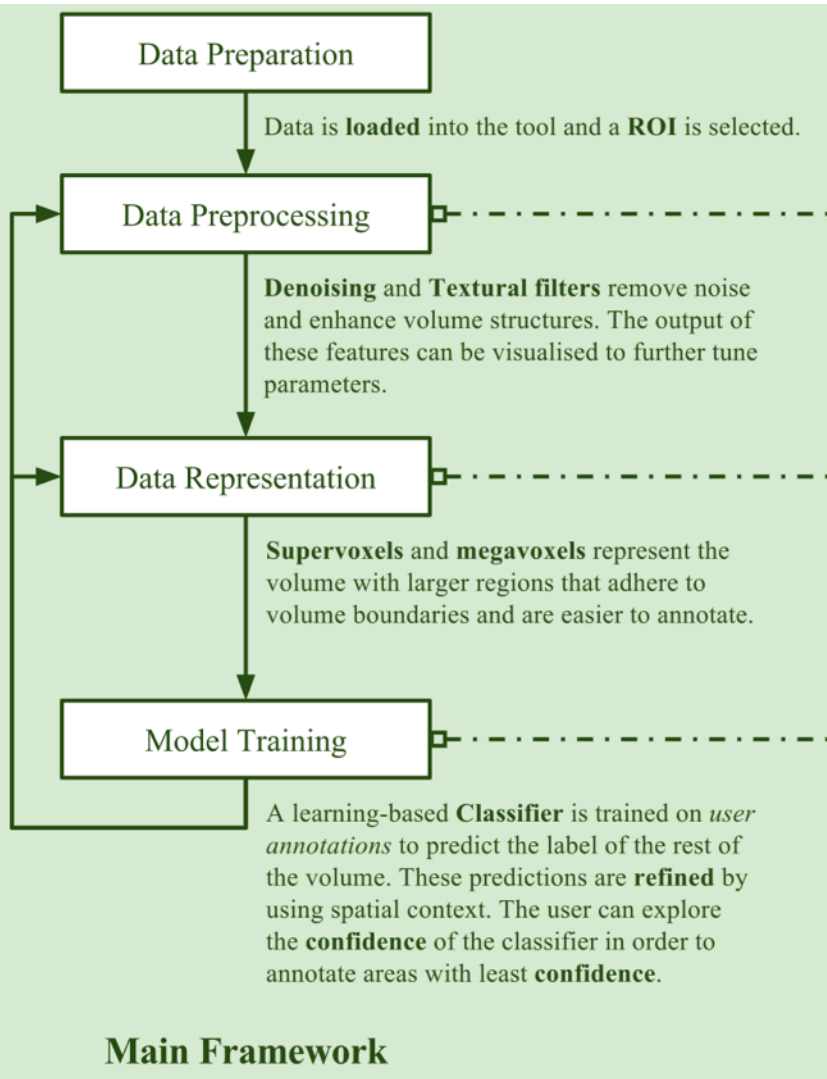


Practical Uses of SuRVoS Workbench

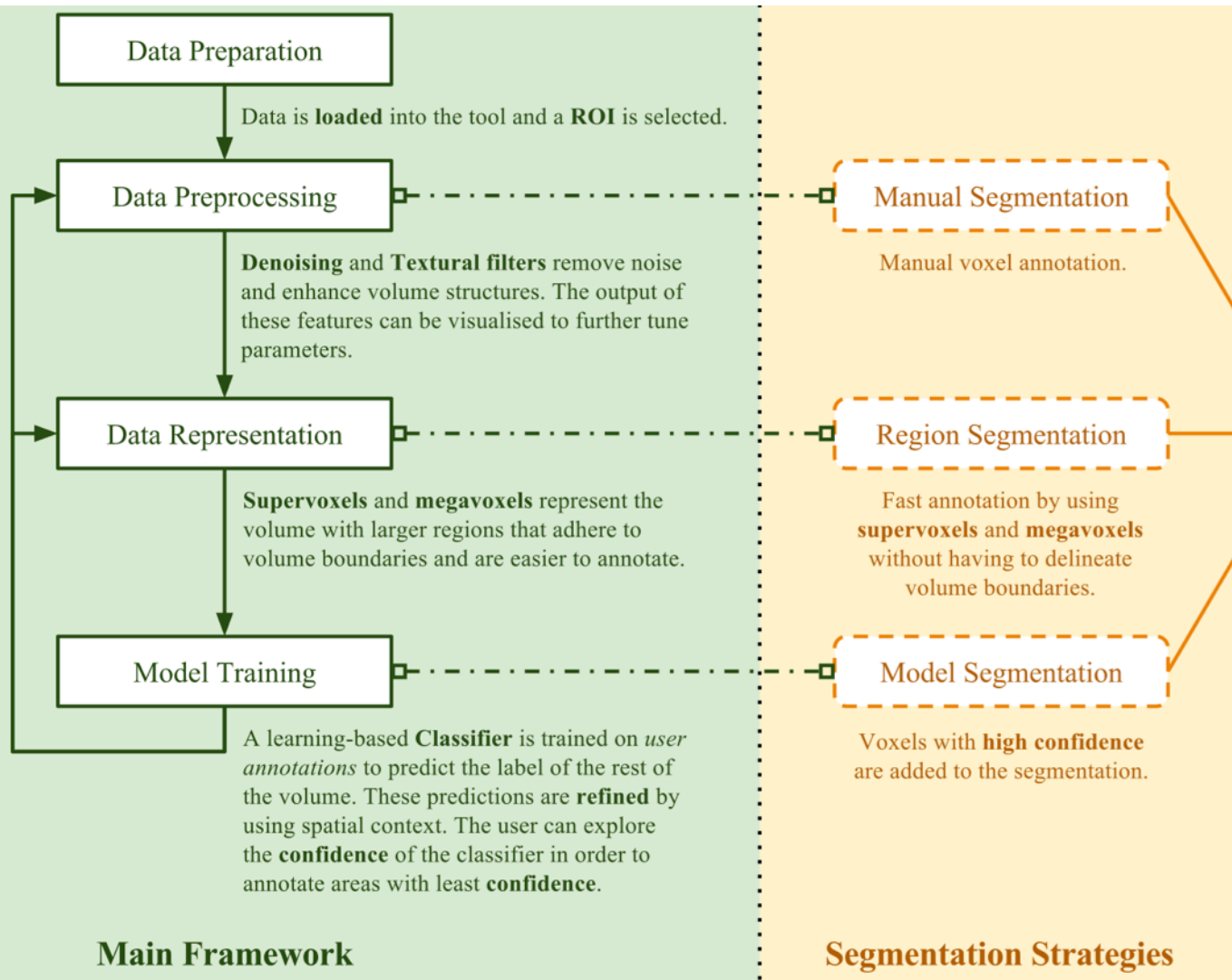
Michele C. Darrow & Matt Spink

Beamline B24
Diamond Light Source

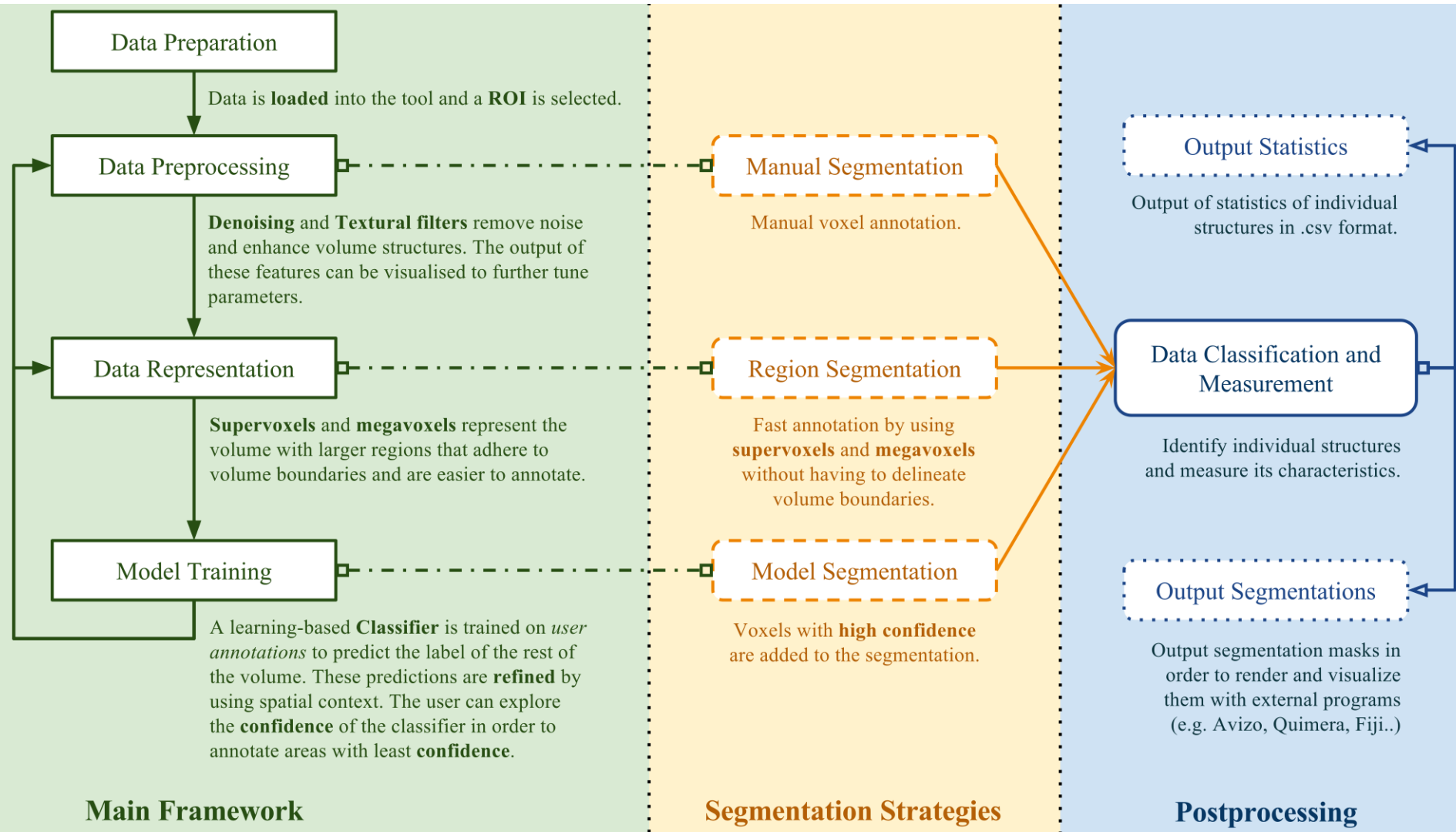
Main Framework



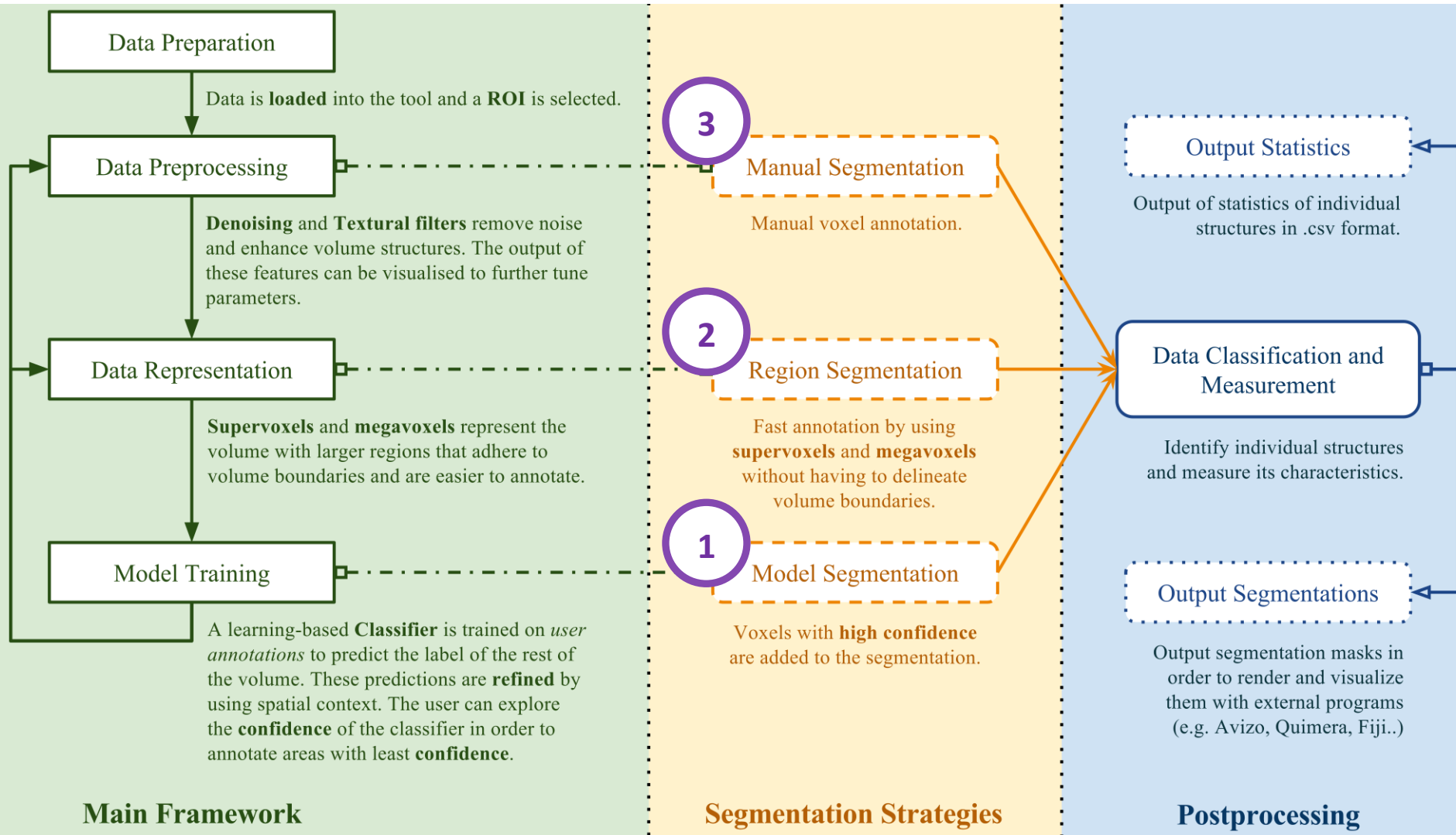
Main Framework & Segmentation Strategies



Main Framework, Segmentation Strategies & Postprocessing



The Plan for Today:



Starting SuRVoS

- In NoMachine, open a terminal (right click on the desktop and choose “open in terminal”)
- To log on to the cluster:
 - Type: `module load global/cluster`
 - Hit enter
 - Type: `qlogin -q medium.q@@com10 -l exclusive -l gpu=1`
 - Hit enter
 - When prompted, put in your password, then hit enter
- To start SuRVoS
 - Type: `module load survos`
 - Hit enter
 - Type: `survos`
 - Hit enter

Quick Notes

We would like to use the segmentations you complete today. They will be used to test new features of SuRVoS and to assess subjectivity in segmentation. Please let us know if you do not wish your segmentations to be used in these ways. We appreciate your help to further develop SuRVoS.

We will show slides with examples for each task and detailed instructions, followed by some time to complete the task. Matt and I will circulate throughout the room to help and answer individual questions during the hands-on time. Feel free to work along with the presentation if you're confident, but rest assured that there will be time built in to complete each task we talk about.

