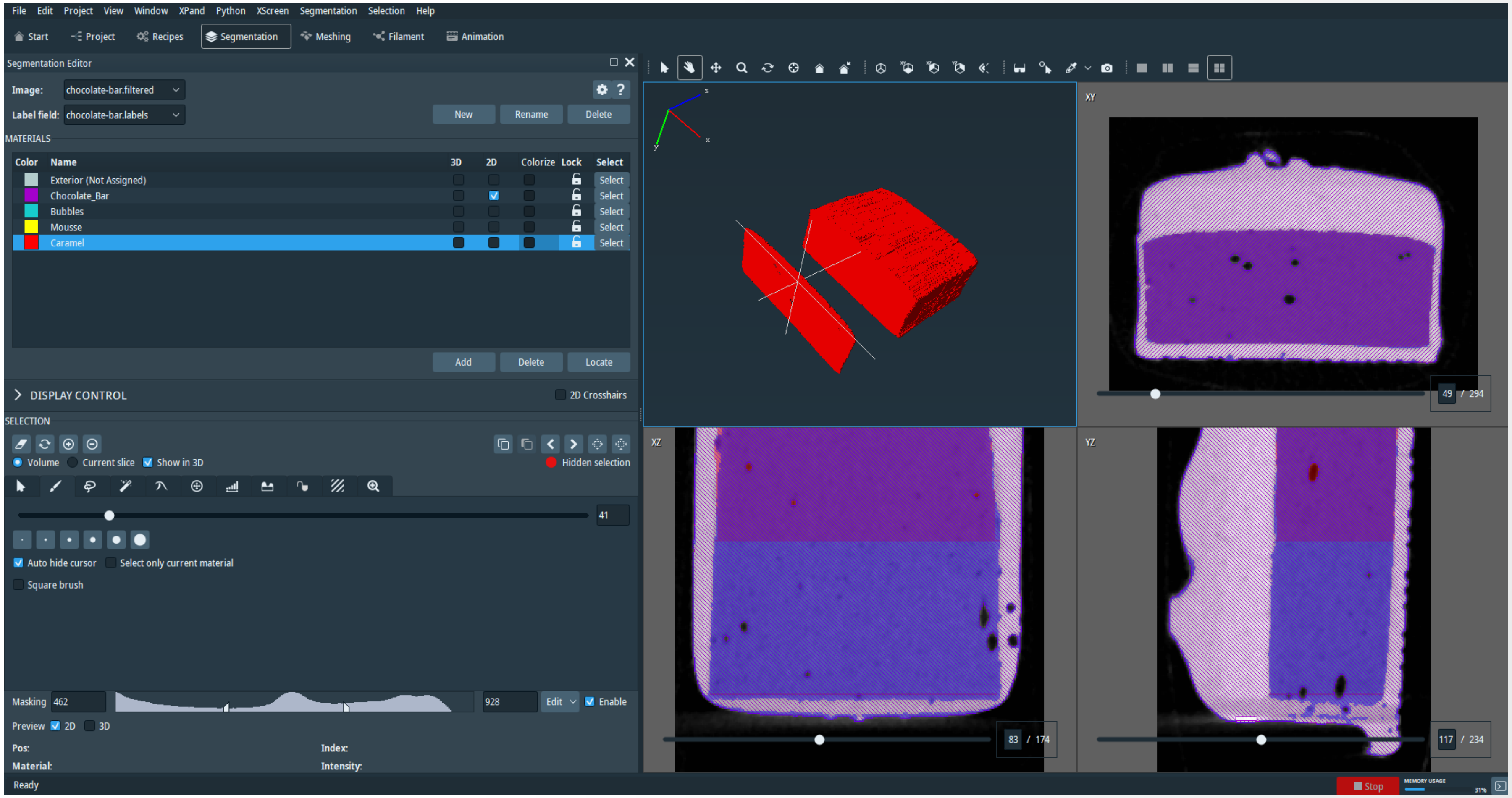


Segmentation Editor: solution to exercises

Tutorial: https://youtu.be/lQsKXRr9Njs

For setting the Viewer position as in the illustration, go to Edit-> Preferences->Segmentation->

ViewerLayout-3D Viewer Position: Upper Left



Visualization of segmentation maps

Visualization of segmentation maps: main modules

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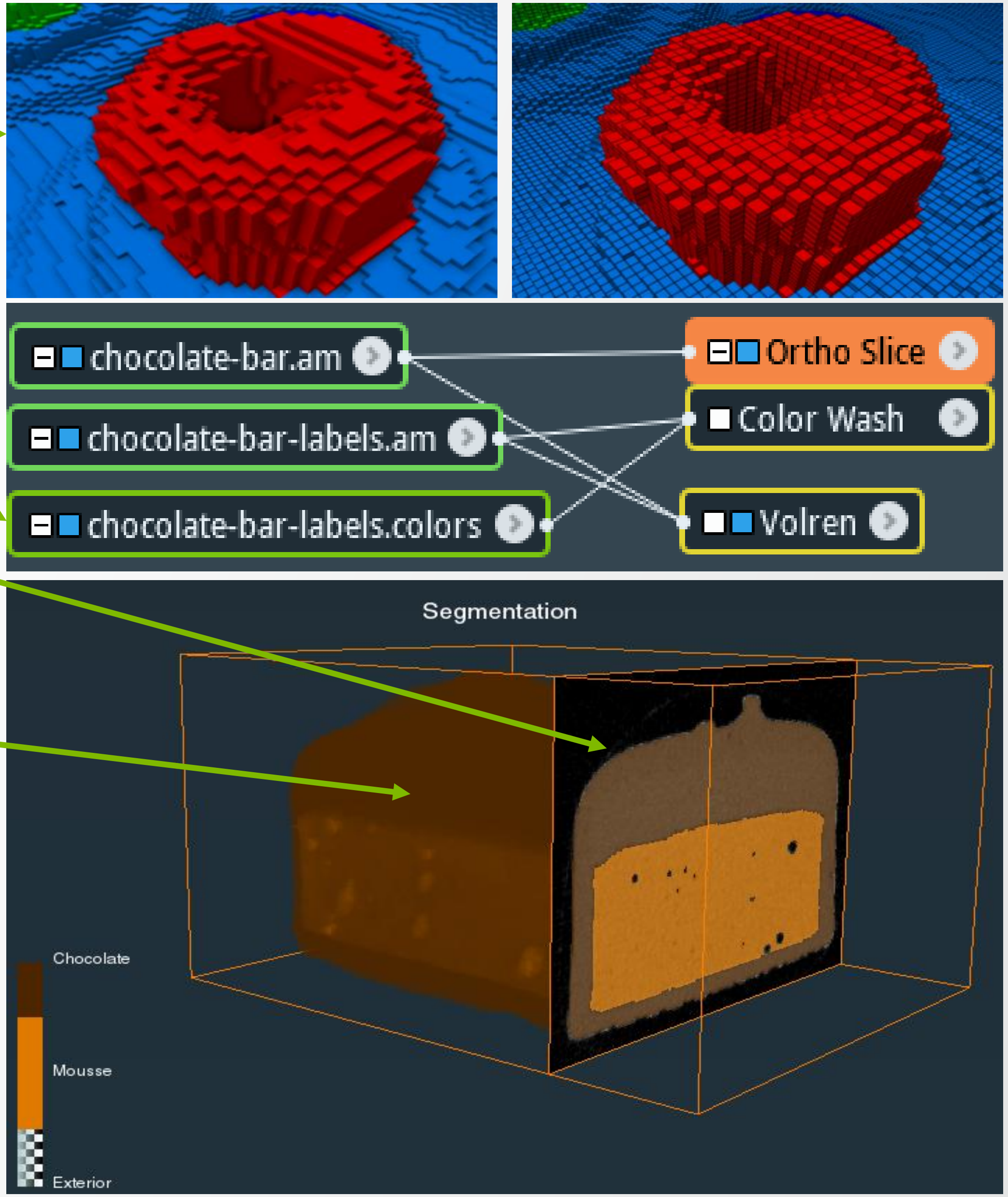
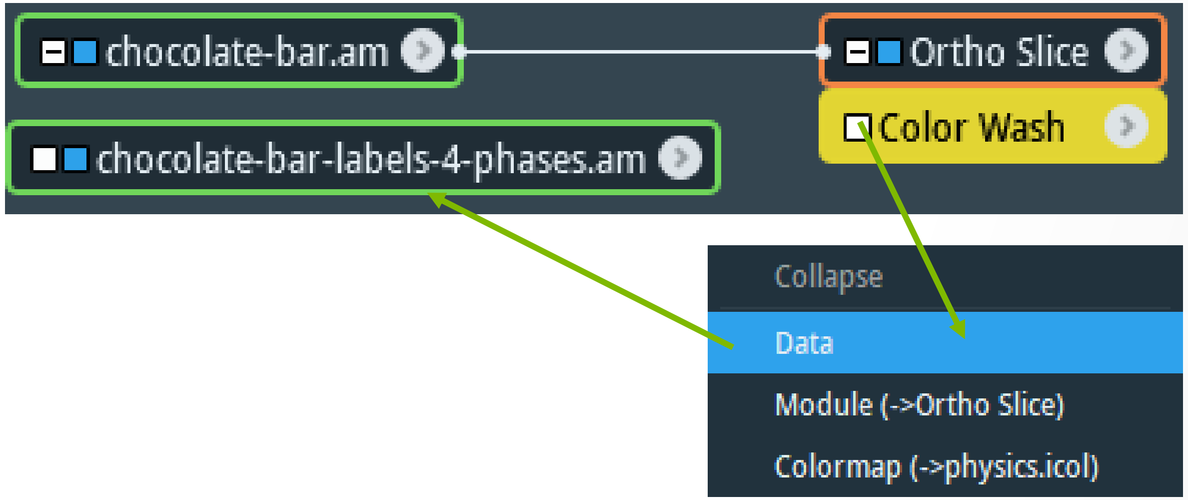
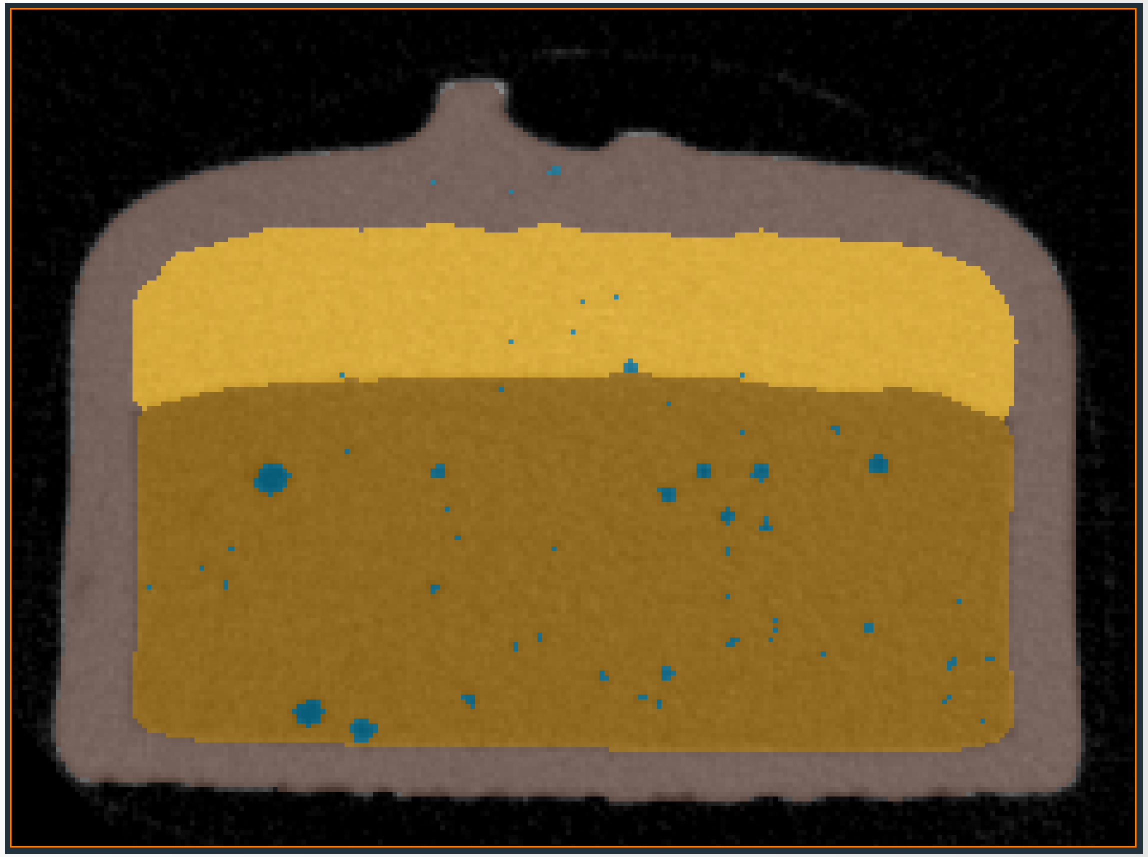
- Voxelized Rendering: displays the boundaries of voxels in a 3D volume,
- Create Label Colormap: Generates a label colormap with the colors defined in the Segmentation Editor
- Colorwash: details on next slide.
- Volren: displays 3d scalar fields volumes.
  - Connect a greyscale volume to ‘Data’ port, optionally a labeled image to port ‘Labels’
  - Tune Transfer functions and materials.



Visualization of segmentation maps: Color Wash

- Configure an Orthoslice to visualize the greyscale image
- Attach the Colorwash to Orthoslice
- Connect a label image to the port 'Data' of Colorwash
  - Adjust label transparency.





- Colorwash can also be connected to a grayscale image, various fusion rules are available

Visualization of segmentation maps: side by side viewers

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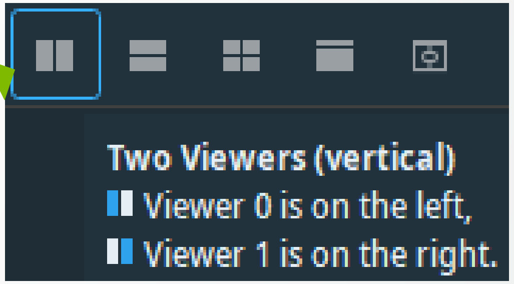
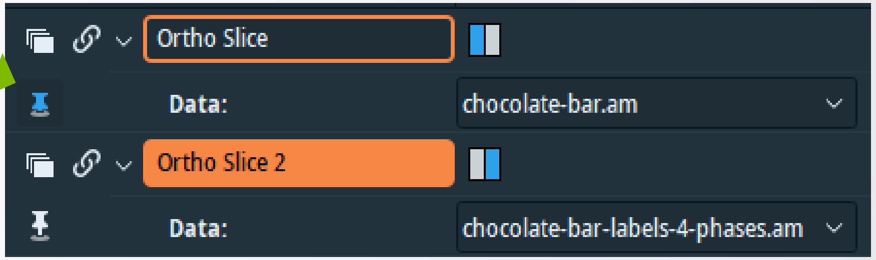
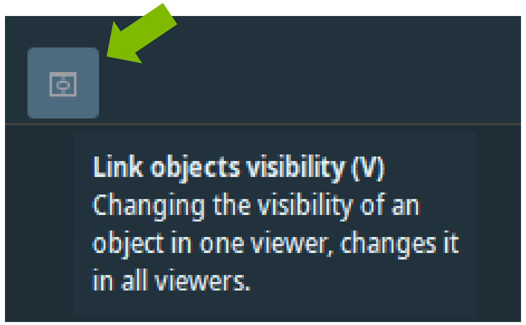
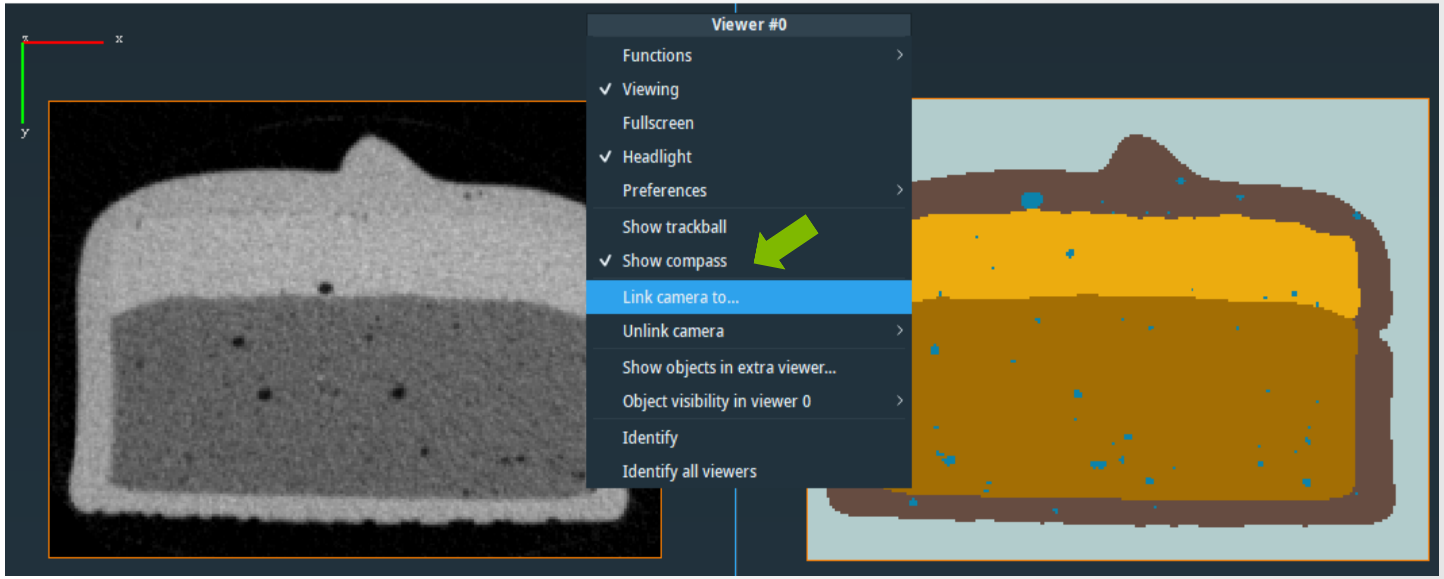
- Attach Ortho Slices on 2 datasets
- Set 2 viewers and visibility of Ortho Slices on each viewer
  Tip: pin "Data" port of one Ortho Slice to have it visible on both Ortho Slices
- Link cameras: right click in a viewer, link camera to... left click on 2nd viewer





Tip: For independent viewers, make sure to switch off "Link object visibility"

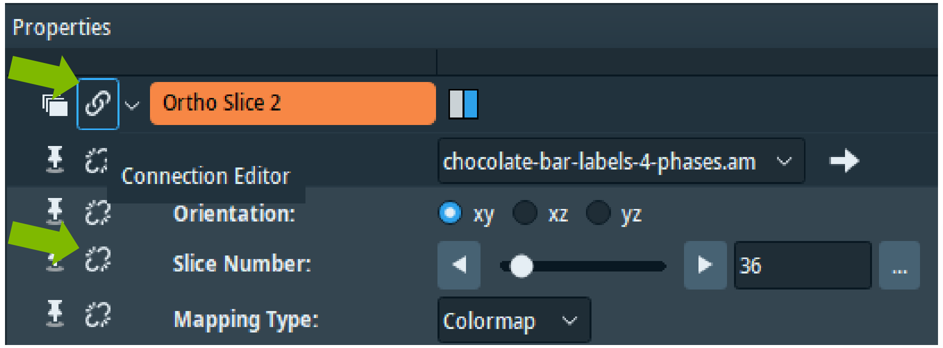


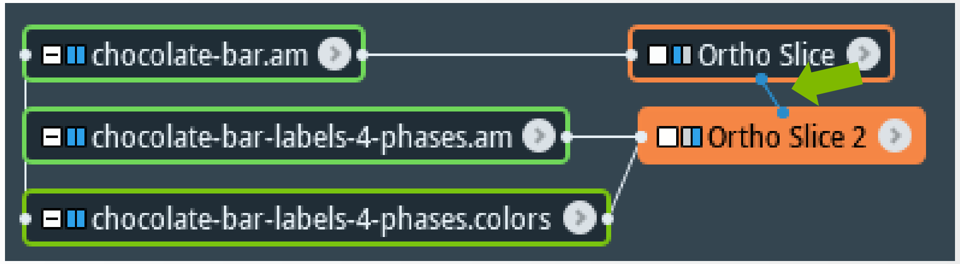


Visualization of segmentation maps: linking ports

Linking ports – e.g. “Slice Number” port:

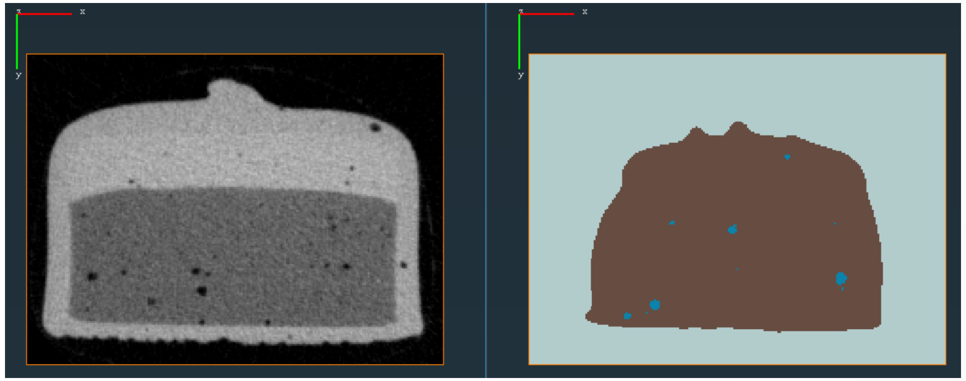
- Activate “Connection Editor” for “Ortho Slice 2”
- Click on the connection icon next to the port and drag over the “Ortho Slice” in the Project View





- Slice number ports are now linked, changing one will change the other simultaneously.

Before linking pots



After linking pots

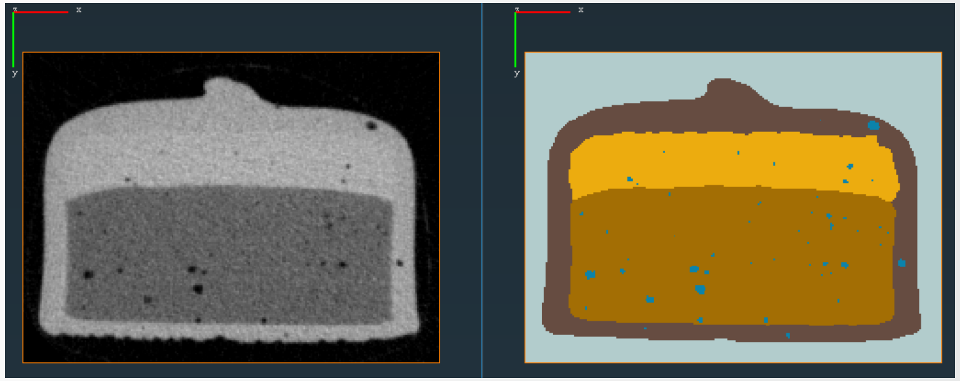
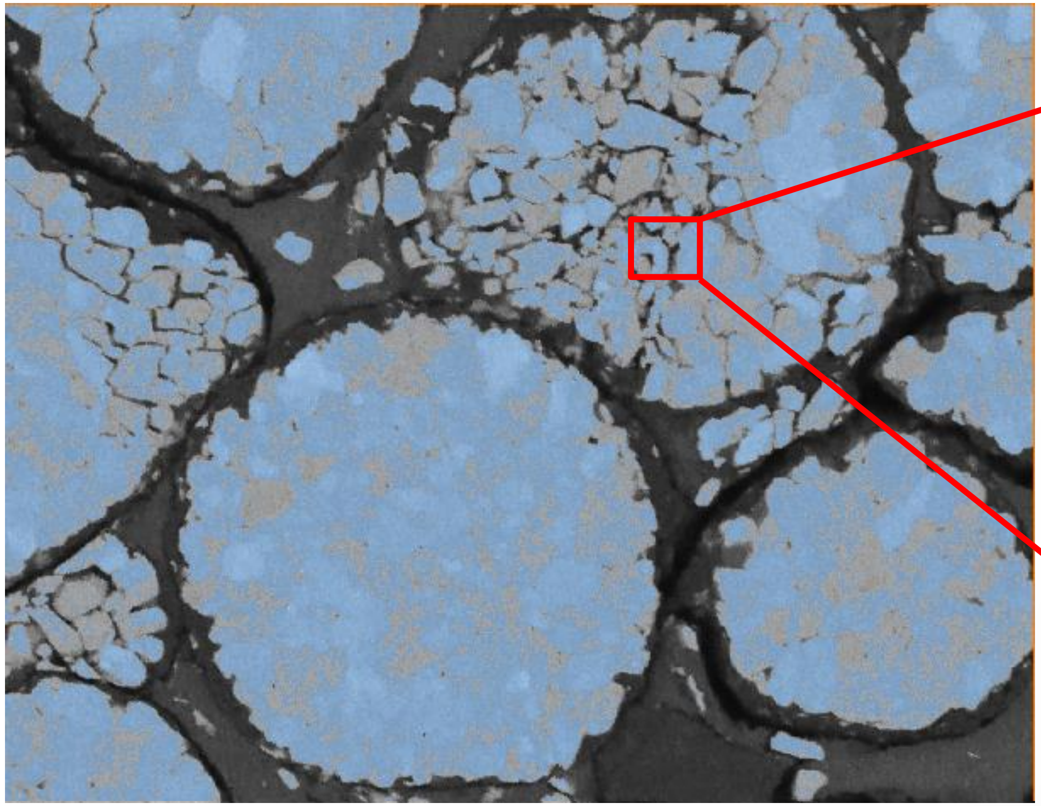
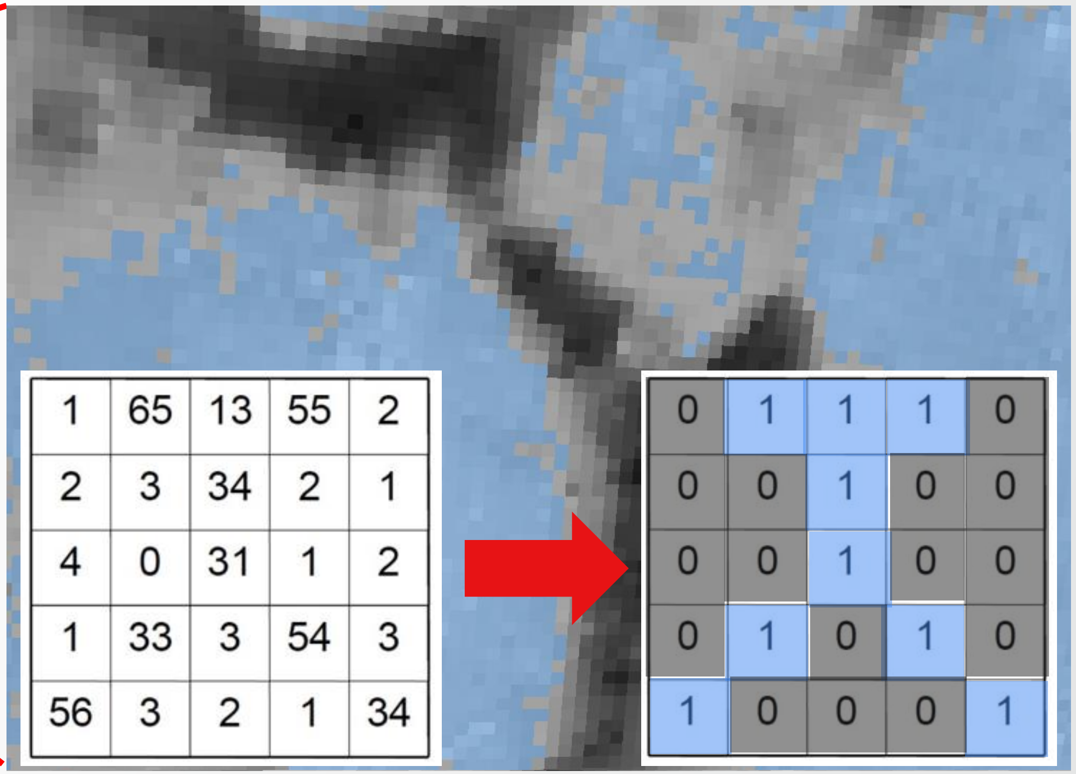


Image segmentation: general principles

Image Segmentation Principles

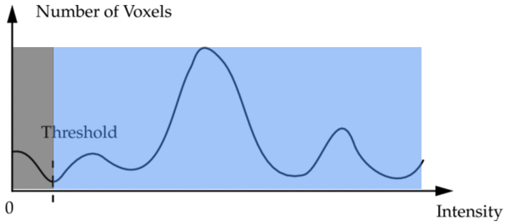
Thresholding: Binarization of graysale image into Label Image





1	65	13	55	2
2	3	34	2	1
4	0	31	1	2
1	33	3	54	3
56	3	2	1	34

0	1	1	1	0
0	0	1	0	0
0	0	1	0	0
0	1	0	1	0
1	0	0	0	1



8-bit image (2^8) intensity values from 0 to 255

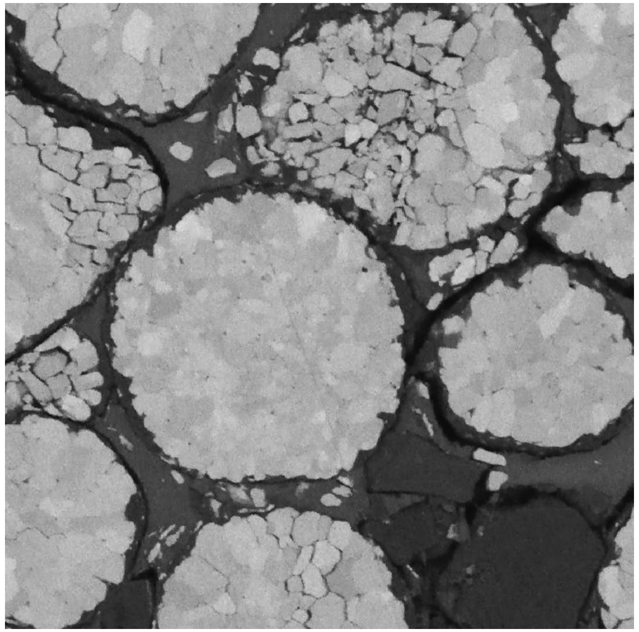
16-bit image (2^{16}) intensity values from 0 to 65,535

32-bit image (2^{32}) intensity values from 0 to 2,147,483,647

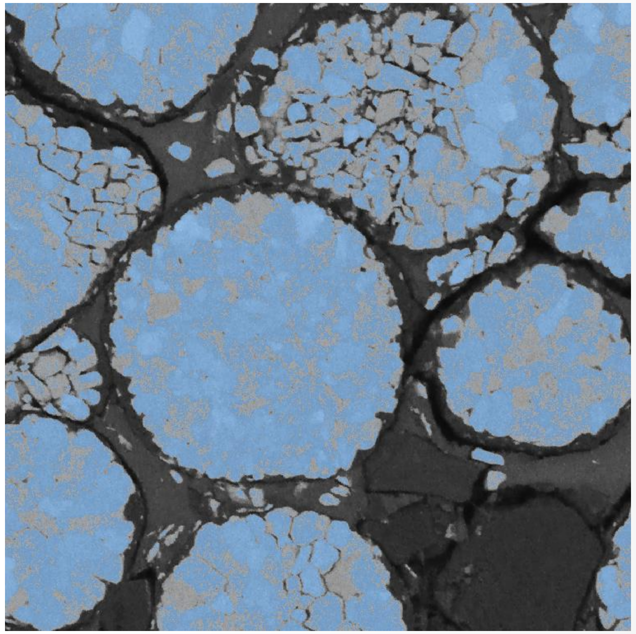
Binary Image (2 possible) values 0 or 1

Image Segmentation Principles

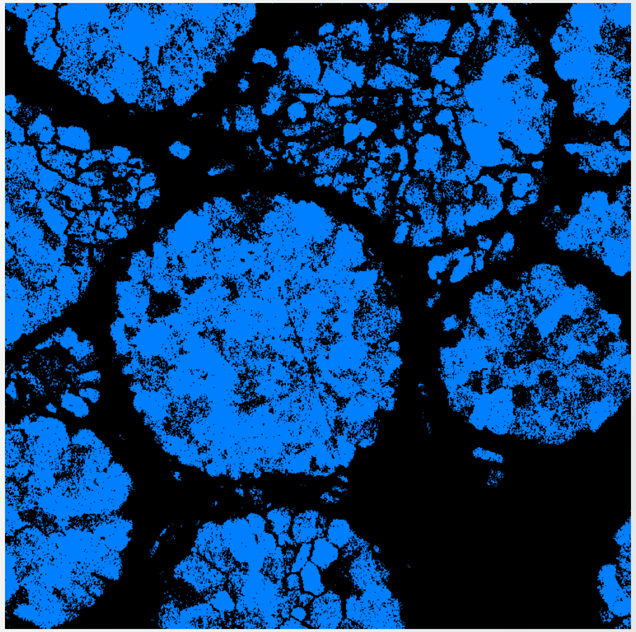
Thresholding: Binarization of graysale image into Label Image



Grayscale (intensity) Image



Thresholding (Binary mask)

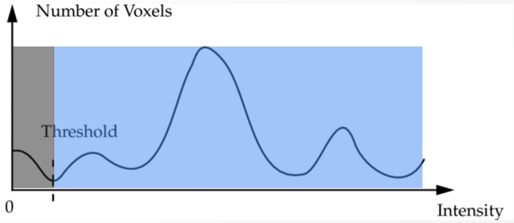


Binary (Label) Image

8-bit image (2^8) intensity values from 0 to 255

16-bit image (2^{16}) intensity values from 0 to 65,535

32-bit image (2^{32}) intensity values from 0 to 2,147,483,647



Binary Image (2 possible) values 0 or 1

Image Segmentation Principles

Binarization and Separation Methods

- **Thresholding**

  Global Thresholding, Local Thresholding, Multi-Thresholding, Auto-Thresholding

- **Watershed segmentation**

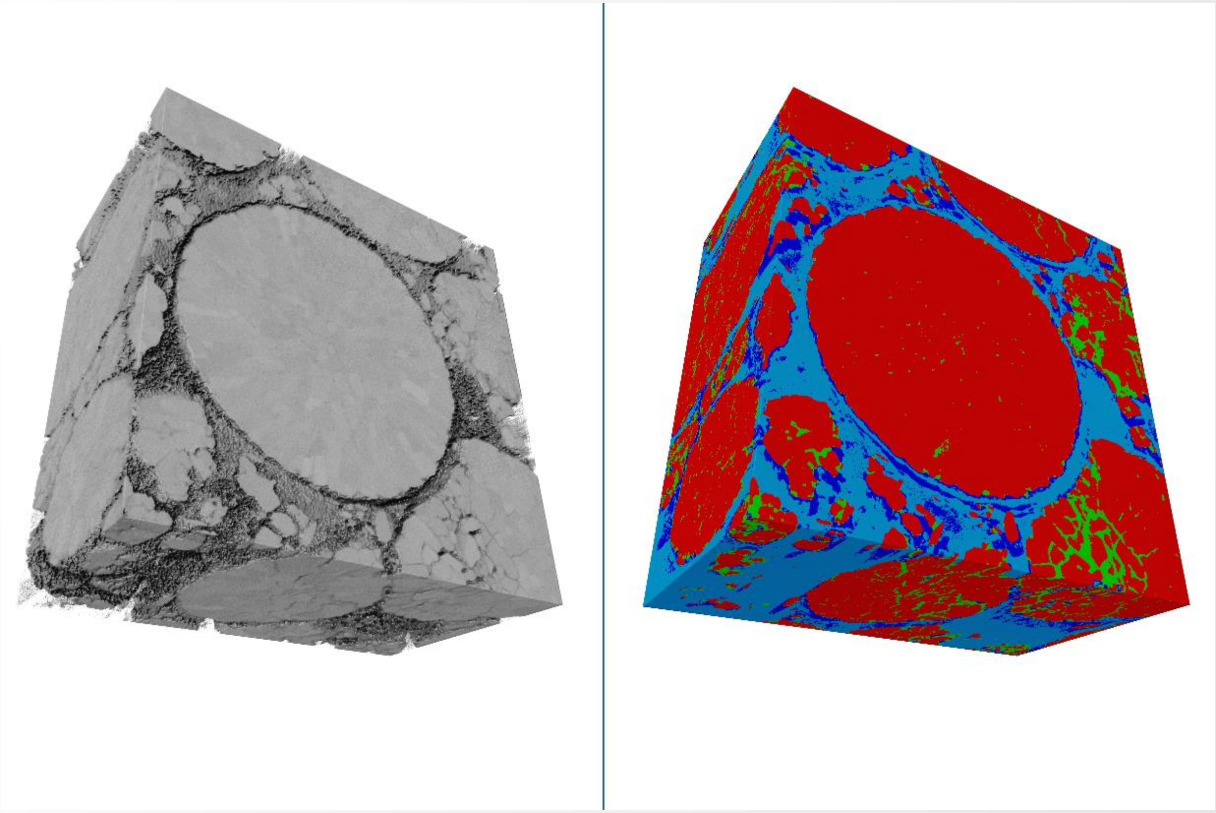
  Automatic edge detection
  Marker-based watershed with interactive / automatic markers

- **Mathematical morphology**

  Grayscale/binary morphology, variety of structuring elements
  Structure enhancement filter
  Membrane enhancement filter

- **Correlation-based Segmentation**

  Segmentation of regions that are co-localized in different channels of a multi-channel image



Data courtesy: Cell data: Mason Dean & Ronald Seidel (MPI Postdam-Golm), David Knoetel (Zuse Institute Berlin)
Brain data: Leslie Vosshall's Laboratory at HHMI-The Rockefeller University.
Membrane data: of Biophysical Fluorescence Laboratory Center For Bioimaging Sciences Departments of Biological Sciences & Chemistry National University Of Singapore

Segmentation in image processing workflows

Thermo Fisher SCIENTIFIC

Step 1. Optimise image acquisition:
- Lower noise
- Improve contrast
- Remove artefacts

Step 2. Image pre-processing:
- Noise reduction filters
- Background correction
- Deconvolution

Step 3. Segmentation:
- Thresholding
- Mathematical morphology
- Watershed

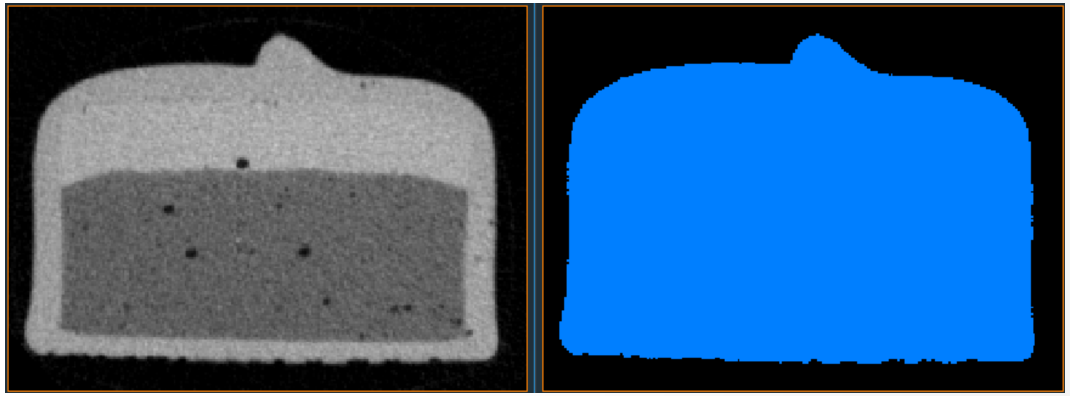
Step 4. Post-processing:
- Separate objects
- Clean segmentation maps

Image segmentation: concepts

Thresholding:

- Binarization: separating the dataset pixels/voxels into object and background.

Input data   Binarization result



- Multi-thresholding: separating the dataset pixels/voxels into several groups.

Filtered input data   Multi-thresholding result

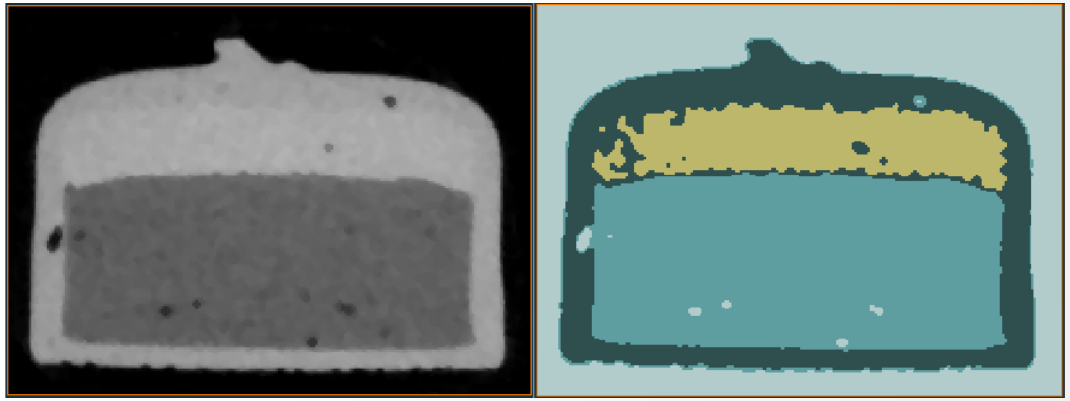
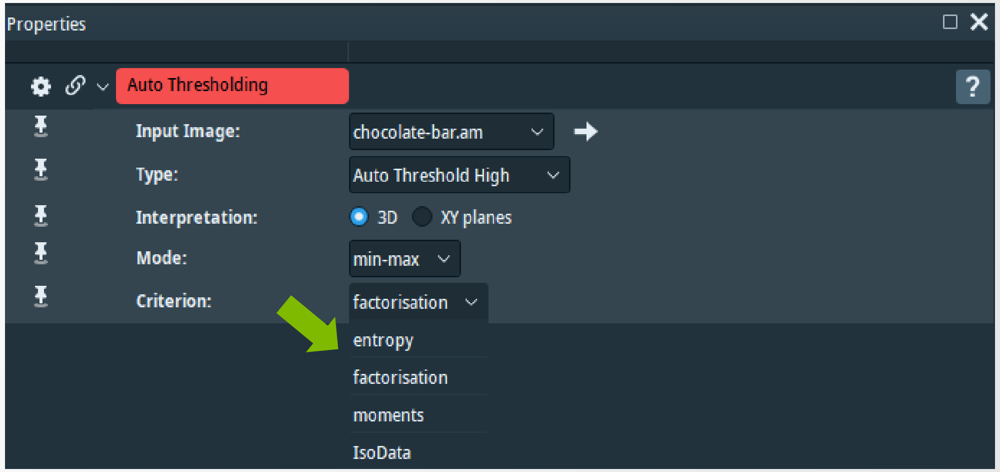


Image segmentation: thresholding methods

Main modules for performing thresholding:

- Interactive modules:
  - Interactive Thresholding
    - Used for binarization, allows setting the threshold value interactively
  - Multi-Thresholding
    - Up to five different regions separated by four different thresholds can be extracted
    - All thresholds are set interactively

- Automatic modules:
  - Auto Thresholding
    - Binary or 3-phase segmentation
    - The threshold or (thresholds for 3-phase mode) are computed automatically
    - 4 methods available for threshold computation



- Local thresholding modules:
  - Local Thresholding
  - Adaptive Thresholding

Image segmentation: thresholding methods example

Multi-thresholding:

- Use Multi-Thresholding module:
  - For setting segmentation **regions**: write a name for each segmentation region
  - For setting **threshold values**: study the histogram – place thresholds between histogram lobes
  - The intensity range between two threshold values defines a region
  - Push **Histo** button to generate the histogram

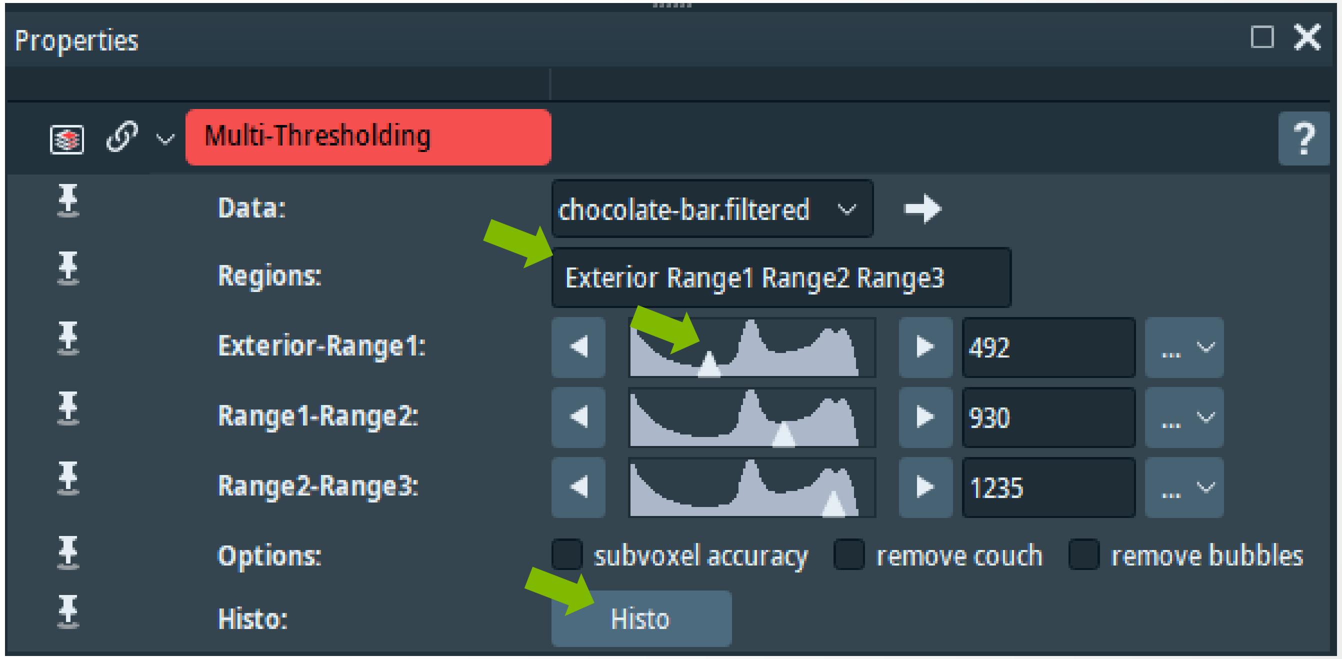
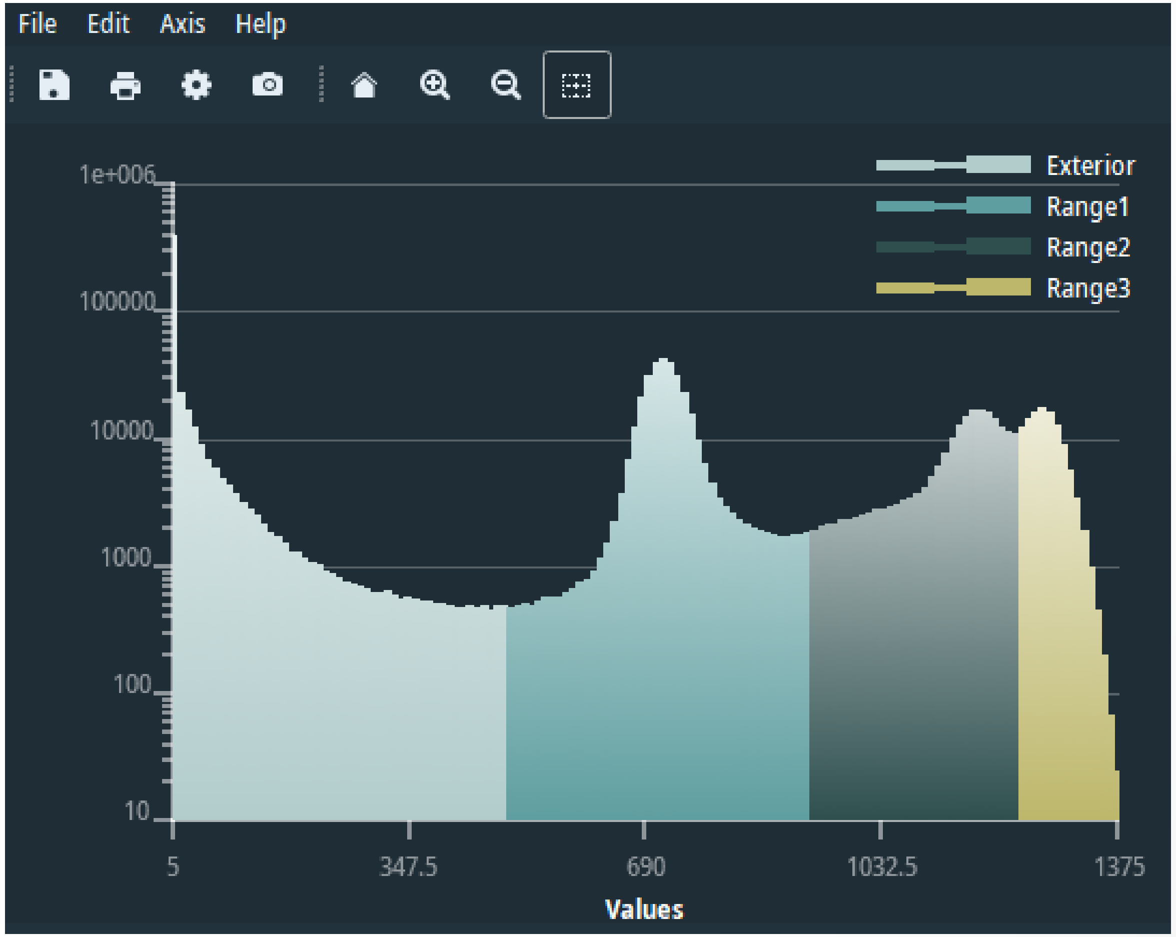


Image segmentation: thresholding methods example

Multi-thresholding:

Histogram



Multi-thresholding result

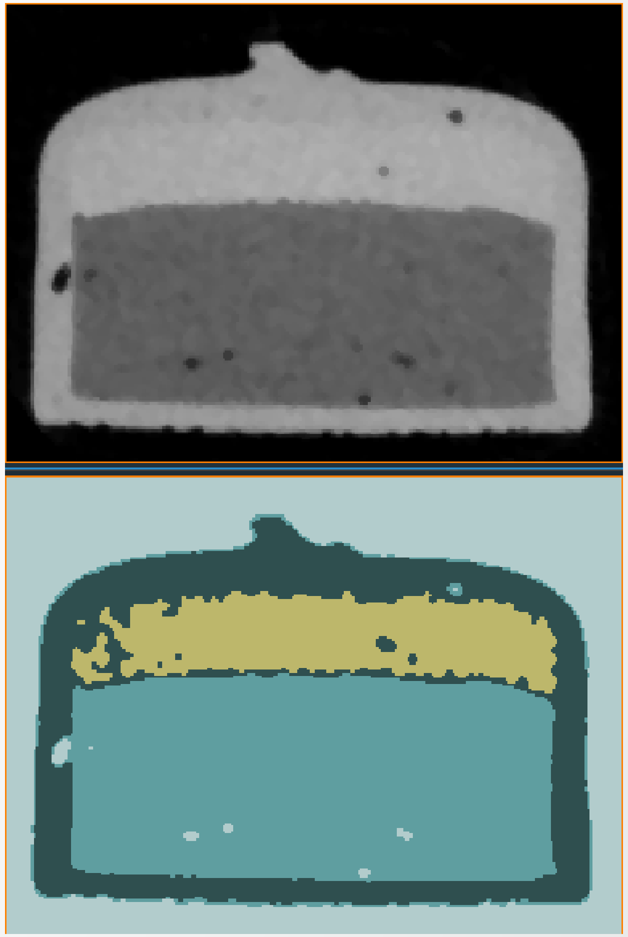
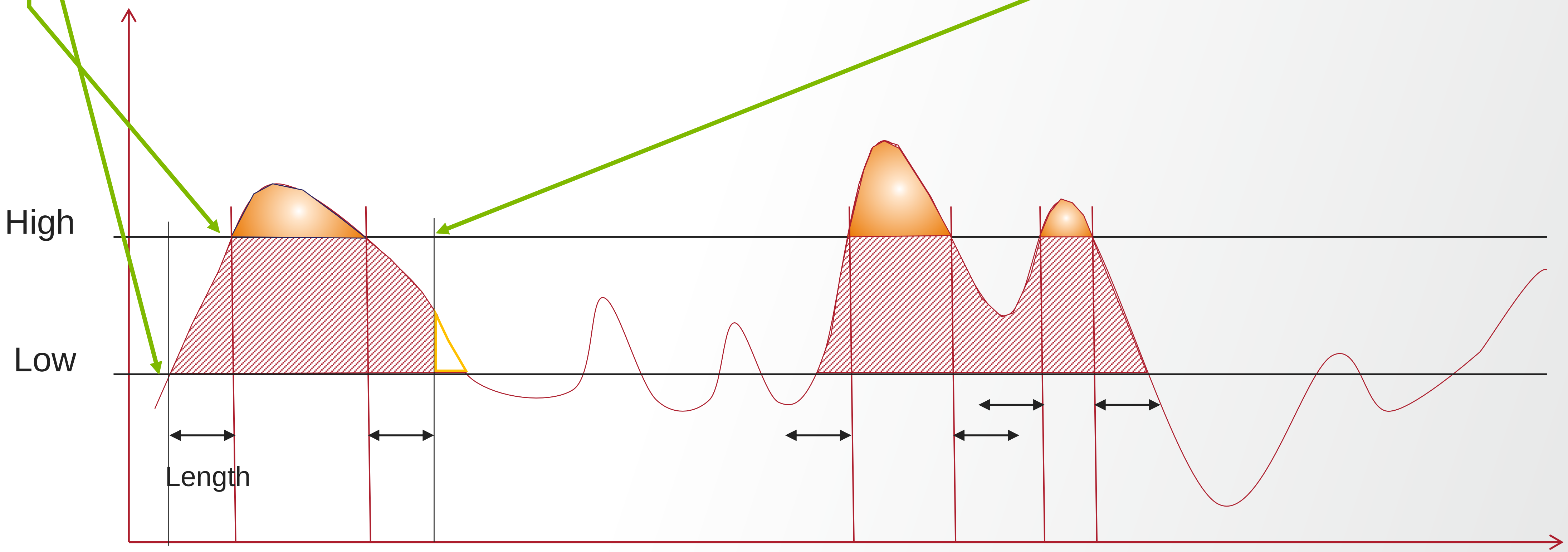


Image segmentation: thresholding advanced

Hysteresis thresholding

- Starts from regions selected with high threshold
- Propagates into voxels with intermediate intensities - up to a given length

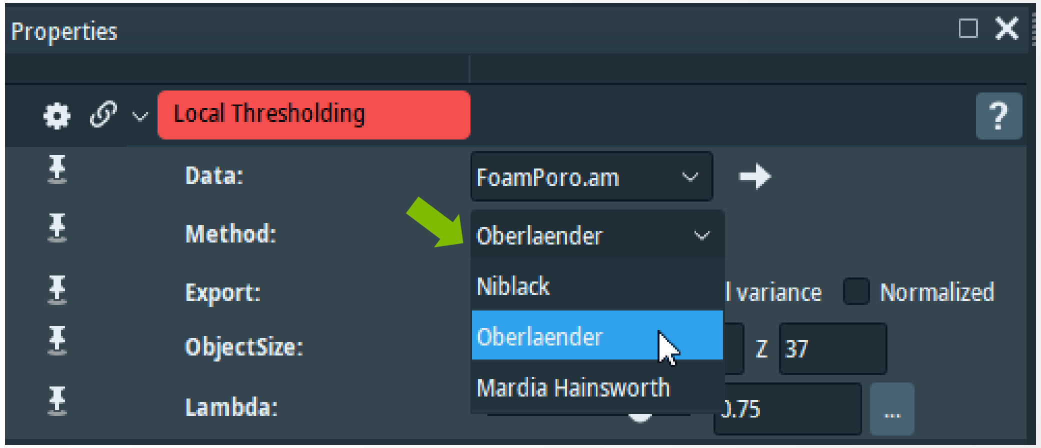


Tip: Use probes, or an interactive thresholding module to help set both threshold values

Image segmentation: thresholding methods example

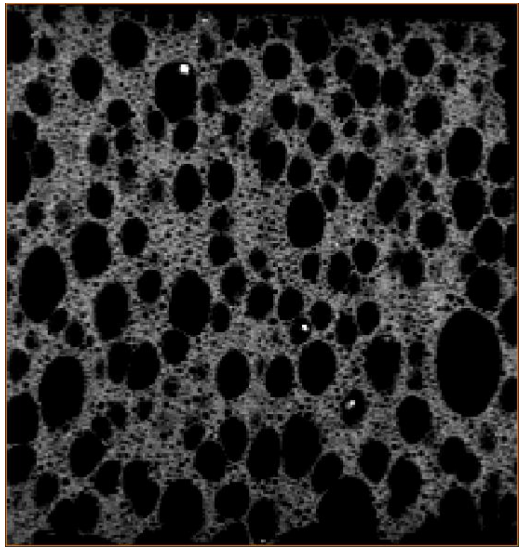
Local thresholding:

- Use Local Thresholding module:
  - For foreground object detection
  - For datasets presenting small background variations
- Three methods are available

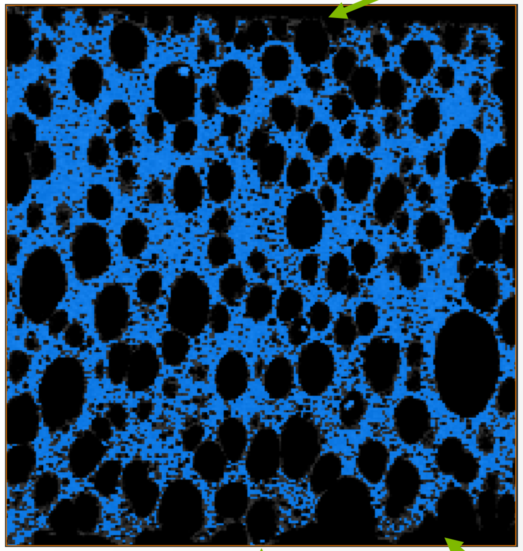


Example on *FoamPoro.am* dataset:

Input slice



Auto-thresholding result



Local-thresholding result

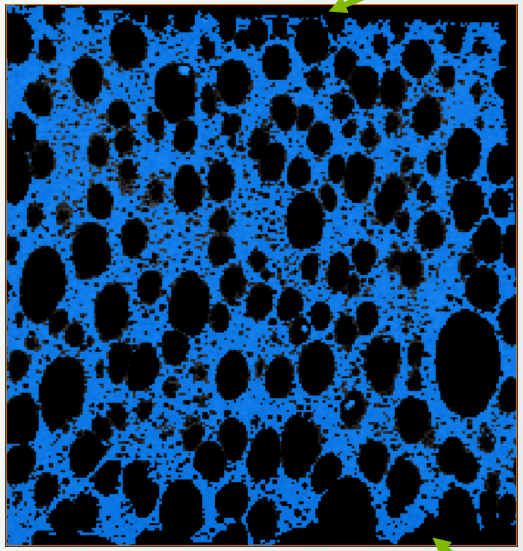
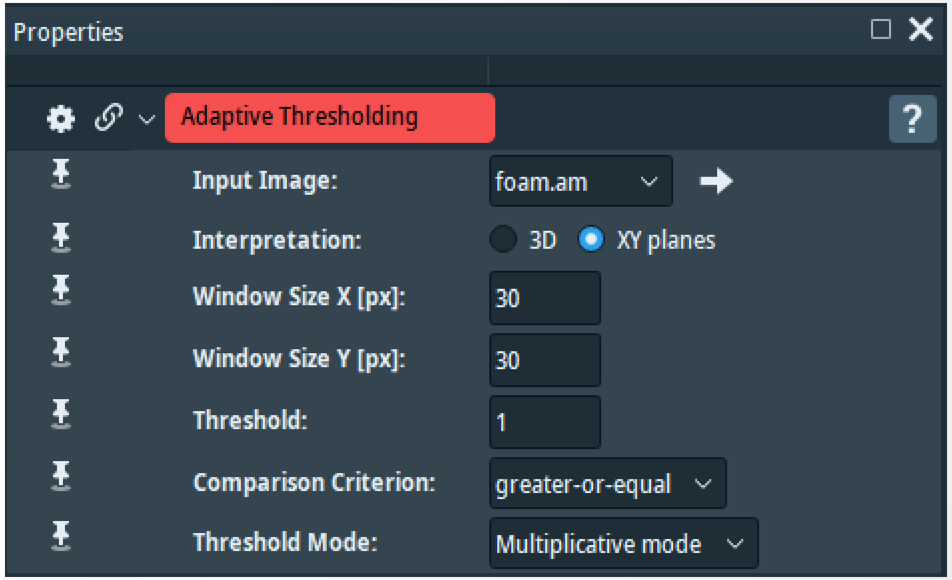


Image segmentation: thresholding methods example

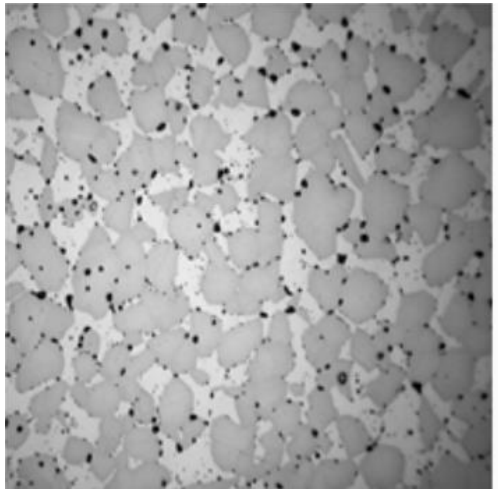
Adaptive-thresholding:

- Use Adaptive-Thresholding module:
    - For thresholding problems that require to adapt the threshold locally e.g. in the case of intensity variation along the data

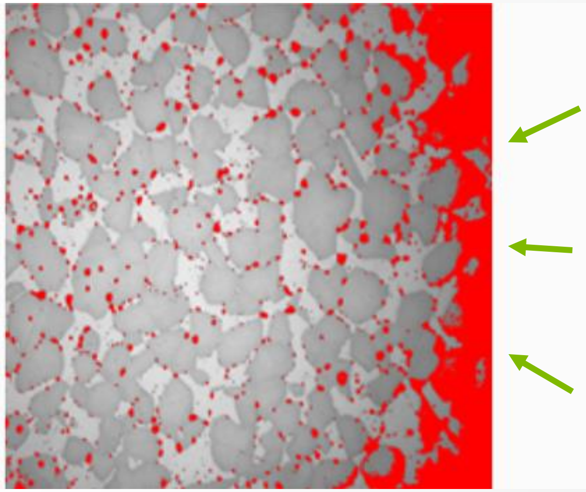
Example:



Input slice



Global-thresholding result



Local-thresholding result

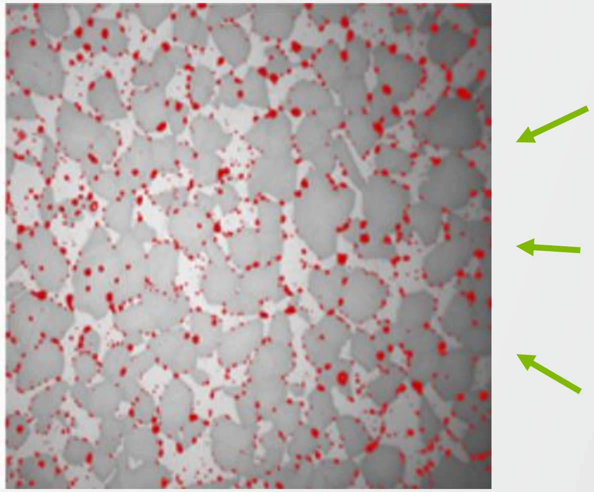


Image segmentation: thresholding methods limitations

Thresholding limitations (e.g. on Multi-thresholding):

- Segmentation artefacts at the boundary between regions (alternative: watershed)

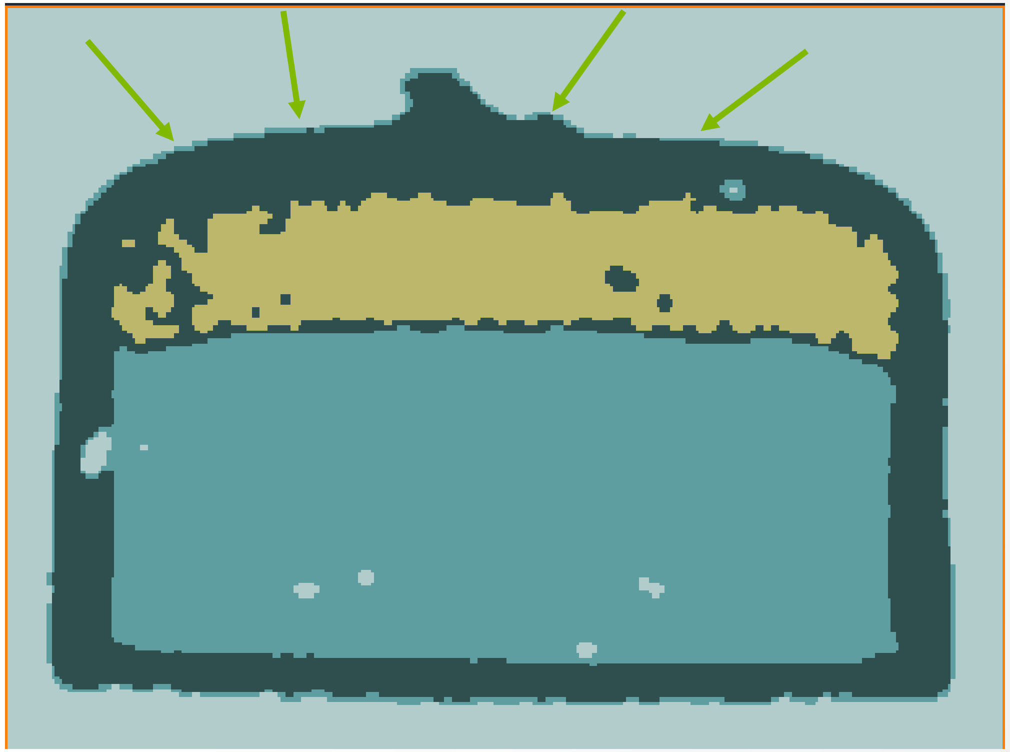
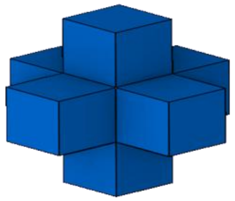


Image segmentation: advanced concepts
Part 1: mathematical morphology

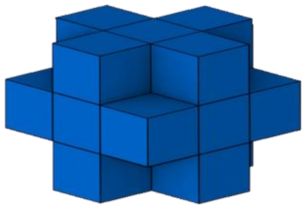
Image segmentation: erosion and dilation - binary

Mathematical morphology (mm):

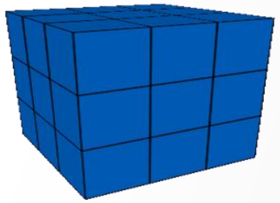
- Structuring element – neighborhood of voxels, defined by:
  - Size
  - Shape (cube, line, disk, ball)
  - Connectivity type – reminder for a cube neighborhood of size 1:



6 (common faces)



18 (common edges)



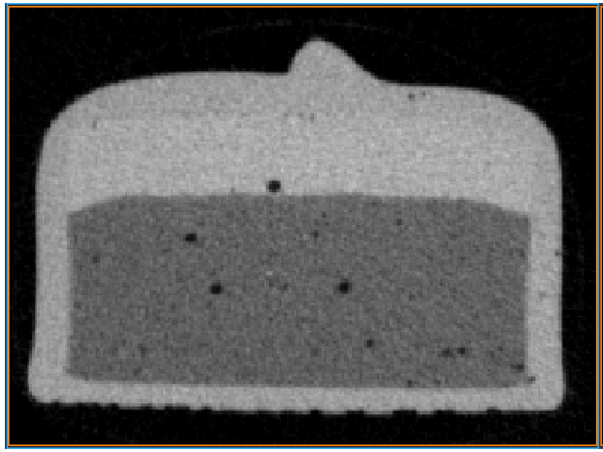
26 (common vertices)

- Basic mm operations:
  - Erosion – shrinks the object
    - If any voxel in the neighborhood is 0, the voxel is set to 0 in the eroded image, else to 1
  - Dilation – grows the object
    - If any voxel in the neighborhood is 1, the voxel is set to 0 in the dilated image, else to 1

Image segmentation: erosion and dilation – binary

Binary Erosion and Dilation exemple:

Input dataset



Binarized input

Erosion result

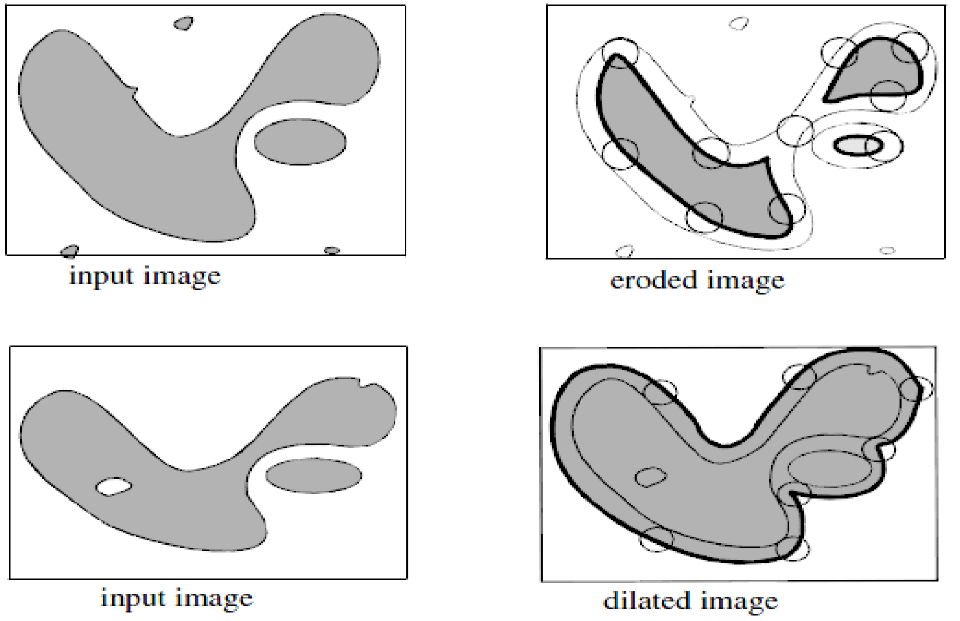
Dilation result

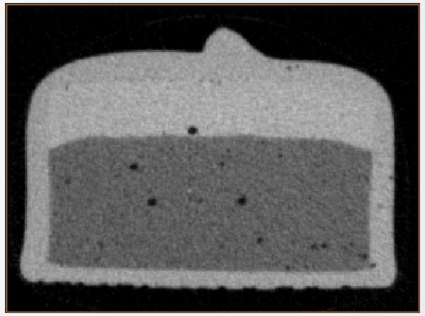
Image segmentation: erosion and dilation – grayscale

Erosion and Dilation on grayscale data:

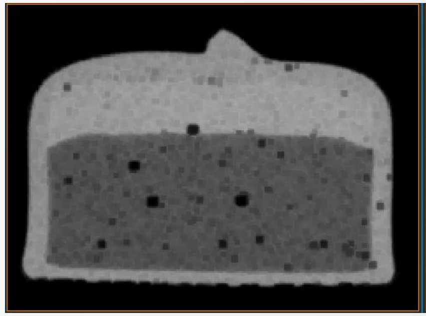
- Erosion
  - Replace voxel value by the minimum intensity value in neighborhood
  - Shrinks bright objects

- Dilation
  - Replace voxel value by the maximum intensity value in neighborhood
  - Expands bright objects





Erosion result



Dilation result

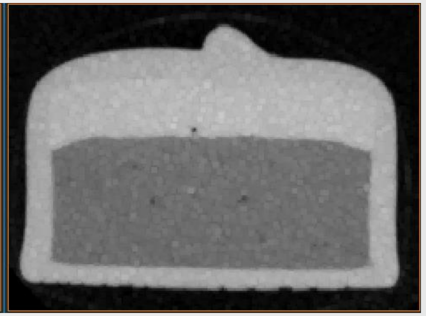
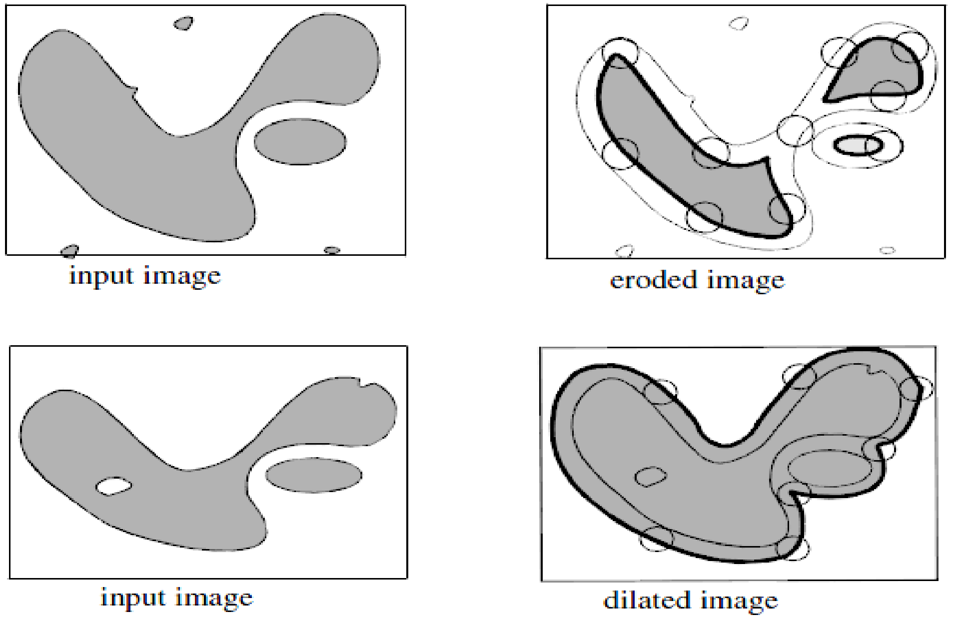


Image segmentation: erosion and dilation – grayscale

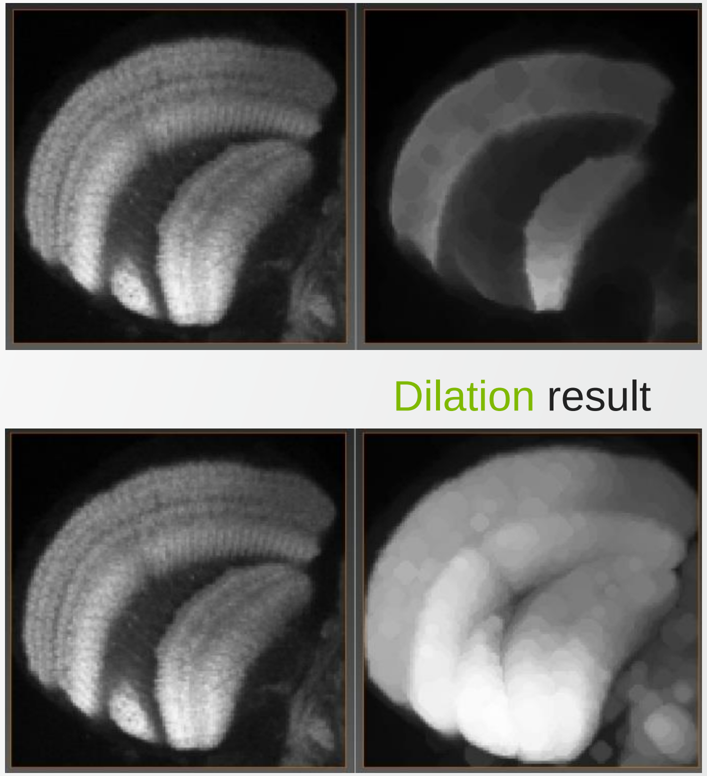
Erosion and Dilation on grayscale data:

- Erosion
  - Replace voxel value by the minimum intensity value in neighborhood
  - Shrinks bright objects

- Dilation
  - Replace voxel value by the maximum intensity value in neighborhood
  - Expands bright objects



Erosion result



Dilation result

Image segmentation: opening and closing - binary

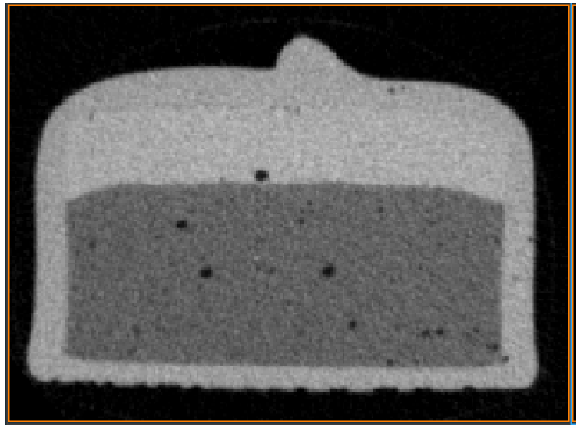
Mathematical morphology operations derived from Erosion and Dilation:

- Opening:
  - Erosion + Dilation
    (using the same structuring element - SE)
  - All detection objects smaller than the size of the structuring element are removed

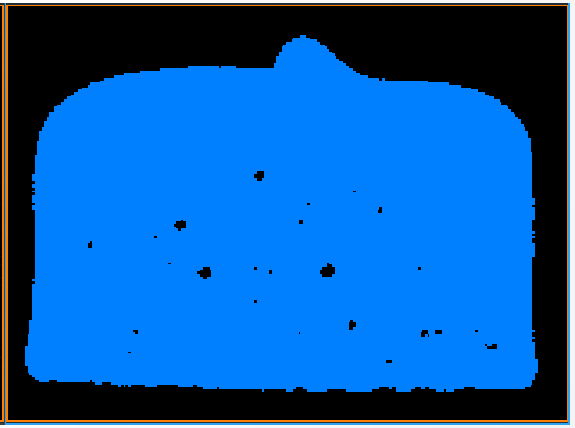
- Closing:
  - Dilation + Erosion
    (using the same SE)
  - All void regions (label=0) smaller than the size of the structuring element are filled

Image segmentation: closing example - binary

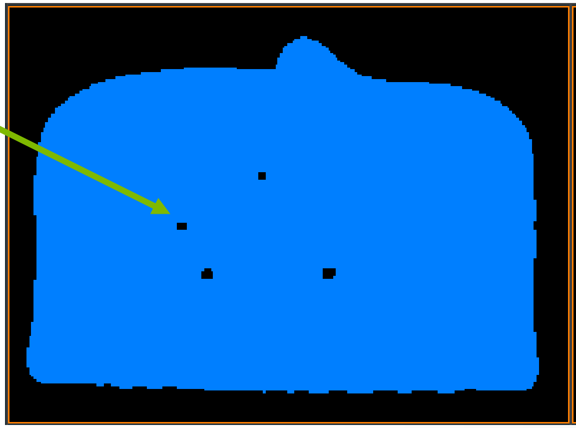
Input dataset



Thresholding result



Closing result
SE of size 1



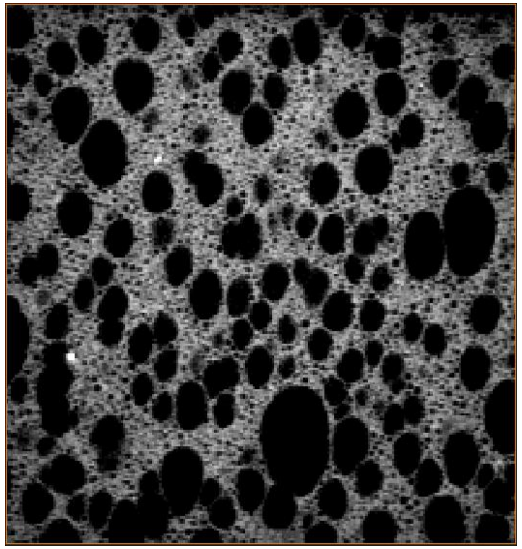
Closing result
SE of size 3

Note: the SE size is too small for filling all porosities.

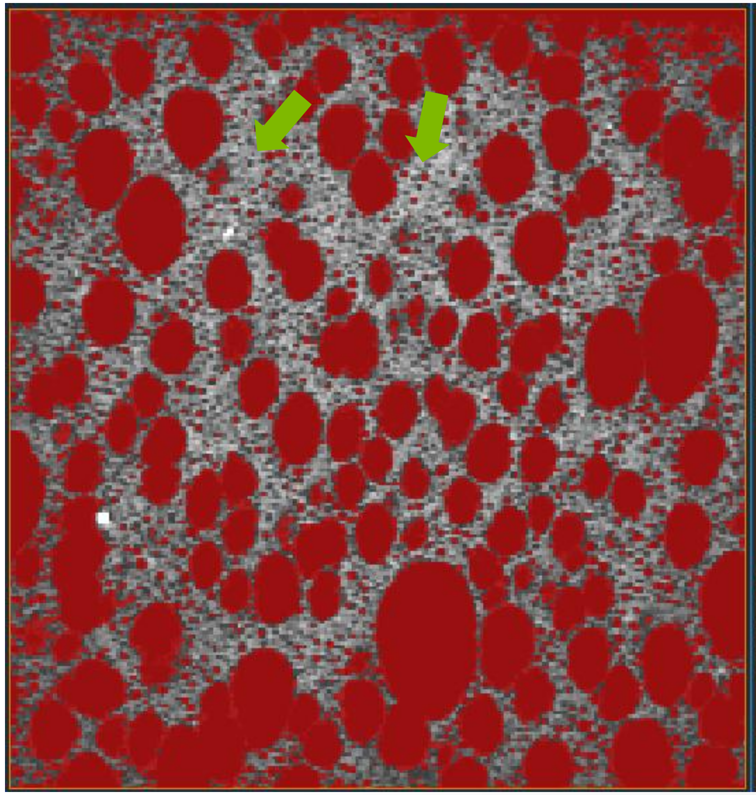
Closing: fills small holes (e.g. porosities) and connects detection objects that are close to each other.

Image segmentation: opening example - binary

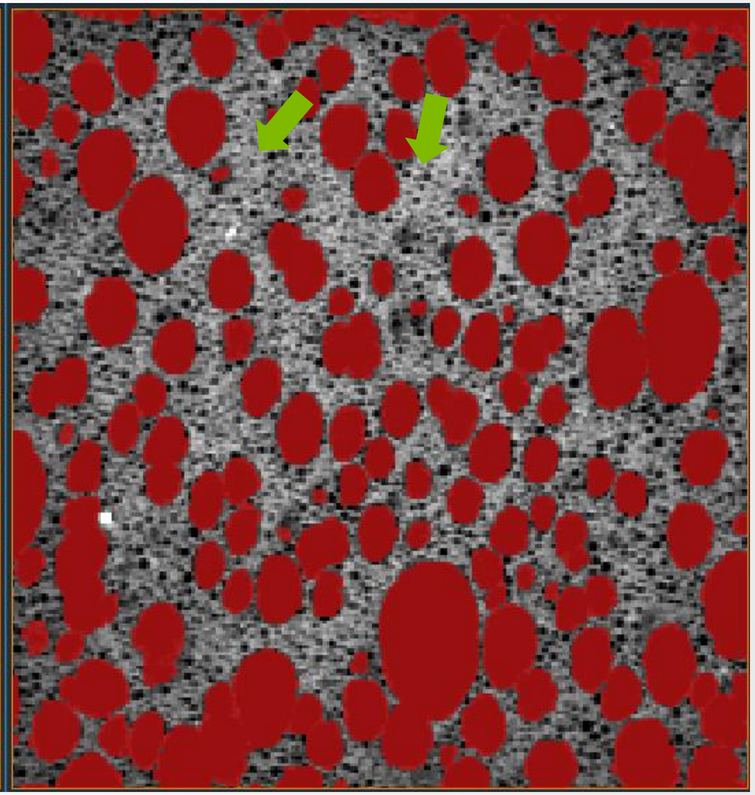
Input dataset



Thresholding result

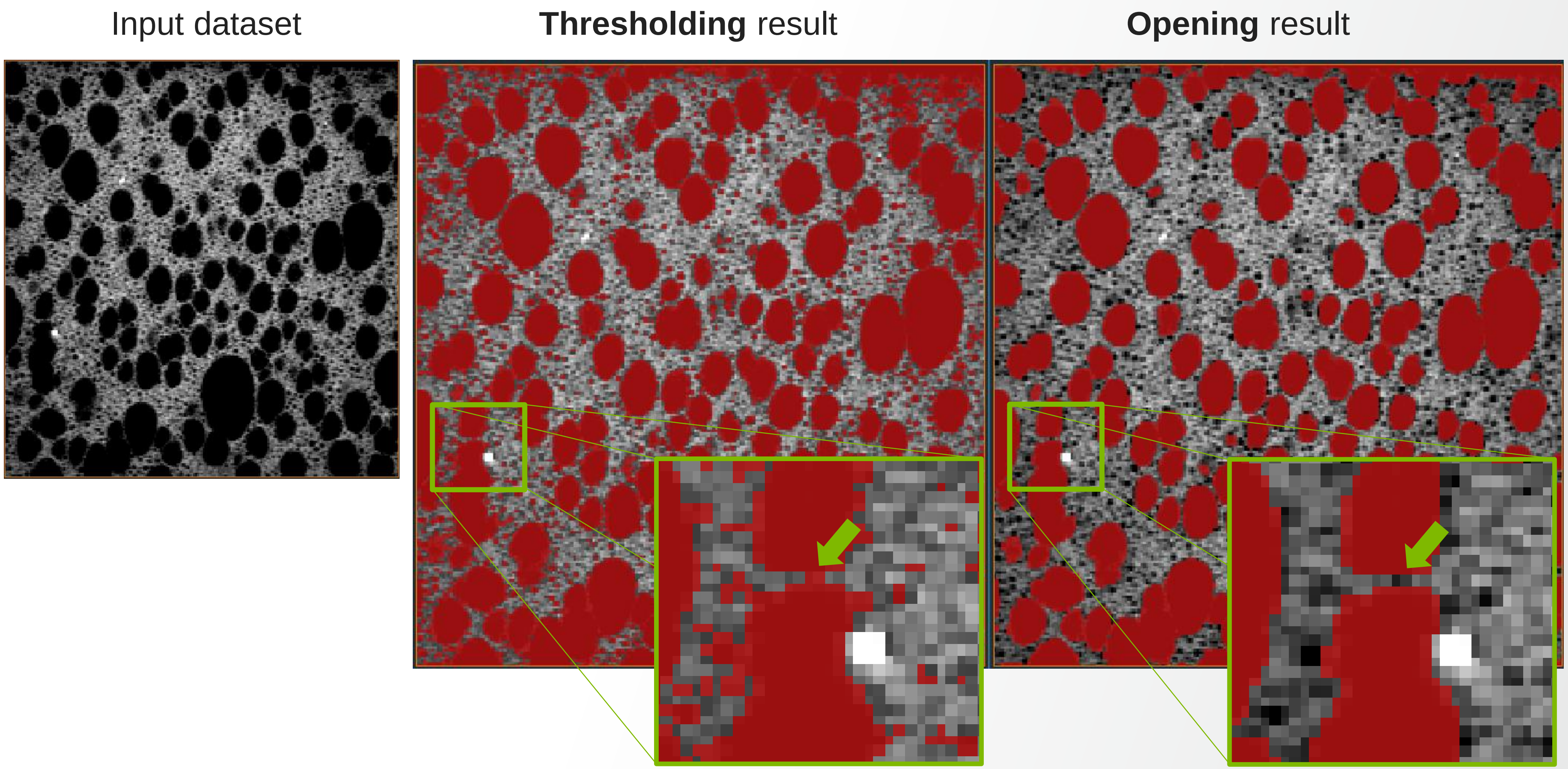


Opening result



Opening: removes small structures (clean segmentation results – remove artefacts of small size).

Image segmentation: opening example- binary



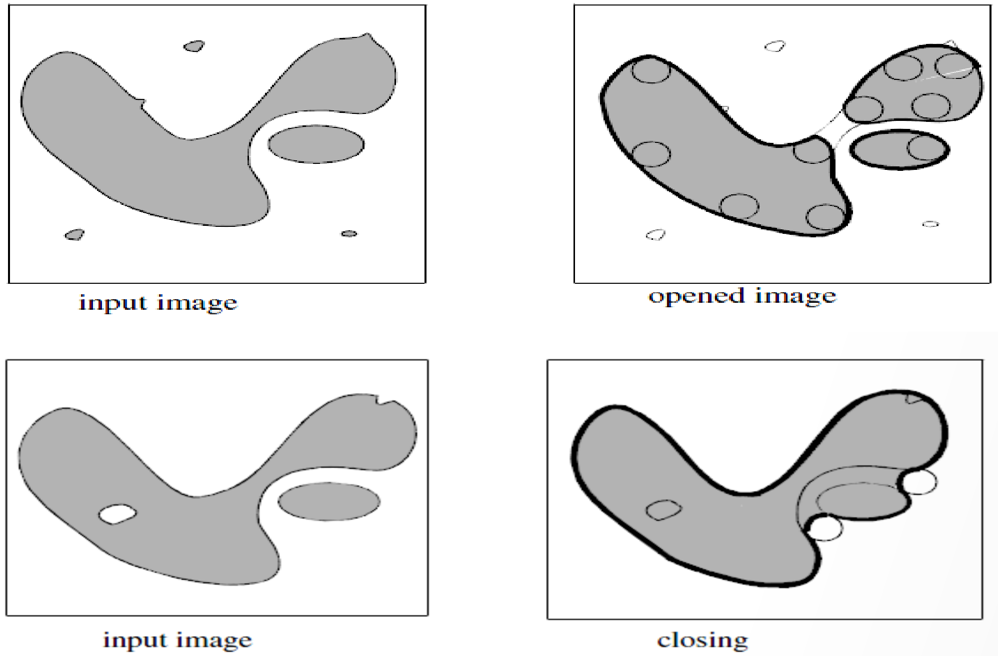
Opening: removes links (connections) of small size (e.g. separate detection object).

Image segmentation: opening and closing – grayscale

Opening and Closing on grayscale data:

- Opening
  - Removes small bright structures.

- Closing
  - Removes small dark structures.



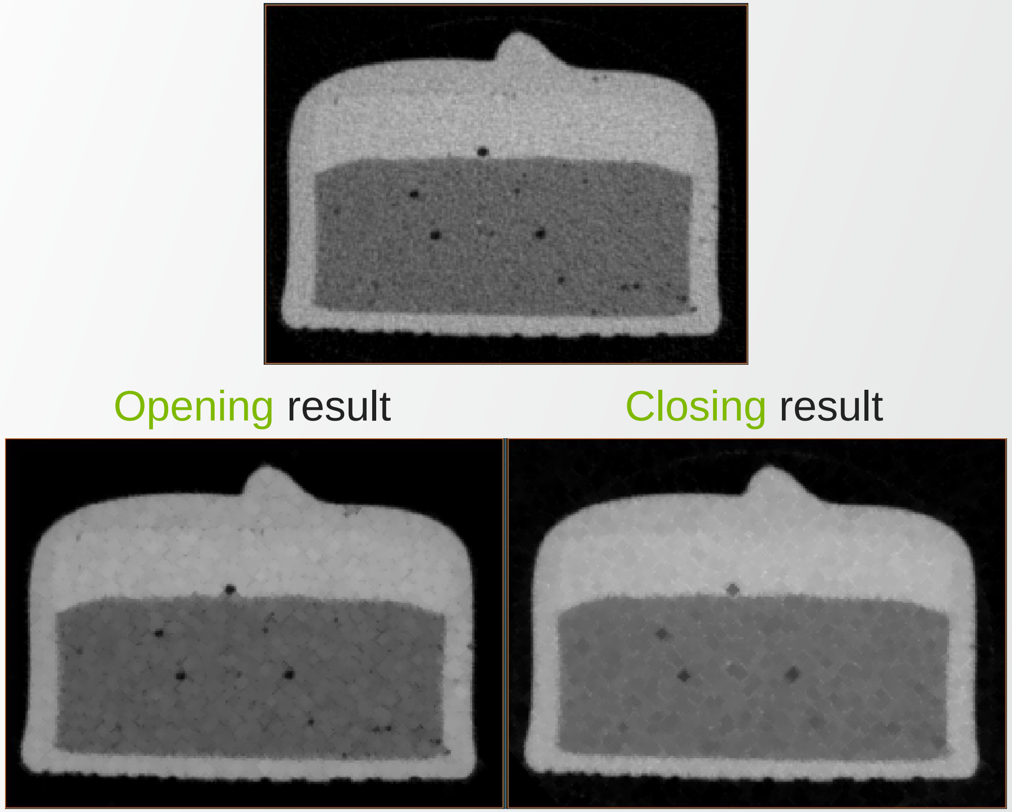
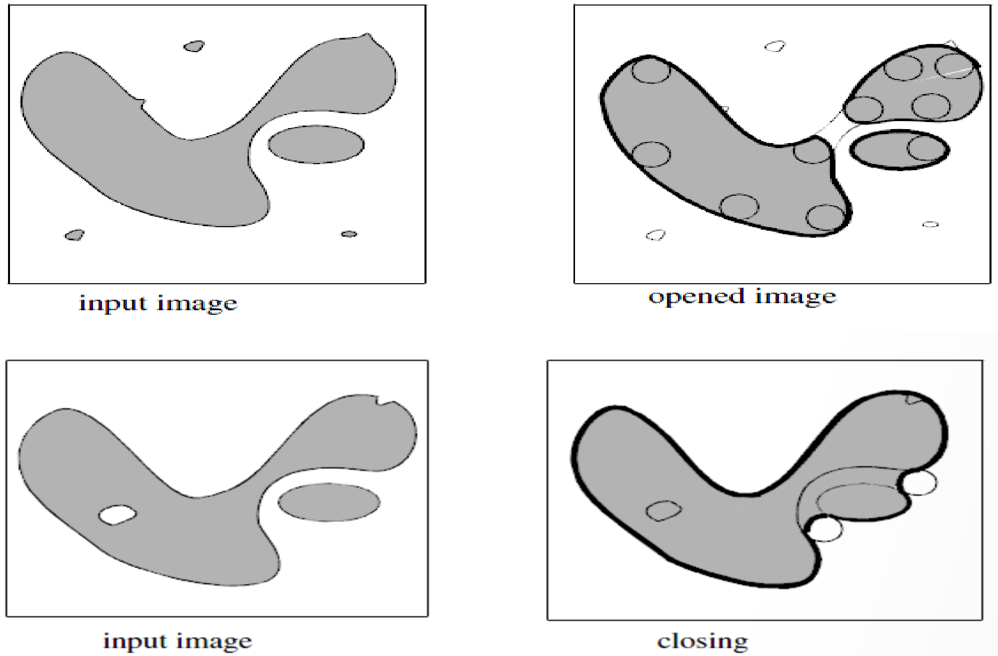


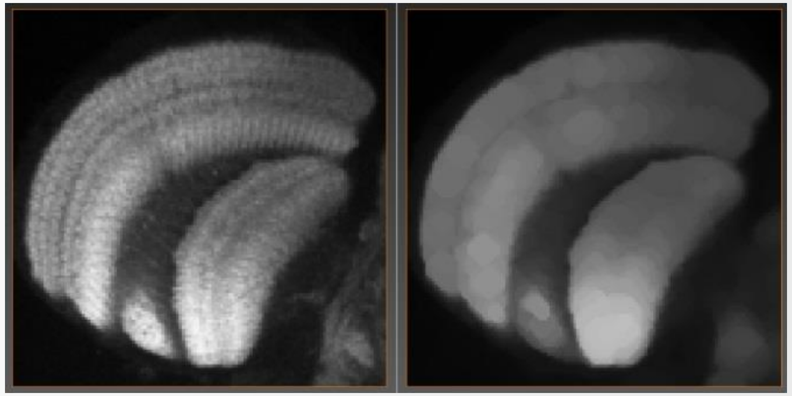
Image segmentation: opening and closing – grayscale

Opening and Closing on grayscale data:

- Opening
  - Removes small bright structures.
- Closing
  - Removes small dark structures.



Opening result



Closing result

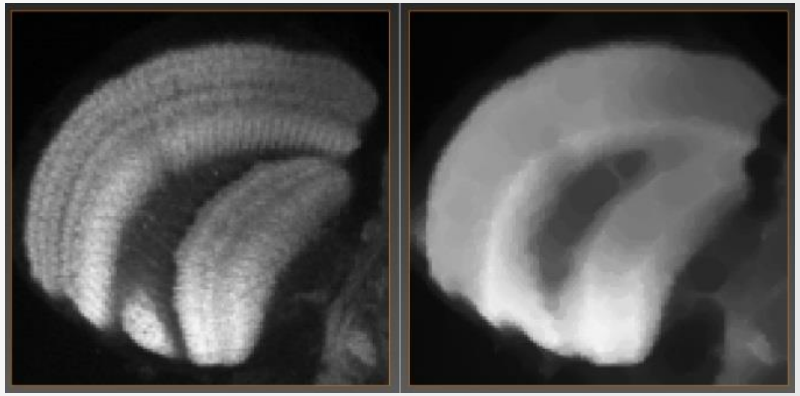


Image segmentation: when thresholding does not work

Q: How can we segment the small spots only ?