Starting or Continuing Project

SuRVoS: Super-Region Volume Segmentation workbench

Source Repository

Find the latest version of the software, contribute or suggest improvements.

Documentation

Discover how does **SuRVoS** work and how to get the most out of it.

Issues and Help

Did you have any trouble or did you find any bug? We will try to help.

Open Dataset

Load an existing dataset of supported file formats:

IMOD (.mrc, .rec), HDF5 (.h5, .hdf5), Tiff Stacks (.tif, .tiff)

Load workspace

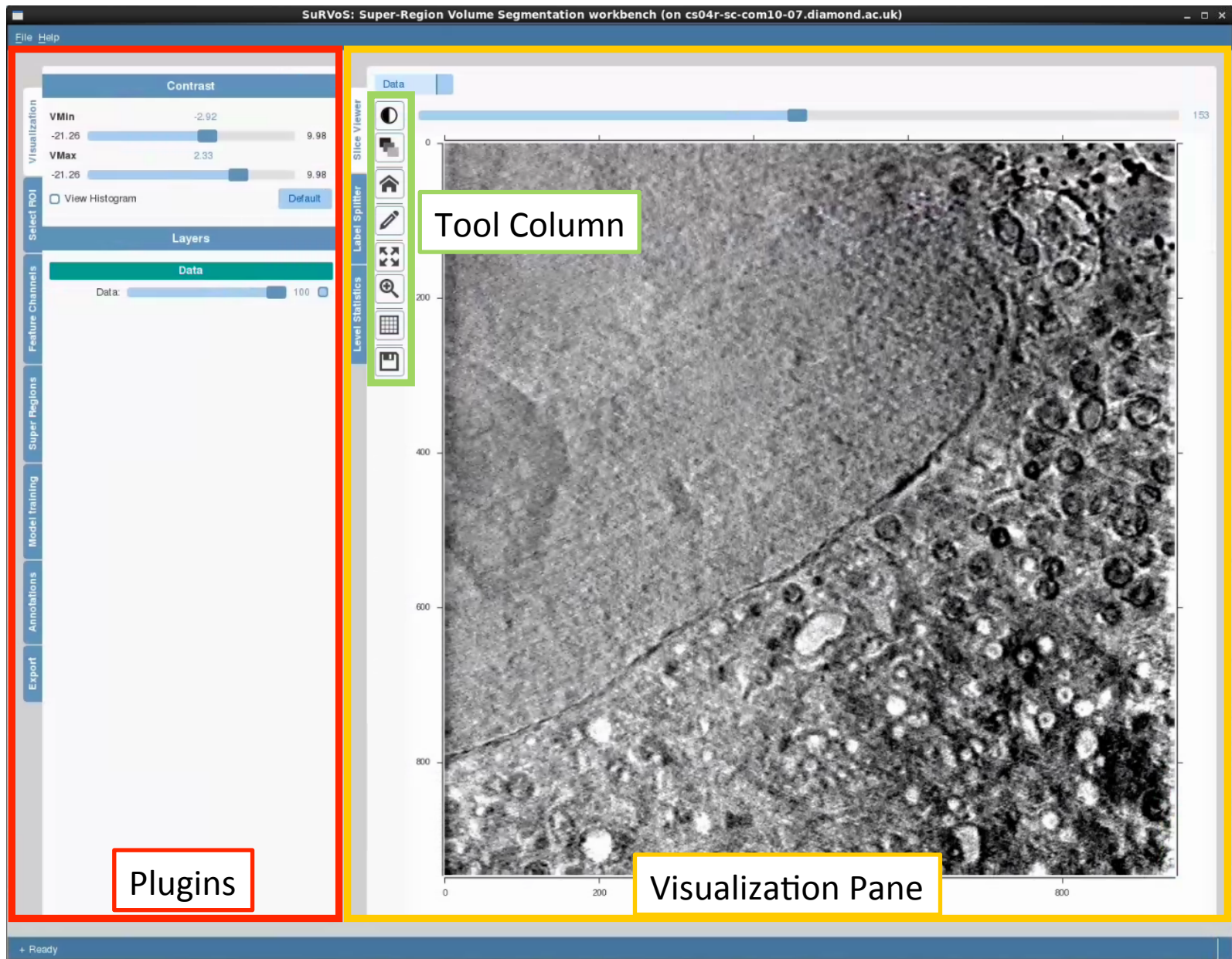
Load a workspace previously created with SuRVoS.

All the feature channels, super-regions and annotations will be recovered.

Starting a New Project

Continuing a Project

Orientation to SuRVoS



Tool Column, Shortcuts



Adjust contrast



Viewer window



Home (go back to original view)



Annotate



Pan Axis with the left mouse button, zoom with right



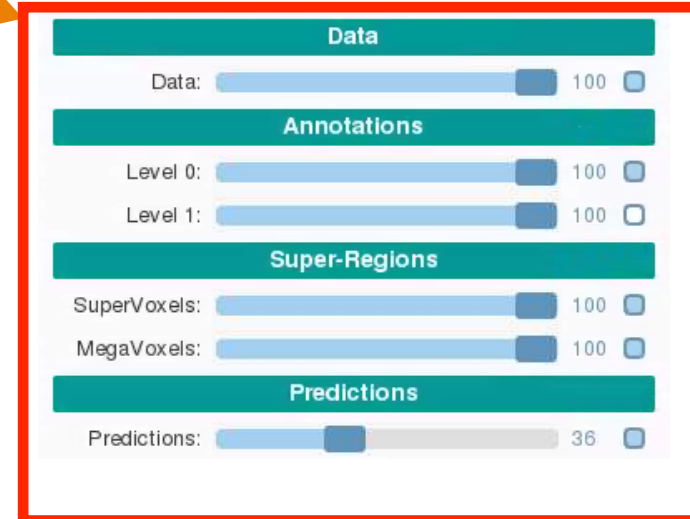
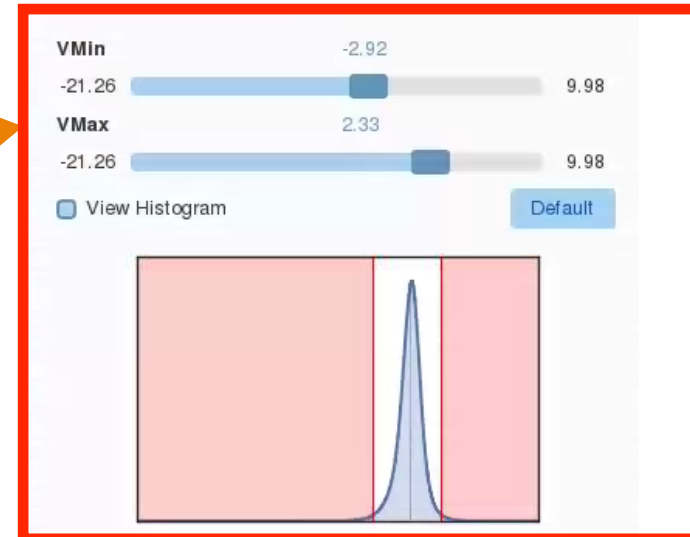
Draw a rectangle and zoom to that area




Turn on/ off a grid



Save current image



Visualization Tab in GUI



The image shows the 'Visualization' tab selected in the left sidebar of a GUI. The sidebar contains five tabs: 'Visualization', 'Select ROI', 'Feature Channels', 'Super Regions', and 'Model training'. The 'Visualization' tab is highlighted with a purple star.

The main content area of the 'Visualization' tab is divided into several sections:

- Contrast**: This section contains two sliders for 'VMin' and 'VMax'. The 'VMin' slider is set to -2.77, and the 'VMax' slider is set to 2.63. Both sliders have a range from -7.45 to 8.92. Below the sliders is a checkbox labeled 'View Histogram' and a button labeled 'Default'.
- Layers**: This section contains three sub-sections, each with a slider and a checkbox:
 - Data**: The slider is set to 100, and the checkbox is checked.
 - Super-Regions**: The slider is set to 100, and the checkbox is unchecked.
 - Annotations**: The slider is set to 100, and the checkbox is unchecked.

Region of Interest Tab in GUI

Note: order is Z, Y, X

Filled in means selected

All downstream calculations will be performed on the selected region.

We suggest you start with a small, representative region of interest to optimize parameters. Then, select the whole area to be segmented and reapply appropriate parameters.

Select Region of Interest

Shape: (50, 946, 946)

From: z 0 y 0 x 0

To: z 50 y 946 x 946

Add

Available ROI

	z [0, 50)	y [0, 946)	x [0, 946)	<input checked="" type="checkbox"/>
X	z [30, 40)	y [0, 946)	x [0, 946)	<input type="checkbox"/>
X	z [20, 30)	y [0, 946)	x [0, 946)	<input type="checkbox"/>

- 1) Insert the desired Z, Y and X values and click Add
- 2) Select the created Region of interest (ROI) that you have just created

Group A ROI

Visualization

Select Region of Interest

Shape: (306, 946, 946)

From: z 95 y 440 x 250

To: z 145 y 780 x 590

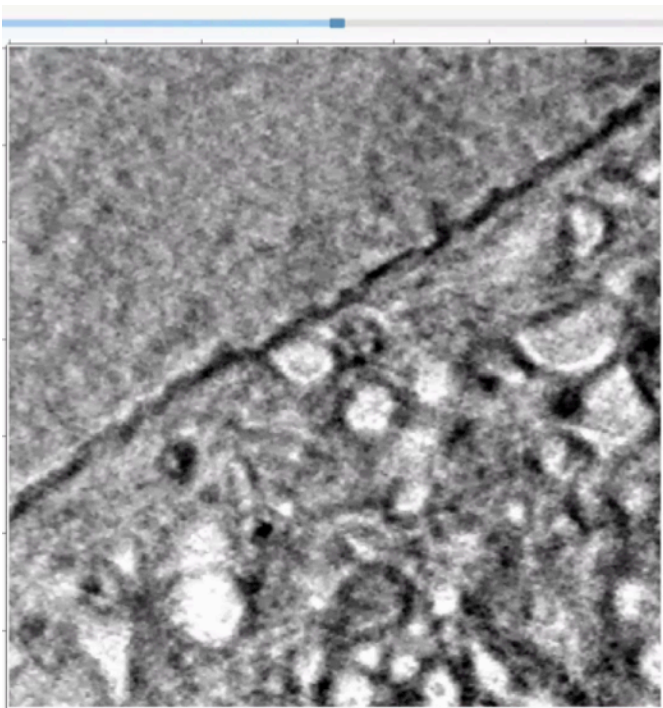
ROI

Add

1

X z [95, 145] y [440, 790] x [250, 590] ☒

2



Group B ROI

Visualization

Select Region of Interest

Shape: (306, 946, 946)

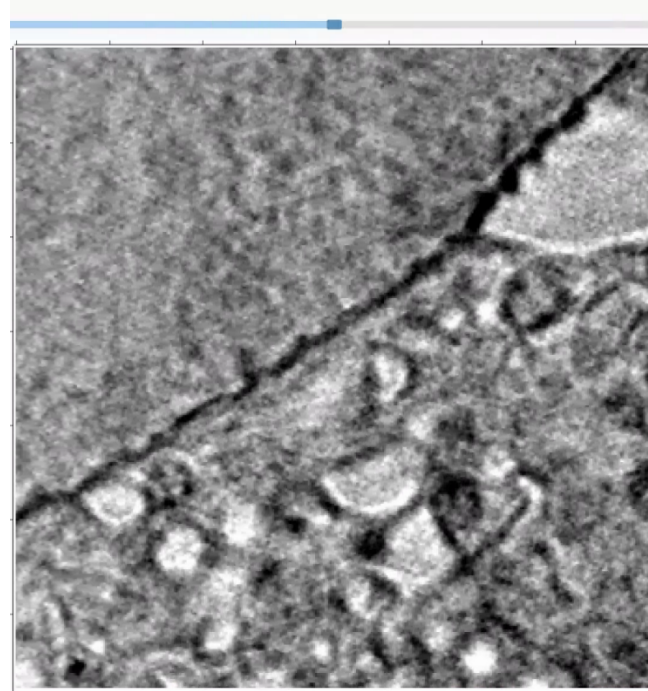
From: z 95 y 360 x 350

To: z 145 y 700 x 690

ROI

Add

X z [95, 145] y [360, 700] x [350, 690] ☐



Time – 2 minutes

Available Filter and Feature Algorithms

- Raw
 - Threshold
 - Invert Threshold
- Denoising
 - Gaussian Filter
 - Total Variation Filter
- Local Features
 - Local Mean
 - Local Standard Deviation
 - Local Normalization
 - Local Gradient Magnitude
 - Local Gradient Orientation
- Gaussian Features
 - Gaussian Normalization
 - Gaussian Gradient Magnitude
 - Gaussian Gradient Orientation

- Blob Detection
 - Difference of Gaussian
 - Laplacian of Gaussian
 - Determinant of Hessian
 - Determinant of Structure Tensor
- Texture and Structure
 - Hessian Eigenvalues
 - Structure Tensor Eigenvalues
 - Gabor Filter
- Robust Features
 - (SI) Gaussian
 - Derivative Rotation Invariant
 - (SI) Difference of Gaussians
 - (SI) Laplacian of Gaussian
 - (SI) Determinant of hessian
 - (SI) Frangi Filter
- Activation Layers
 - Maximum Response
 - Rectified Linear Unit

Available Filter and Feature Algorithms

- **Raw**

Binary selection of data

- **Denoising**

Removes noise

- **Local Features**

Uses info from neighboring voxels based on a cube

- **Gaussian Features**

Uses info from neighboring voxels based on a Gaussian neighborhood

- **Blob Detection**

Uses info from neighboring voxels based on a Laplacian neighborhood

- **Texture and Structure**

Highlights textural differences

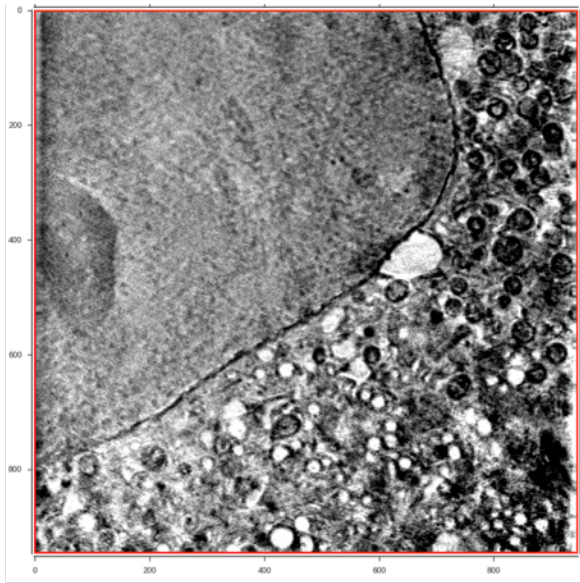
- **Robust Features**

Uses Gaussian, Blob and Texture and Structure Features with varying parameters and identifies the largest response

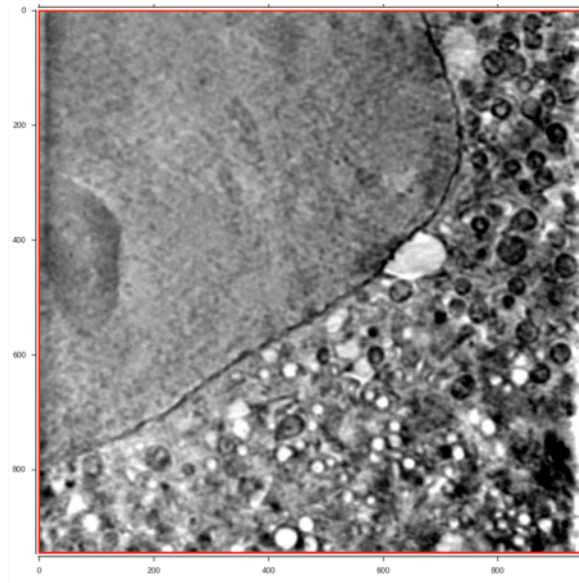
- **Activation Layers**

In development

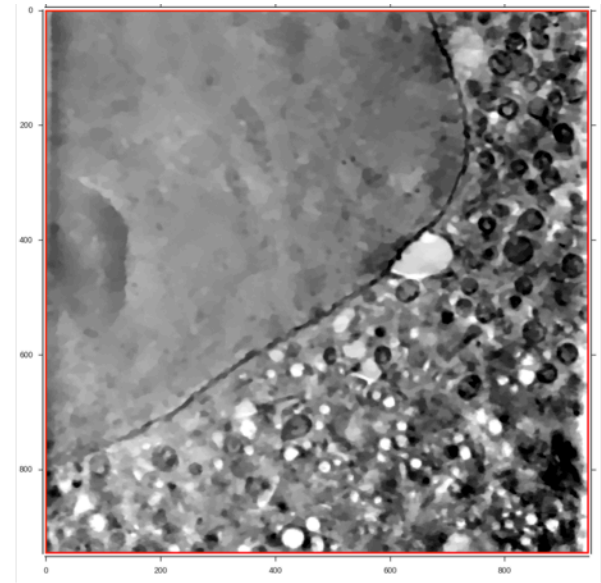
Available Filter Algorithms



(a) Raw SIRT reconstruction



(b) Gaussian Smooth



(c) Total Variation

Recommend

Sigma: 2.0 (range 1-5)

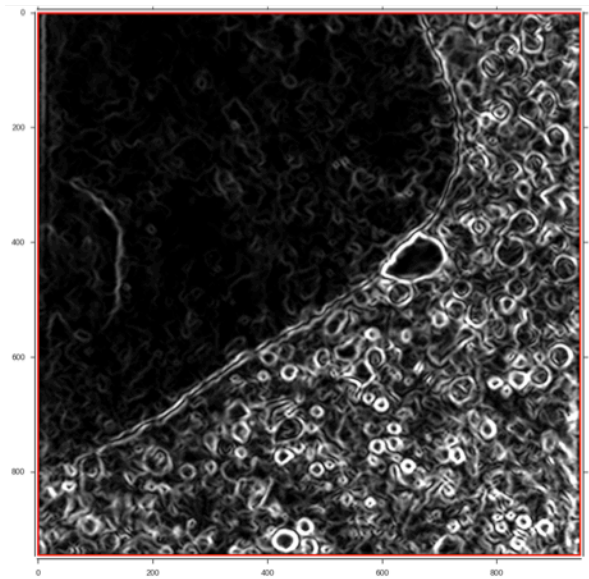
Recommend

Lambda: 10 (range 1-15)
(lower more denoising)

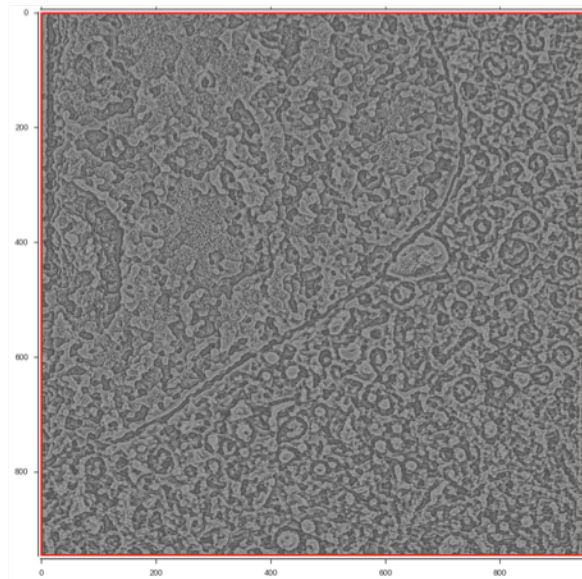
Maxiter: 100 (range 50-500)
(more iterations more denoising)

Note: Recommend to use Gaussian filter for supervoxel and megavoxel calculations, total variation for filter and feature calculations.