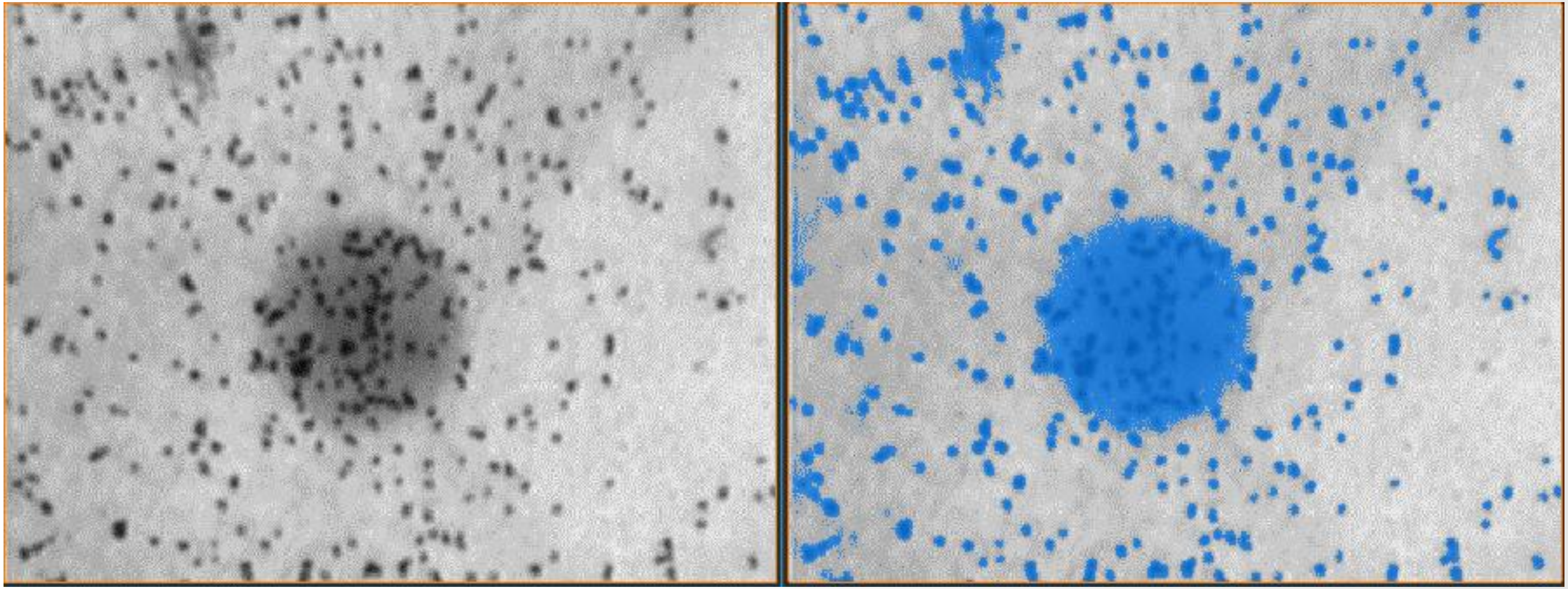


Image segmentation: Top-Hat Transform

R: Apply Top-Hat transform and then thresholding

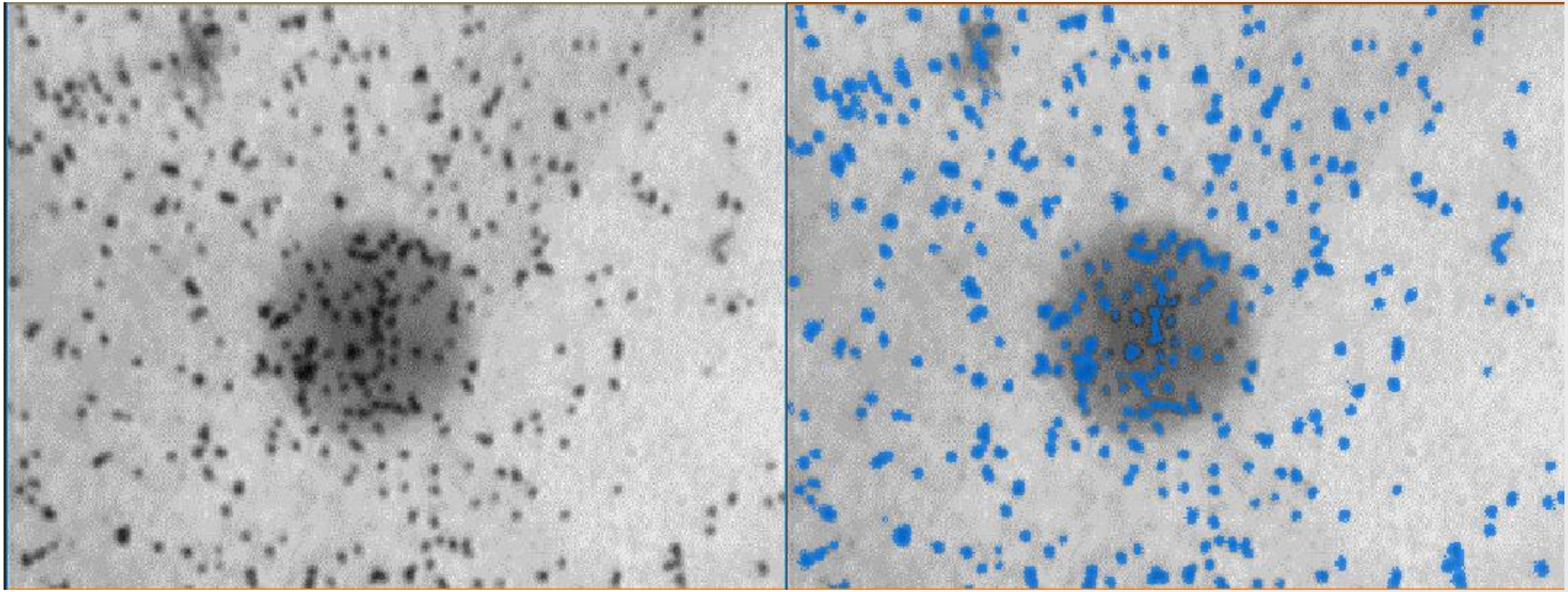


Image segmentation: Top-Hat Transform

Top-Hat (TH) Transform:

- Derived from Opening and Closing
- Highlights small size structures
- Two types of TH transform:
    - White TH:
        - Highlights bright structures
        - Mathematical expression: Input Data - Opening result
    - Black TH:
        - Highlights dark structures
        - Mathematical expression: Closing result - input data
- Good practice: apply before thresholding in order to corrects non-uniform lighting

Image segmentation: Top-Hat Transform example

Q: How does one get from A to B

A B

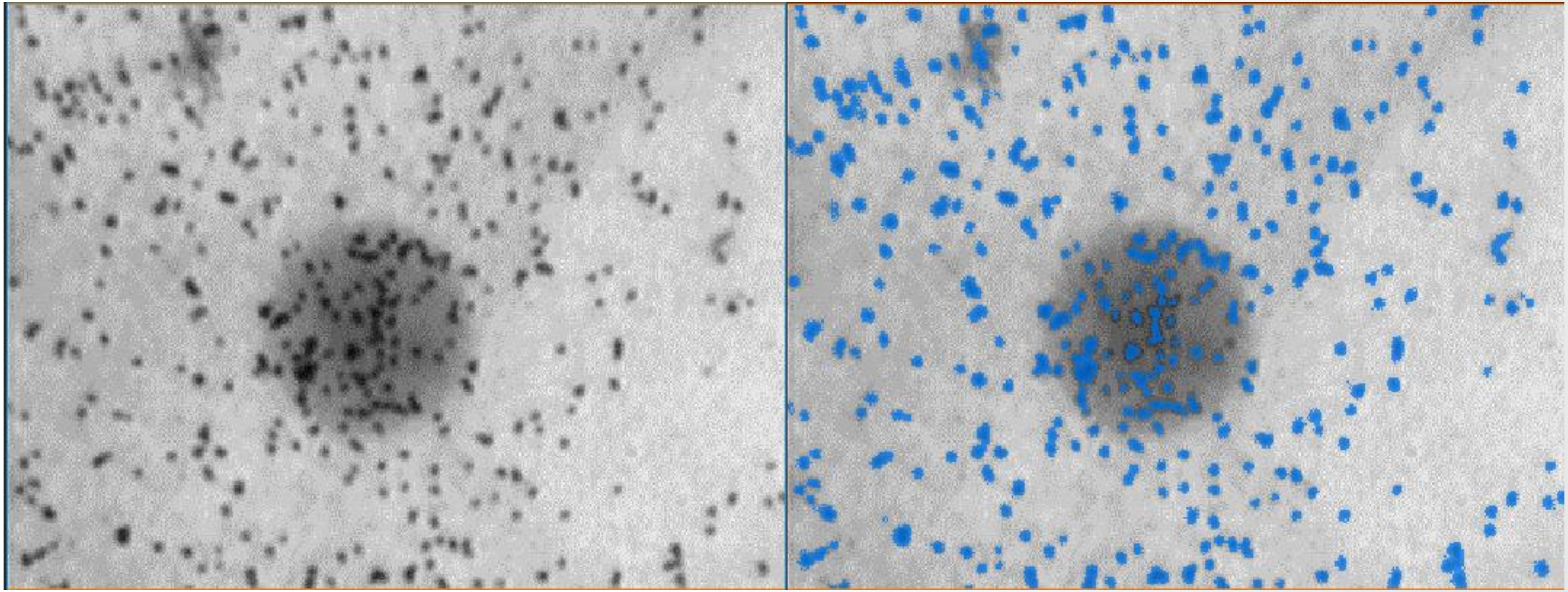
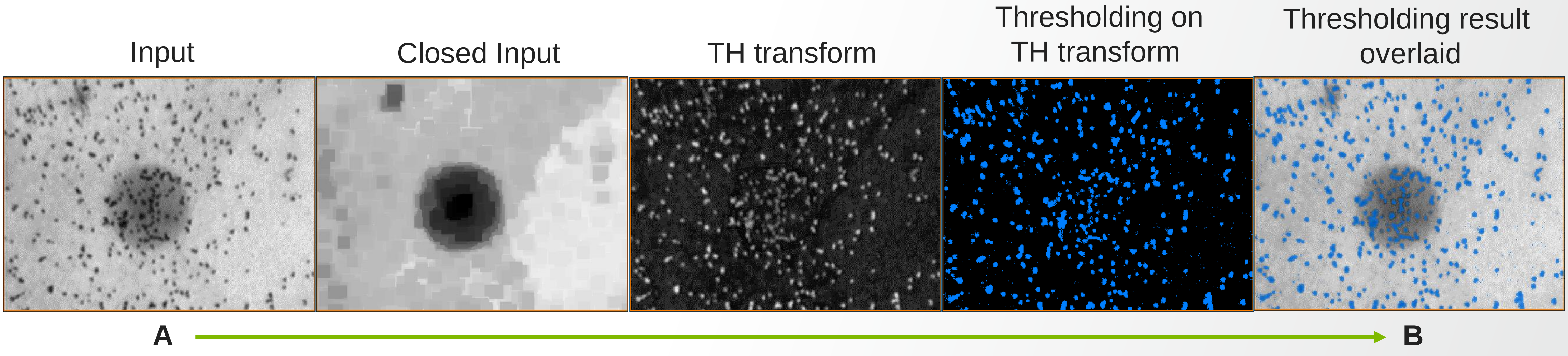


Image segmentation: Top-Hat Transform example



Getting from A to B:

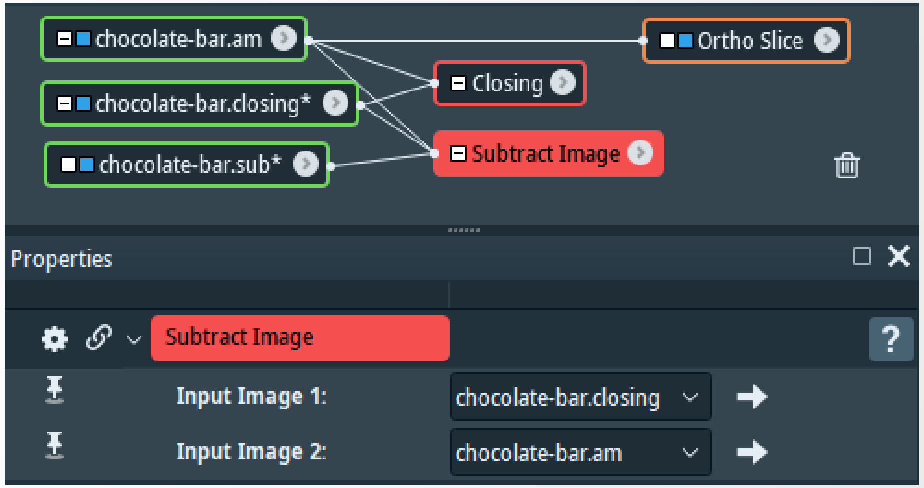
- Apply Closing to the Input
- Subtract Input from Closing Result → TH transform image
- Apply thresholding on the TH transform image
- Overlay result on Input (Colorwash)

Image segmentation: Top-Hat in Avizo

Ways of applying (TH) Transform in Avizo:

- Dedicated module
  - Interactive Top-Hat

- Use the modules that correspond to the operations composing the TH transform
  - Opening, Closing, Arithmetic/Subtract Image
  - Advantage – more flexibility in the choice of the:
    - Structuring element
    - Thresholding method



- Segmentation Editor:
  - Top-hat selection tool
    - TH transform image computation
    - Thresholding on the TH transform image

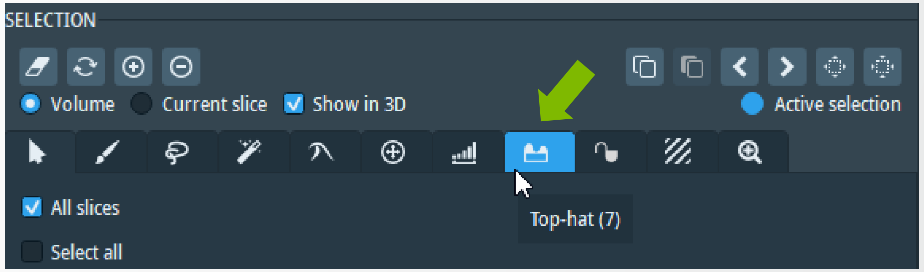


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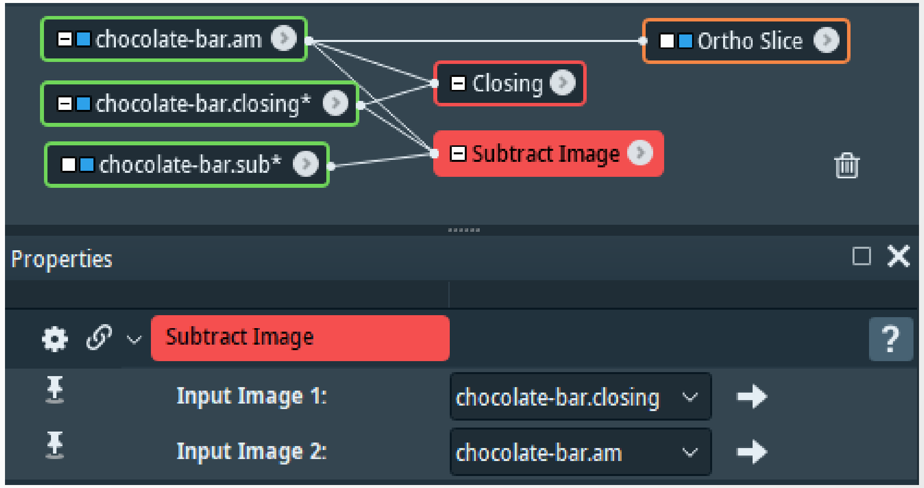
Image segmentation: Top-Hat in Amira

Ways of applying (TH) Transform in Amira:

- Dedicated module
  - Interactive Top-Hat

- Use the modules that correspond to the decomposed TH transform
  - Opening, Closing, Arithmetic/Subtract
  - Advantage – more flexibility in the choice of the:
    - Structuring element
    - Thresholding method



- Segmentation Editor:
  - Top-hat selection tool
    - TH transform image computation
    - Thresholding on the TH transform image

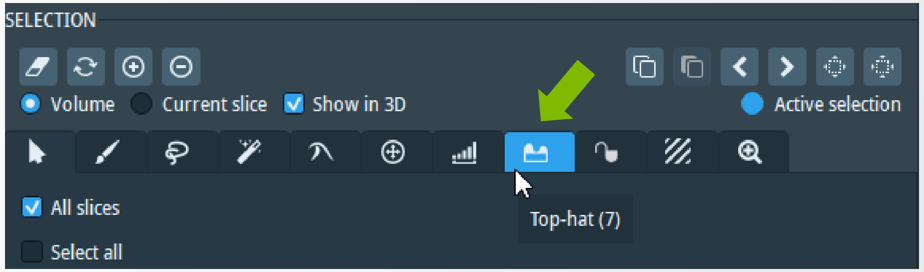


Image segmentation: Top-Hat exercise

Bubble detection in chocolate bar

Apply the necessary module(s) and ports parametrization to create a similar view:

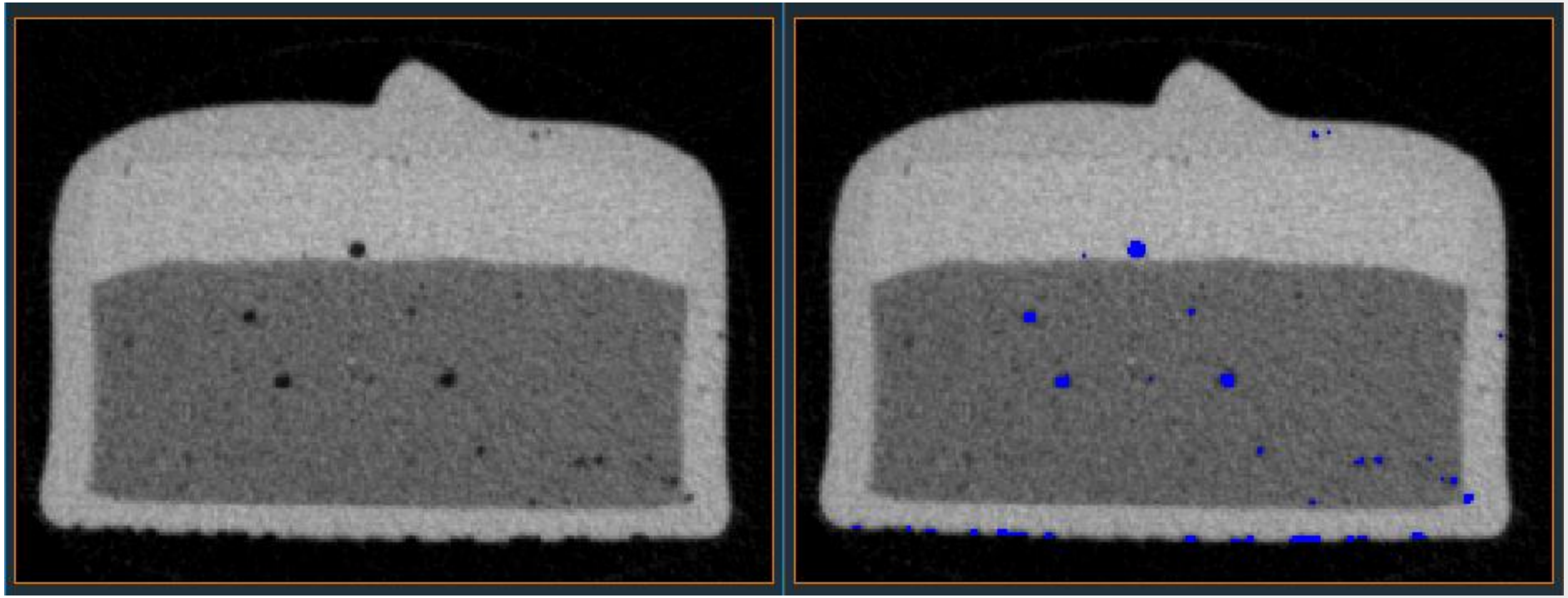


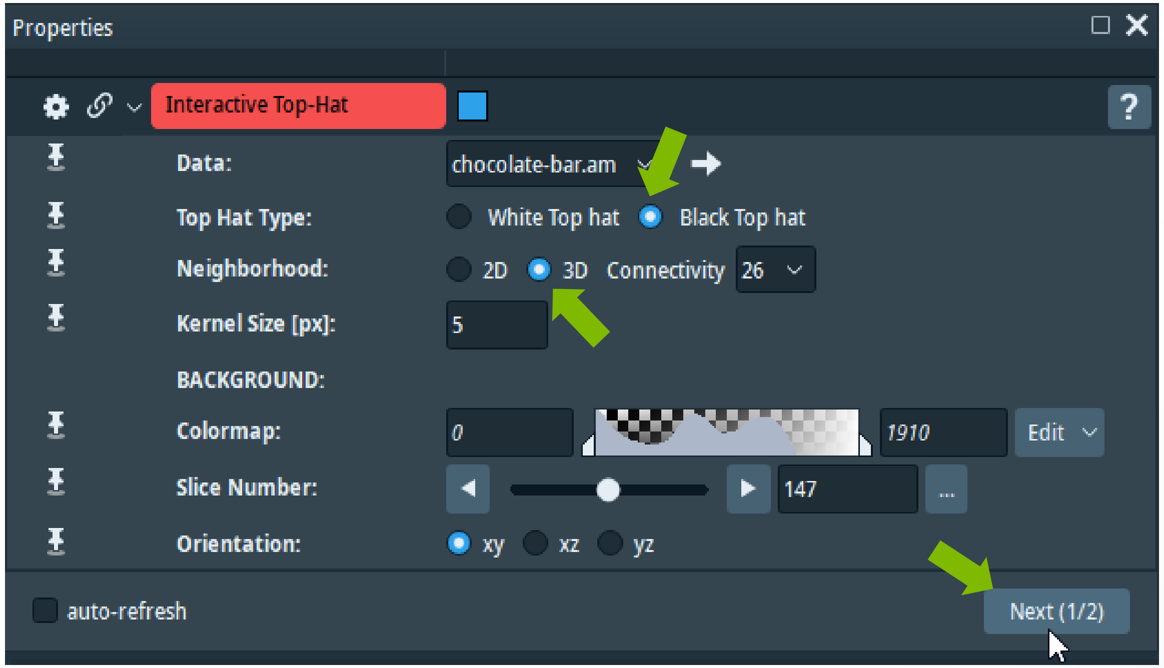
Image segmentation: Top-Hat exercise

Solution

e.g. Interactive Top-Hat module – 2 steps:

- (1/2): Computation of the Black TH transform
- (2/2): Thresholding of the TH transform image

TH transform image histogram



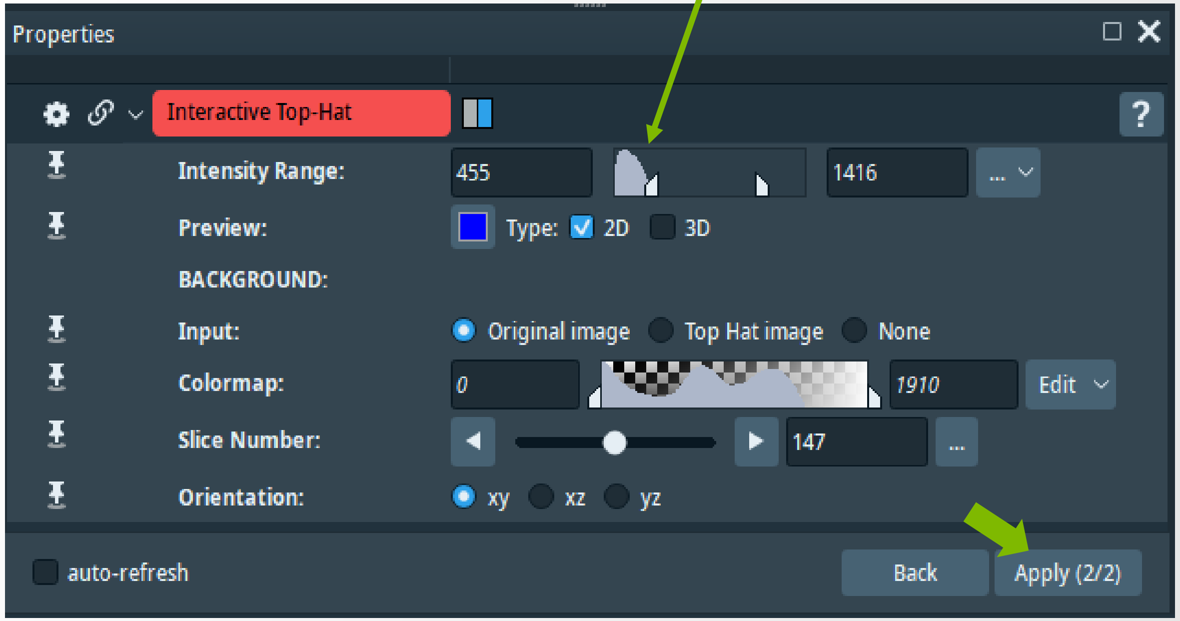
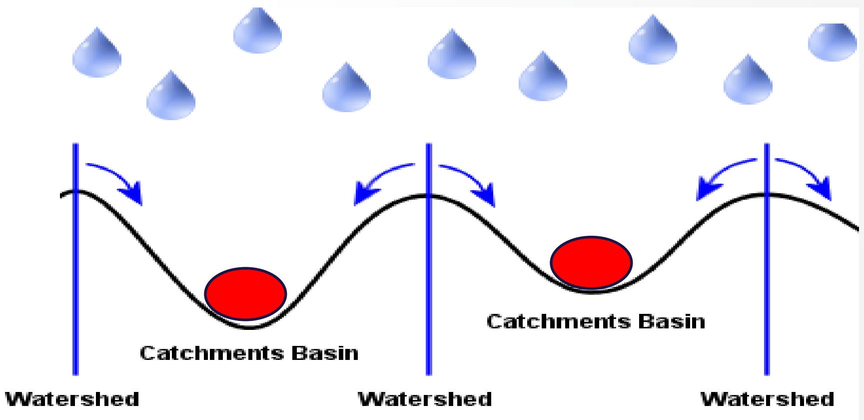
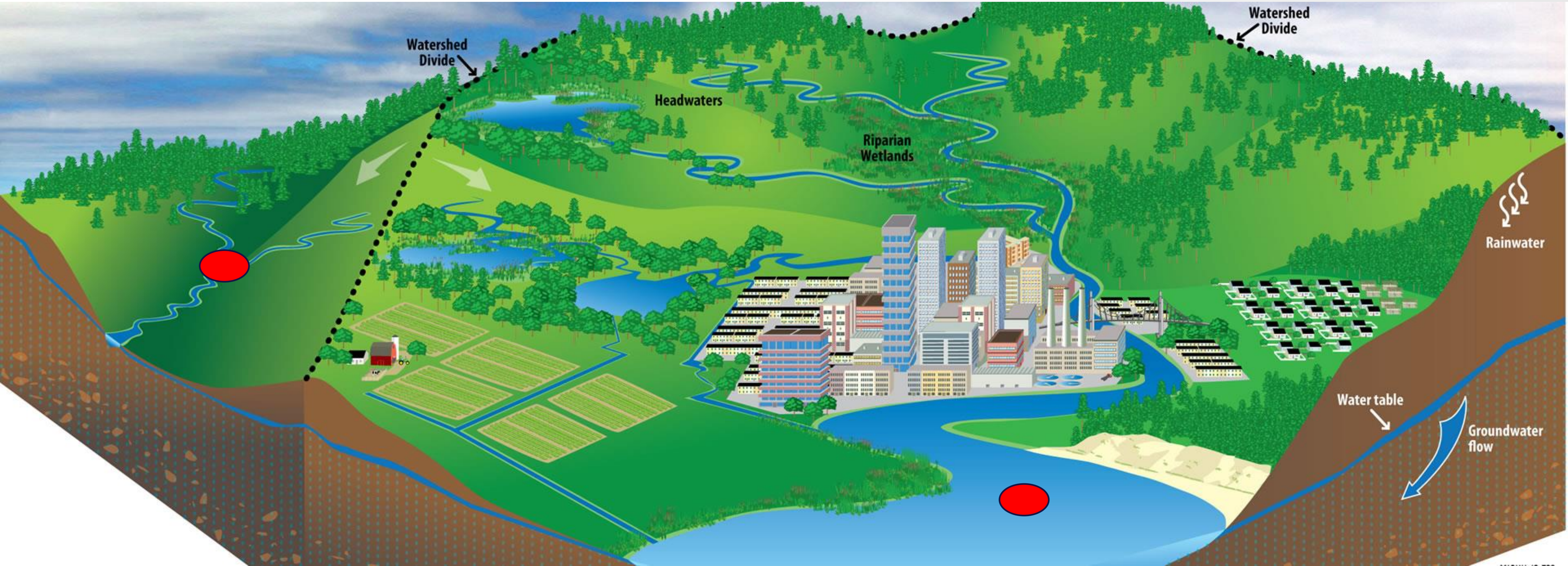


Image segmentation: advanced concepts
Part 2: watershed segmentation

Introduction to Watershed

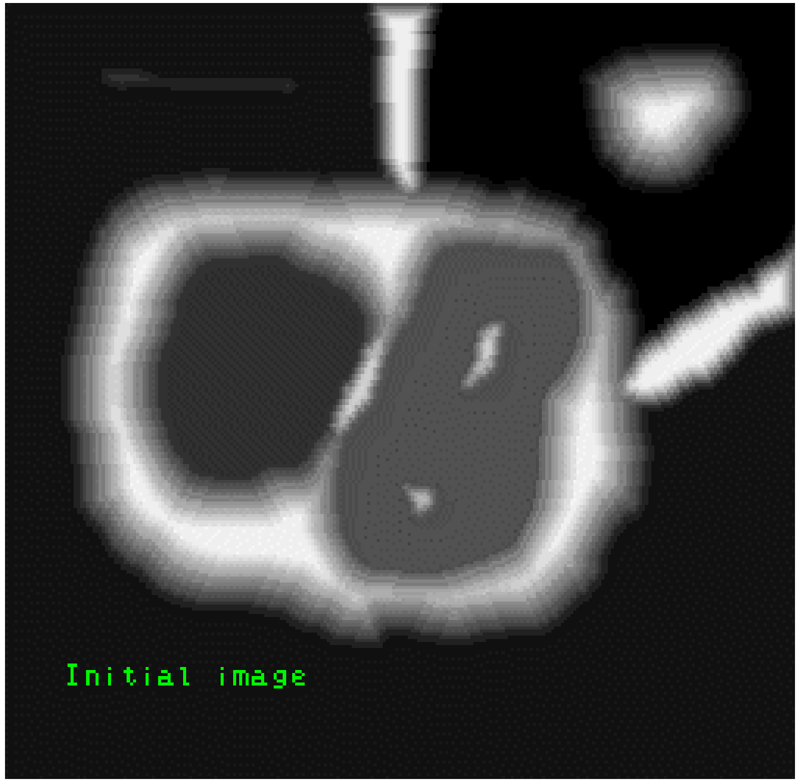
Watershed is the area of land that drains into catchment basins

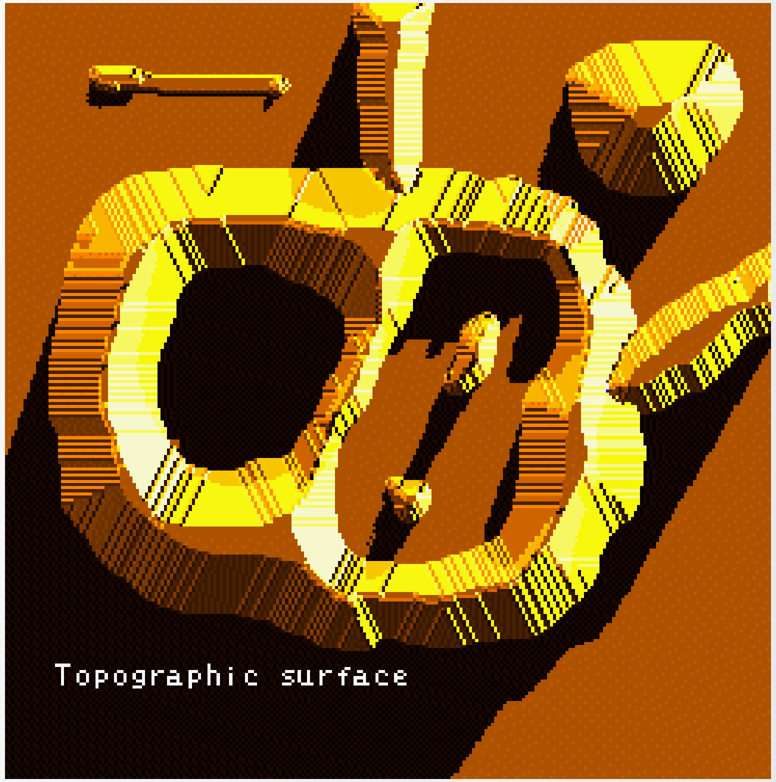




Watershed Transformation

Any grayscale image can be considered as a topographic surface.





Watershed Transformation

If we flood this surface from its minima and, if we prevent the merging of the waters coming from different sources, we partition the image into two different sets: the catchment basins and the watershed lines.

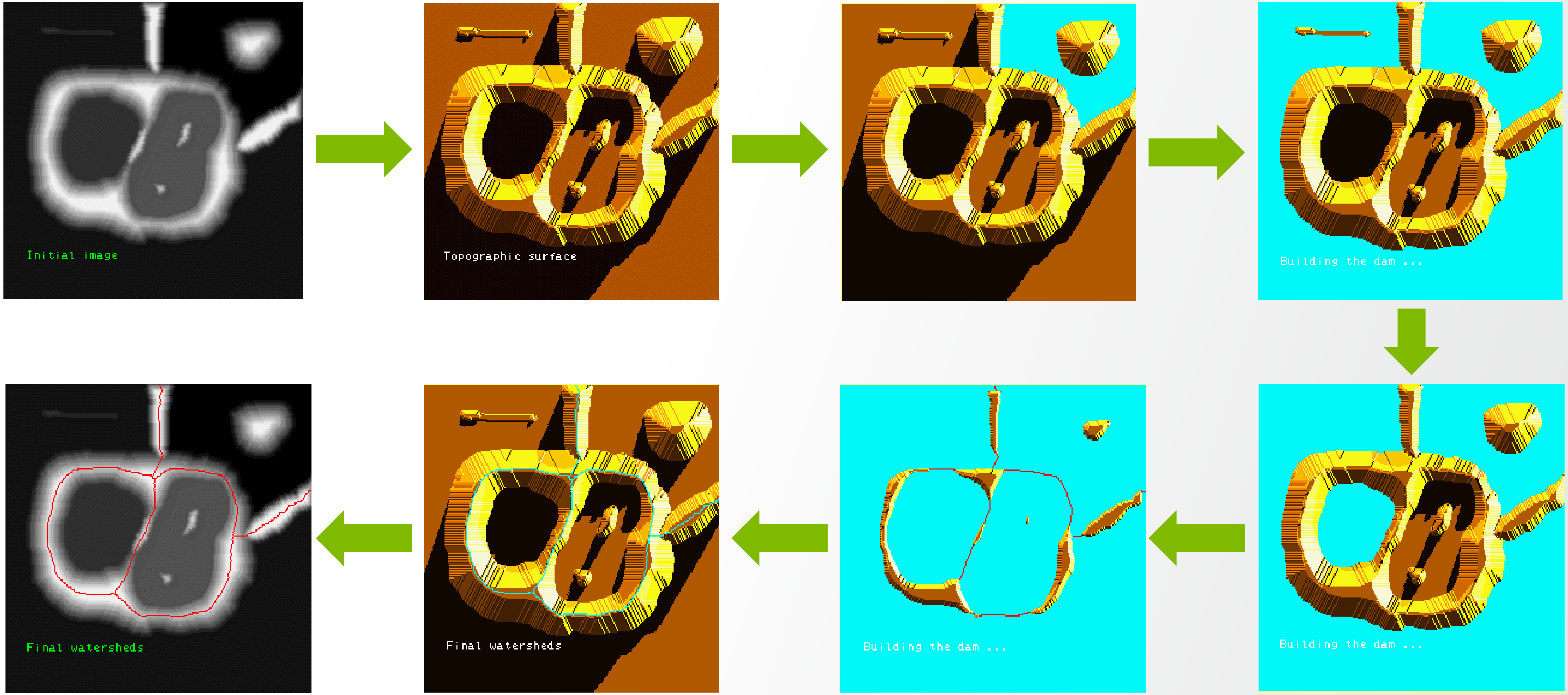
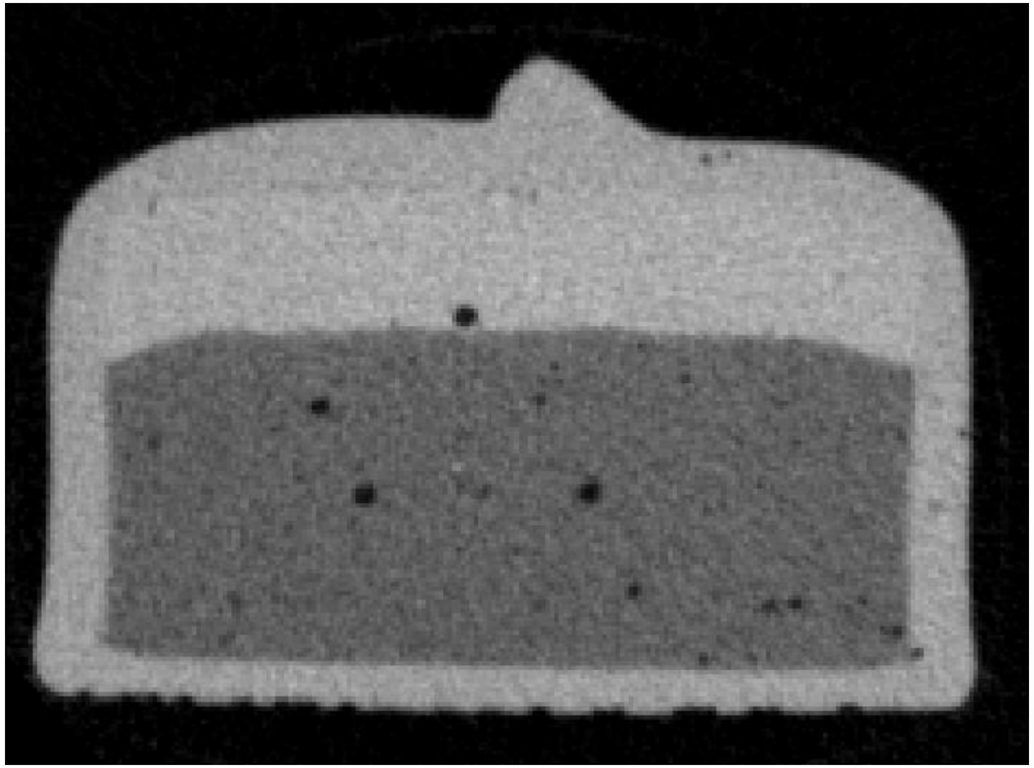


Image gradient

If we apply this transformation to the image gradient, the catchment basins should theoretically correspond to the homogeneous grey level regions of this image.



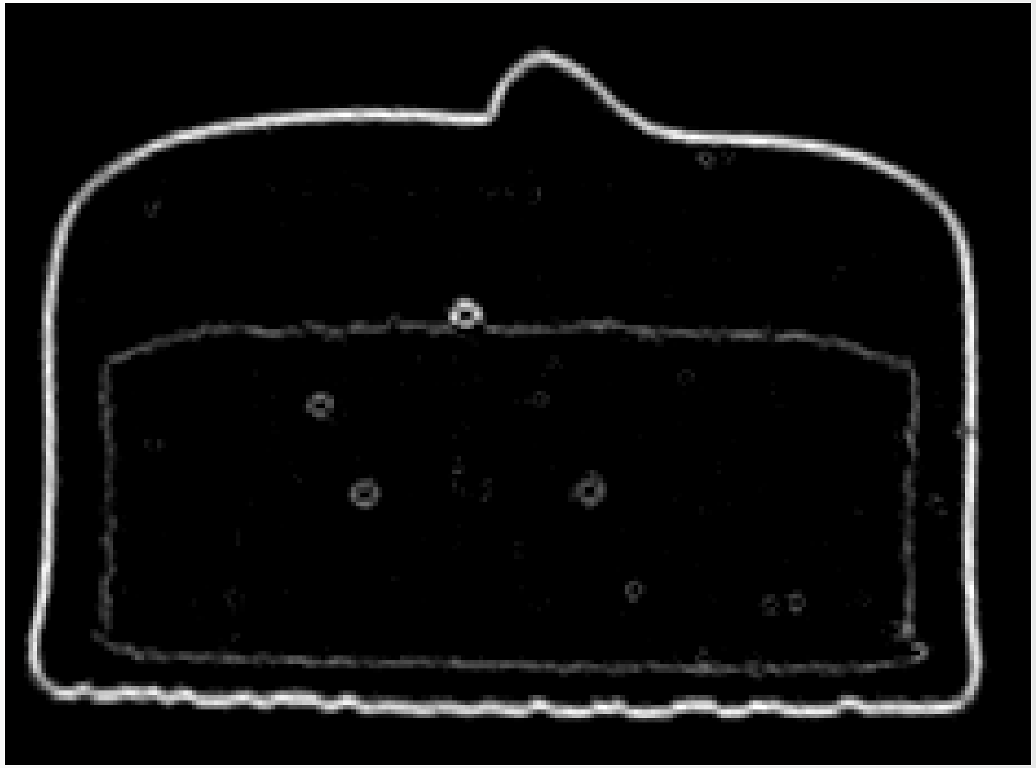
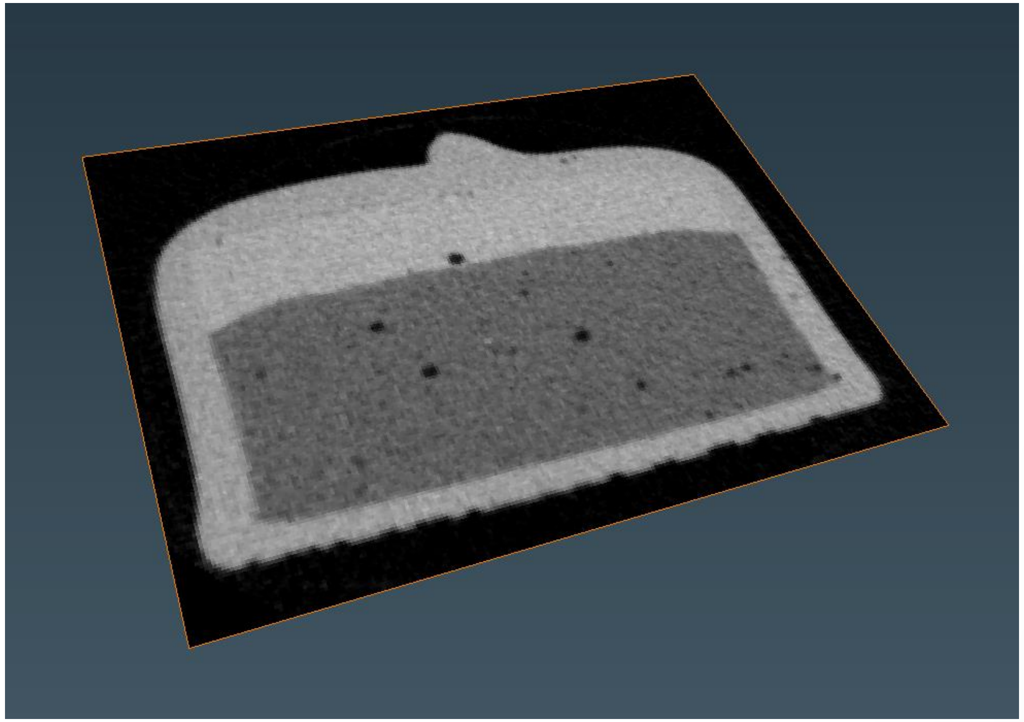


Image gradient

A 2D Image can be viewed as a Height Map. The gradient of an image, computed in each point as the first order spatial derivatives along the x and y directions (dx and dy) gives an estimation of the slope of the equivalent landscape that the image might represent.

Grayscale Image



Gradient = First Order Derivatives

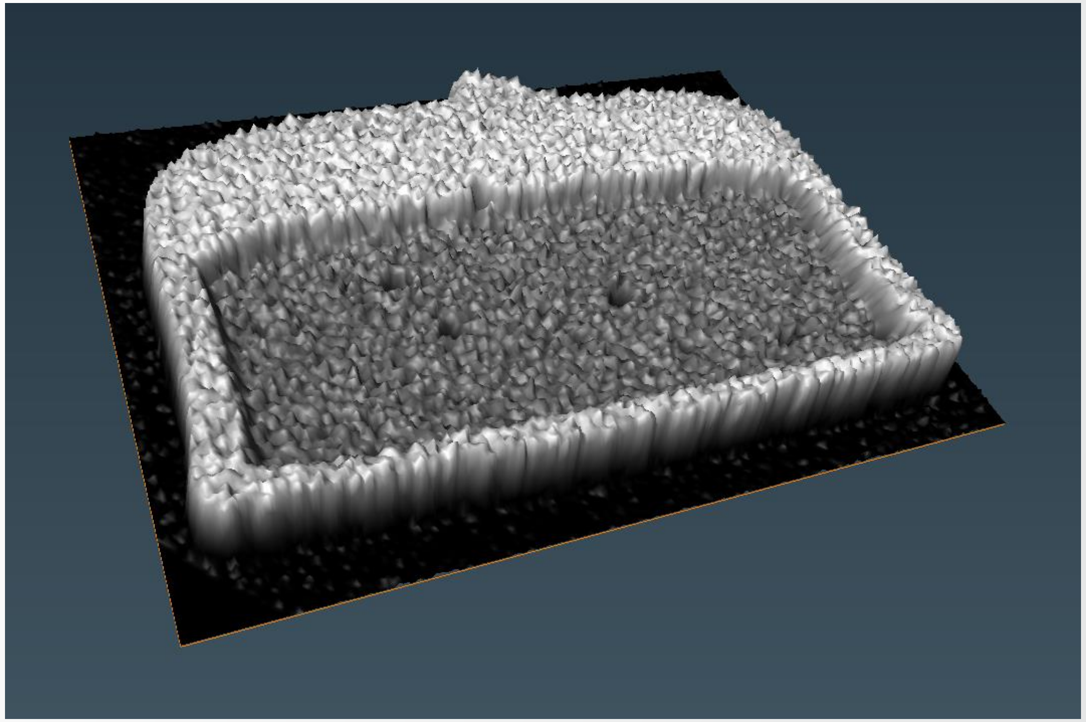


Image gradient

The gradient magnitude – computed as the squared root of the sum of the squared spatial derivatives allows an estimation of the slope steepness.

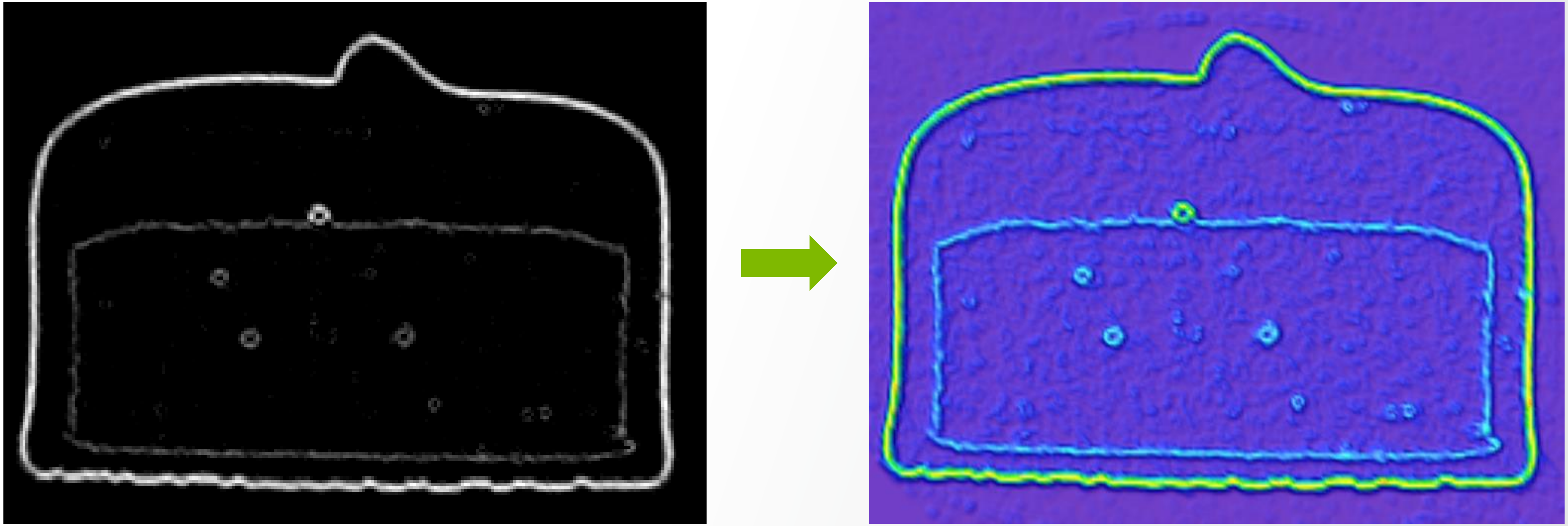
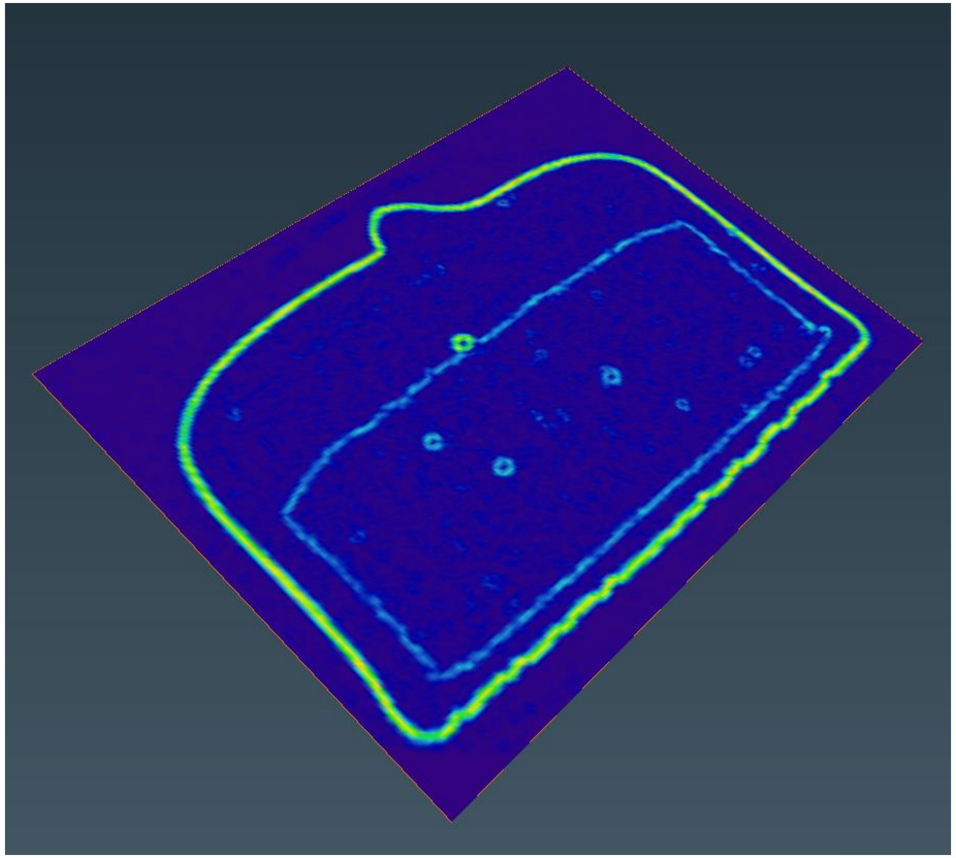
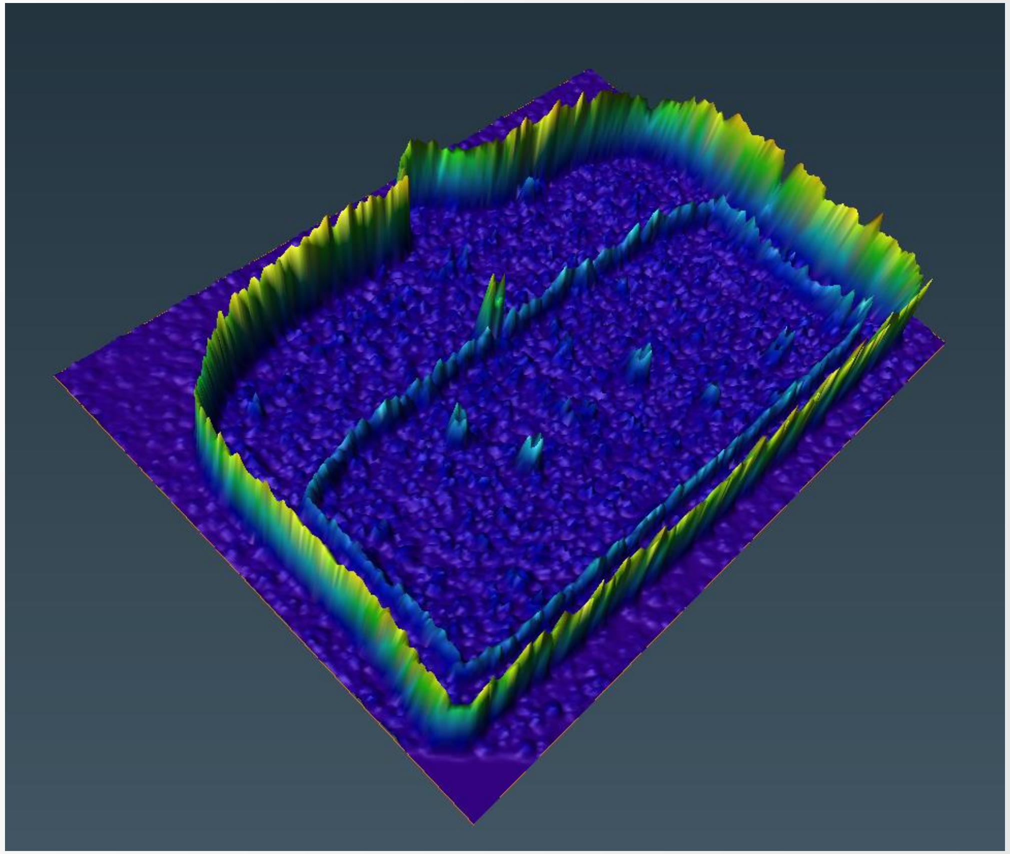


Image gradient

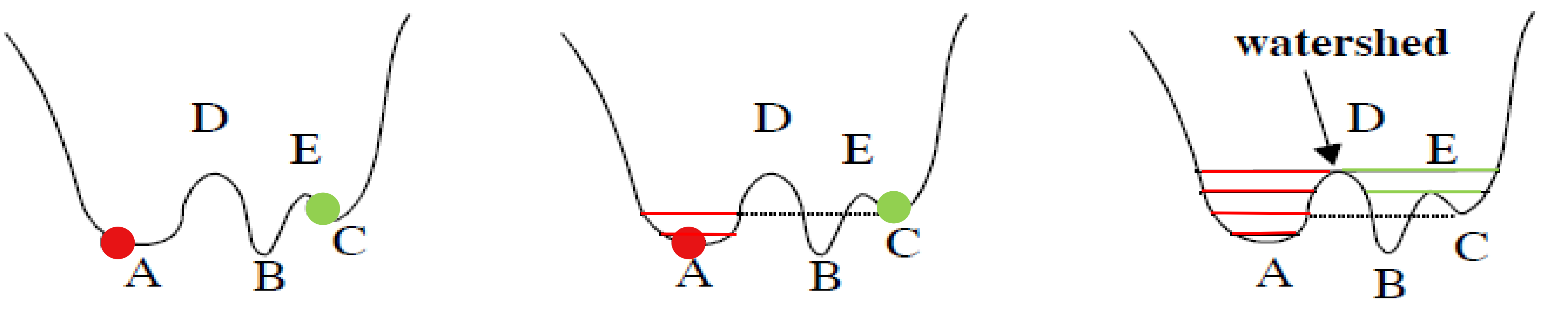
Gradient Magnitude = local steepness ~= contours





Watershed for image segmentation

- Transform grayscale image into gradient image (topographic surface)
- Typically: use the gradient magnitude as landscape image
- Simulate flooding of water (markers) in the landscape image
- Start from low level landscape (local minima)
- Fill the watershed into the basins with respective markers until reach the watershed line (local maxima)



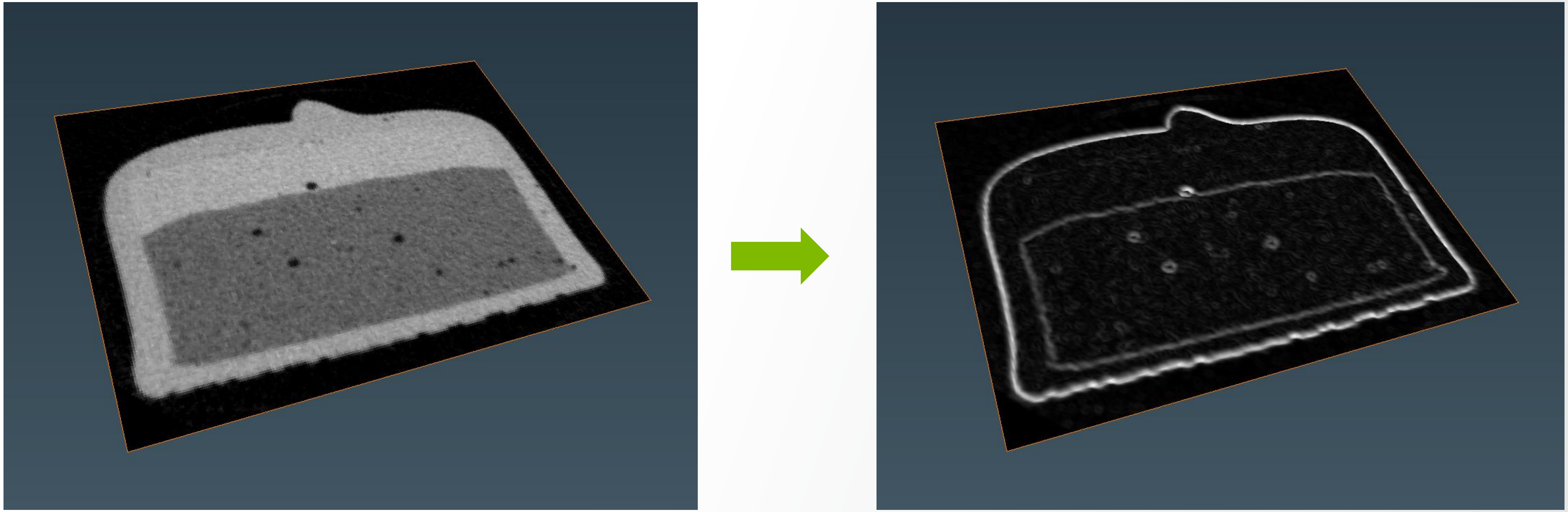
Markers

B,C: Local minima in the landscape image

D,E: Ridges (local maxima) in the landscape image

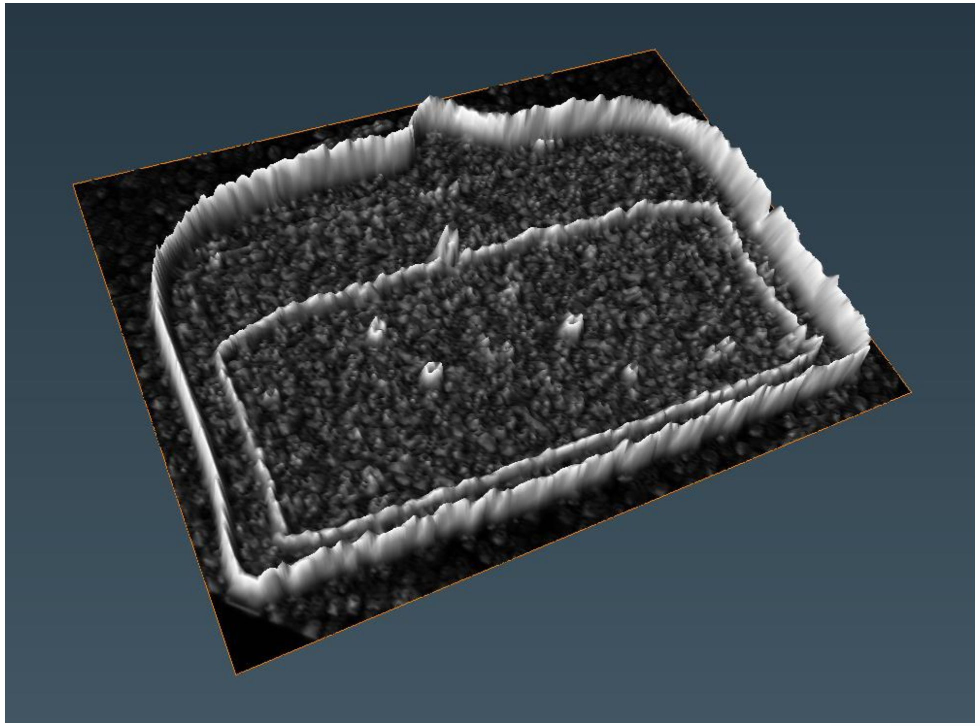
Watershed for image segmentation

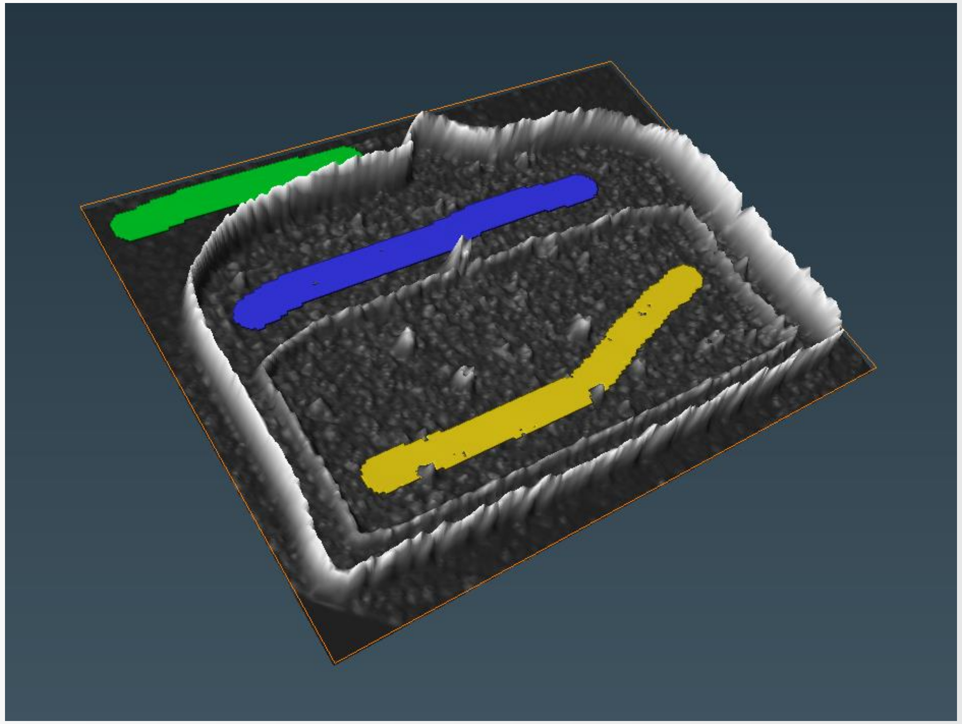
- Transform grayscale image into gradient image (topographic surface)
- Typically: use the gradient magnitude as landscape image



Watershed for image segmentation

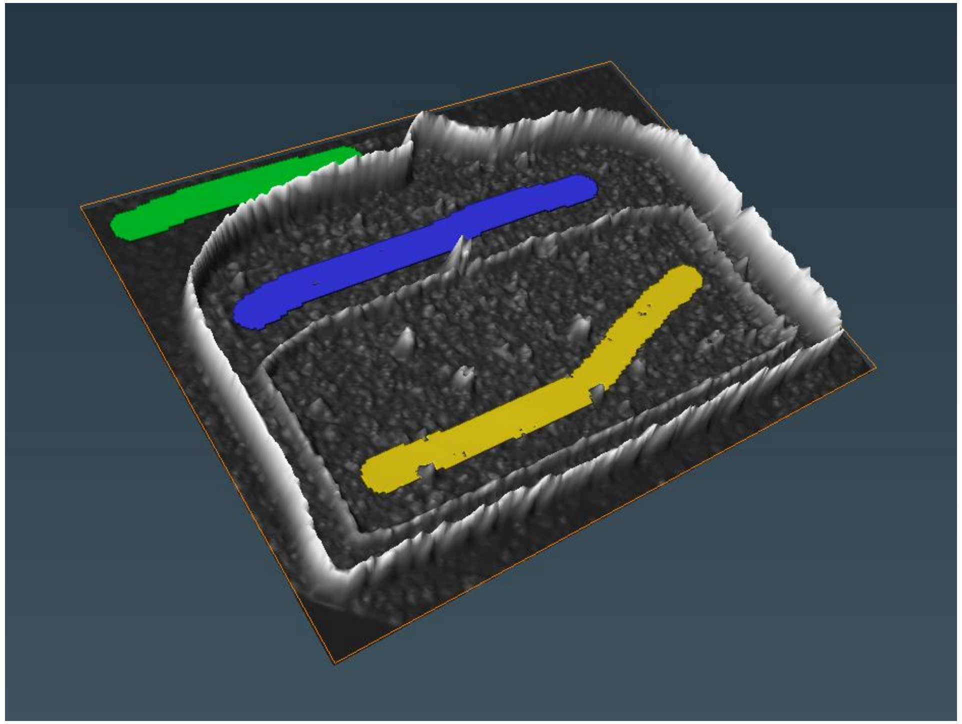
- Simulate flooding of water (markers: Green, Blue, Yellow) in the landscape image
- Start from low level landscape (local minima)

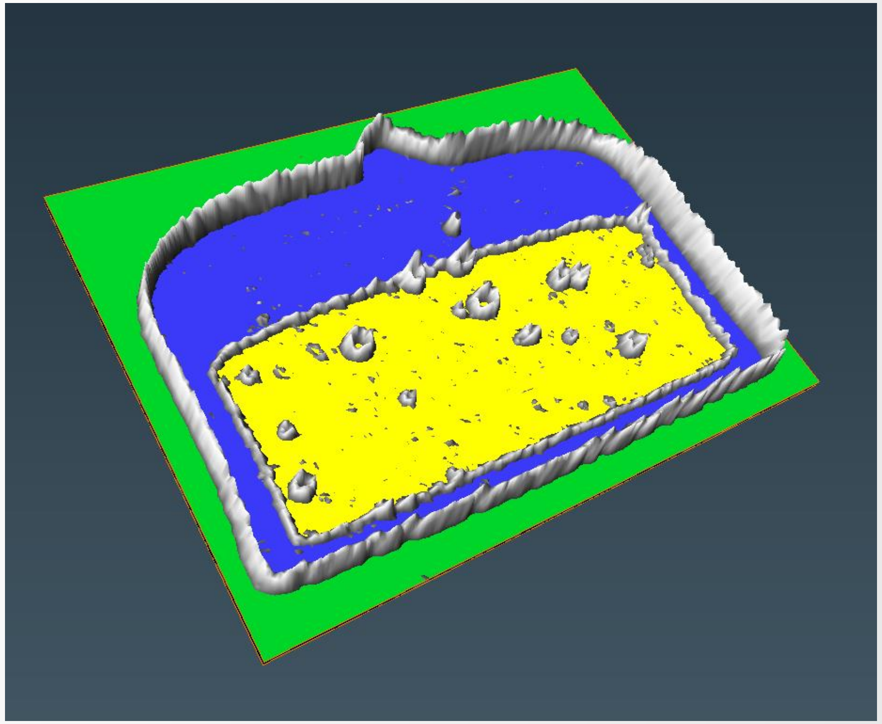




Watershed for image segmentation

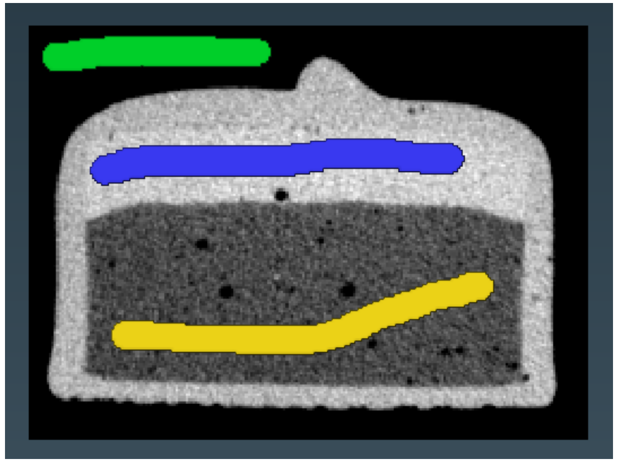
- Fill the watershed into the basins with respective markers (Green, Blue, Yellow) until reach the watershed line (local maxima) where two marker sources meet

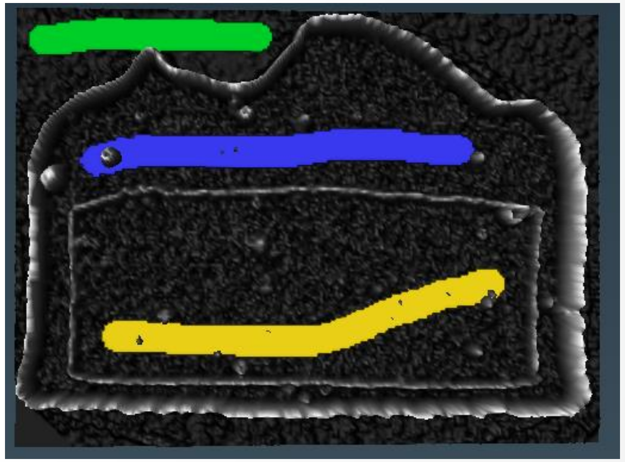


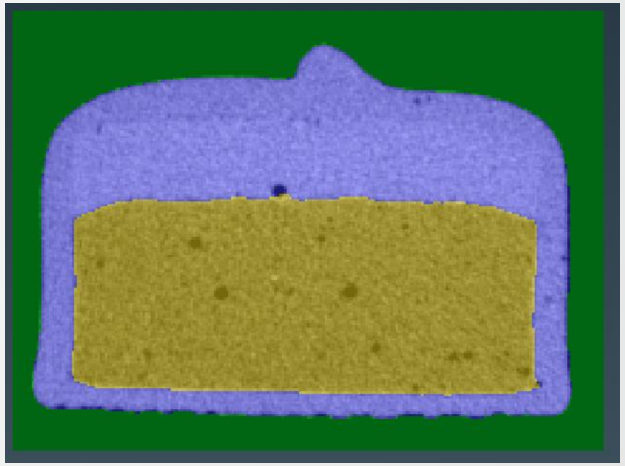


Marker-based Watershed: Segmentation Editor

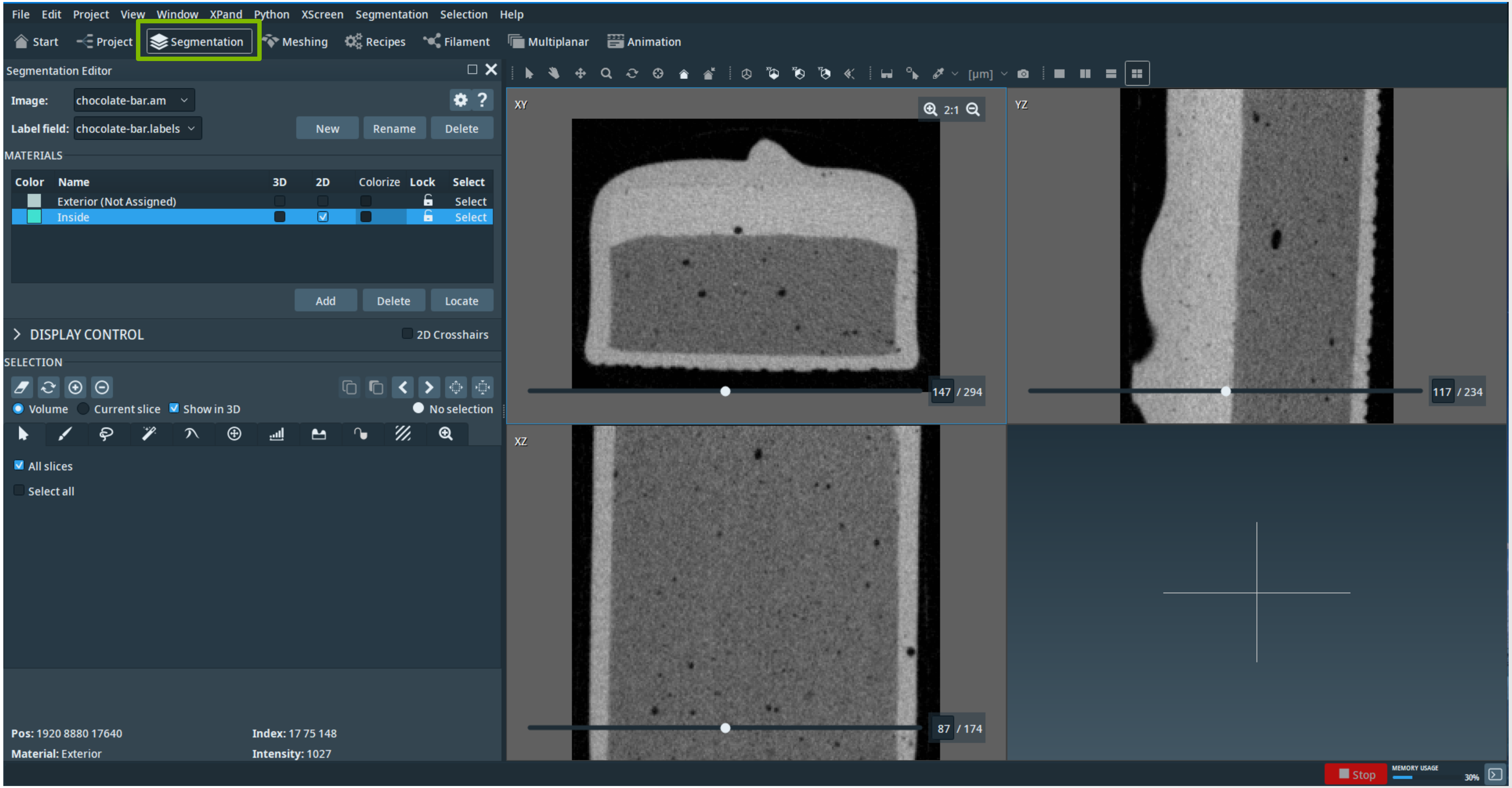
- Start with user-defined markers (e.g., brush tool)
- Compute the watersheds (basins) separating the markers (seeds)
- Create a landscape image (Gradient Image) once
- Grow the markers in each Watershed basin







Marker-based Watershed: Segmentation Workroom



Segmentation Editor: 3D Viewer position

The default **3D Viewer position** in Avizo is “Upper Left”. You can switch it to “Bottom Right” from: Edit -> Preferences ->Segmentation