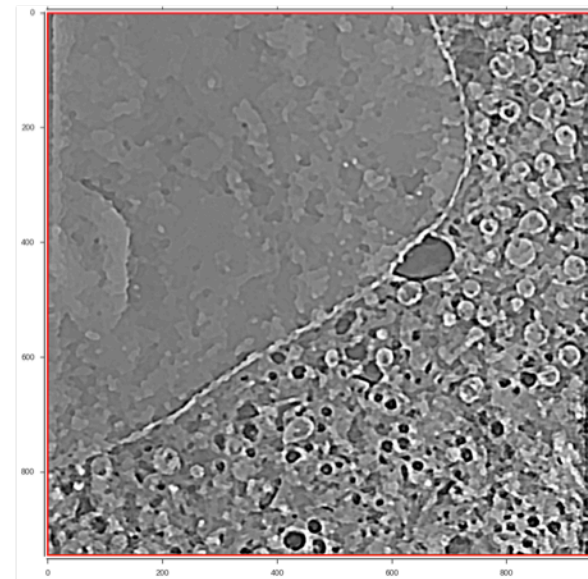
Selected Feature Filters



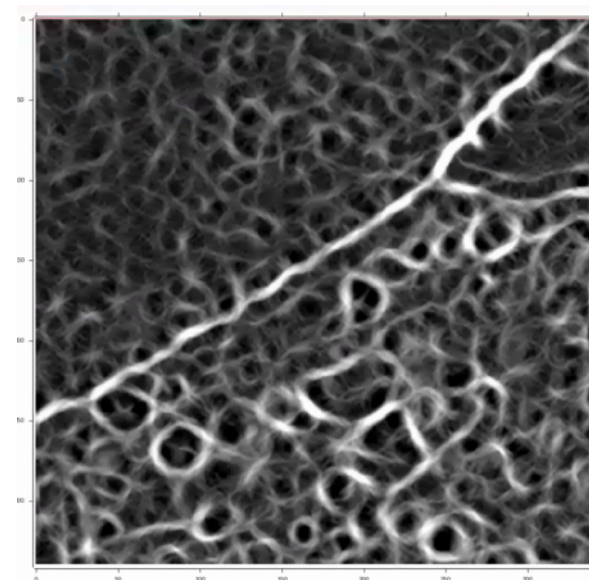
(d) Gradient Magnitude



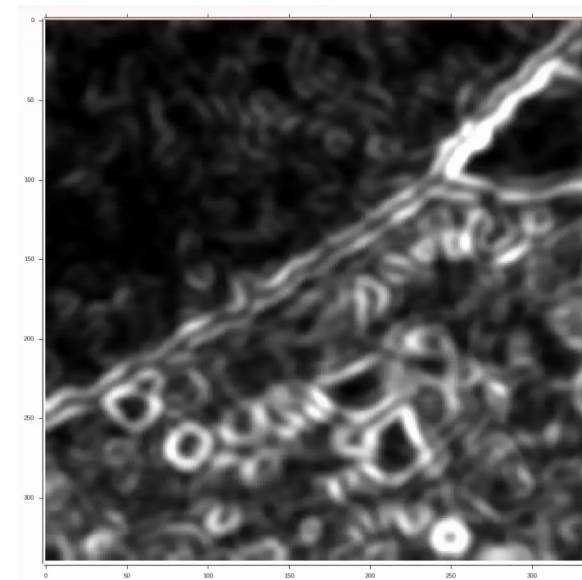
(e) Gaussian Local Normalization



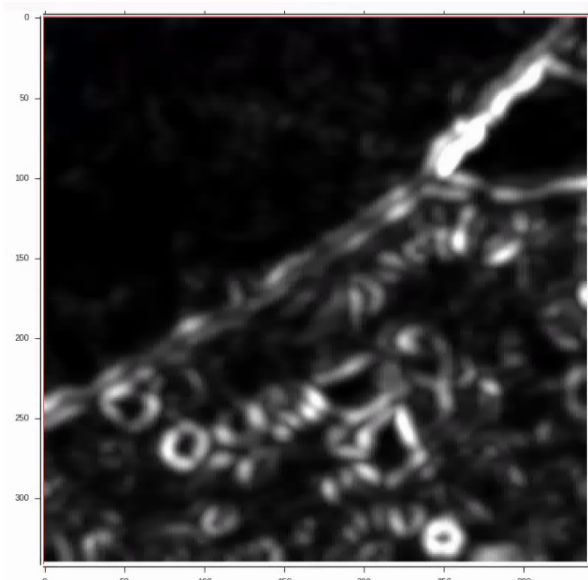
(f) Laplacian of Gaussian



(g) Hessian Eigenvalues

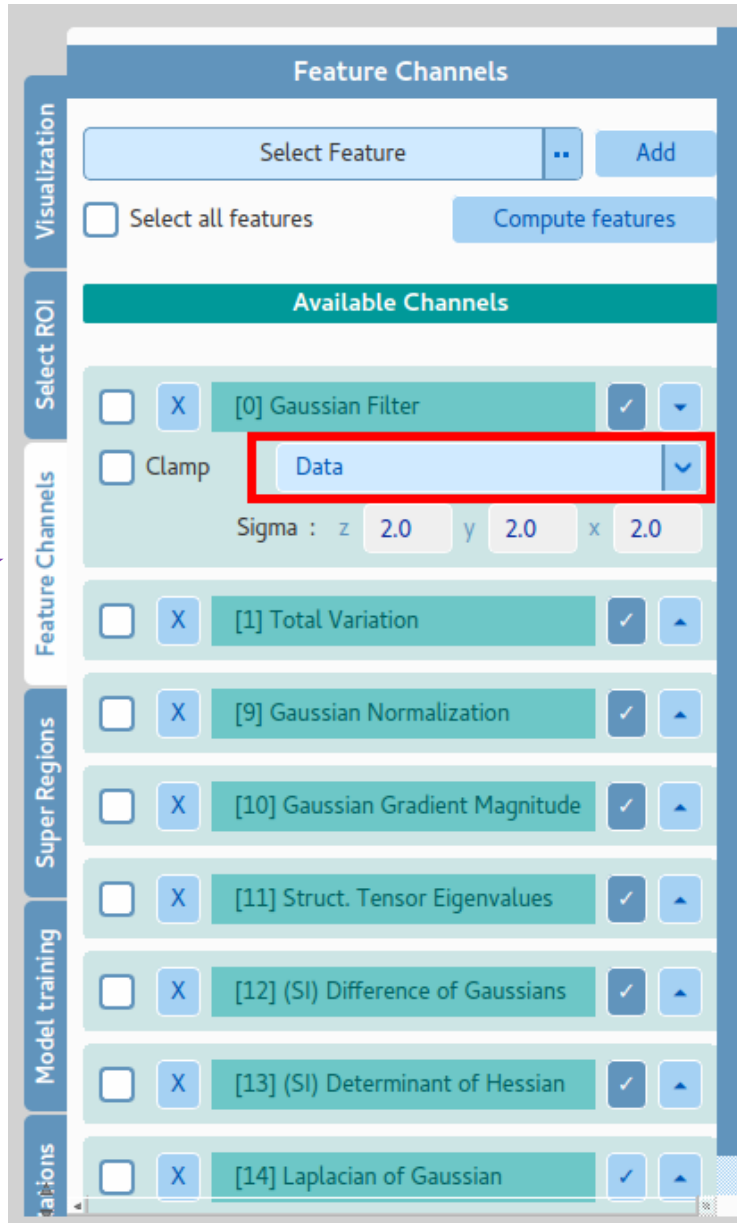


(h) Local Gradient Magnitude



(i) Structure Tensor Eigenvalues

Available Filter Algorithms



Select feature, then add
Click on name, or arrow to see details
Change input and algorithm values if needed
Click tick mark to run

To compute multiple features at once, select all (using box at top) or by selecting individual channel boxes to left of name, then click compute features (at top)

Note: For filters that use them, coordinate order is Z, Y, X

Time – 10 minutes

Introduction to Super Regions

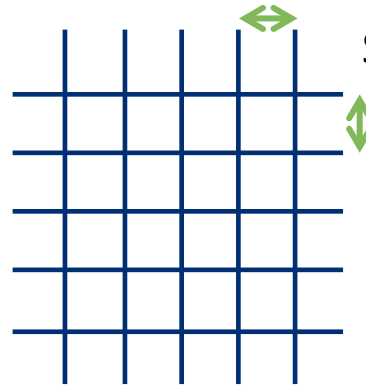


- Supervoxels and megavoxels are **edge preserving**, and **three-dimensional**.
- Segmentations are **less time and labor intensive** and should be **less subjective**.

Super Region Parameters: Supervoxels

SuperVoxels:

- SP shape
- Spacing
- Compactness

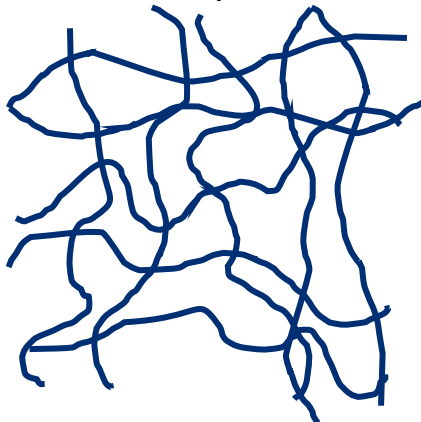


SP Shape: i.e. # of voxels to include in supervoxel grid

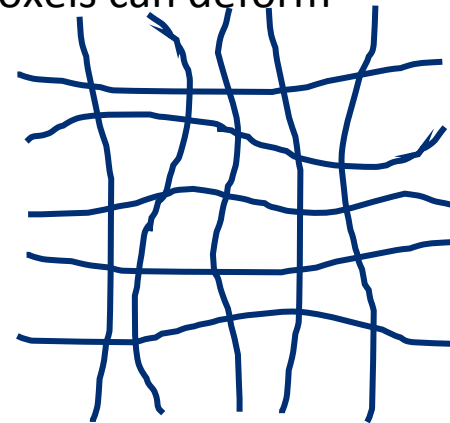
Note: order is Z, Y, X

Spacing: Importance of boundaries in each direction.

Compactness: how much supervoxels can deform



Low compactness #



High compactness #

Super Region Parameters: Megavoxels

Megavoxels:

- Lambda
- NumBins
- Gamma

Size of
megavoxels

Boundary
adherence



High lambda



Low lambda

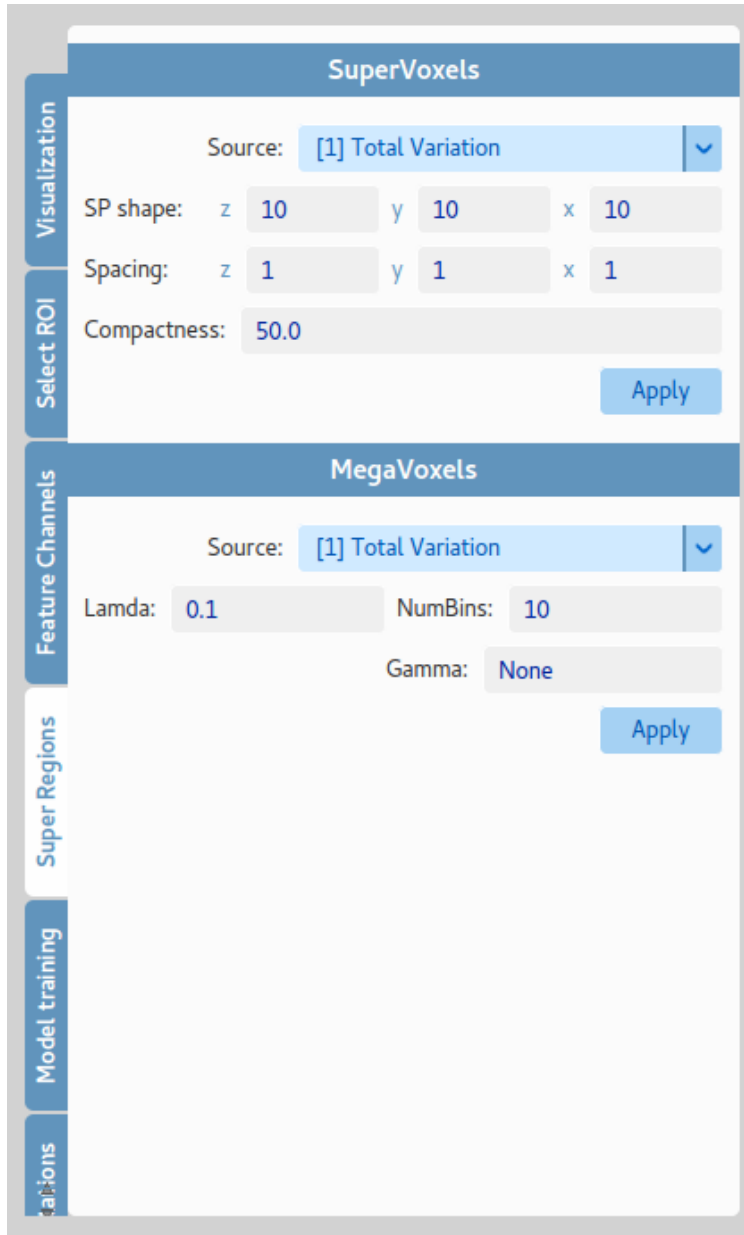
Low NumBins



High NumBins

Gamma: controls the cost of merging two supervoxels together

Super Region Parameters: Recommendations



The screenshot shows a software interface for configuring Super Region parameters. On the left is a vertical sidebar with five tabs: 'Visualizations' (highlighted with a purple star), 'Select ROI', 'Feature Channels', 'Super Regions', and 'Model training'. The main panel is divided into two sections: 'SuperVoxels' and 'MegaVoxels'. The 'SuperVoxels' section has a 'Source' dropdown set to '[1] Total Variation', 'SP shape' fields for z=10, y=10, and x=10, 'Spacing' fields for z=1, y=1, and x=1, and a 'Compactness' field set to 50.0. The 'MegaVoxels' section has a 'Source' dropdown set to '[1] Total Variation', 'Lamda' set to 0.1, 'NumBins' set to 10, and 'Gamma' set to 'None'. Both sections have an 'Apply' button at the bottom right.

SuperVoxels

Source: [1] Total Variation

SP shape: z 10 y 10 x 10

Spacing: z 1 y 1 x 1

Compactness: 50.0

Apply

MegaVoxels

Source: [1] Total Variation

Lamda: 0.1 NumBins: 10

Gamma: None

Apply

SuperVoxels:

- Source: Data channel
- SP shape: z=10 y=10 x=10
- Spacing: 1x1x1 (range 1-5)
- Compactness: 20 (range 10-100)

MegaVoxels:

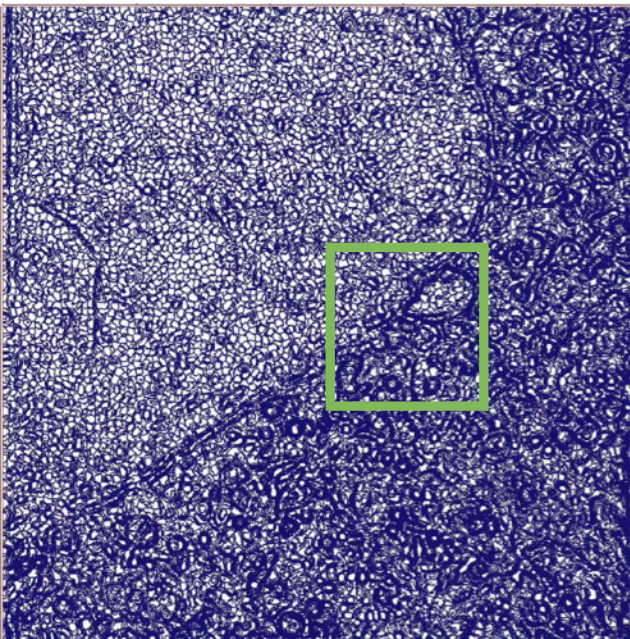
- Source: Data channel
- Lamda: 0.1 (range 0.001-0.5)
- NumBins: 20 (range 10-50)
- Gamma: Either “None” or “auto”

Notes:

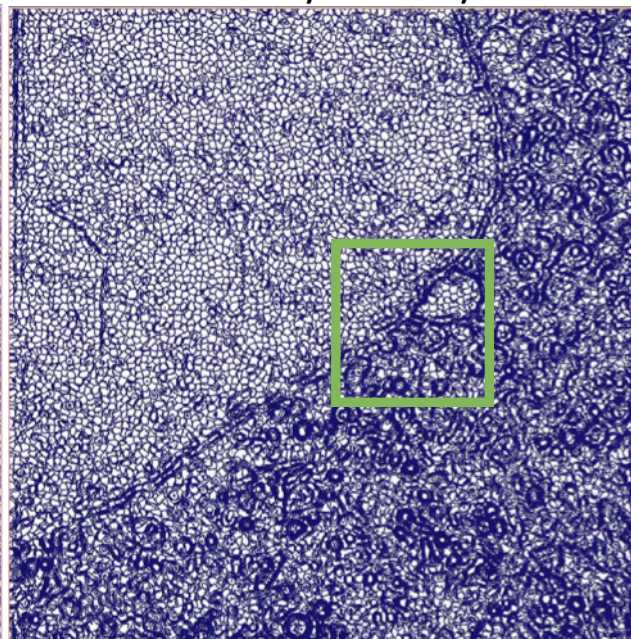
- SP shape parameters multiplied together have to equal less than 1024
- Order is Z, Y, X

Default:

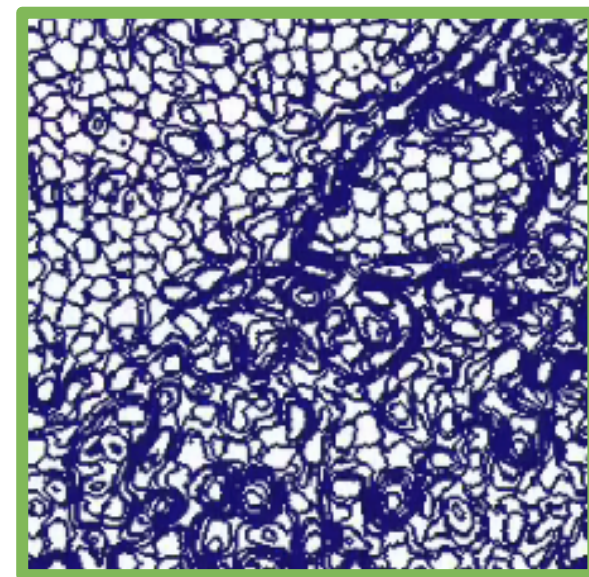
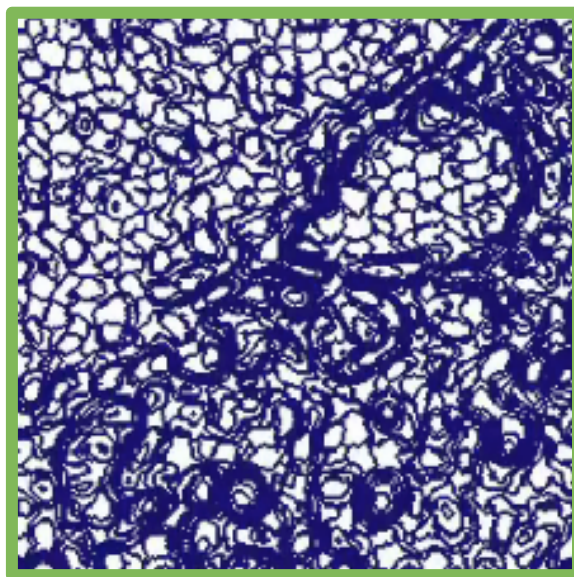
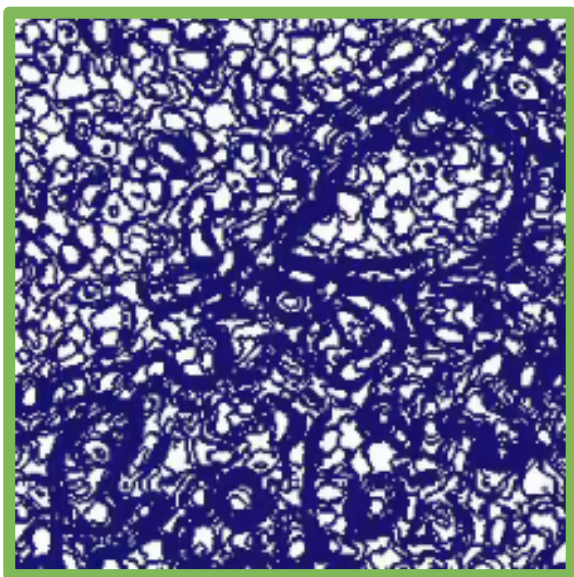
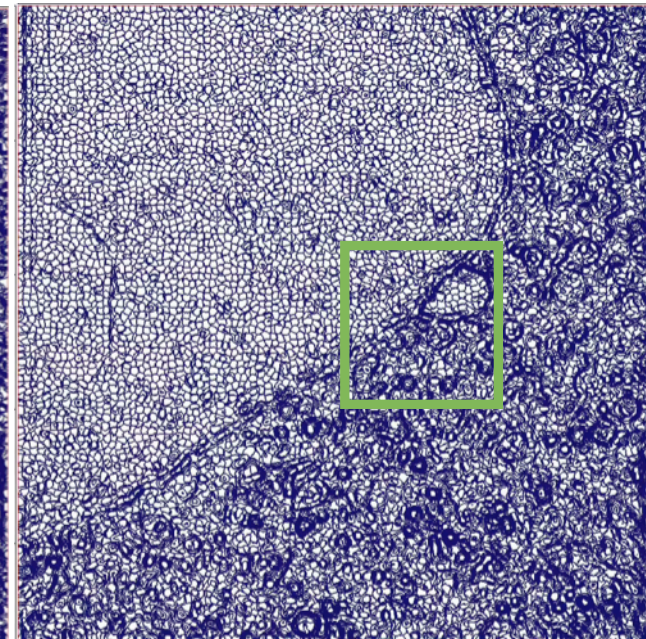
10x10x10 / 1x1x1 / **10**