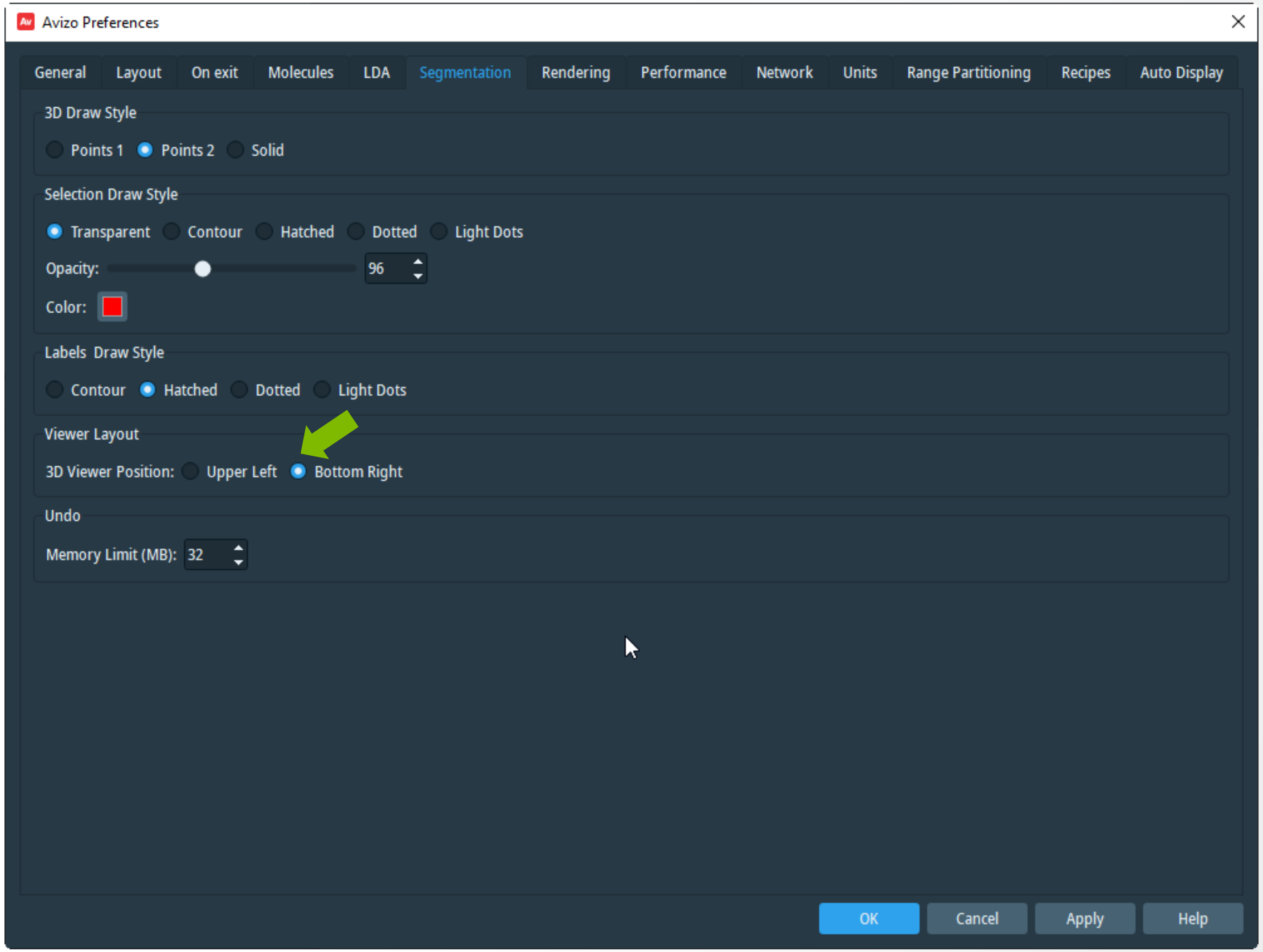


Image segmentation: Marker-based Watershed

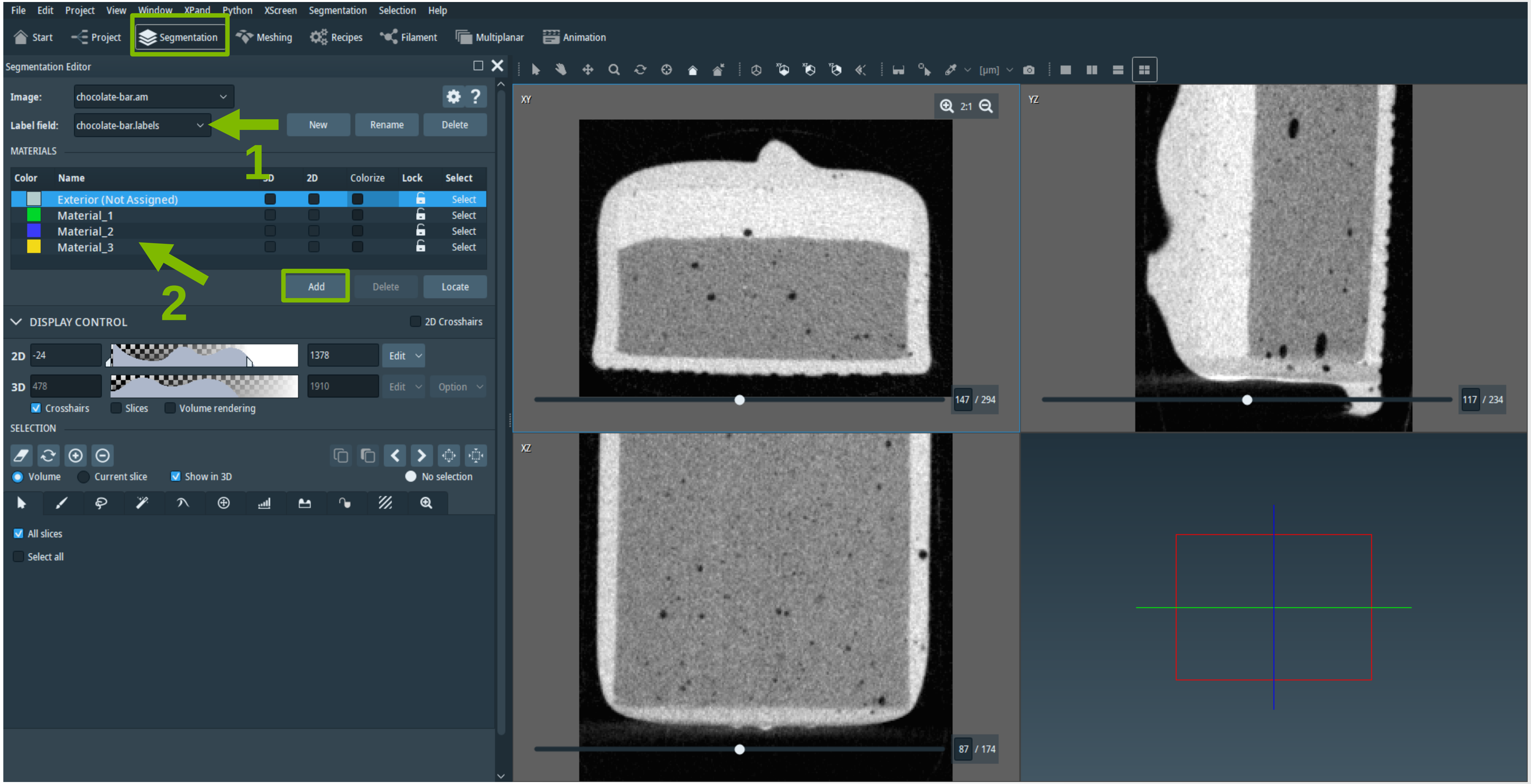


Image segmentation: Marker-based Watershed

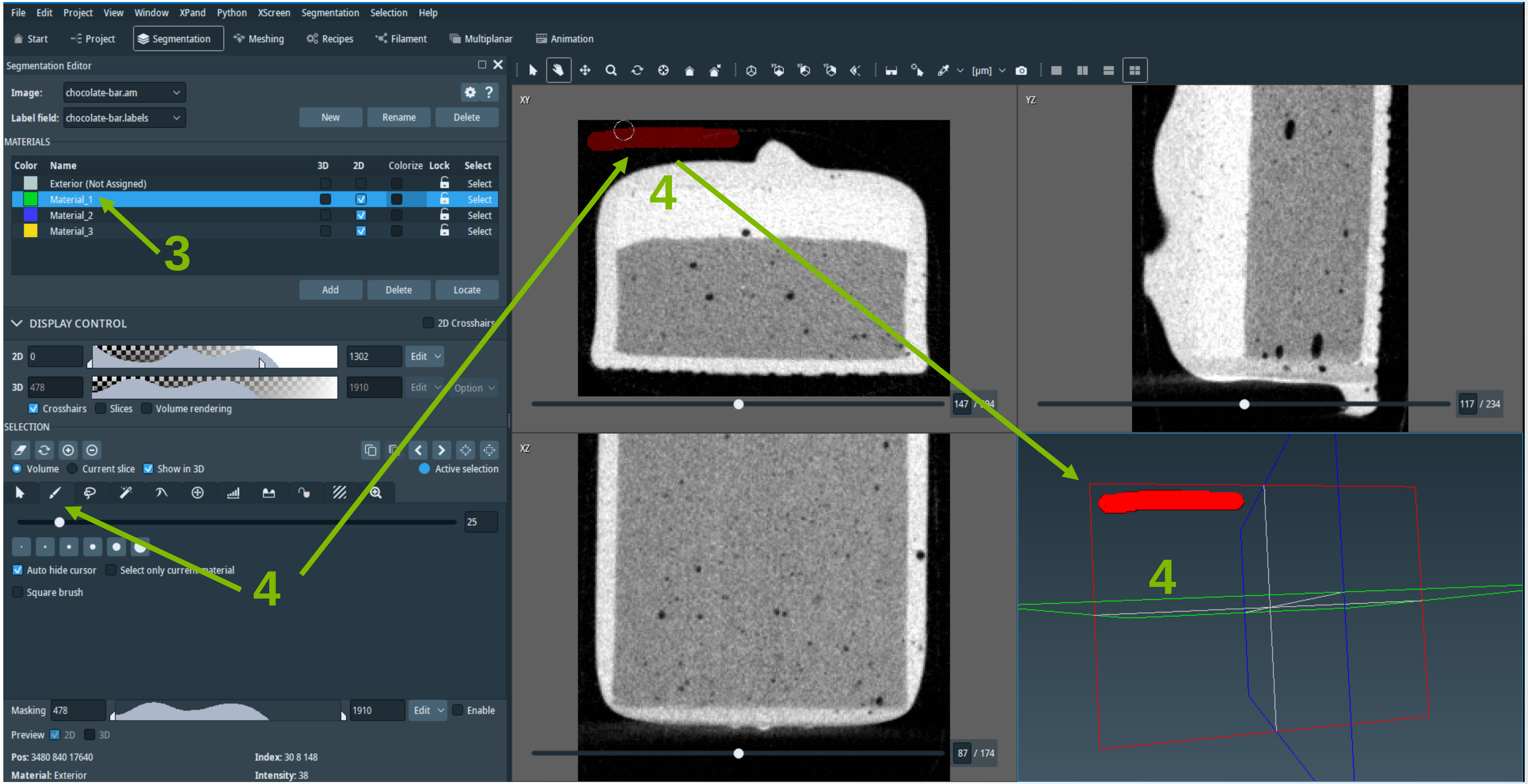


Image segmentation: Marker-based Watershed

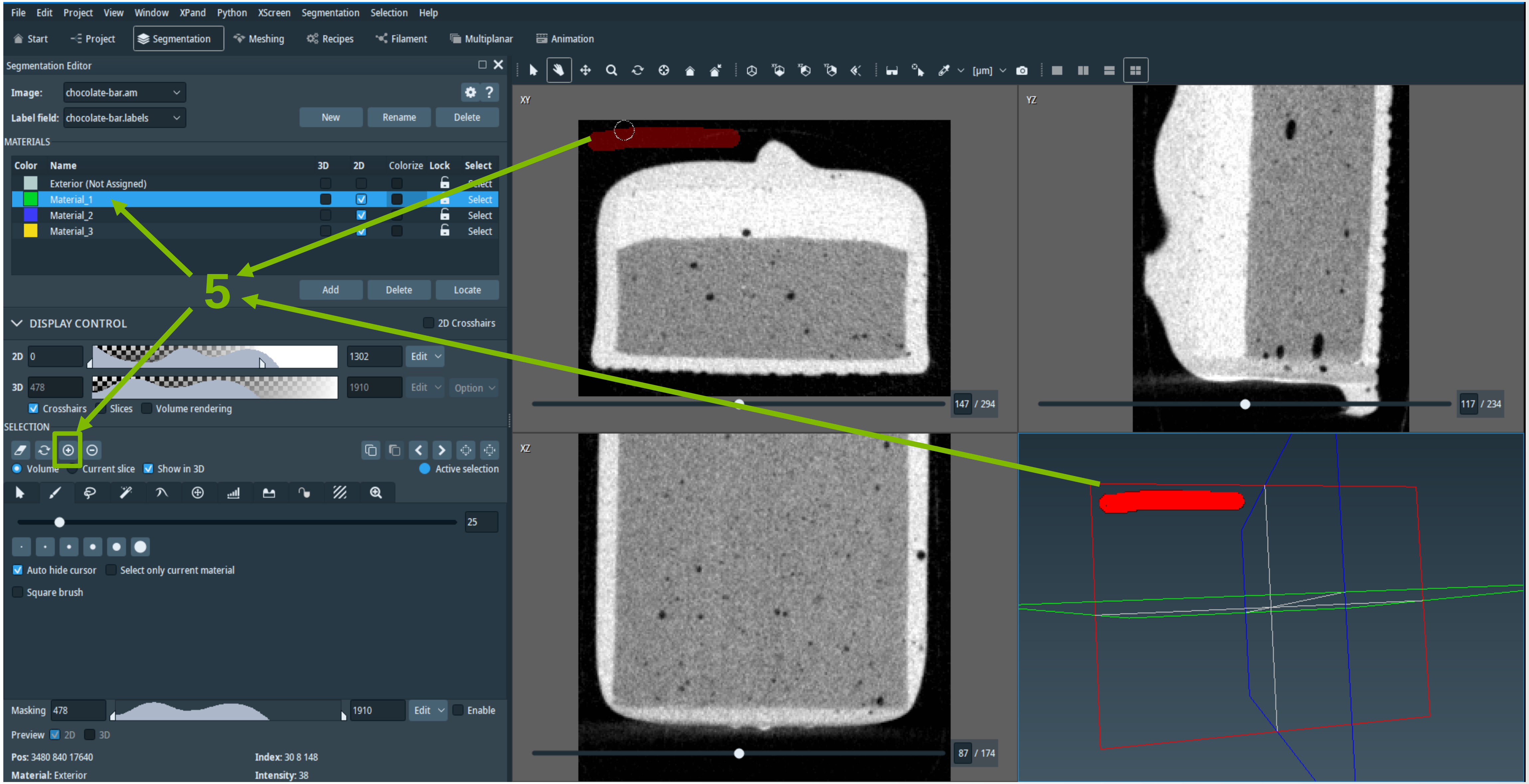


Image segmentation: Marker-based Watershed

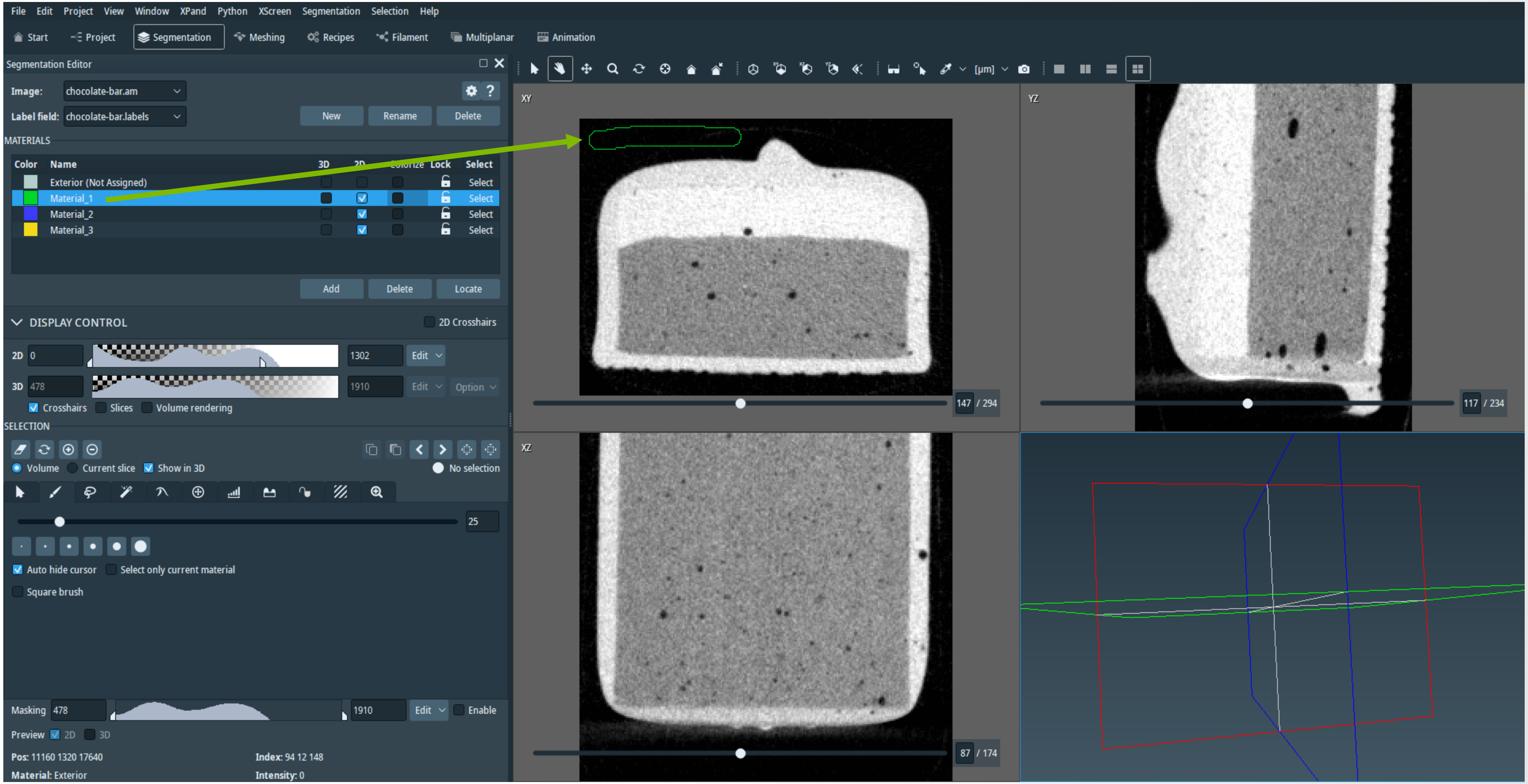


Image segmentation: Marker-based Watershed

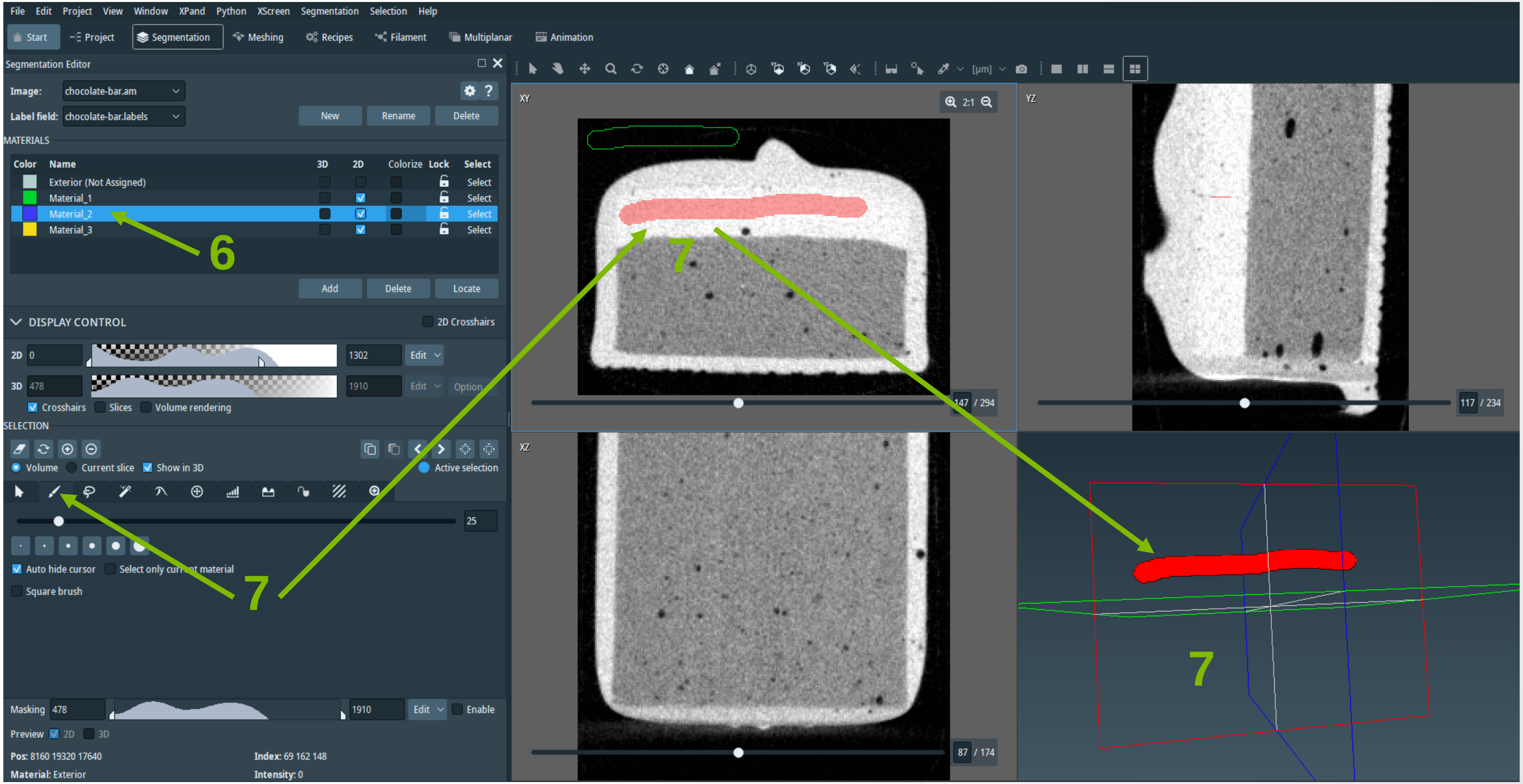


Image segmentation: Marker-based Watershed

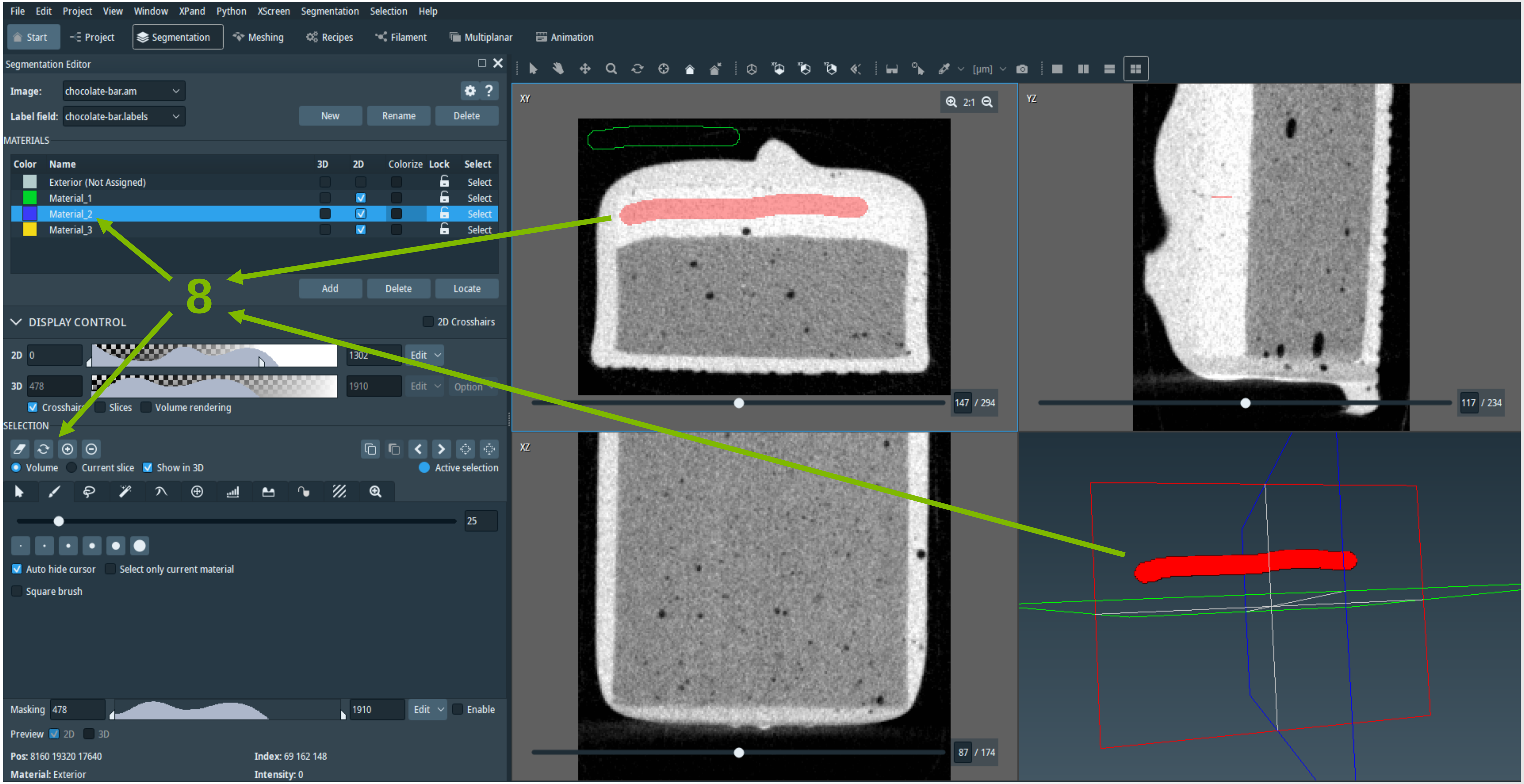


Image segmentation: Marker-based Watershed

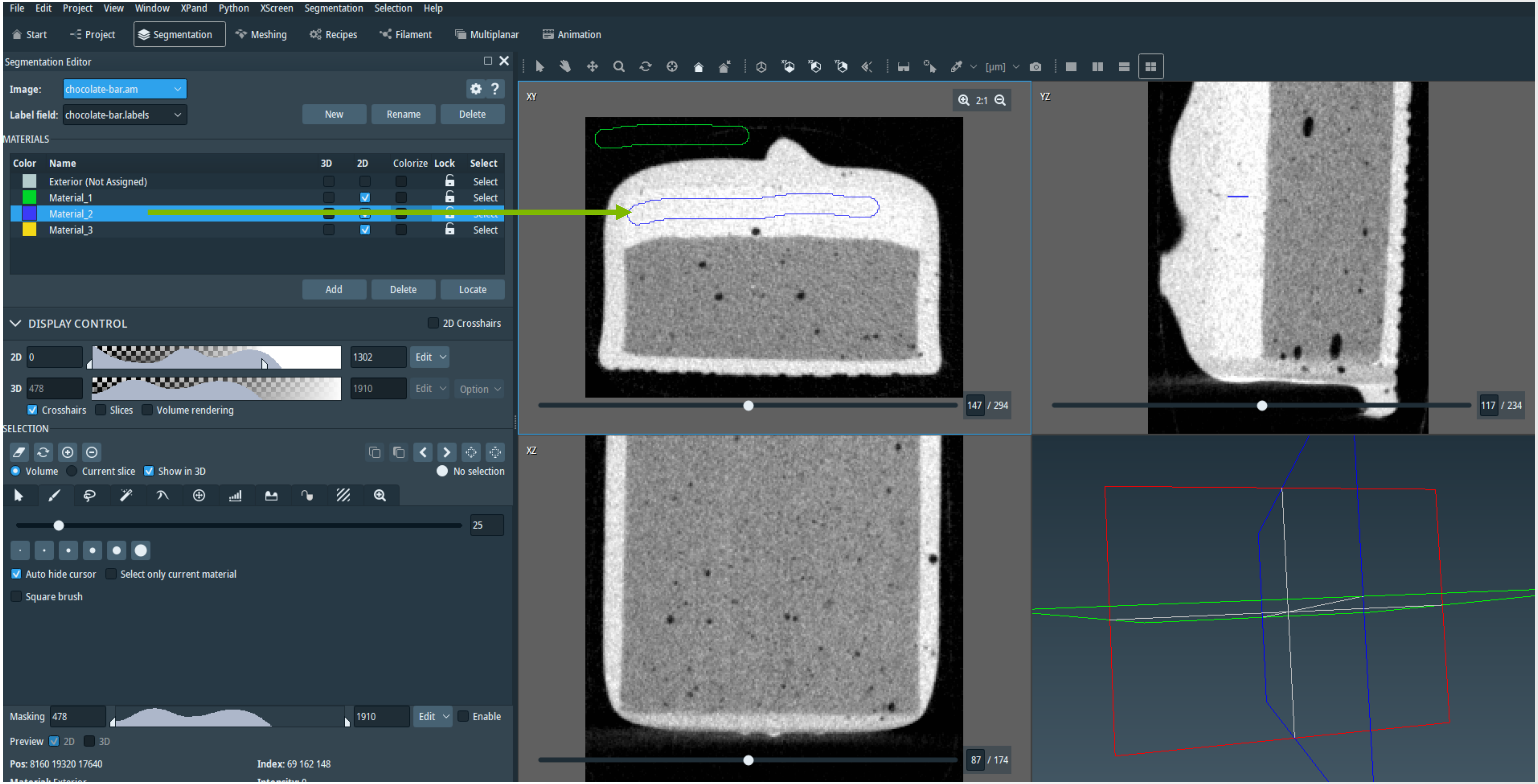


Image segmentation: Marker-based Watershed

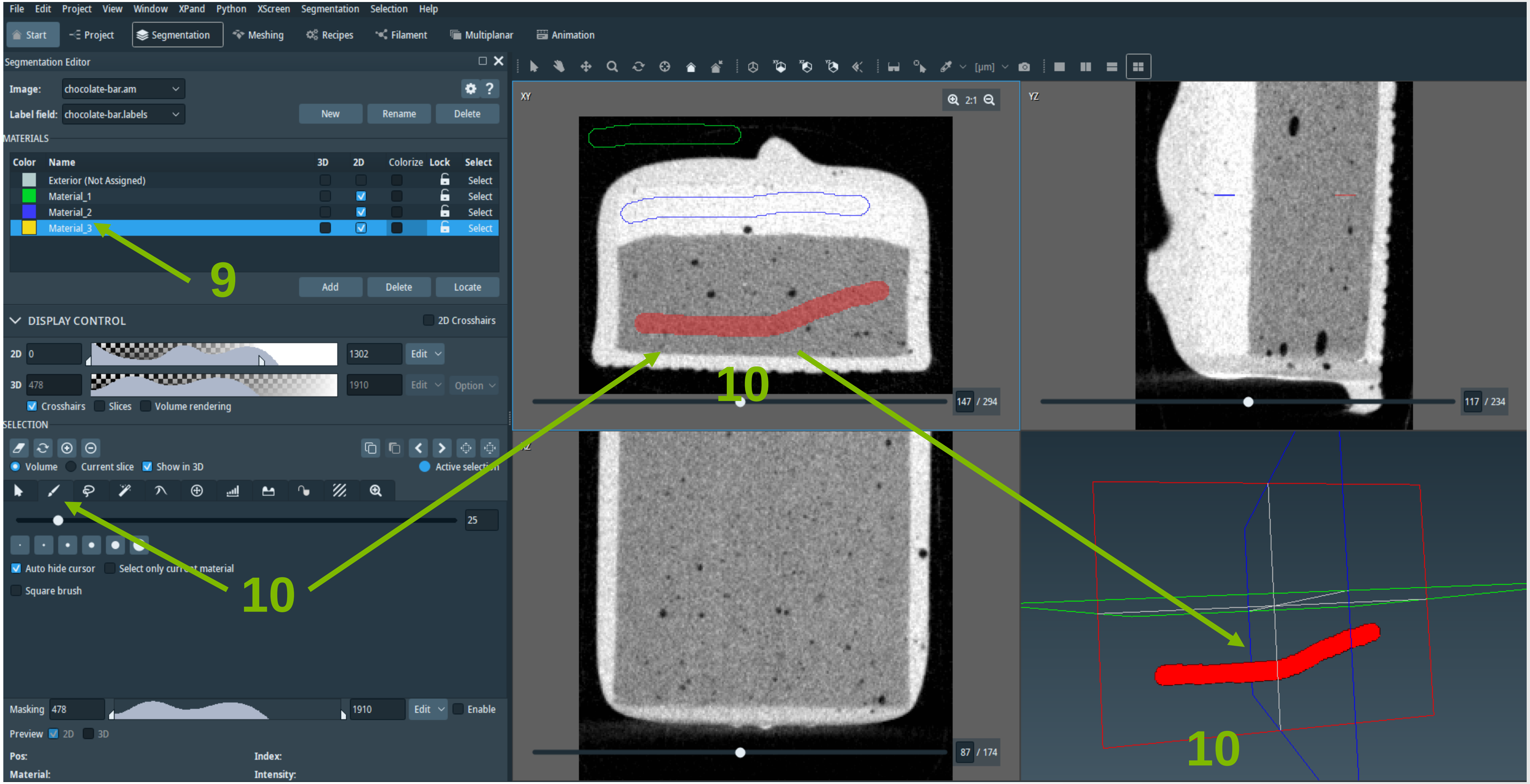


Image segmentation: Marker-based Watershed

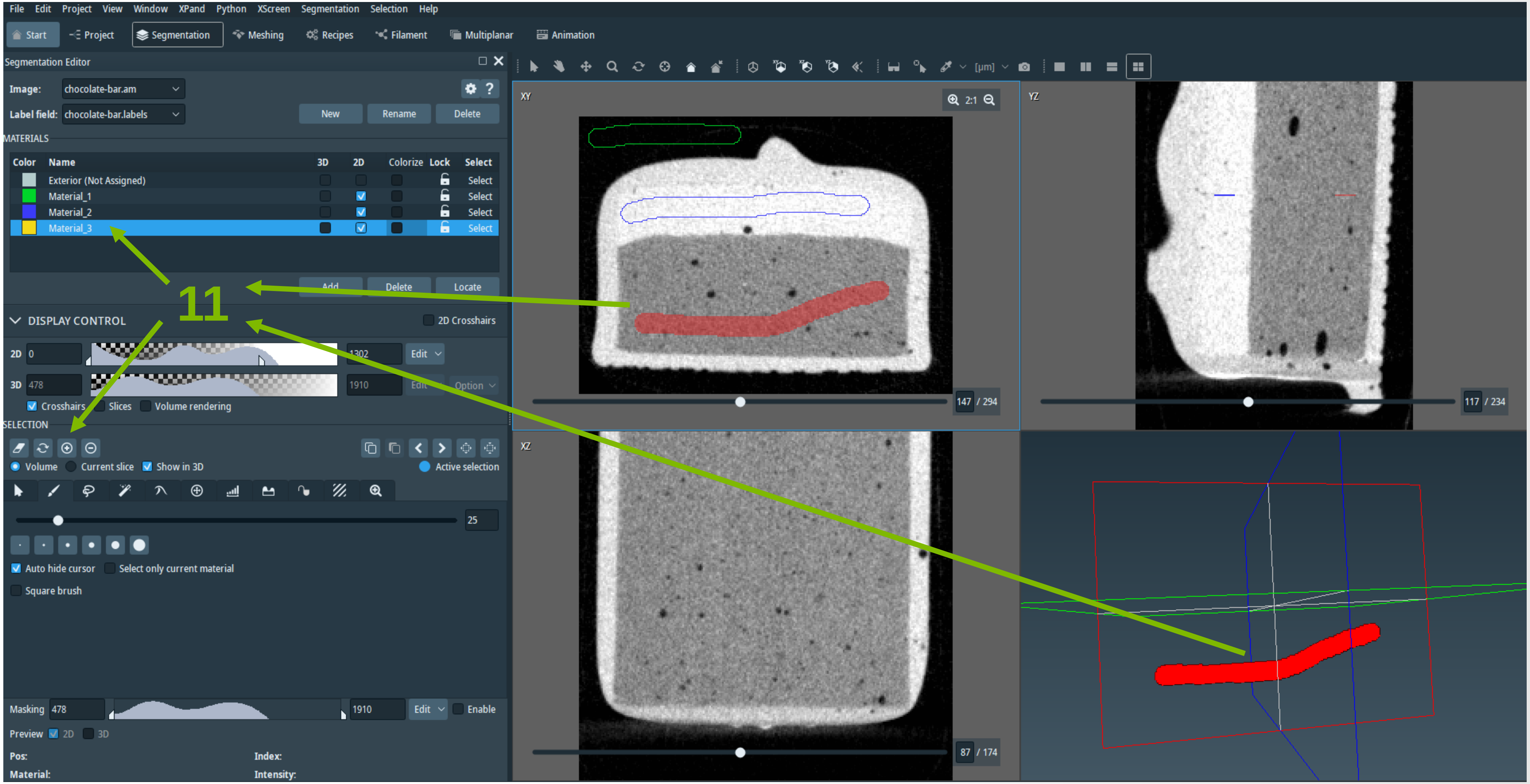


Image segmentation: Marker-based Watershed

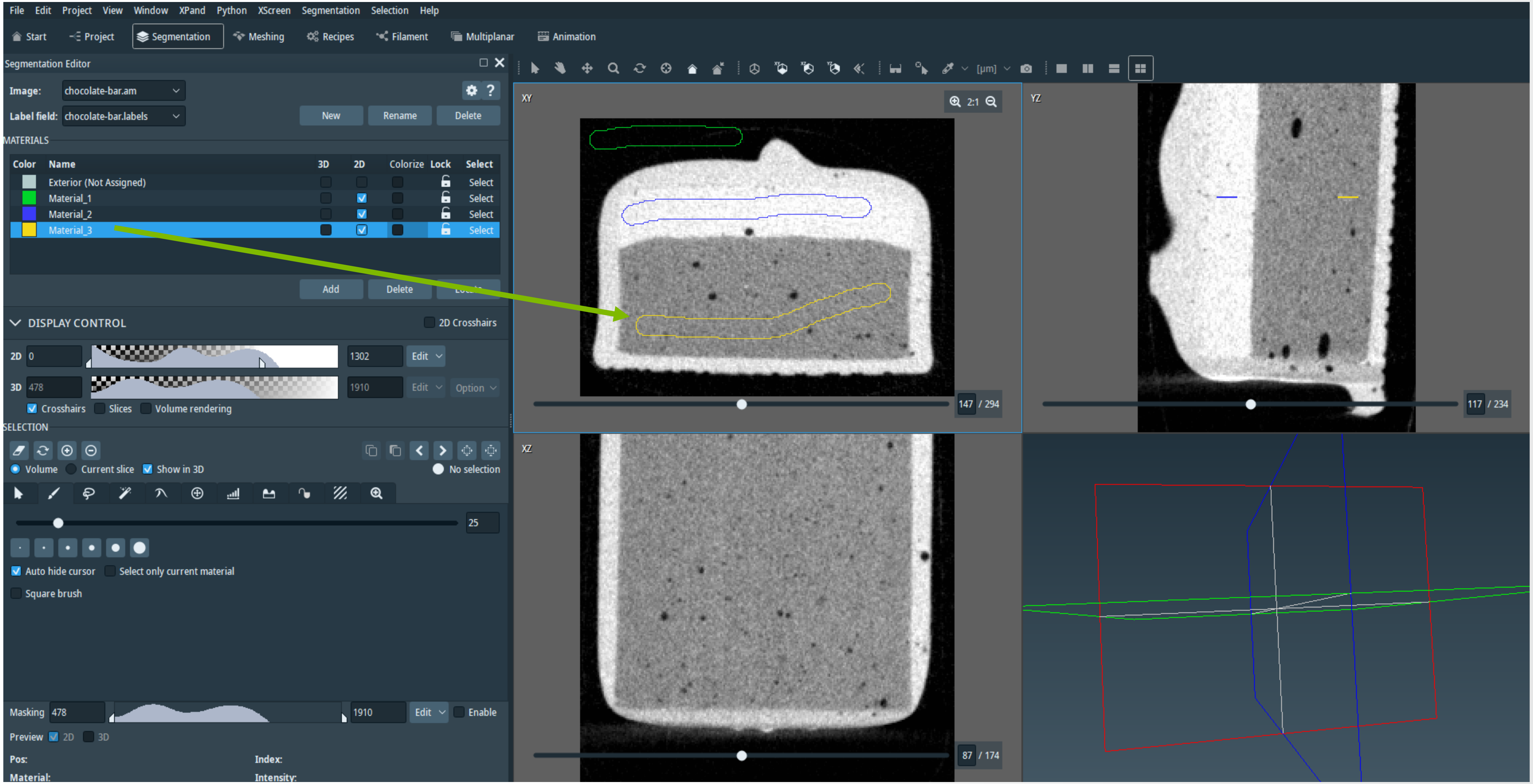


Image segmentation: Marker-based Watershed

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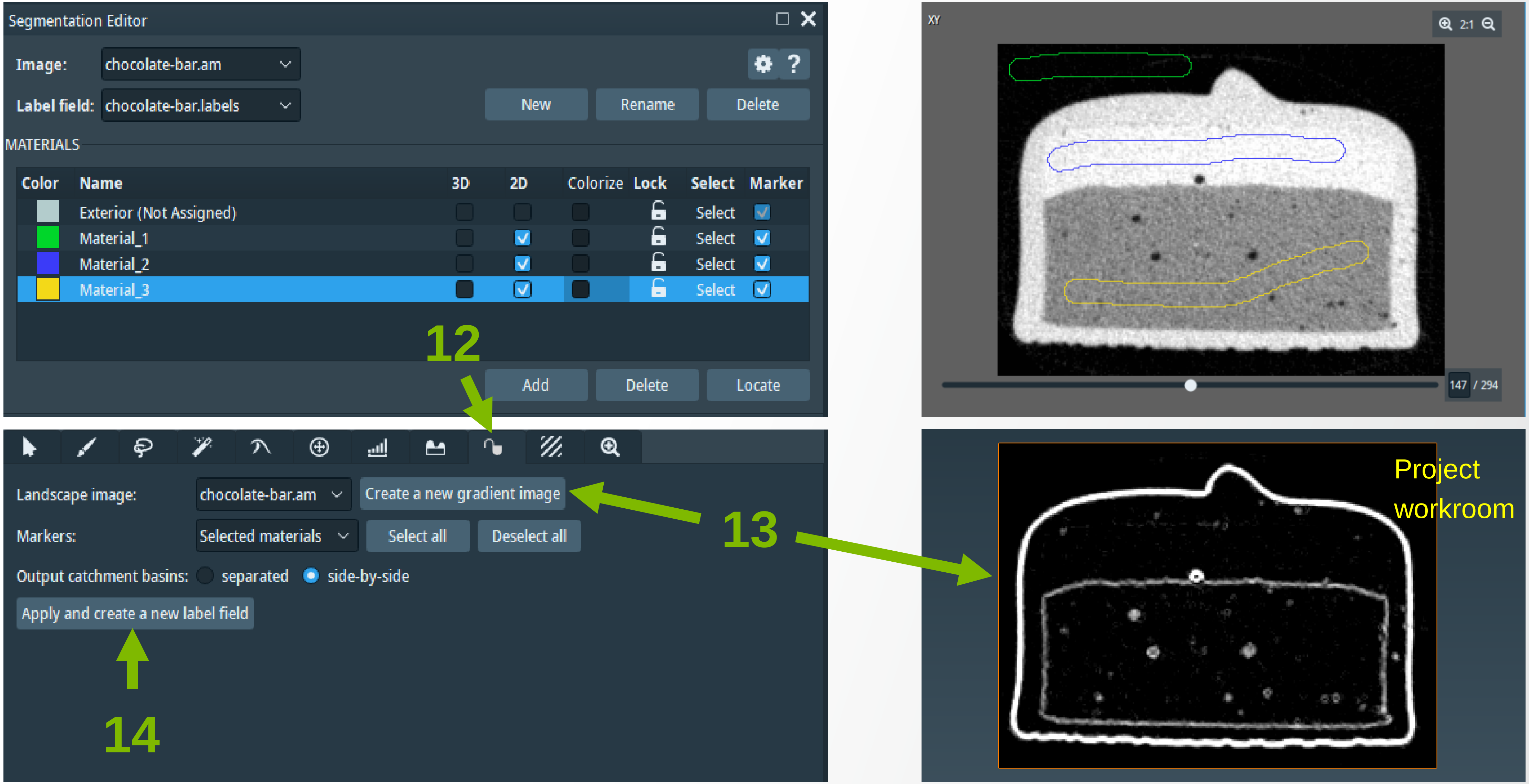


Image segmentation: Marker-based Watershed

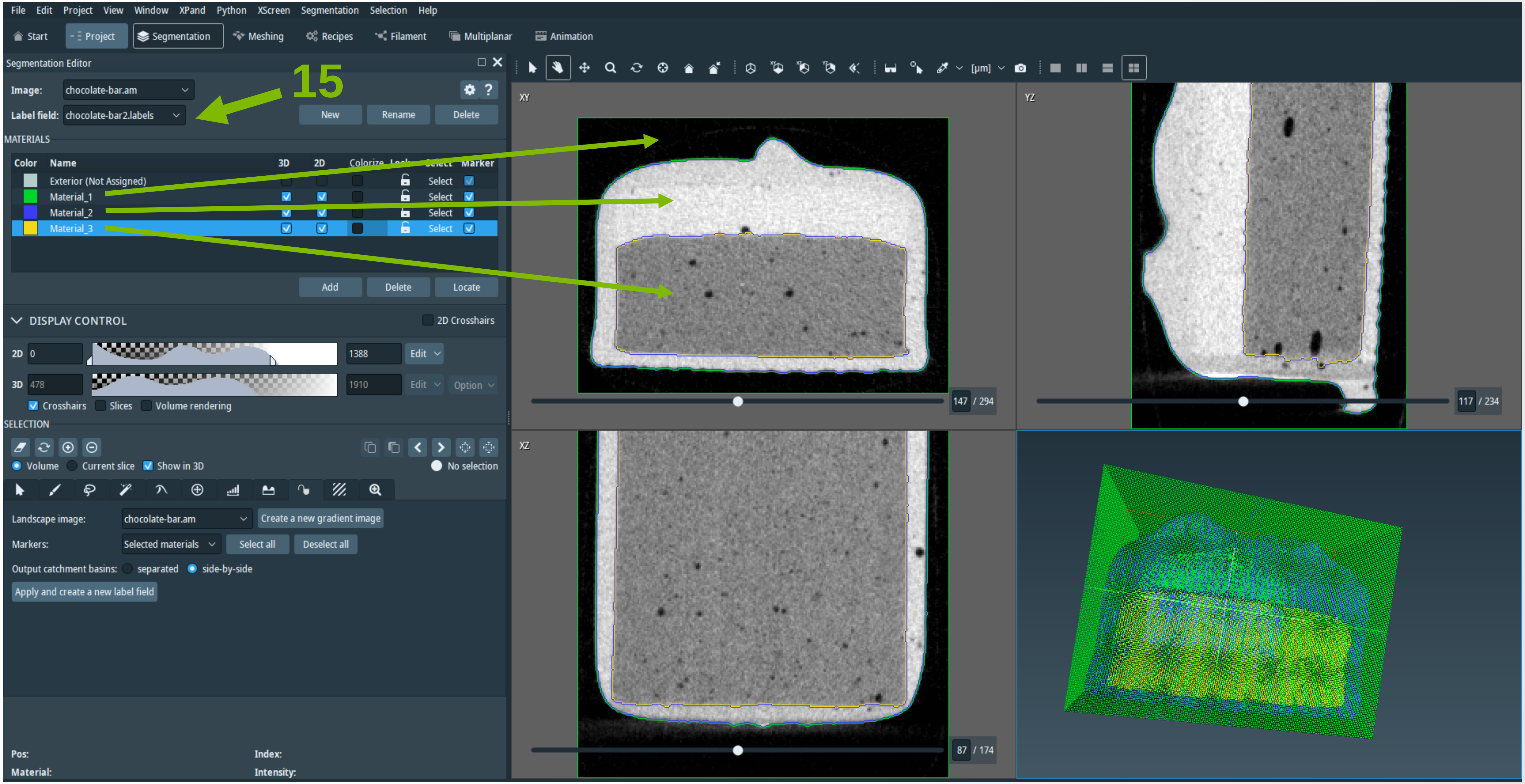
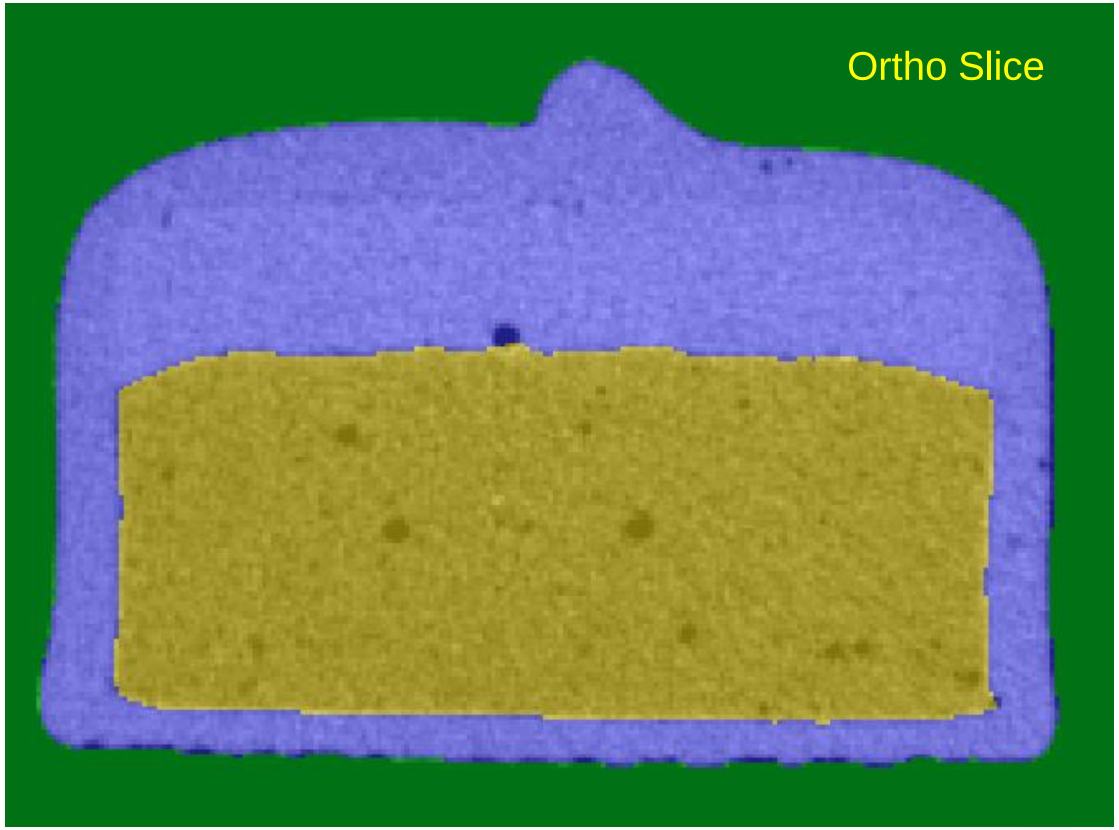
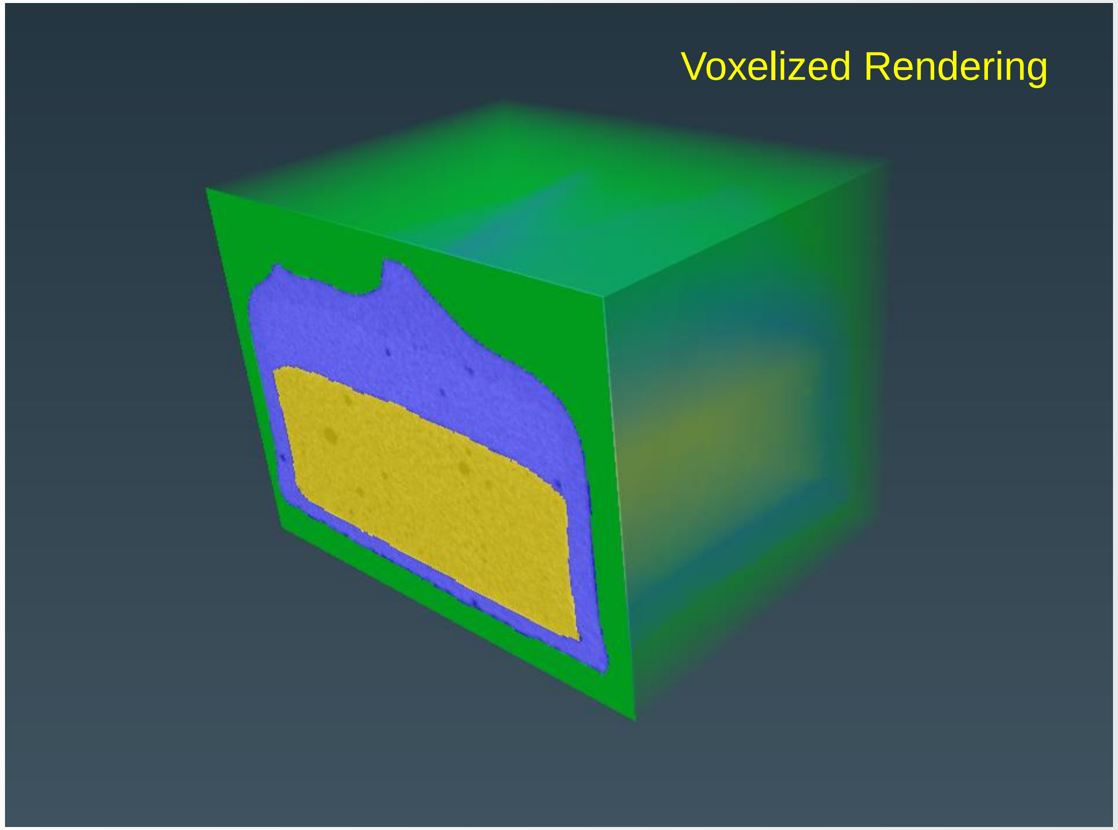


Image segmentation: Marker-based Watershed





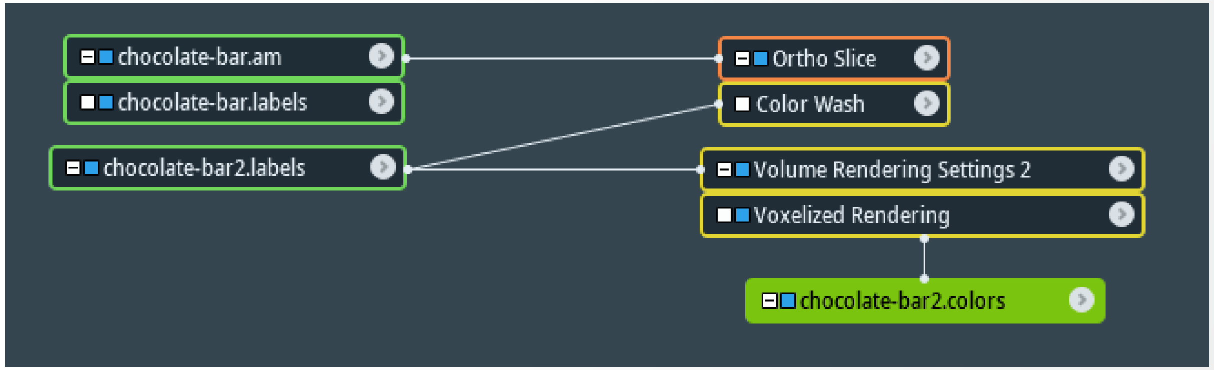
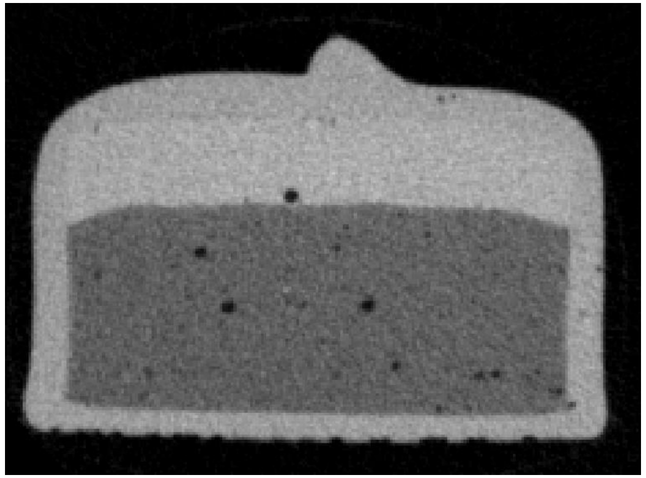
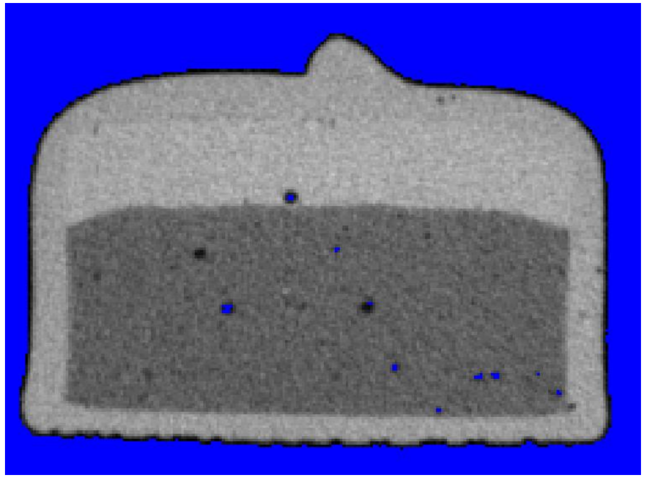
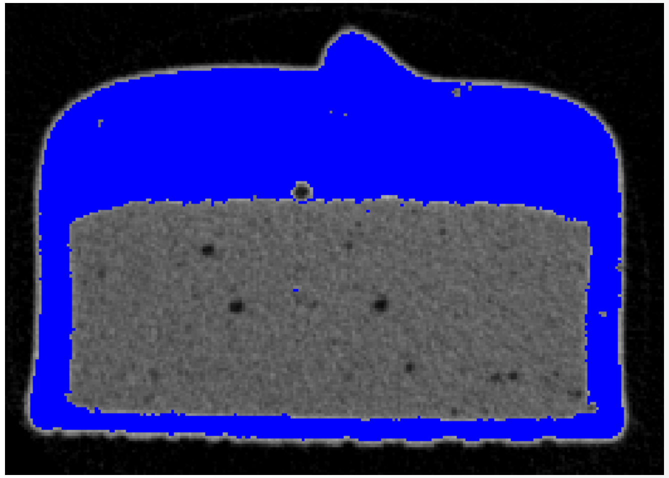


Image segmentation: Watershed Segmentation Wizard



- Define markers for phases via thresholding
- Mask out regions with high gradient magnitude
- Expand markers with watershed





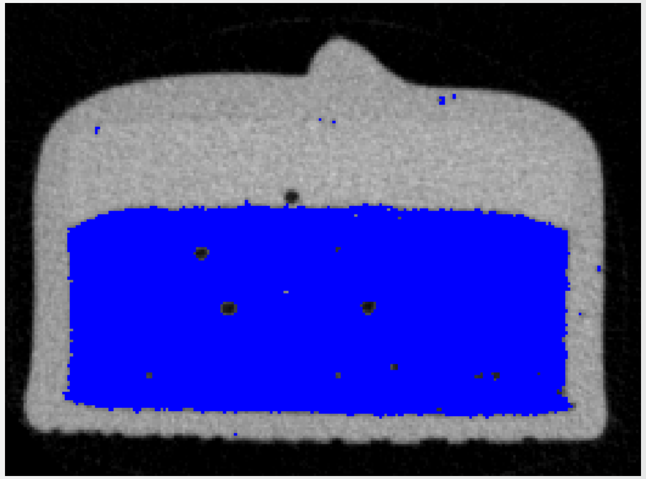
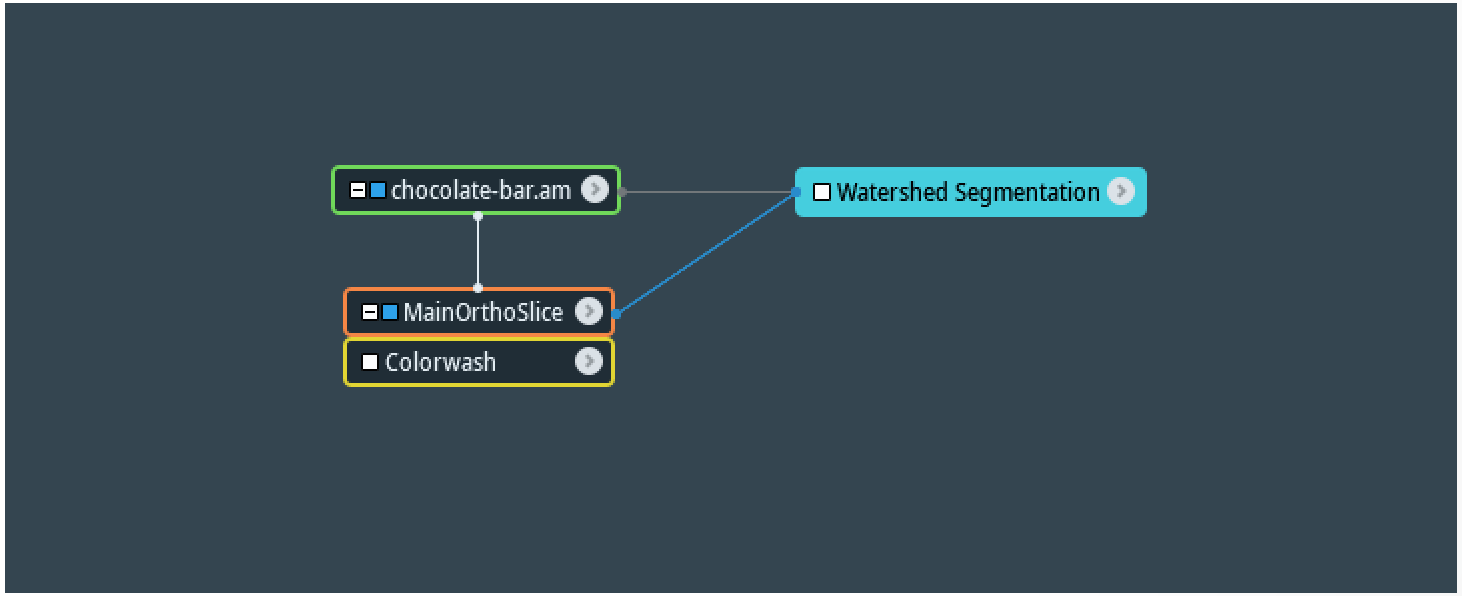
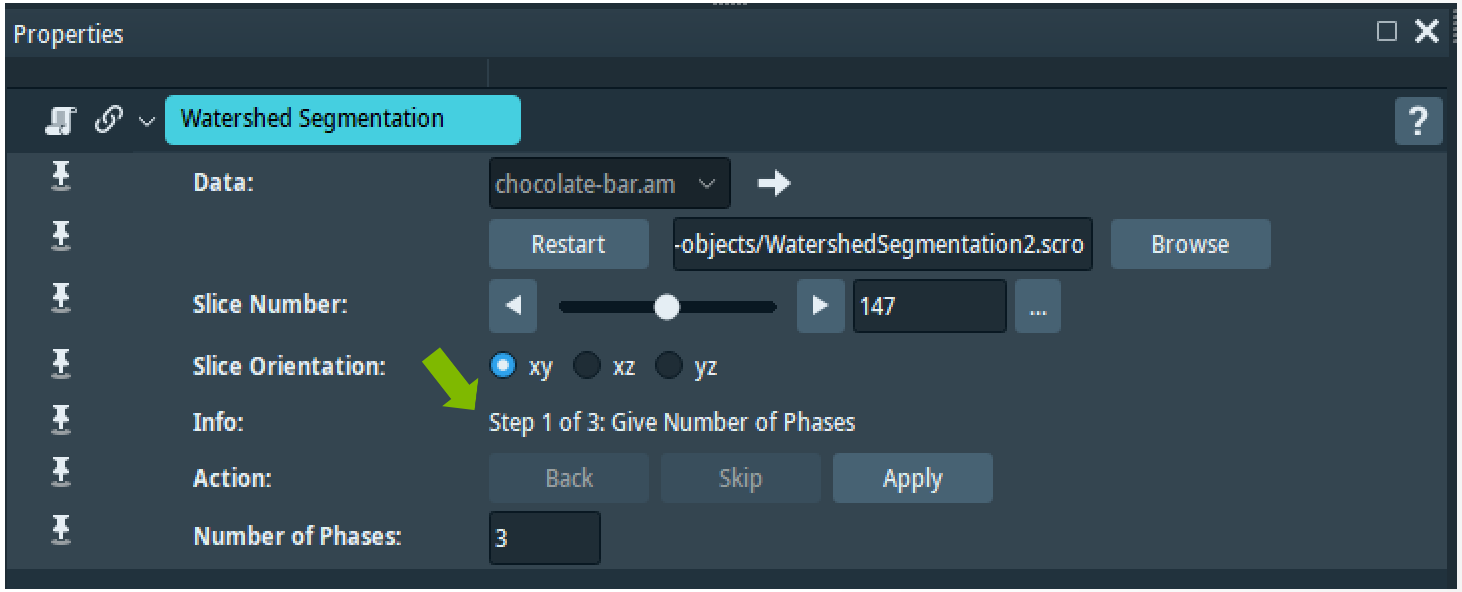


Image segmentation: Watershed Segmentation wizard





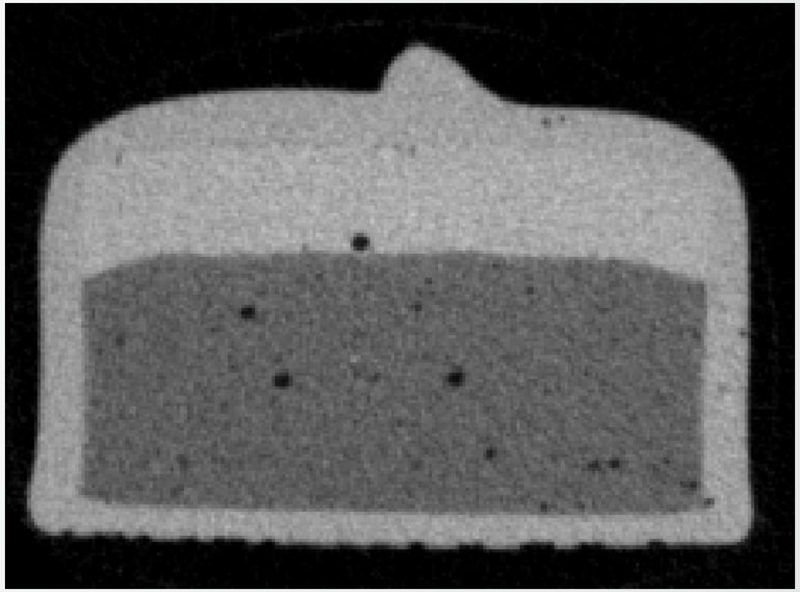
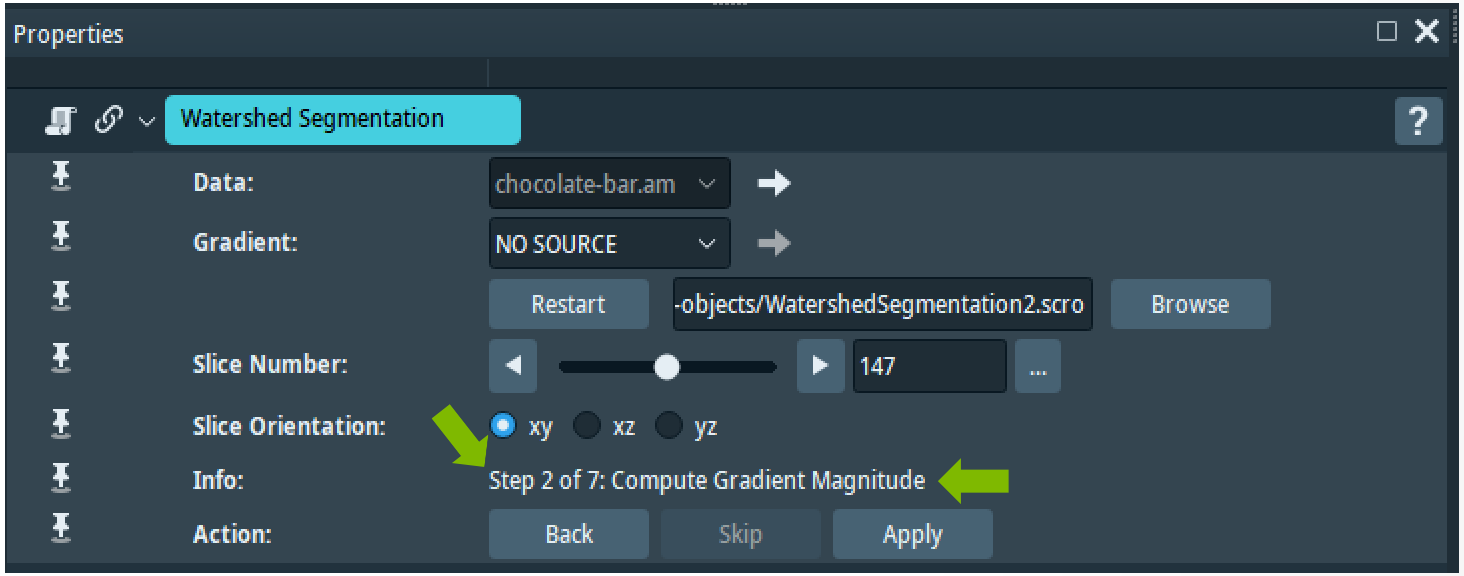


Image segmentation: Watershed Segmentation wizard



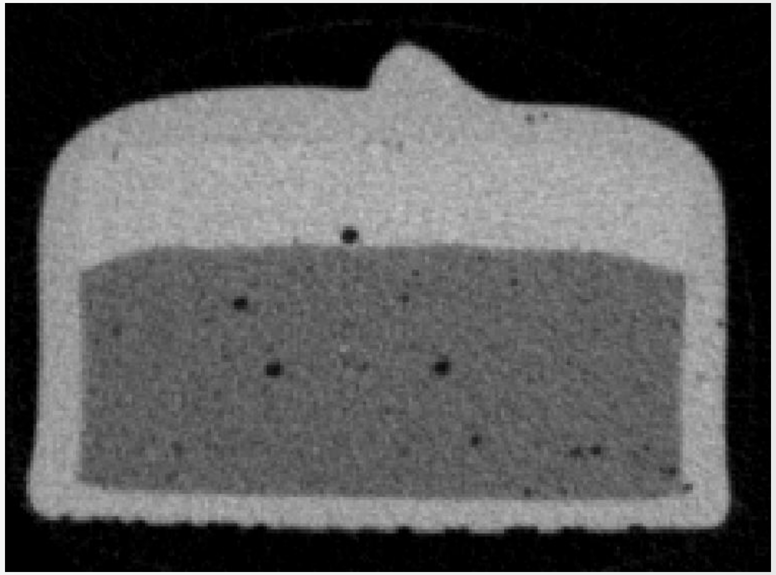
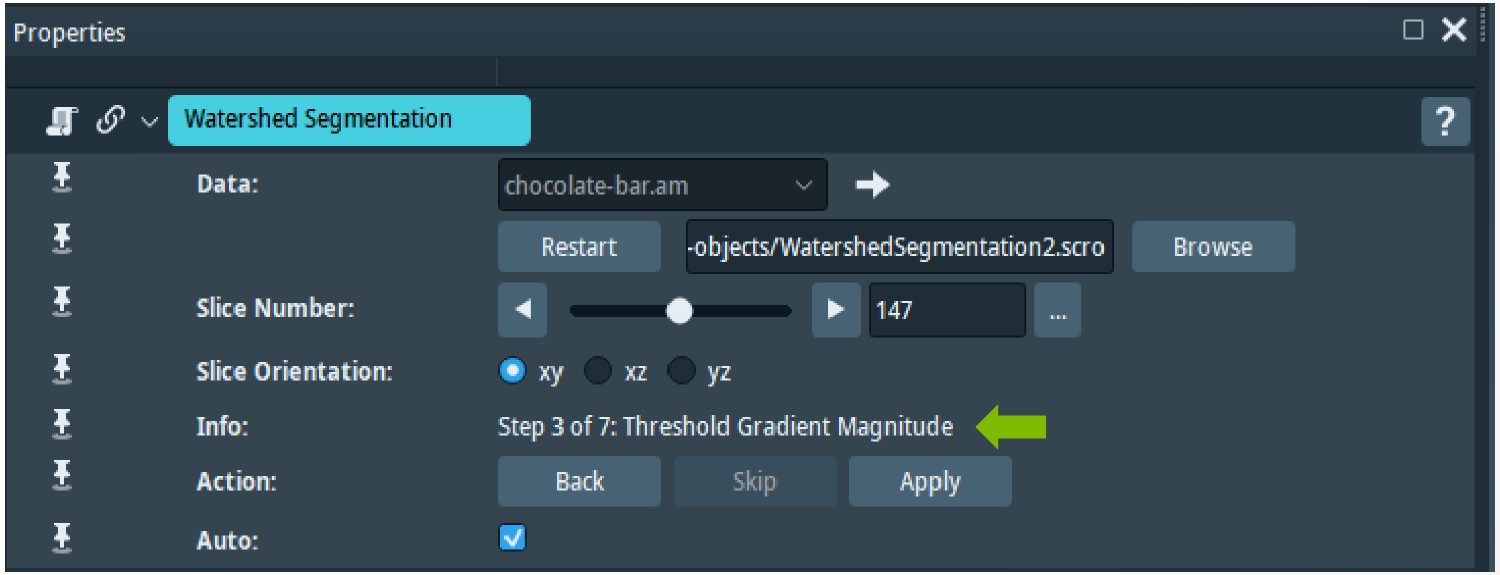


Image segmentation: Watershed Segmentation wizard



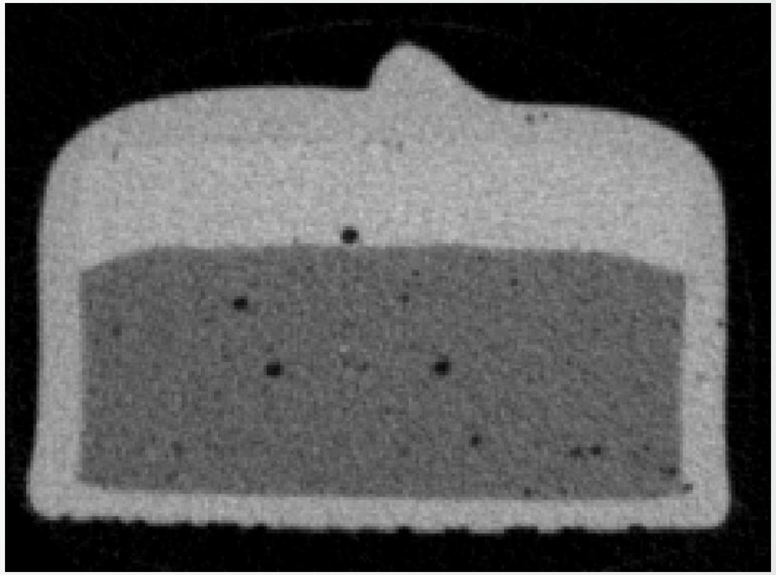
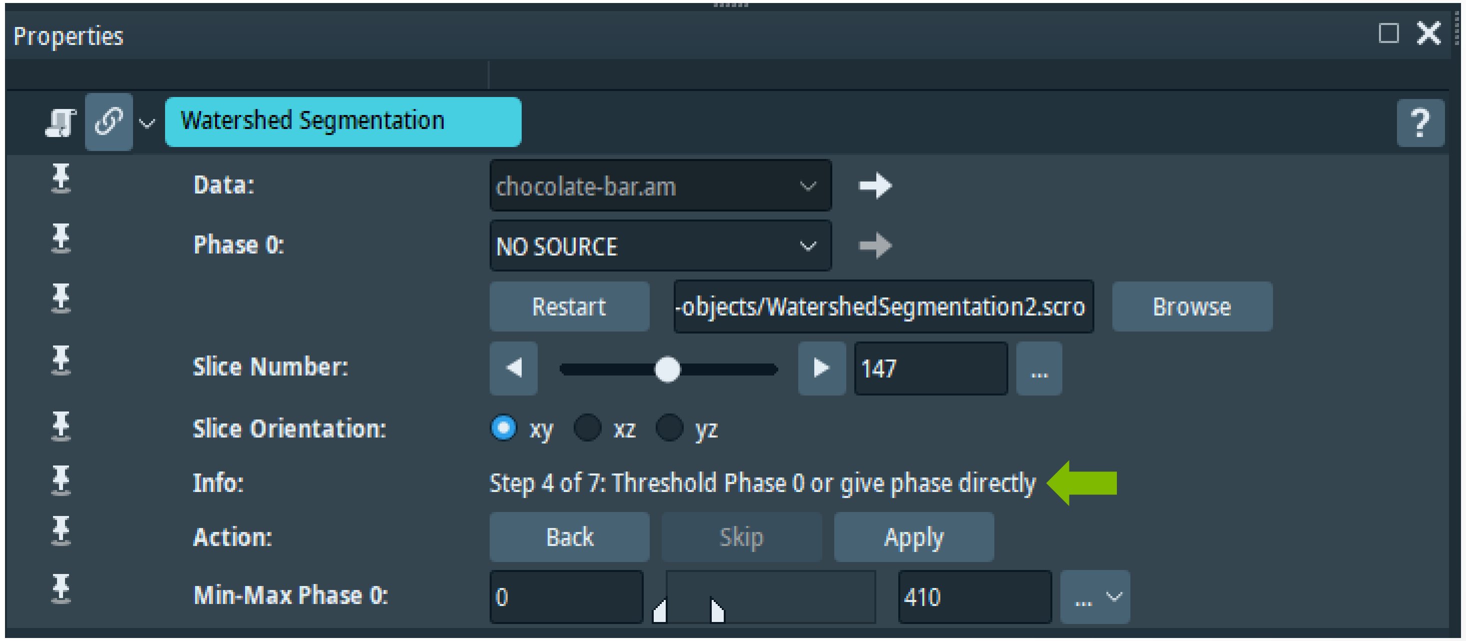


Image segmentation: Watershed Segmentation wizard



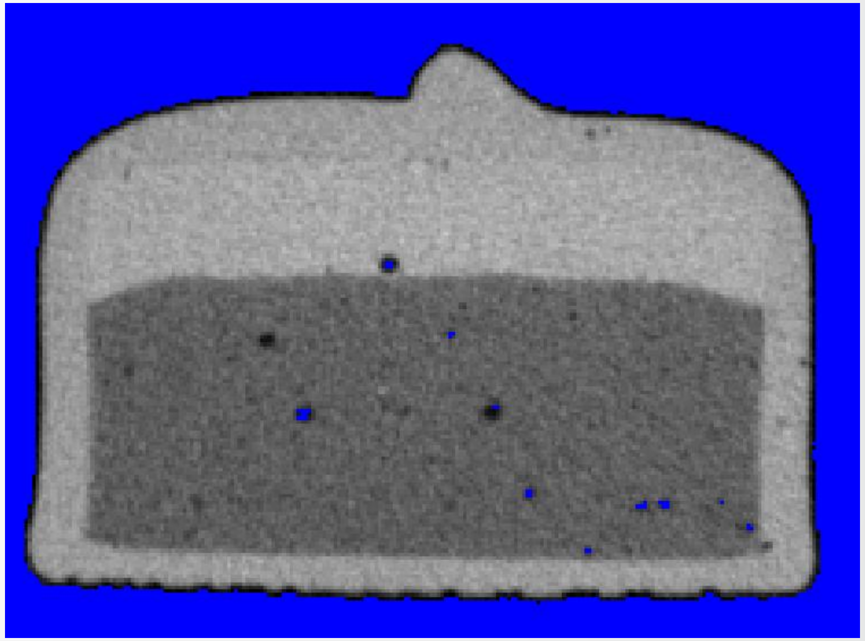
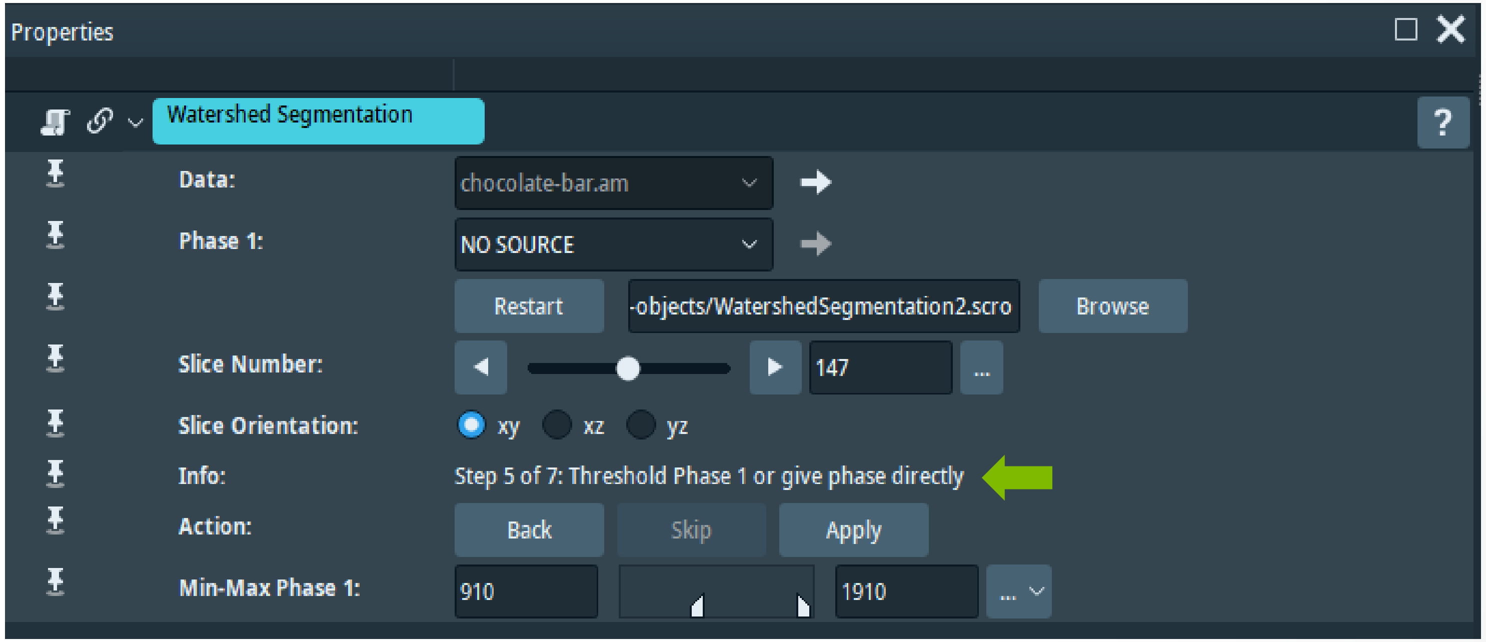


Image segmentation: Watershed Segmentation wizard



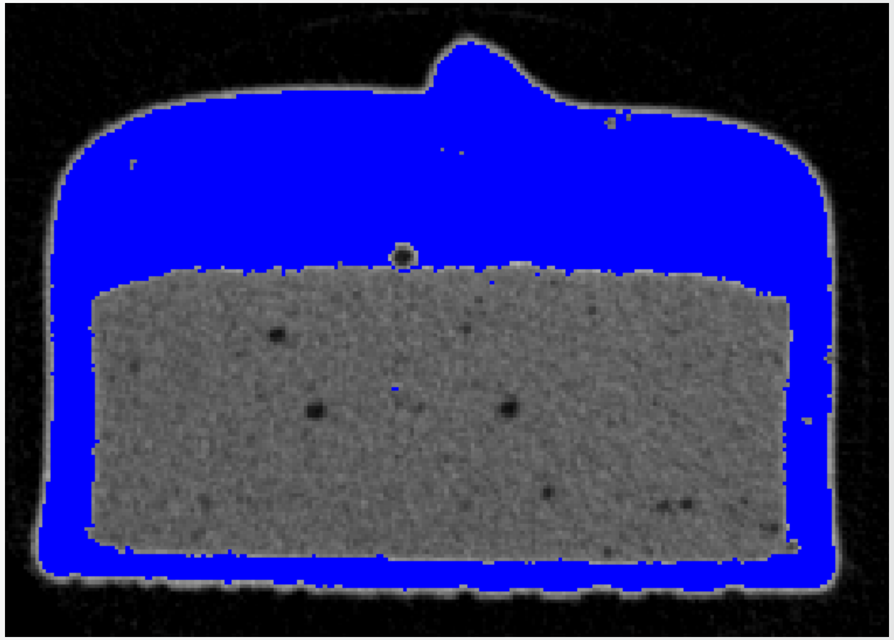
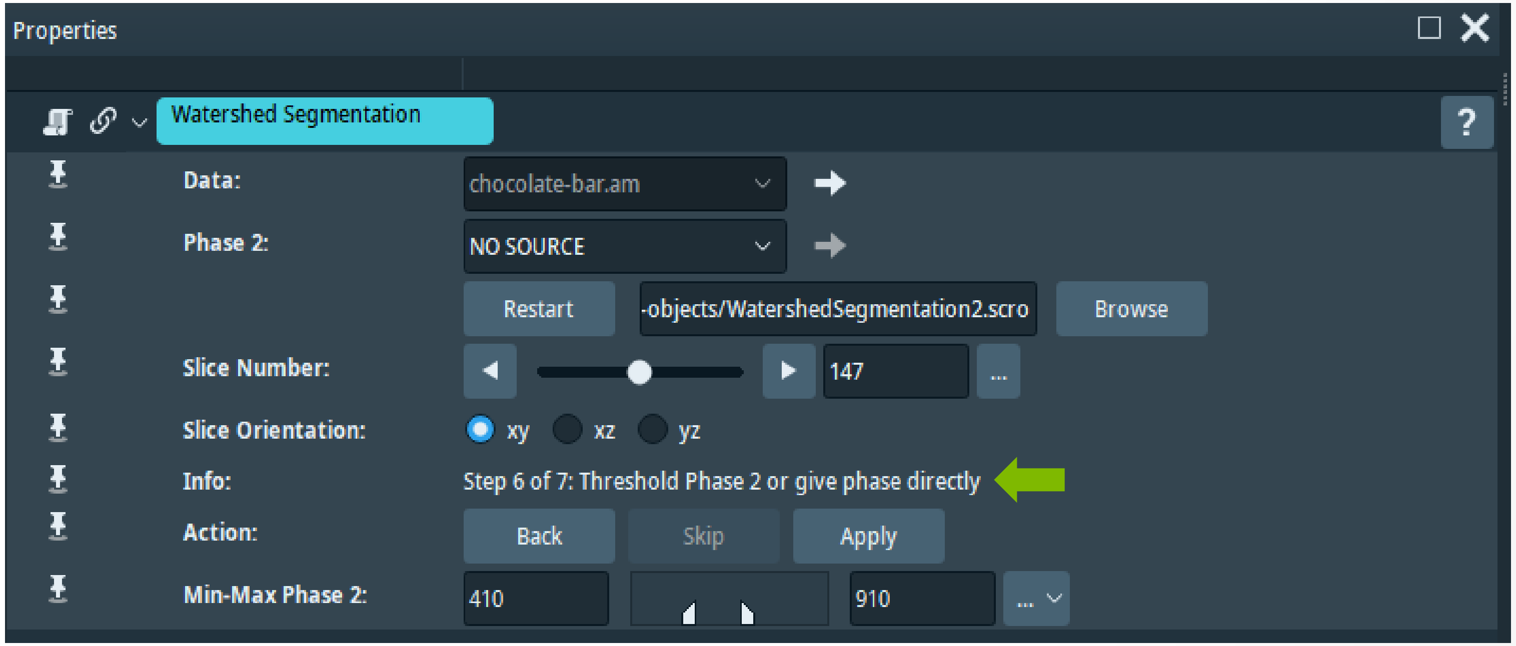


Image segmentation: Watershed Segmentation wizard



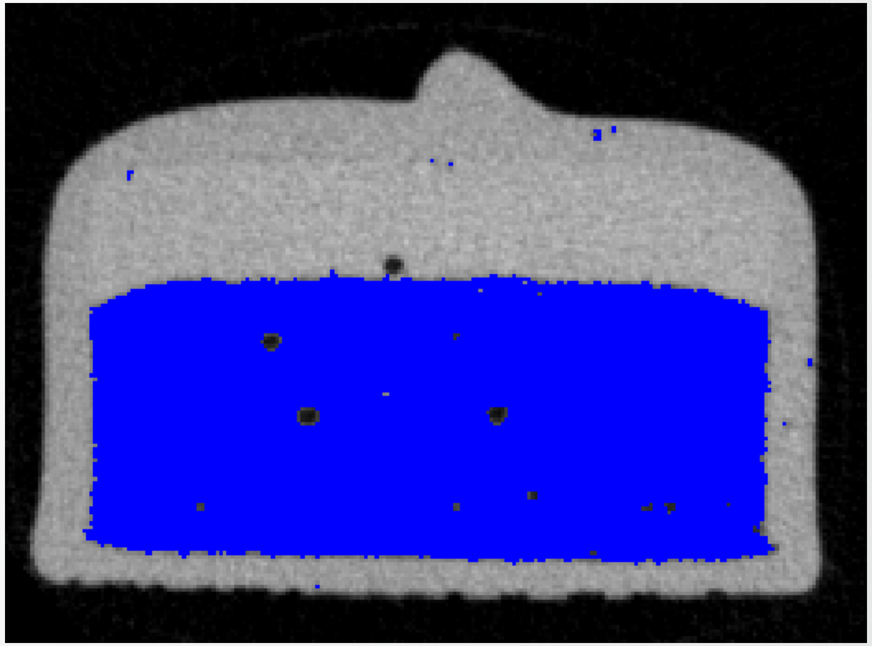
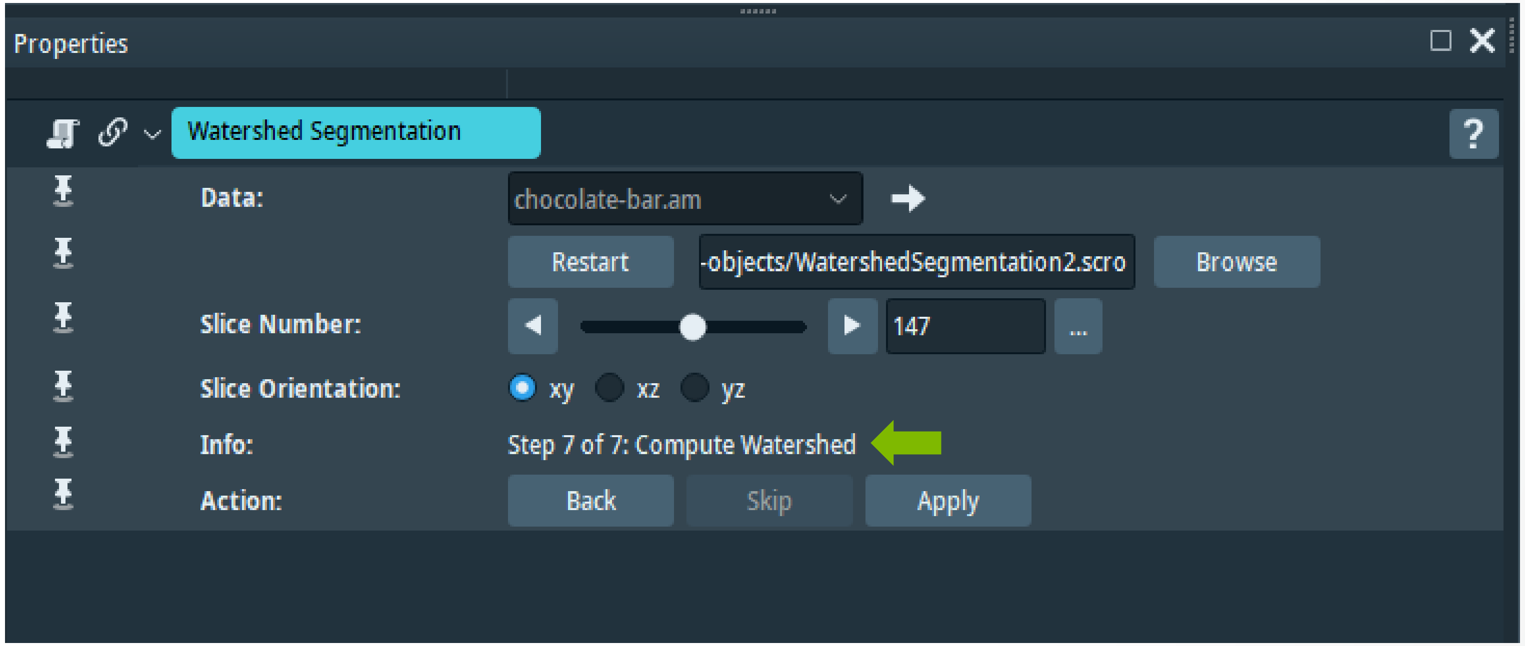


Image segmentation: Watershed Segmentation wizard



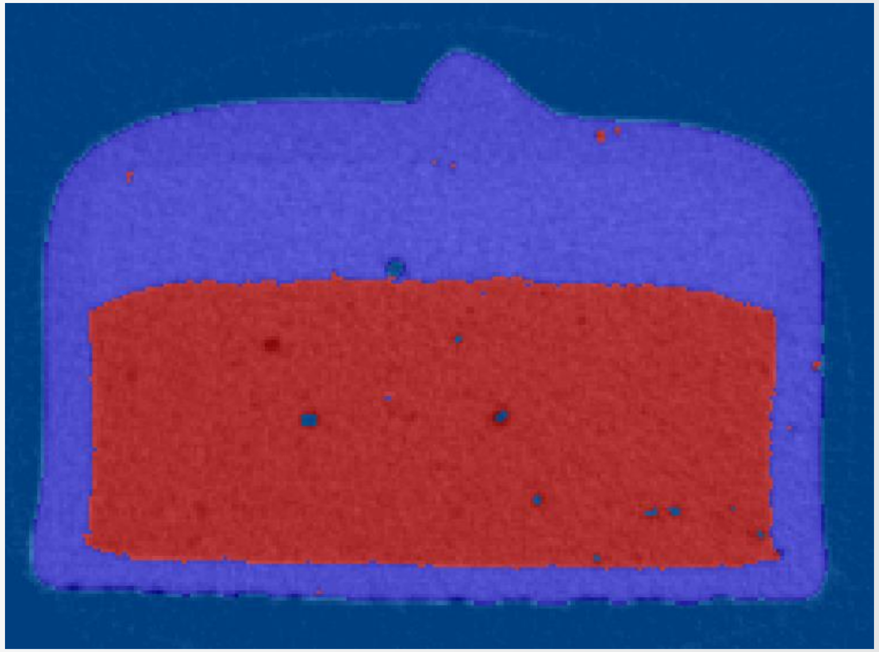
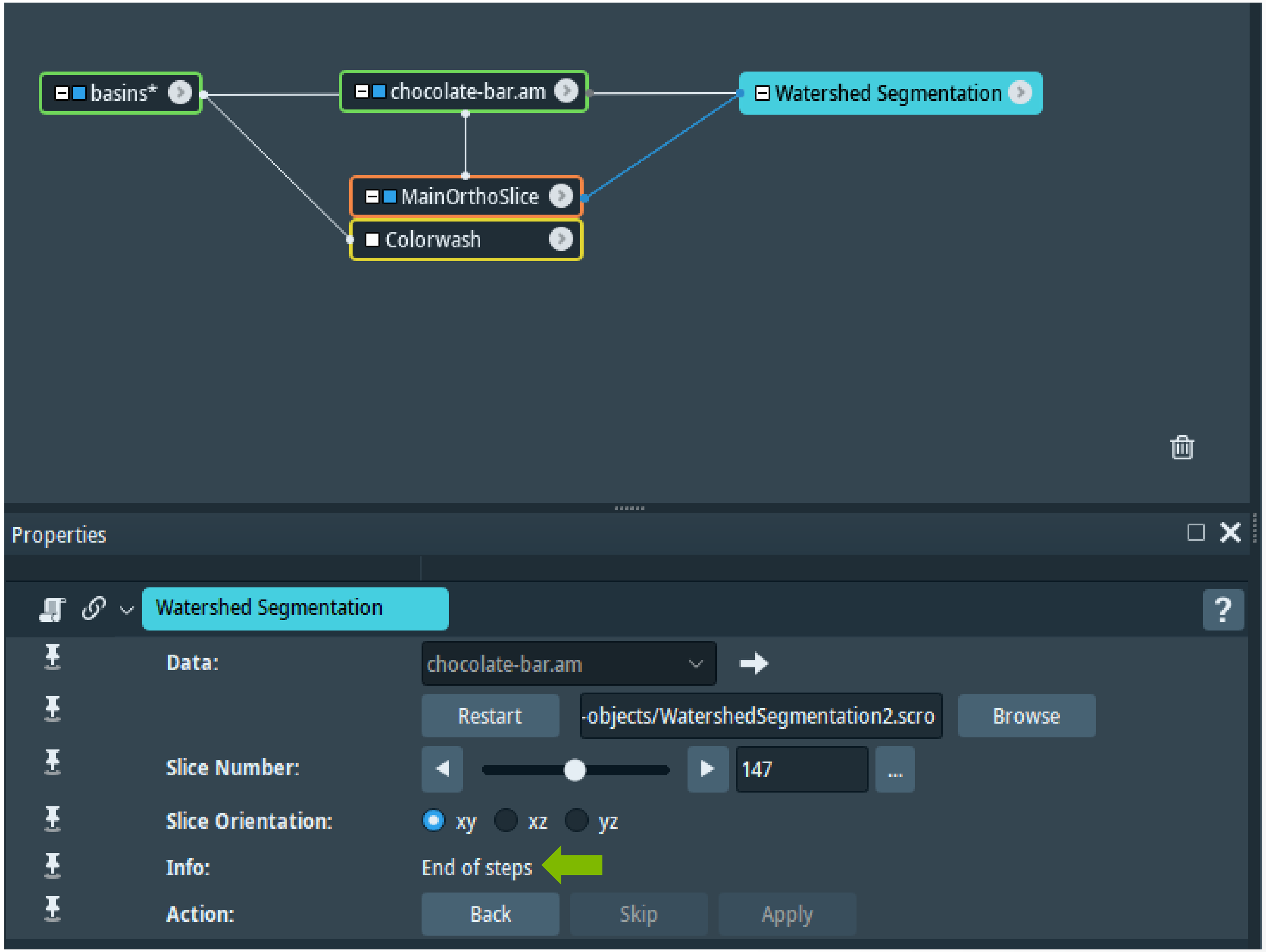
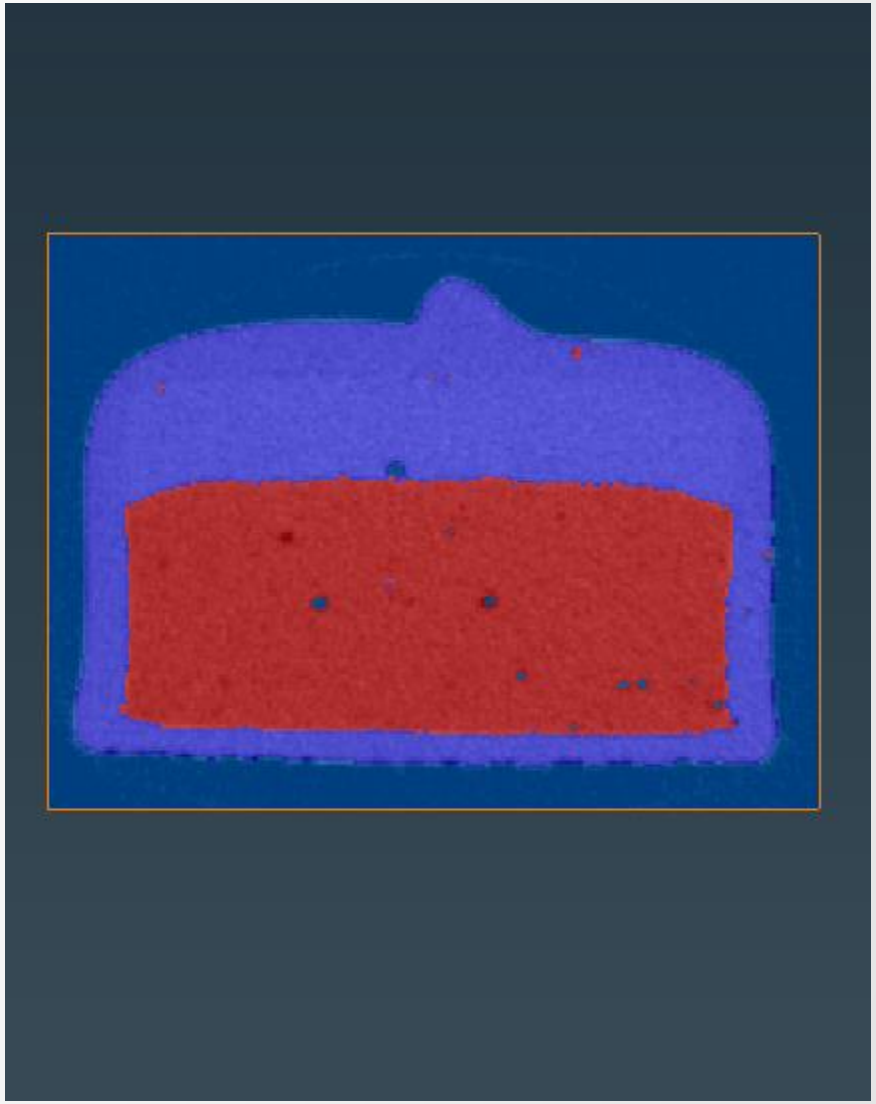


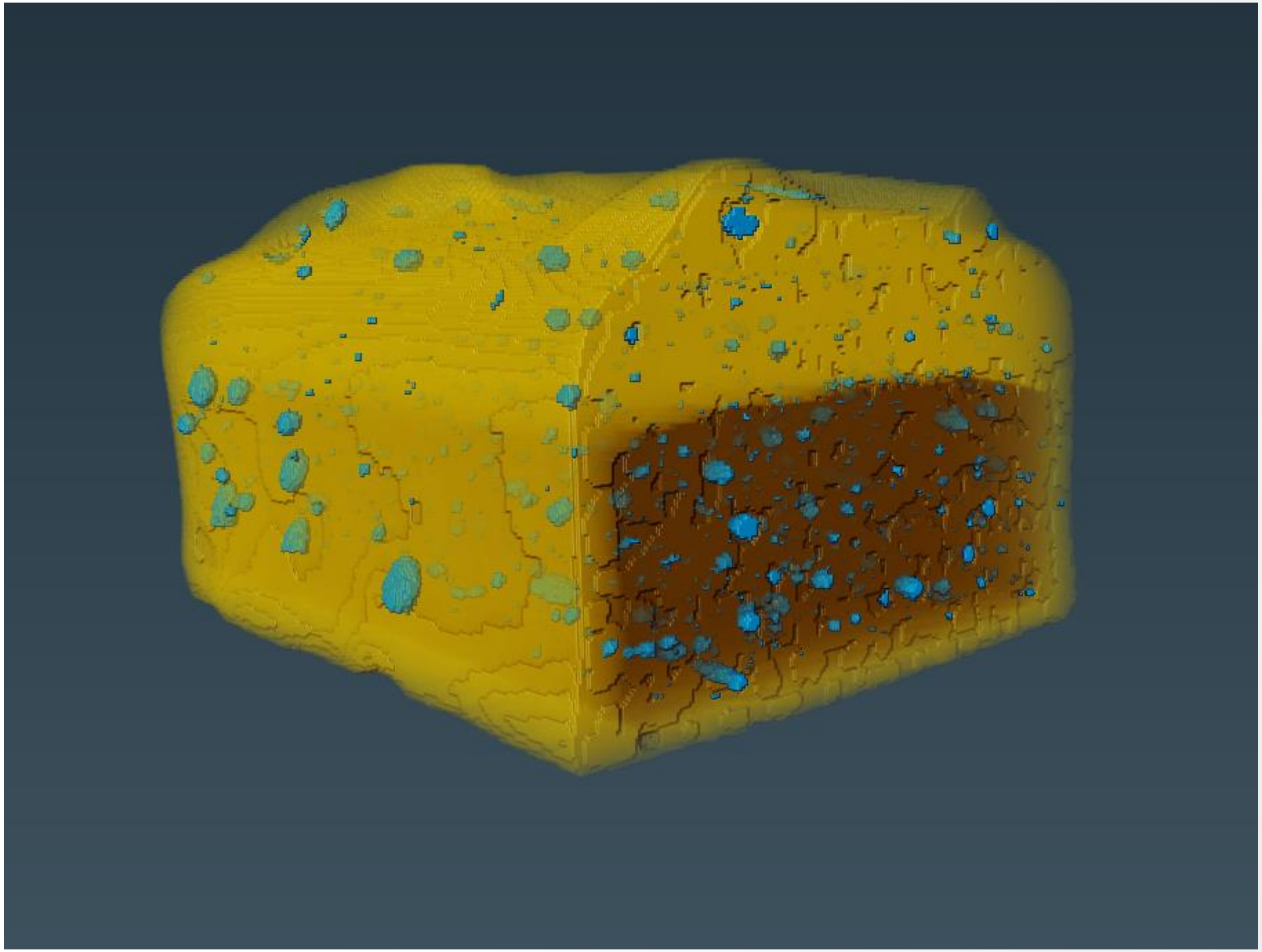
Image segmentation: Watershed Segmentation wizard





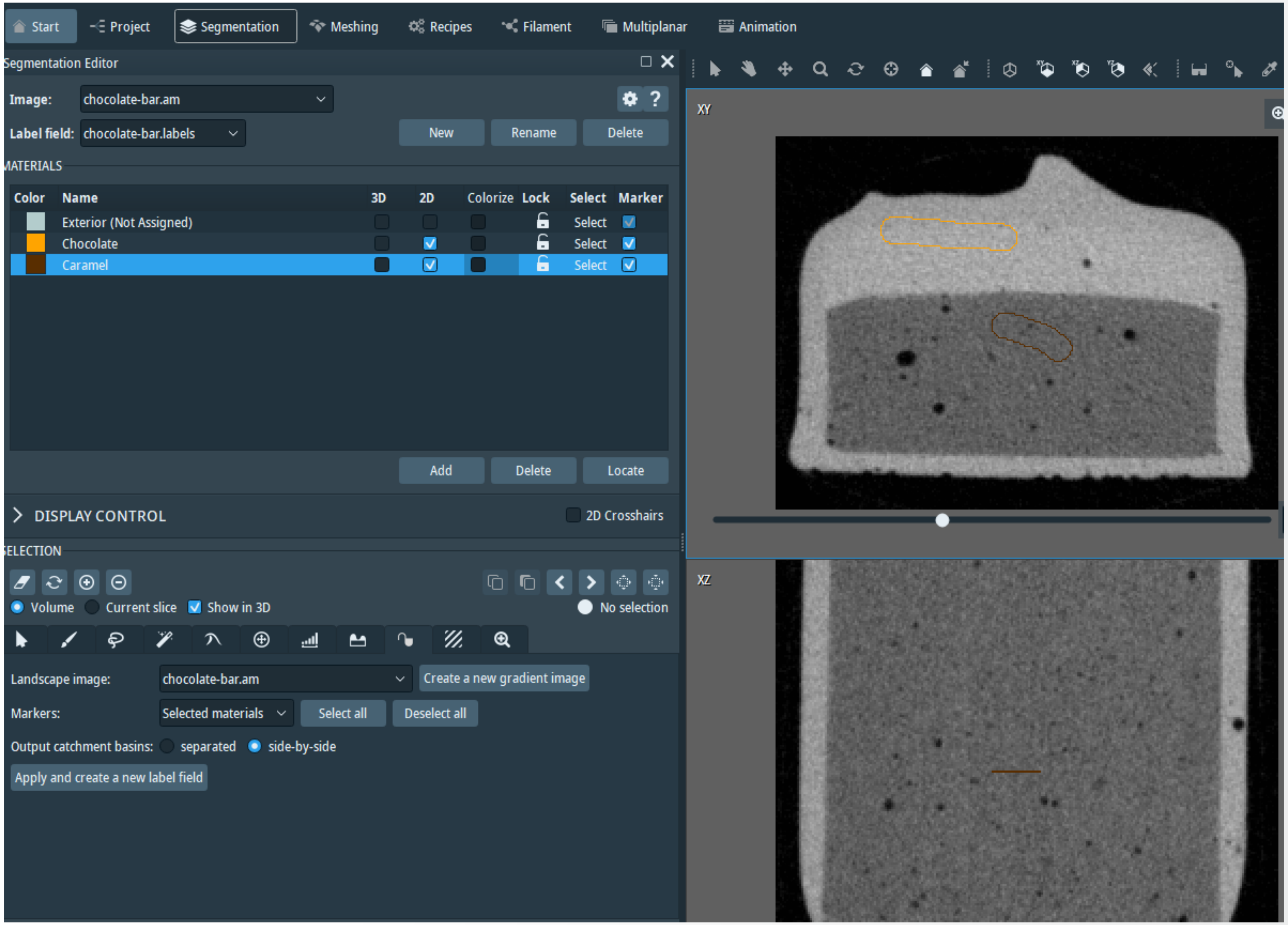
Watershed in Segmentation Editor: exercise

Multi-phase segmentation of chocolate bar



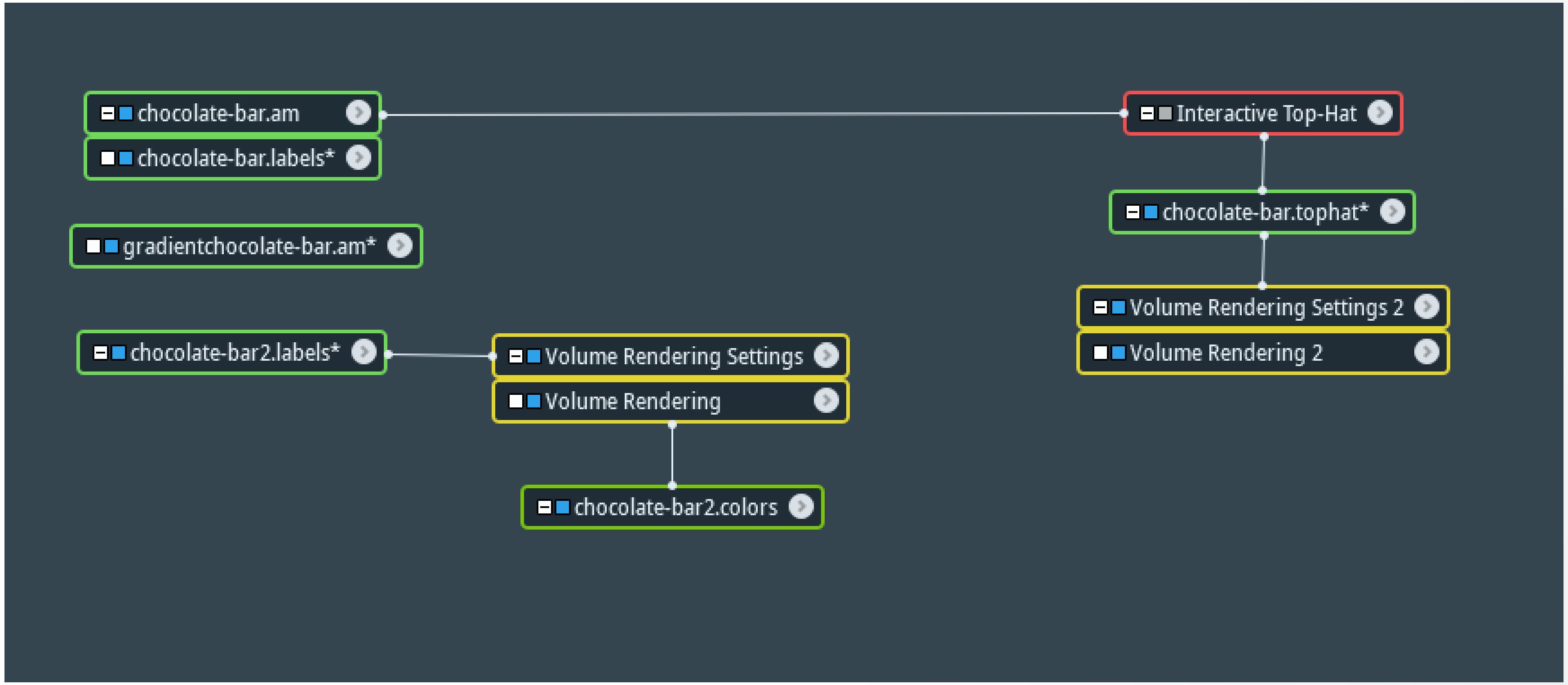
Watershed in Segmentation Editor: exercise

Solution



Watershed in Segmentation Editor: exercise

Solution



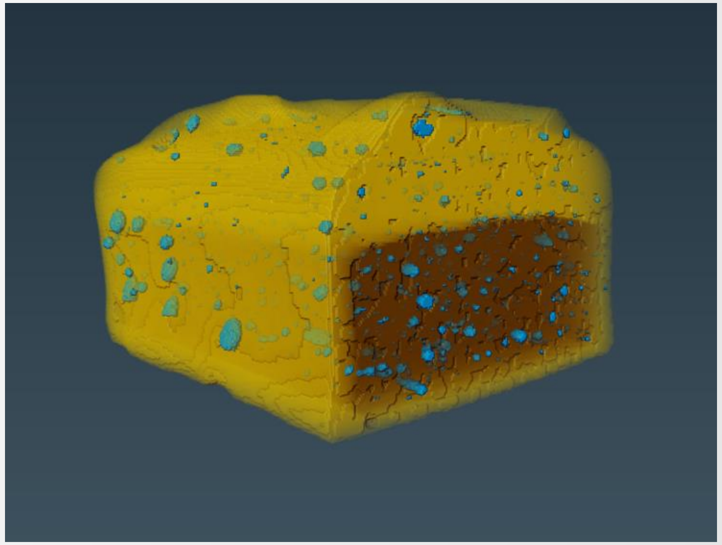


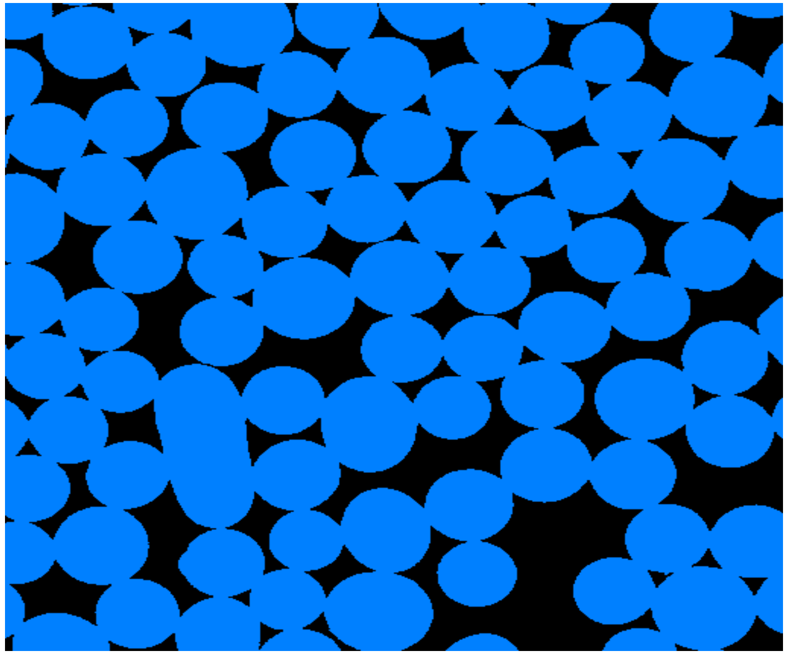
Image segmentation: post-processing

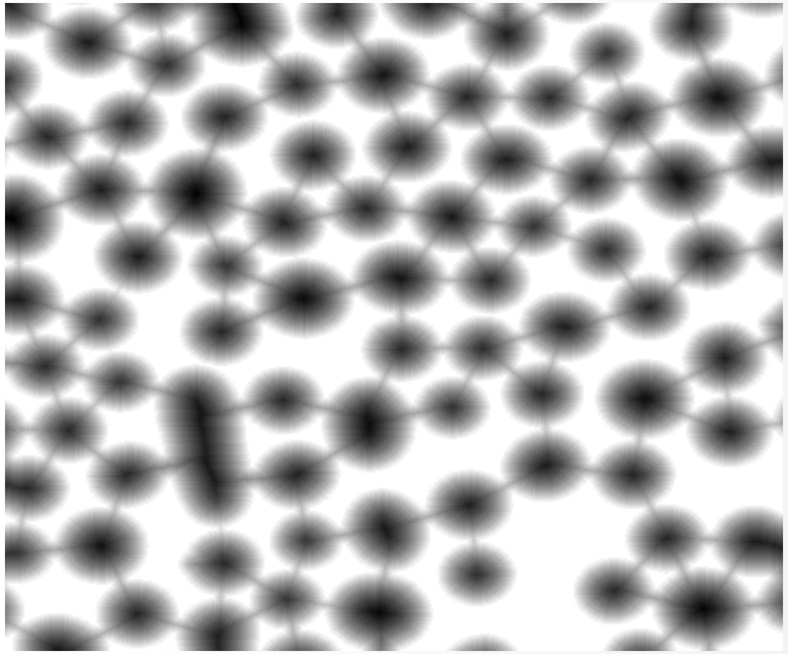
Segmentation post-processing: morphological filtering

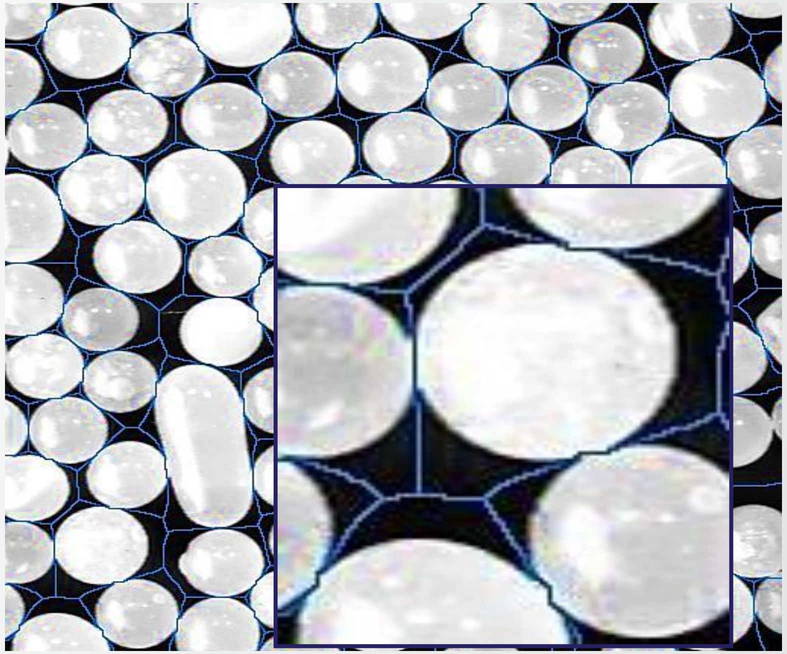
- Binary mathematical morphology
  - Interactive Shrink & Grow in Segmentation Editor
  - Opening and Closing
- Fill Holes
- Dilate + Fill Holes + Erode: may close more open cavities/pores
- Remove Small Spots
- Border Kill: removes objects touching image bounding box

Segmentation post-processing: object separation

- Separate Objects
  - Smaller ‘Extent’ value means more separation
  - Criterion relates to convexity of the particles
  - See tutorial **“Separating, Measuring and Reconstructing -> Separation using Watershed step by step”**



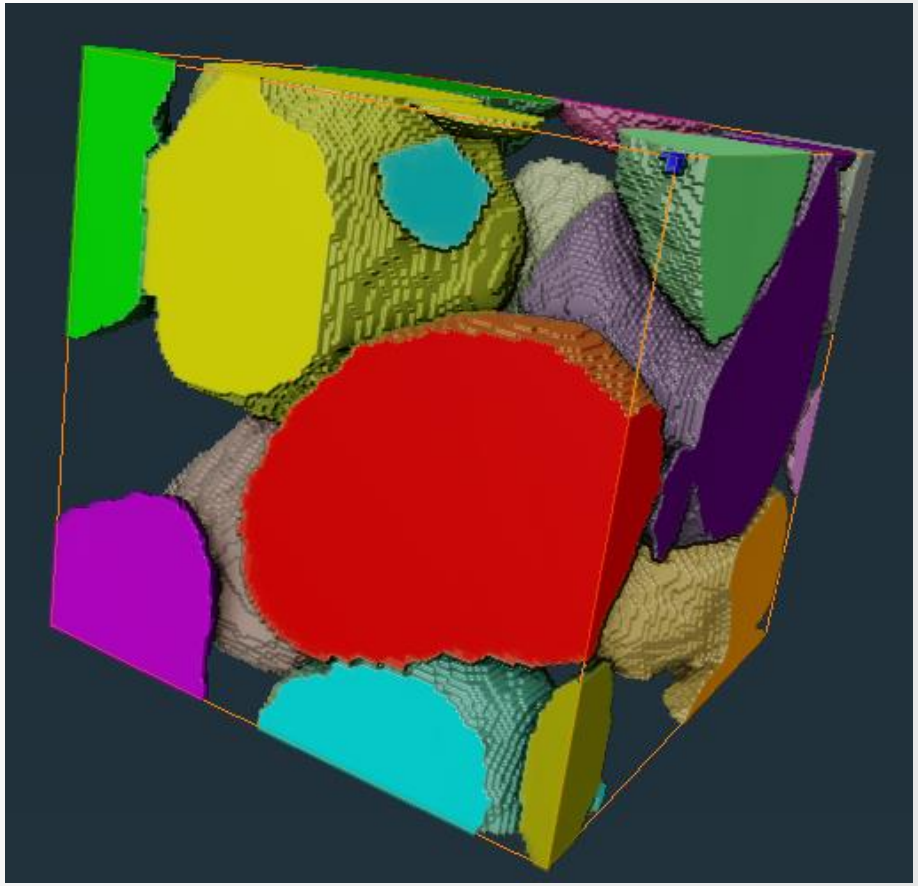




Segmentation post-processing: exercise

Separate objects after sand-pack segmentation

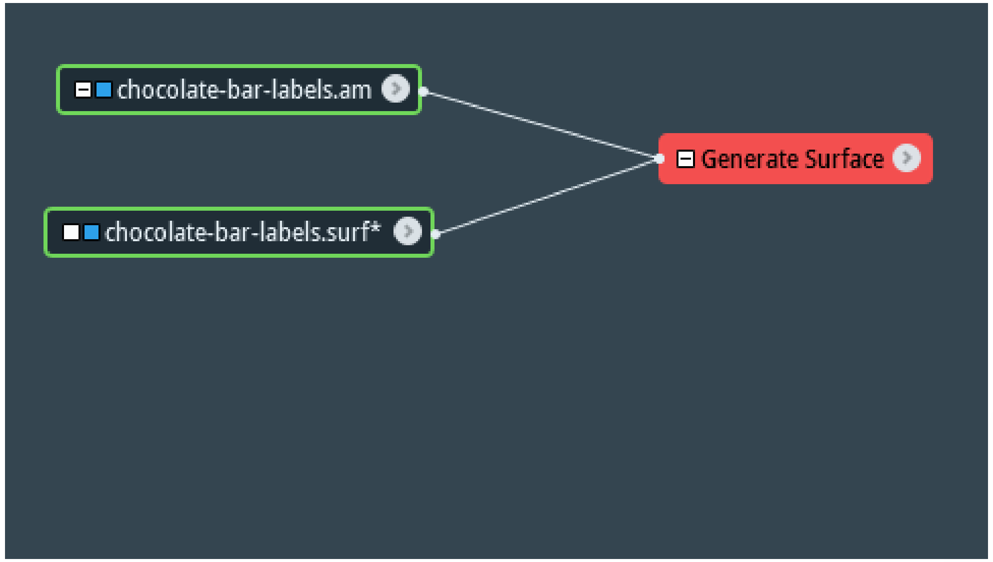
- Data to use is Data/Sandpack/SandPack128.am
- Follow steps given in tutorial **“Separating, Measuring and Reconstructing -> Separation using Watershed step by step”**

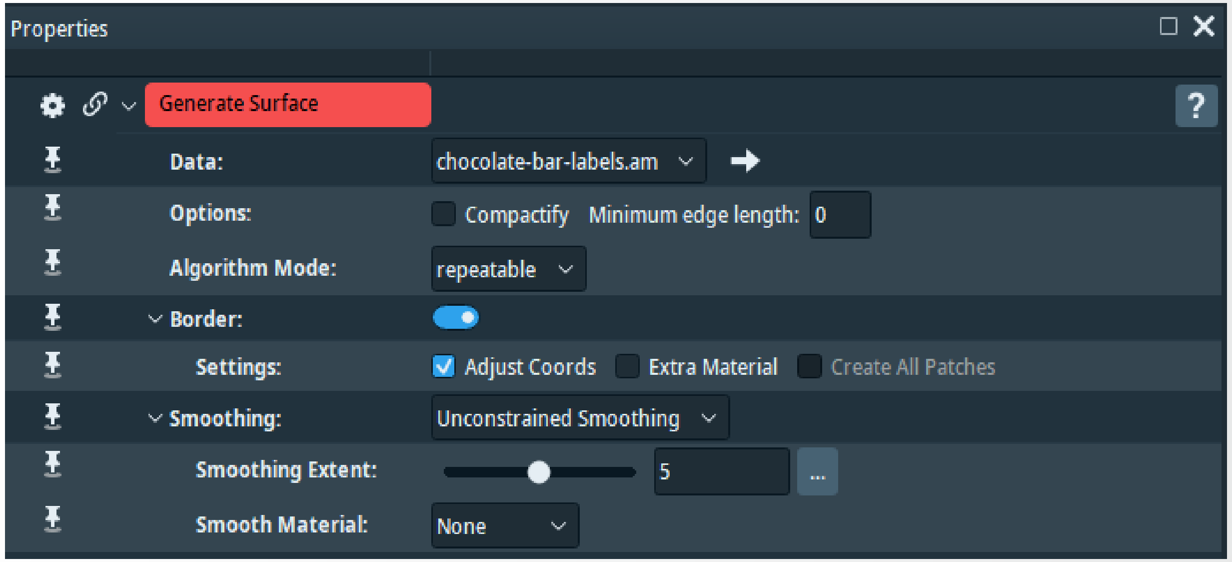


Surface generation

Surface reconstruction

Open the label image chocolate-bar-labels (data>tutorials) then attach Generate Surface module to the label image. In the properties window, using the default parameters:

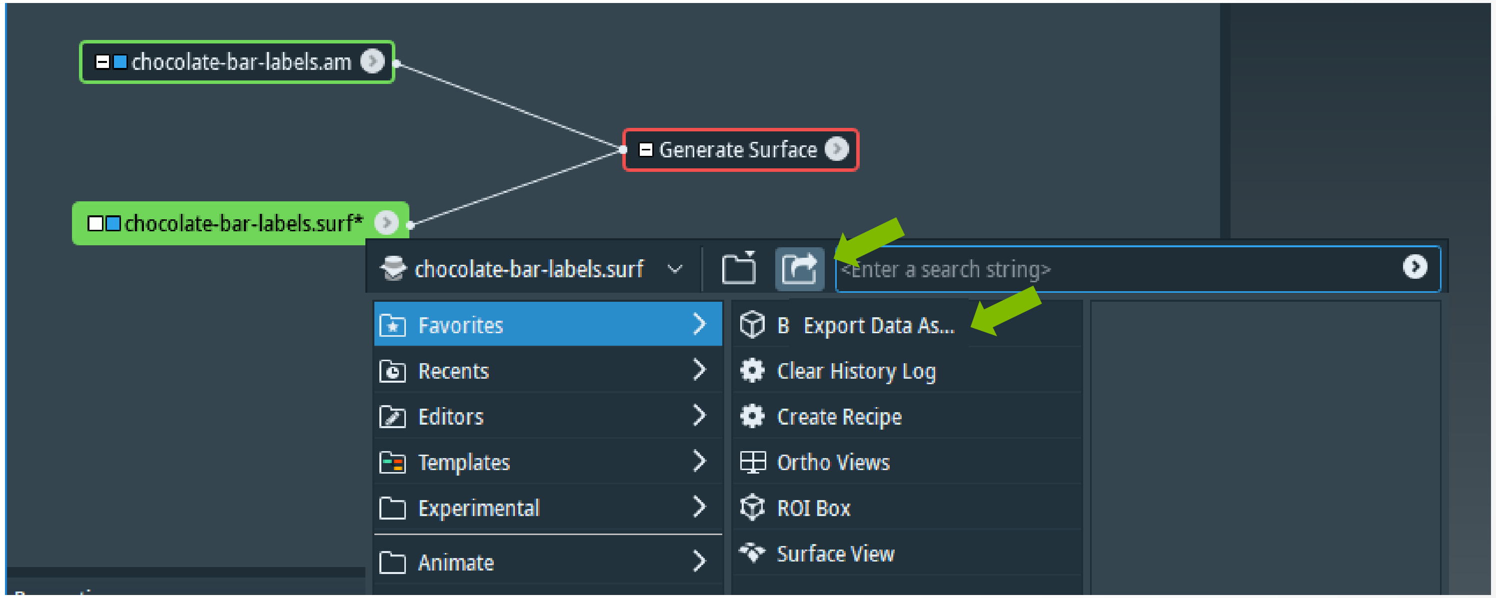


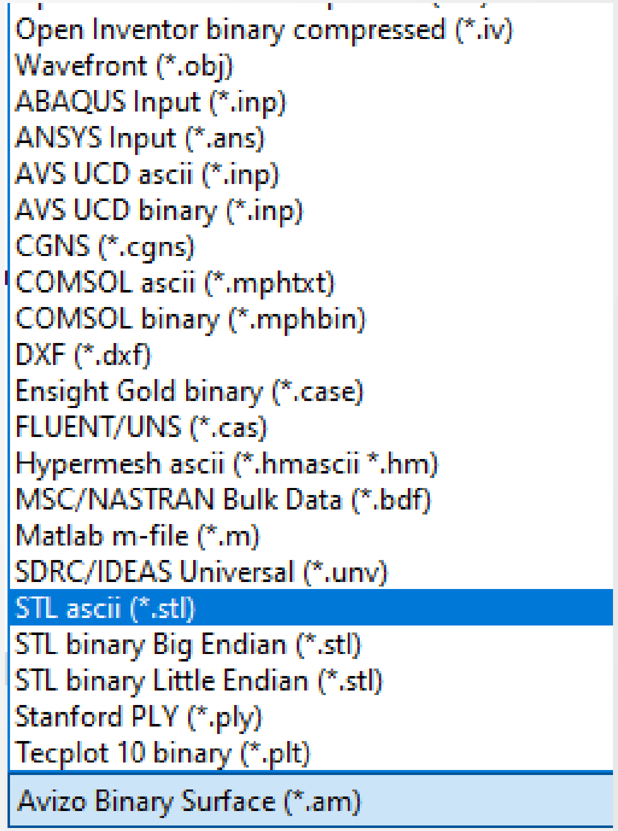


- Data: chocolate-bar-labels.am (label image)
- Border Settings: Adjust Coords
- Algorithm Mode : repeatable
- Smoothing: Unconstrained Smoothing (use None/Constrained smoothing to preserve thin structures)
- Smoothing Extent: 5

Surface data export

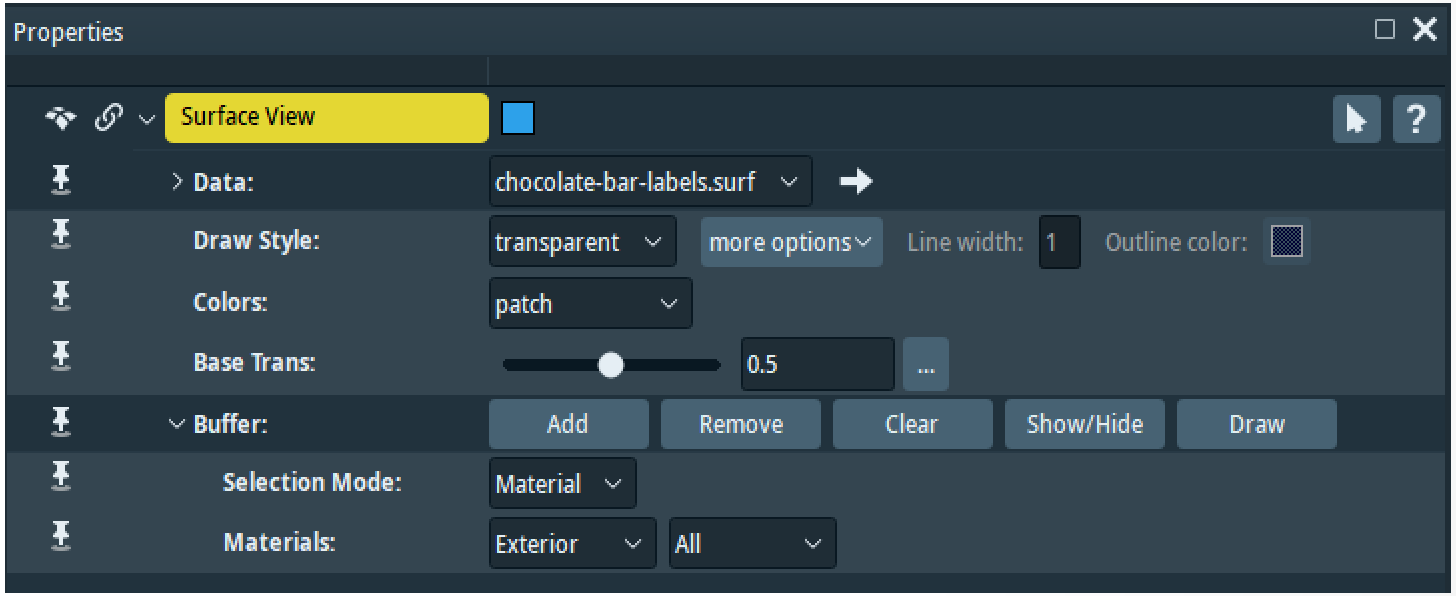
The result surface chocolate-bar-labels.surf can be exported by right click at the surface (or left click and then go to > File) and select Export Data As then select the format (e.g. .stl or .obj) to export.