10x10x10 / 1x1x1 / **20**

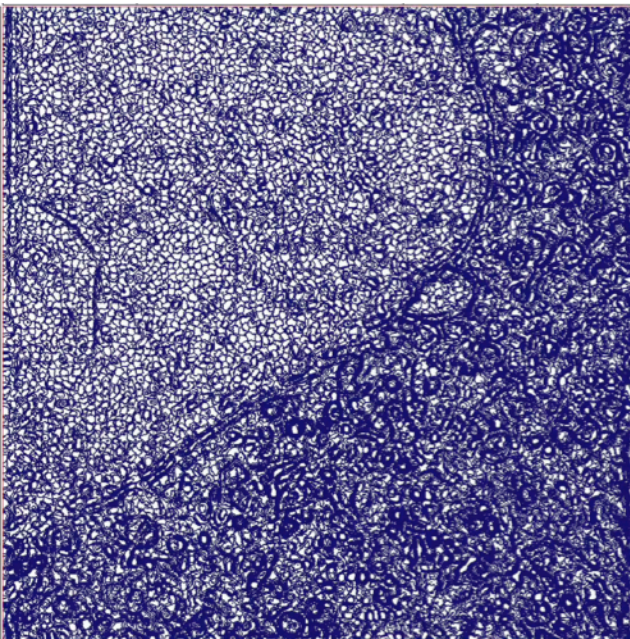


10x10x10 / 1x1x1 / **30**

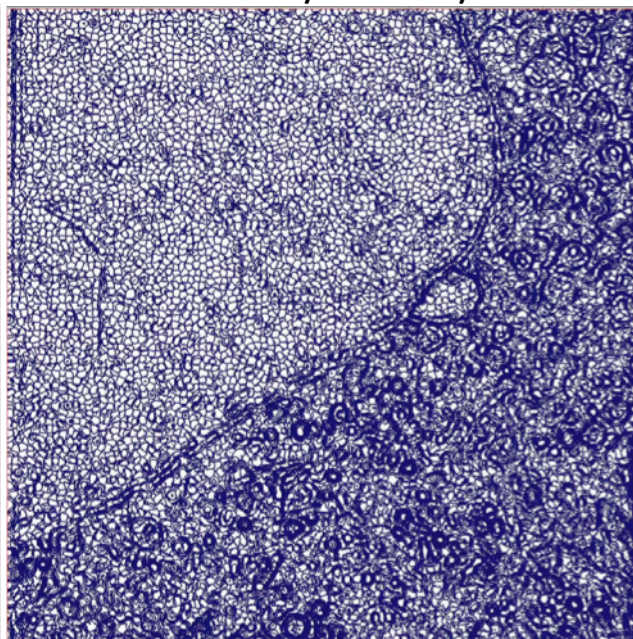


Default:

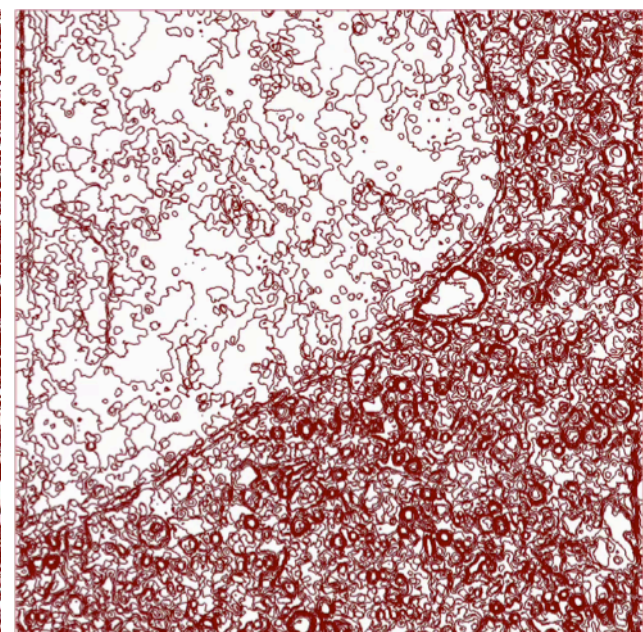
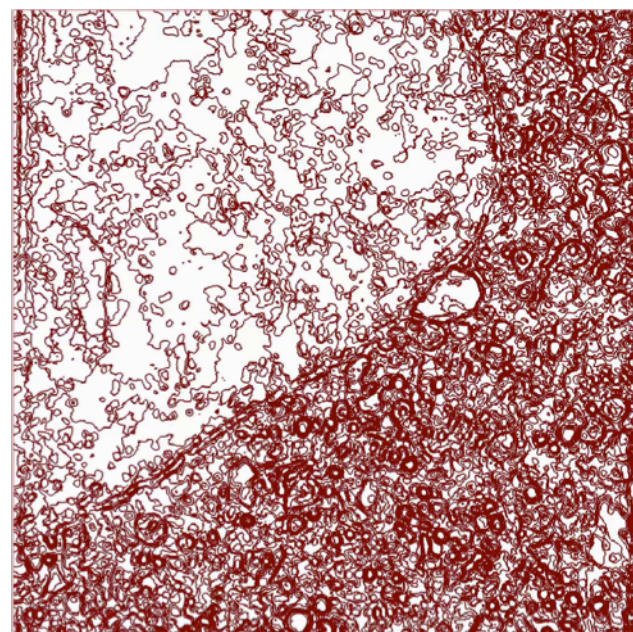
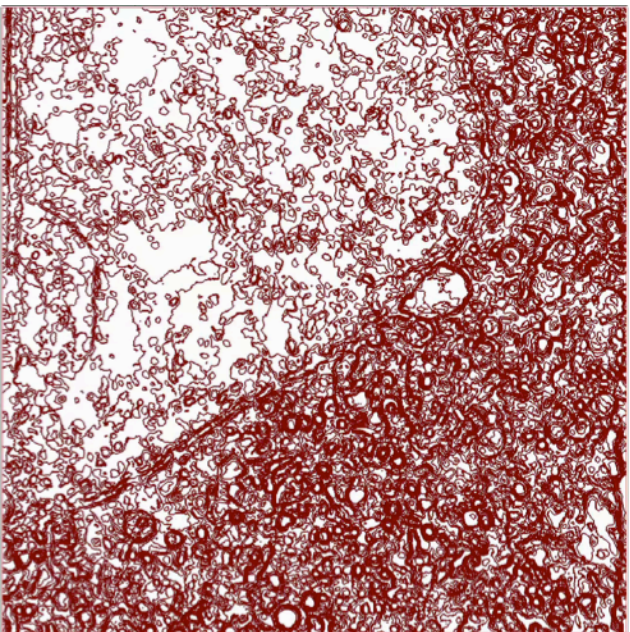
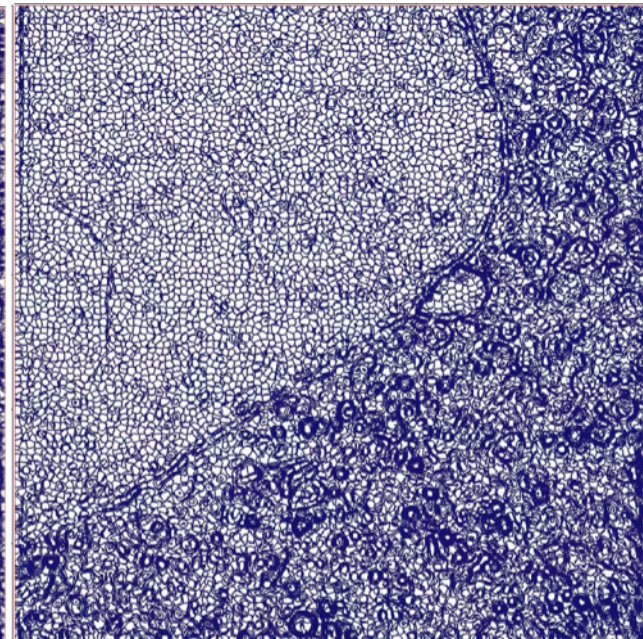
10x10x10 / 1x1x1 / **10**



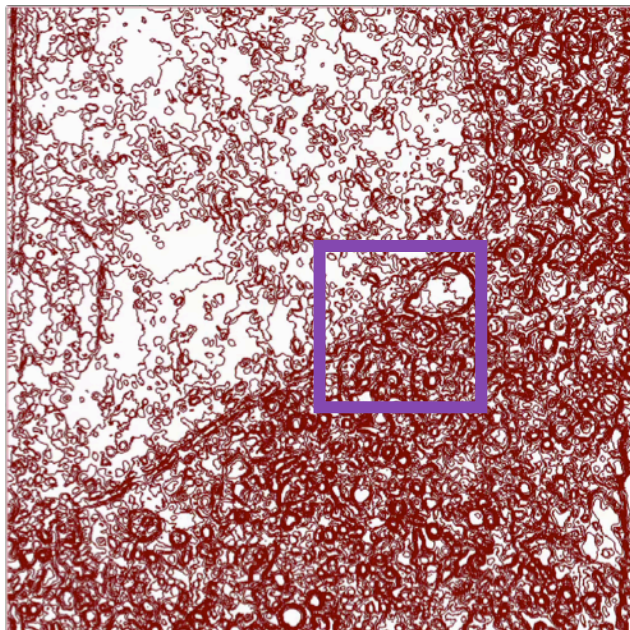
10x10x10 / 1x1x1 / **20**



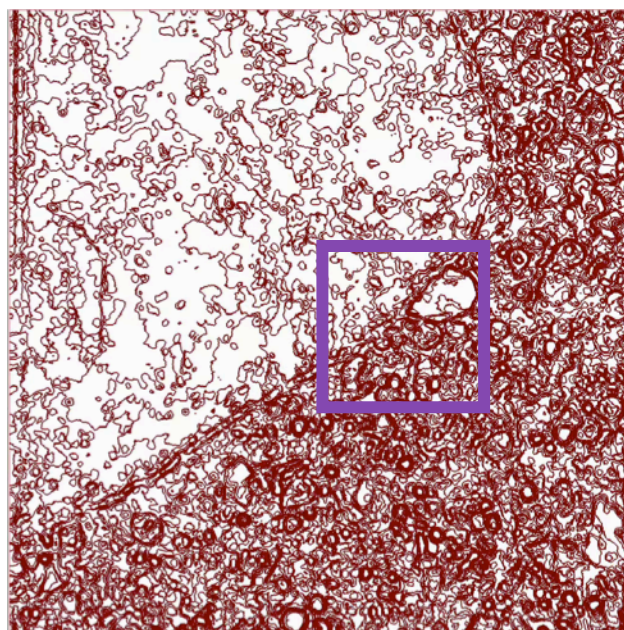
10x10x10 / 1x1x1 / **30**



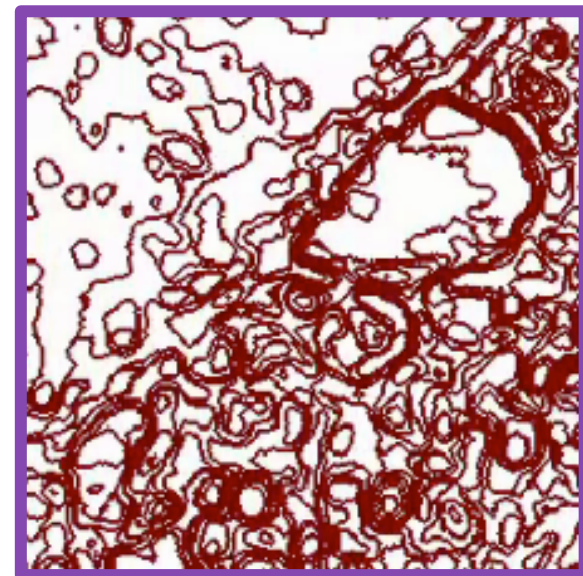
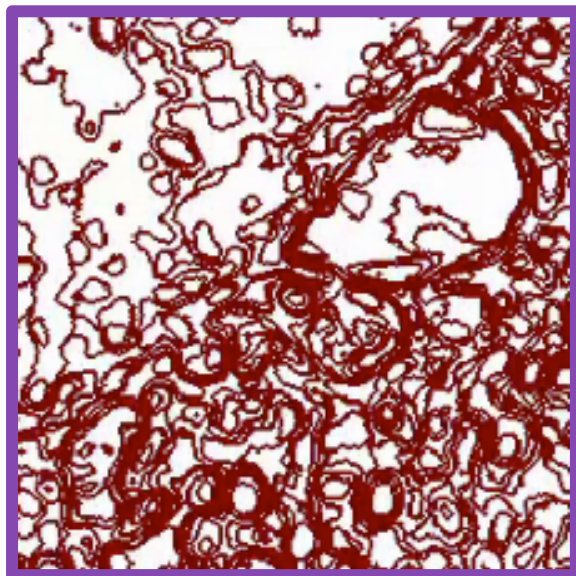
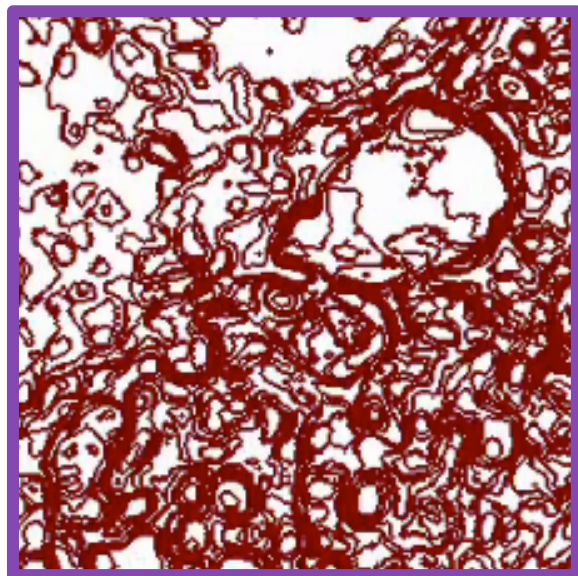
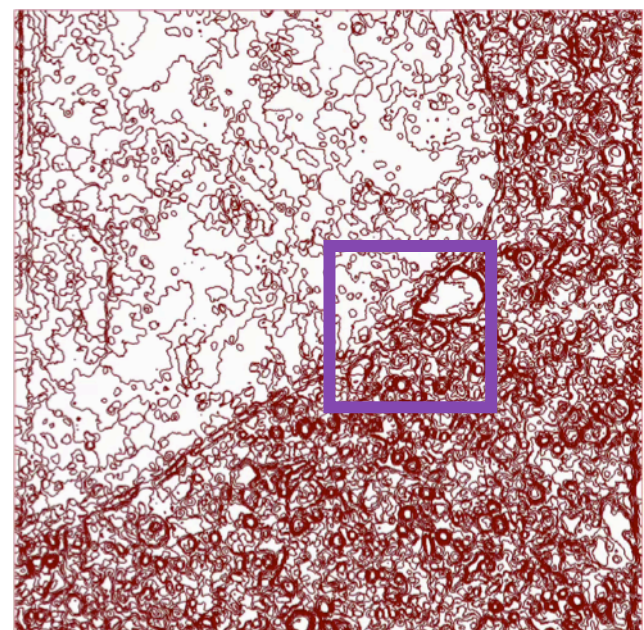
10x10x10 / 1x1x1 / **10**



Default:
10x10x10 / 1x1x1 / 20

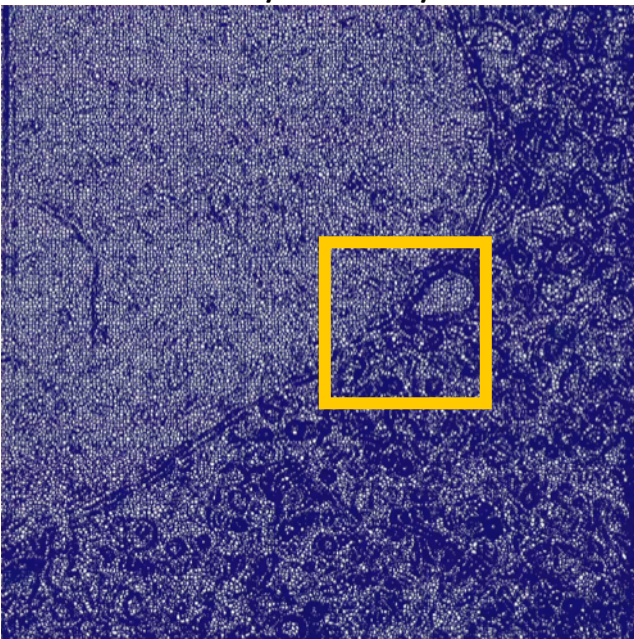


10x10x10 / 1x1x1 / **30**

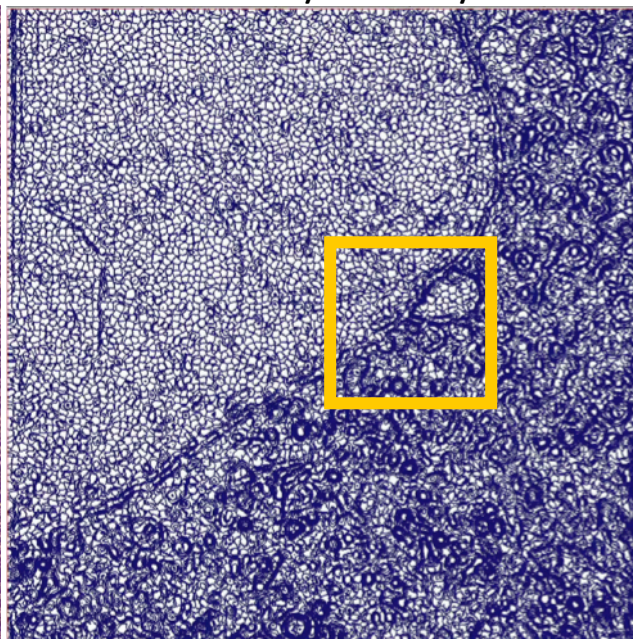


Default:

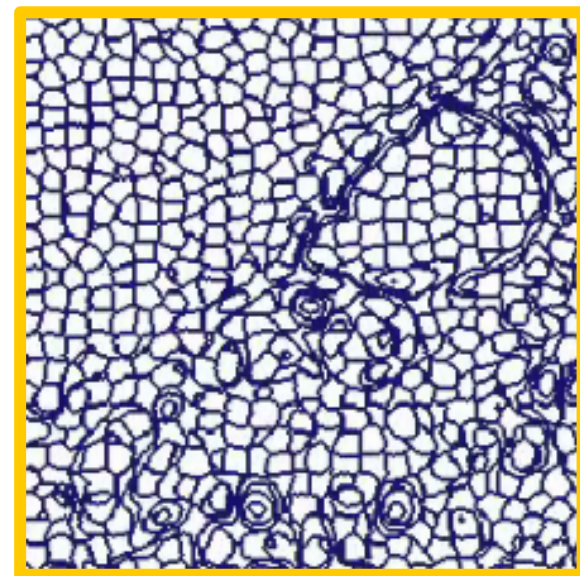
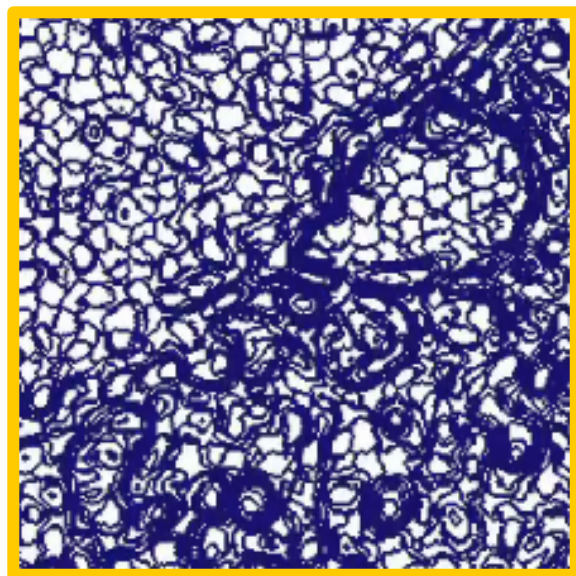
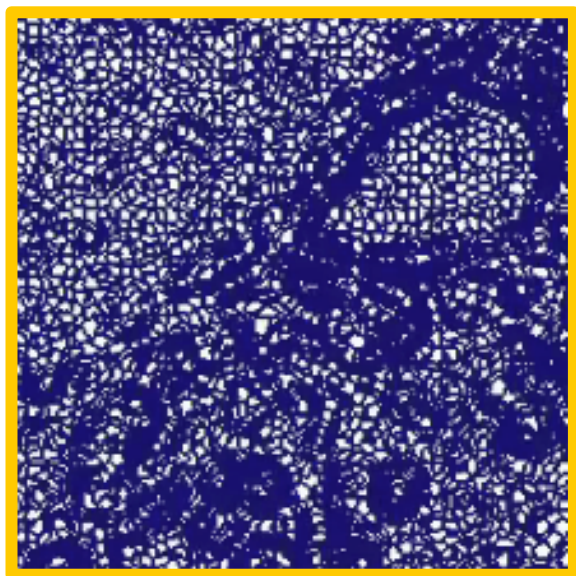
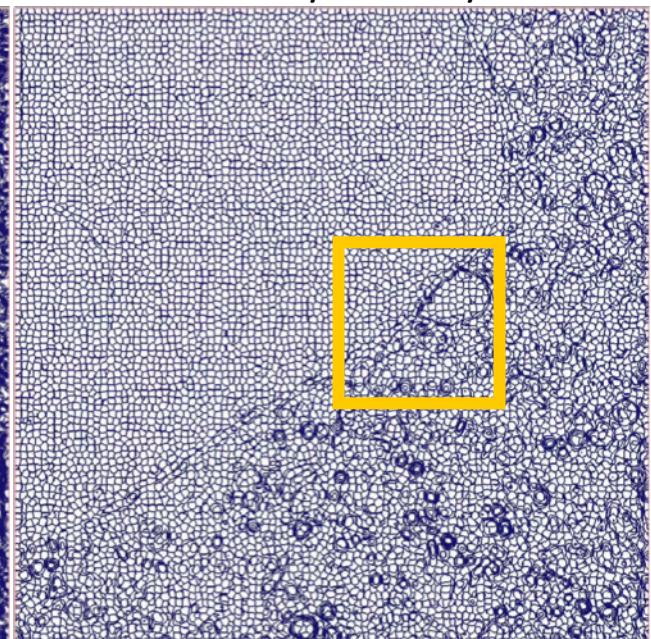
5x5x5 / 1x1x1 / 20