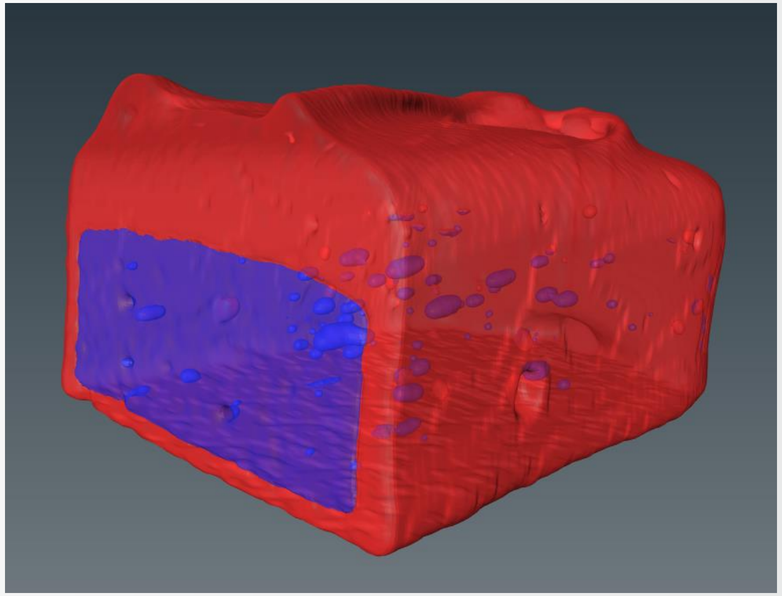




Surface view

Attach Surface View to the chocolate-bar-labels.surf to visualize the surface.



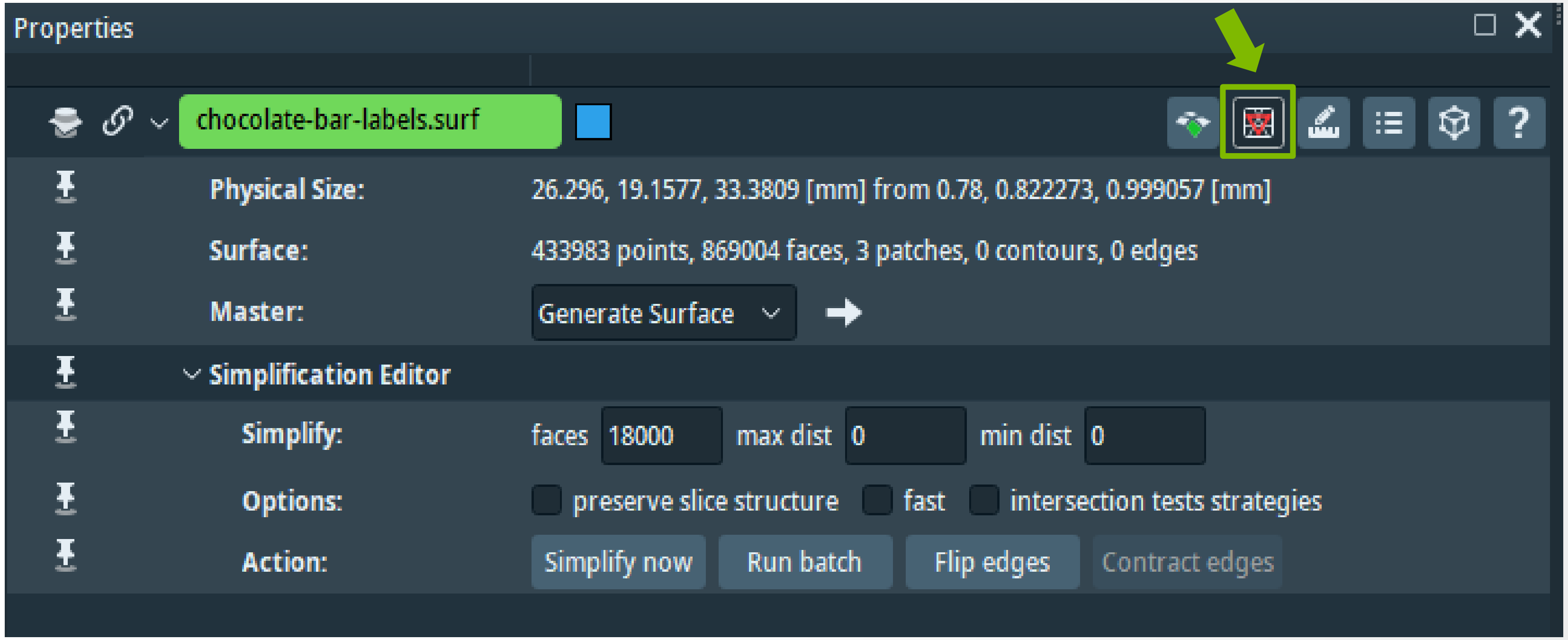


- Data: chocolate-bar-labels.surf
- Draw Style: Transparent
- Colors: patch
- Base Trans: 0.5

Surface View usage, tips & tricks: https://youtu.be/zXq3A4bKcFg

Surface simplification

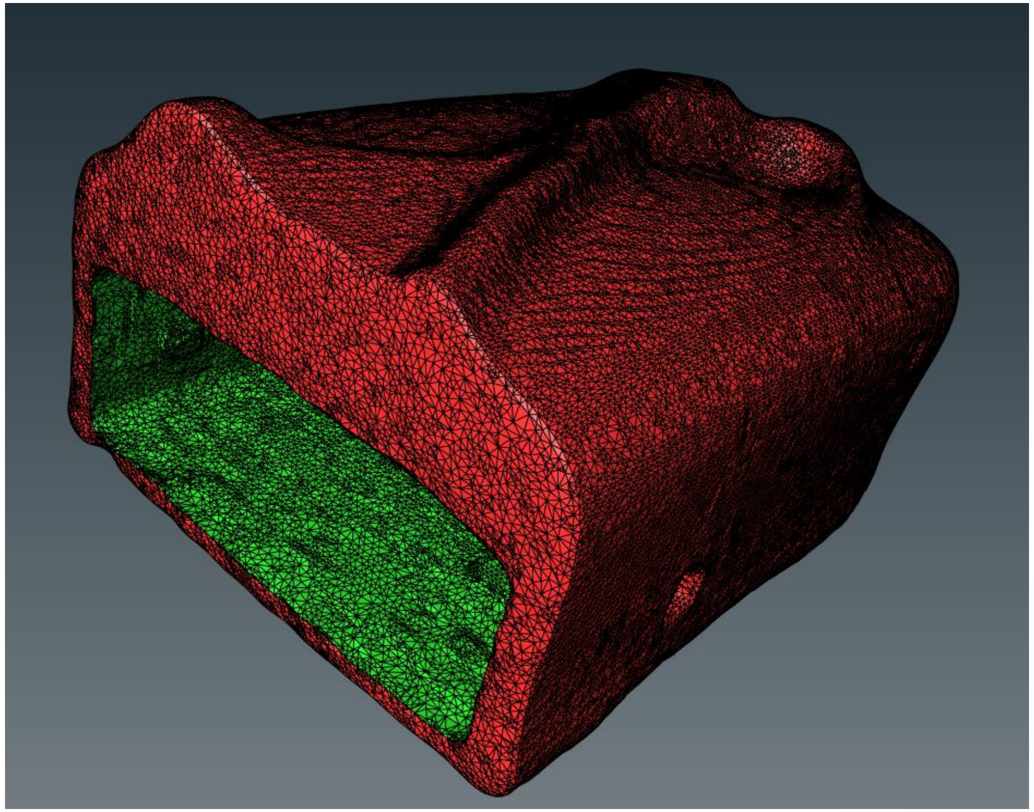
Activate Simplification Editor in the properties port of chocolate-bar-labels.surf.



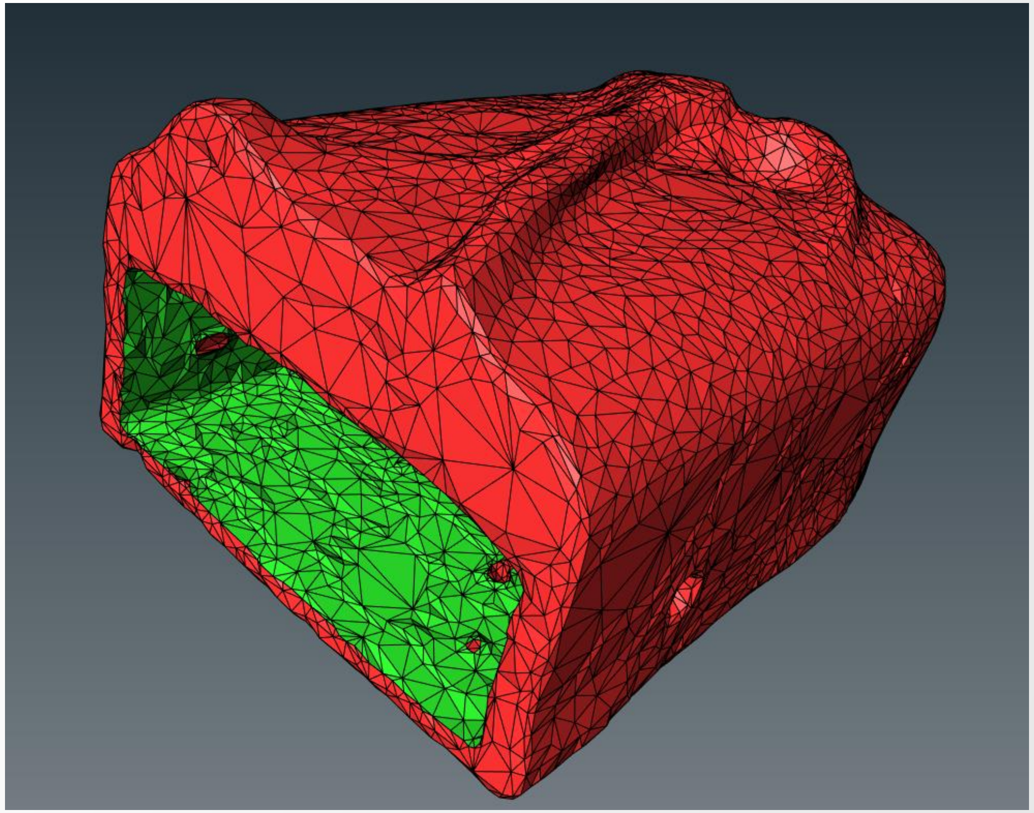
Simplification Editor

- Simplify: faces 18000 (0 max & min dist)
- Action: Simplify now

Surface simplification example



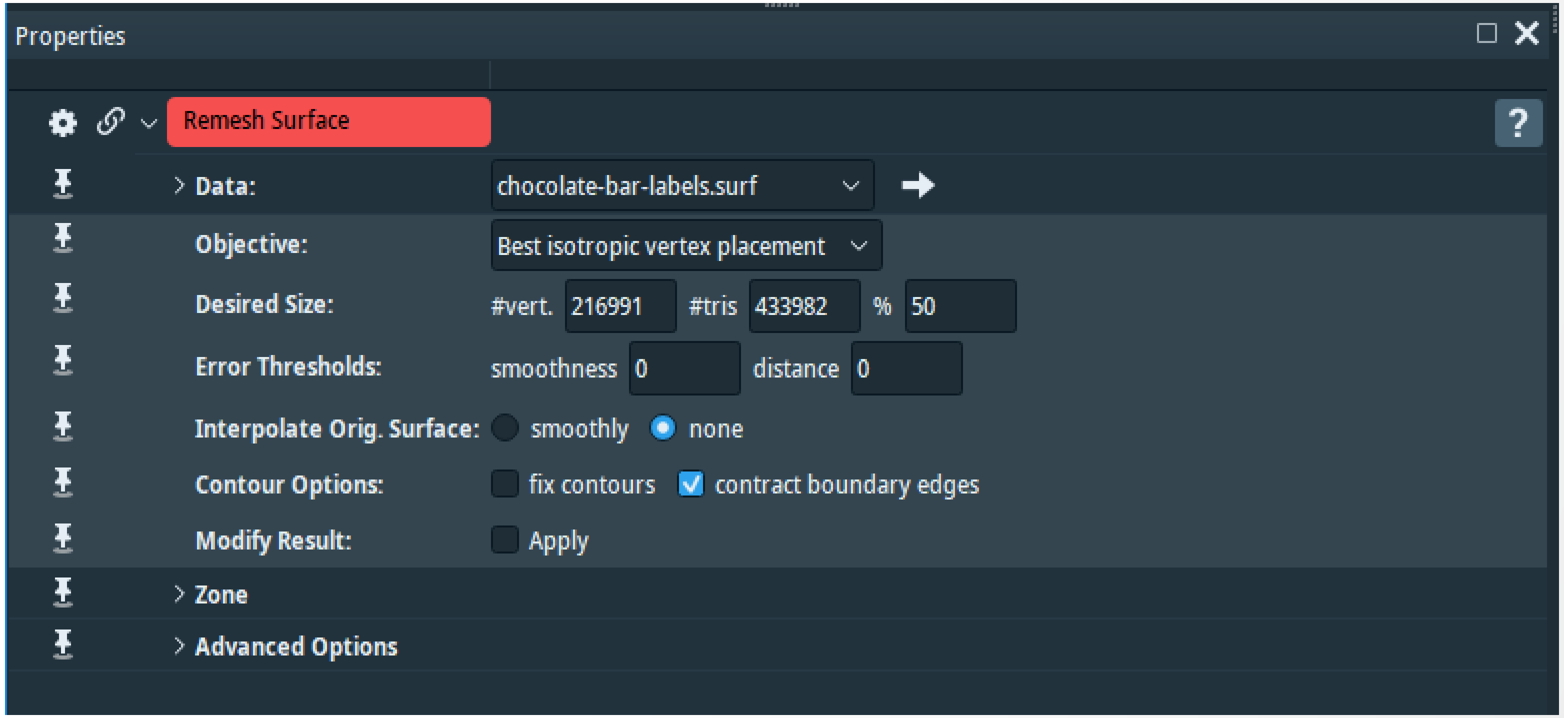
180000 faces



18000 faces

Surface remeshing

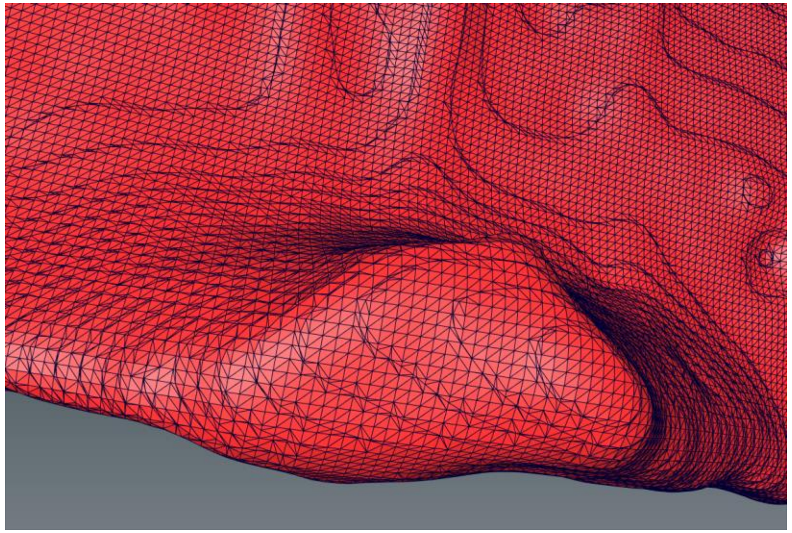
After reconstruction, the surface can be coarse: for refining, remeshing is necessary.
Attach Remesh Surface module to the chocolate-bar-labels.surf.



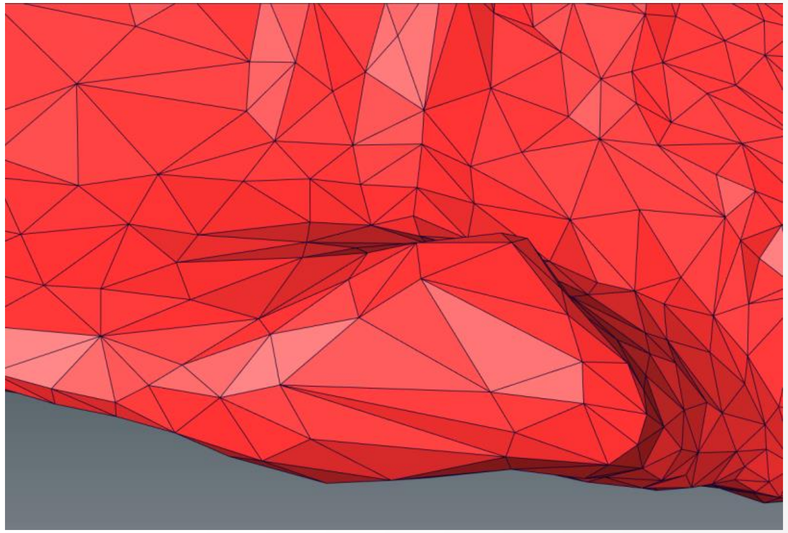
- Data: chocolate-bar-labels.surf
- Objective: Best isotropic vertex placement
- Desired Size: #vertex = 216991, #tris = 433982, % = 50
- Interpolate Original Surface: none
- Contour Options: contract boundary edges

Surface remeshing

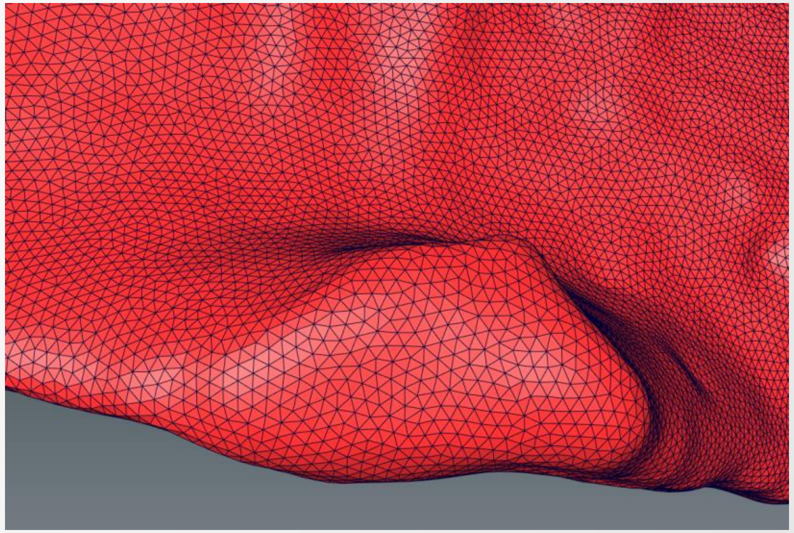
Dataset: *Chocolate-bar-labels.surf*



Original surface



After simplification

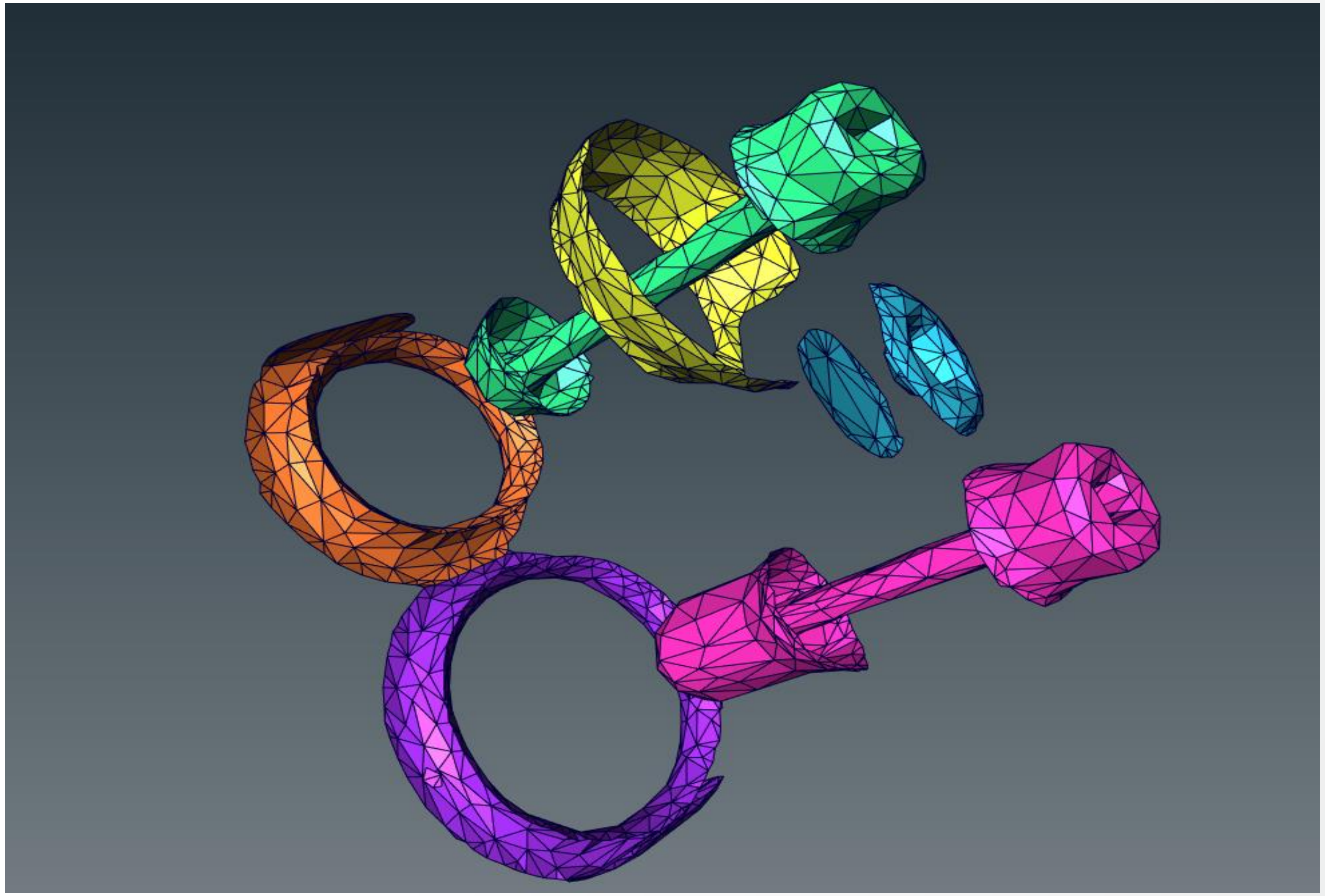


After remeshing

Surface view: exercise

Tuning Surface View module

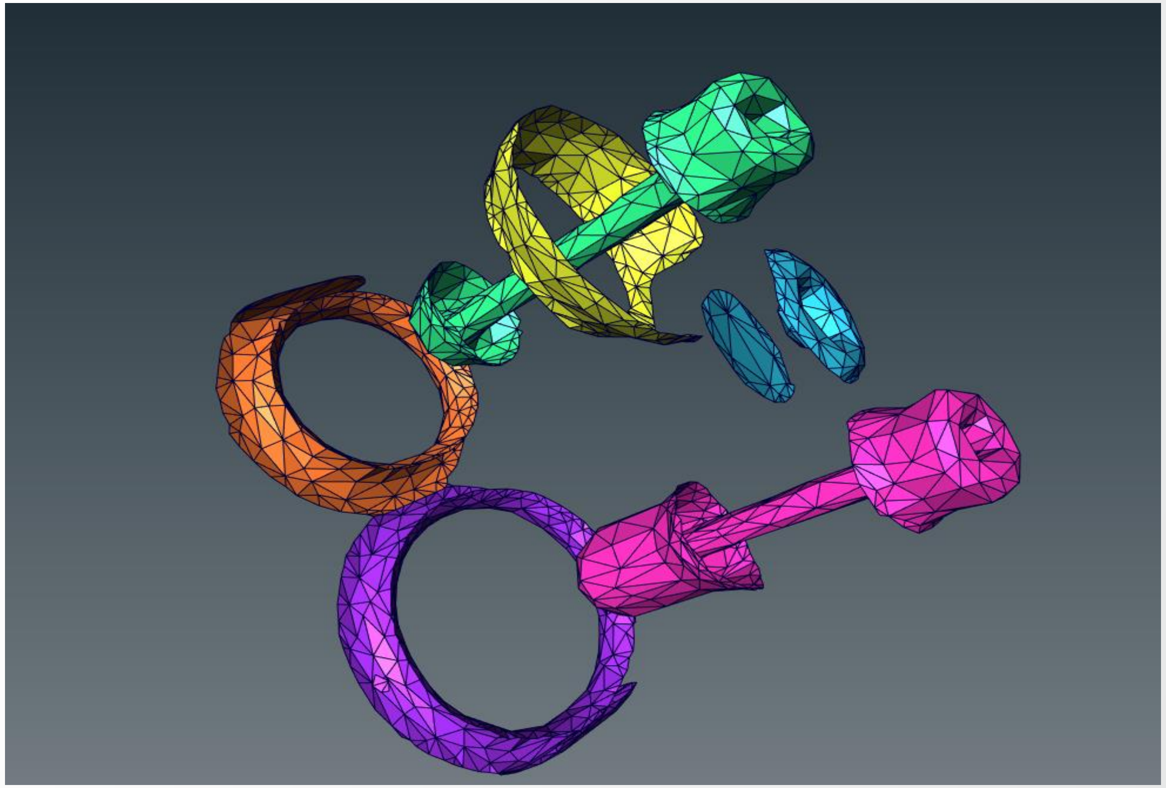
Load *motor.labels* (data->tutorials) then use Generate Surface and Surface View to obtain a similar view.



Data visualization: exercise 3

Solution

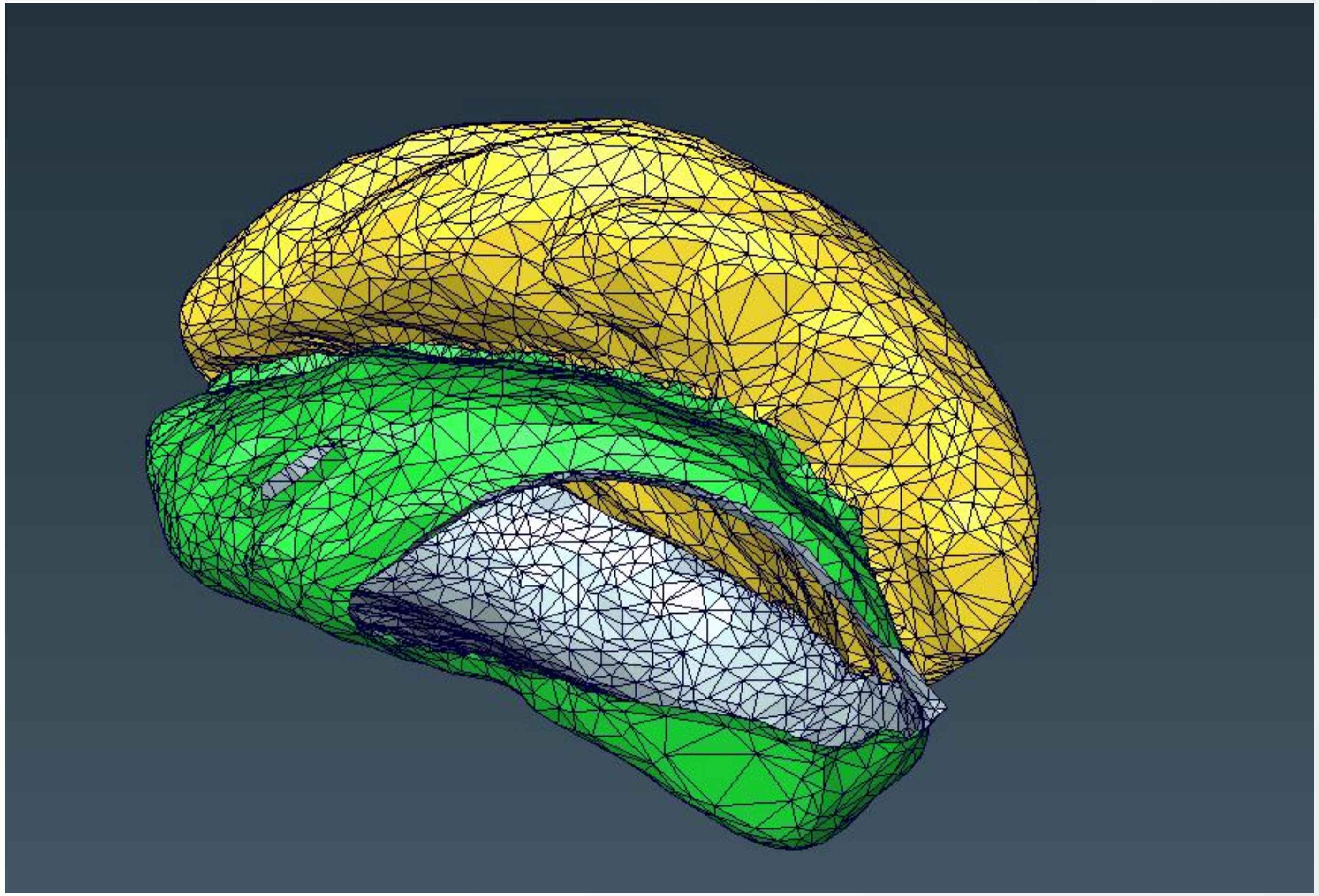
1. Generate Surface:
    - Smoothing: Existing Weights
2. Simplify Surface:
    - 18000 faces
3. Surface View:
    - Draw Style: Outline
    - Colors: Patch
    - Buffer: Remove Material3



Surface view: exercise

Tuning Surface View module

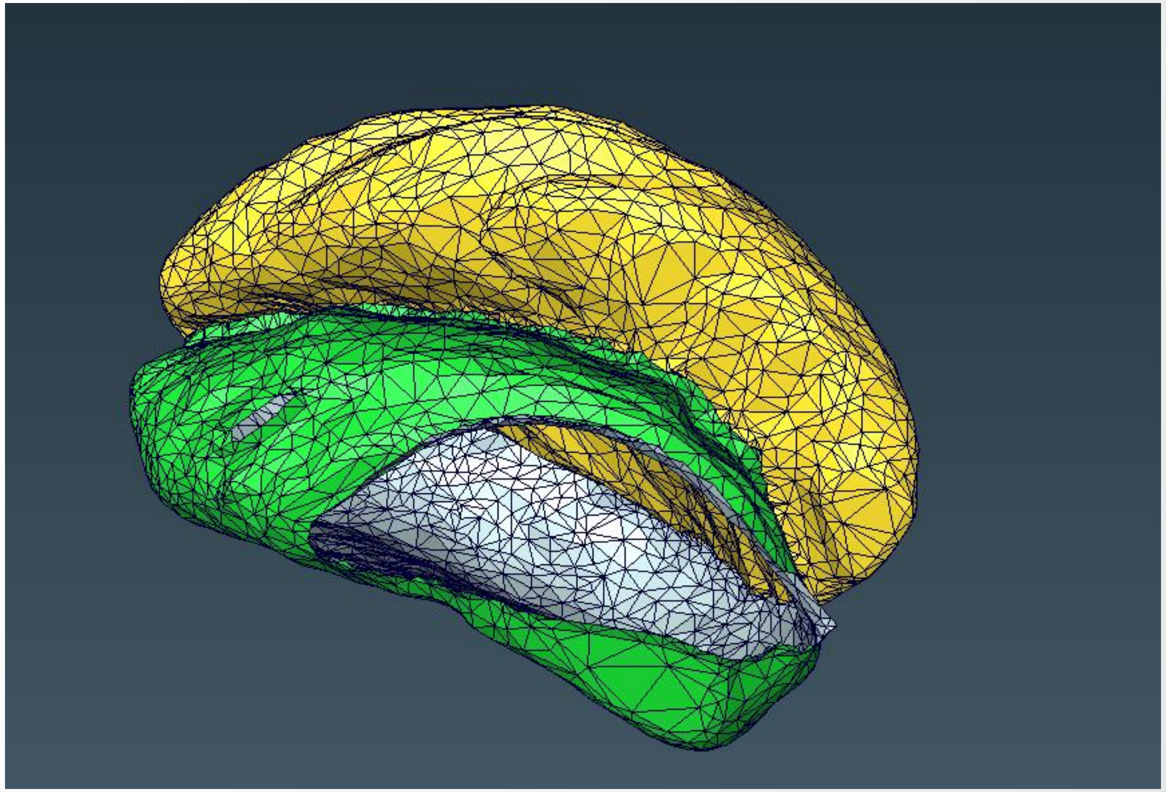
Load lobus.labels (data>tutorials) then use Generate Surface and Surface View to obtain a similar view.



Data visualization: exercise 3

Solution

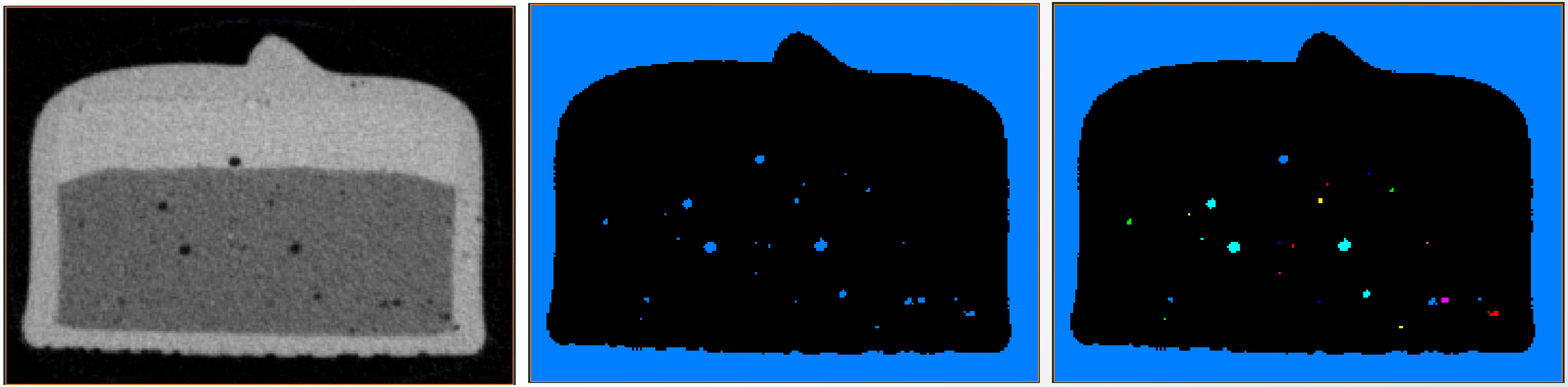
1. Generate Surface:
   - Smoothing: Existing Weights
2. Simplify Surface:
   - 18000 faces
3. Surface View:
   - Draw Style: Outline
   - Colors: normal
   - Buffer: Remove Medulla



Quantification

Quantification on segmentation results

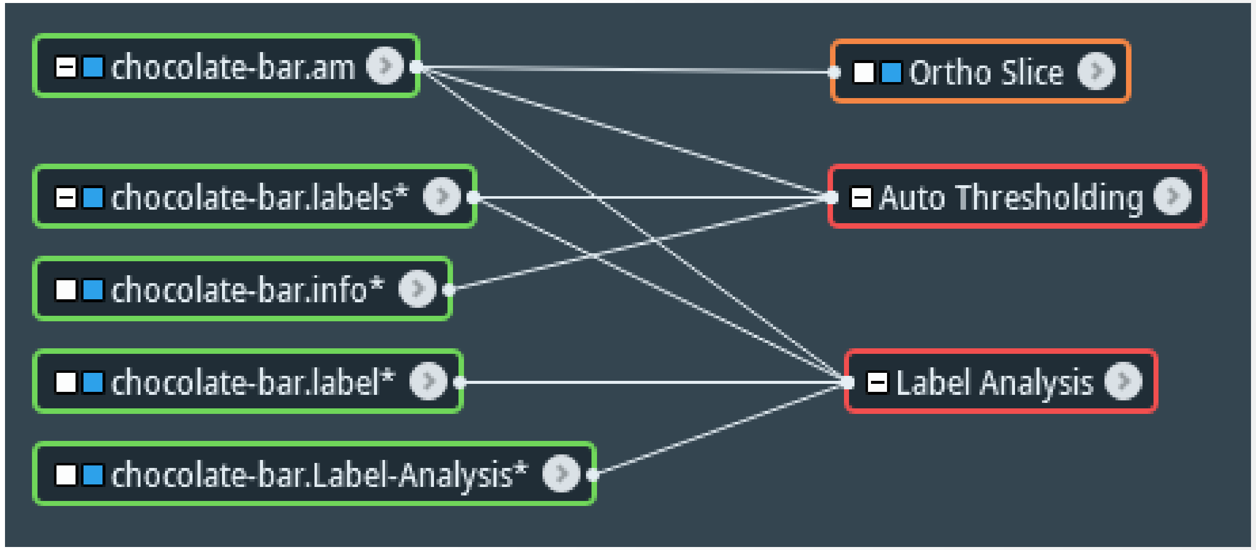
Q: How can one identify segmentation objects and extract measurements and statistics ?



Label segmentation objects and extract measures

A: Label Analysis module. It

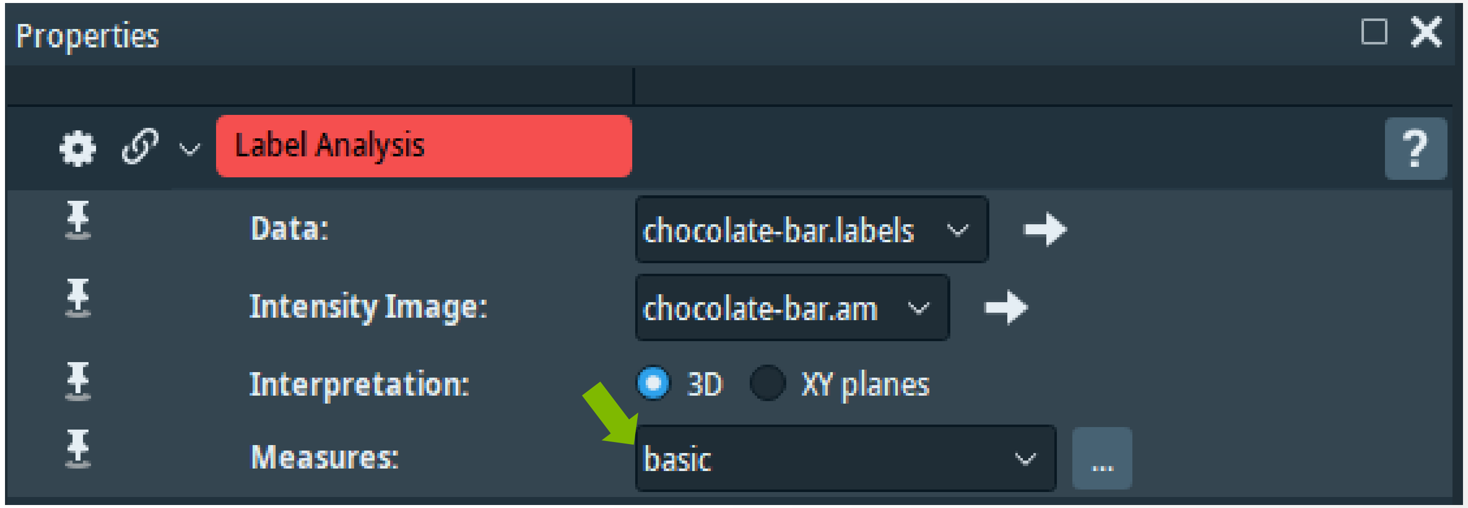
- Generates a label image: a unique label is assigned to each connected component (if input is binary)
- Allows extracting individual measures for each label object
- Allows extracting global statistics
- Intensity input (optional): allows extracting gray level statistics (e.g. mean, min, max)



Label Analysis

Thermo Fisher SCIENTIFIC

A: Label Analysis module. Default port initialization:

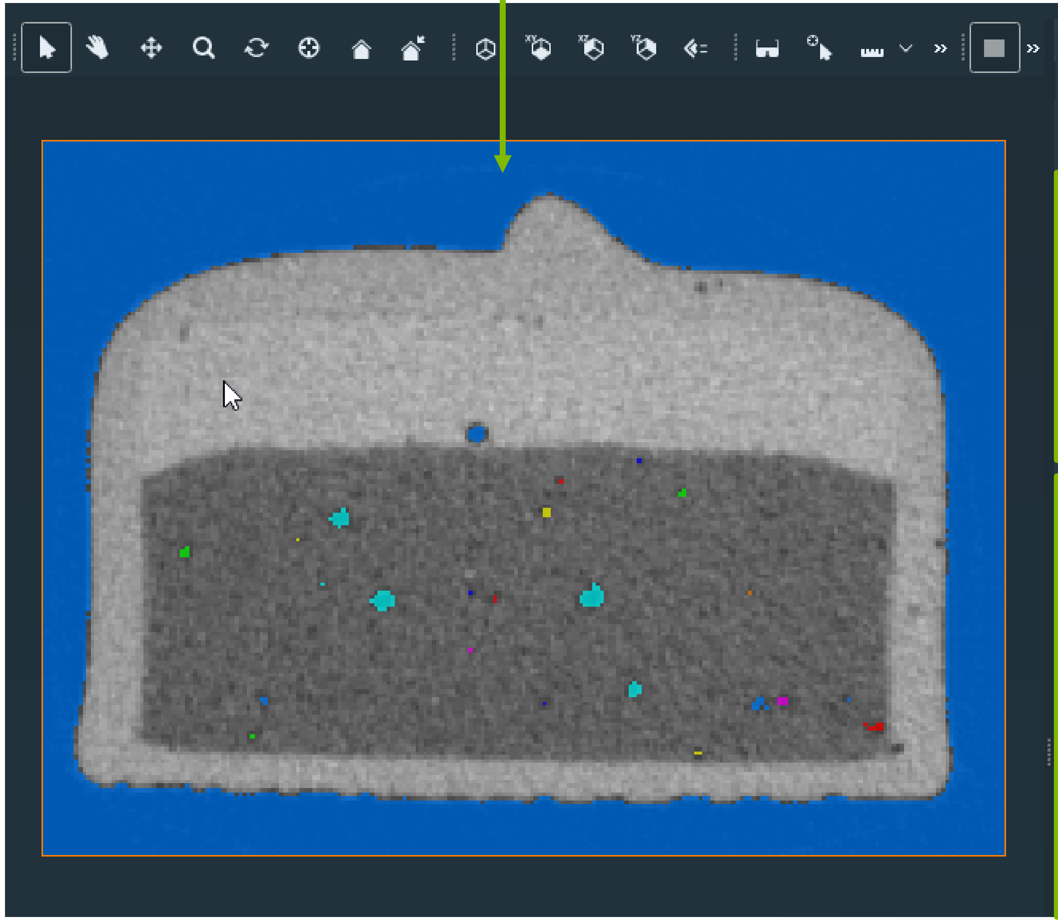


- "basic" Measures – group of pre-defined measures:
    - Volume3d
    - Area3d
    - BaryCenterX
    - BaryCenterY
    - BaryCenterZ
    - Mean

Label Analysis

Label Analysis results for default ports initialization.

Labeled image (overlaid on input)



Global statistics

Tables

chocolate-bar.Label-Analysis

	Volume3d	Area3d	BaryCenterX	BaryCenterY	BaryCenterZ	Mean	index
Mean	2.71364e-09	2.47505e-06	0.0136737	0.0137114	0.0199017	429.141	1725.5
Min	1.728e-12	4.32603e-08	0.00174	0.00267667	0.002088	28.1531	1.0
Max	9.25943e-06	0.00750127	0.02604	0.01944	0.0342	490.0	3450.0
Median	4.24511e-12	6.36654e-08	0.0135711	0.0138603	0.0194694	442.907	1725.0
Variance	2.48438e-14	1.63043e-08	3.85969e-05	7.94986e-06	7.23928e-05	3173.69	991875.1
Kurtosis	3445.0	3444.8	-1.1465	0.188536	-1.25042	3.28613	-1.19996
Skewness	58.7111	58.7085	0.0599537	-0.524767	-0.0307468	-1.59478	-2.07946e-05

Individual measures

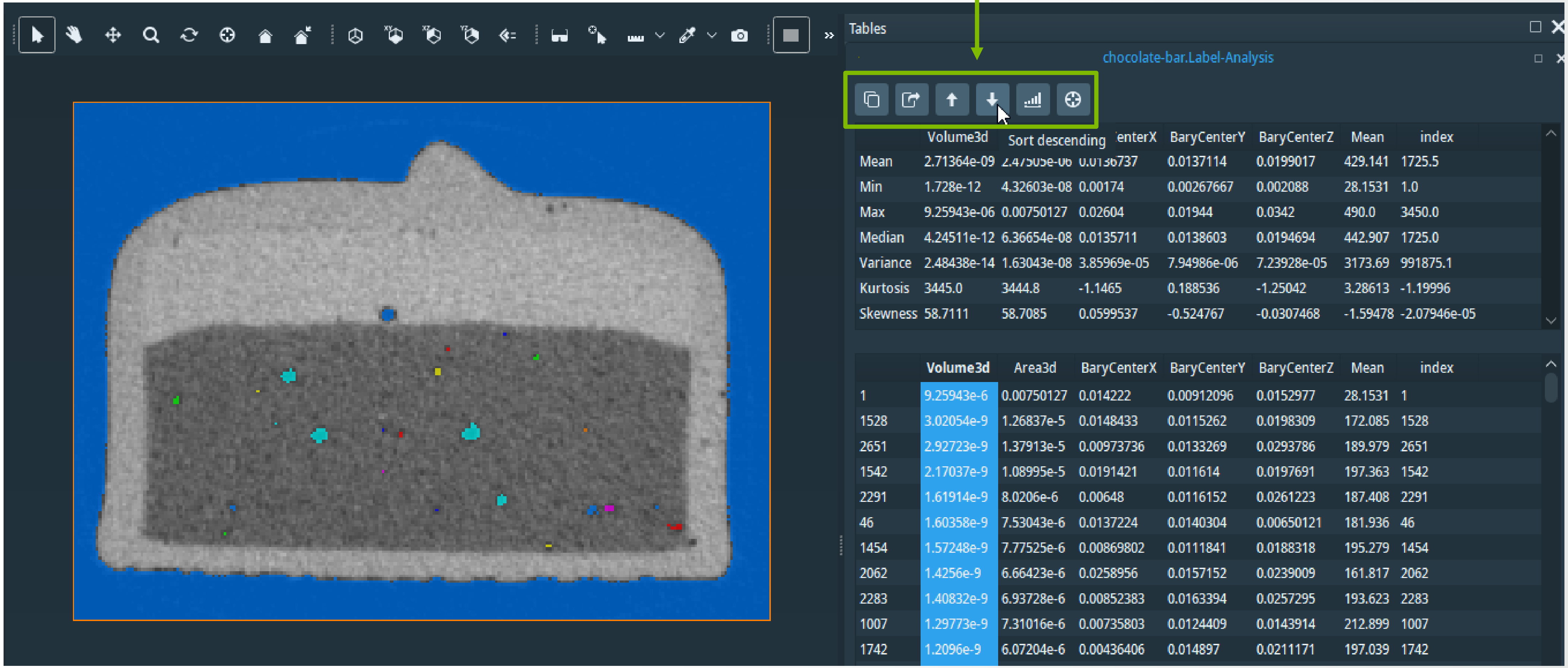
	Volume3d	Area3d	BaryCenterX	BaryCenterY	BaryCenterZ	Mean	index
1	9.25943e-6	0.00750127	0.014222	0.00912096	0.0152977	28.1531	1
2	8.64e-12	1.75547e-7	0.011616	0.018168	0.002088	430.6	2
3	3.456e-12	8.12381e-8	0.01392	0.01782	0.00312	423.5	3
4	9.8496e-11	1.01297e-6	0.0167053	0.0162358	0.00393053	211.404	4
5	2.2464e-11	3.52903e-7	0.0161354	0.01344	0.00403385	368.692	5
6	1.728e-12	4.32603e-8	0.00888	0.01596	0.00396	400.0	6
7	2.0736e-11	3.36868e-7	0.01493	0.01792	0.00409	352.833	7
8	5.184e-12	1.16227e-7	0.01272	0.01748	0.00412	453.0	8
9	1.48608e-10	1.35403e-6	0.00850884	0.0168614	0.00453767	213.105	9
10	4.1472e-11	5.46083e-7	0.00662	0.01764	0.00433	293.667	10
11	1.728e-12	4.32603e-8	0.02064	0.0114	0.00432	485.0	11

Label Analysis

Thermo Fisher
SCIENTIFIC

Different tools are available for manipulating the spreadsheet measures:

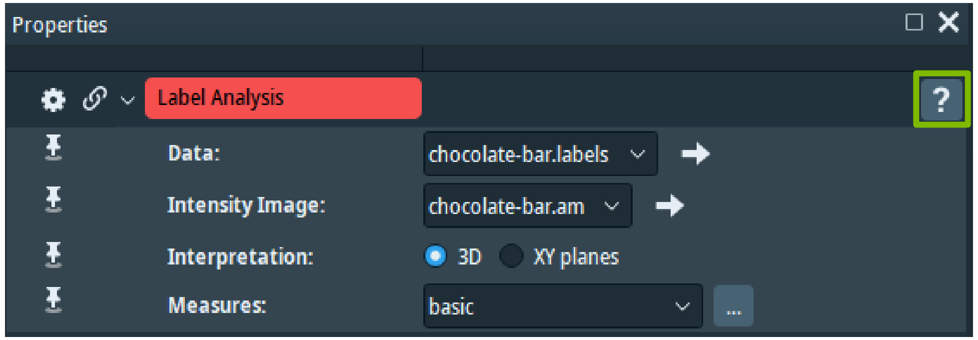
- E.g. “Sort descending” sorts the values of a column in descending order.

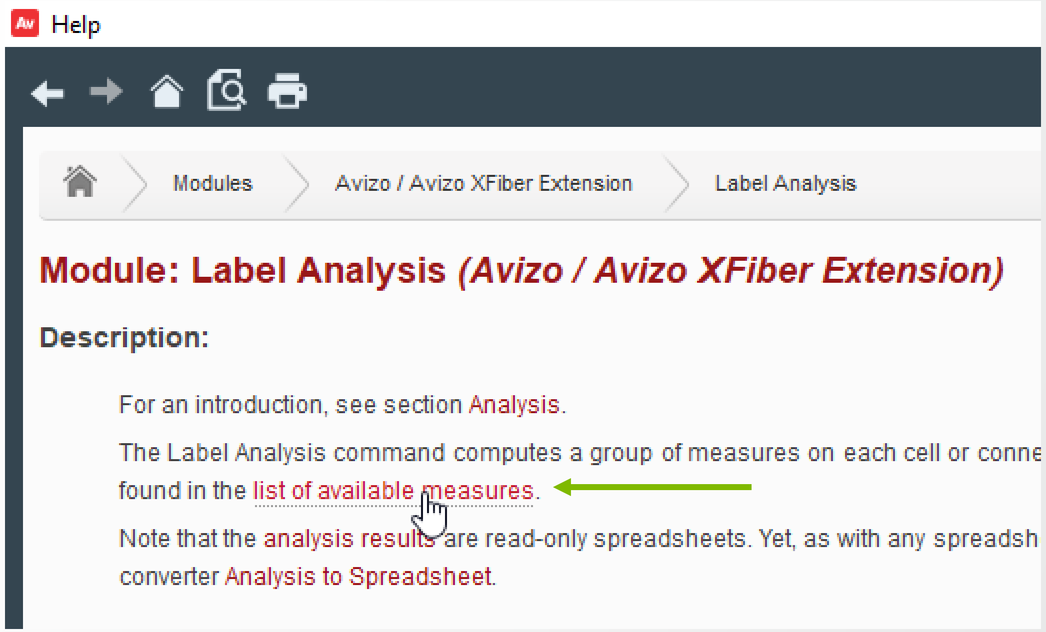


Label Analysis: pre-defined measures list

Some measures are pre-defined and ready to use by “Label Analysis” module.
For checking the list of pre-defined measures:

- Go to “Label Analysis” Help page
- Click on “list of available measures”





Label Analysis: pre-defined measures list

Help page with the list of pre-defined individual label measures.

Help

Search Help: <Enter a search string>

List of individual label measures

Cooccurrence

Computing Co-occurrence matrix is a common method for extracting texture attributes.

Notes:

- Co-occurrence measurements rely in a distribution of different vectors that can be set in the Cooccurrence tab of the Label Measures Attributes Editor.
- All of these measurements are based on the intensity input image. Using them with a label or binary image as intensity image is not relevant.

Measurements usable only with 2D interpretation:

- **CooccurrenceASM**: The Co-occurrence Angular Second Moment, also called uniformity, gives high values when image pixels present strong local uniformity.

$$ASM = \sum_{i,j=1}^{N} M(i,j)^2 \text{ with M the cooccurrence matrix and N the number of gray level.}$$

- **CooccurrenceCon**: The Co-occurrence Contrast gives high values for great gray level variations.

$$Con = \sum_{i,j=1}^{N} M(i,j) \times (i-j)^2$$

- **CooccurrenceCor**: The Co-occurrence Correlation measures the dependency between gray levels and those of neighboring pixels.

$$Cor = \sum_{i,j=1}^{N} M(i,j) \times \frac{(i-\mu_i)(j-\mu_j)}{\sqrt{\sigma_i^2 \sigma_j^2}}$$

$$\text{with } \mu_i = \mu_j = \sum_{i,j=1}^{N} i \times M(i,j)$$

$$\text{and } \sigma_i^2 = \sigma_j^2 = \sum_{i,j=1}^{N} (i-\mu_i)^2 \times M(i,j)$$

- **CooccurrenceDEn**: Co-occurrence Difference Entropy.

$$DEn = -\sum_{k=0}^{N-1} p_{x-y}(k) \times log(p_{x-y}(k))$$

$$\text{with } p_{x-y}(k) = \sum_{|i-j|=k} M(i,j)$$

- **CooccurrenceDirX**: X offset defining the direction used for co-occurrence computation (dx).
- **CooccurrenceDirY**: Y offset defining the direction used for co-occurrence computation (dy).
- **CooccurrenceDVa**: Co-occurrence Difference Variance.

$$DVa = variance(p_{x-y})$$

- **CooccurrenceEnt**: Co-occurrence Entropy.

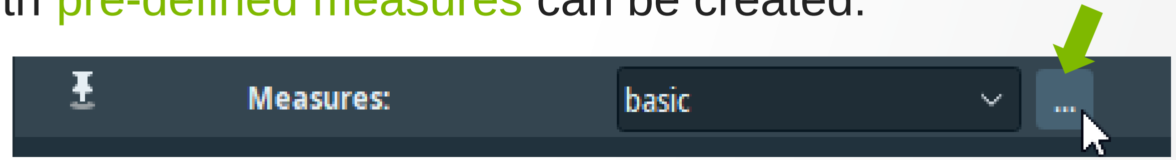
$$Ent = -\sum_{i,j=1}^{N} M(i,j) \times log(M(i,j))$$

- **CooccurrenceIC1**: Co-occurrence Information measure of Correlation 1.

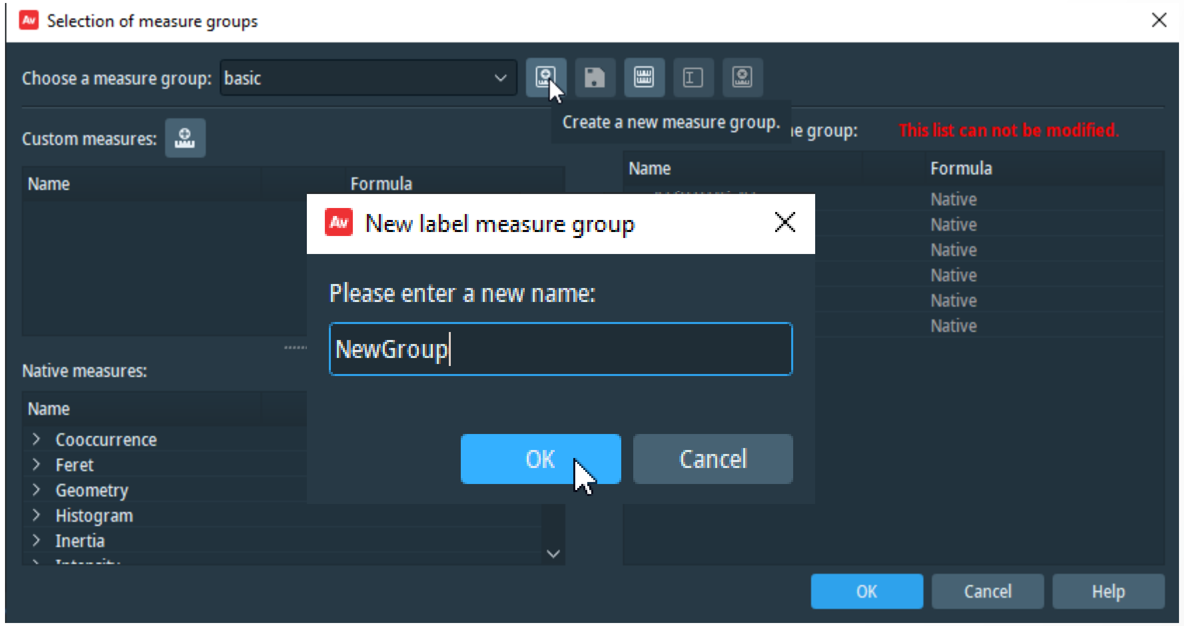
$$IC1 = \frac{HXY - HXY1}{max(HX, HY)}$$

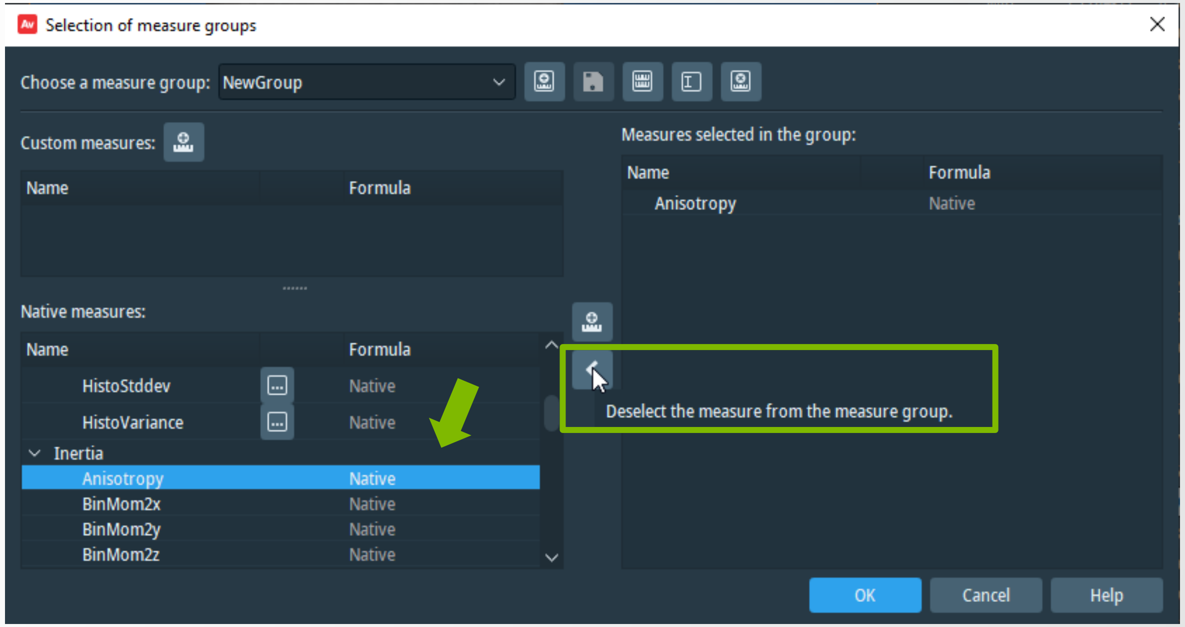
Label Analysis: custom measures list, custom measures

A custom measures list with pre-defined measures can be created:



- Click on a measure on the left side to add it to the group
- Select a measure added to the group (right side) and click on “Deselect the measure form the measure group” to remove it from the group.

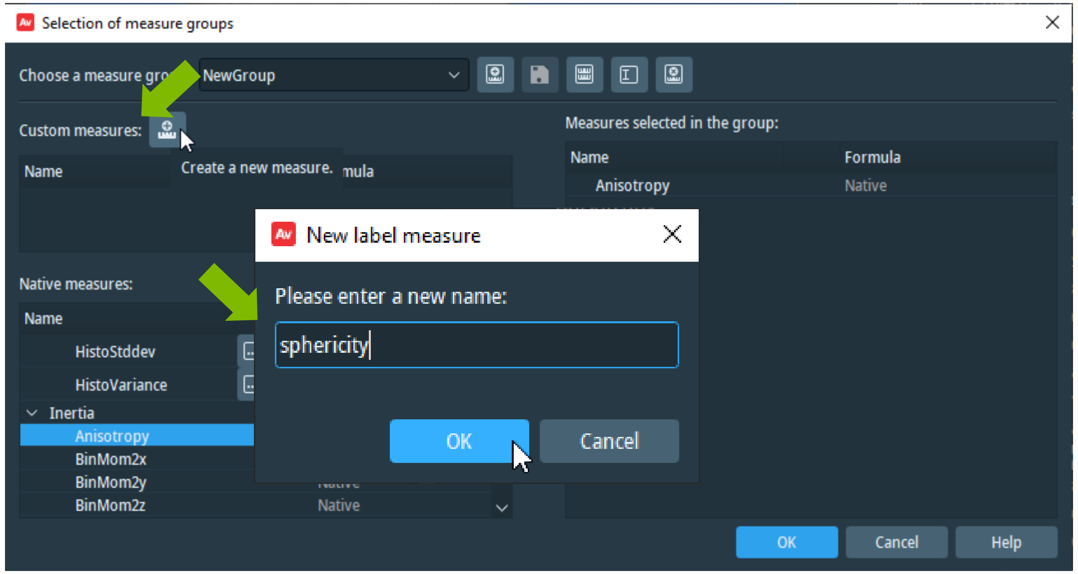


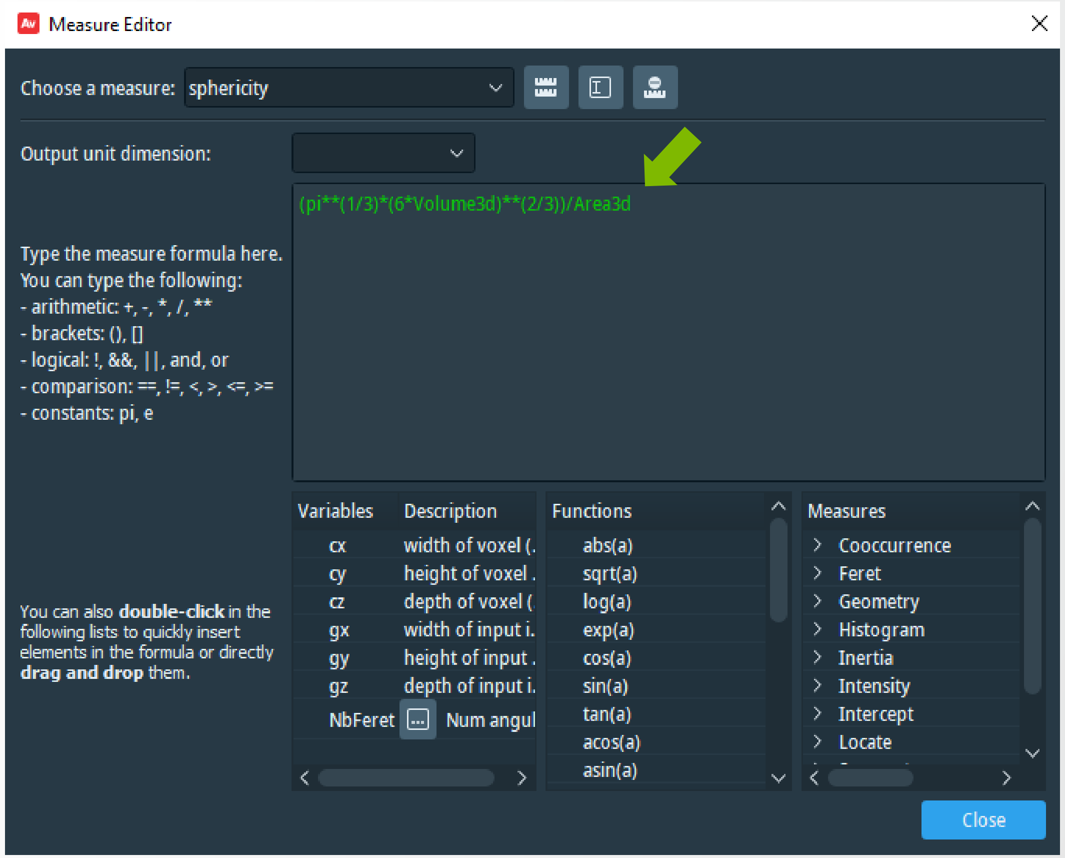


Label Analysis: custom measures list

Custom measures can also be created and added to the custom measures list. E.g. sphericity:

- Click on “Create a new measure” icon
- Type the name of the custom measure
- Type the measure in the “Measure Editor” (it’s green if valid, red if not).

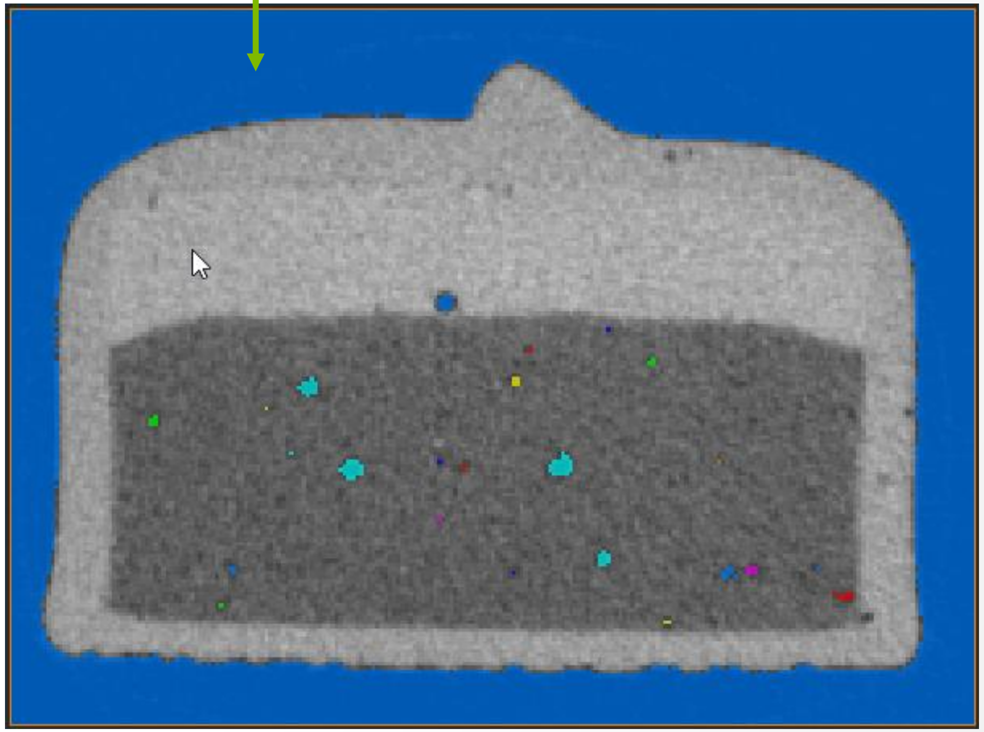




Quantification: removing unwanted detection

Q: how can one remove parasite detection from a label image and its corresponding measures in the measures spreadsheet ?
E.g.: for the segmentation example on chocolate bar, remove the label corresponding to background and keep only the porosity labels and measures

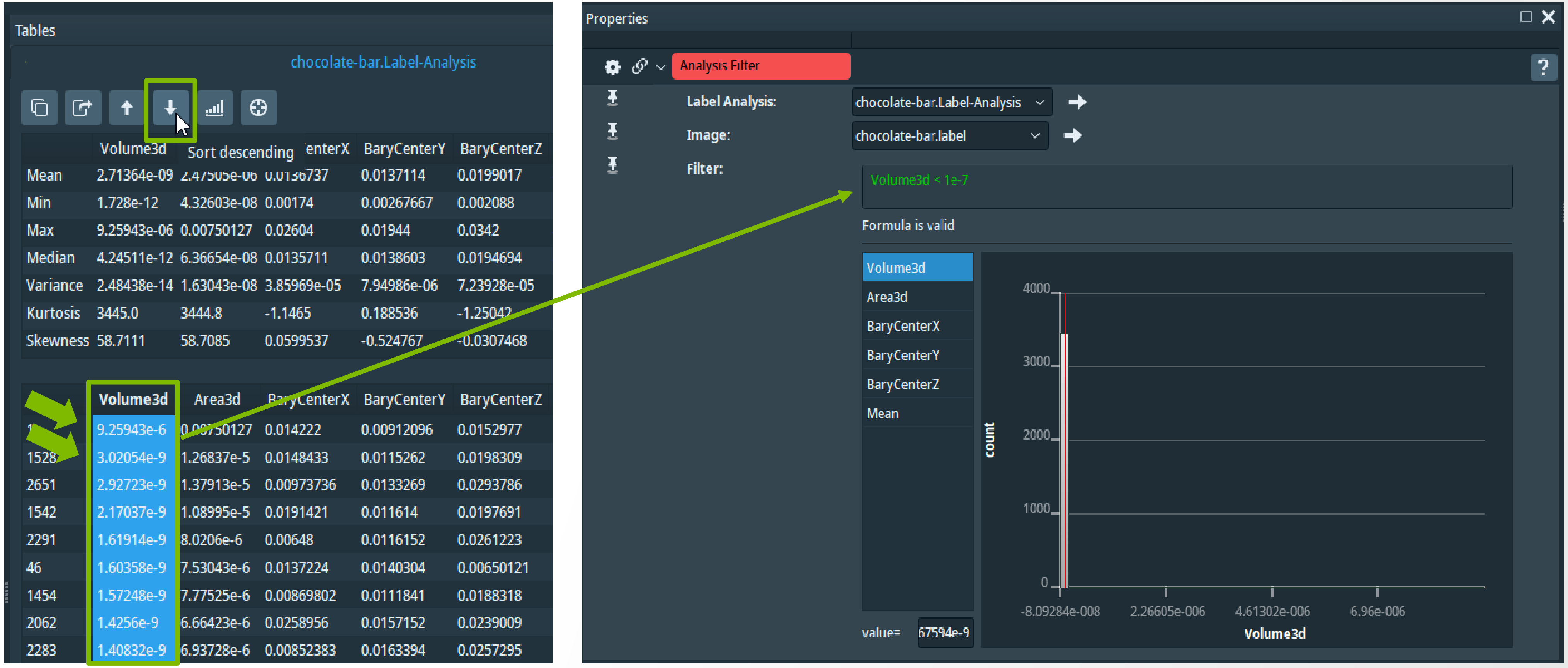
Unwanted detection to be removed



Quantification: removing unwanted detection

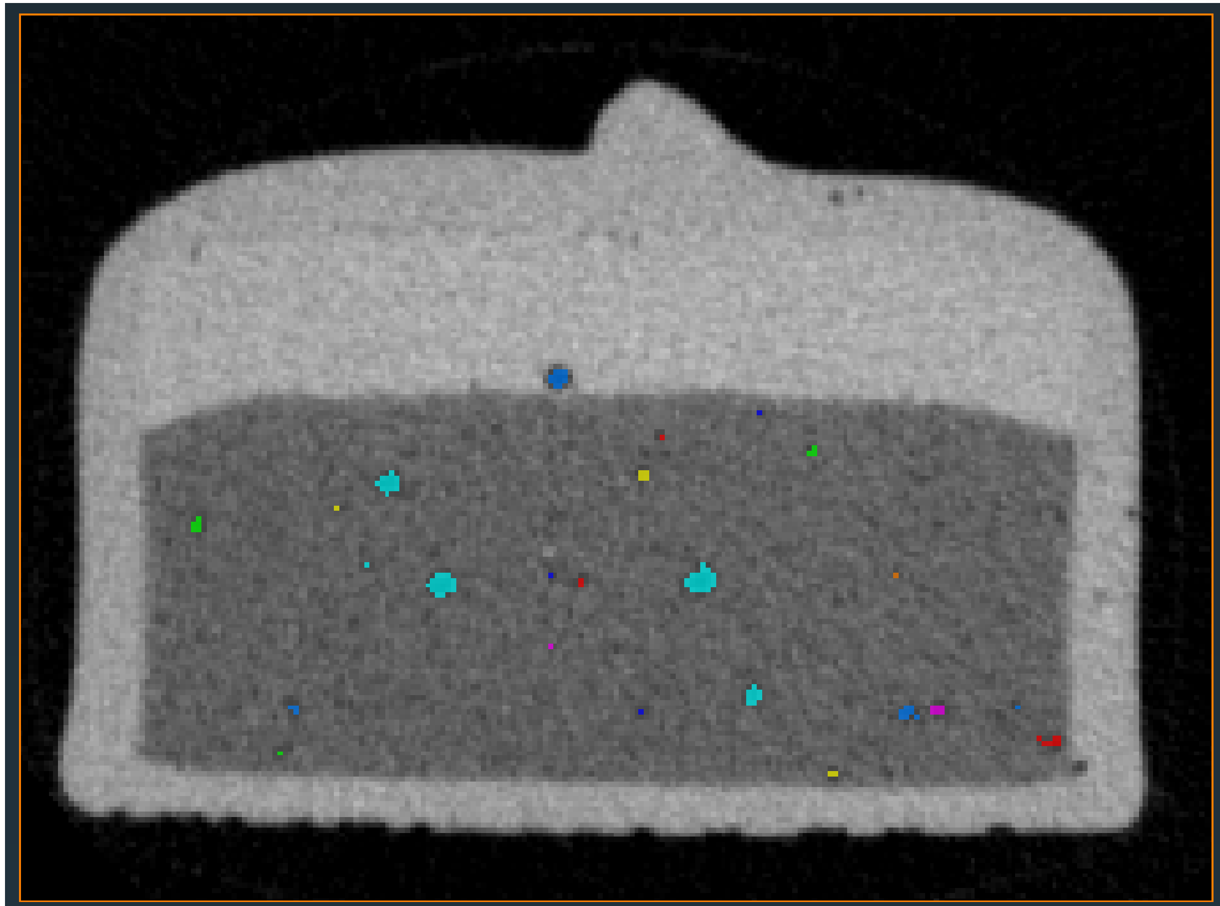
A: Analysis Filter module

- Filters out from the measures spreadsheet, labels that do not fulfill a filtering criterion
- Same behavior on the label image label image (when provided as input – optional)
- Filtering criterion: choose one (or more) measures that allow to discriminate the parasite detection and write filtering formula.



Quantification: removing unwanted detection

Analysis Filter result for the filtering formula “Volume3d < 1e-7”:



	Volume3d	Area3d	BaryCenterX	BaryCenterY	BaryCenterZ	Mean	index
Mean	2.97003e-11	3.00971e-07	0.0136736	0.0137128	0.0199031	1.0	1726.0
Min	1.728e-12	4.32603e-08	0.00174	0.00267667	0.002088	1.0	2.0
Max	3.02054e-09	1.37913e-05	0.02604	0.01944	0.0342	1.0	3450.0
Median	3.82821e-12	9.26204e-08	0.0135757	0.0138634	0.0192277	1.0	1726.0
Variance	1.70541e-20	5.5529e-13	3.86081e-05	7.94606e-06	7.24077e-05	0.0	991300.1
Kurtosis	207.952	92.851	-1.14702	0.191573	-1.25063	--	-1.19997
Skewness	12.3213	8.00959	0.0600184	-0.525469	-0.0311717	--	-2.10824e-05

	Volume3d	Area3d	BaryCenterX	BaryCenterY	BaryCenterZ	Mean	index
1527	3.02054e-9	1.26837e-5	0.0148433	0.0115262	0.0198309	1.0	1528
2650	2.92723e-9	1.37913e-5	0.00973736	0.0133269	0.0293786	1.0	2651
1541	2.17037e-9	1.08995e-5	0.0191421	0.011614	0.0197691	1.0	1542
2290	1.61914e-9	8.0206e-6	0.00648	0.0116152	0.0261223	1.0	2291
45	1.60358e-9	7.53043e-6	0.0137224	0.0140304	0.00650121	1.0	46
1453	1.57248e-9	7.77525e-6	0.00869802	0.0111841	0.0188318	1.0	1454
2061	1.4256e-9	6.66423e-6	0.0258956	0.0157152	0.0239009	1.0	2062
2282	1.40832e-9	6.93728e-6	0.00852383	0.0163394	0.0257295	1.0	2283
1006	1.29773e-9	7.31016e-6	0.00735803	0.0124409	0.0143914	1.0	1007
1741	1.2096e-9	6.07204e-6	0.00436406	0.014897	0.0211171	1.0	1742
524	1.14048e-9	5.93751e-6	0.0252216	0.0146733	0.00993727	1.0	525

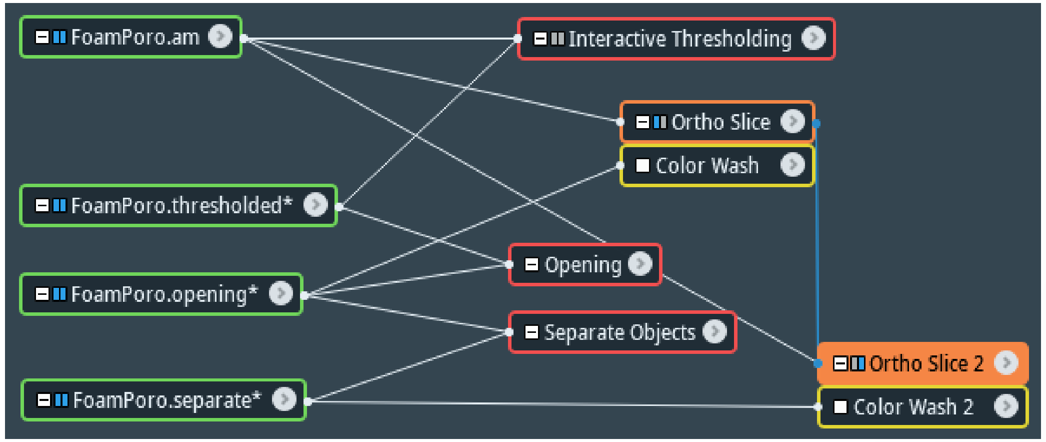
Shape analysis: represent objects as ellipsoids

Example: porosities analysis in *FoamPoro.am*

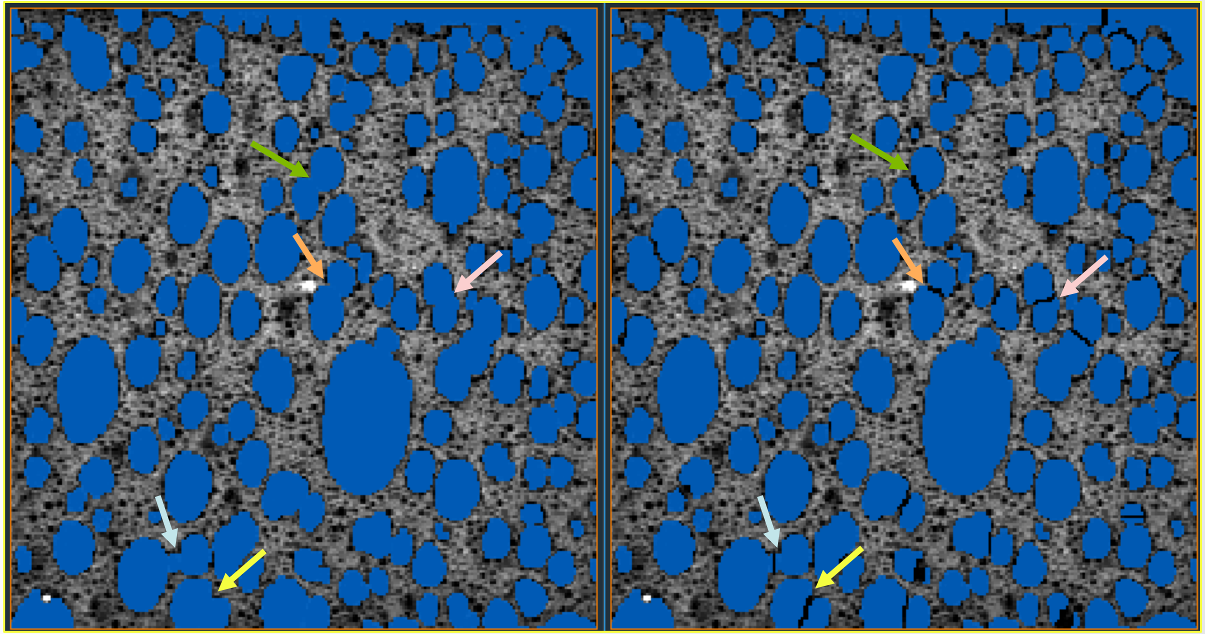
Step 1:

- Do a binary segmentation of the porosities

Segmentation workflow



Porosities before and after object separation

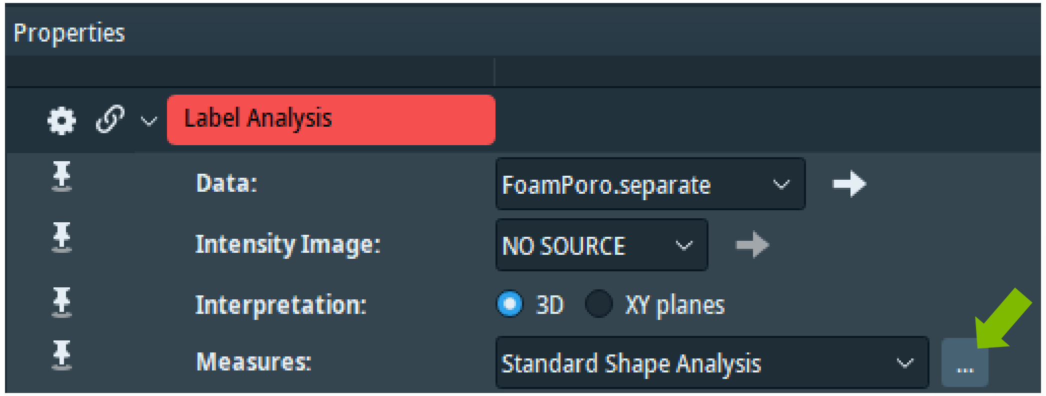


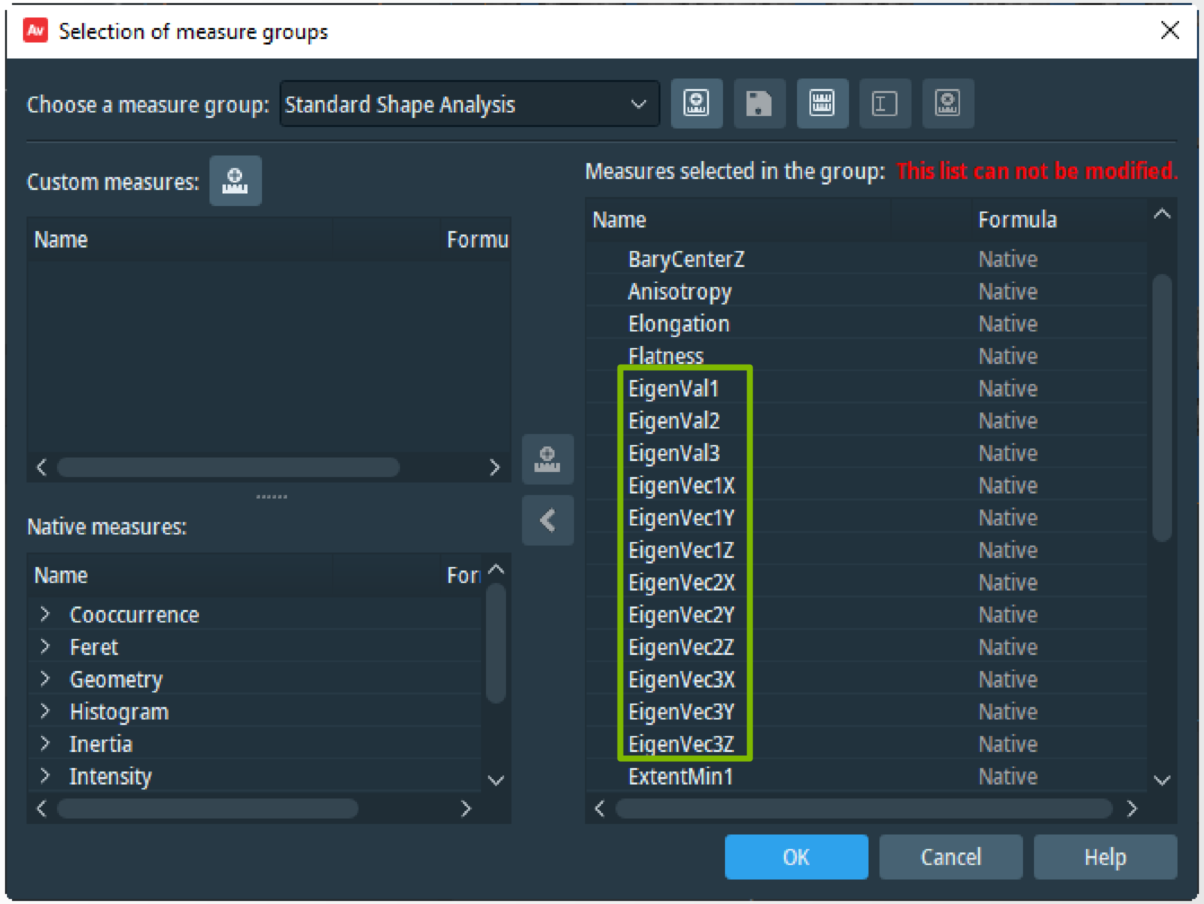
Shape analysis: represent objects as ellipsoids

Example: porosities analysis in *FoamPoro.am*

Step 2:

- Apply Label Analysis with "Standard Shape Analysis"
- Measures for shape analysis:
  - Eigenvectors
  - Eigenvalues



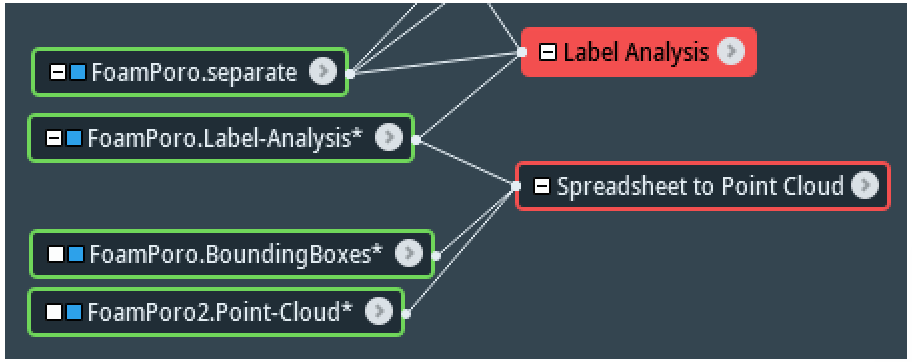


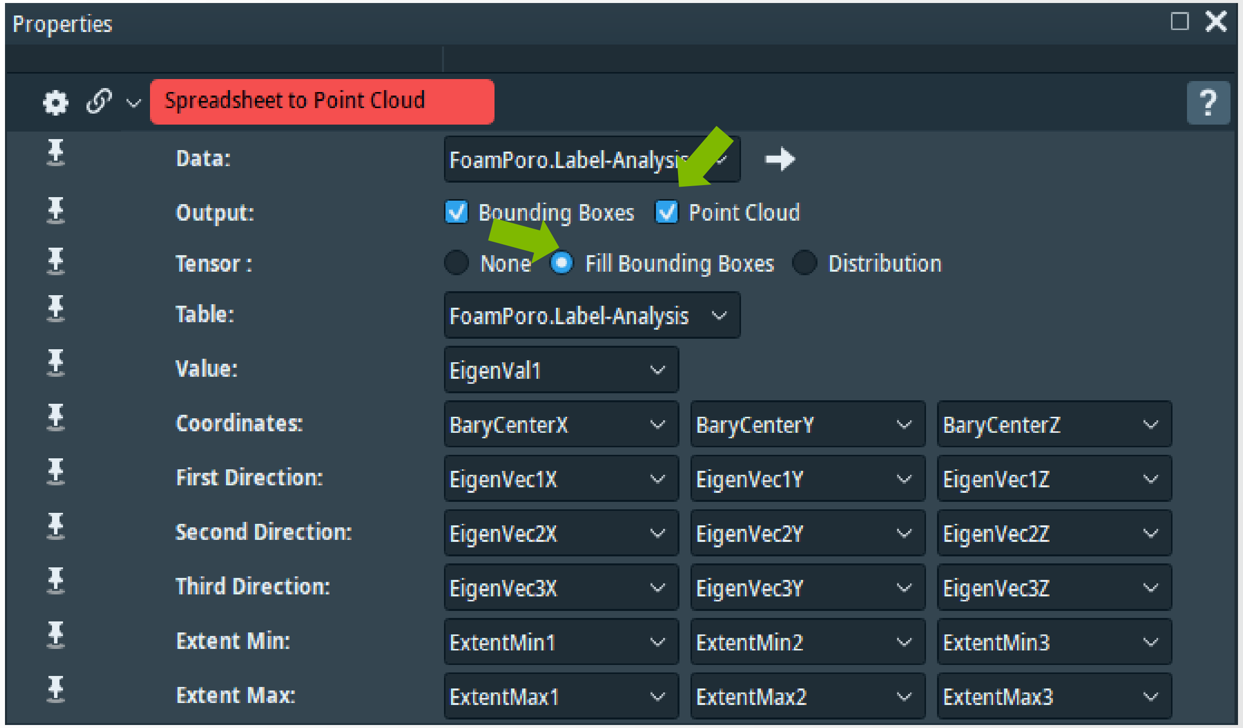
Shape analysis: represent objects as ellipsoids

Example: porosities analysis in *FoamPoro.am*

Step 3:

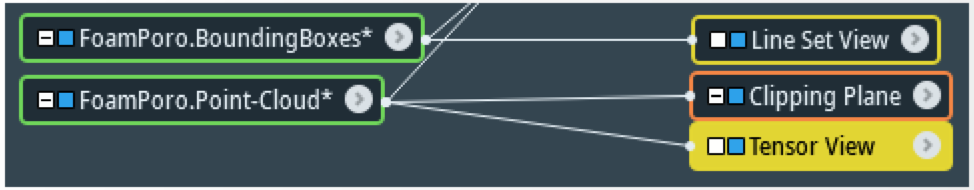
- Generate Bounding Box and Ellipsoid representation for individual labels via “Spreadsheet to Point Cloud” module
  - Check “Bounding Boxes” Output
  - Check “Point Cloud” Output and “Fill Bounding Boxes” for ellipsoid representation





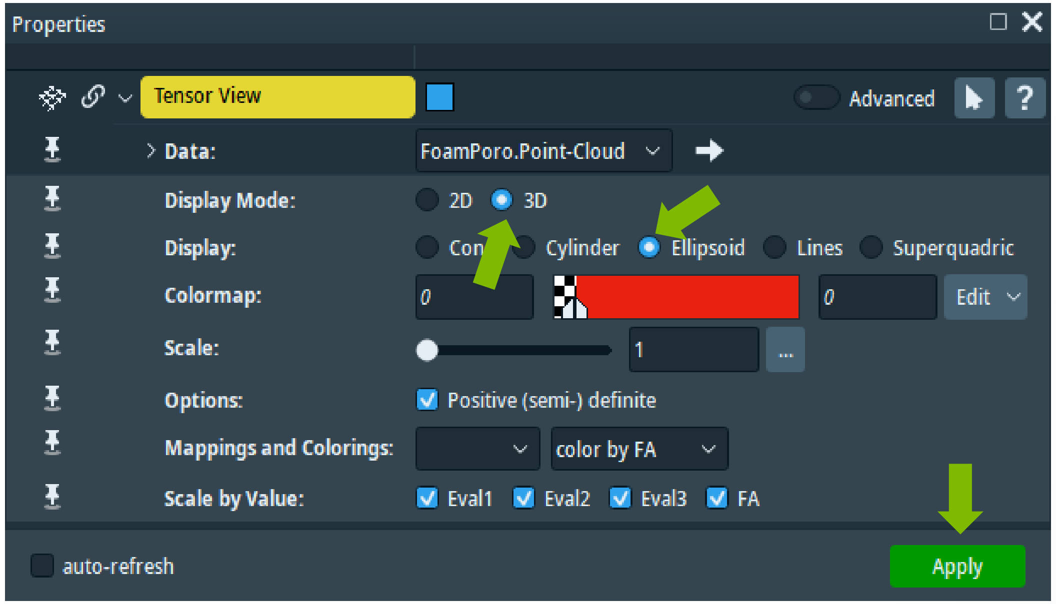
Shape analysis: represent objects as ellipsoids

Example: porosities analysis in *FoamPoro.am*

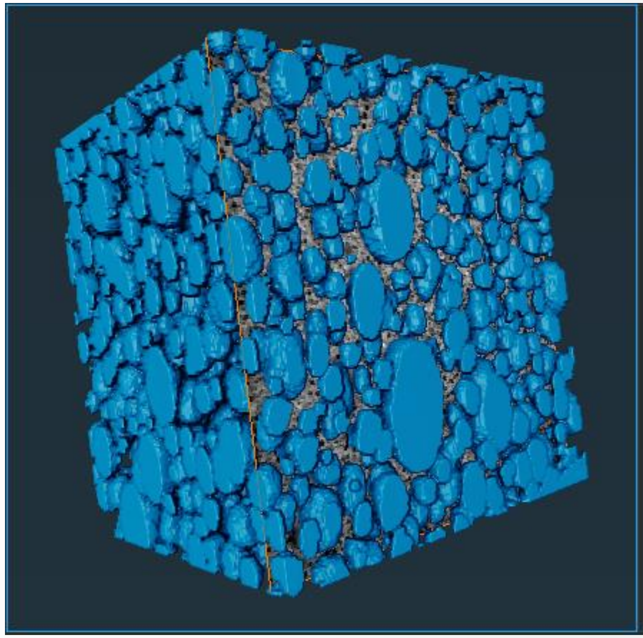


Step 4:

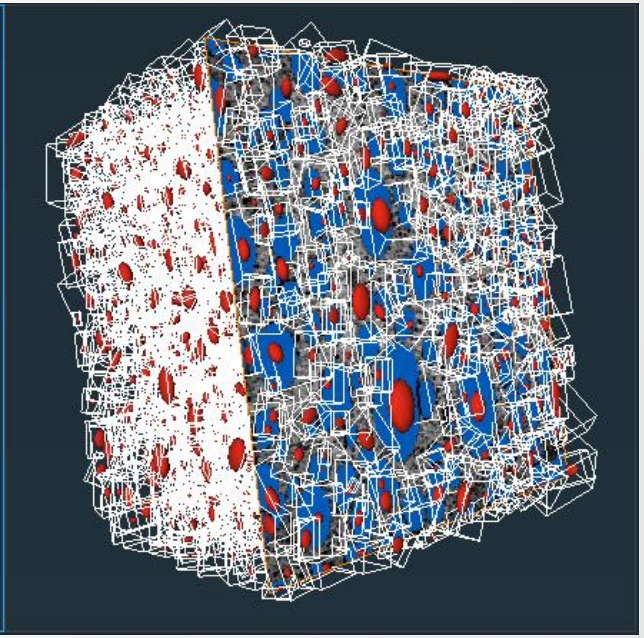
- Visualization:
    - Line Set View for Bounding Boxes
    - Tensor View for the ellipsoids (click on “Apply” for generating the visualization)



Binarisation representation

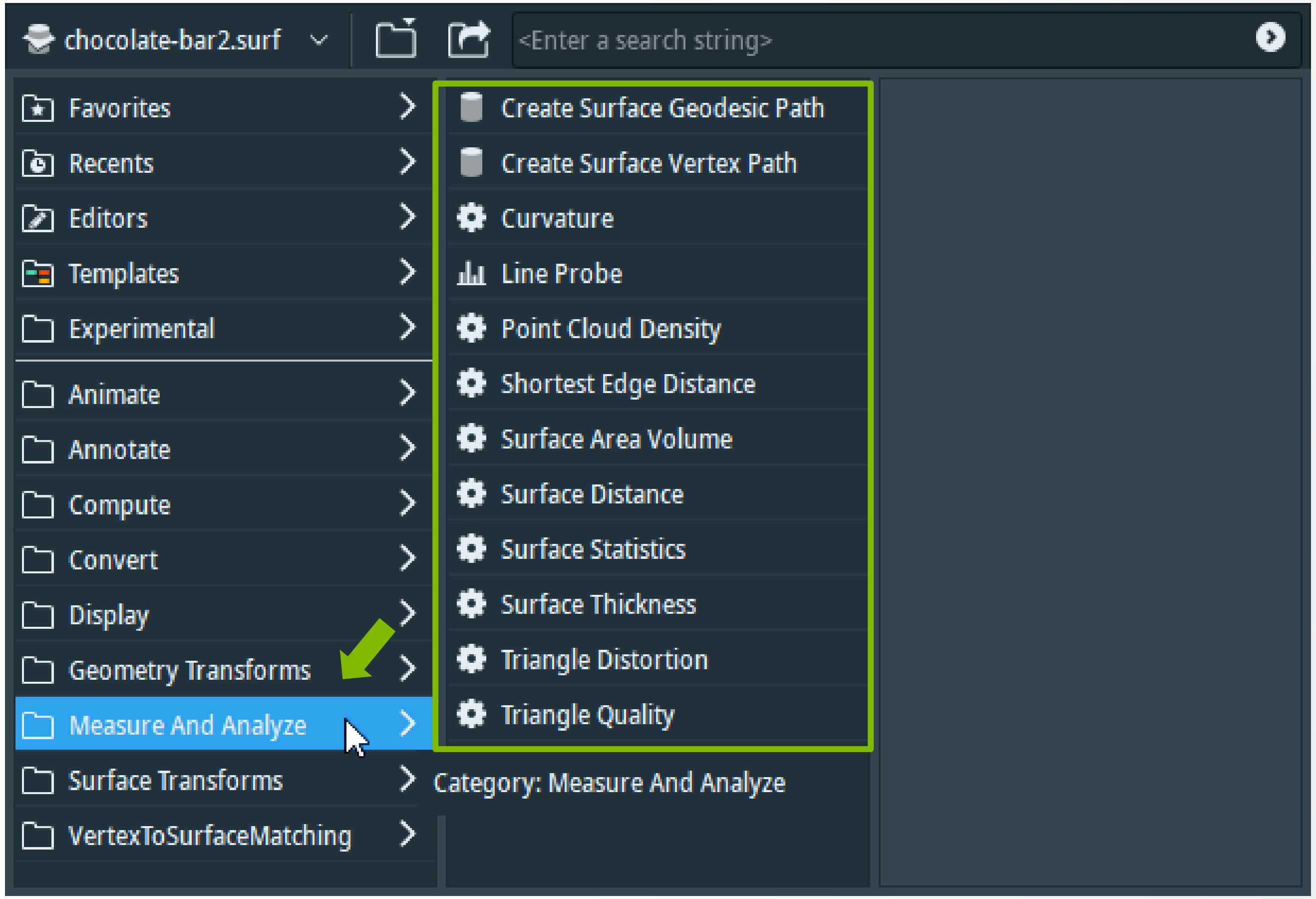


Bounding Boxes and Ellipsoid representation



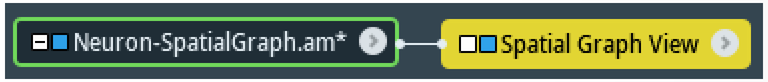
Surface measurements and statistics

Other Surface measurements and statistics modules are available in the “Measure and Analyze” object category (for access: right click on the surface object in the pool).



Extract skeletons and graphs

For filamentous data, automatic extraction of centerlines with local thickness can be done via Auto Skeleton module. It generates a spatial graph data:

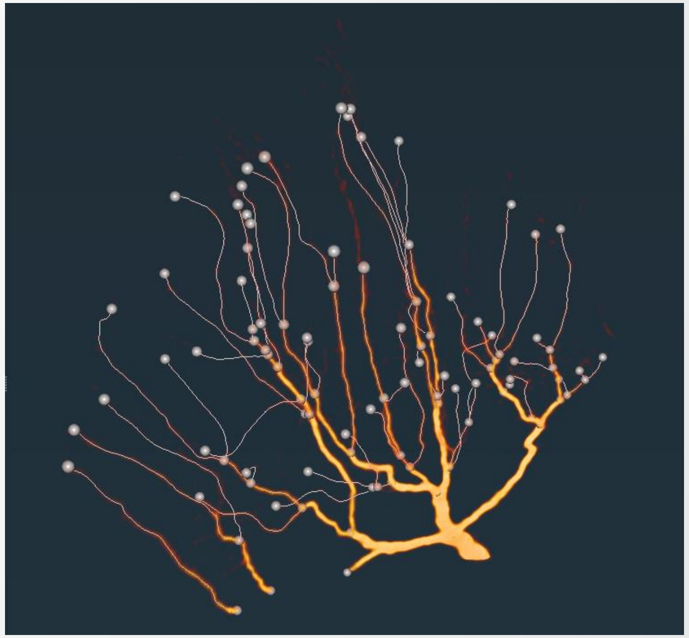
- Spreadsheet with information on nodes, points (thickness info available too), and segments
- Can be visualized with: 

Example: *neuron.am* and *Neuron-SpatialGraph.am* data in …\data\tutotials\neuron

Neuron data

Neuron spatial graph

Neuron data and spatial graph superposed



Measurements and annotations

Units management

- Units are necessary to interpret numbers as physical values.

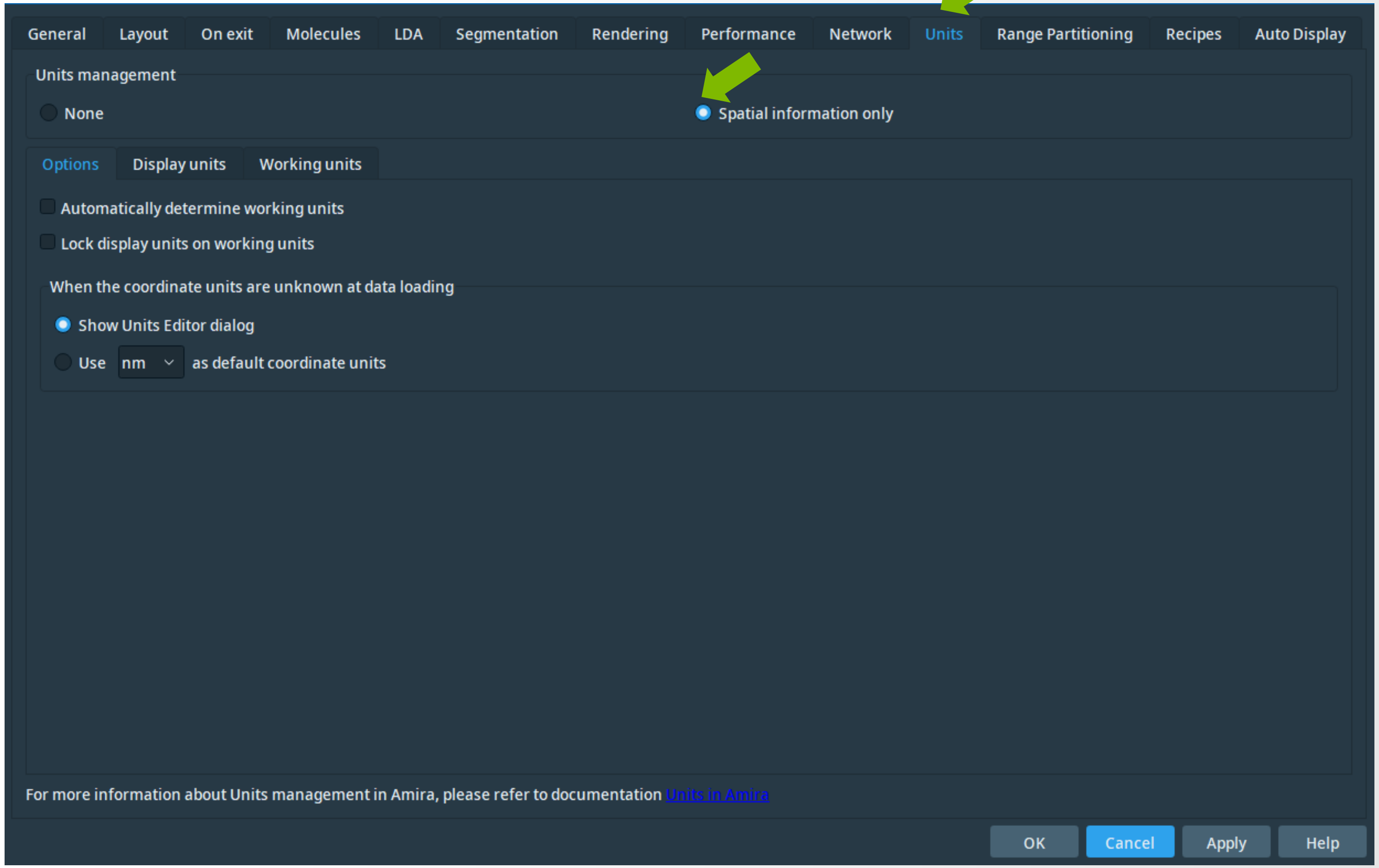
- Two “types” of units must be distinguished:
  - **Working units**:
    - All calculations are done in those units.
    - Can only be changed before loading the first data-set in the project.
  - **Display units**:
    - Used to display numerical values.
    - Can be changed anytime, independently of working units

    Note: Display Units do not change data.

Use **adequate working** units because of the **impact on numerical precision!**

Units management

- Units management is implemented for spatial size only (coordinate and angle units).
- Units management settings can be accessed in preferences:



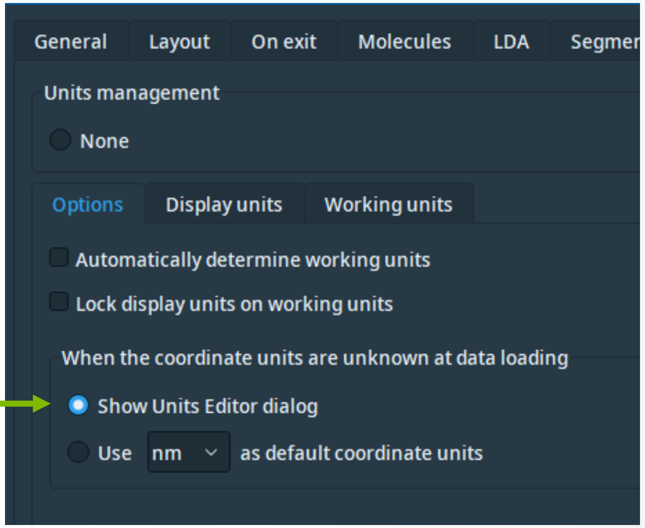
- Default is:
  - enabled ("Spatial information only")
  - default unit: nm

Units management

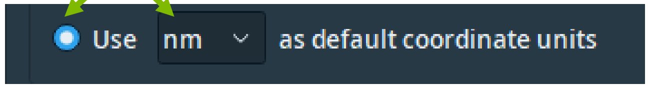
Loading data

- When loading data, either spatial information is given in the same unit (default coordinate units), or the units of each data-set must be set correctly independently.
  - Default setting
  - Recommended:

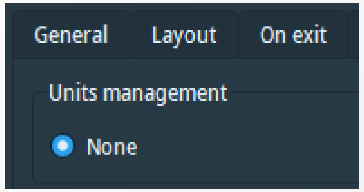
Set to your most likely unit, as this will be pre-selected in the **Units Editor dialog**.



- If you are always using data with the same unit, set the appropriate unit here:



- Alternatively, switch off units management.



Units management

Beware:

- If you save a project, the information about working units is saved with it
- If you load a project having working units set differently from your current ones, the settings from the project are loaded and applied permanently, until you explicitly change it back!

Units management

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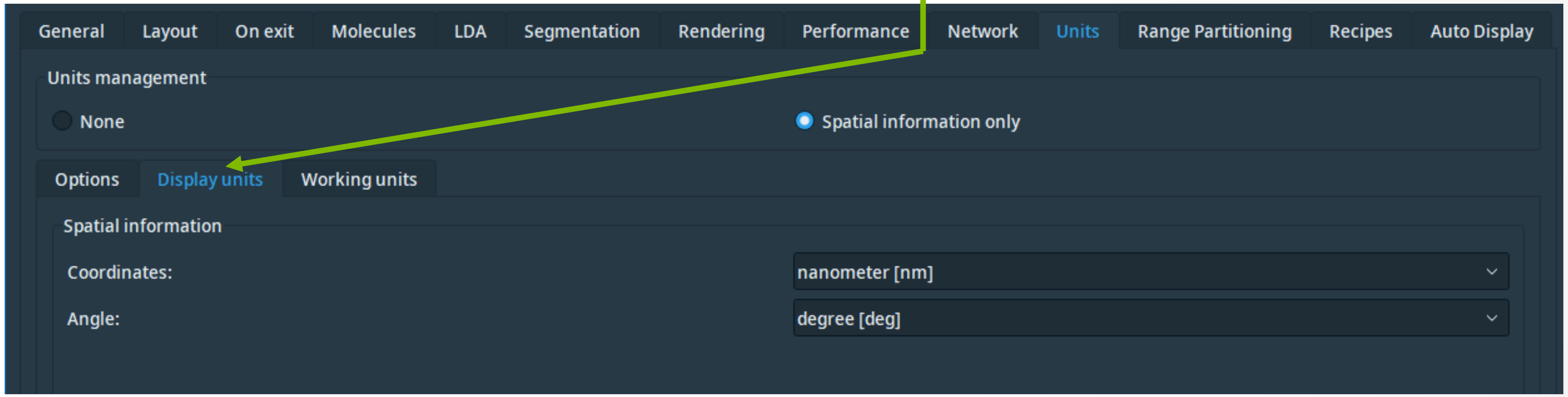
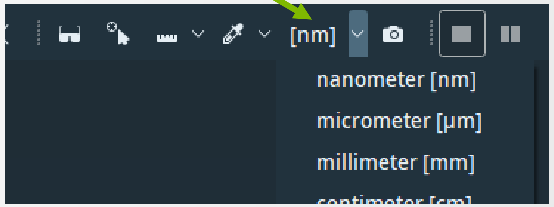
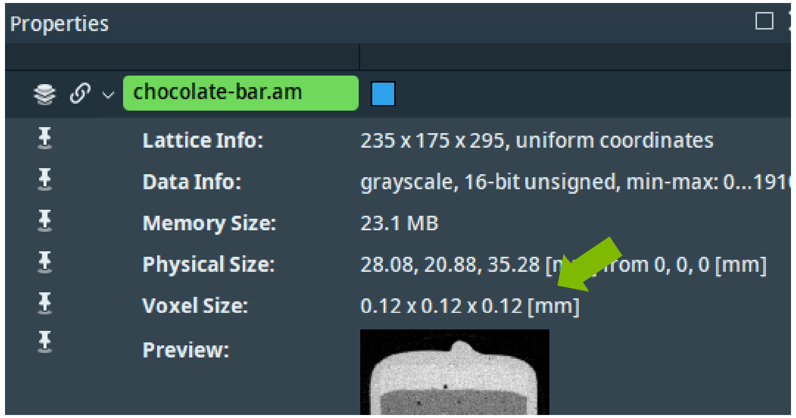
Display units:

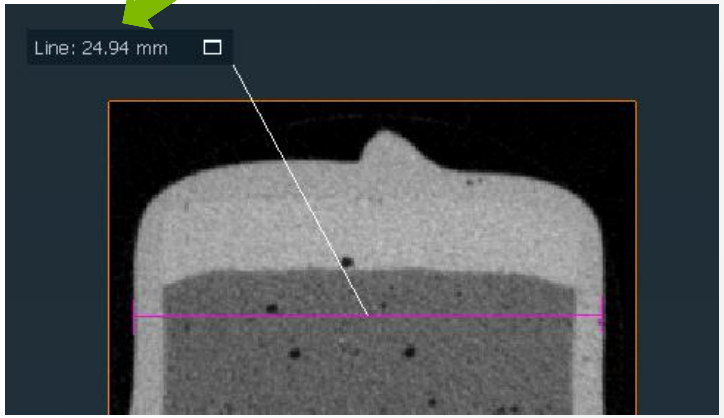
- May be changed anytime, either via the preferences, or via the Viewer Window settings.

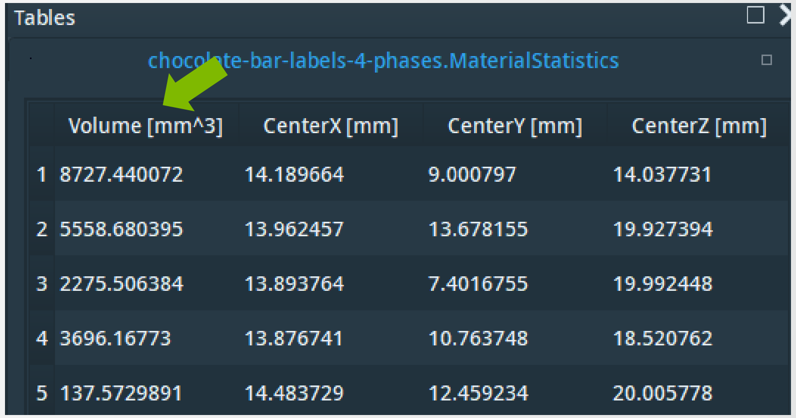




- Affect all measures with units management, e.g.:



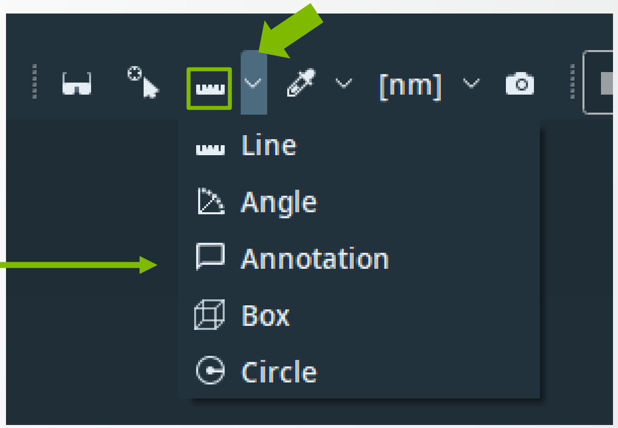




Performing measurements

Measurement tool:

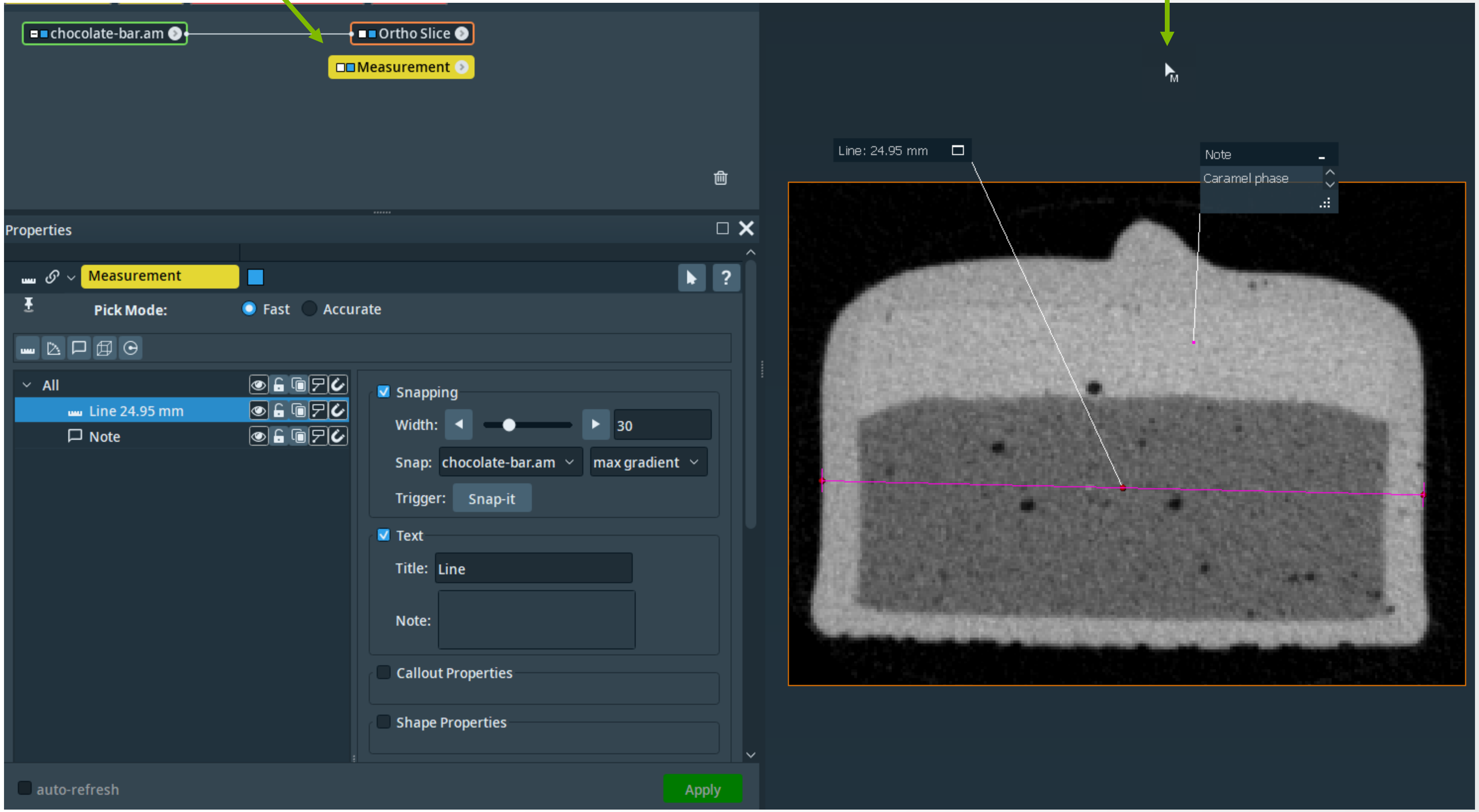
- invoke with
  - Measure button in viewer toolbar (shortcut “M”)
  - Via “Create object … → Annotations → Measurement”
- Different measurements types available
- works on visualization modules in
  - 2D, e.g. Ortho Slice, Slice
  - 3D, e.g. Surface Rendering, Volume Rendering, Voxelized Rendering



Performing measurements

Measurement tool:

- is active when Measurement module is selected

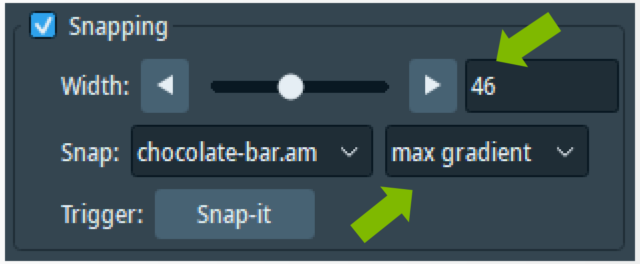


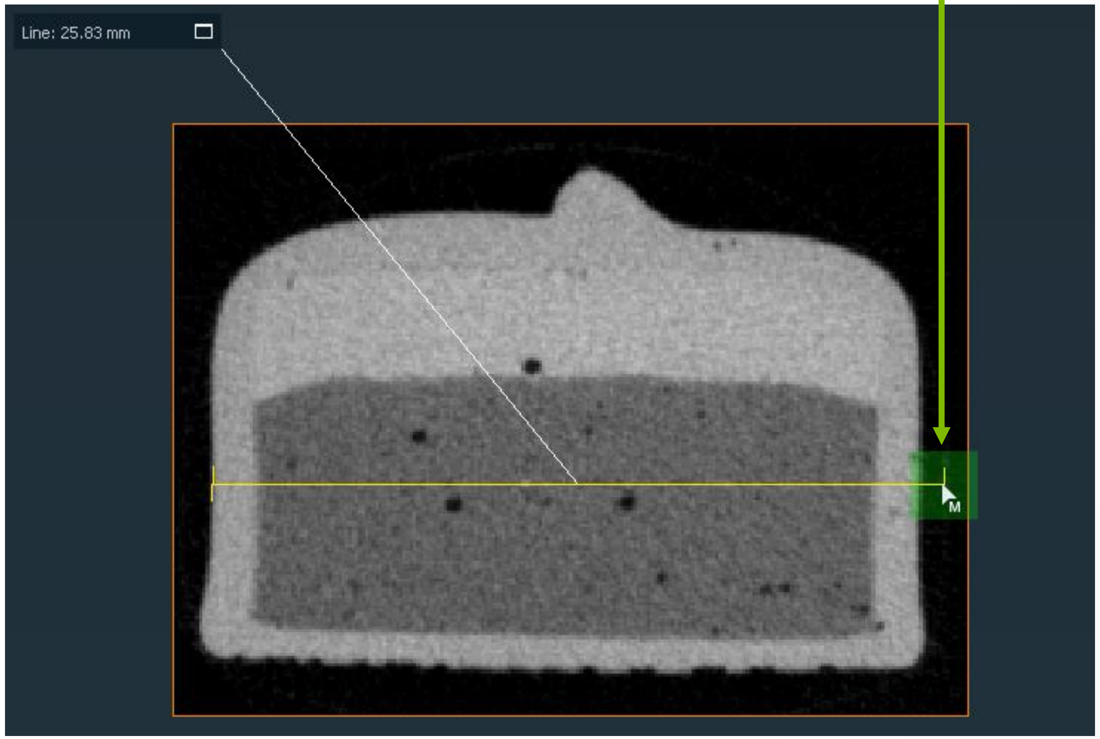
Performing measurements

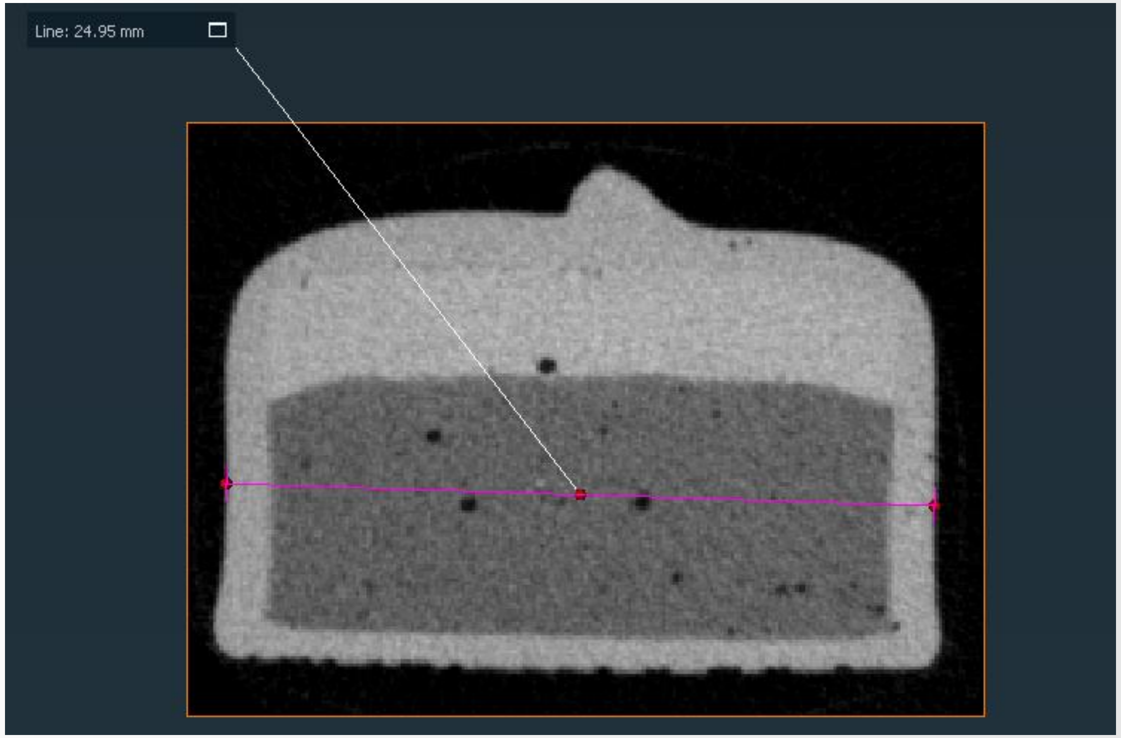
Measurement module:

- snapping possible – click on a point
  - within search window (semi-transparent square)
  - to min, max, or gradient (min or max)

Snapping example



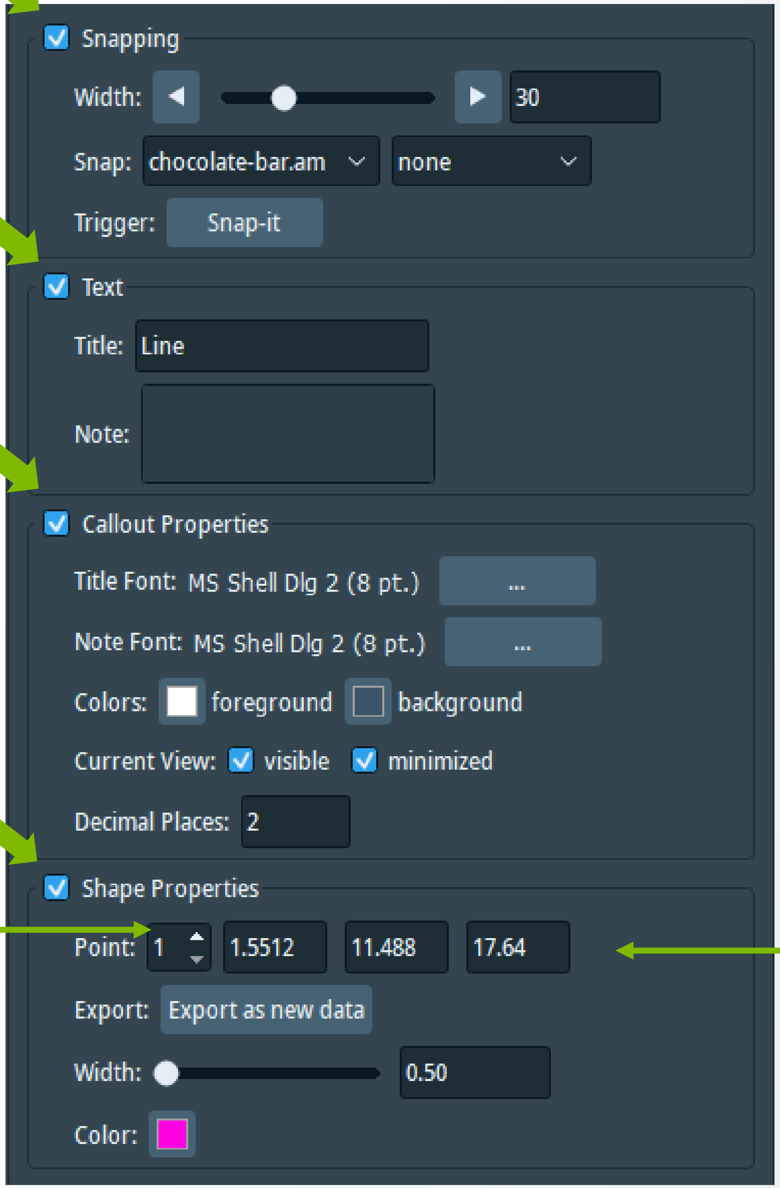




Performing measurements

Measurement module:

- editable properties:
  - snapping
  - text (title and note)
  - callout and shape properties
    - measure points (colors, font, etc.)



Select point to modify (#1 or #2)

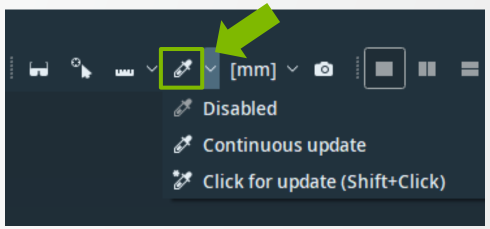
Edit x, y, z coordinated of the selected point

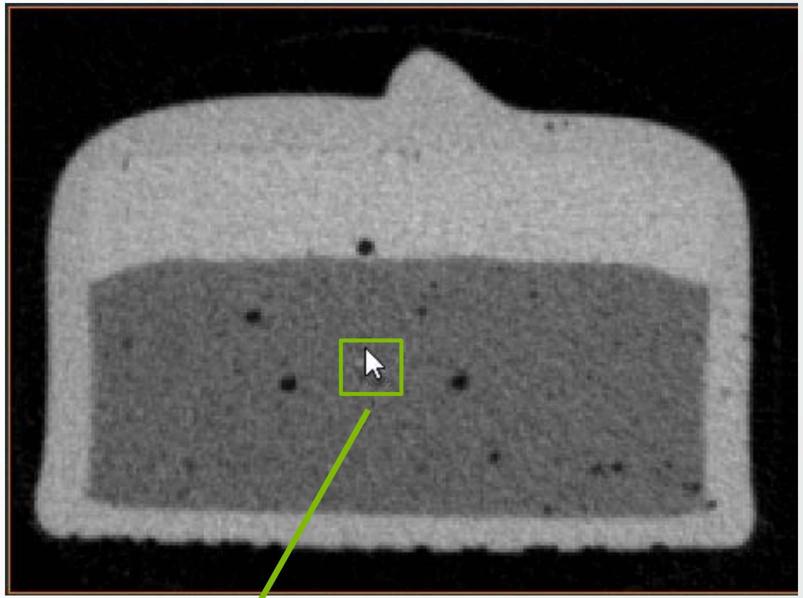
Probing data value

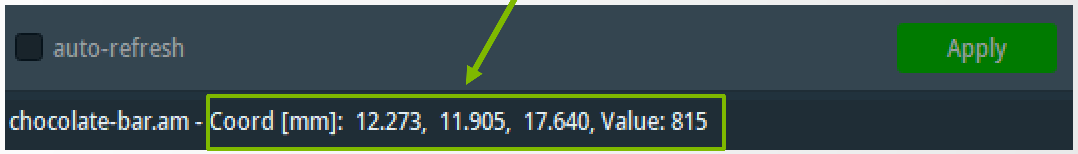
Quick Probe

- show value of data at mouse position (interactive mode should be on)
- works with e.g. Slice, Ortho Slice, and Volume Rendering
- 2 modes:
  - Continuous update
  - Click for update (Shift + Click)

  ➔ prefer “Click for update”
- value is shown in status bar



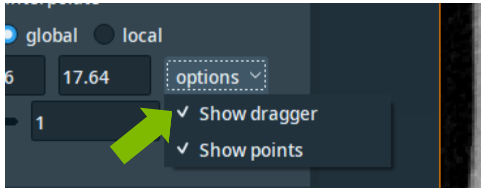


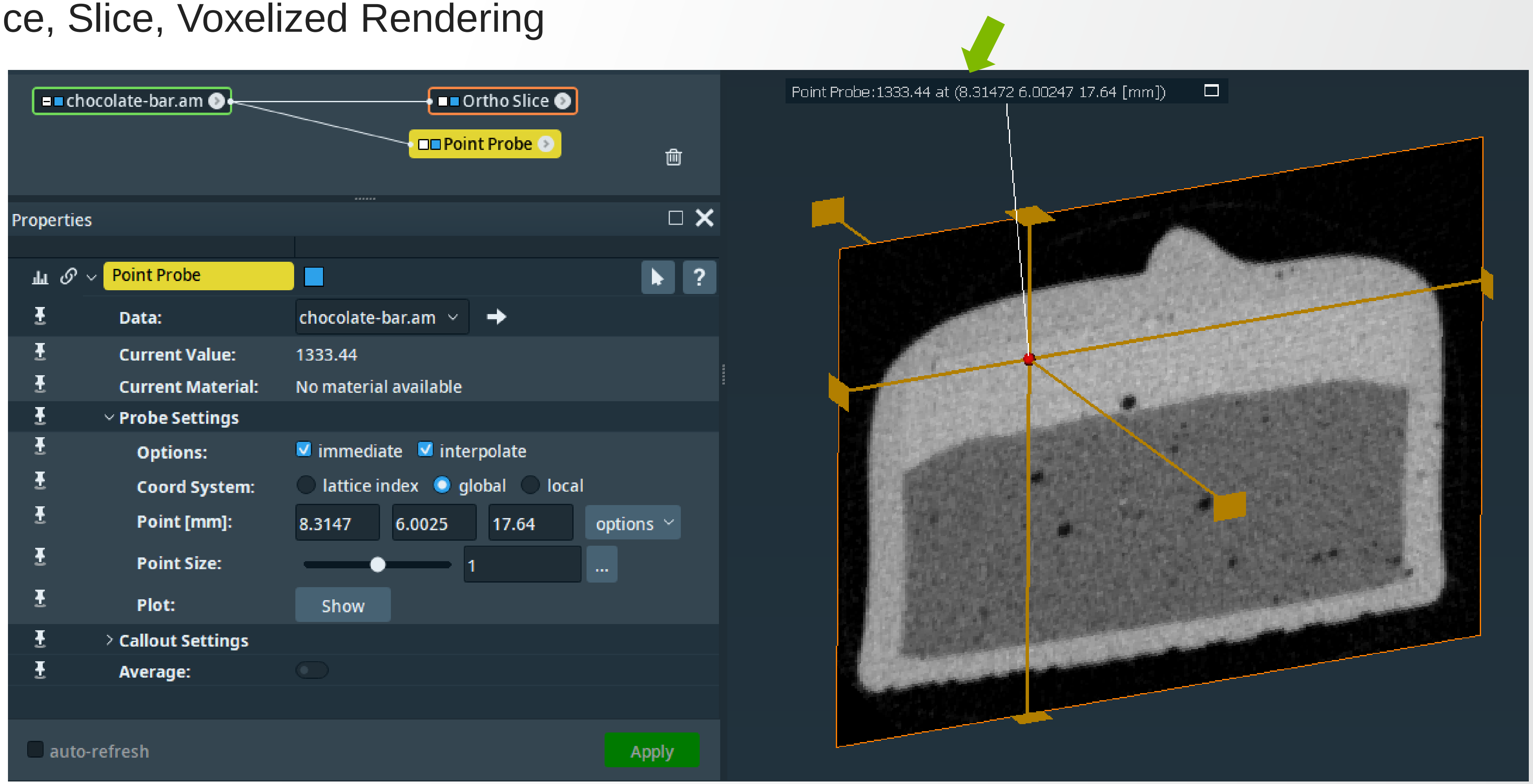


Probing data value

Point Probe

- get callout with position and value
- select Point Probe and click with middle mouse button to pick a location, or move the handles
- works with e.g. Ortho Slice, Slice, Voxelized Rendering
- editable callout settings
- local averaging
- hide dragger:





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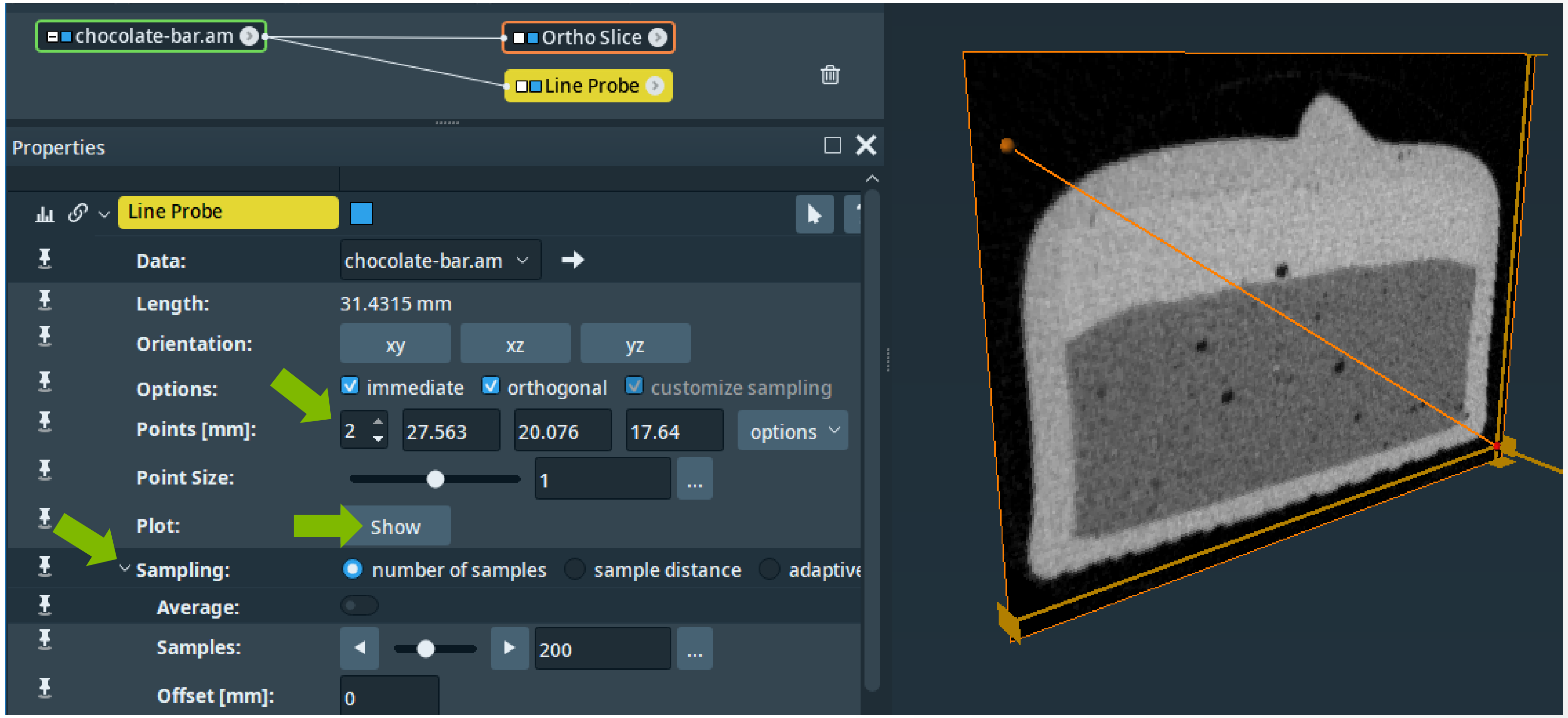
Probing data value

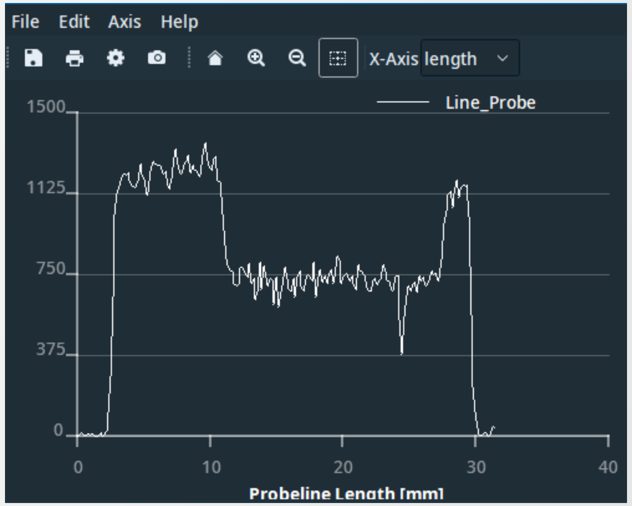
Line Probe (2D and 3D)

- evaluate the data-values along a straight line
- select point to modify (#1 or #2) – to change the coordinates in the text boxes or
- click with middle mouse button to pick new location or use handles to position the points
- for arbitrary orientation de-select “orthogonal”



Display line-profile in plot window:

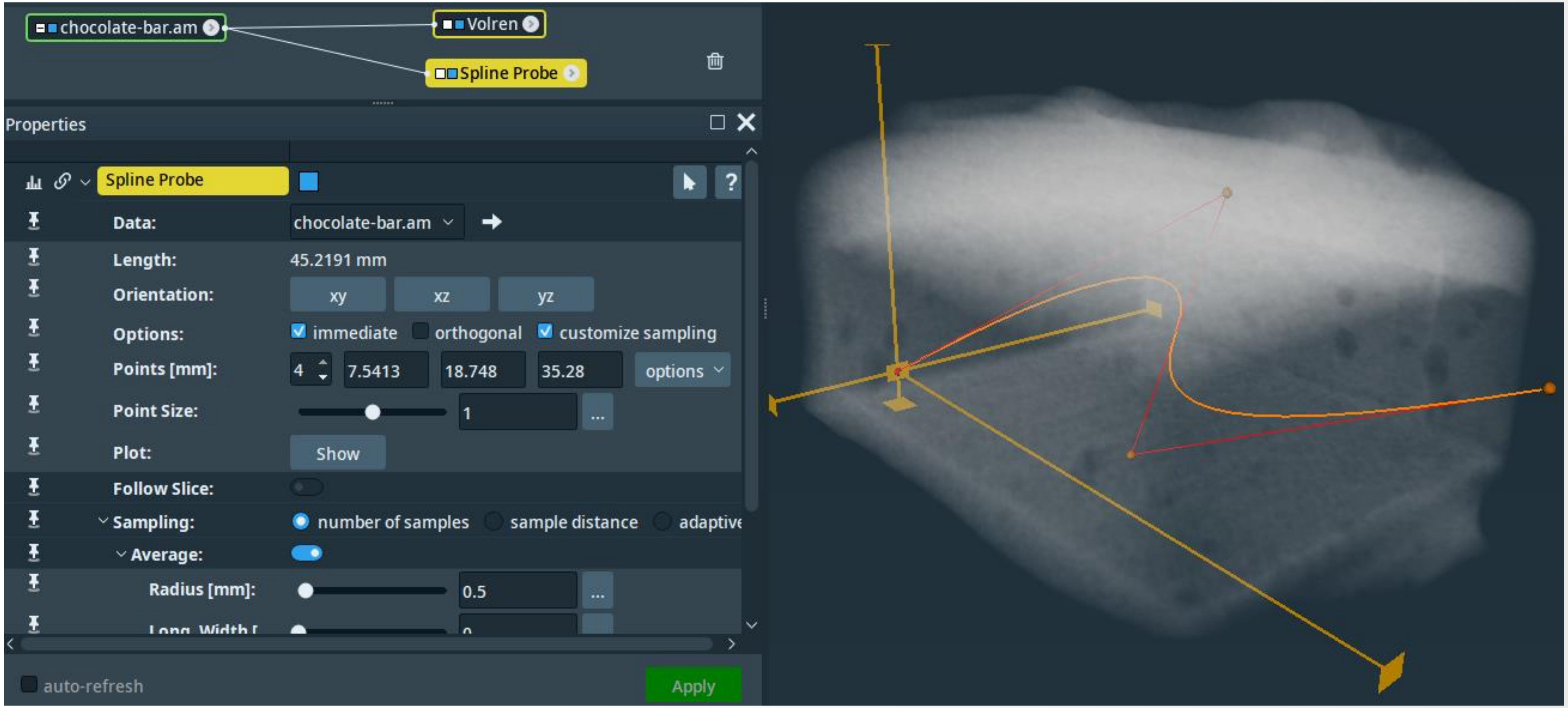
- adjust number of sample-points
- possibility for local averaging



Probing data value

Spline Probe

- similar to Line Probe, but:
  - arbitrary number of control points
  - sampling along smooth, curved Spline

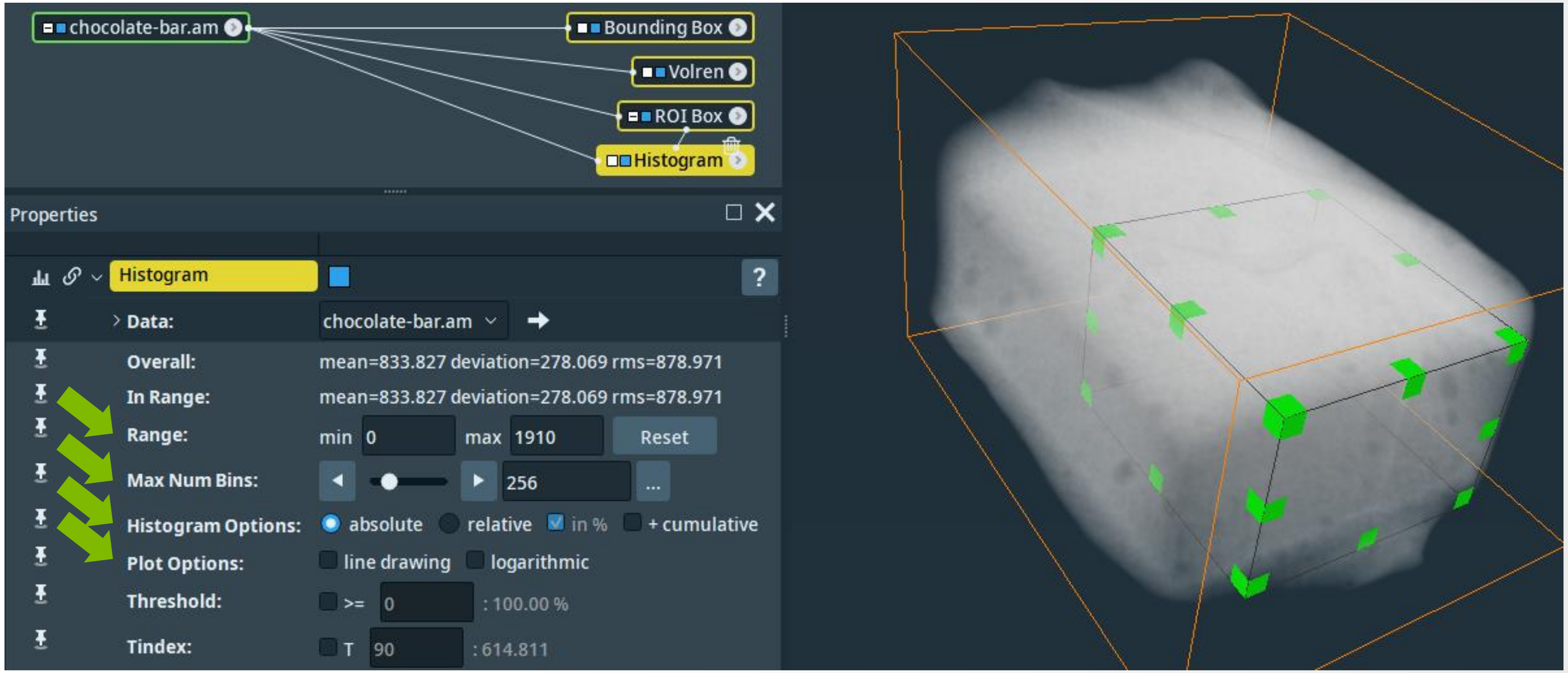


Probing data value

Histogram

- distribution of values in the data-set
- optionally limited to ROI or mask

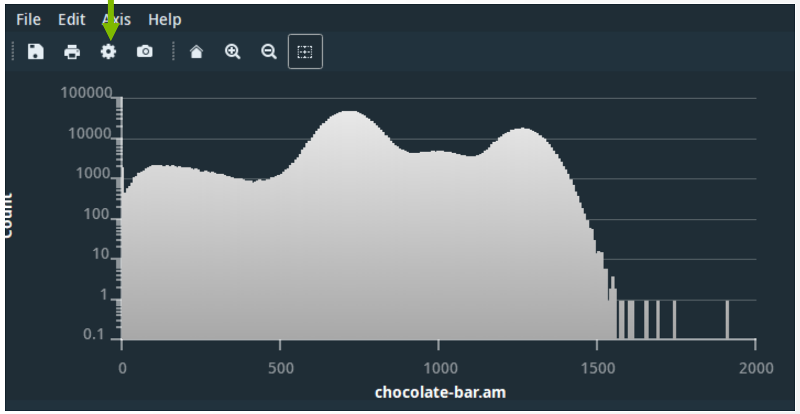
- adjust settings:
  - range
  - number of bins
  - absolute/relative counting
  - linear/logarithmic Y-axis

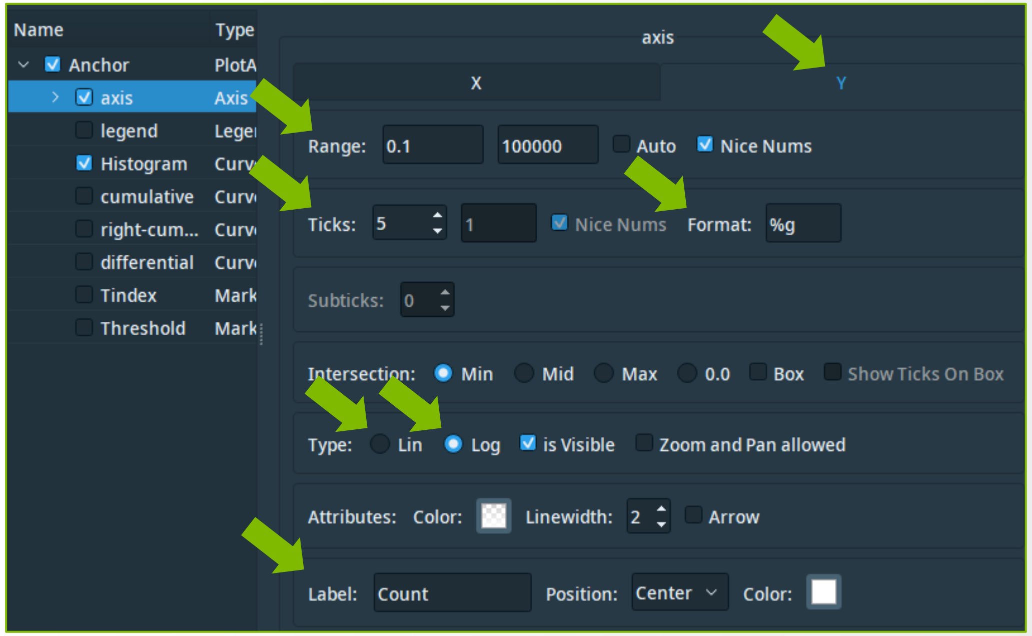


Probing data value

Histogram

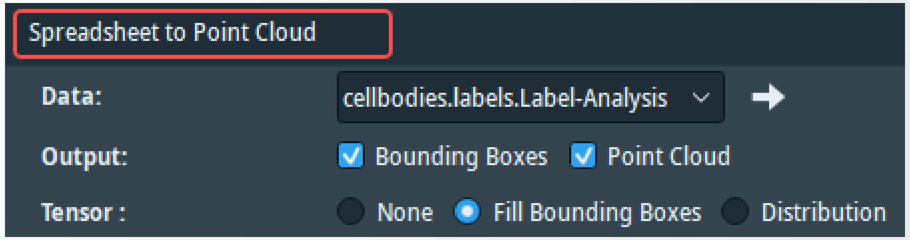
- further settings:
  - axis control for X and Y:
    - range
    - number format
    - tick-marks
    - linear/log
    - labels
    - …

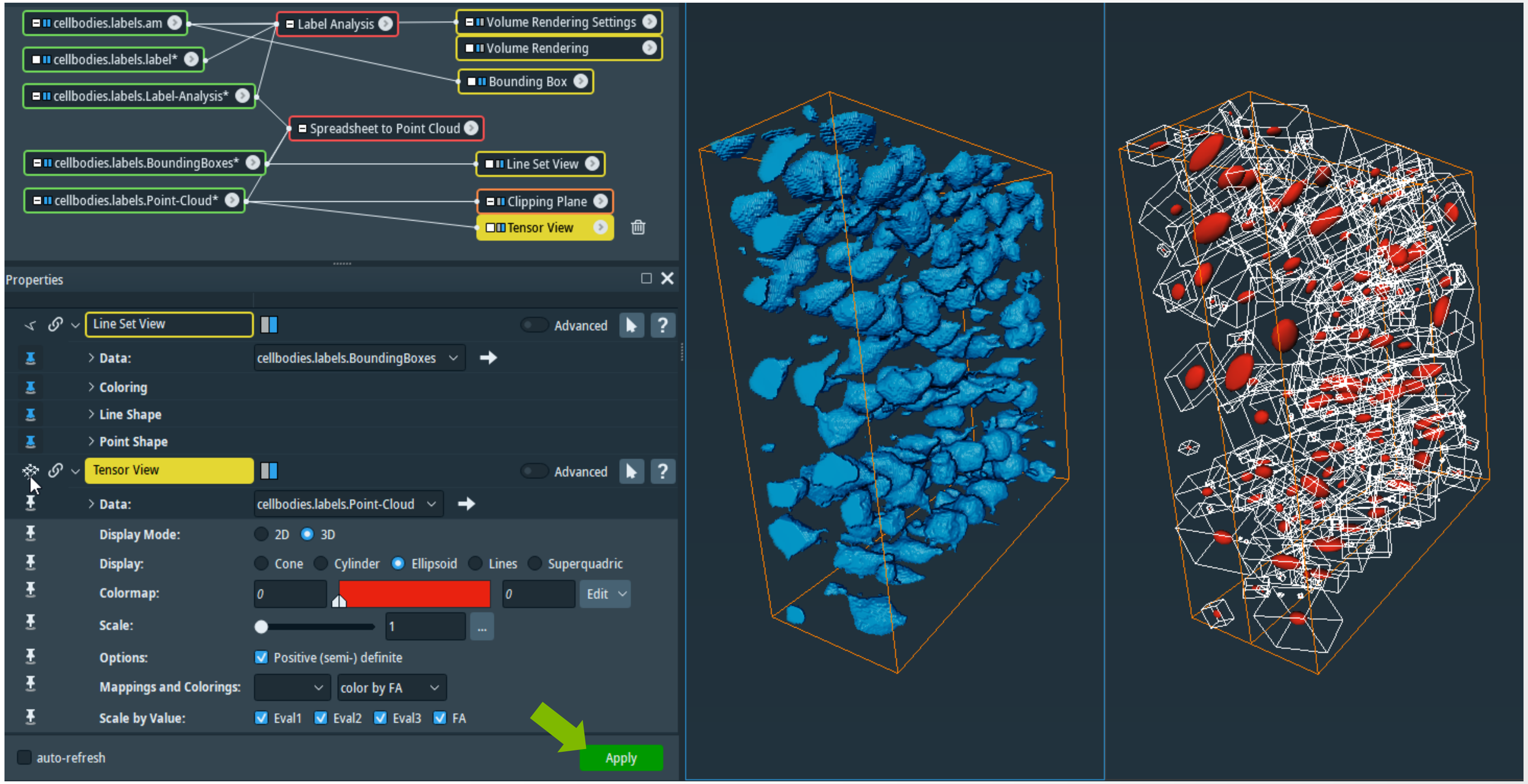




Spreadsheet visualization

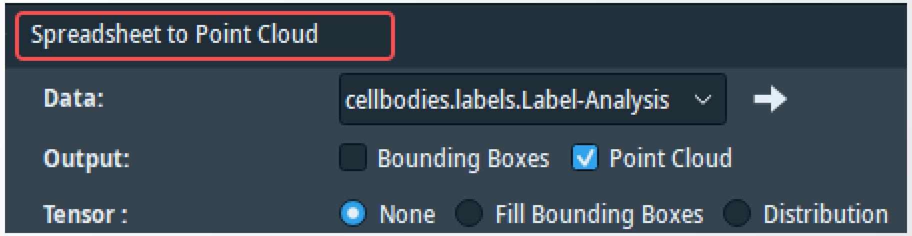
- Plot Spreadsheet
- Histogram
- Spreadsheet To Point Cloud + Point Cloud View for the display
  - e. g. create bounding-box information and orientation tensors

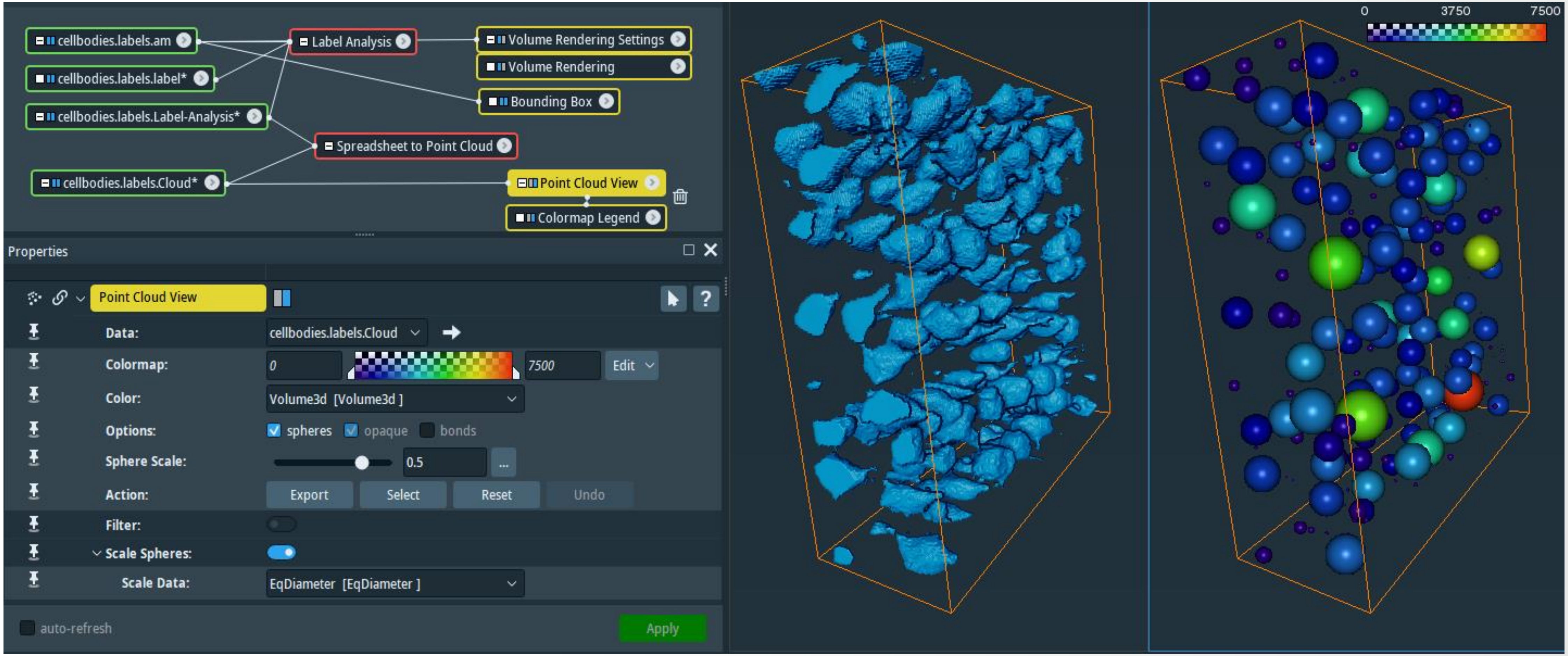




Spreadsheet visualization

- **Spreadsheet To Point Cloud** + Point Cloud View for the display
  - e. g. point cloud as sphere visualization



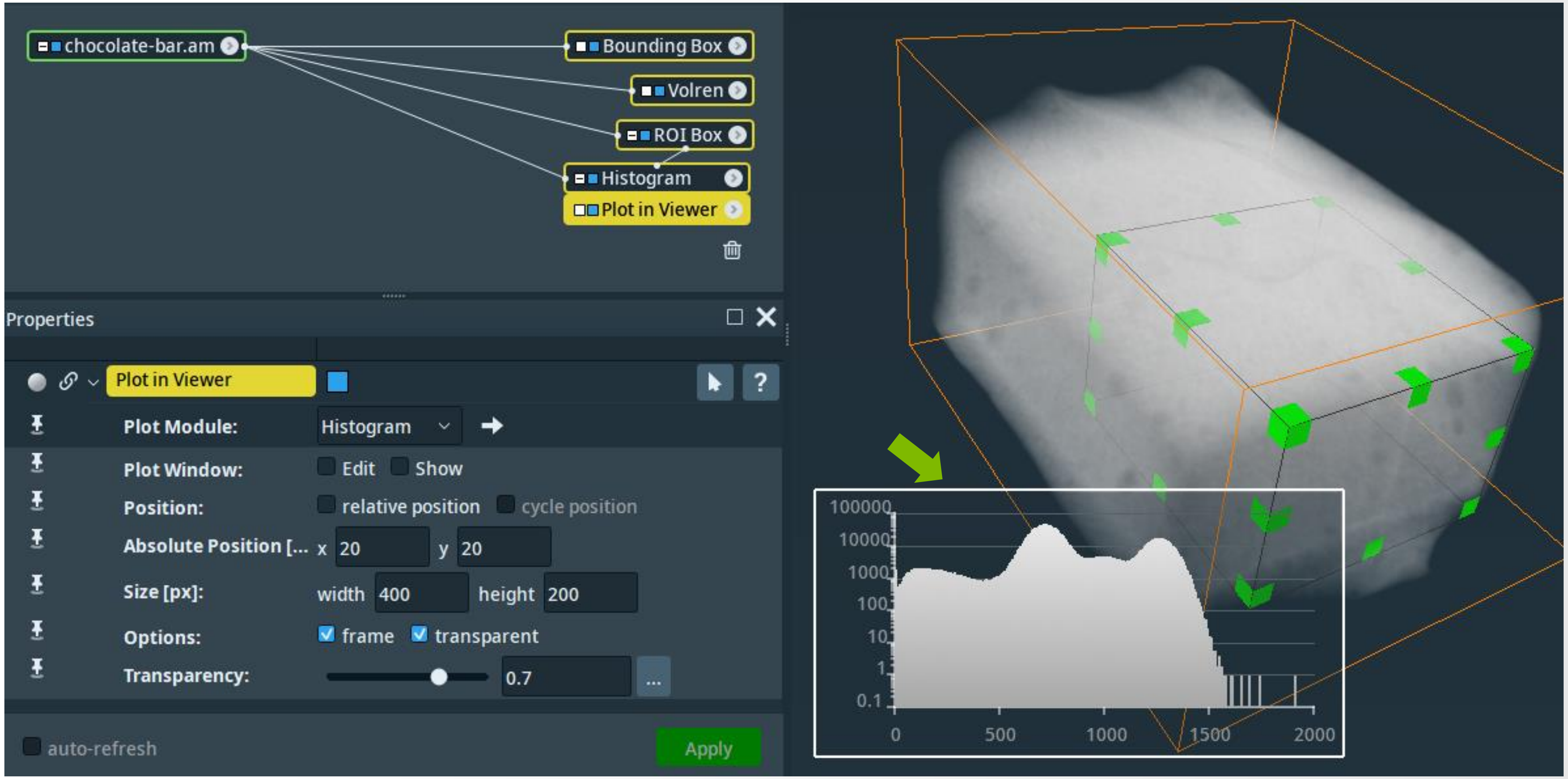


Displaying plots in the main viewer

Normally, all kinds of plots are displayed in a separate viewer window.

With **Plot In Viewer**, plots can be displayed in the main viewer window(s).

- can be attached to:
  - Histogram
  - Point Probe
  - Line Probe
  - Spline Probe
  - Plot Spreadsheet
- options:
  - position
  - size
  - transparency
  - frame

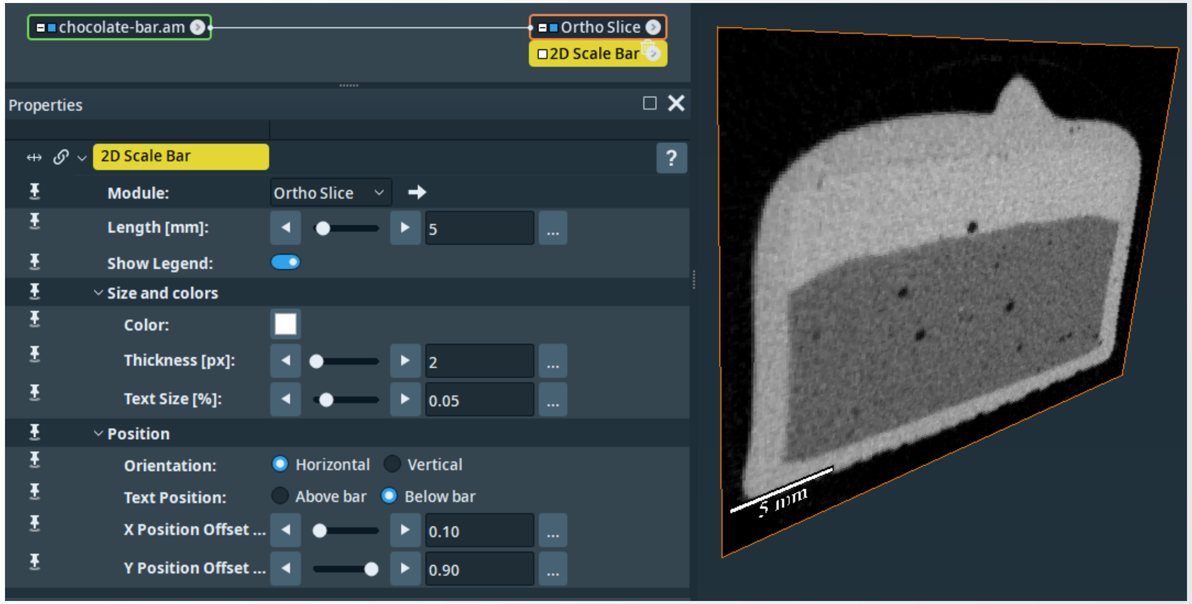


Annotation: Scale Bar and 2D Scale Bar

There are two types of scale-bars available:

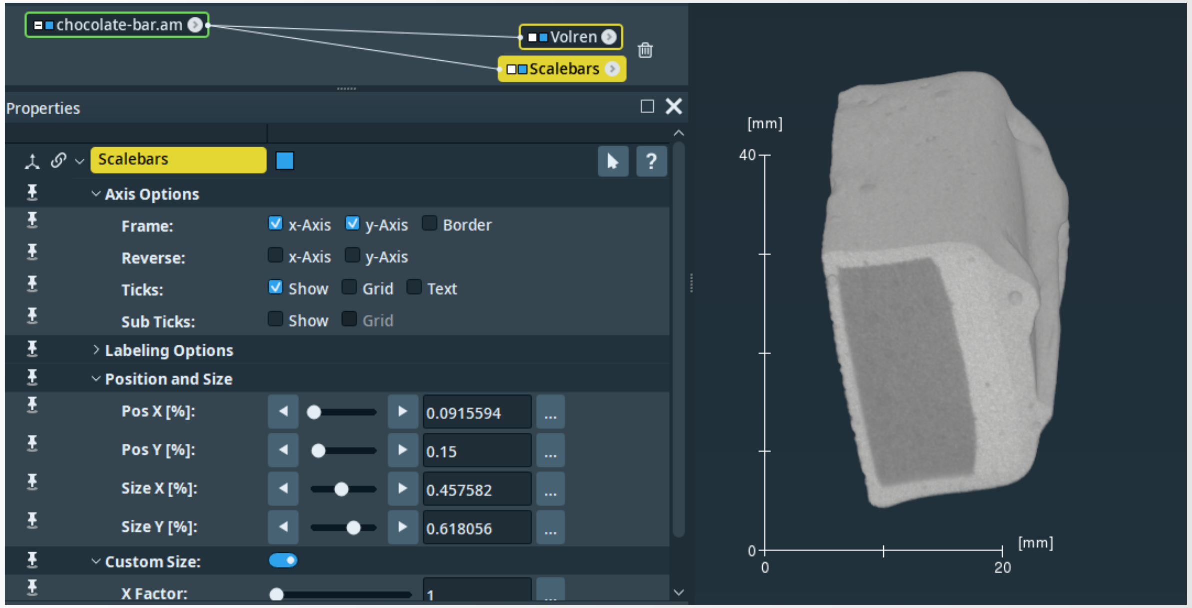
2D Scale Bar

- attached to a slice object
- correct also in perspective ( ) view!
- options: length, position, color, …



Scale Bar

- located in the **3D** viewer
- invoked via click on background and “Create object → Annotations → Scalebars”
- only meaningful in orthographic (, parallel) view! (because of perspective shortening)
- options: length, position, color, label, font, ticks, …

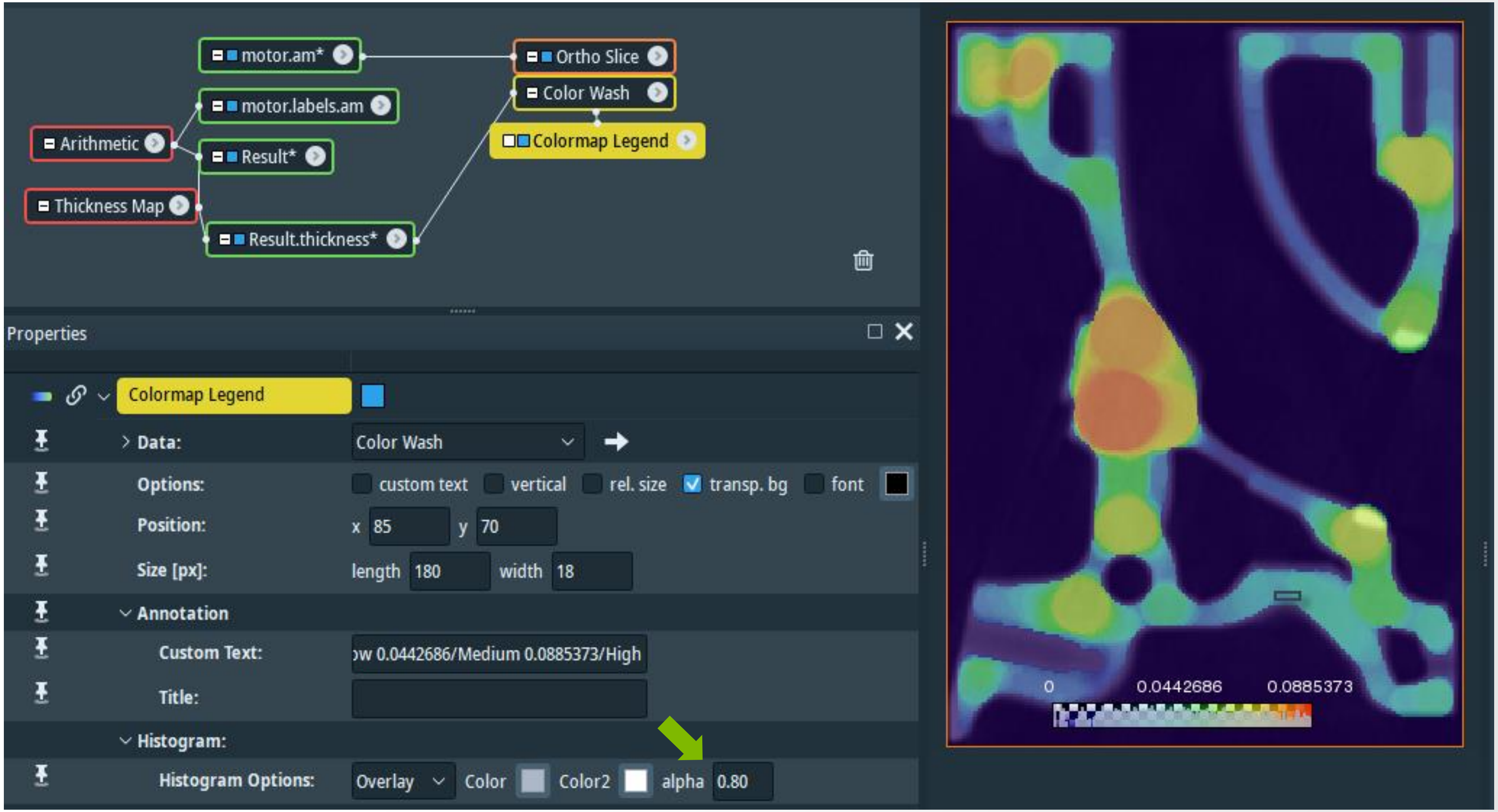


Annotation: Colorbar

Attach **Colormap Legend** to the display module.

- options:
  - size
  - vertical
  - background
  - font
  - title
  - custom text
  - Histogram

Tip: set alpha=0 to hide the histogram



Annotation: Colorbar

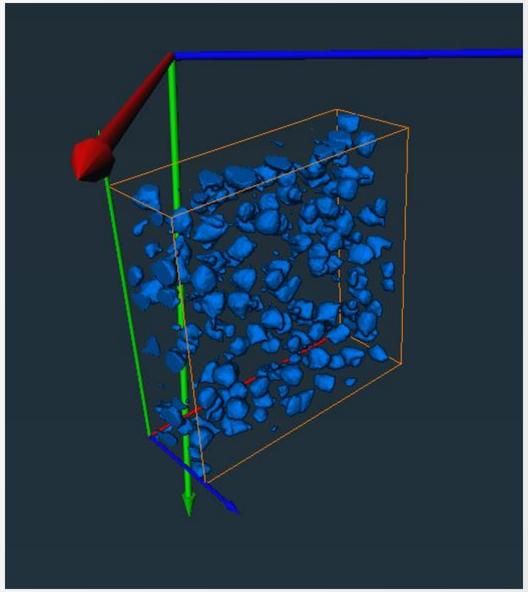
If you want to get rid of the checkerboard-pattern:

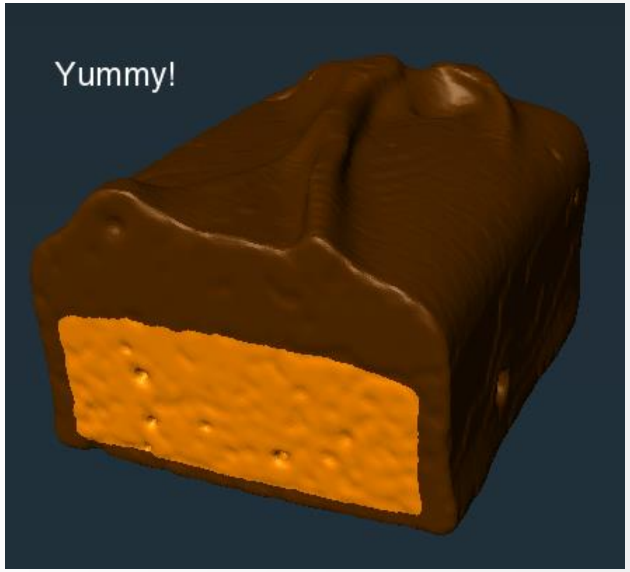
- in the display-module’s (e.g. Color Wash) colormap port: select “Options → Edit colormap”
- in the Colormap Editor: set transparency to “Opaque”

Annotation: Axes, Caption

Axes

- visualization of global coordinate system:
  - no data-set connected
  - invoked via click on background and “Create object → Annotations → Axes”
- visualization of local coordinate system:
  - connected to a data object
  - invoked via the object’s context menu (“Annotate → Axes”)
- default coloring convention: X: red, Y: green, Z: blue





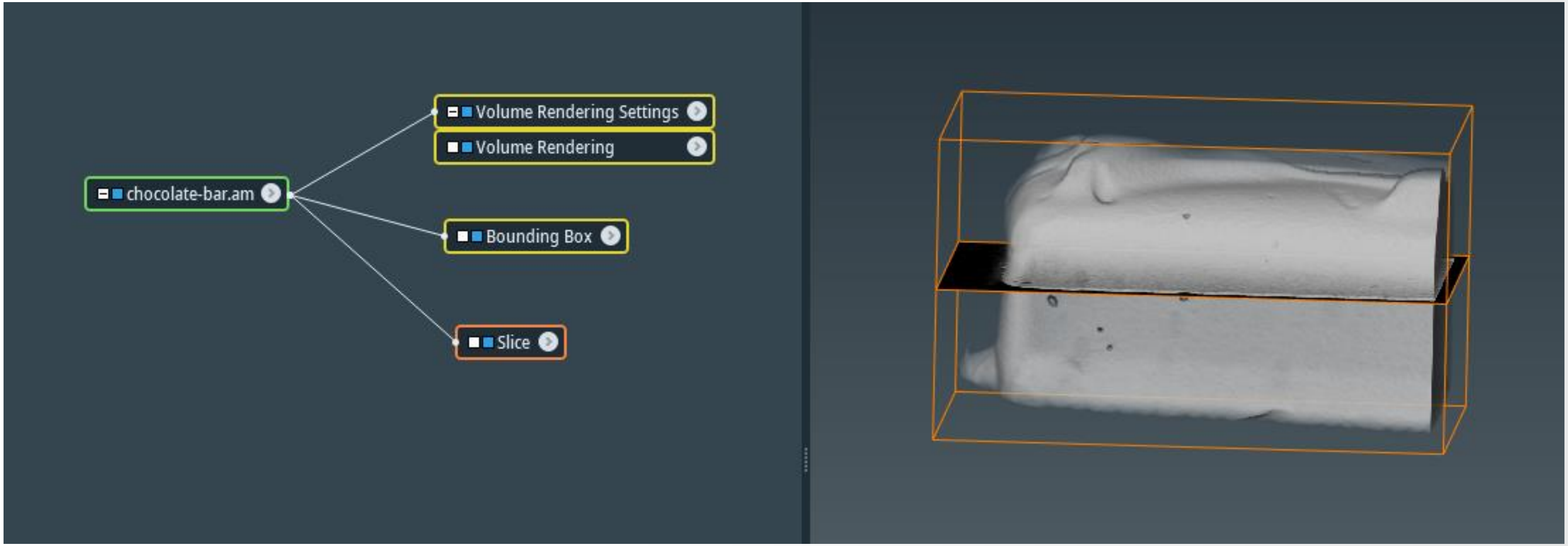
Caption

- any text in the viewing plane of the viewer
- invoked via click on background and “Create object → Annotations → Caption”
- options: position, text, color, font

Data registration and alignment

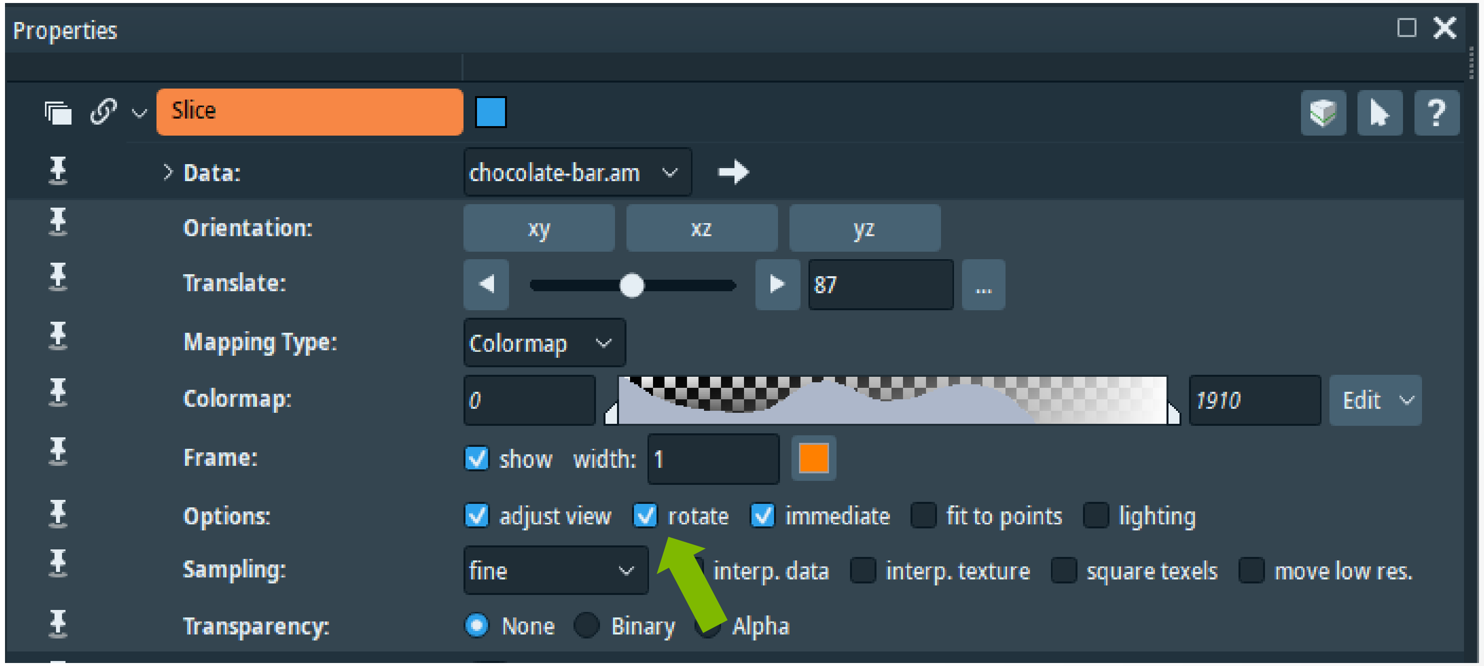
Alignment to an oblique plane: example

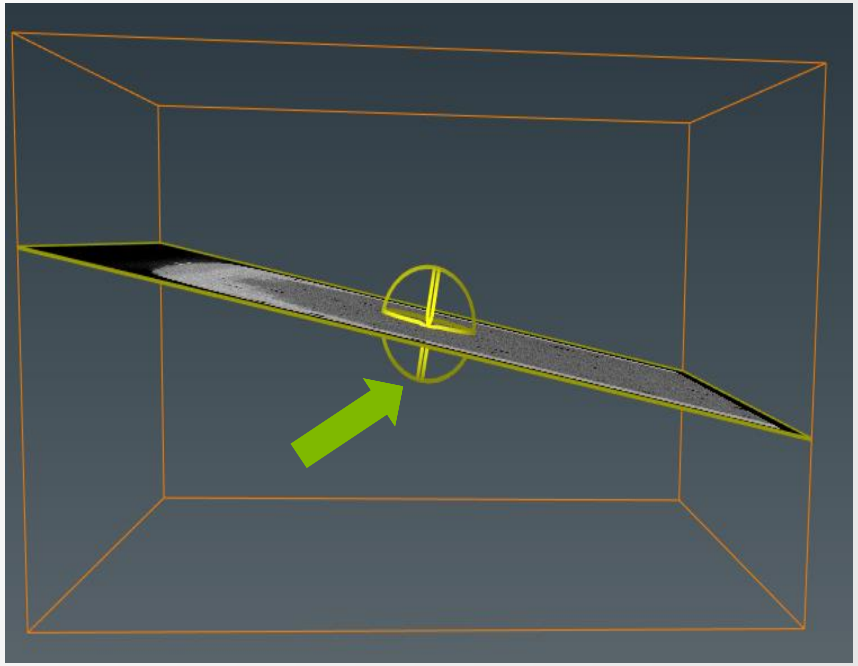
Load chocolate-bar.am then attach Volume Rendering, Bounding Box and Slice to the dataset (oblique view).



Alignment to an oblique plane: example

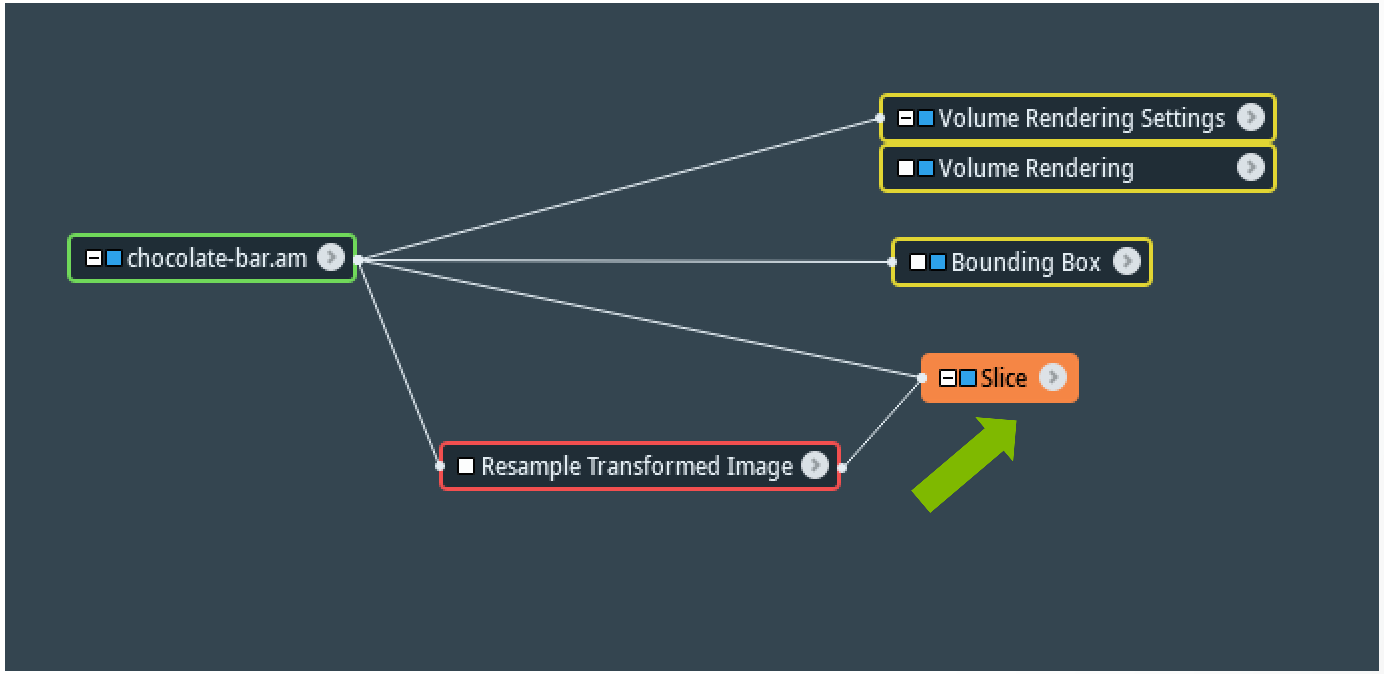
Rotate Slice using rotate mode in Slice properties port (activate trackball) to rotate to the desired tilt angle.

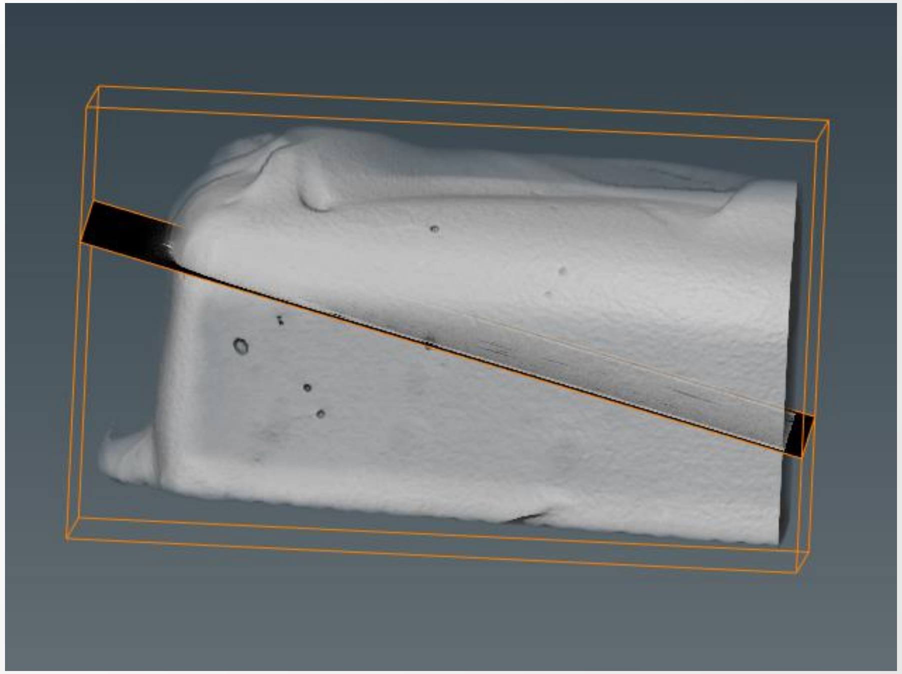




Alignment to an oblique plane: example

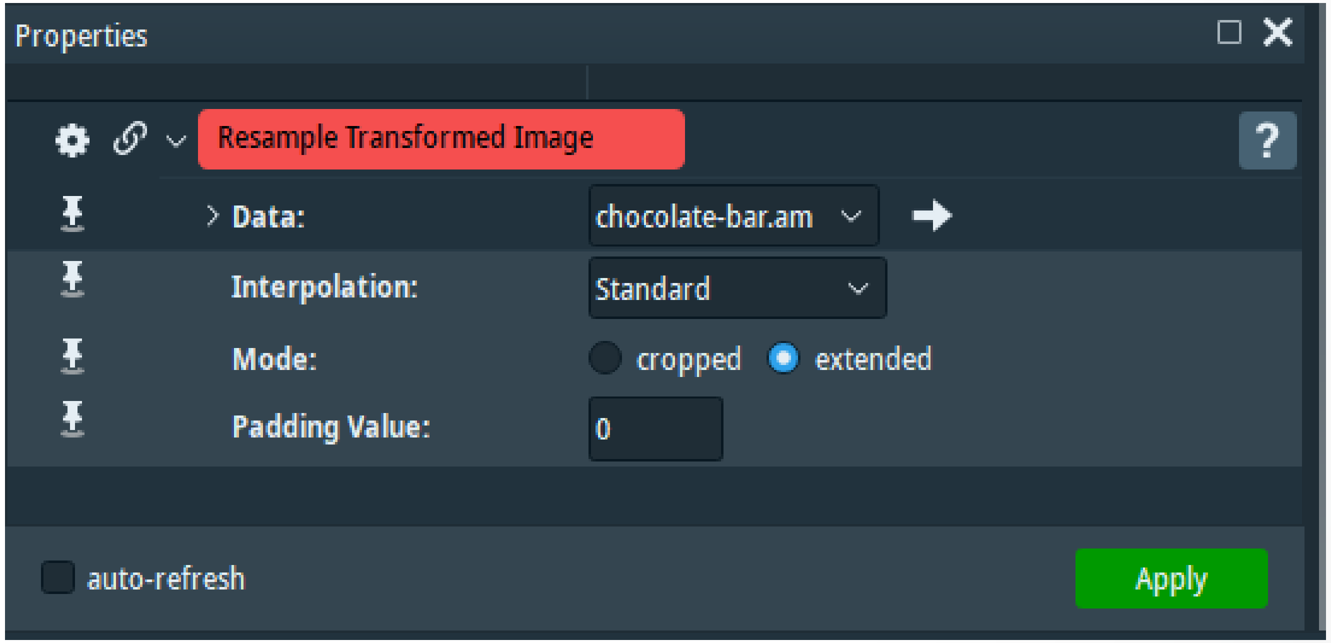
Attach Resample Transformed Image to chocolate-bar.am and set reference to Slice.

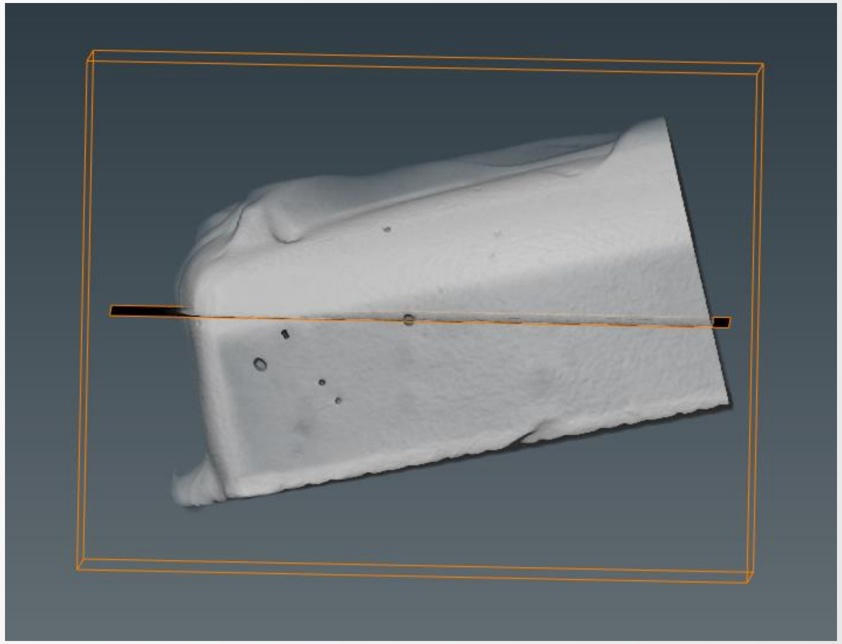




Alignment to an oblique plane: example

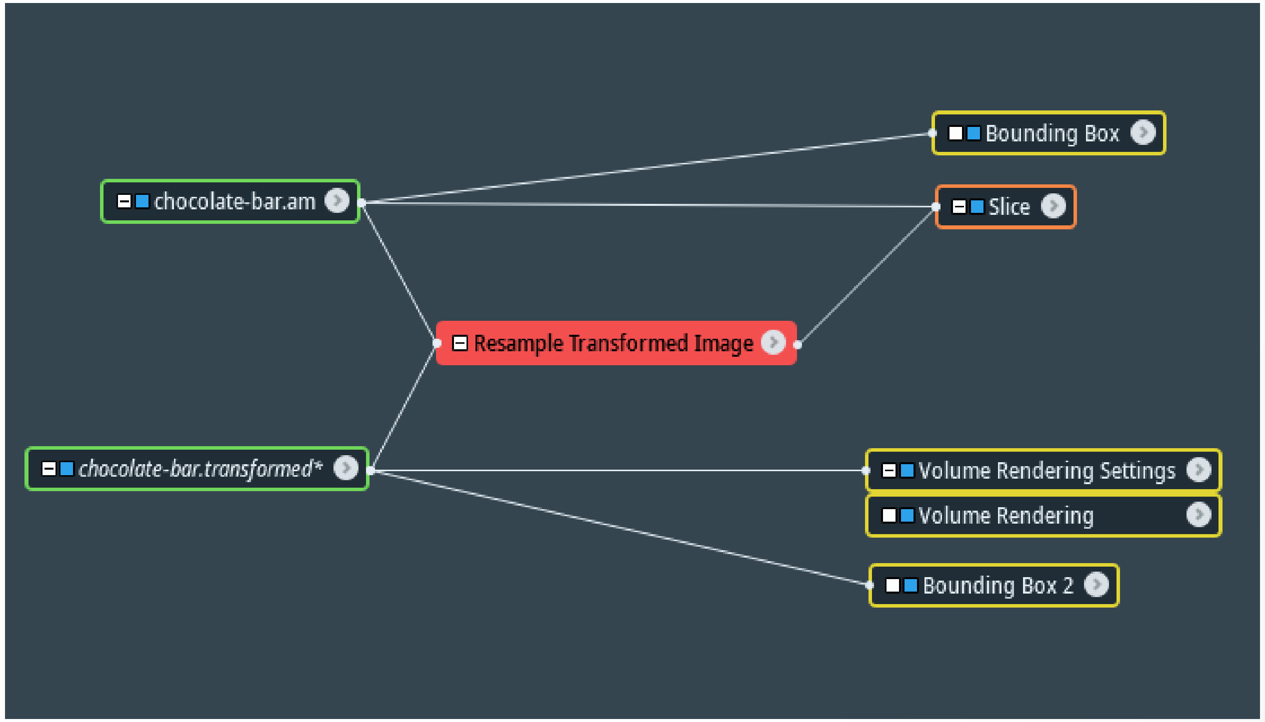
At Resample Transformed Image properties select Interpolation: Standard, Mode: extended then click Apply.

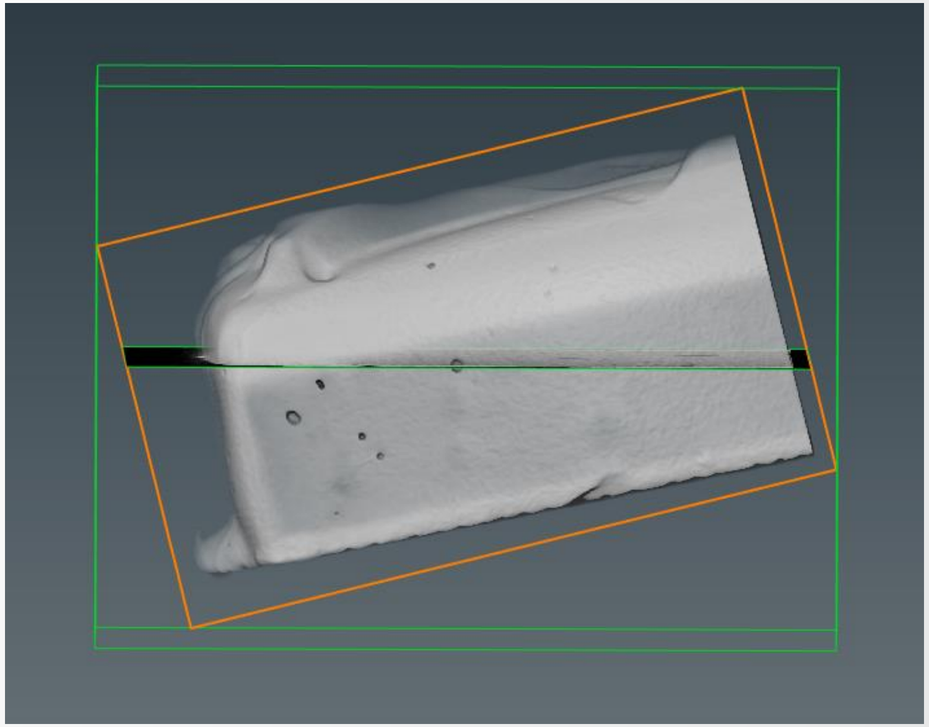




Alignment to an oblique plane: example

Attach another Bounding Box to chocolate-bar.transformed and visualize with Volume Rendering.
The transformed result is now aligned with Slice (in green).





Data registration: introduction

- General concepts
  - All datasets are positioned in ‘physical’ space
  - This position is control by a ‘Transform’

- Registration
  - Optimization of the ‘alignment’ with respect to the degrees of freedom given by the ‘transform’
  - Difficult mathematical problem, sensitive to the initialization

- Avizo can register:
  - Volume to volume (grayscale or label images)
  - Surface to surface
  - Using Linear transform: translation, rotation, optionally scaling and shearing

Data registration: introduction

- General concepts
  - All datasets are positioned in ‘physical’ space
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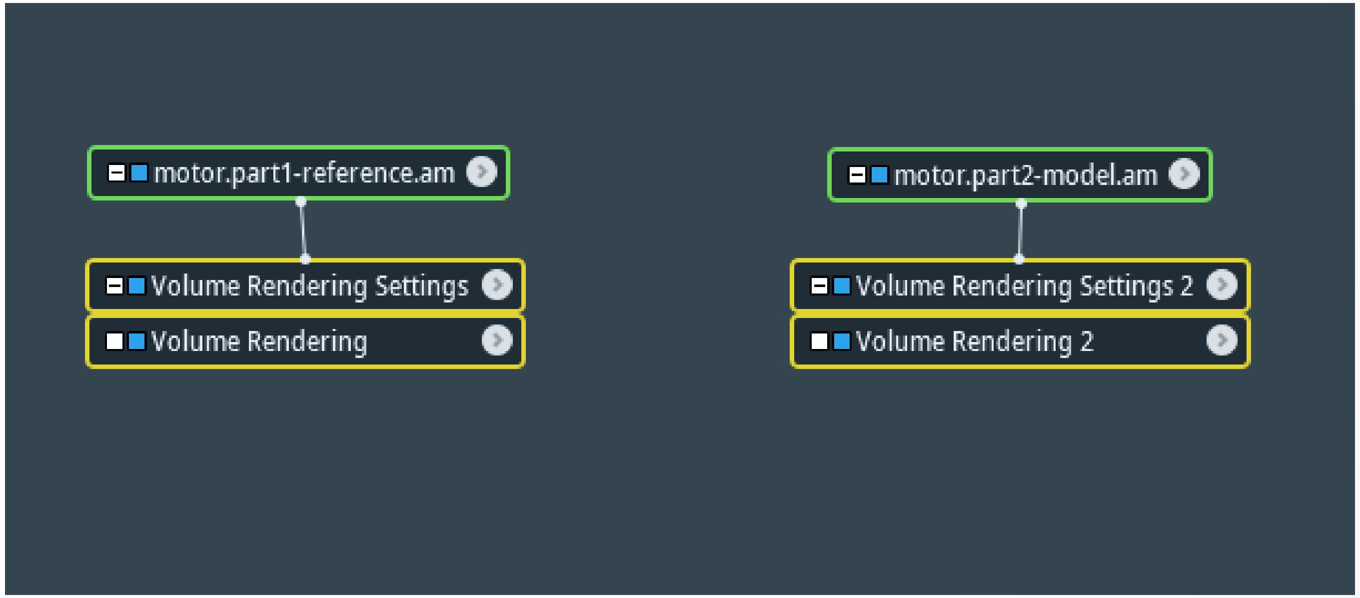
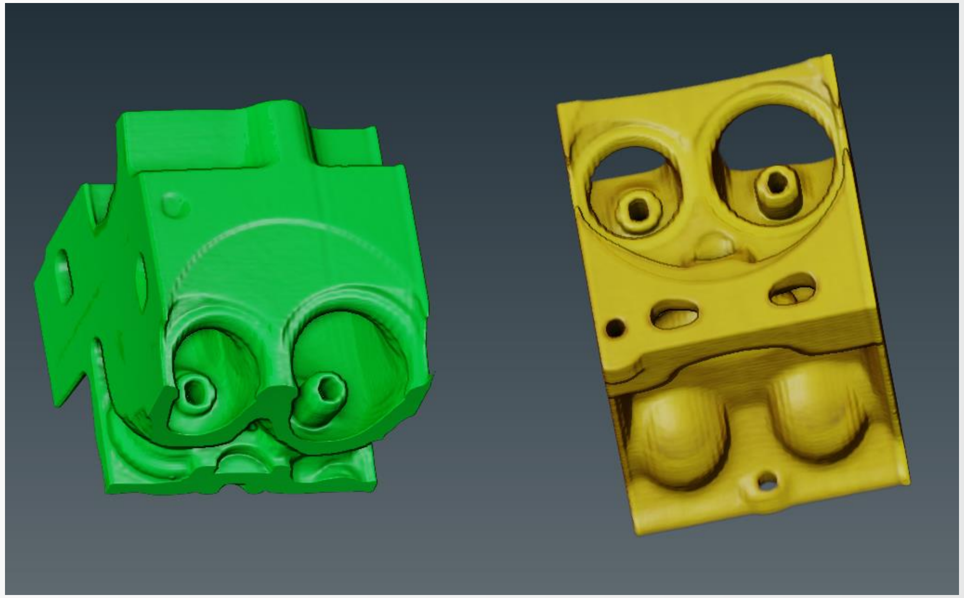
  - Optimization of the ‘alignment’ with respect to the degrees of freedom given by the ‘transform’
  - Difficult mathematical problem, sensitive to the initialization

- Amira can register:
  - Volume to volume (grayscale or label images)
  - Surface to surface
  - Using Linear transform: translation, rotation, optionally scaling and shearing

Data registration: Transform Editor module

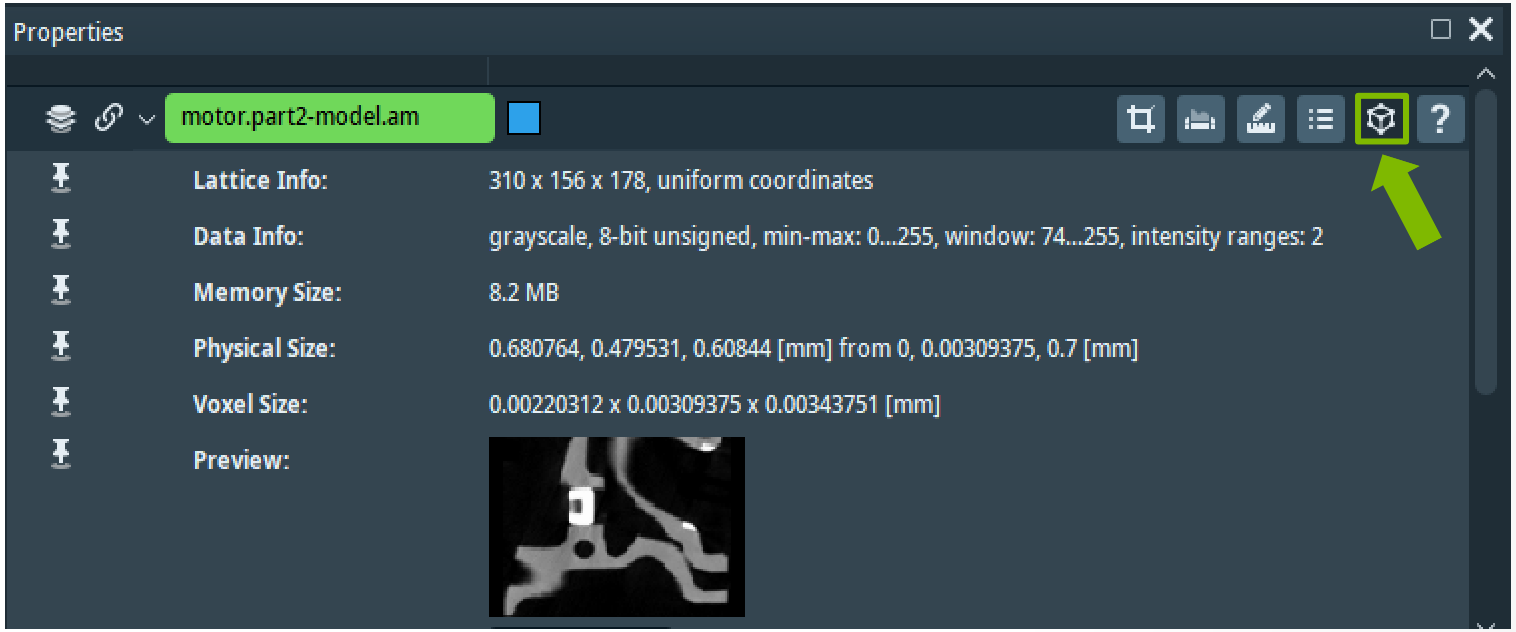
Open motor.part1-reference.am and motor.part2-model.am (data -> registration) then attach Volume Rendering to each dataset.

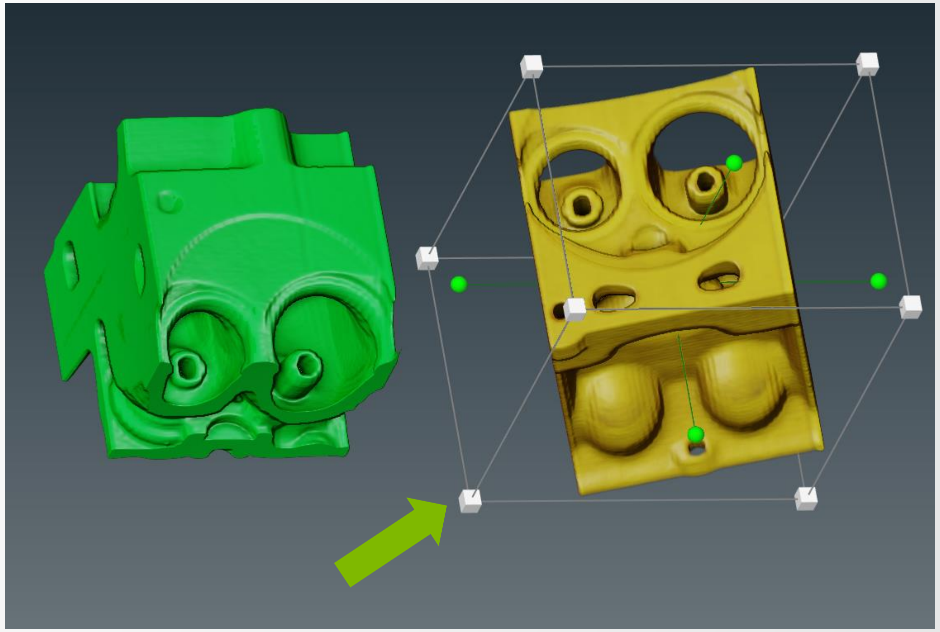




Data registration: Transform Editor module

In the properties window of motor.part2-model.am, activate Transform Editor, the transform box will appear.





Data registration: Transform Editor module

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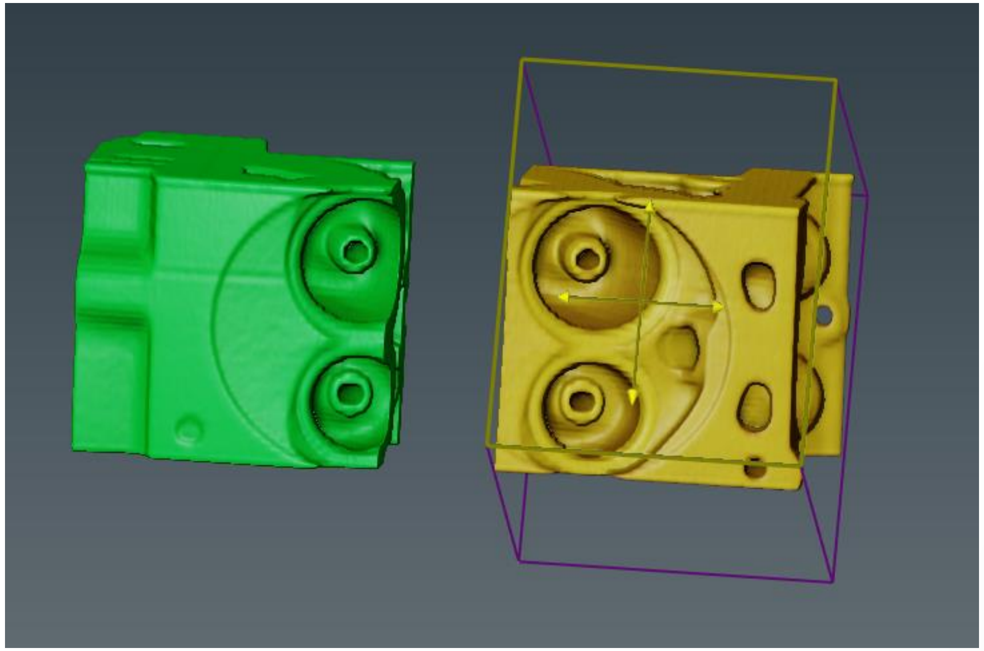
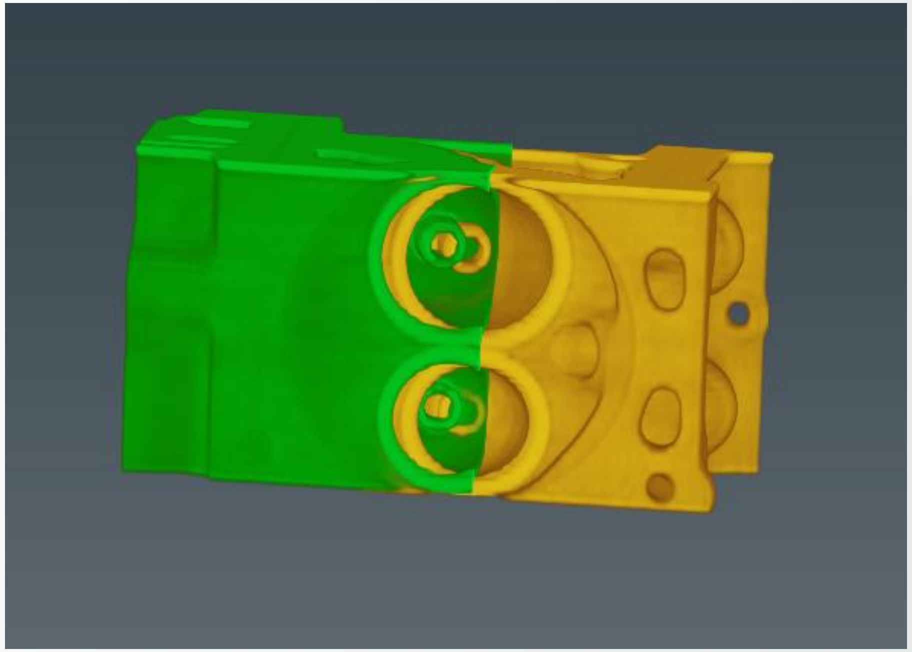
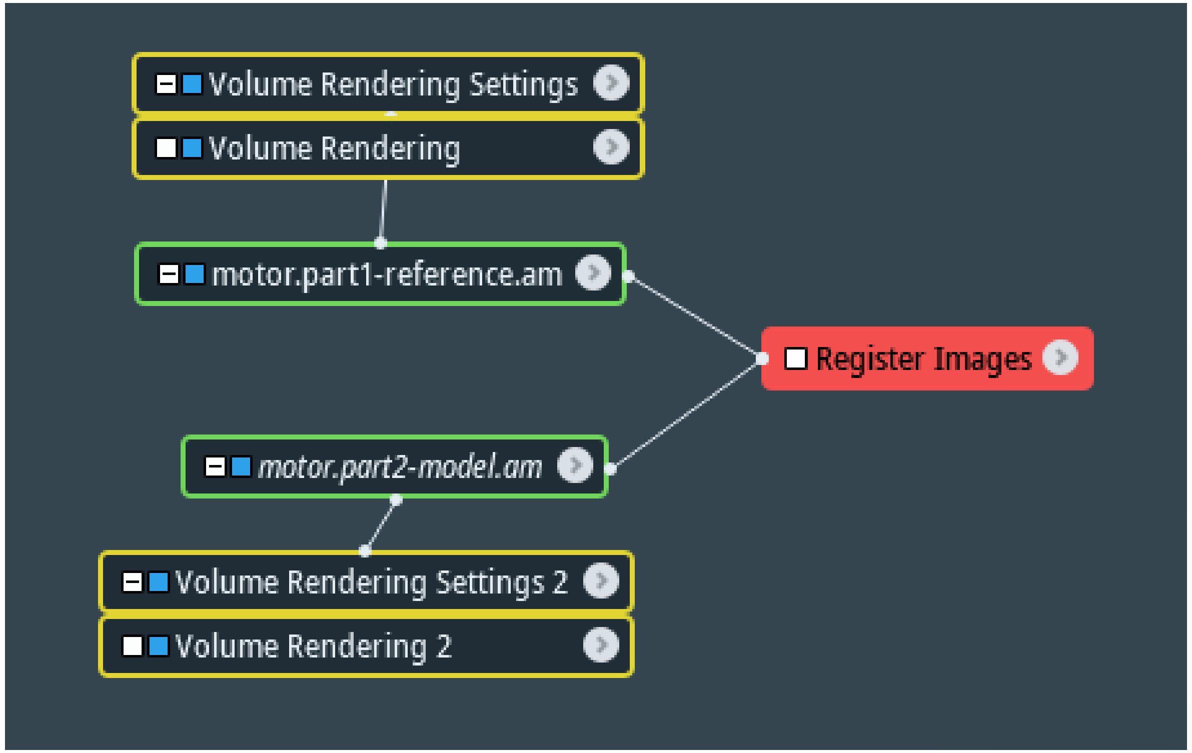
Move the handle box and bring motor.part2-model.am (in yellow) to overlap with motor.part1-reference.am (in green) as much as possible.

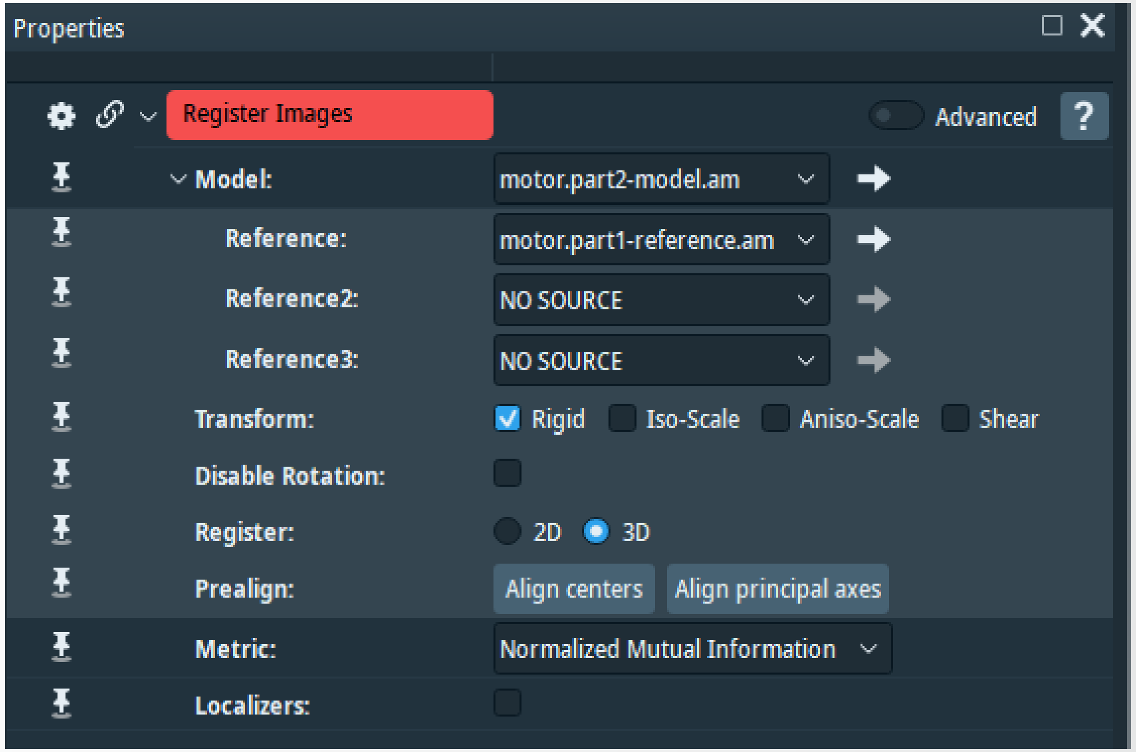




Data registration: Register Images module

Attach Register Images to motor.part2-model.am and set the reference to motor.part1-reference.am.





- Transform: Rigid
- Register: 3D
- Prealign: Align centers & Align principal axes
- Metric: Normalized Mutual Information

Data registration: Resample Transformed Image module

Attach Resample Transformed Image to motor.part2-model.am to apply the transformation (otherwise the transformation will be available for visualization only).

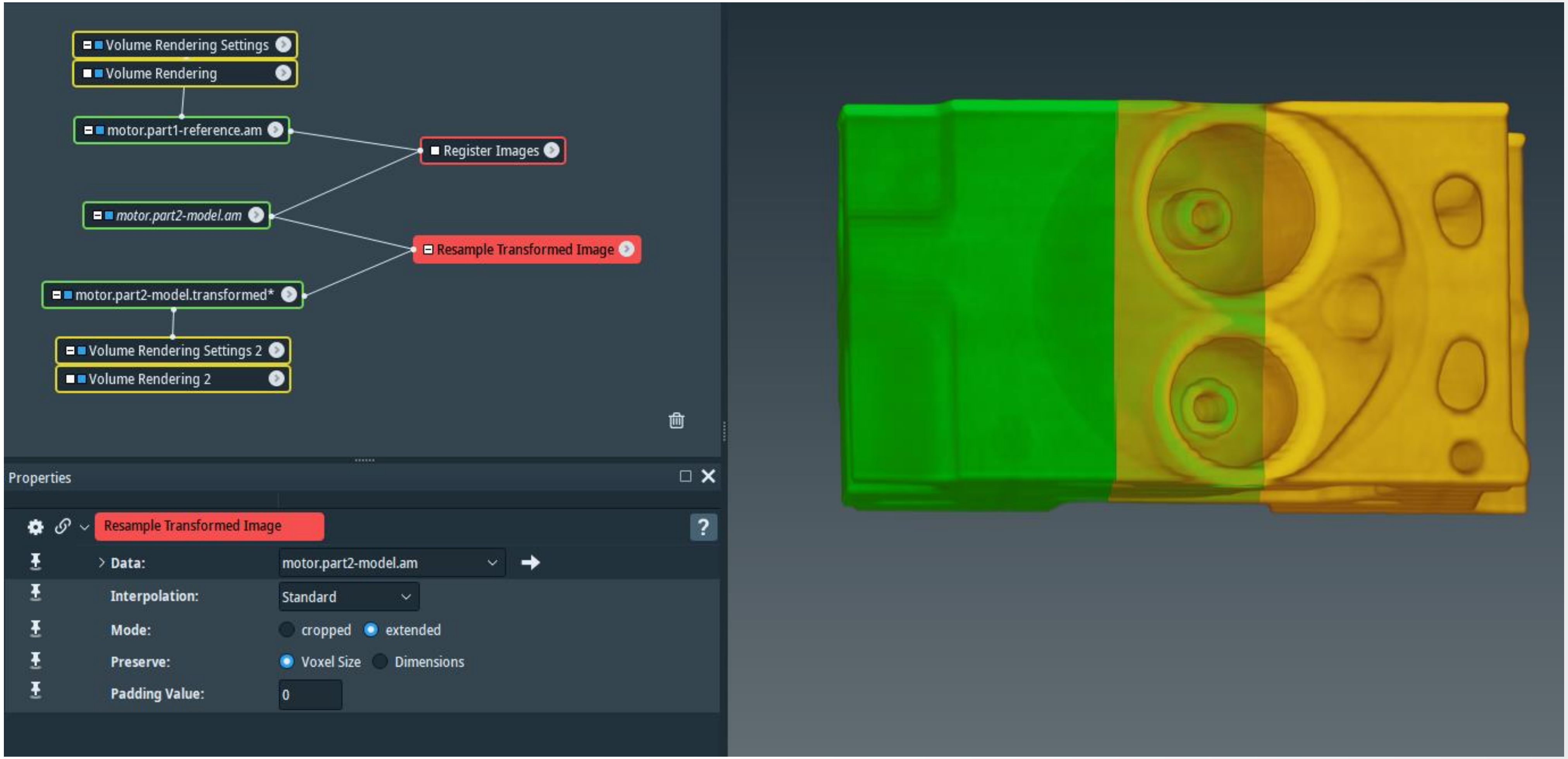
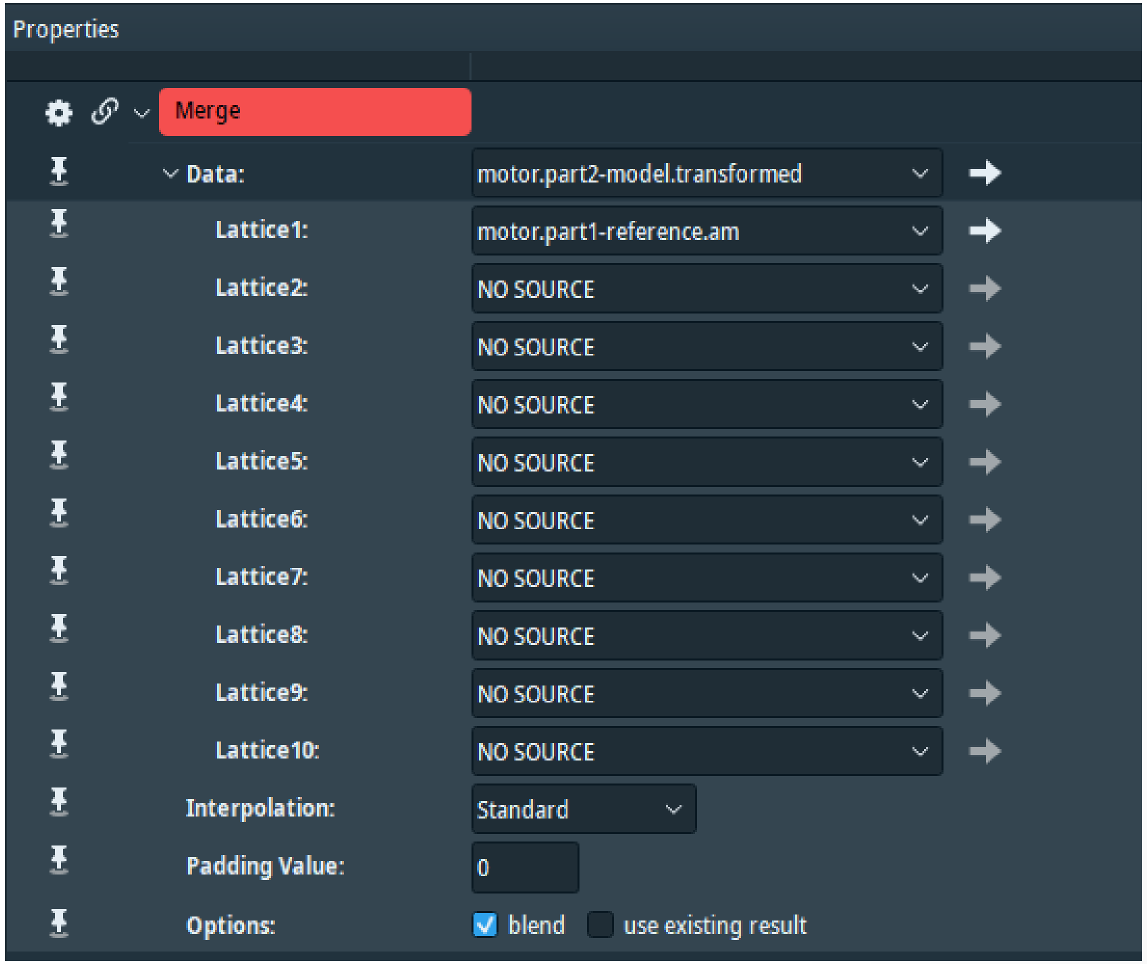


Image fusion & stitching

Attach Merge module to motor.part2-model.transformed and set motor.part1-reference.am as a reference. Merge using standard interpolation with blend option.



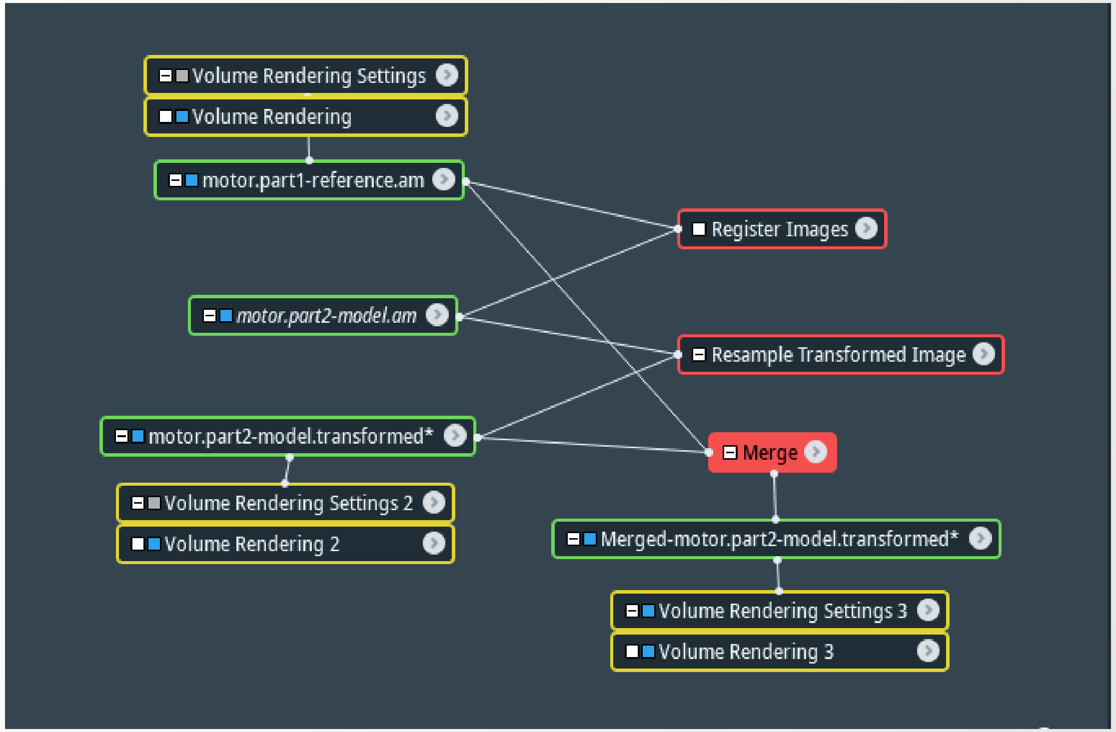
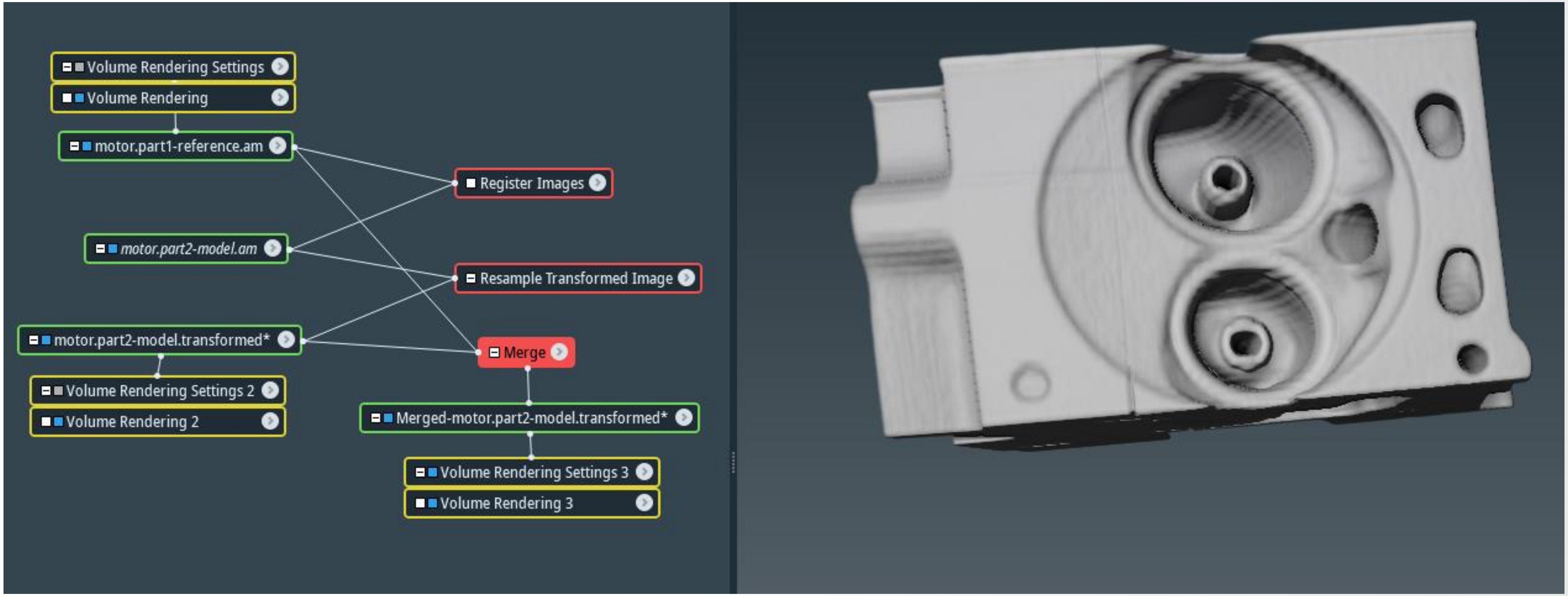
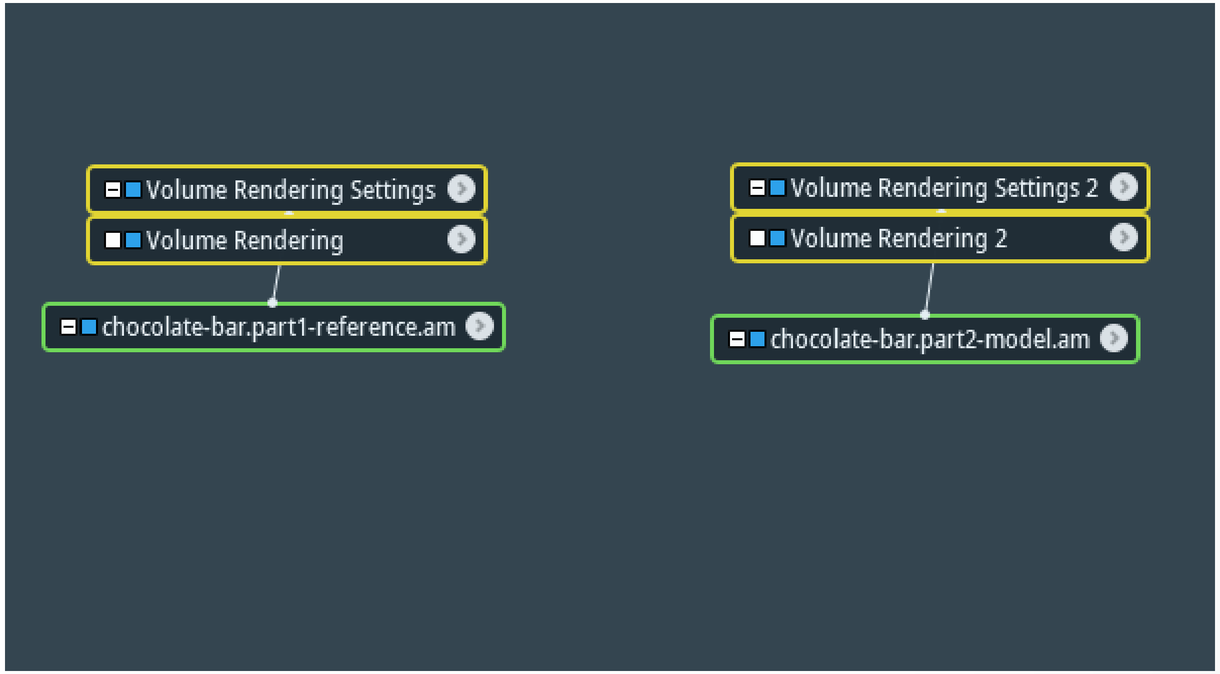


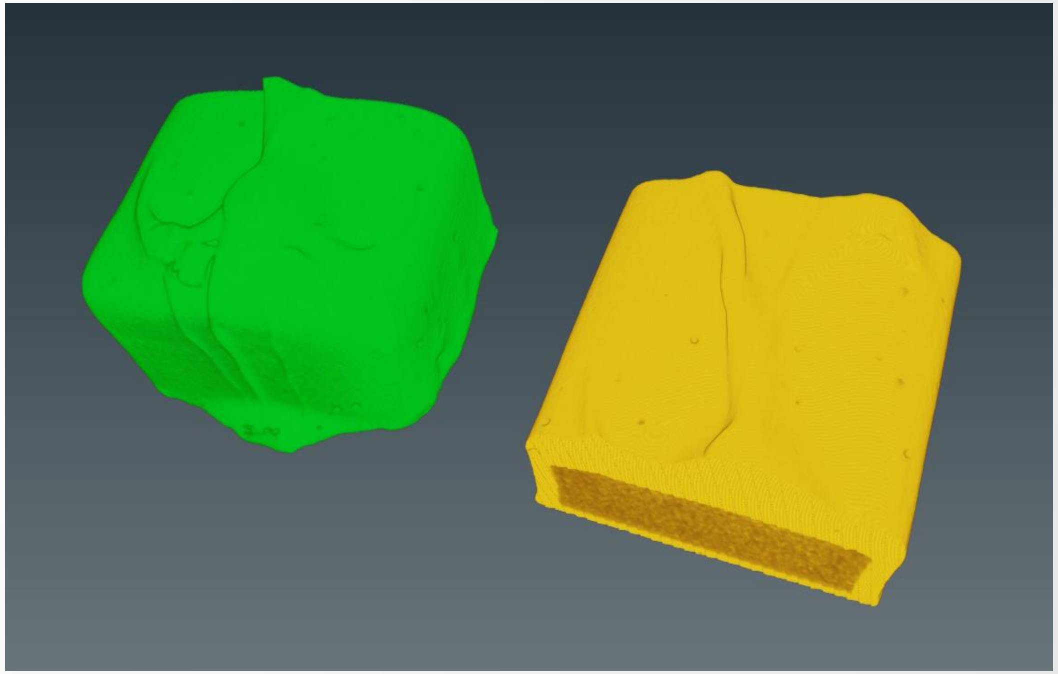
Image fusion & stitching



Data registration: Transform Editor module

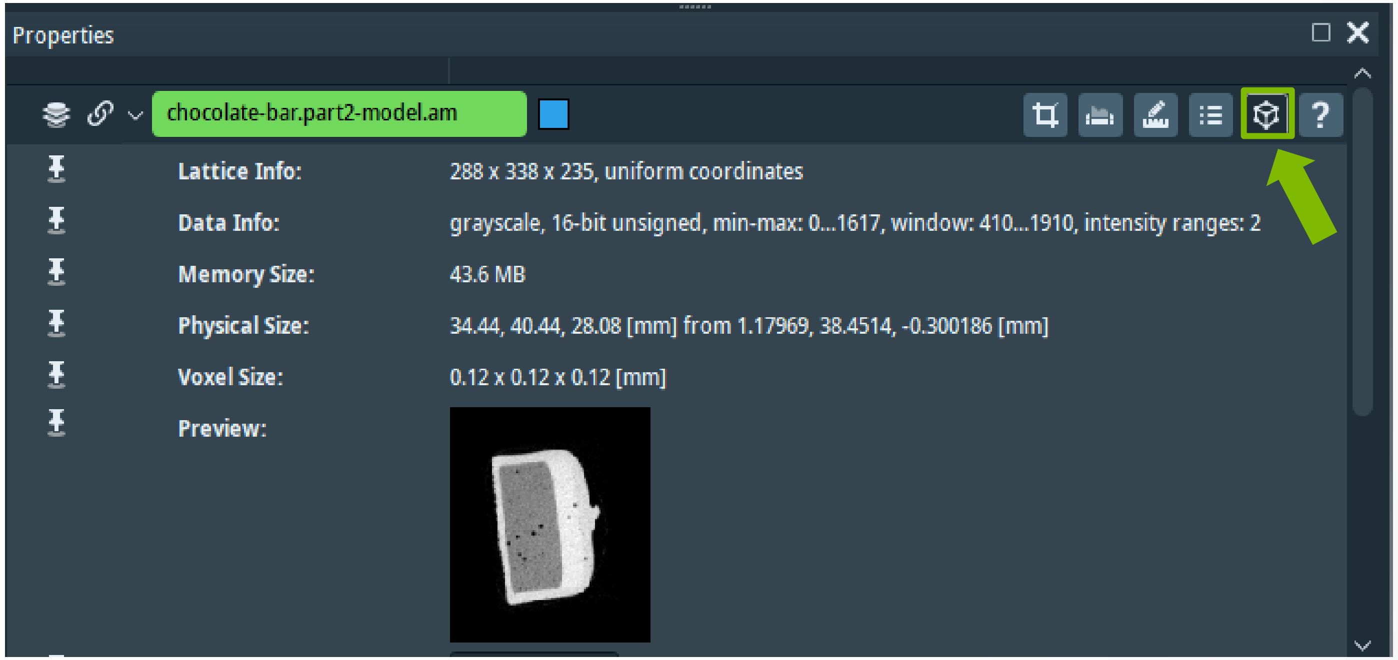
Open chocolate-bar.part1-reference.am and chocolate-bar.part2-model.am (data > registration) then attach Volume Rendering to each dataset.

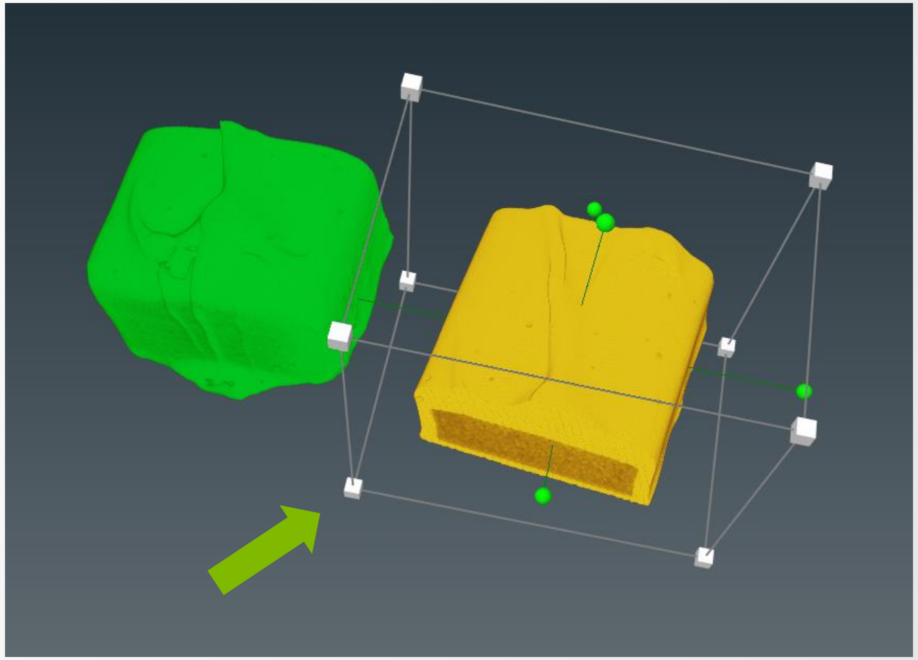




Data registration: Transform Editor module

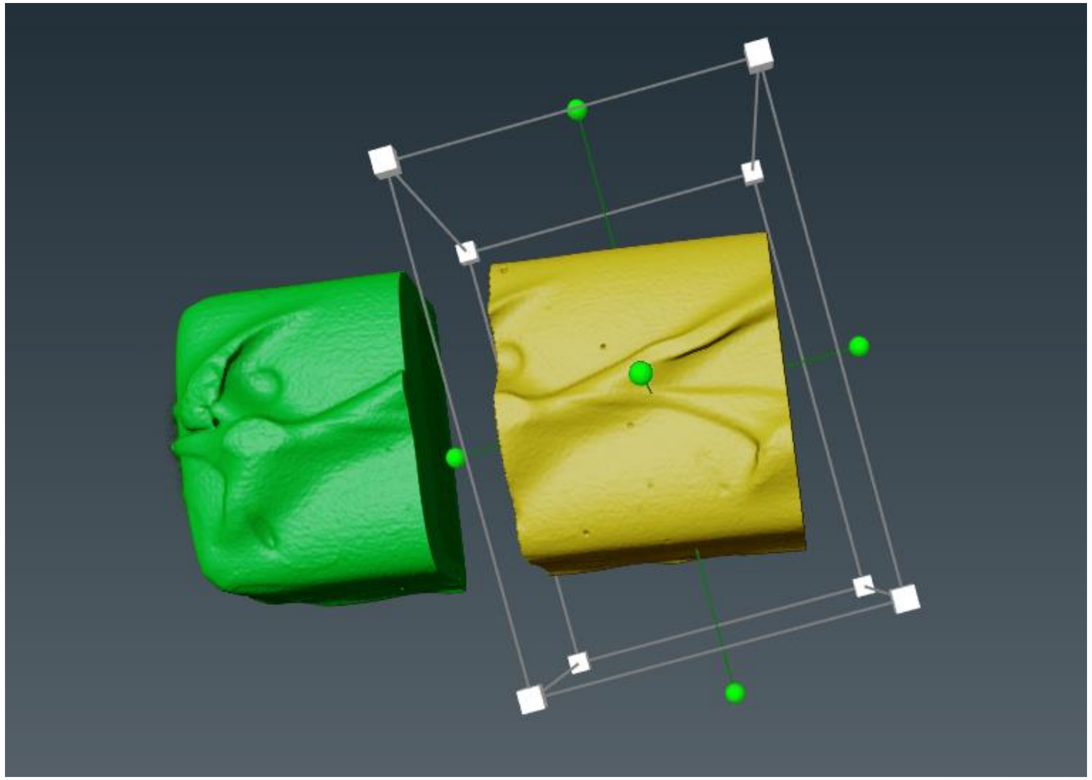
In the properties window of chocolate-bar.part2-model.am, activate Transform Editor, the transform box will appear.

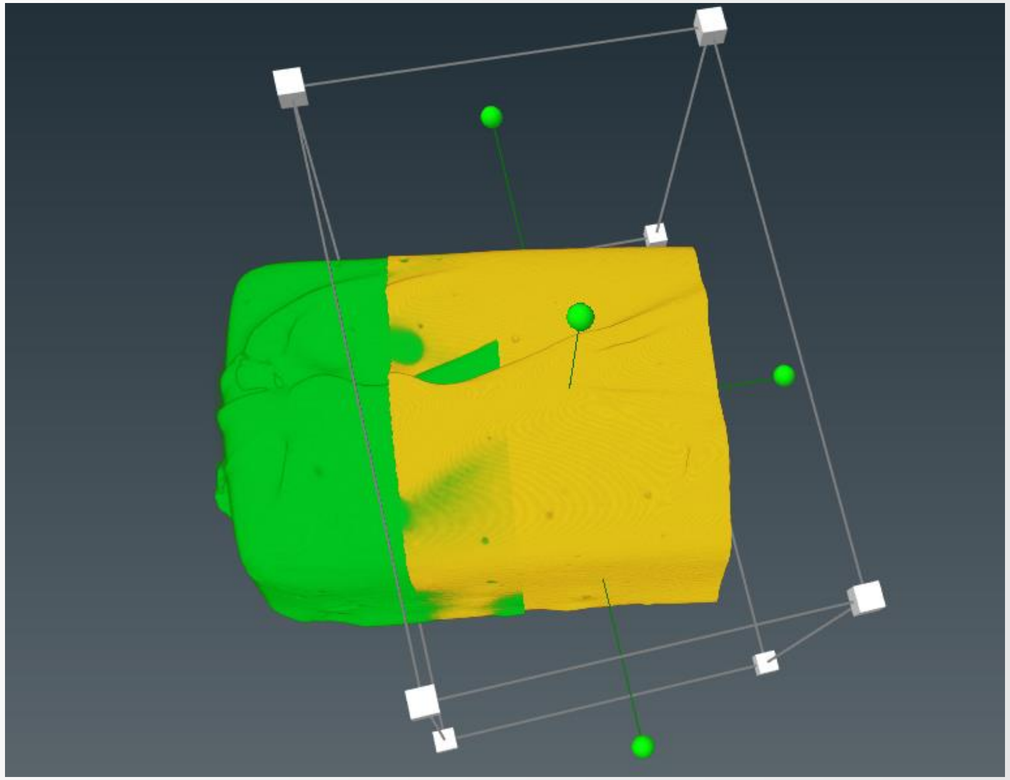




Data registration: Transform Editor module

Move the handle box and bring chocolate-bar.part2-model.am (in yellow) to overlap with chocolate-bar.part1-reference.am (in green) as much as possible.





Data registration: Register Images module

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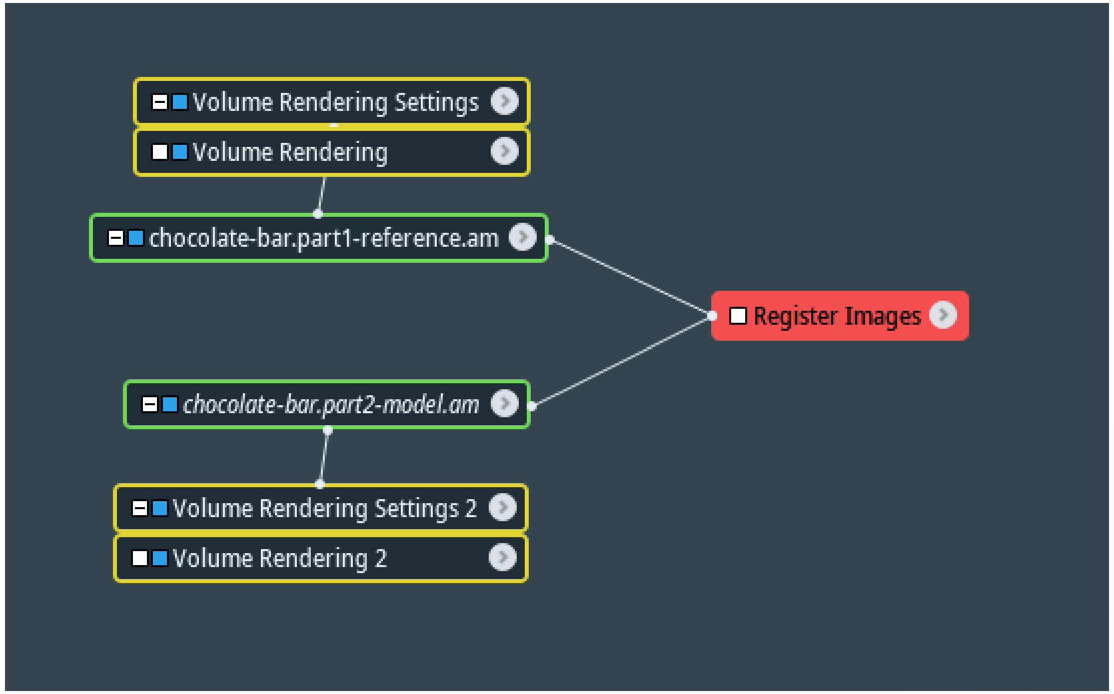
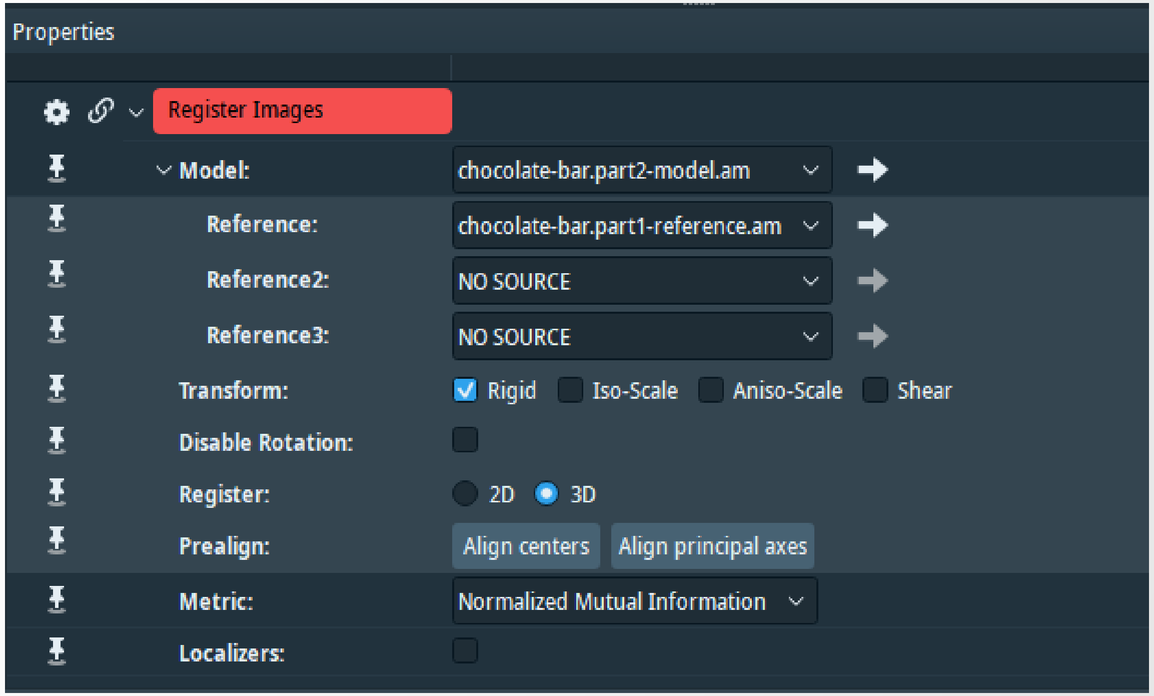
Attach Register Images to chocolate-bar.part2-model.am and set the reference to chocolate-bar.part1-reference.am.





- Transform: Rigid
- Register: 3D
- Prealign: Align centers & Align principal axes
- Metric: Normalized Mutual Information

Data registration: Resample Transformed Image module

Attach Resample Transformed Image to chocolate-bar.part2-model.am to apply the transformation (otherwise the transformation will be available for visualization only).

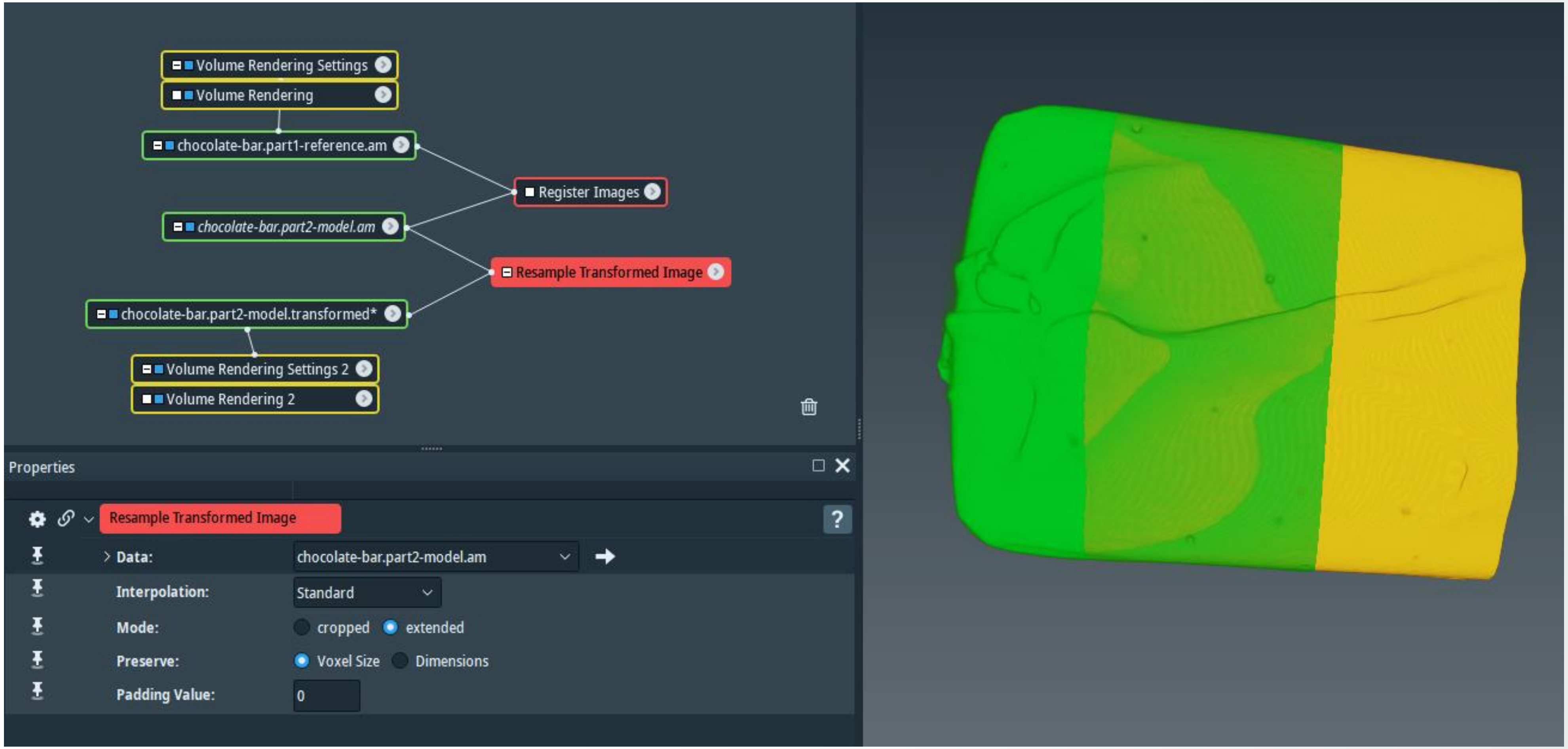
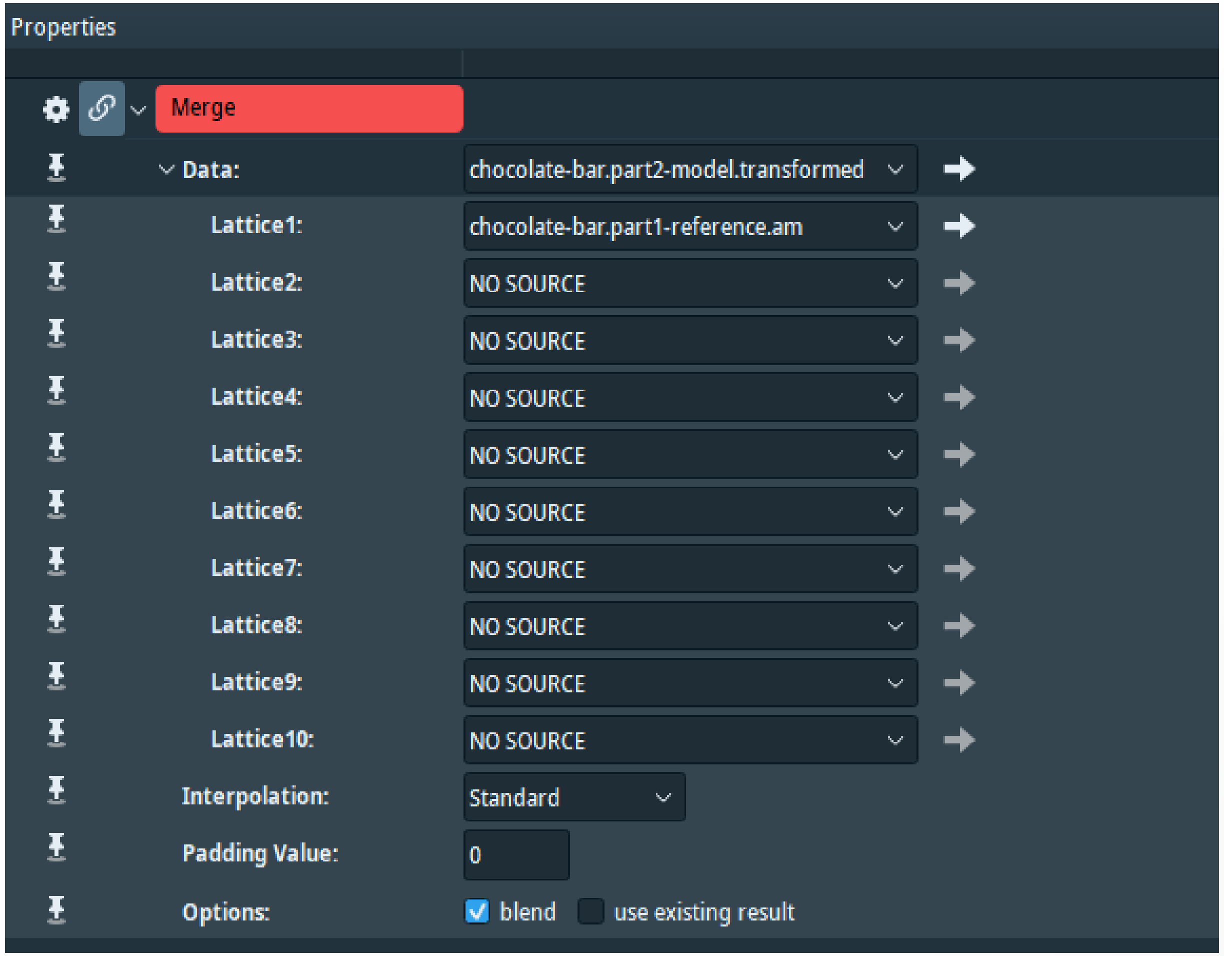


Image fusion & stitching

Attach Merge module to chocolate-bar.part2-model.transformed and set chocolate-bar.part1-reference.am as a reference. Merge using standard interpolation with blend option.



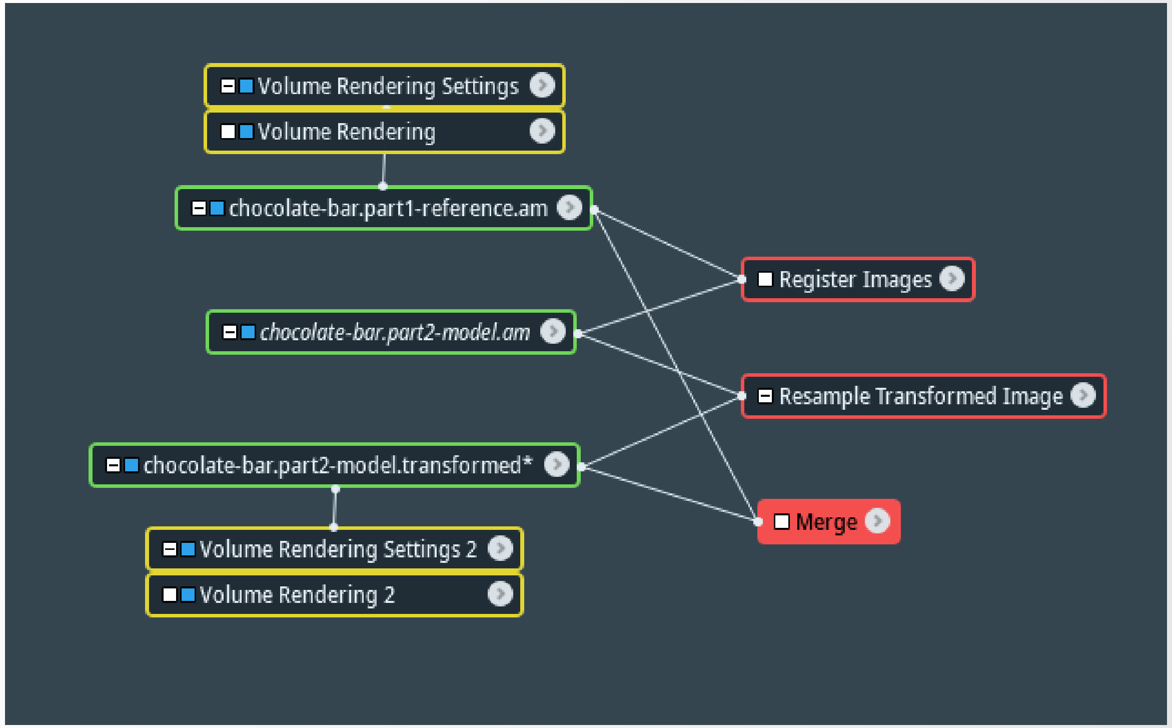
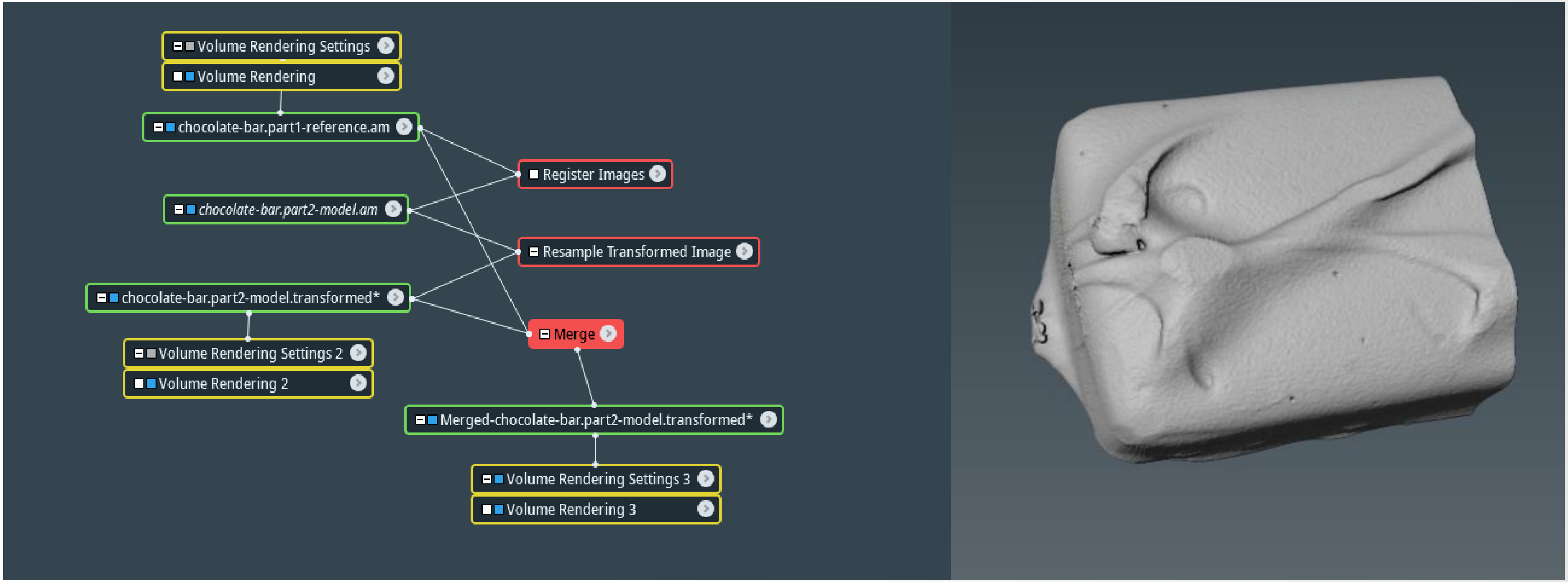


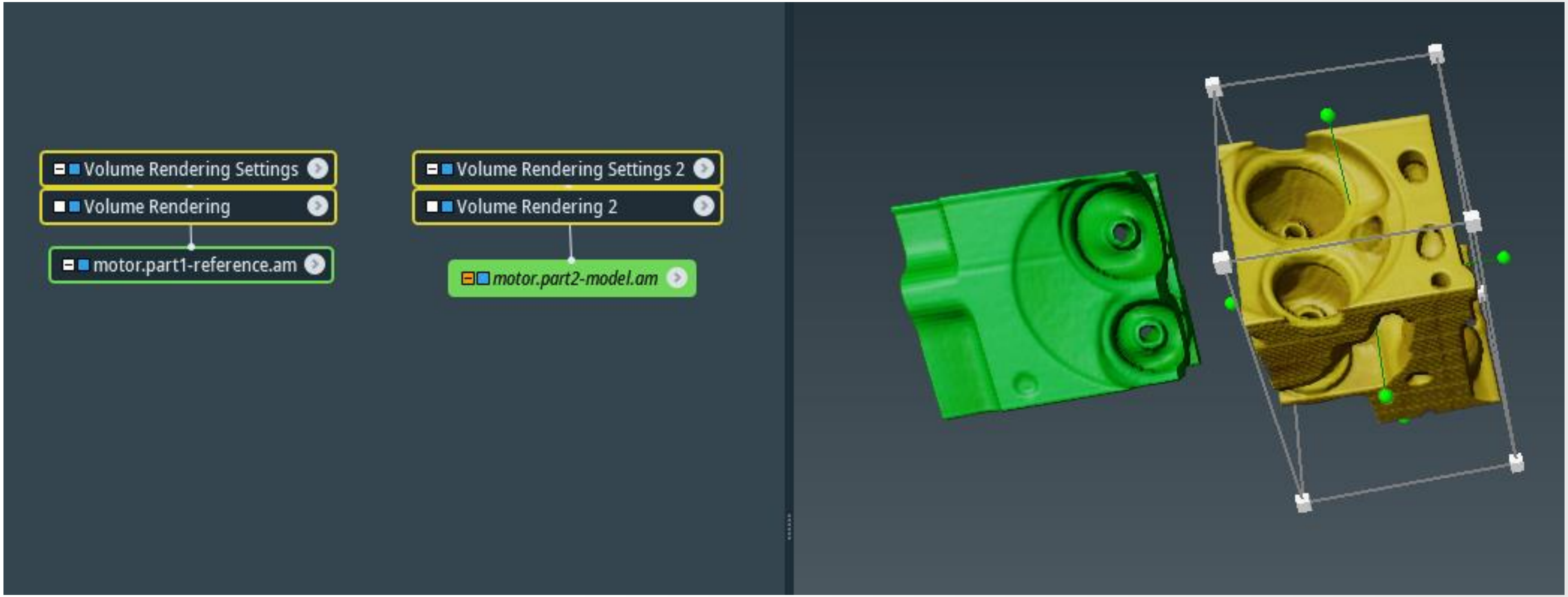
Image fusion & stitching



Data registration: exercise

Register and merge parts of motor data

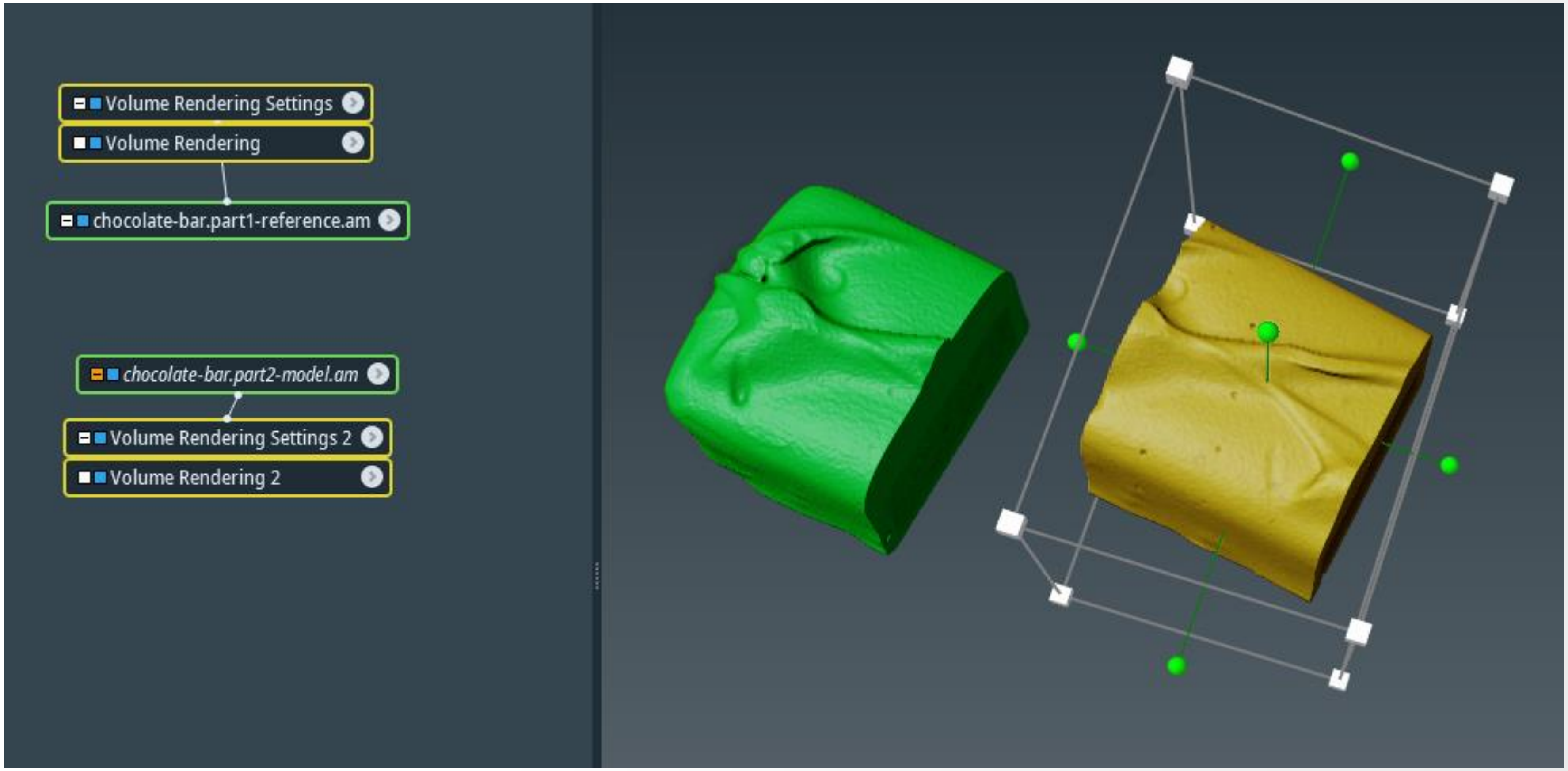
Register and merge the motor parts (…data/registration/motor.part1-reference.am & motor.part2-model.am)



Data registration: exercise

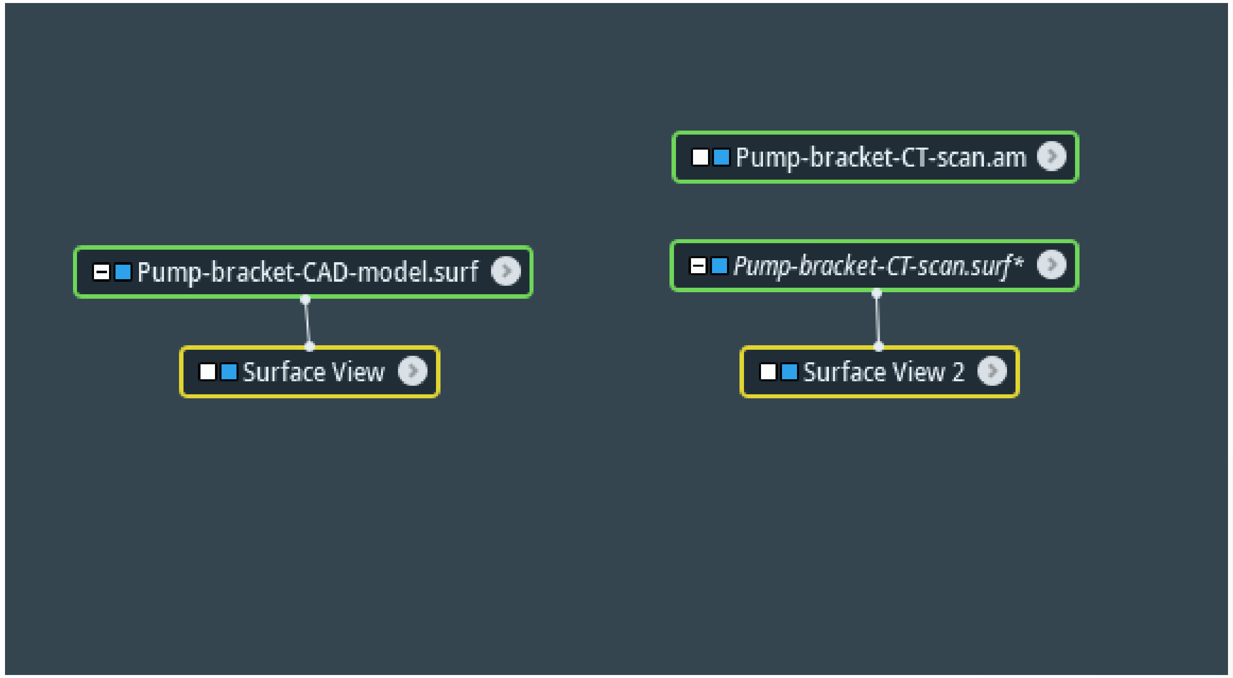
Register and merge parts of chocolate bar data

Register and merge the chocolate bar parts
(…data/registration/chocolate-bar.part1-reference.am chocolate-bar.part2-model.am)



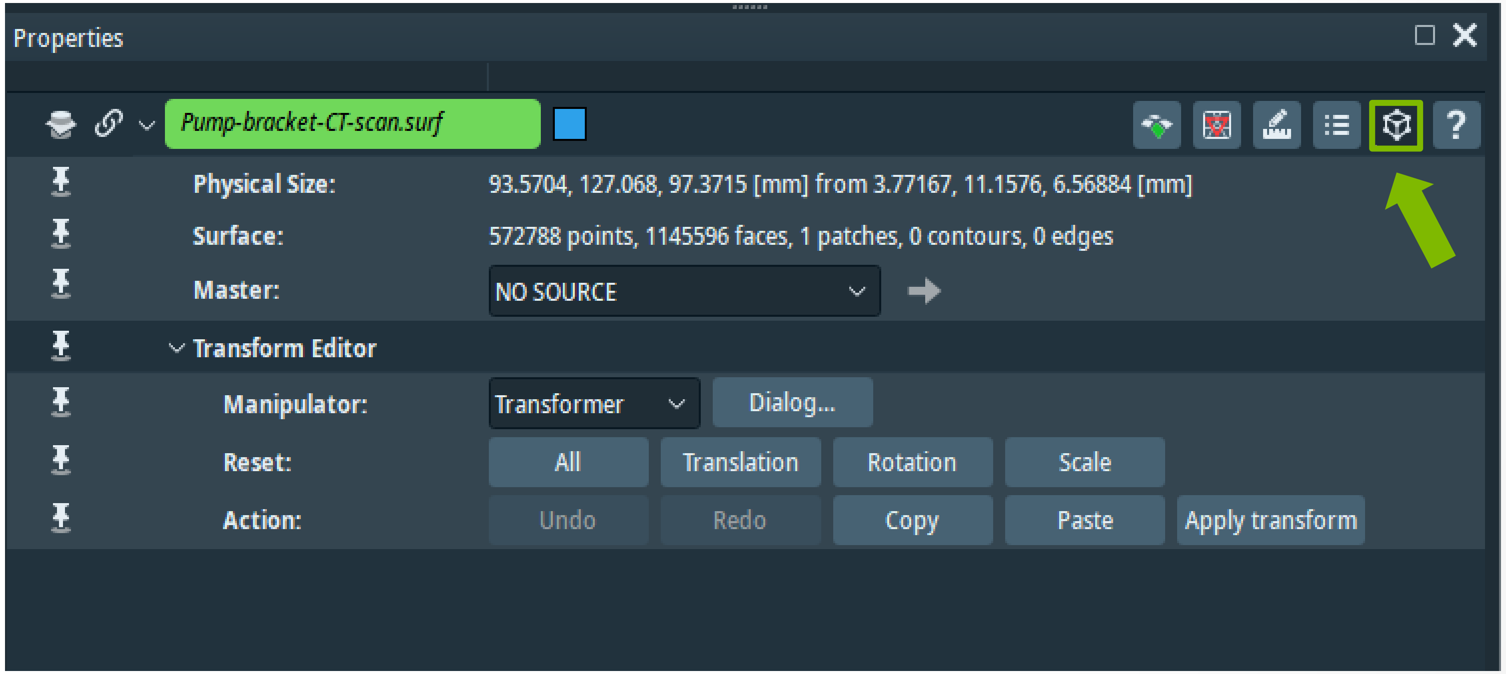
Nominal-Actual comparison

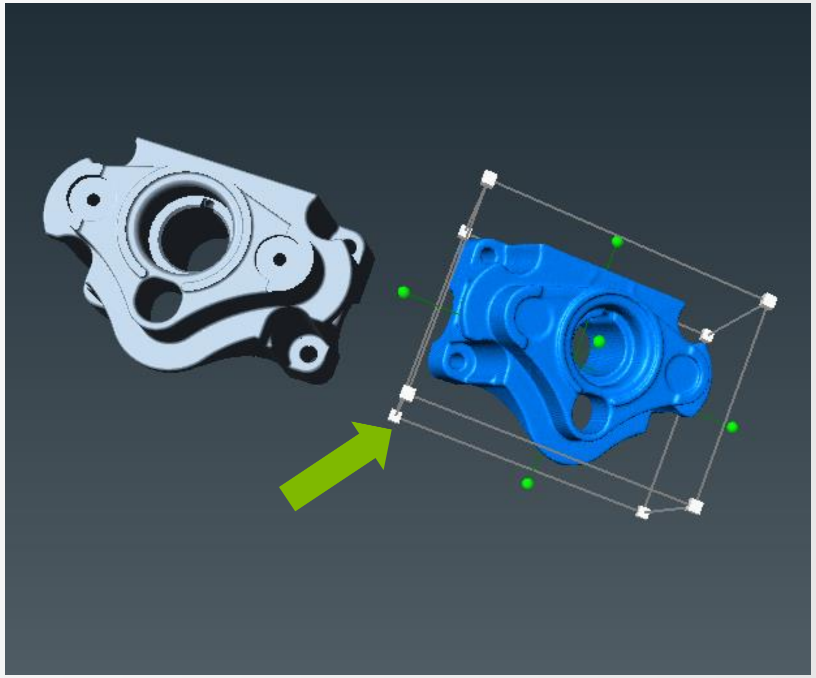
Open Pump-bracket-CAD-model.surf and Pump-bracket-CT-scan.am (…data/pump-bracket) then generate a binary image (thresholding) and then generate surface from Pump-bracket-CT-scan.am and attach Surface View to both surfaces.



Nominal-Actual comparison example

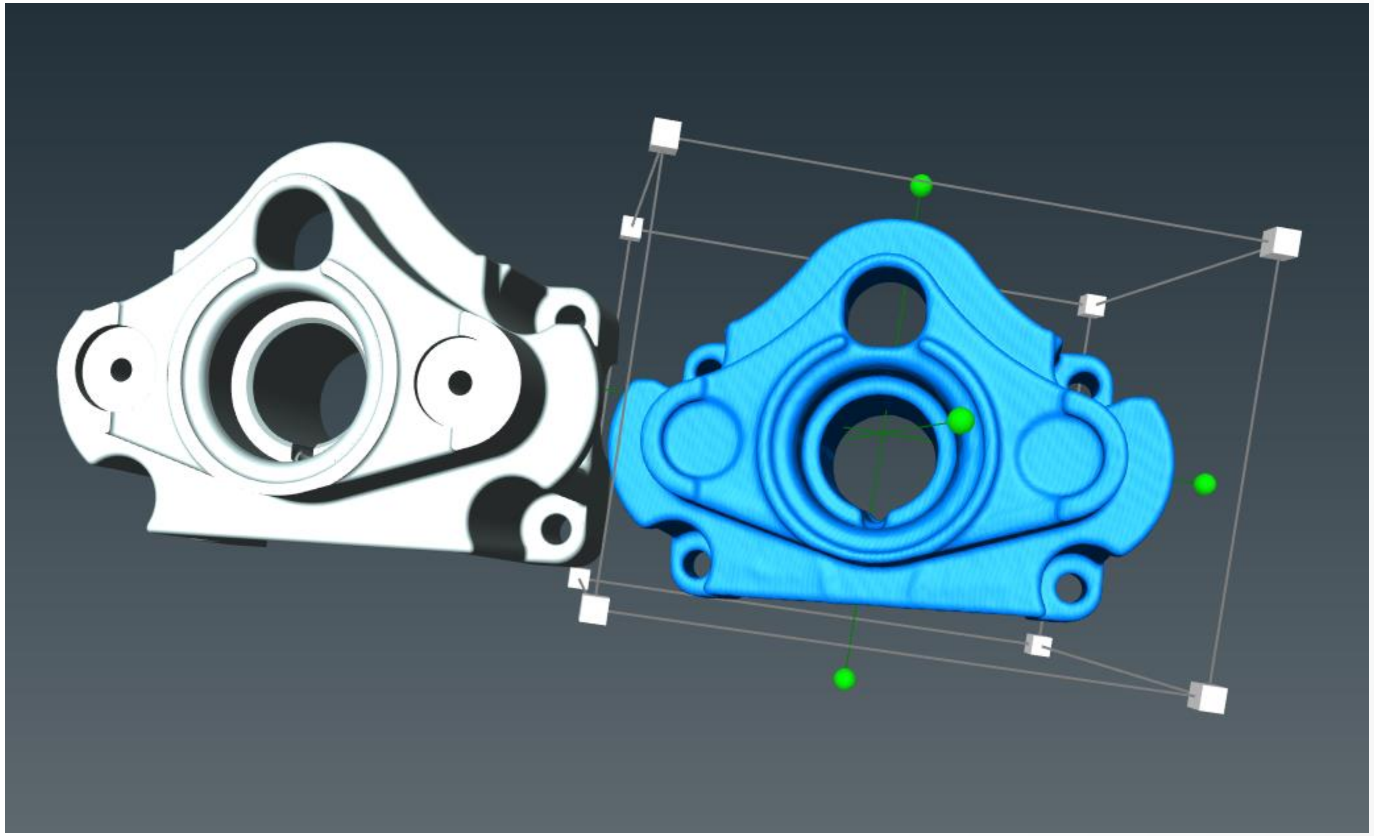
In the properties window of *Pump-bracket-CT-scan.surf*, activate Transform Editor, the transform box will appear.

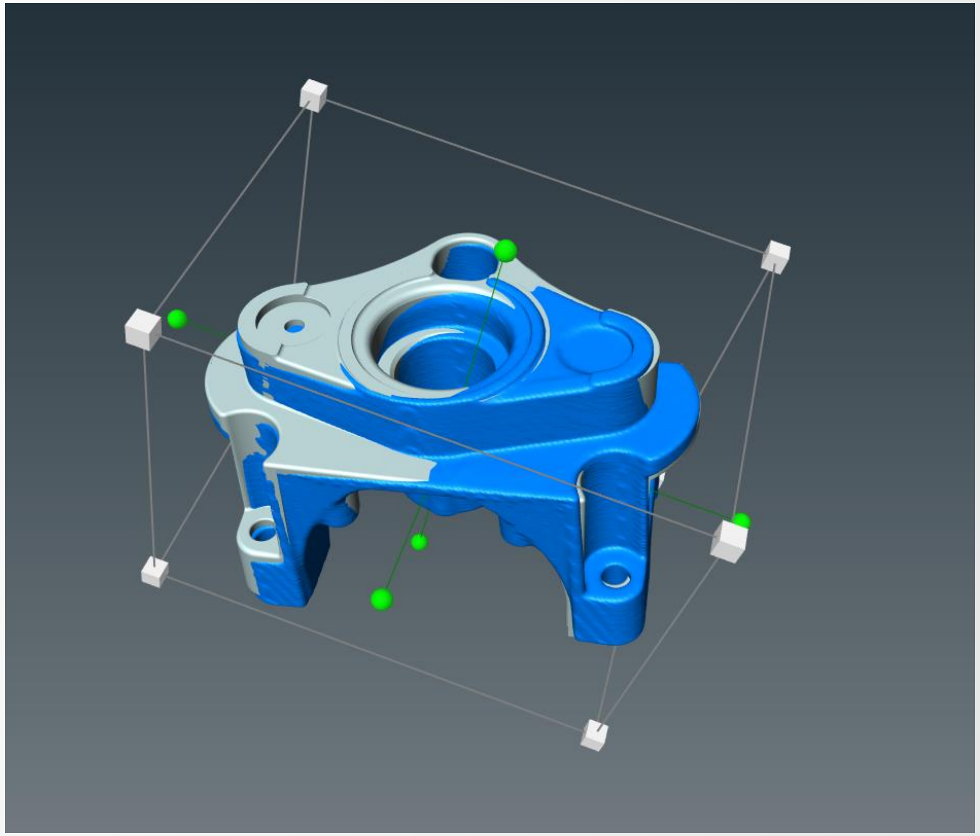




Nominal-Actual comparison example

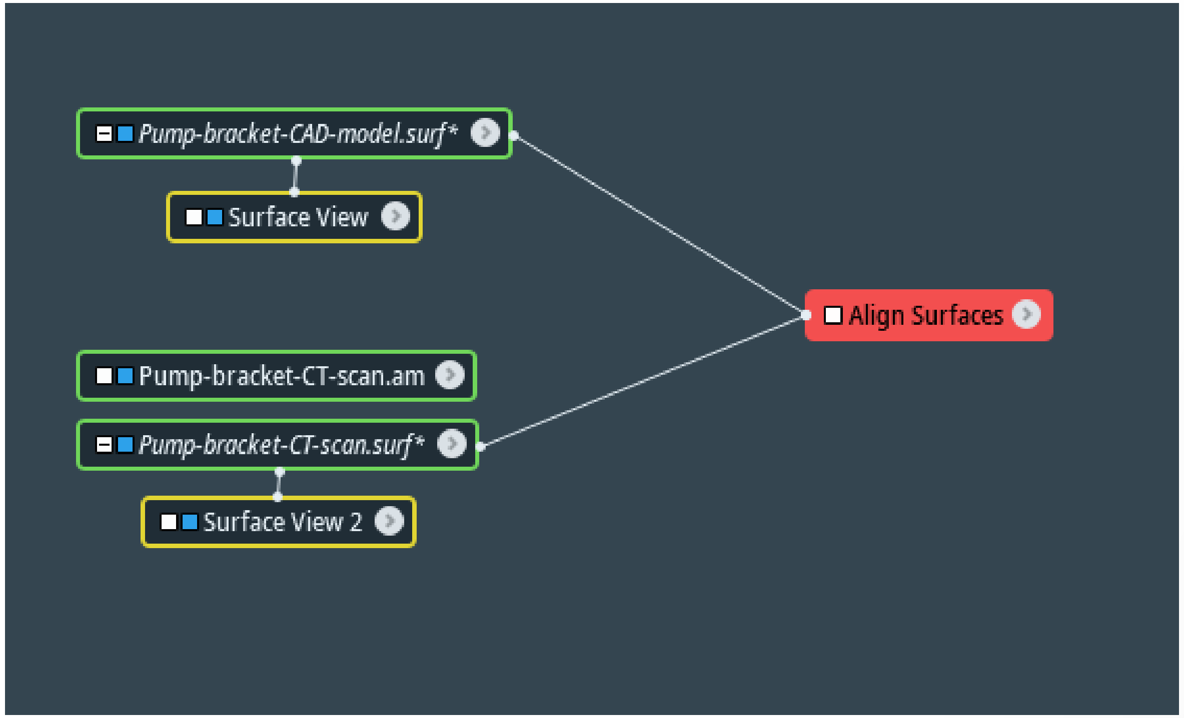
Move the handle box and bring *Pump-bracket-CT-scan.surf* to overlap with *Pump-bracket-CAD-model.surf* as much as possible.

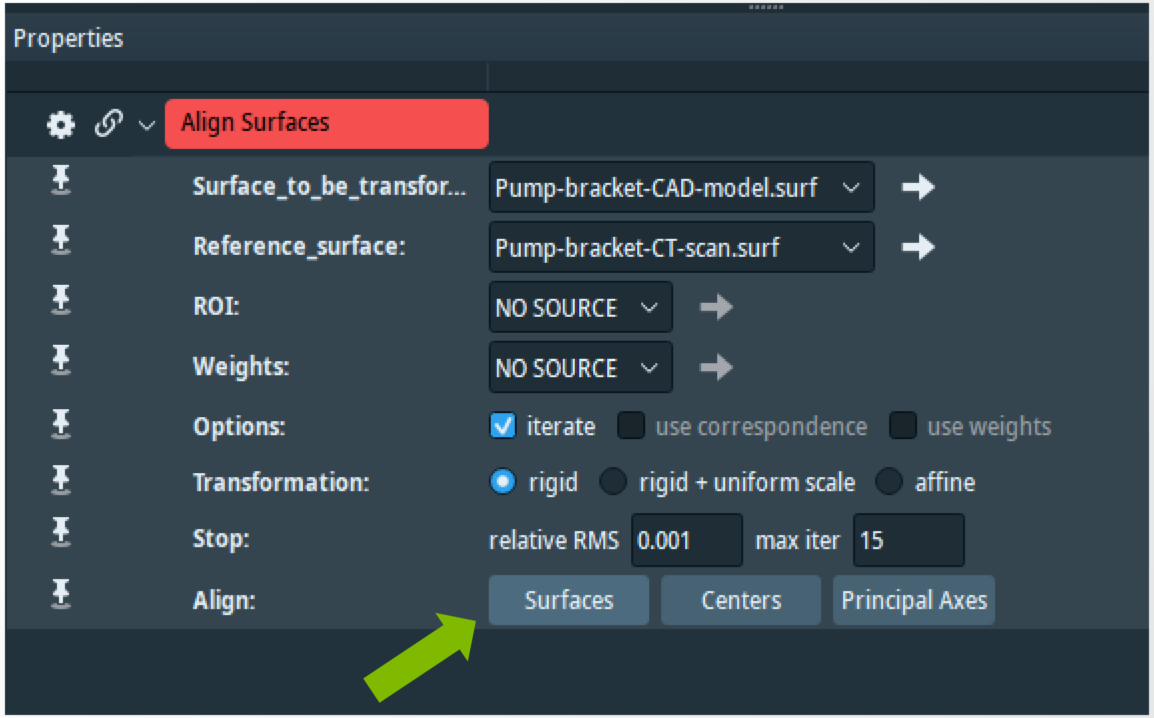




Nominal-Actual comparison example

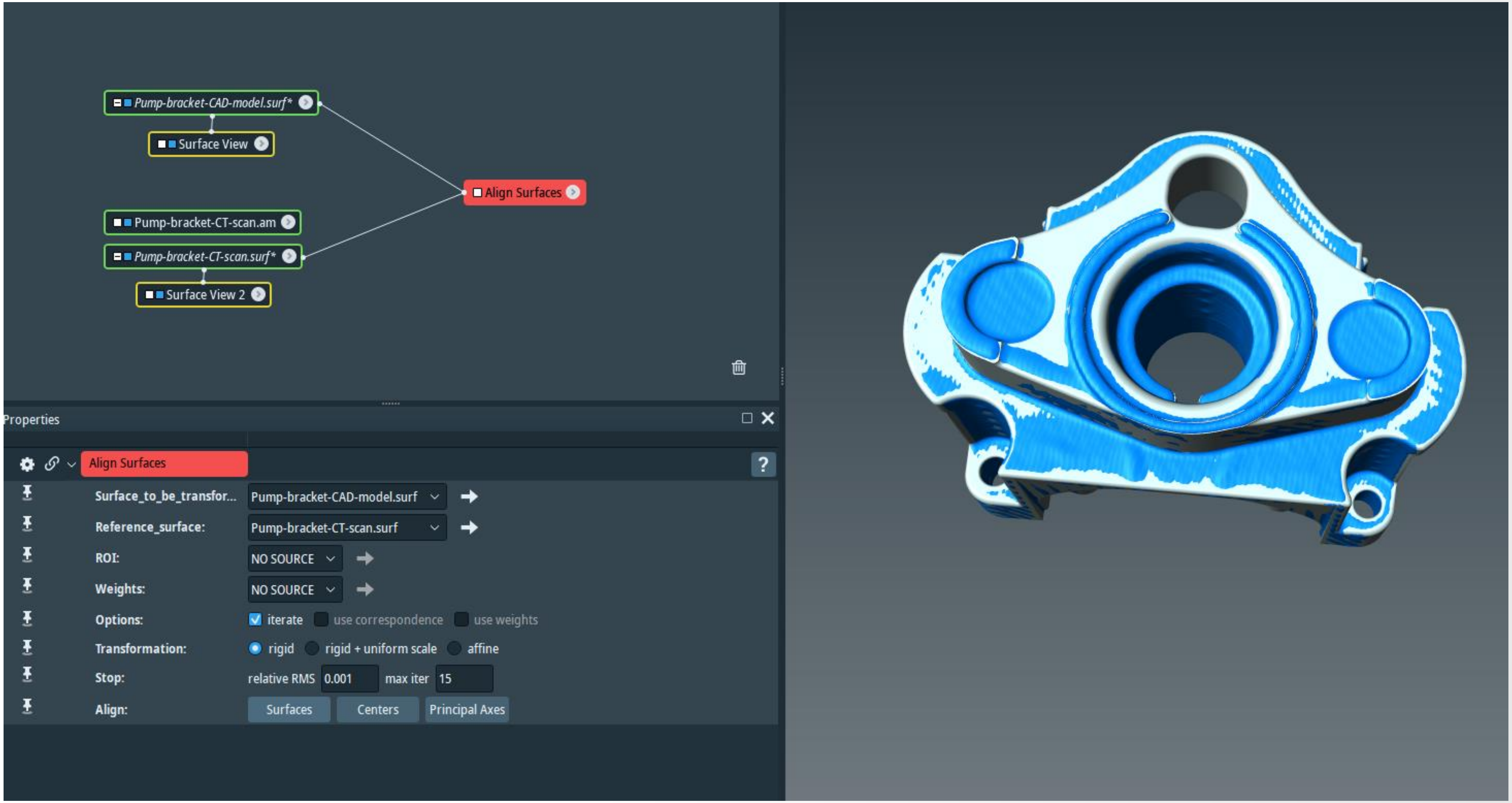
Attach Align Surfaces to *Pump-bracket-CAD-model.surf* and set the reference to *Pump-bracket-CT-scan.surf*.





- Options: iterate
- Transformation: rigid
- Stop: relative RMS = 0.001, max iter = 15
- Align: Surfaces

Nominal-Actual comparison example



Nominal-Actual comparison: e.g. Surface Distance

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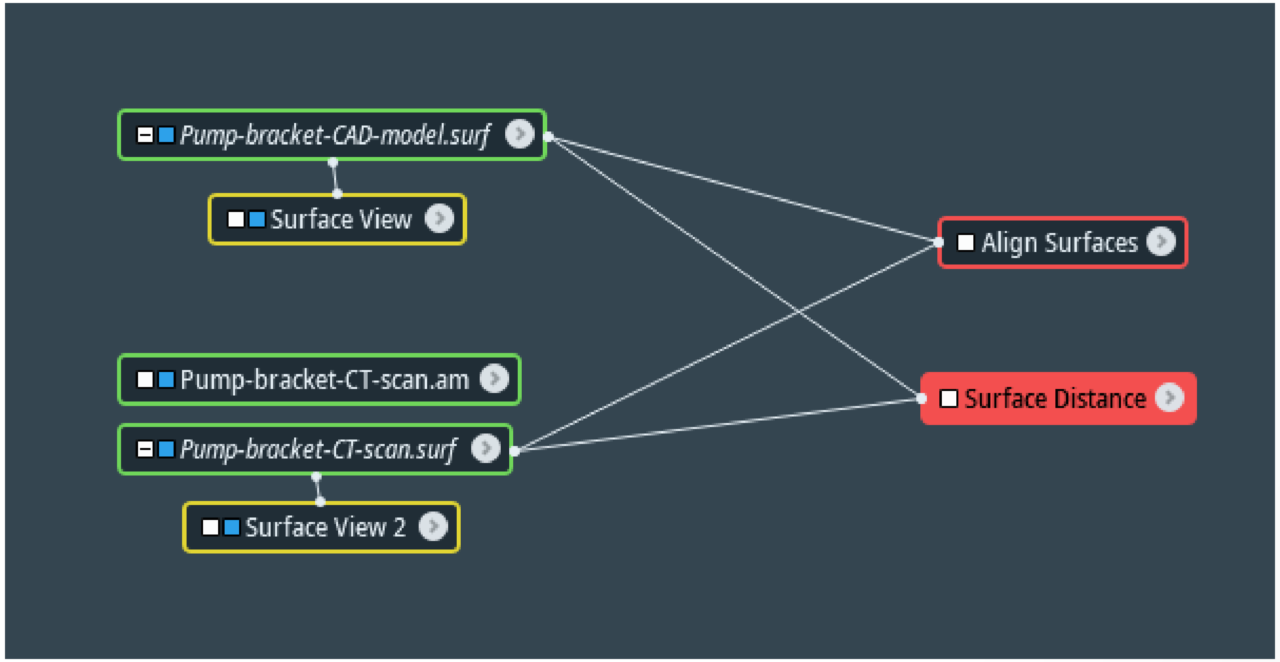
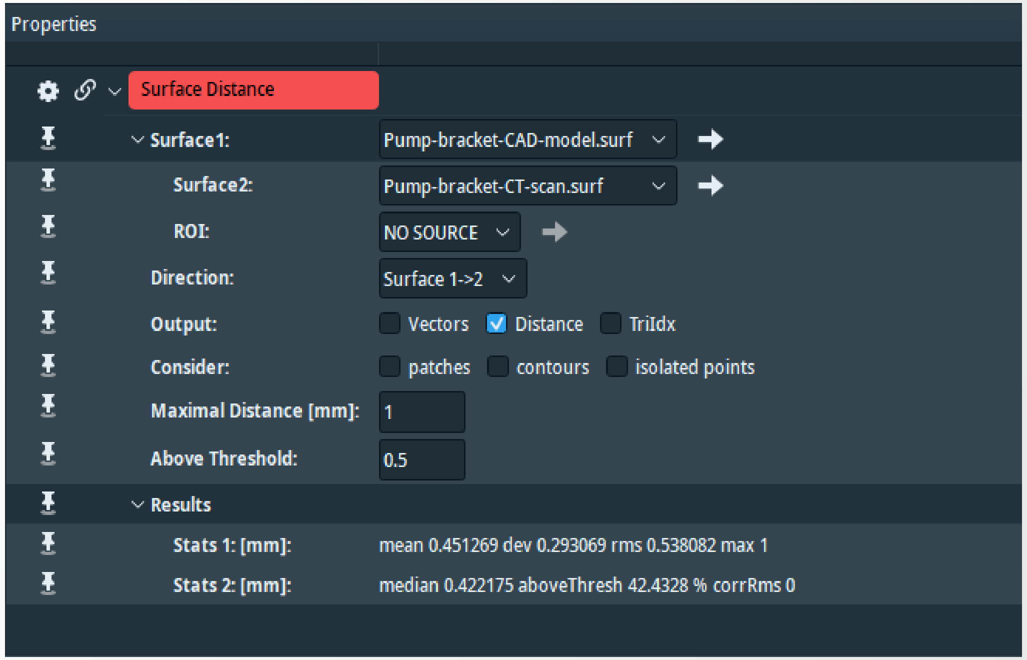
Attached Surface Distance module to Pump-bracket-CAD-model.surf (surface 1) and Pump-bracket-CT-scan.surf (surface 2).

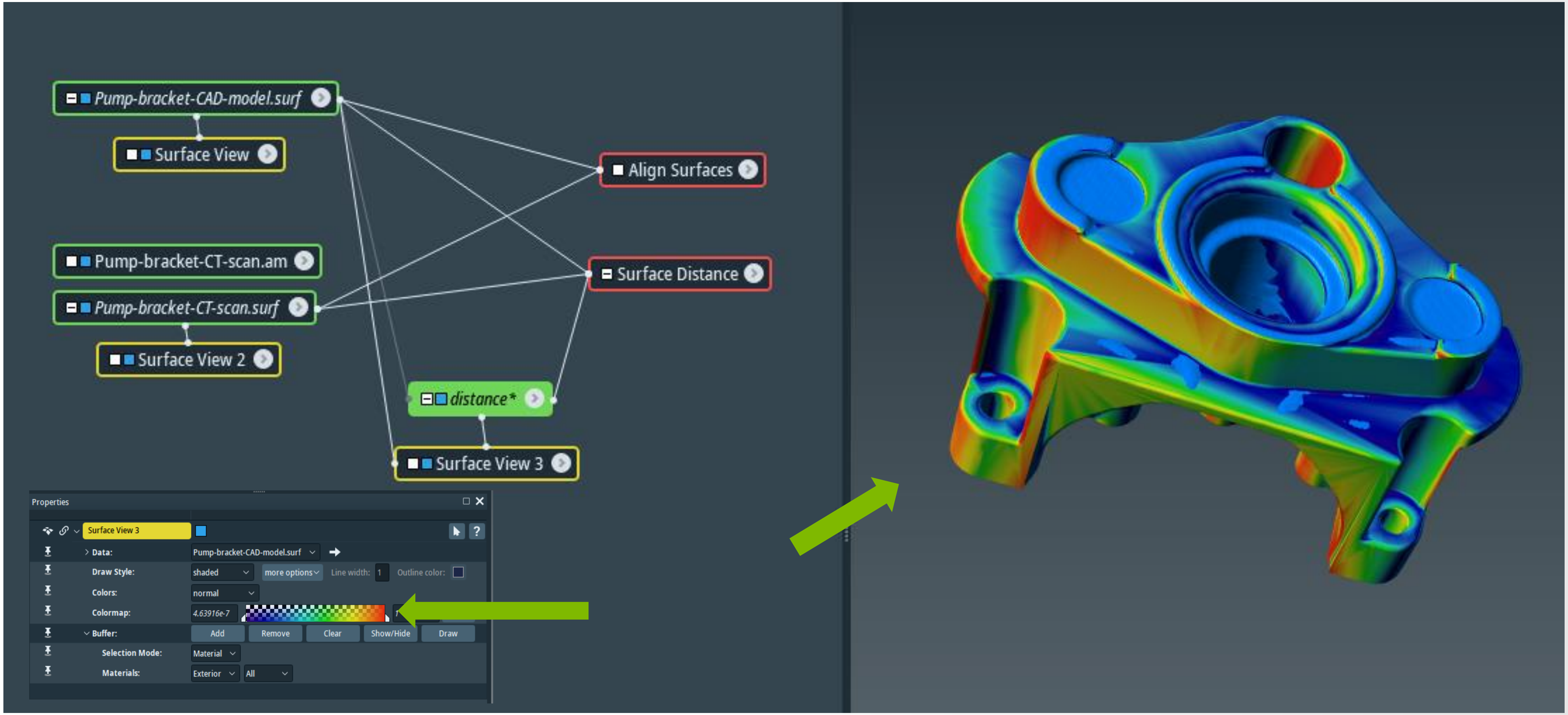




- Direction: Surface 1->2
- Output: Distance
- Maximal Distance: 1
- Above Threshold: 0.5

Nominal-Actual comparison: e.g. Surface Distance

Visualize distance with Surface View, set color map to Physics

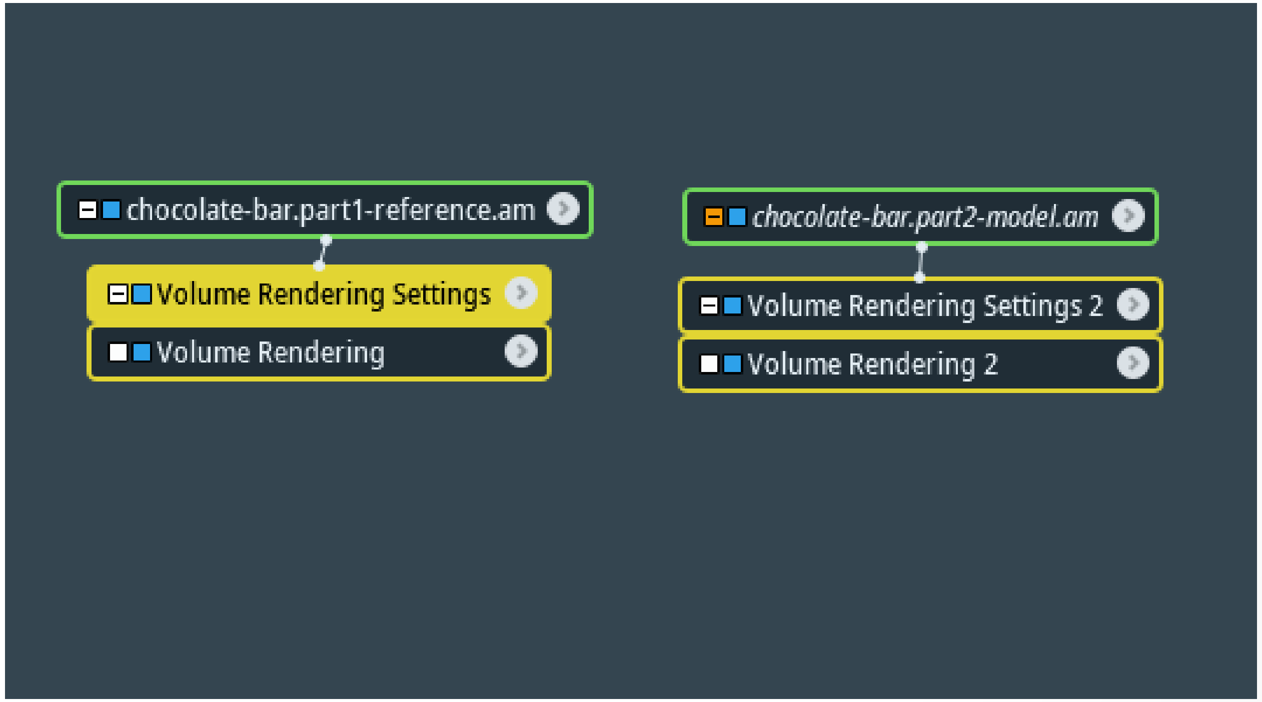


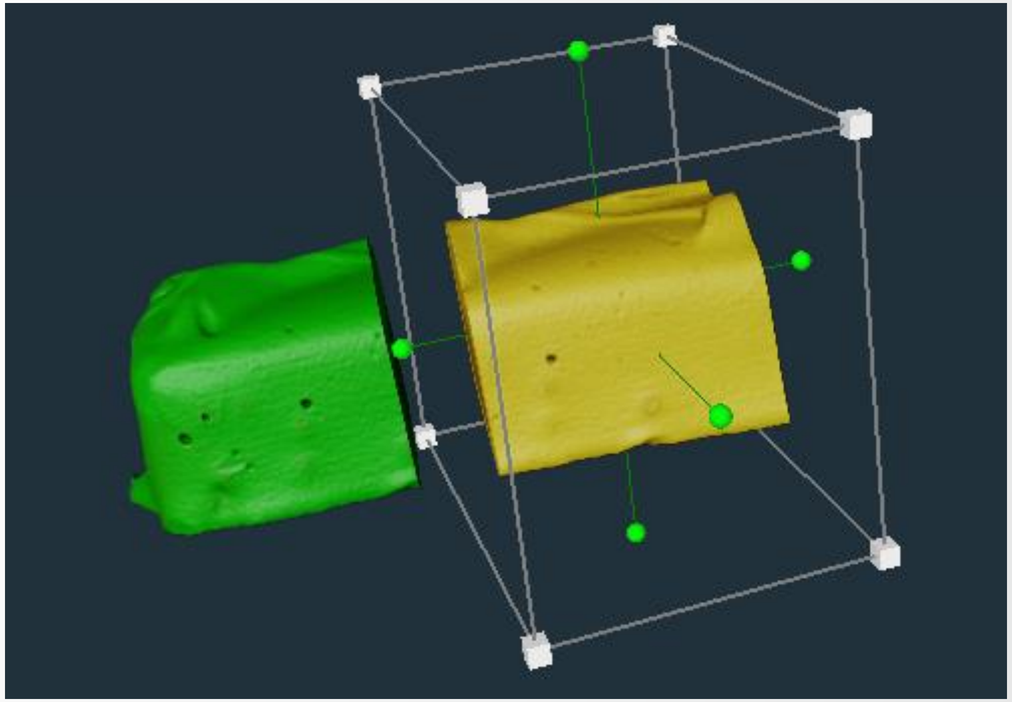
Landmark-based registration: introduction

- Landmarks are useful for registration and alignment of multiple 3D images.
- It allows you to store multiple sets of corresponding marker positions.
- The data type can also be used to represent a simple list of 3D points.

Landmark-based registration: example

Open chocolate-bar.part1-reference.am and chocolate-bar.part2-model.am (data -> registration) then attach Volume Rendering to each dataset.



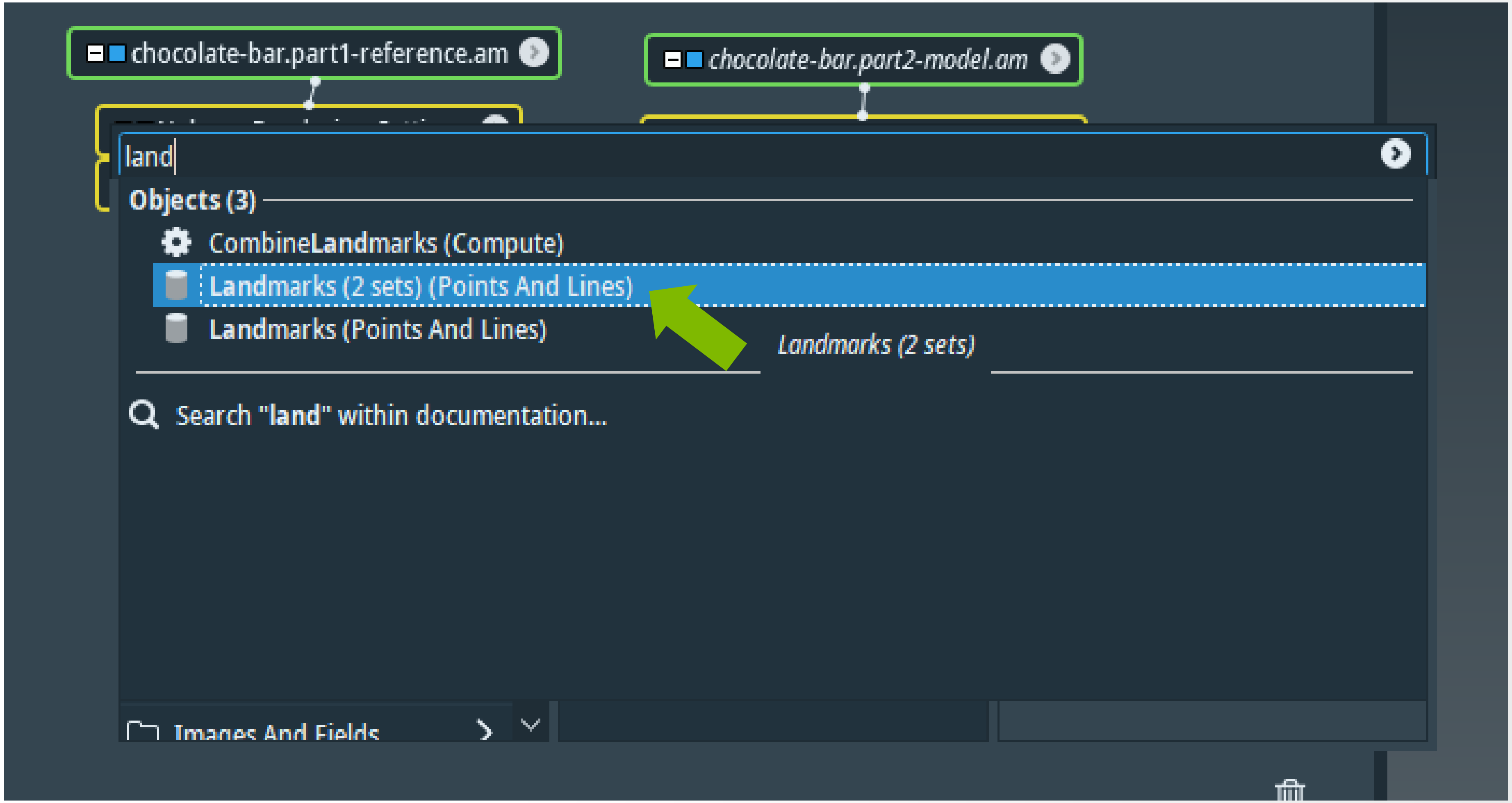


In the properties window of chocolate-bar.part2-model.am, activate Transform Editor, the transform box will appear. Move the handle box and bring chocolate-bar.part2-model.am (in yellow) to align with chocolate-bar.part1-reference.am (in green) as much as possible.

Once satisfied apply Resample transform Image to save the transformed dataset.

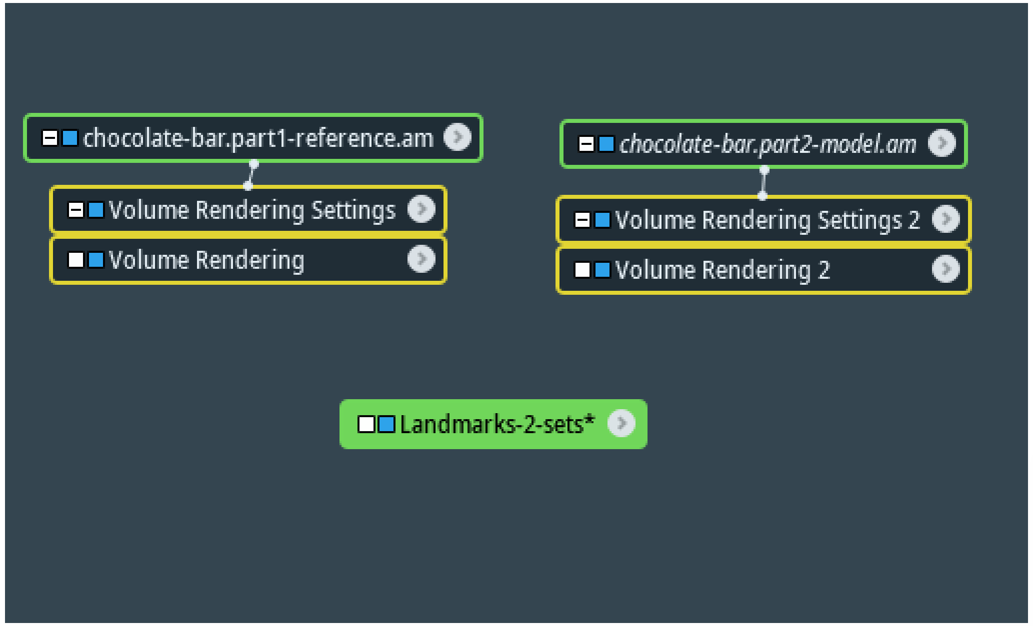
Landmark-based registration: example

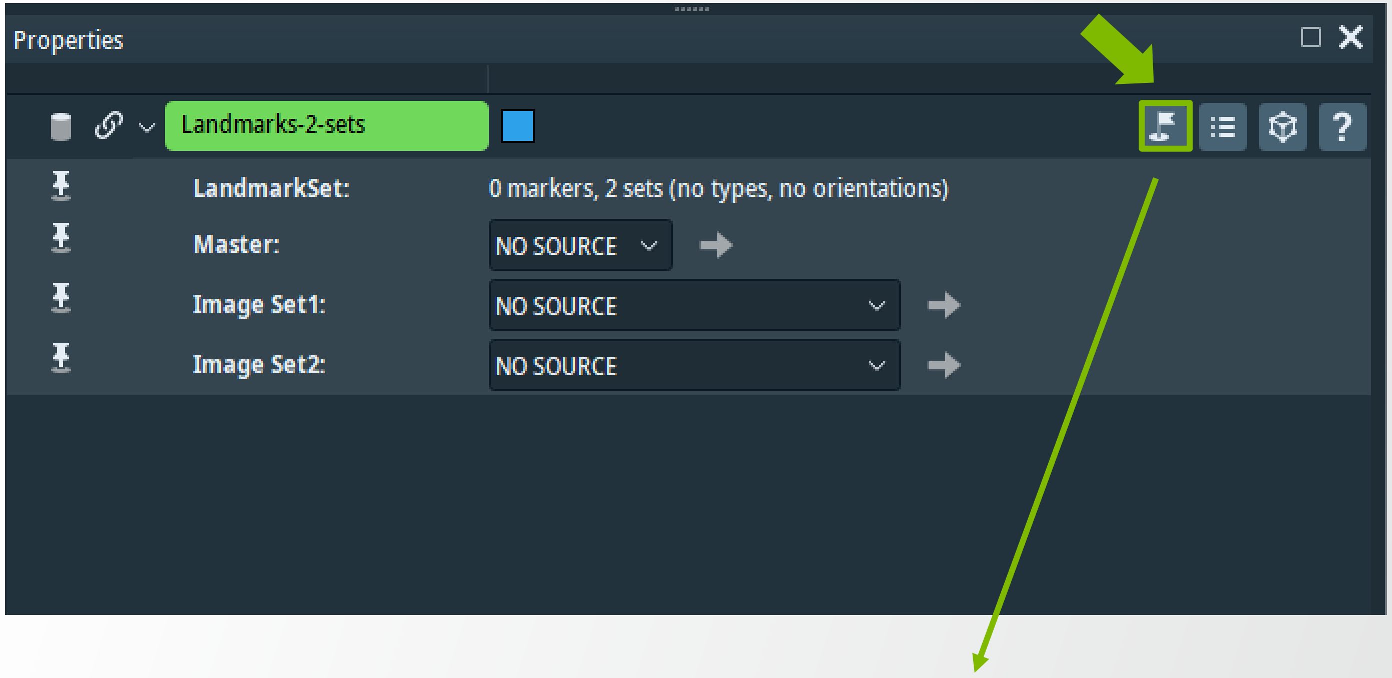
In Project View, right-click and “**Create Object**”, select **Landmarks (2 sets) (Points And Lines)**.



Landmark-based registration: example

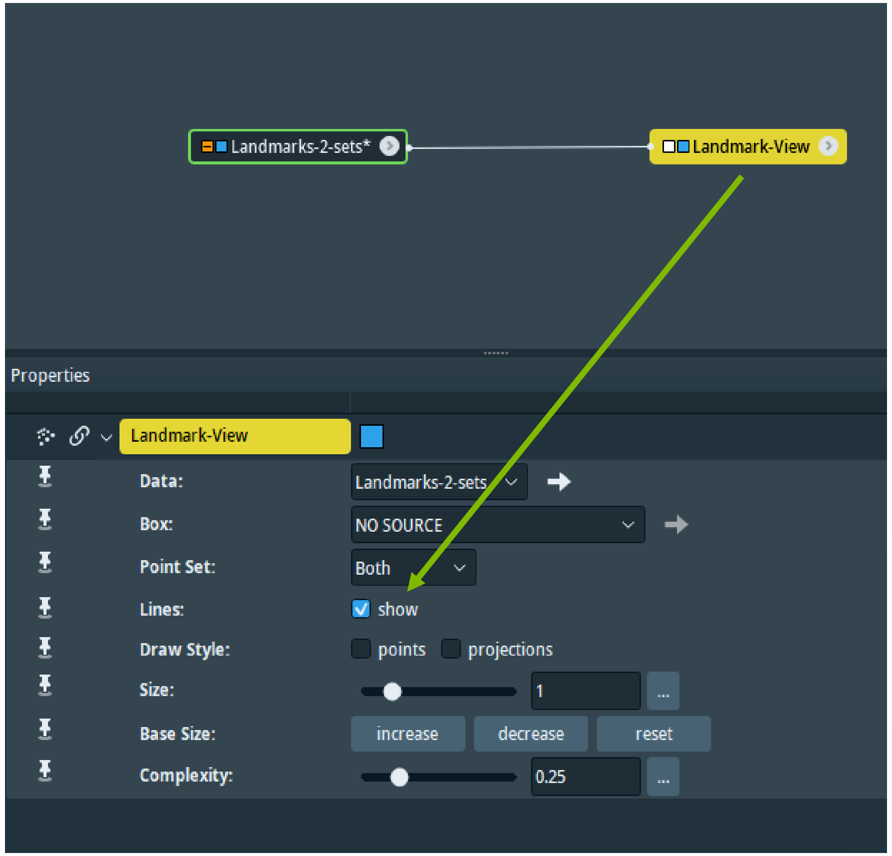
Activate Landmark Editor in the properties port of Landmarks-2-sets, Landmark View will appear.

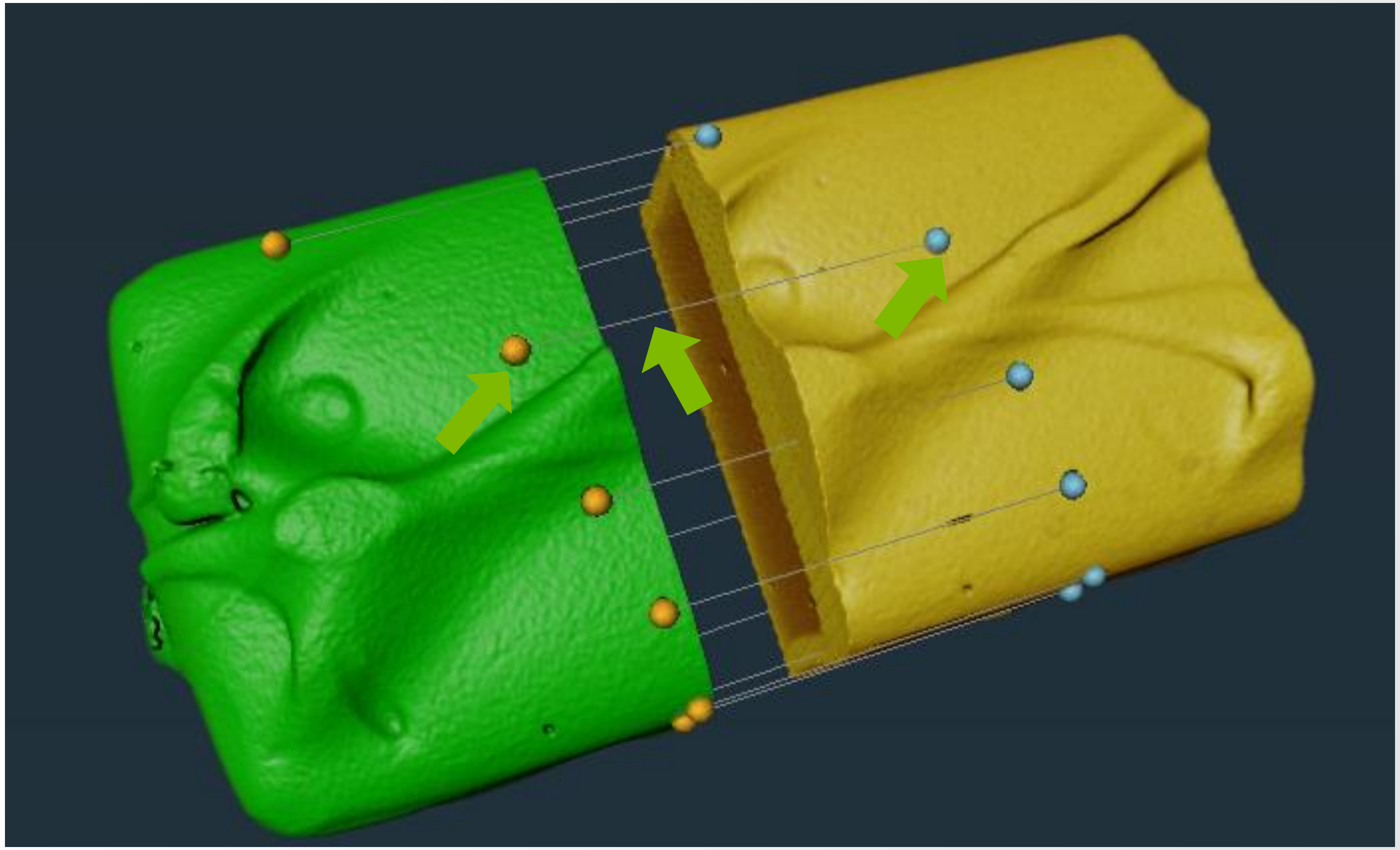




Landmark-based registration: example

In Landmark-View select to show lines. Then go back to Landmarks-2-sets to start adding points by click on chocolate-bar.part1-reference.am (yellow dots) and connect to chocolate-bar.part2-model.am (light-blue dots). Line sets between the two volumes will be shown. Rotate the volumes and add more points and lines to connect common landmarks between the two volumes. Do not make crossing lines.



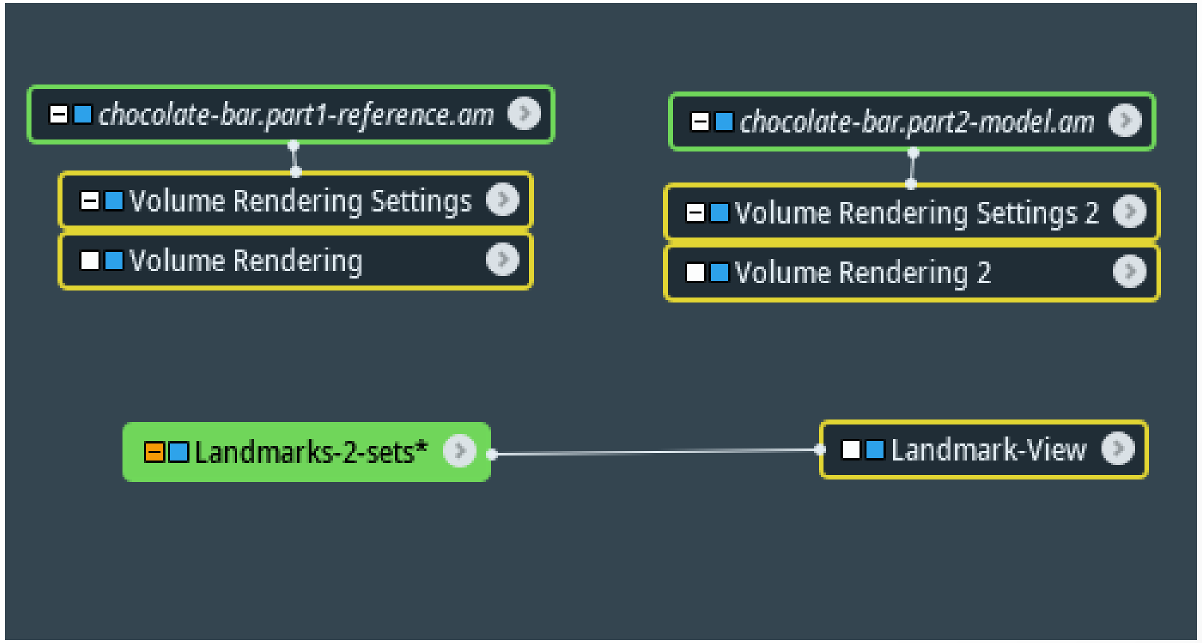


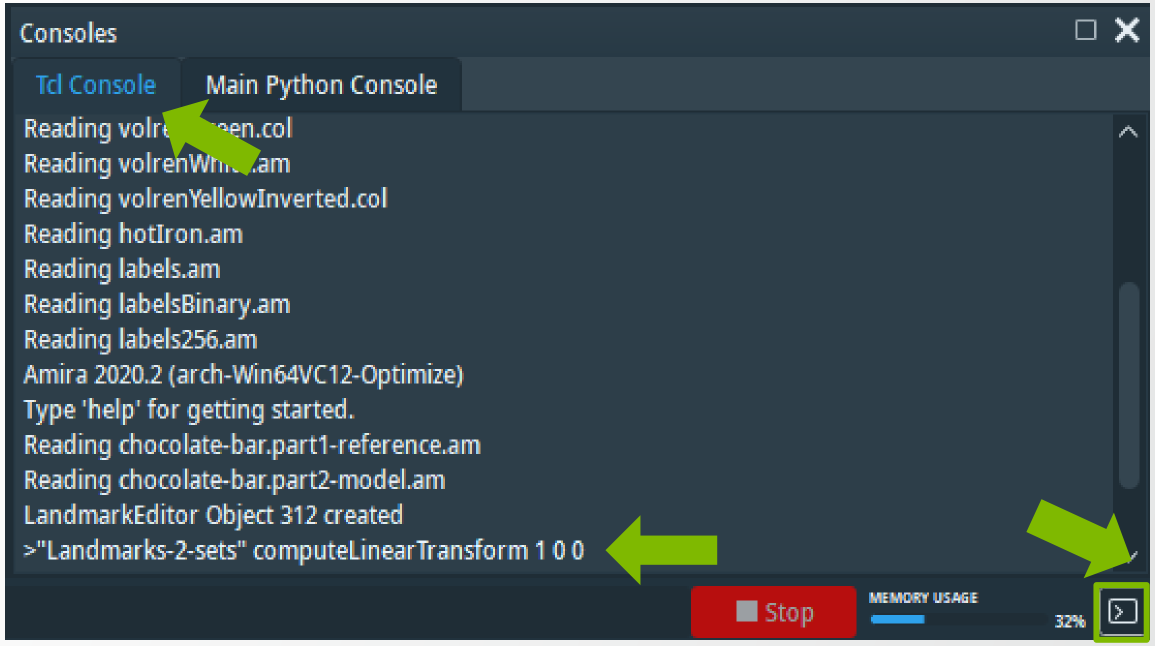
Landmark-based registration: example

Open Tcl console, then come back to “Project View” and click on “Landmarks-2-sets”. Next go back to Tcl Console and then press “Tab” to activate- > “Landmarks-2-sets” and input “computeLinearTransform 1 0 0”:

- “Landmarks-2-sets” computeLinearTransform 1 0 0

Press enter.



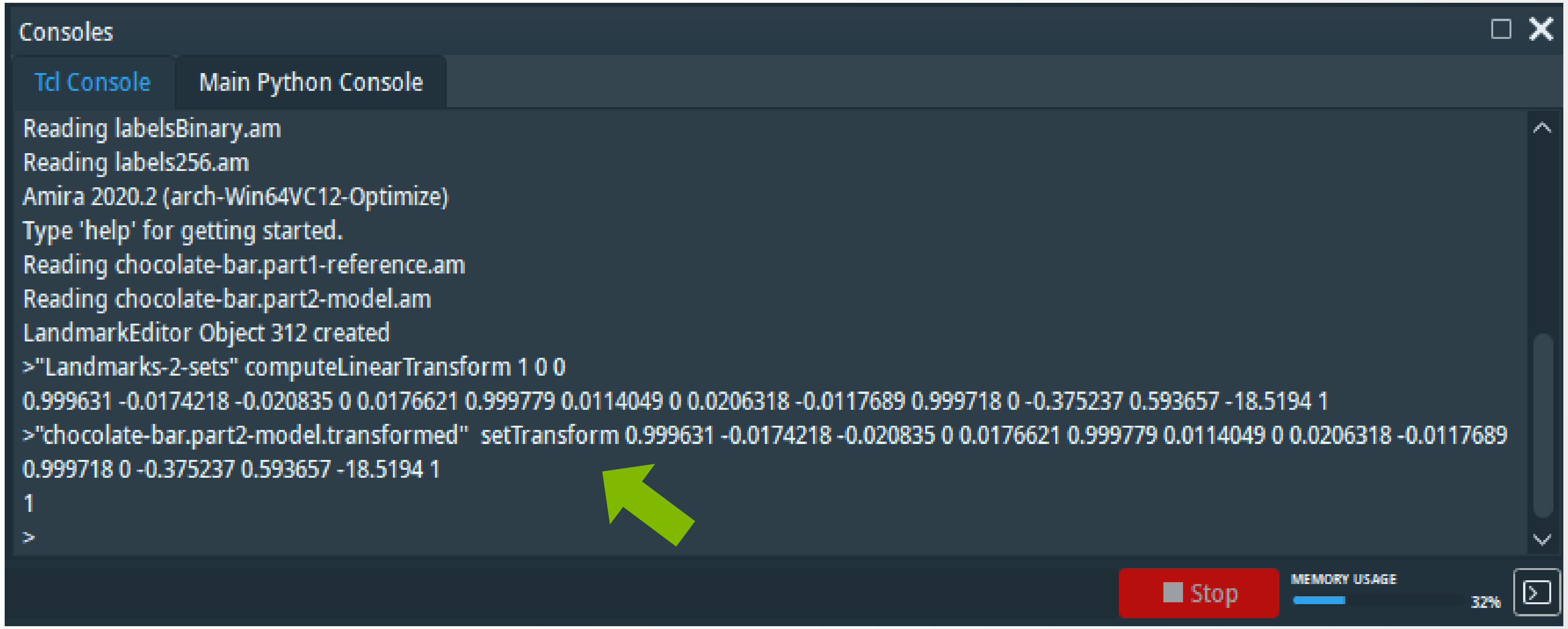


Landmark-based registration: example

Go back to Project View and click on chocolate-bar.part2-model_transformed.am. Then go back to Tcl Console and then press “Tab” to activate -> “chocolate-bar.part2-model-transformed.am” and input setTransform and paste the values from previous step:

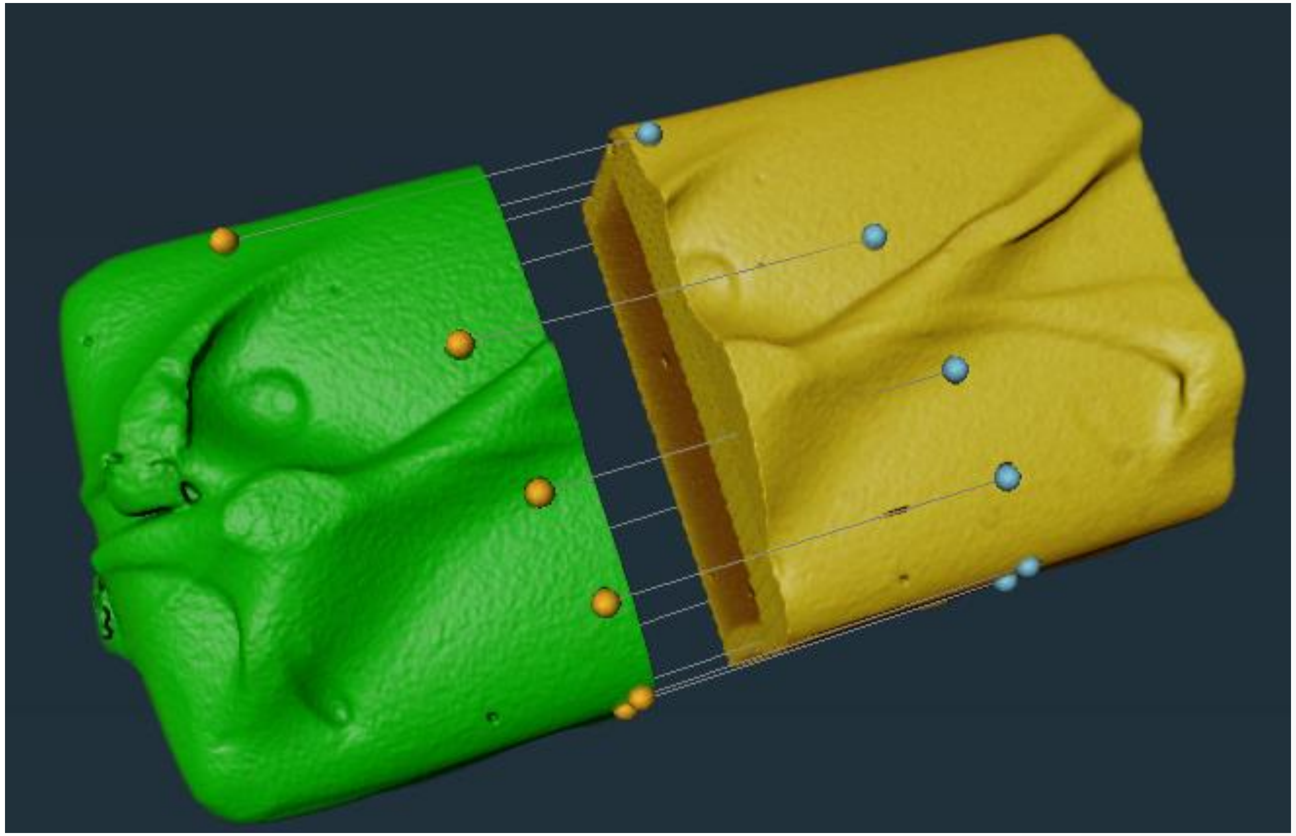
- “chocolate-bar.part1-reference.am” setTransform 0.999631 -0.0174218 -0.020835 0 0.0176621 0.999779 0.0114049 0 0.0206318 -0.0117689 0.999718 0 -0.375237 0.593657 -18.5194 1

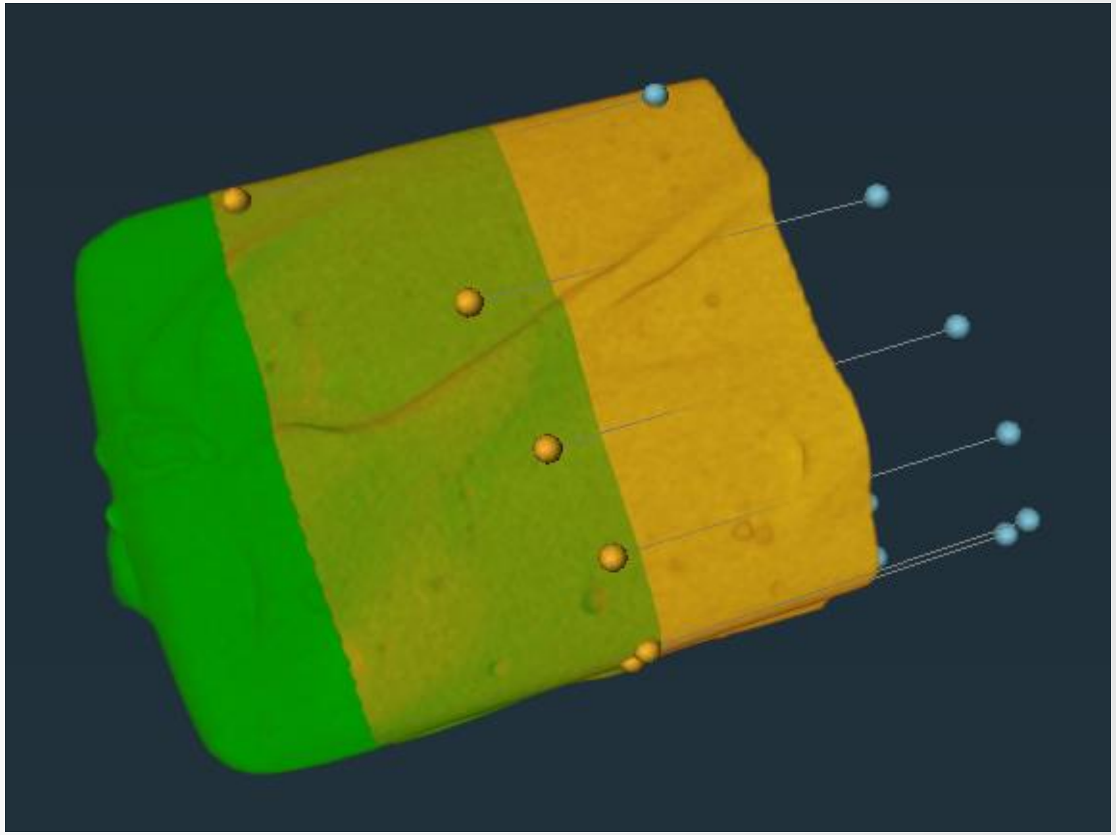
Press enter.



Landmark-based registration: example

Registration result:





Artificial Intelligence

Artificial Intelligence

What is Deep Learning

Deep learning is a machine learning technique that teaches computers to do what comes naturally to humans:

Learn by example

Example Data

has patterns

Train

finds patterns

Model

recognizes patterns

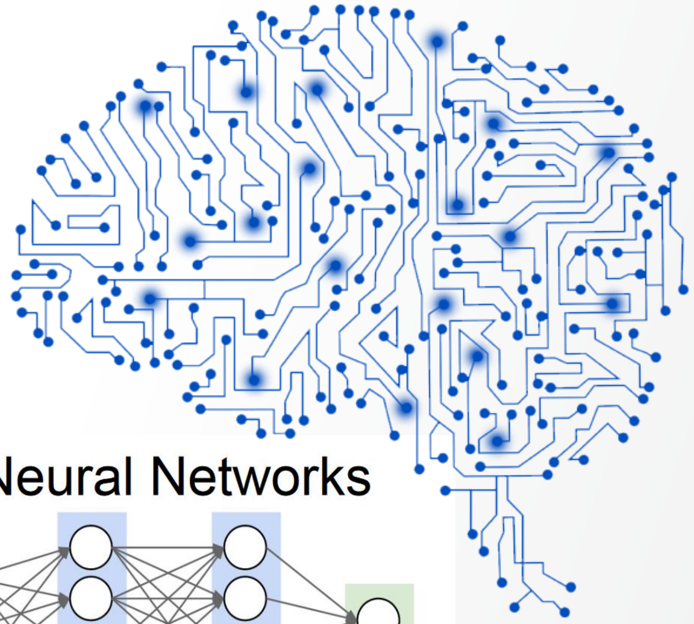
New Data

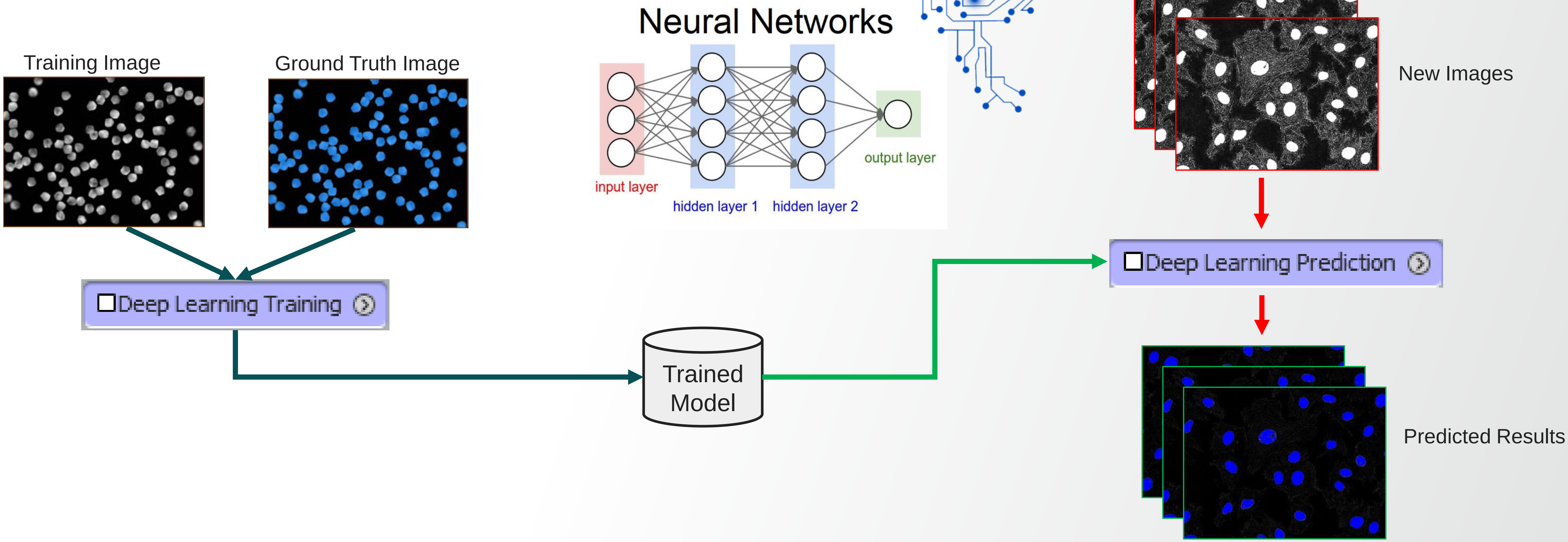
Apply Model

Prediction

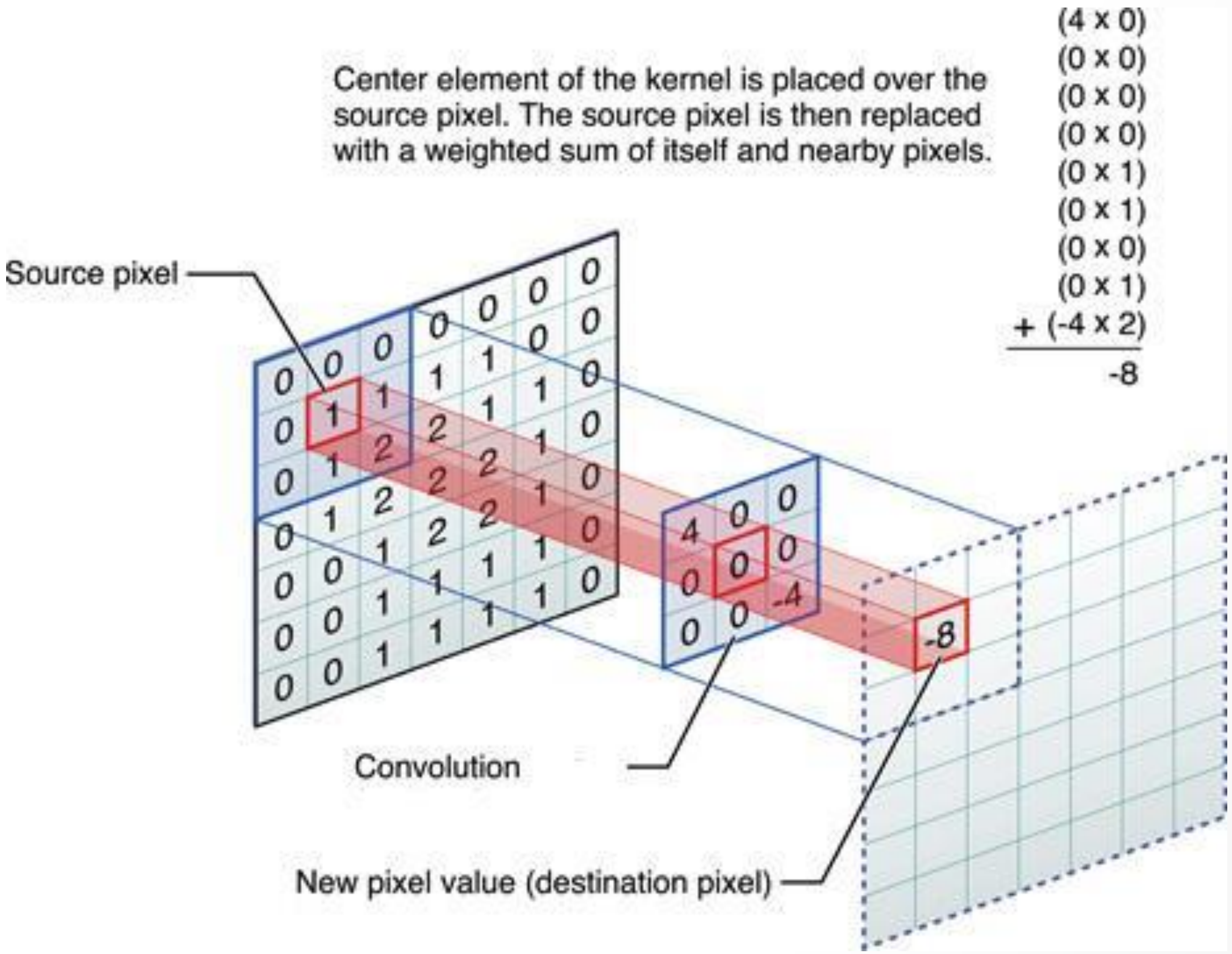
AI-Powered Deep Learning

- Learn by example
- Automate tedious tasks
- Solve challenging feature identification
- Improve accuracy & time to results





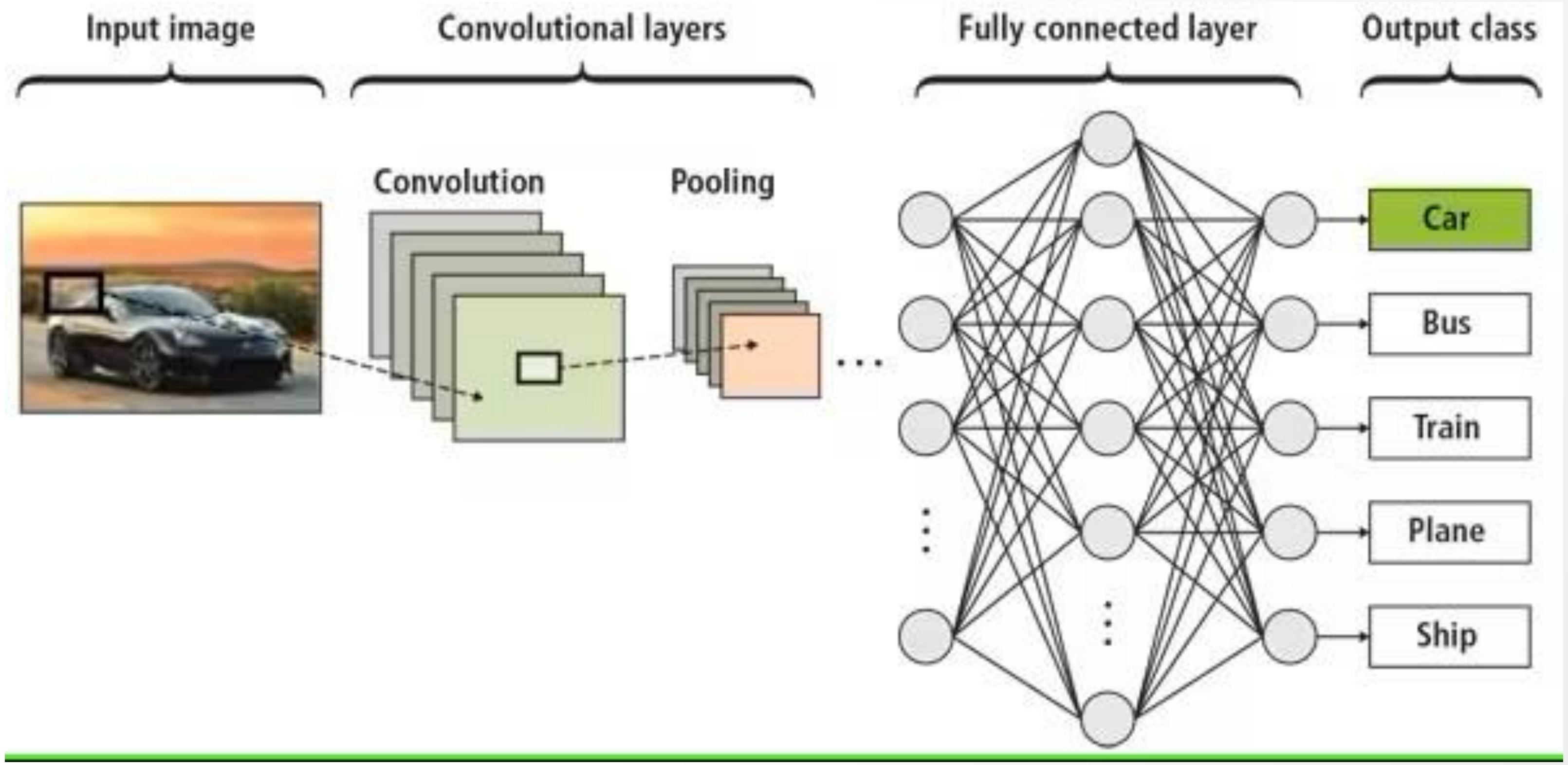
Convolution



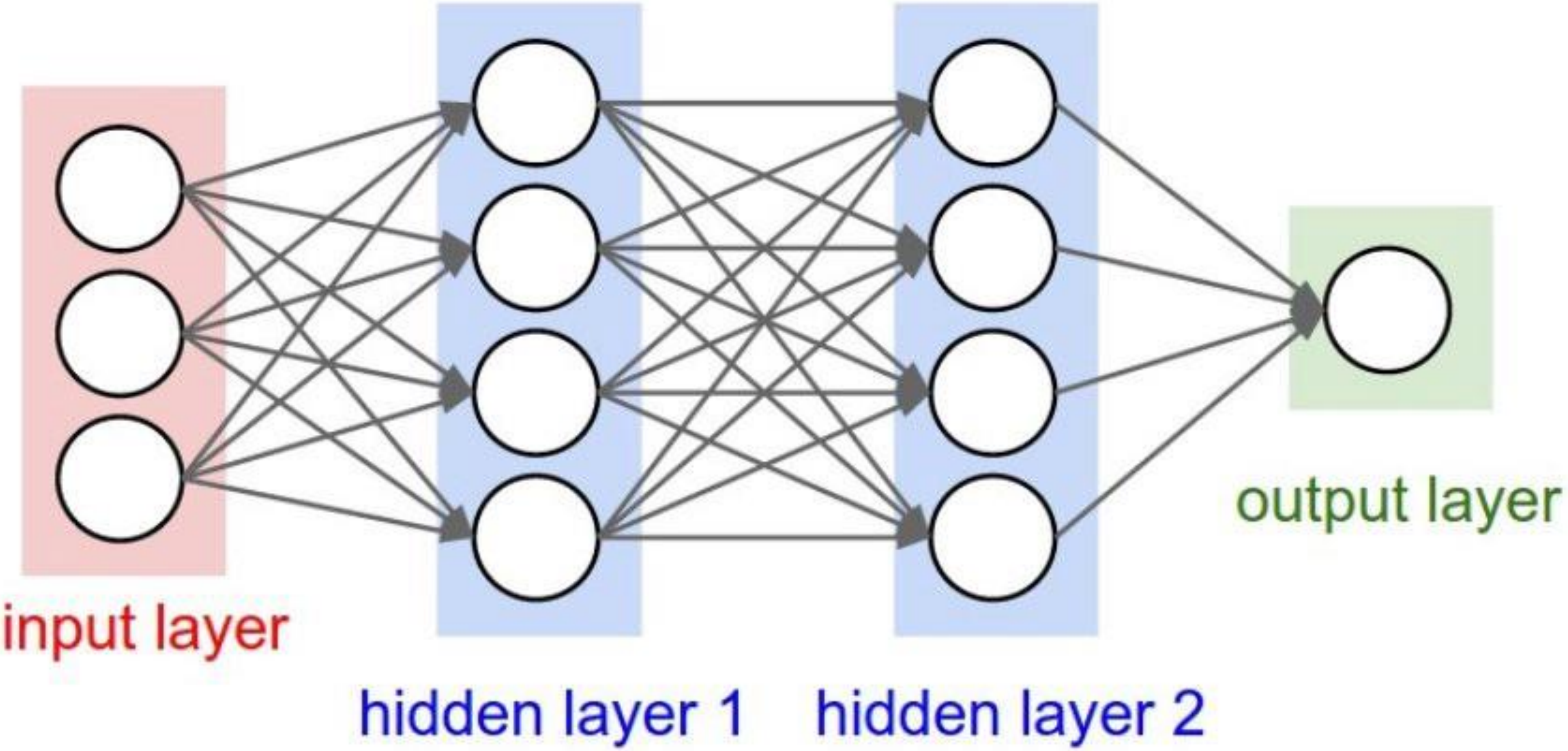
In mathematics, convolution is **a mathematical operation on two functions (f and g) that produces a third function () that expresses how the shape of one is modified by the other**.

The term convolution refers to both the result function and to the process of computing it.

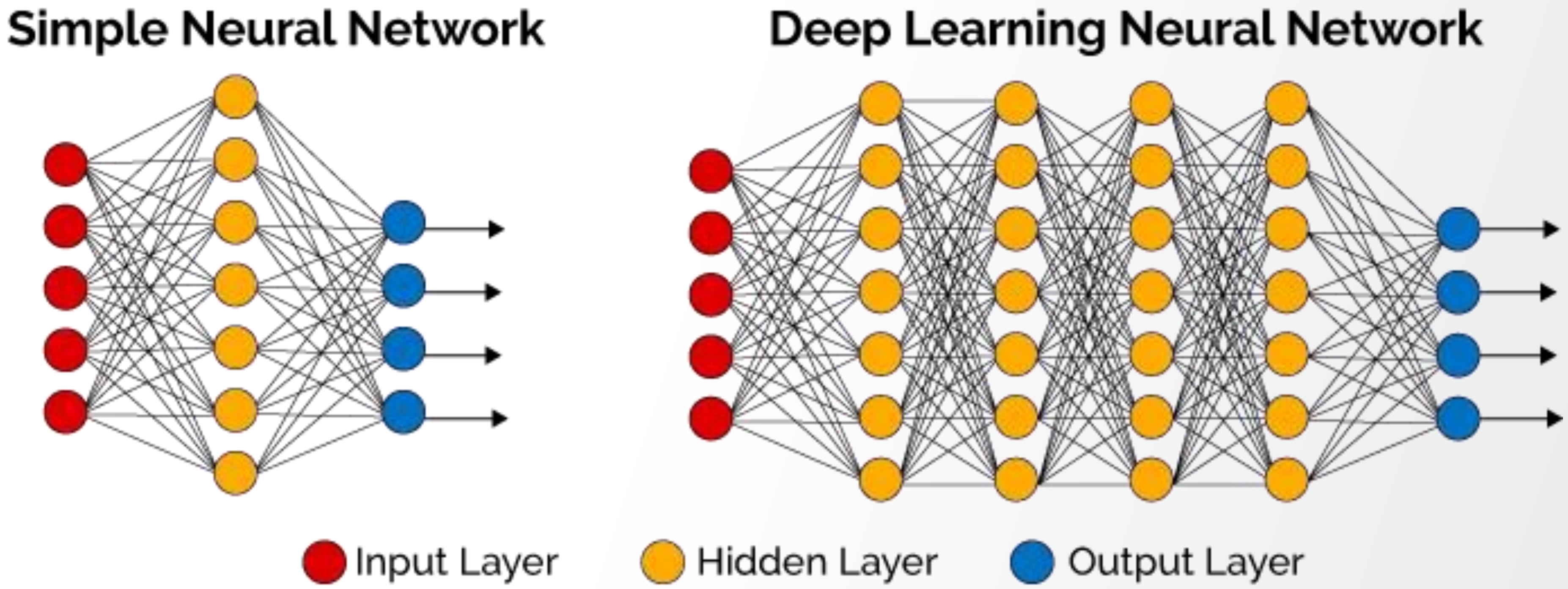
Deep Learning Neural Network



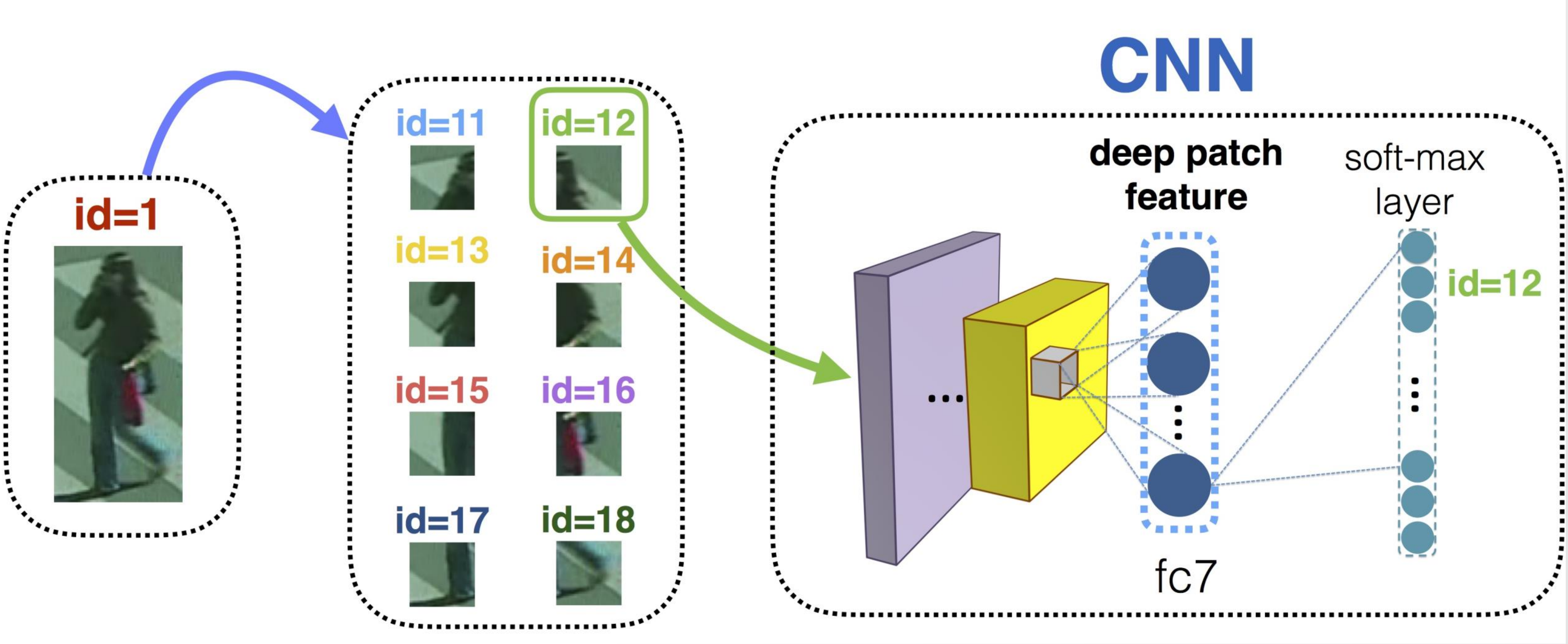
Deep Learning Neural Network



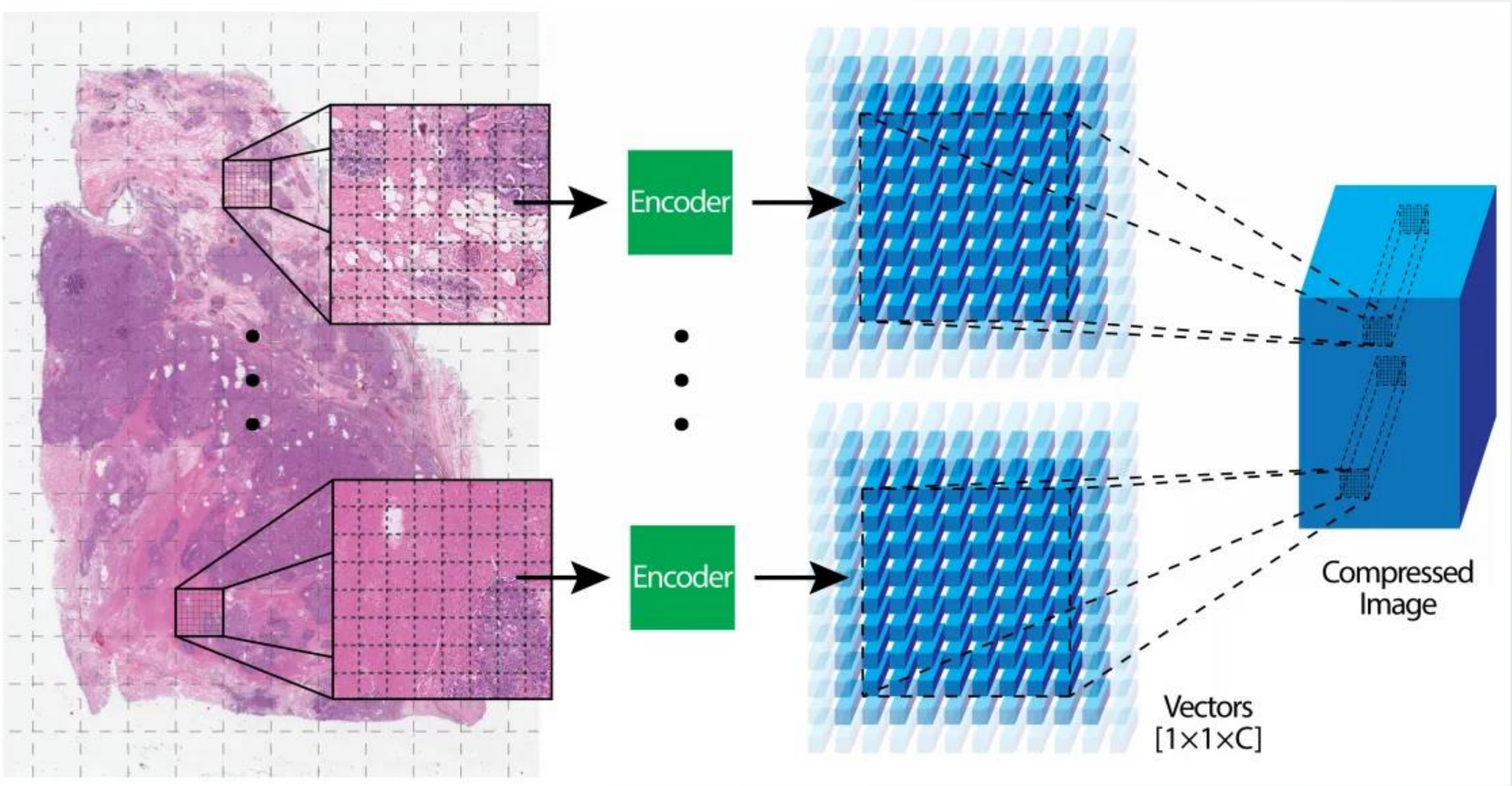
Neural Network



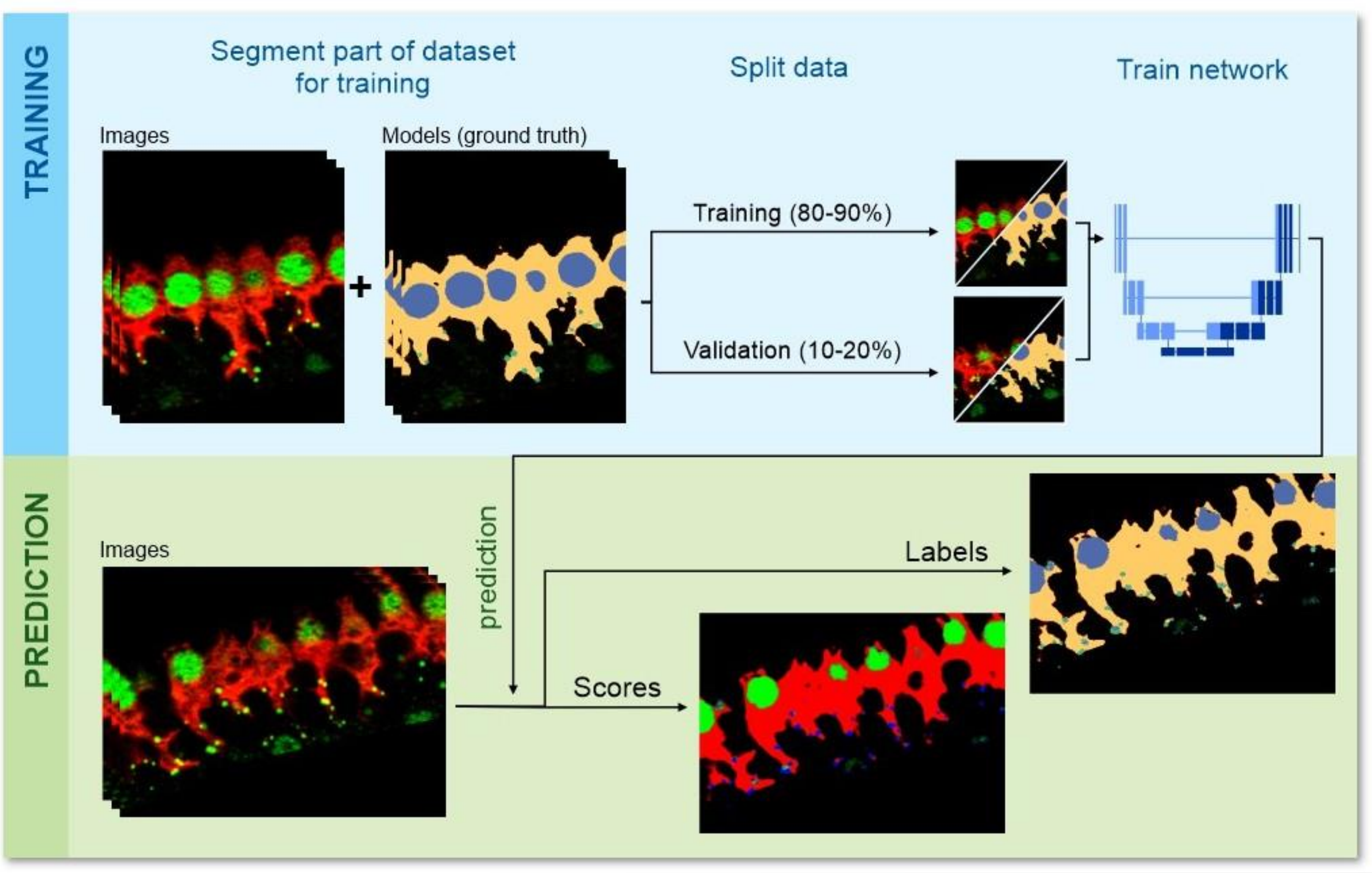
Neural Network



Neural Network

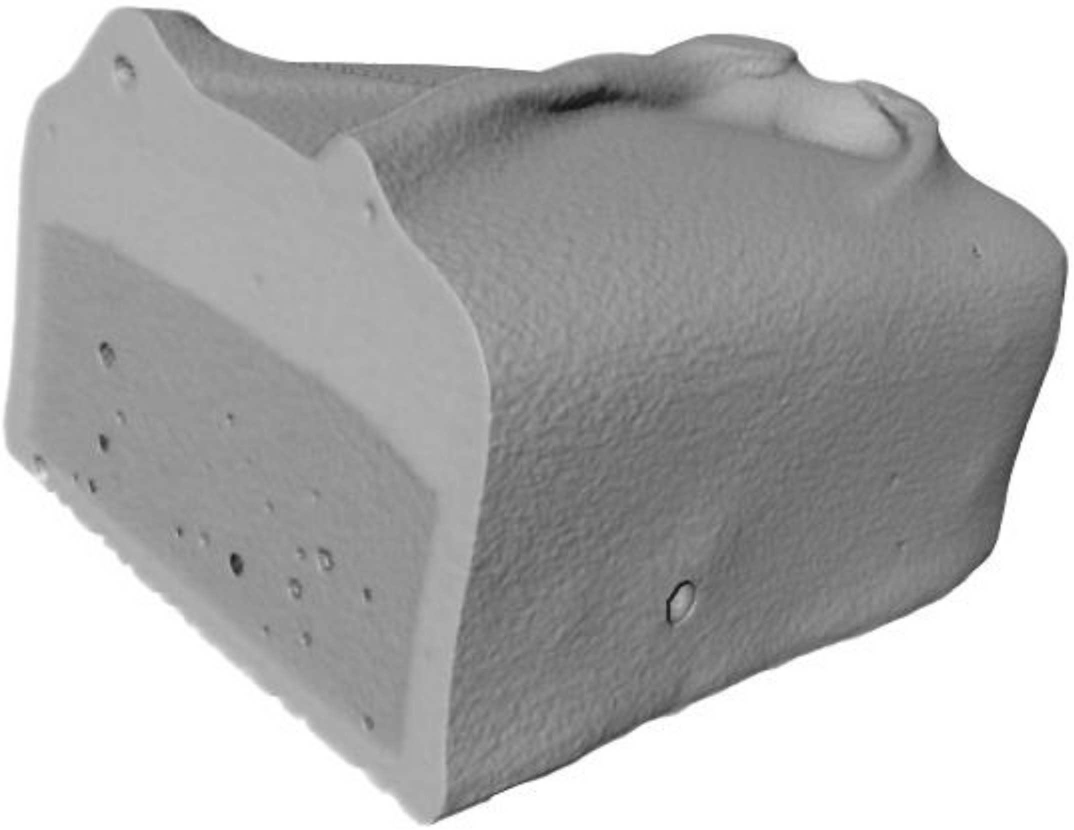


Neural Network



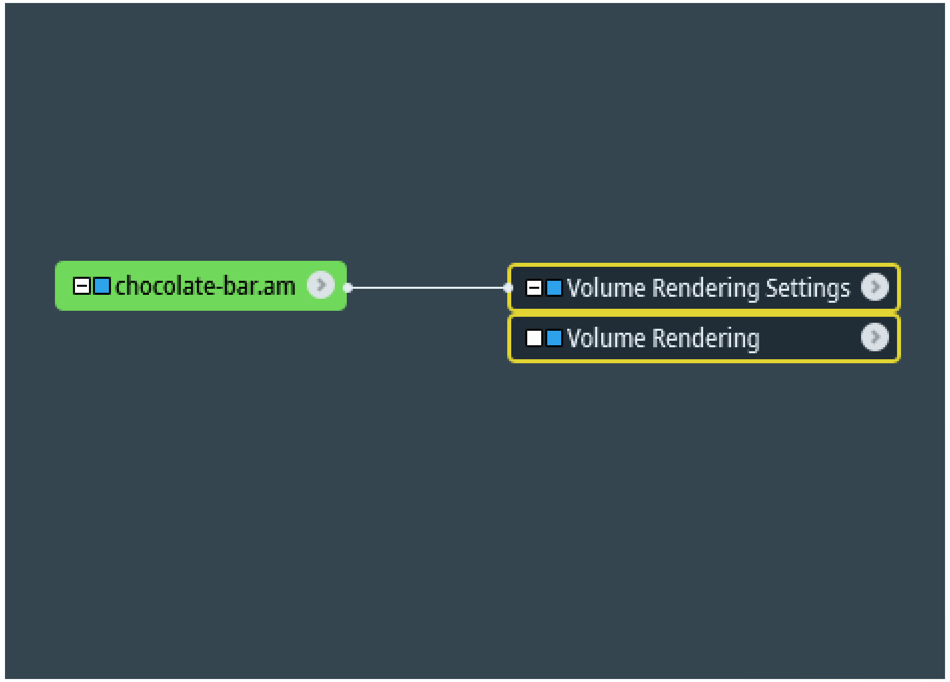
Animation generation

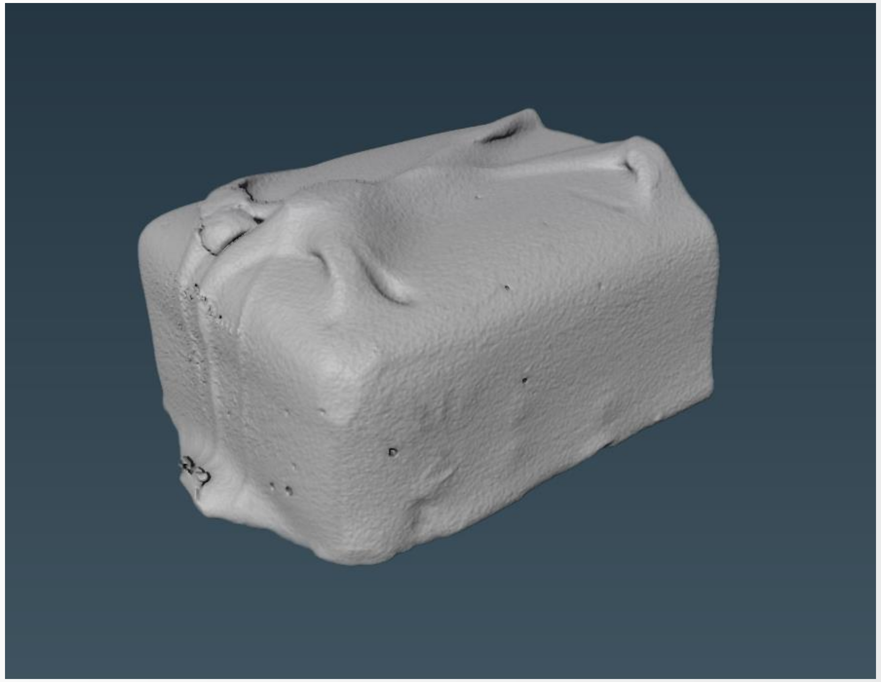
Animation: Camera Path (Rotation)



Animation: Camera Path (rotation)

Open chocolate-bar.am (data > tutorials) then attach Volume Rendering to the dataset.



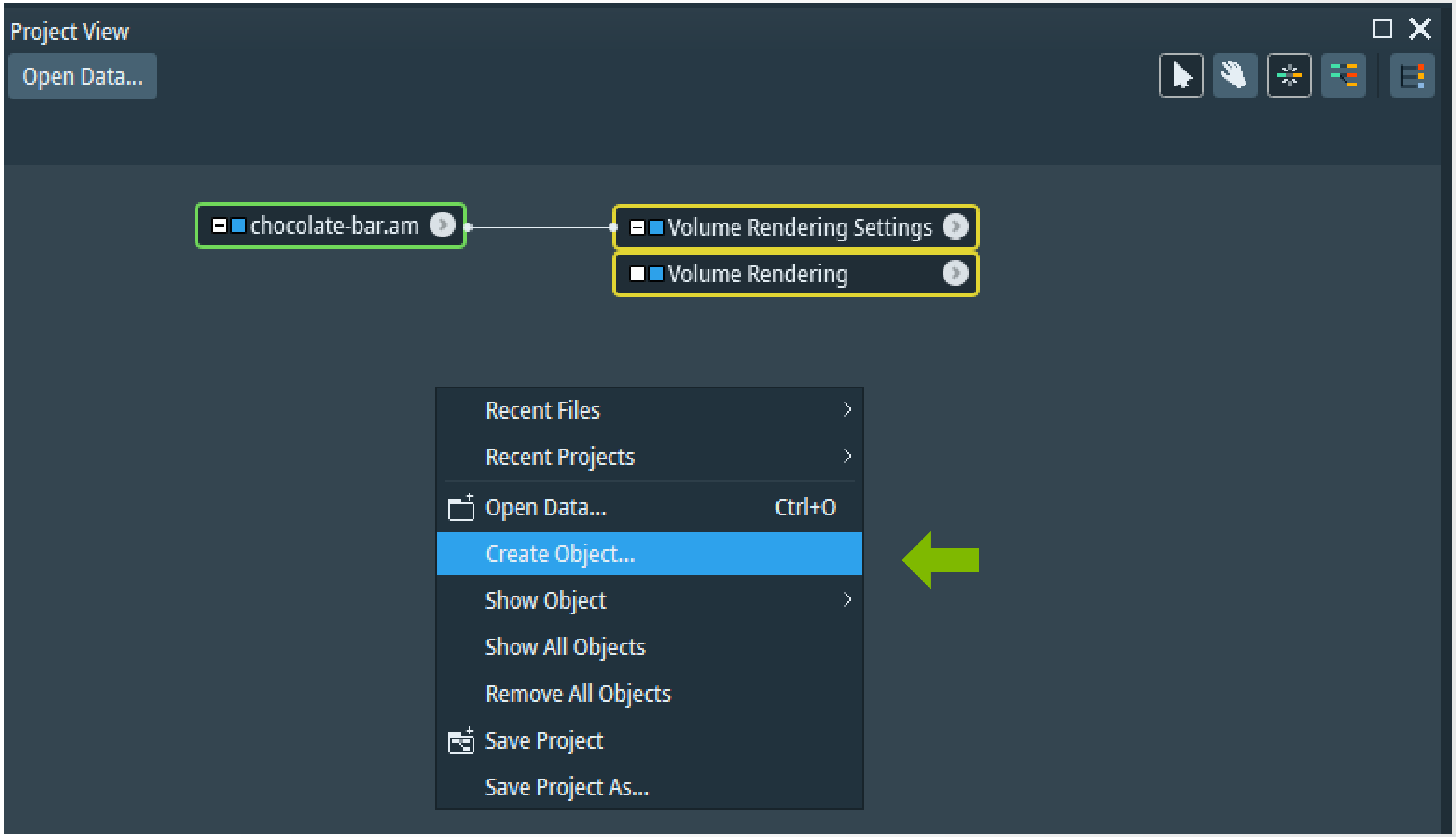


Animation: Camera Path (rotation)

Attach Camera Path

Right-Click anywhere in the project view

- **Create Object**



Animation: Camera Path (rotation)

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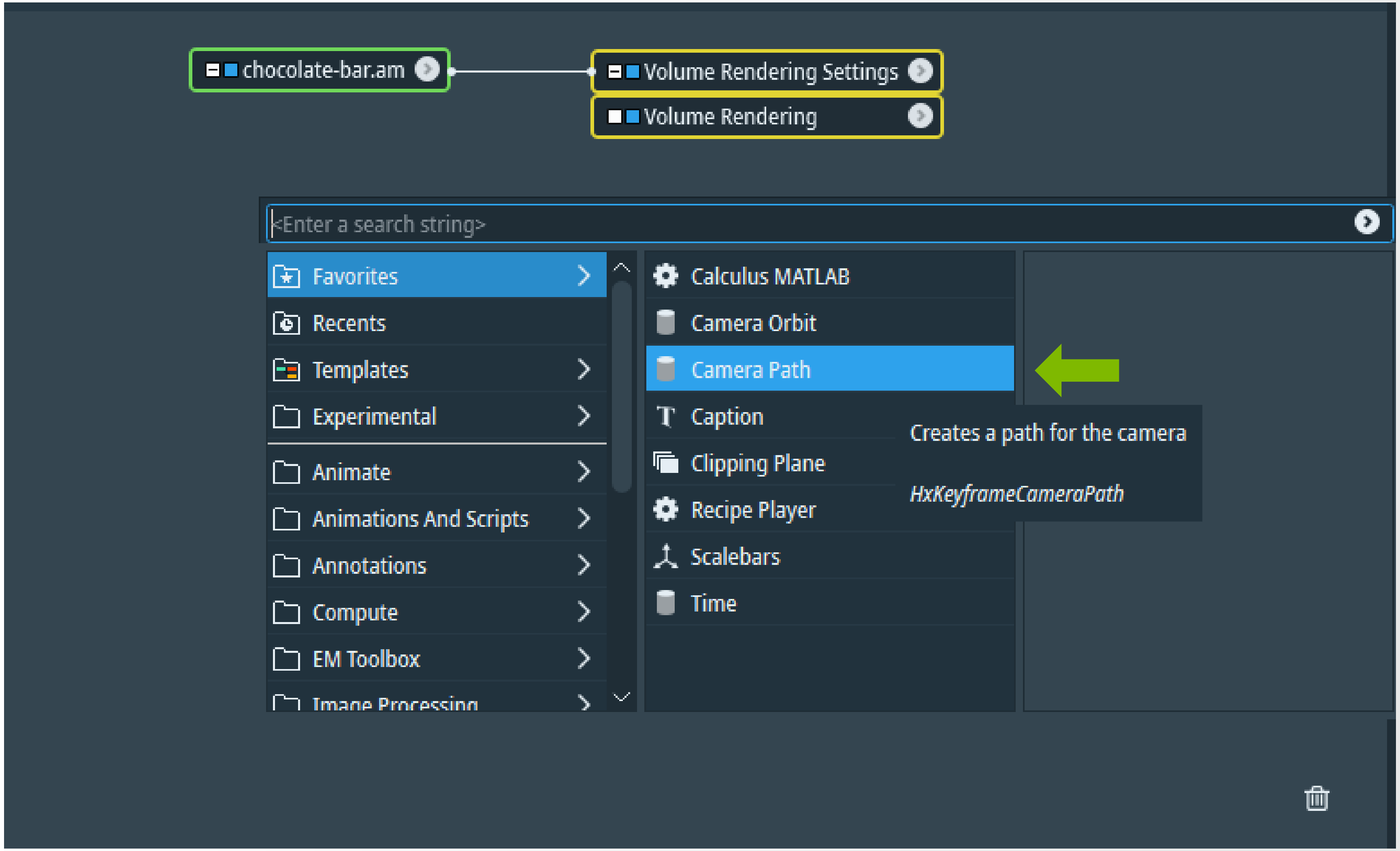
Attach Camera Path

Right-Click anywhere in the project view

- Create Object
- Camera Path

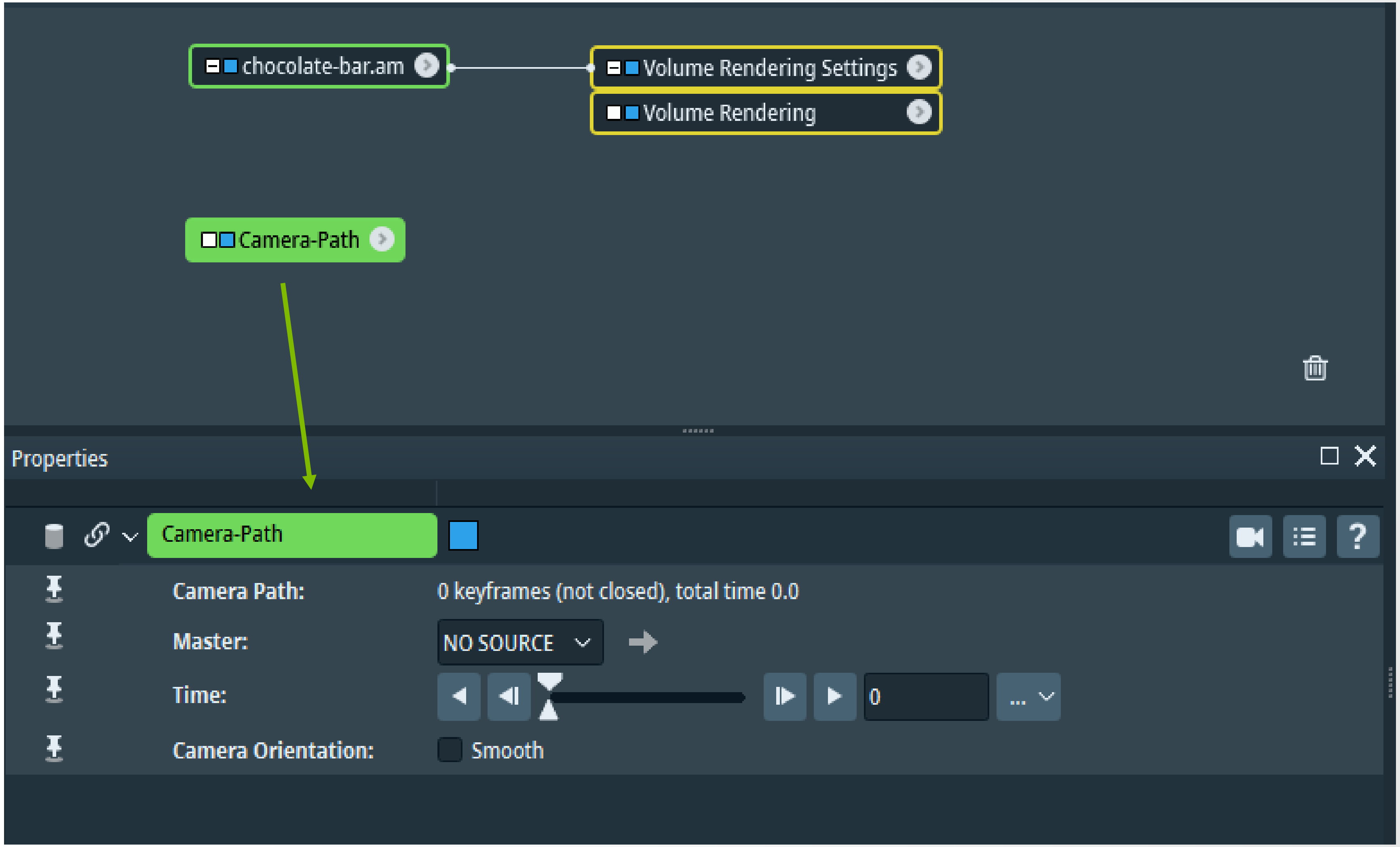


Animation: Camera Path (rotation)

Camera Path

Click on the Camera-Path module

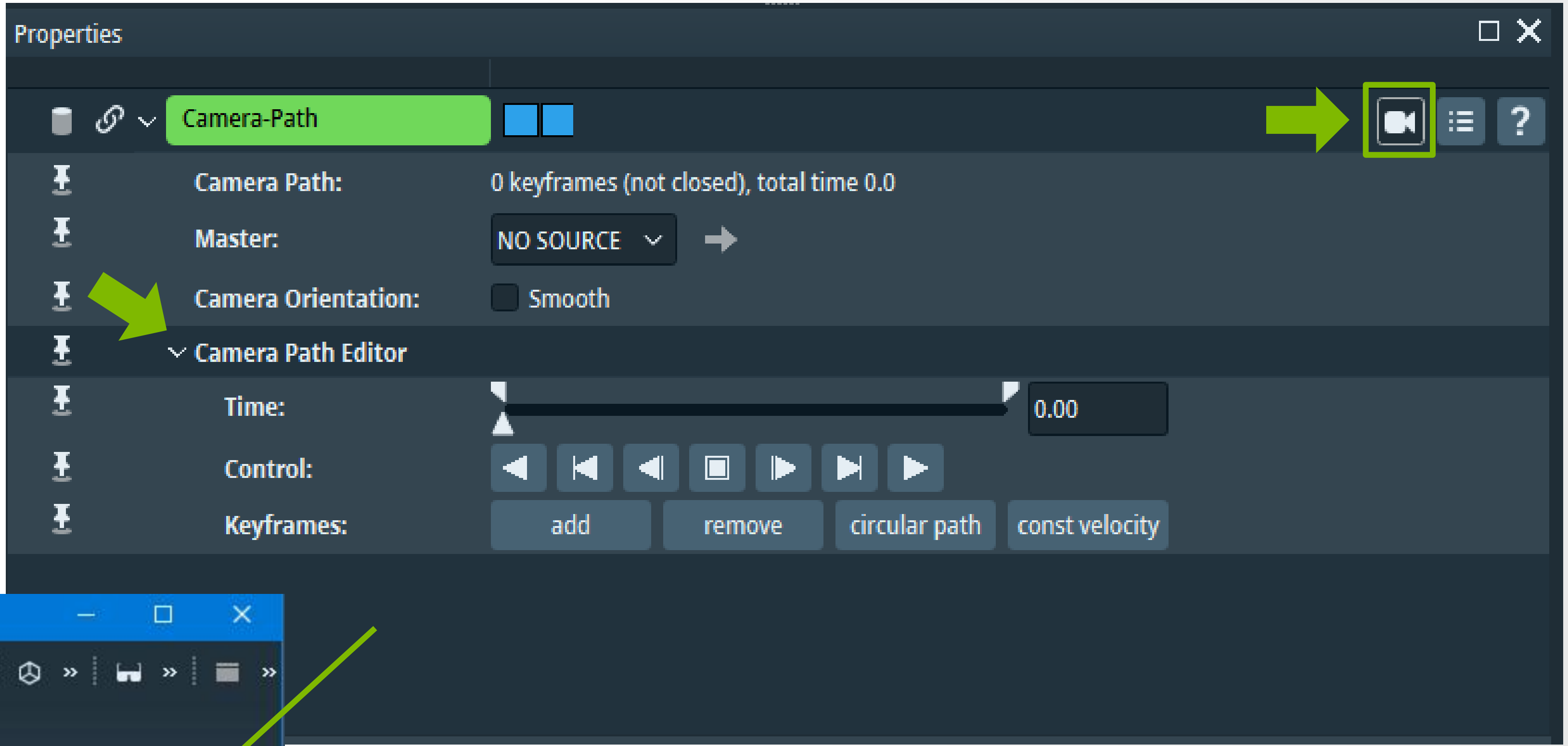
- Properties

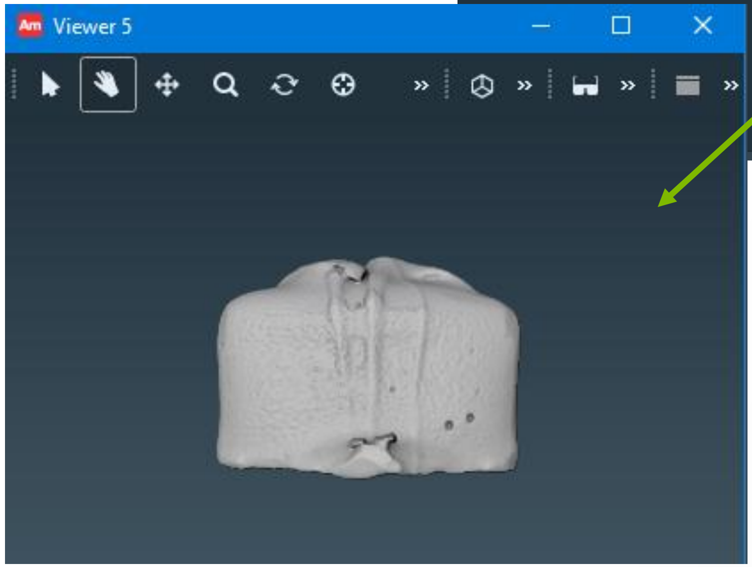


Animation: Camera Path (rotation)

Camera Path

Click Camera Path Editor icon (top-right) to start adding key frames.
A small camera path view will also appear.





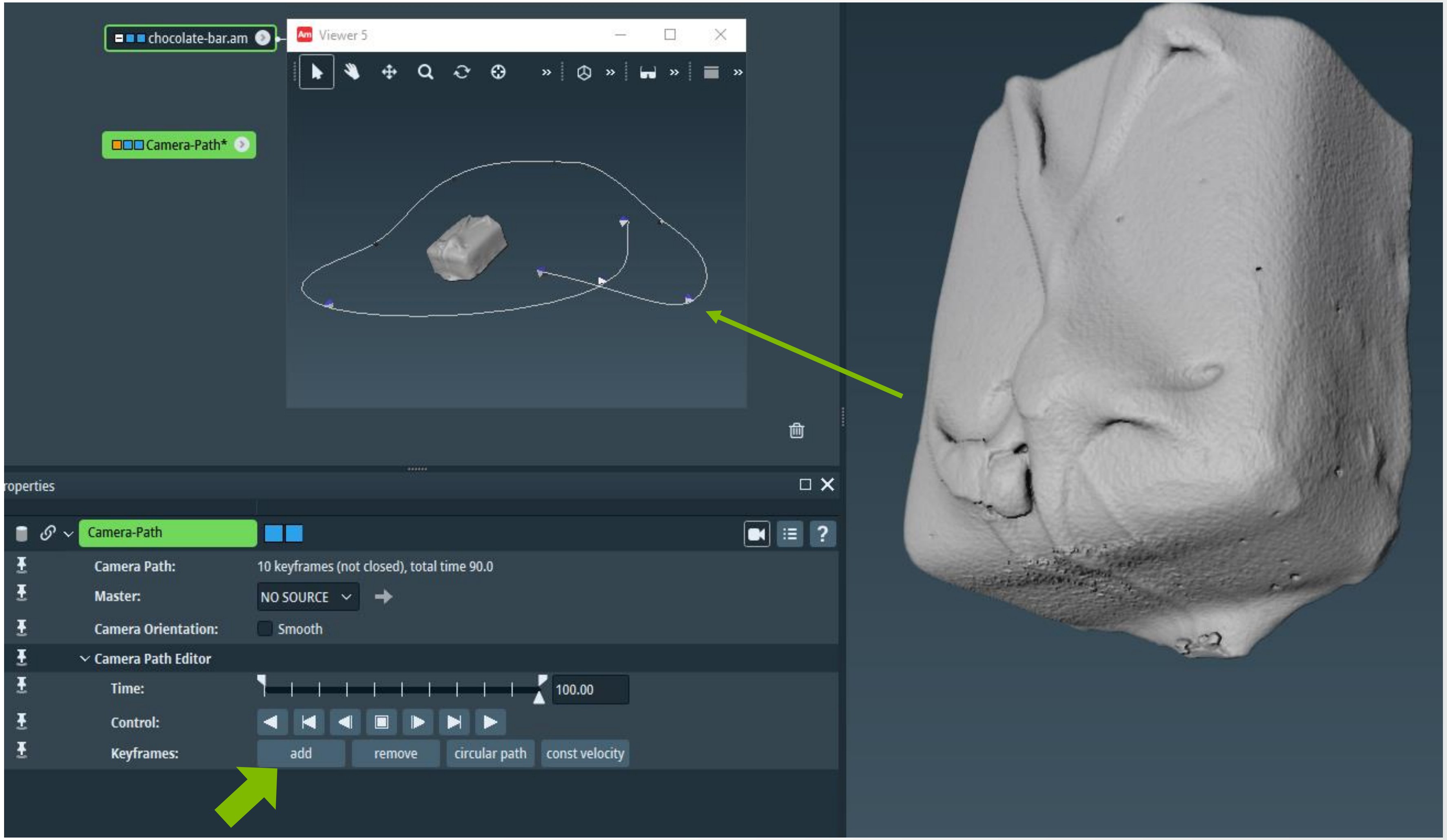
Animation: Camera Path (rotation)

Camera Path

Click Add to start adding key frames

For each key frame;

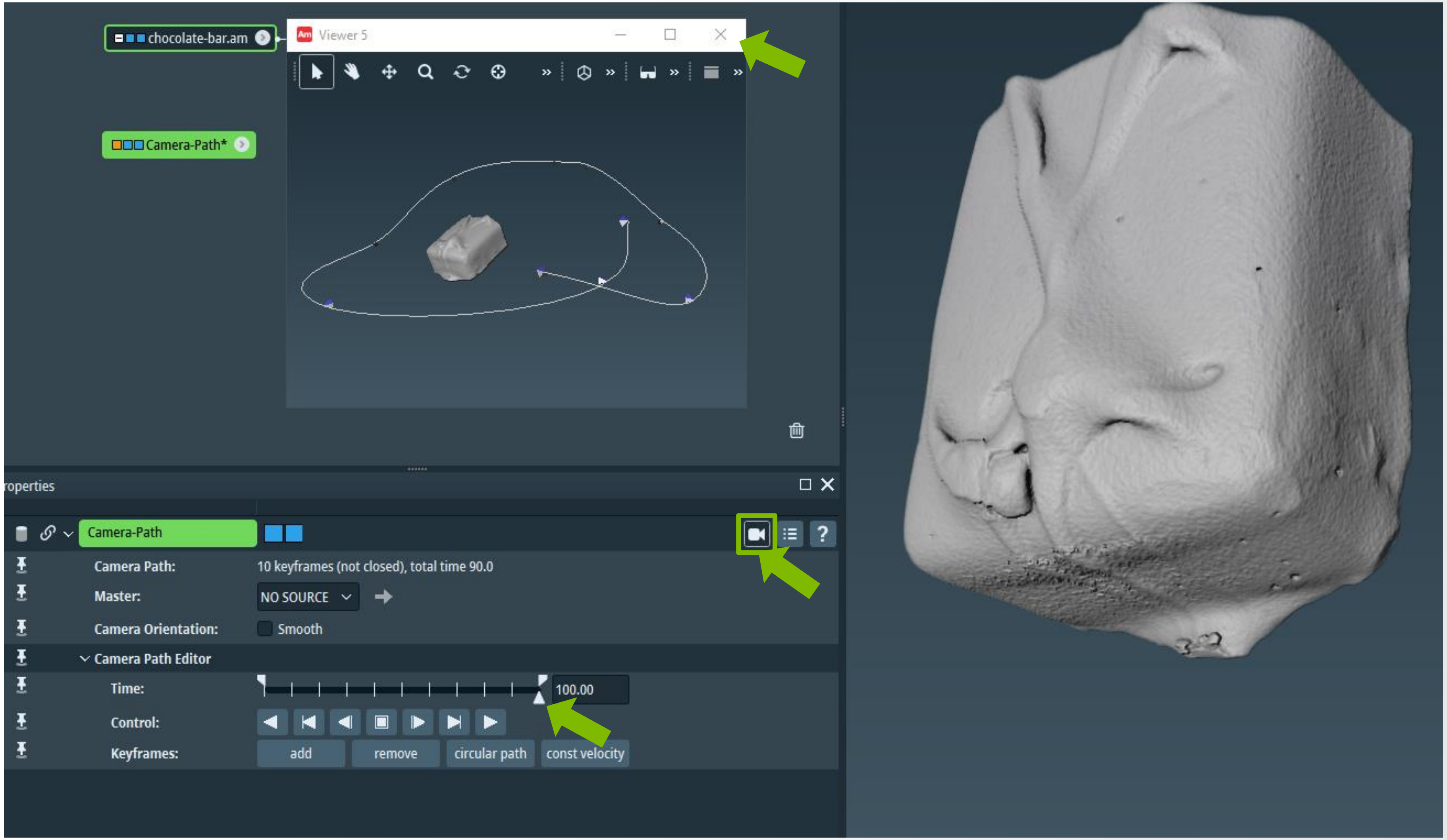
- Turn, rotate, zoom data in or out
- The path of camera rotation will be shown in the small Camera Path Viewer



Animation: Camera Path (rotation)

Camera Path

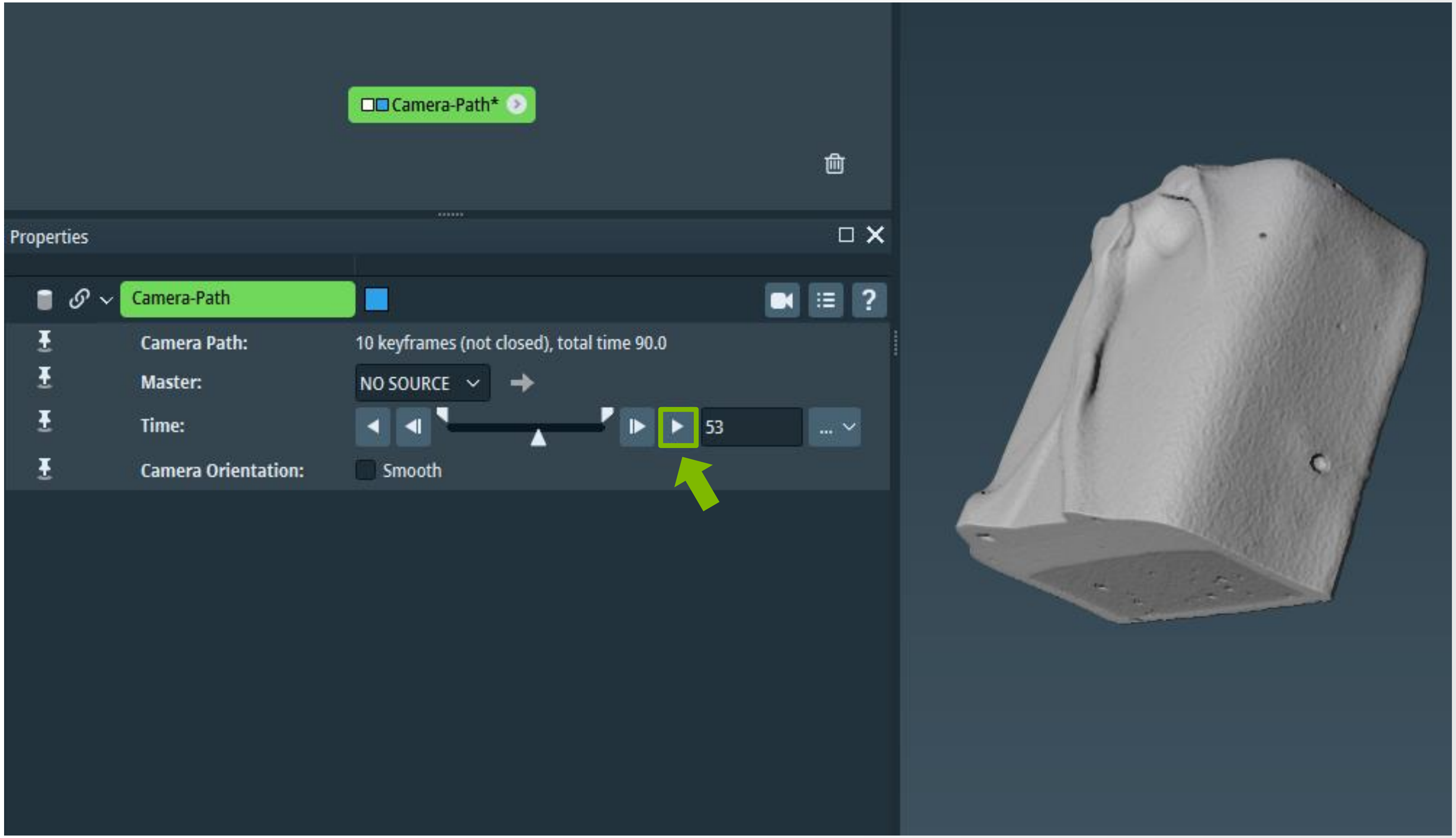
Click the small viewer to close Camera Path Editor when finish with key frames
(or click at the Camera Editor icon one more time).



Animation: Camera Path (rotation)

Camera Path

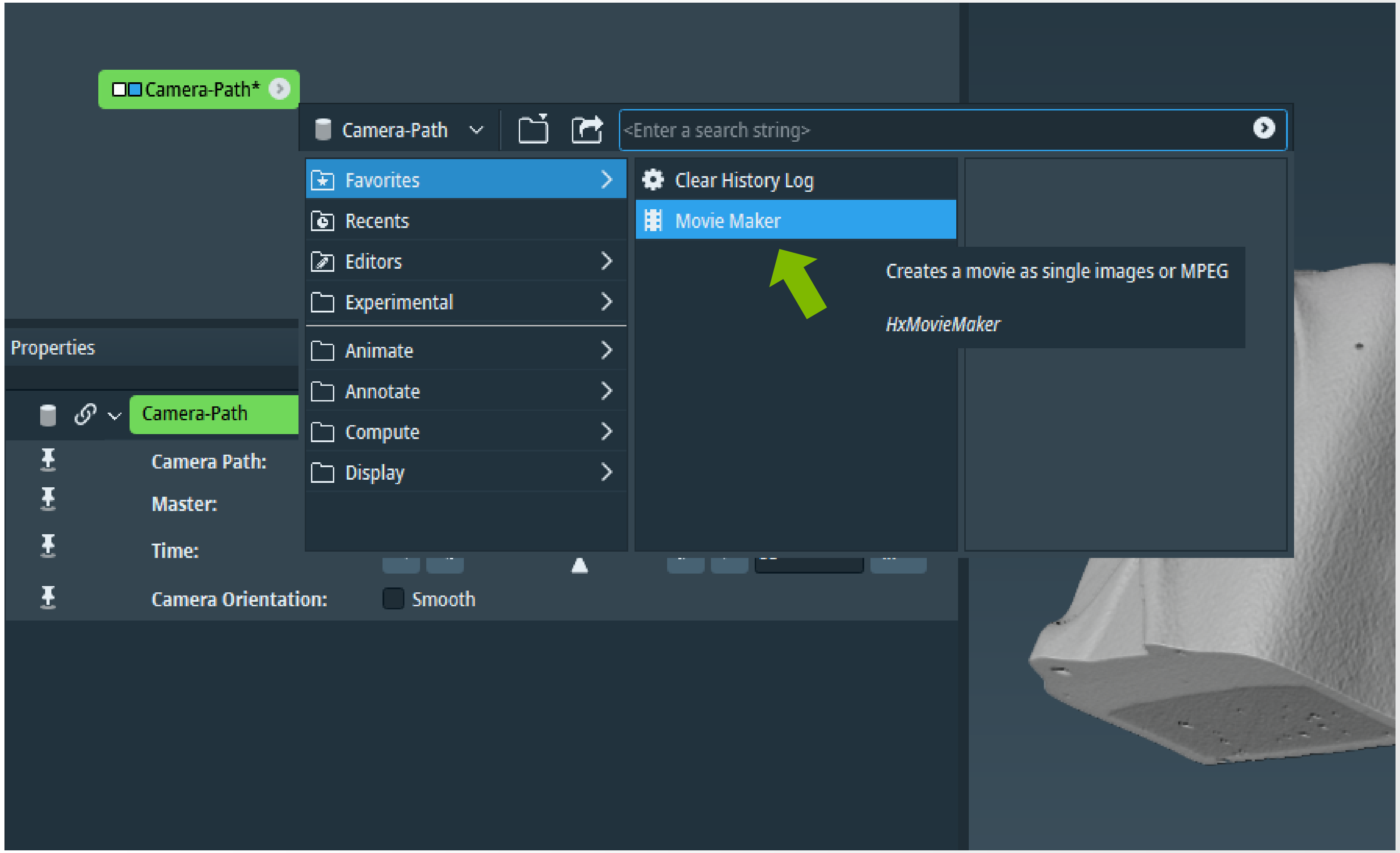
Click Play button to preview the animation



Animation: Camera Path (rotation)

Making movie

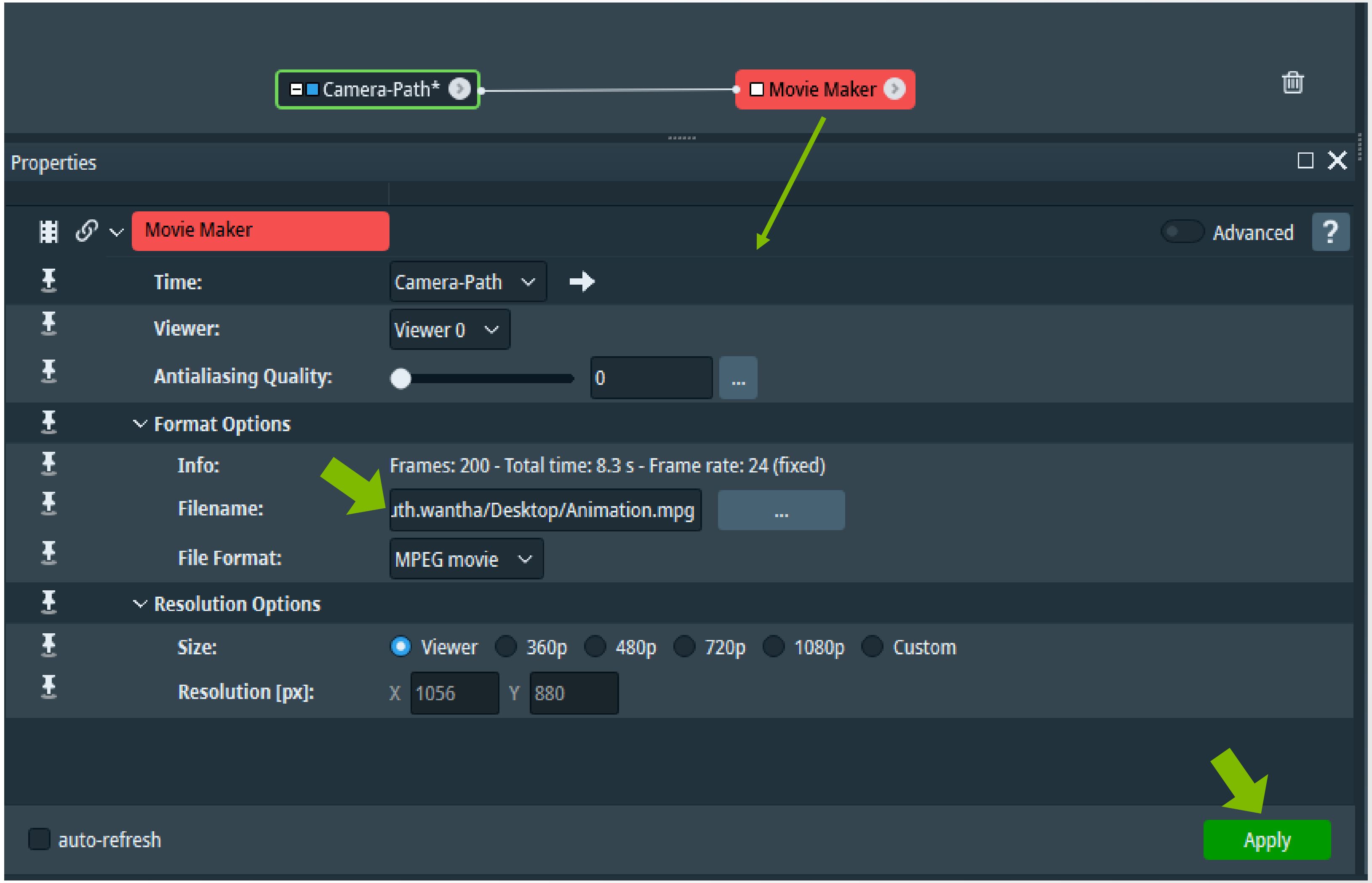
Right click at Camera-Path module to attach Movie Maker module



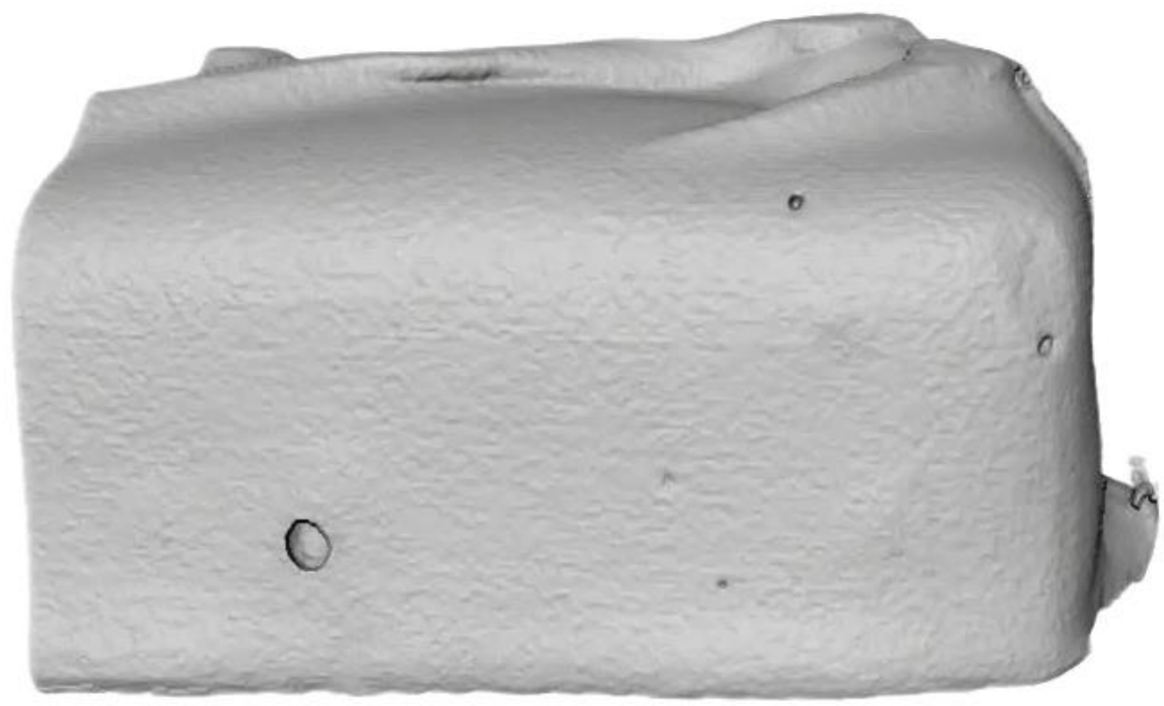
Animation: Camera Path (rotation)

Movie Maker

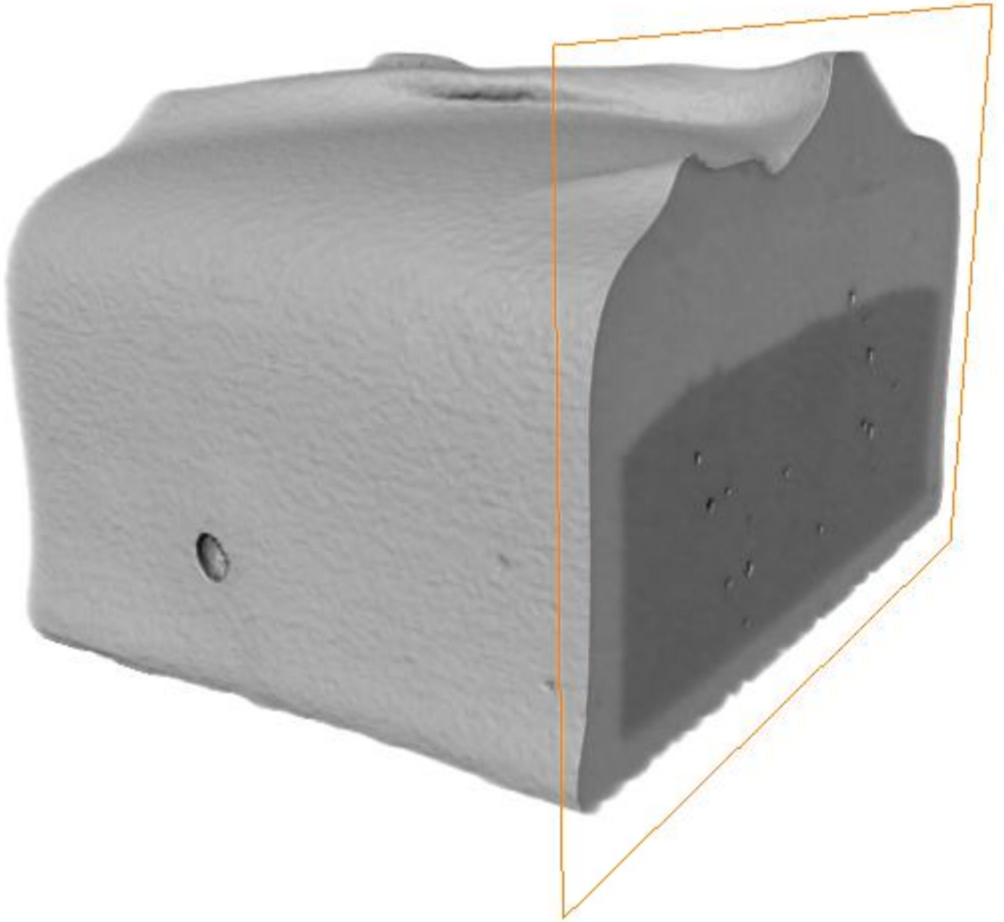
Set the parameters (**Filename** field is **mandatory**) then click Apply to create a movie file.



Animation: Camera Path (rotation) movie example



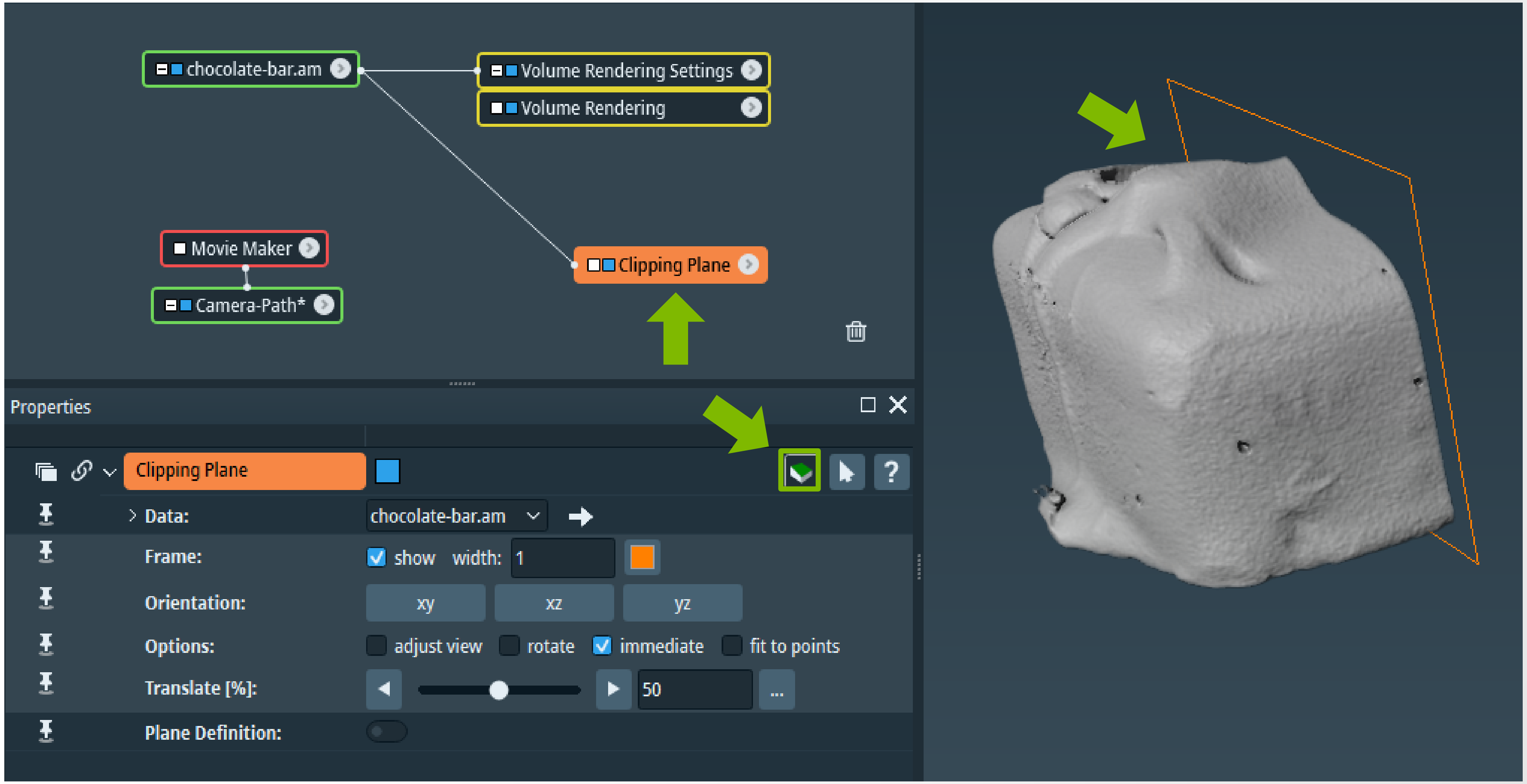
Animation Workroom: Animation Director



Animation Workroom: Animation Director

Animation Director

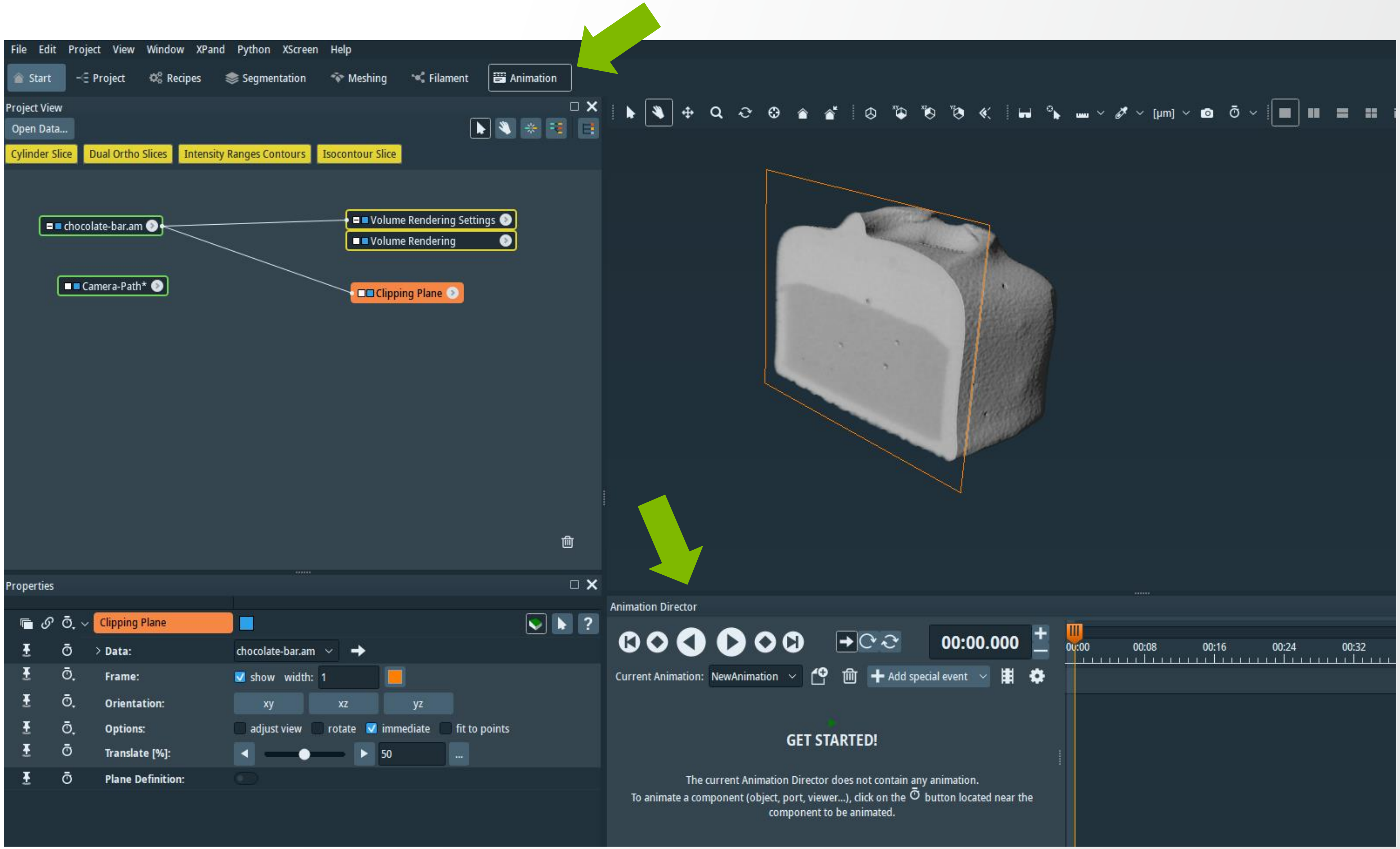
Attach Clipping Plane to the dataset and "clip"



Animation Workroom: Animation Director

Animation Director

Then activate the Animation workroom. The Animation Director will appear.

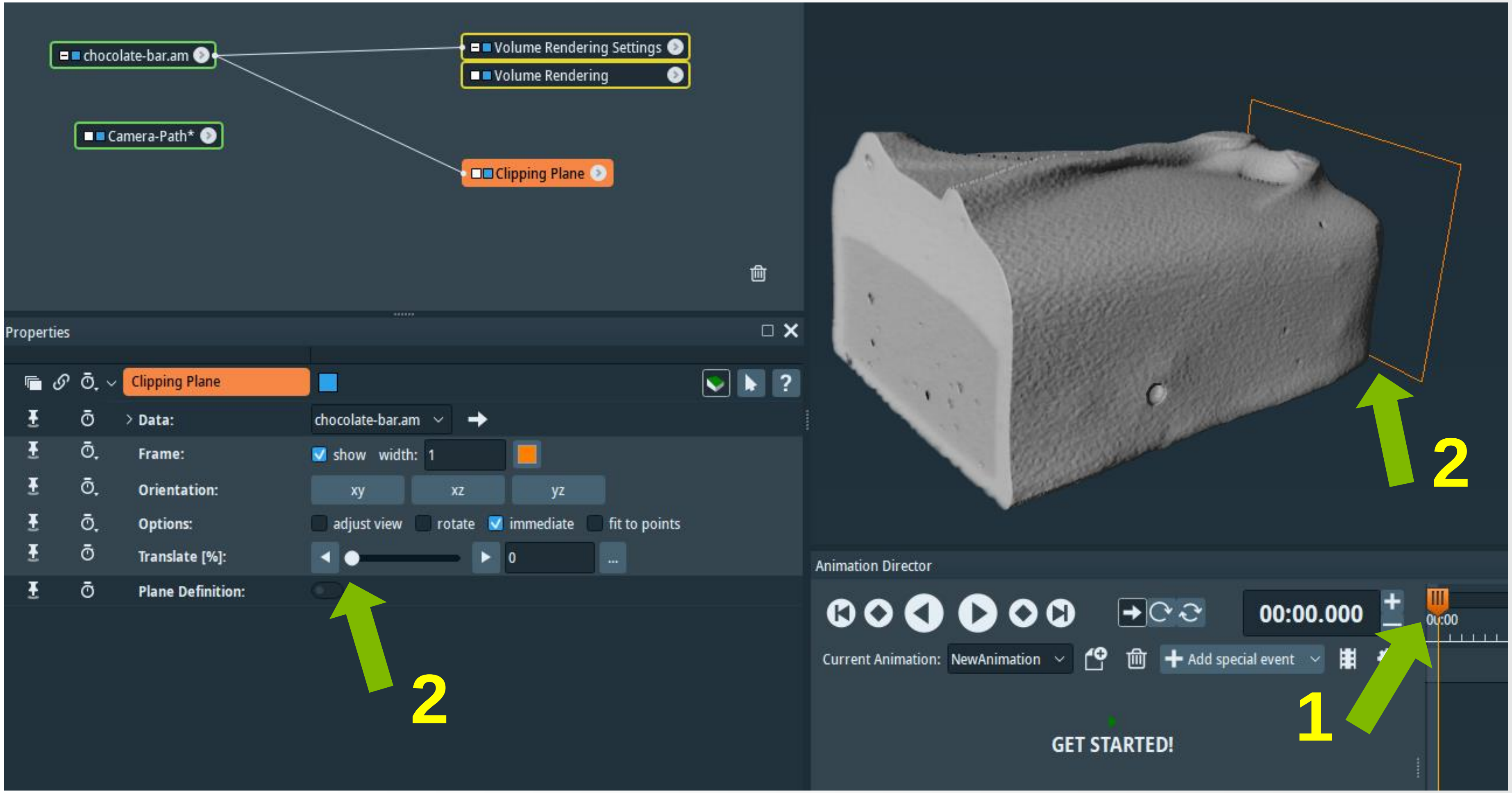


Animation Workroom: Animation Director

Translation Animation

1. Start with the Timeline at 00:00:000

2. In Clipping Plane Properties translate to 0%.
Pay attention to the clipping direction: full volume should be visible at 0% (Translate).



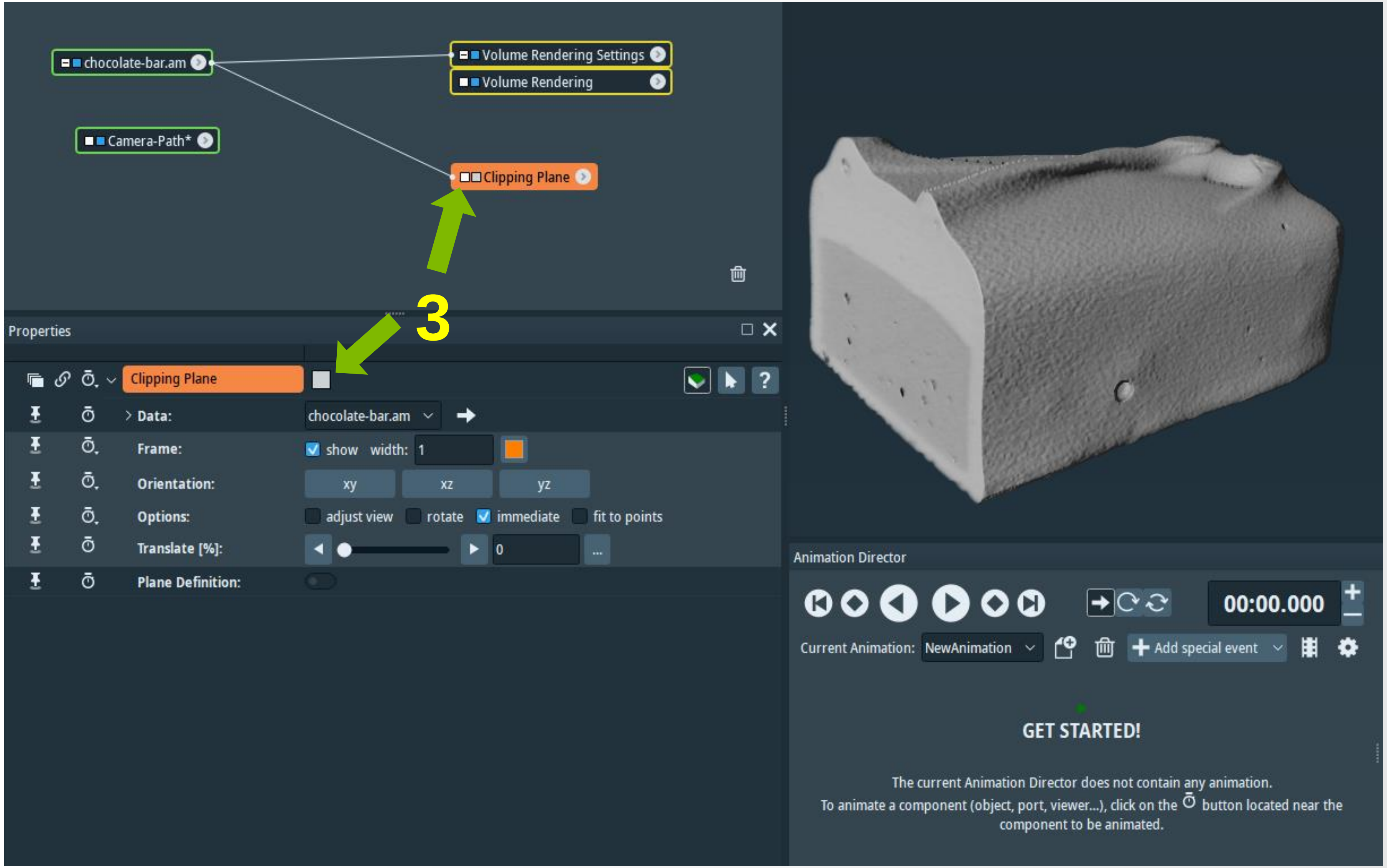
Animation Workroom: Animation Director

Translation Animation

1. Start with the Timeline at 00:00:000

2. In Clipping Plane Properties. Translate to 0%

3. Hide the Clipping Plane bounding box.



Animation Workroom: Animation Director

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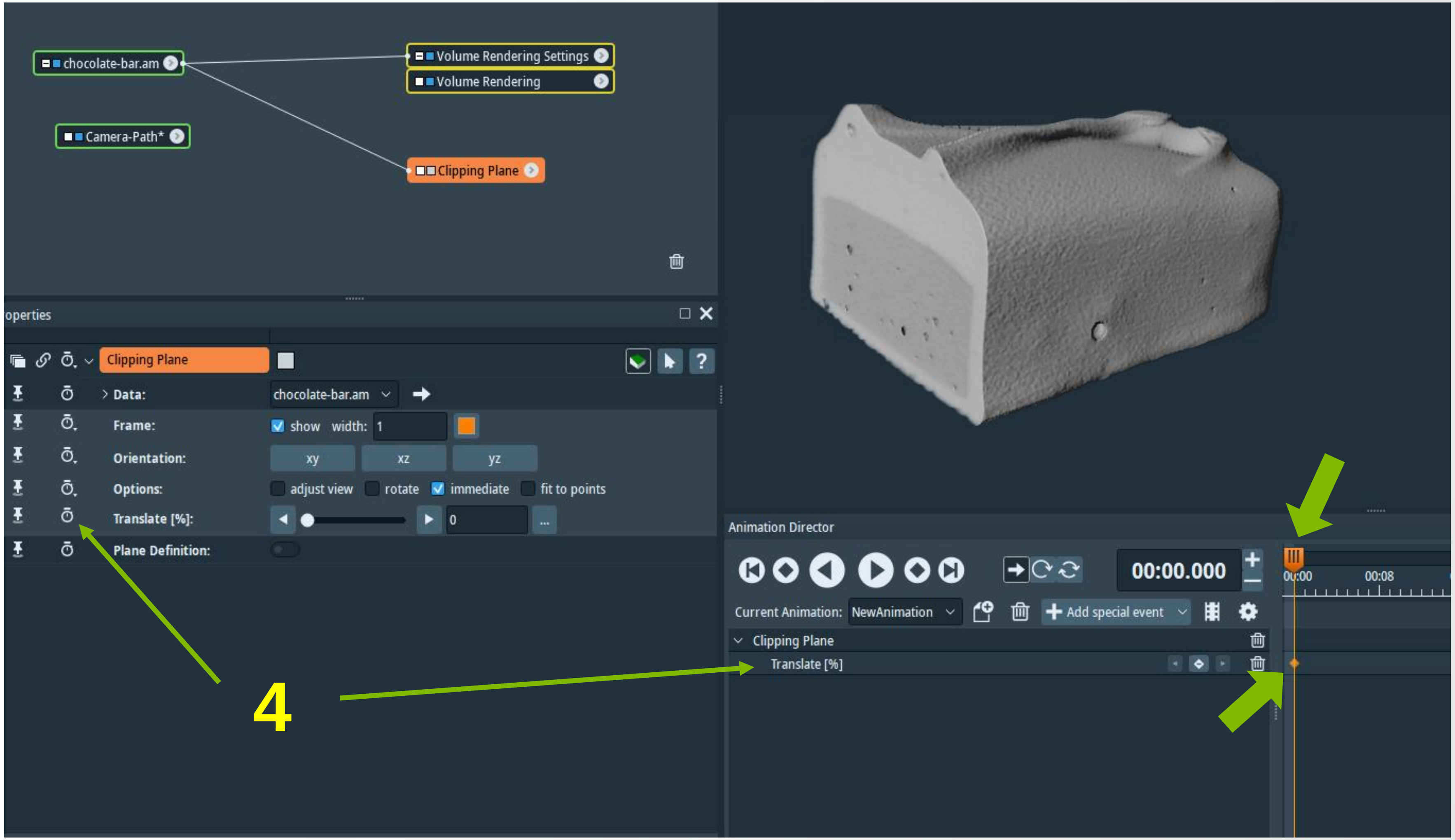
Translation Animation

4. Click the Stopwatch button next to Translate (%) in Clipping Plane properties to start adding the first key frame (Translation) to the timeline at 00:00:000.



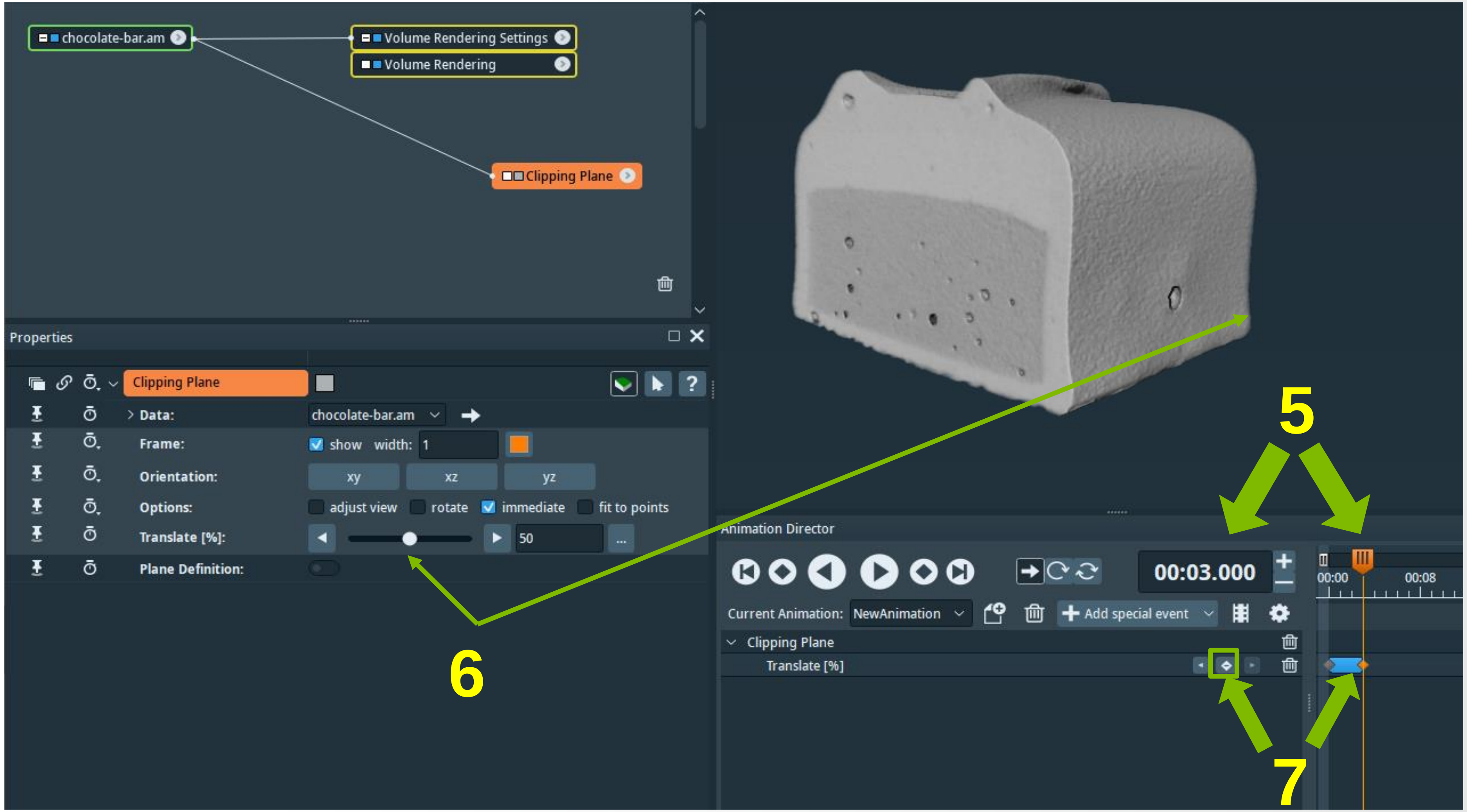
Animation Workroom: Animation Director

Translation Animation

5. Move the timeline bar to 00:03:000

6. Translate to 50 %

7. Add a key frame



Animation Workroom: Animation Director

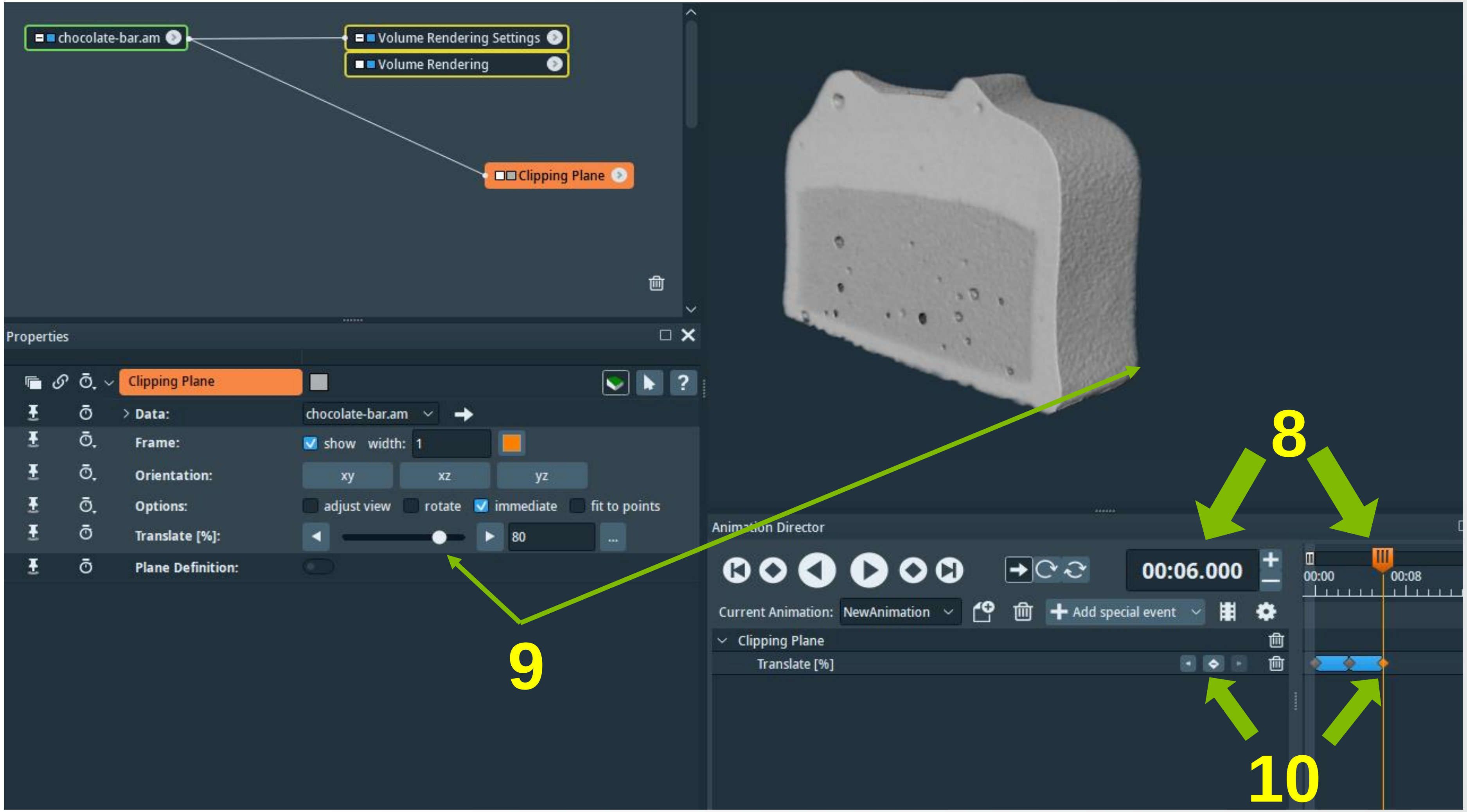
Translation Animation

Repeat the steps

8.Move the timeline bar to 00:06:000

9. Translate to 80 %

10. Add a key frame



Animation Workroom: Animation Director

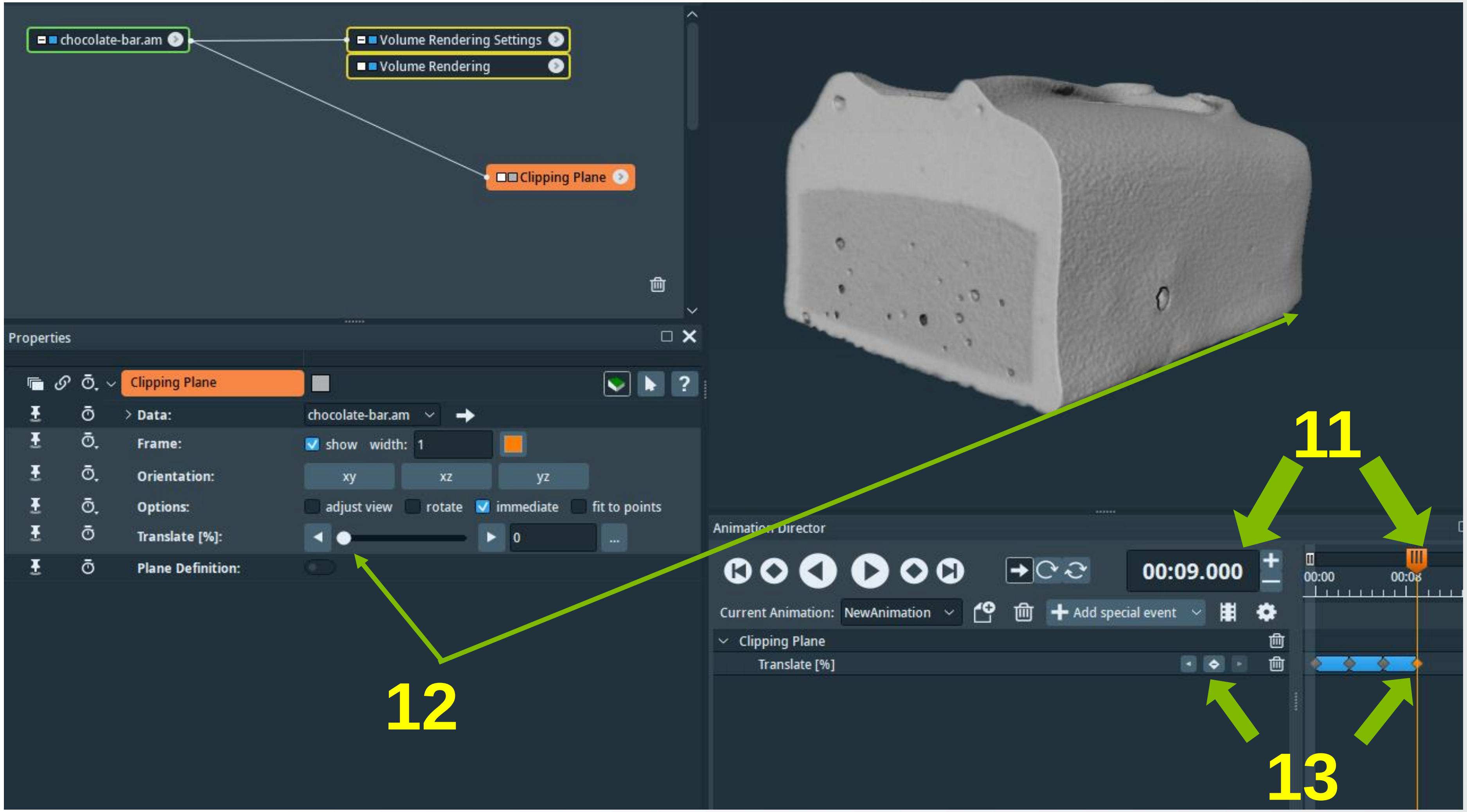
Translation Animation

Repeat the steps

11. Move the timeline bar to 00:09:000

12. Translate to 0 %

13. Add a key frame



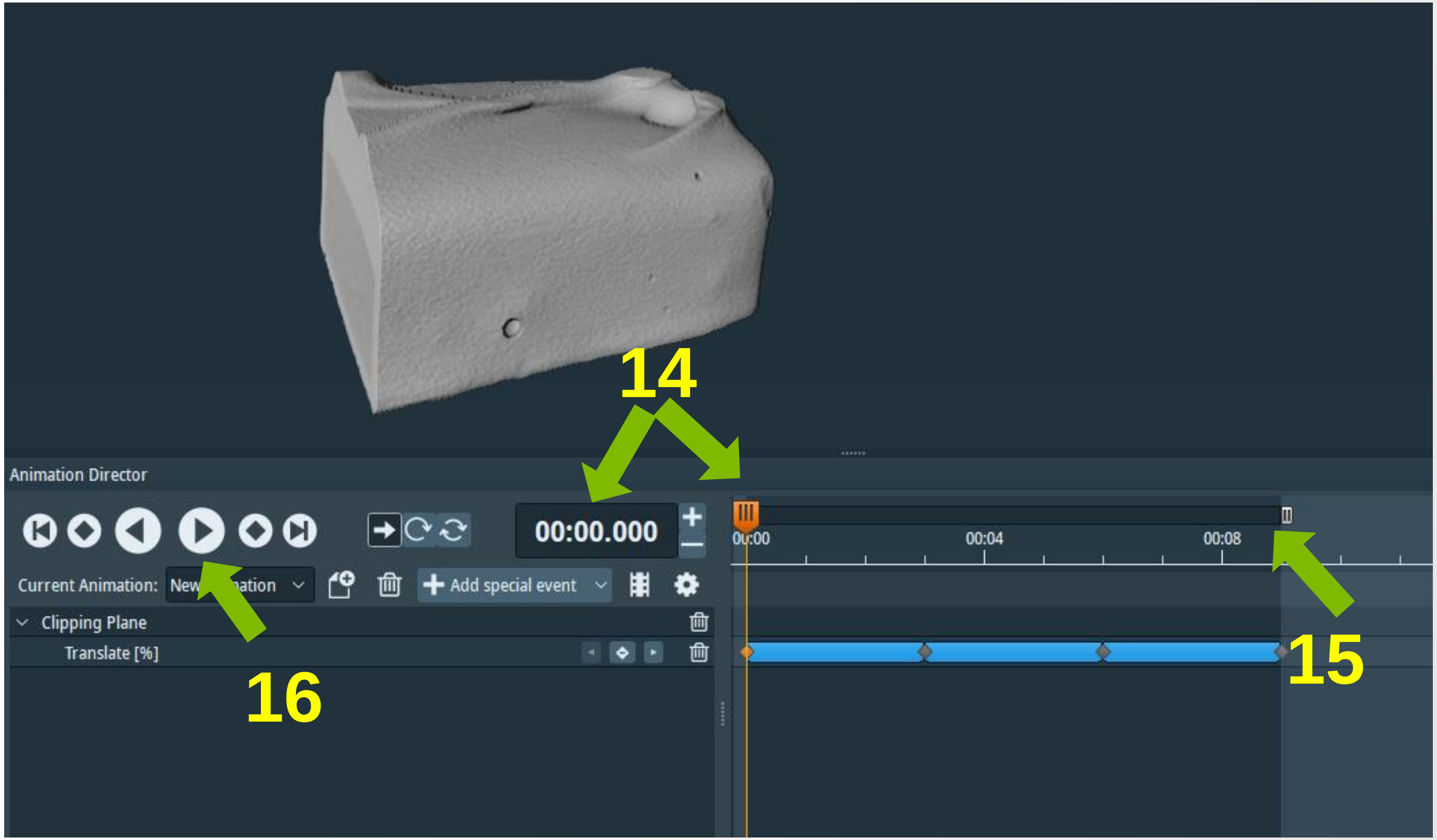
Animation Workroom: Animation Director

Translation Animation

14. When done, bring timeline bar to 00:00:000

15. Set the limit of the animation at 00:09:000

16. Click Play button in Animation Director to preview the animation.



Animation Workroom: Animation Director

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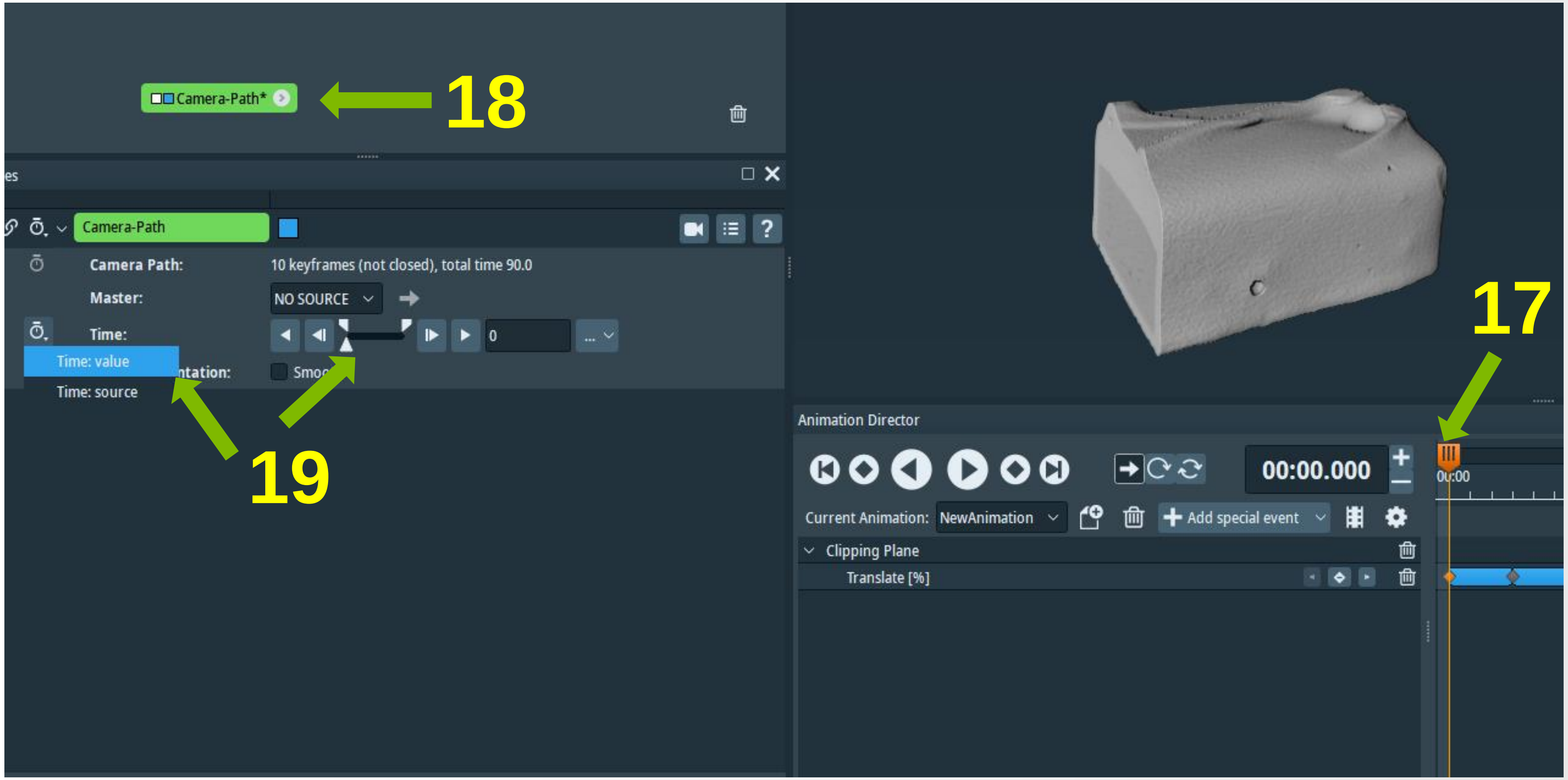
Translation + Rotation Animation

17. At time 00:00:000

18.Click on Camera-Path module (with previously created rotation key frames).

19. Make sure to have Time 0 in Camera Path properties port before clicking on Stopwatch button next to Time.

Select Time value to add to Animation Workroom.



Animation Workroom: Animation Director

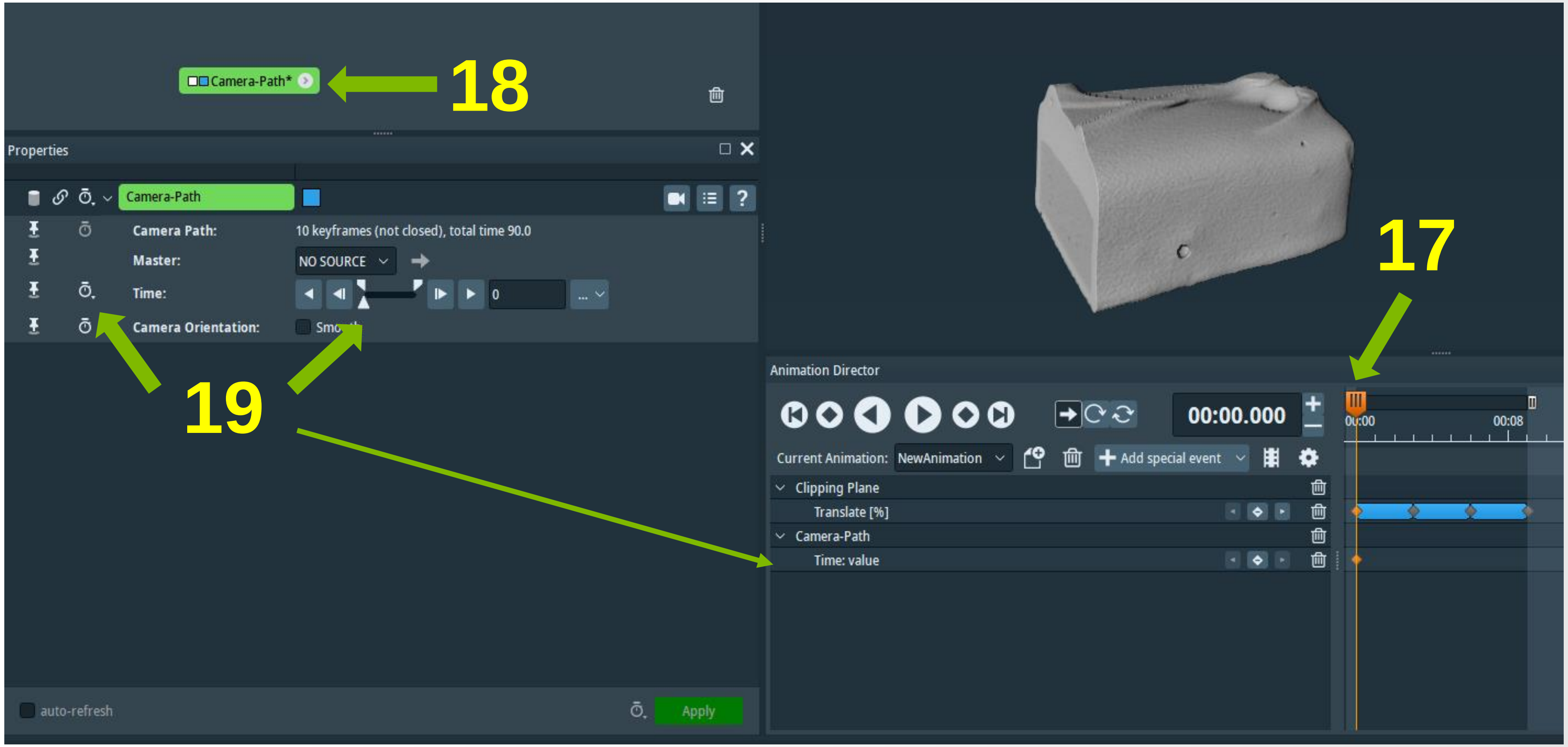
Translation + Rotation Animation

17. At time 00:00:000

18.Click at Camera-Path module (with previously created rotation key frames).

19. Make sure to have Time 0 in Camera Path properties port before clicking on Stopwatch button next to Time.

Select Time value to add to Animation Workroom.



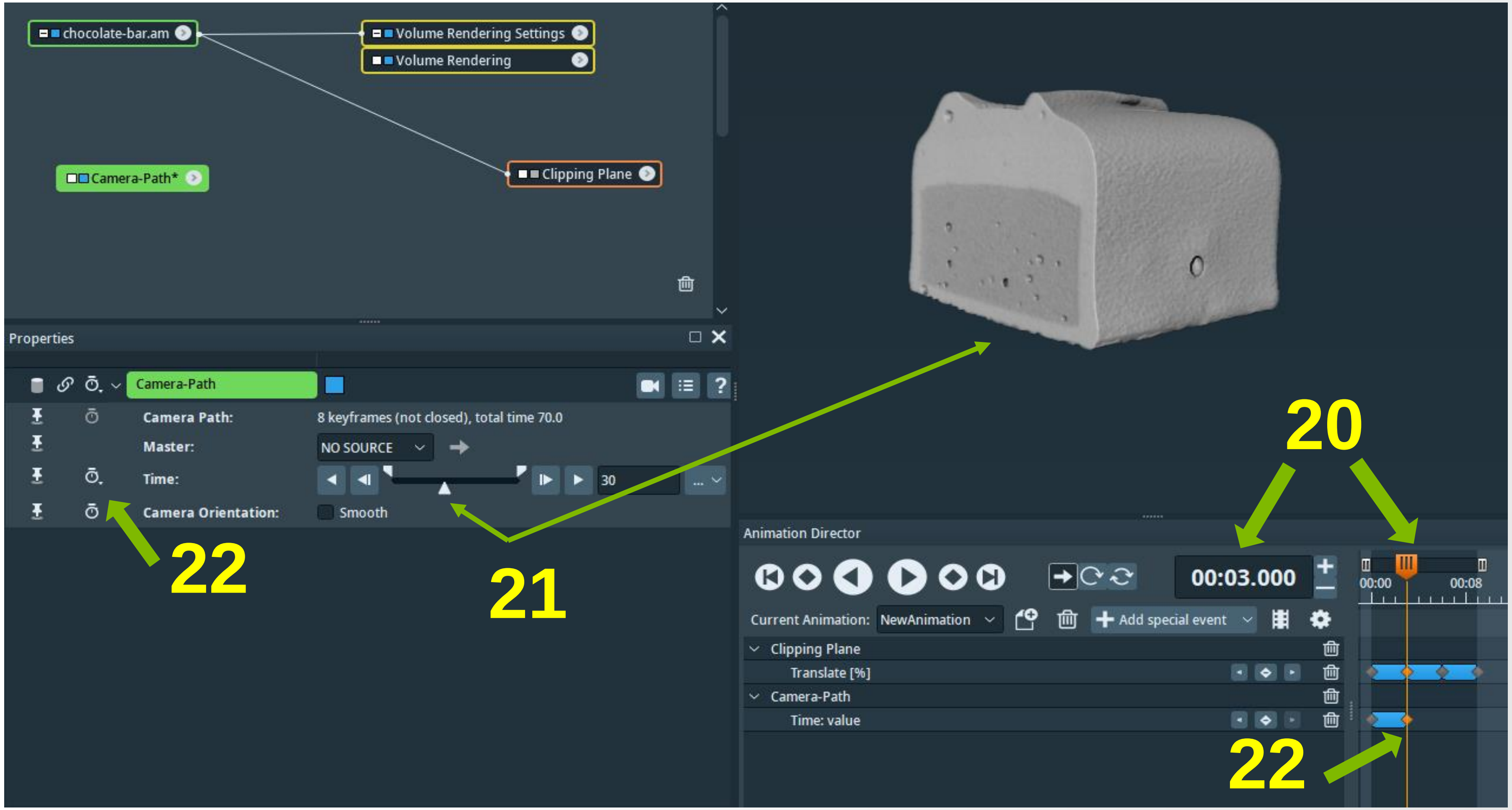
Animation Workroom: Animation Director

Translation + Rotation Animation

20. Move the timeline bar to 00:03:000

21. Move Camera Path Key frame to time 30

22. Add a key frame



Animation Workroom: Animation Director

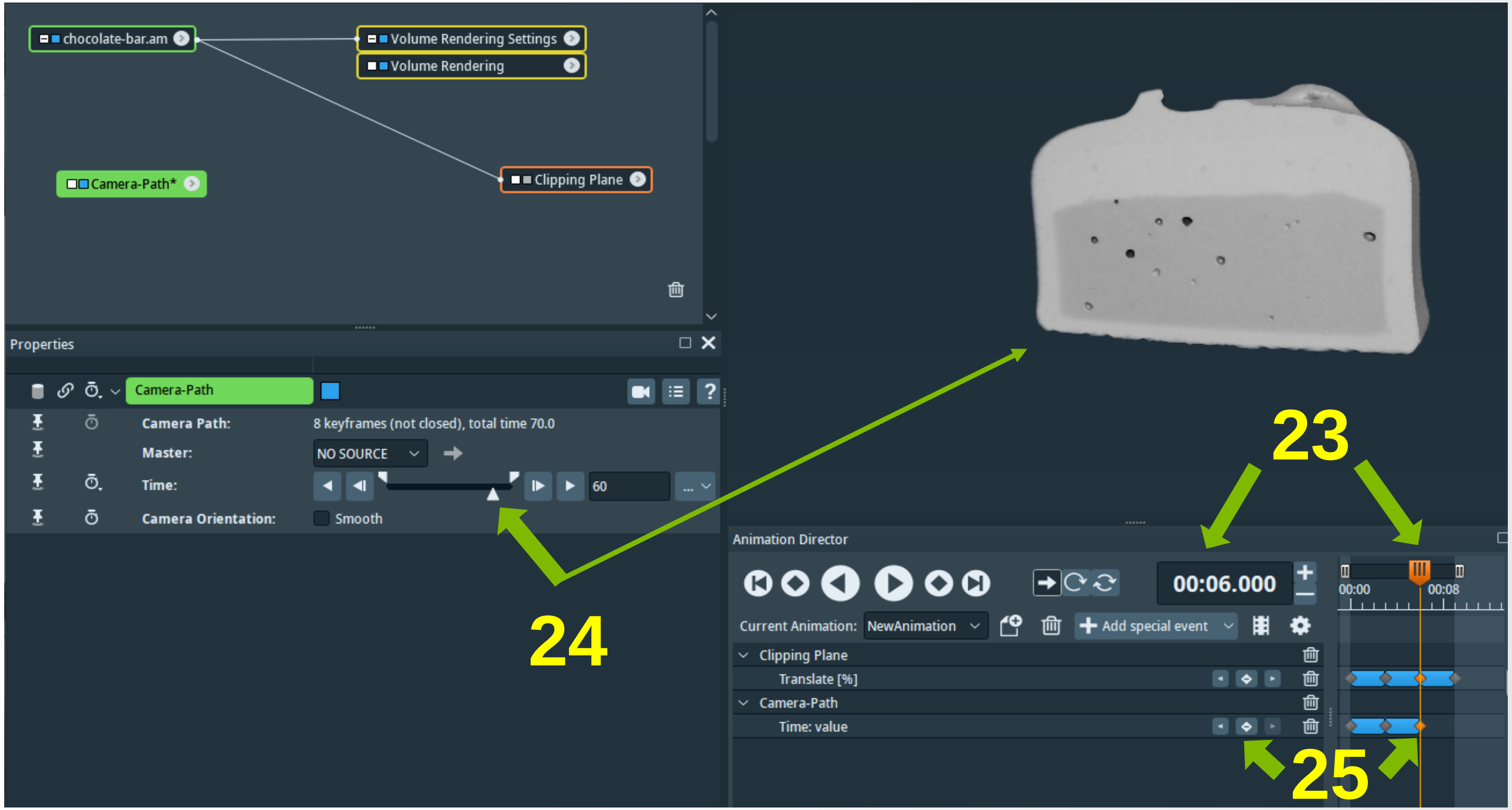
Translation + Rotation Animation

Repeat the steps

23. Move the timeline bar to 00:06:000

24. Move Camera Path Key frame to time 60

25. Add a key frame



Animation Workroom: Animation Director

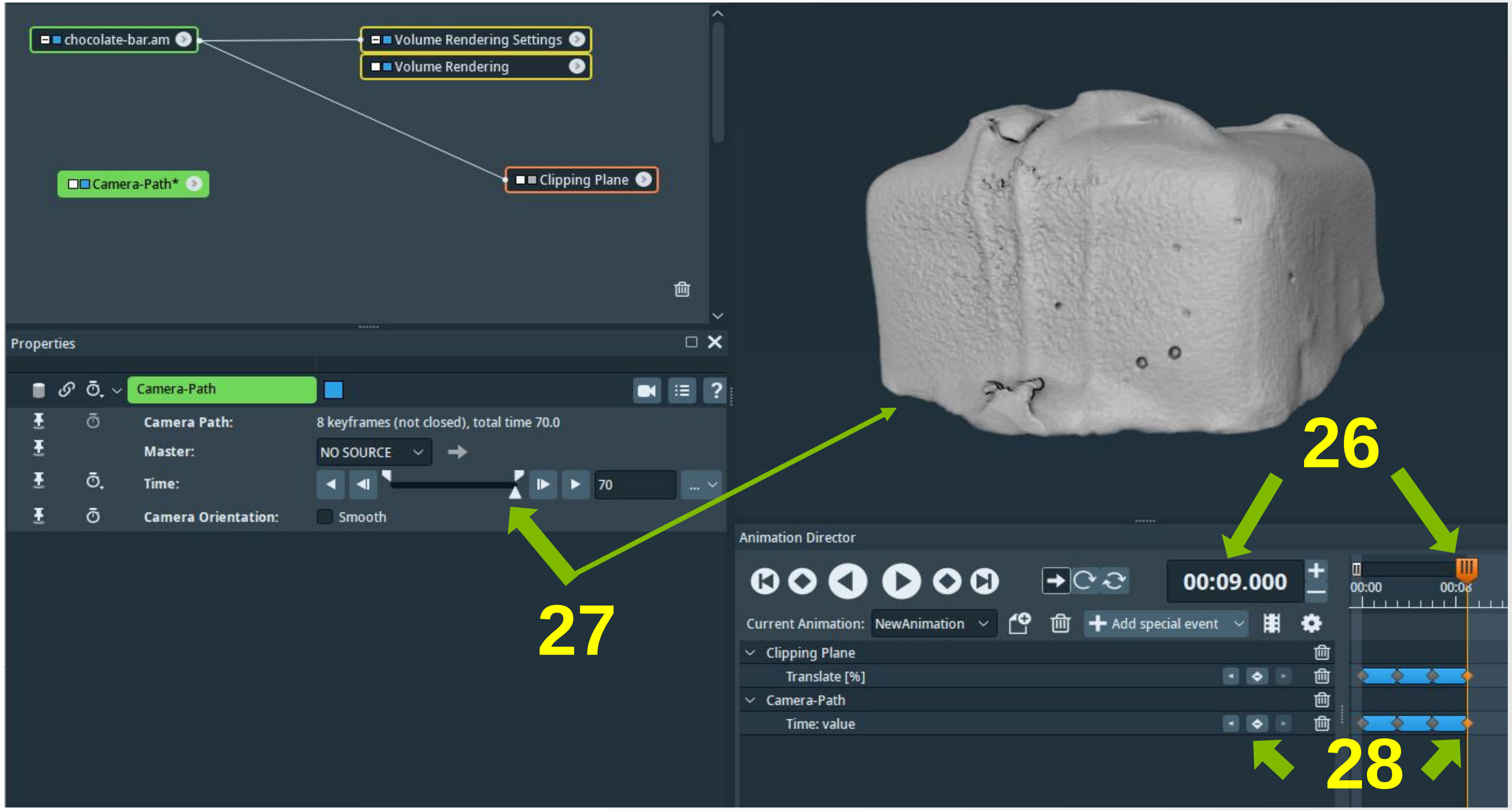
Translation + Rotation Animation

Repeat the steps

26. Move the timeline bar to 00:09:000

27. Move Camera Path Key frame to time 90

28. Add a key frame



Animation Workroom: Animation Director

Translation + Rotation

Animation

29. When done, bring timeline bar to 00:00:000

30. Click Play button in Animation Director to preview the animation.

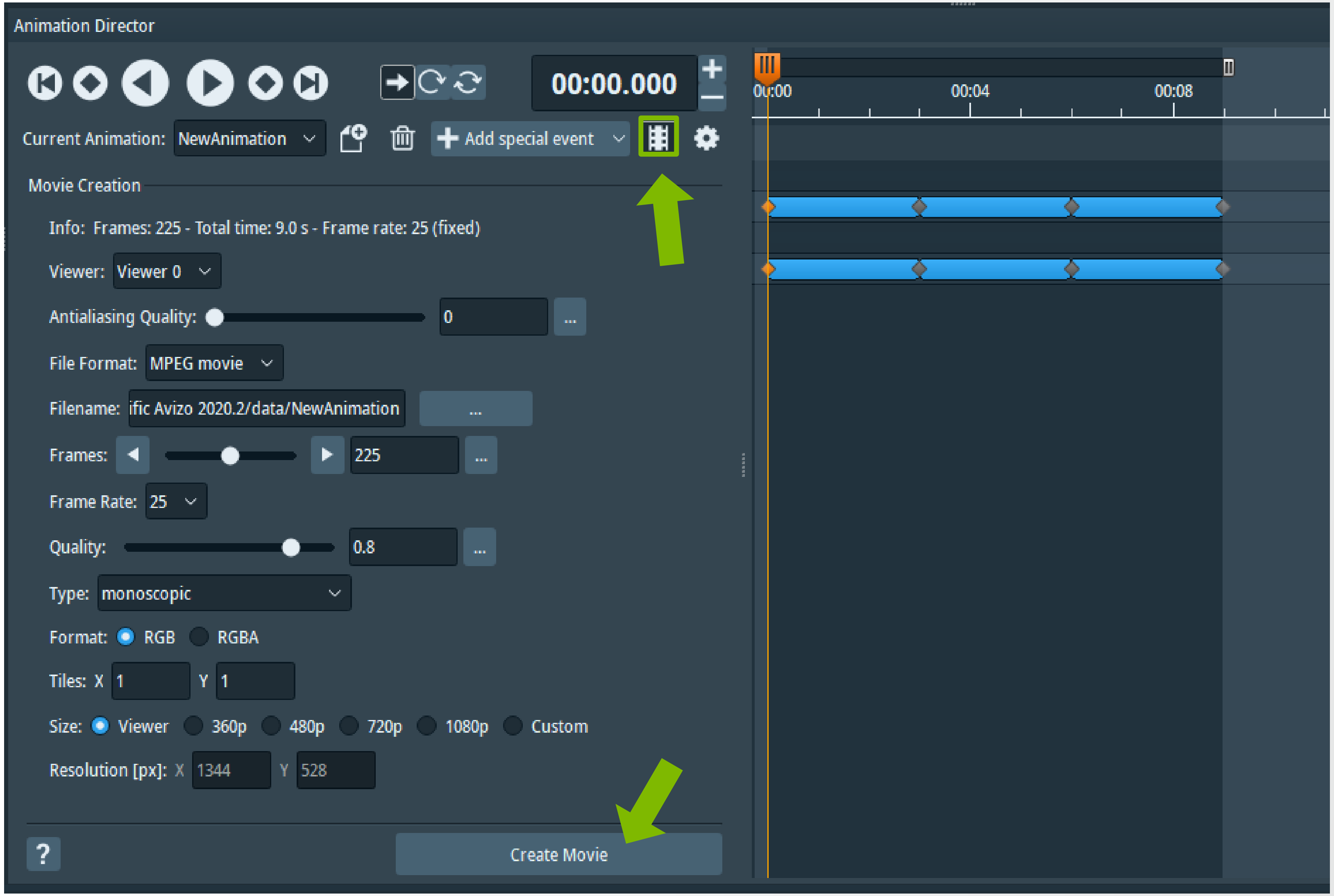
Please note that the animation limit was already set at 00:09:000

Animation director: movie generator

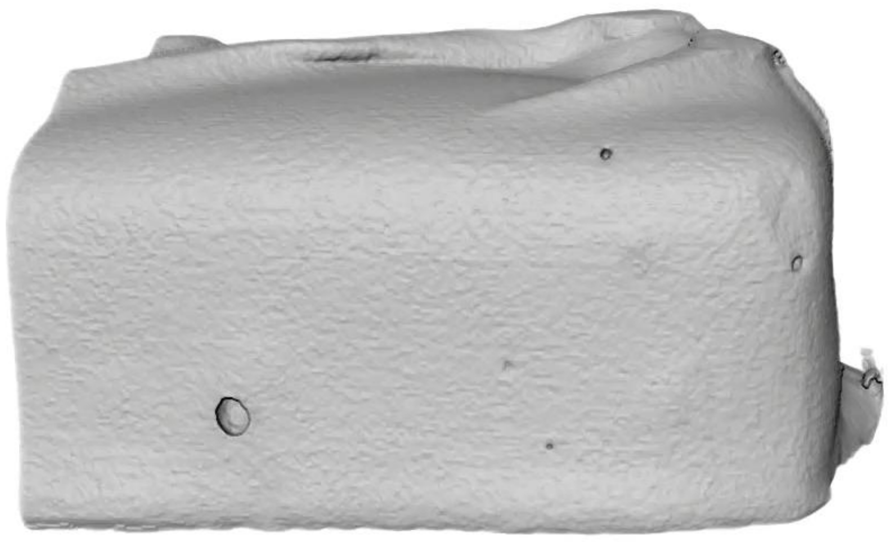
Movie Creation

Activate Movie Creation in Animation Director and input all parameters as needed.

Then click Create Movie.



Animation Workroom: Animation Director movie example



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Thank you!

Find out more at **thermofisher.com/avizo**