10x10x10 / 1x1x1 / 20

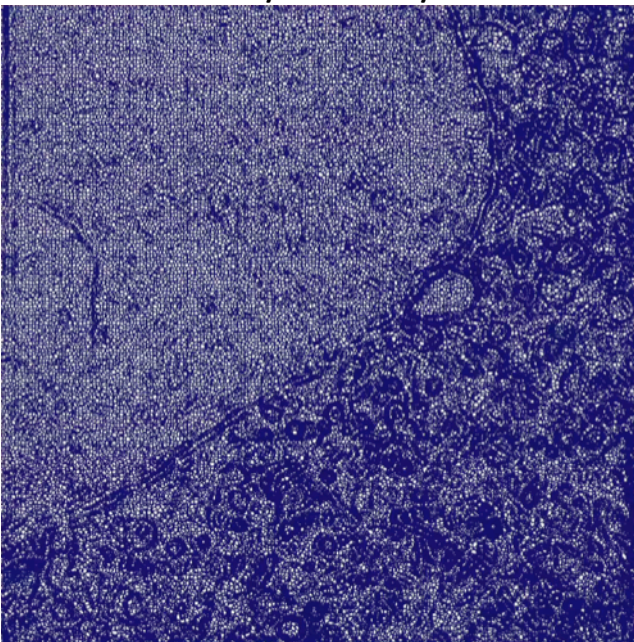


10x10x10 / **3x3x3** / 20

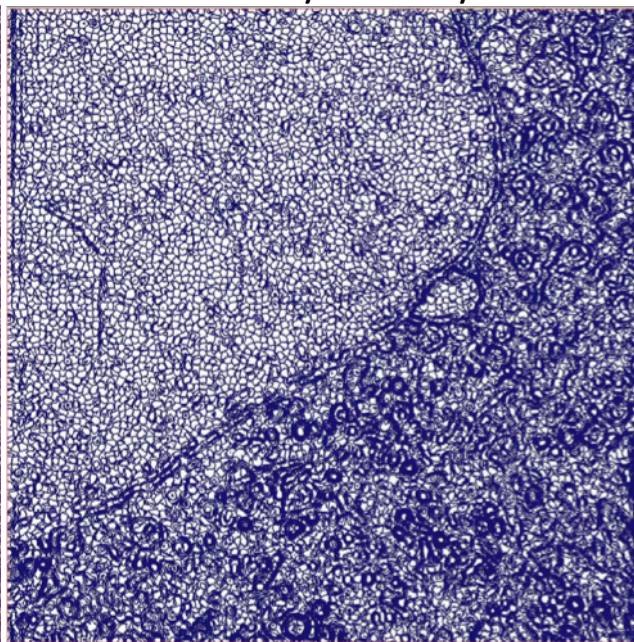


Default:

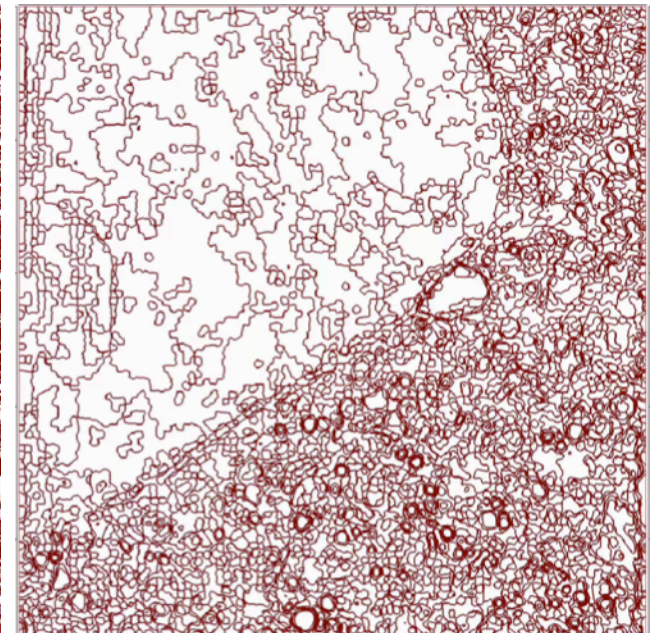
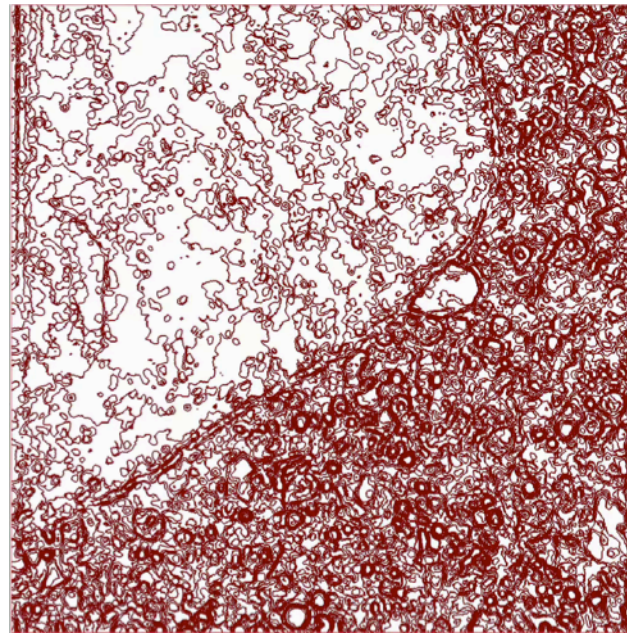
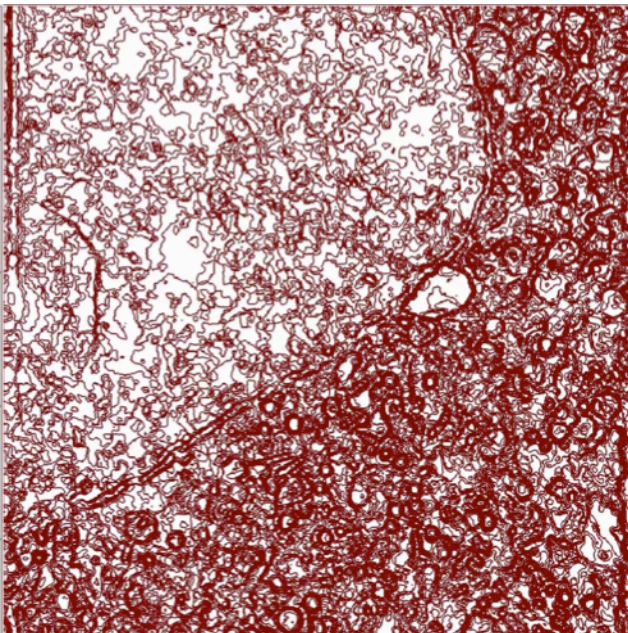
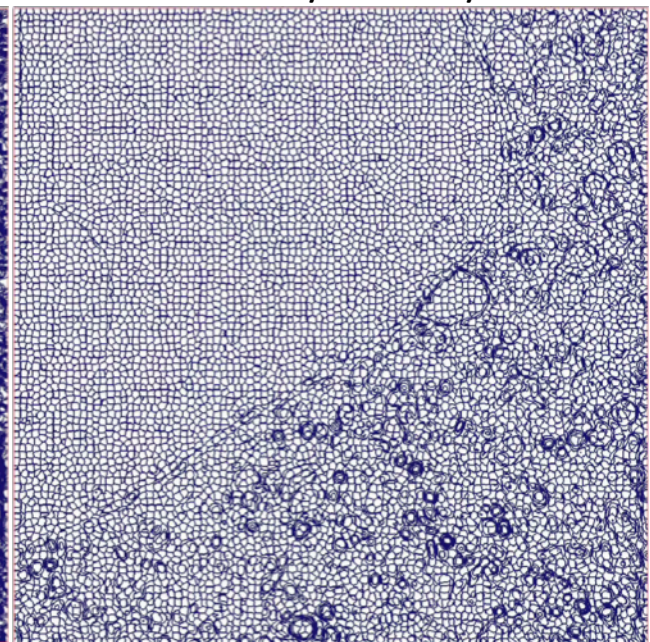
5x5x5 / 1x1x1 / 20



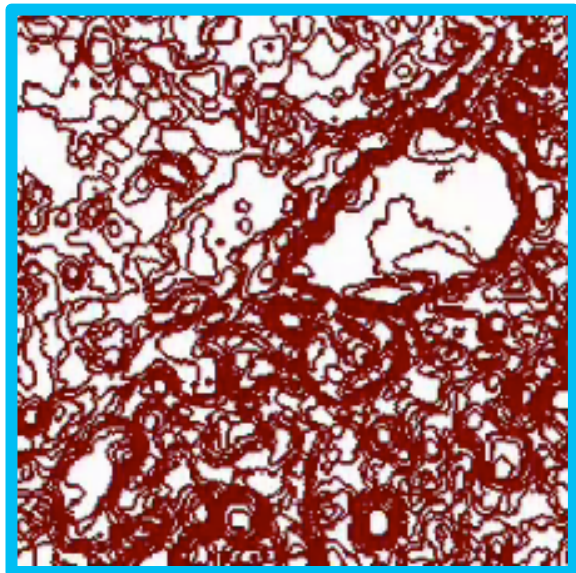
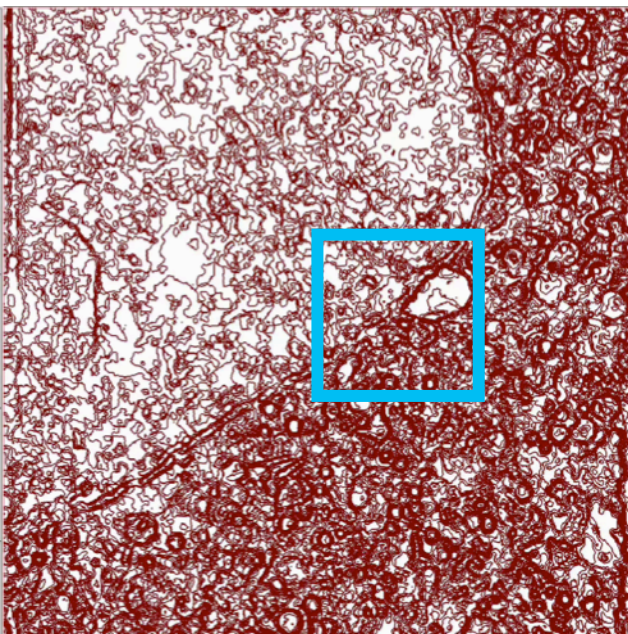
10x10x10 / 1x1x1 / 20



10x10x10 / **3x3x3** / 20

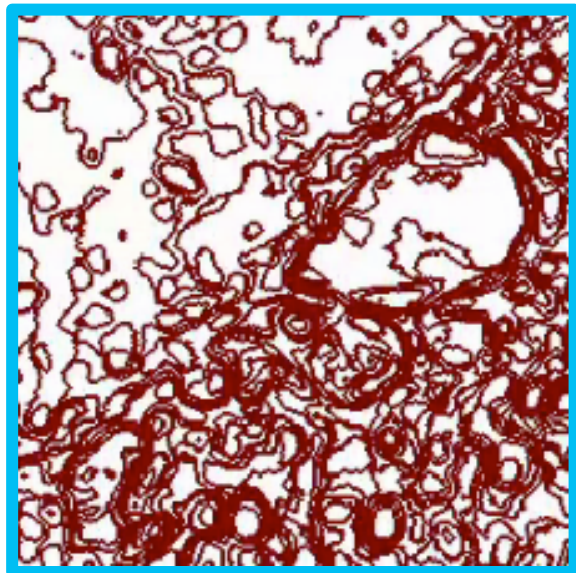
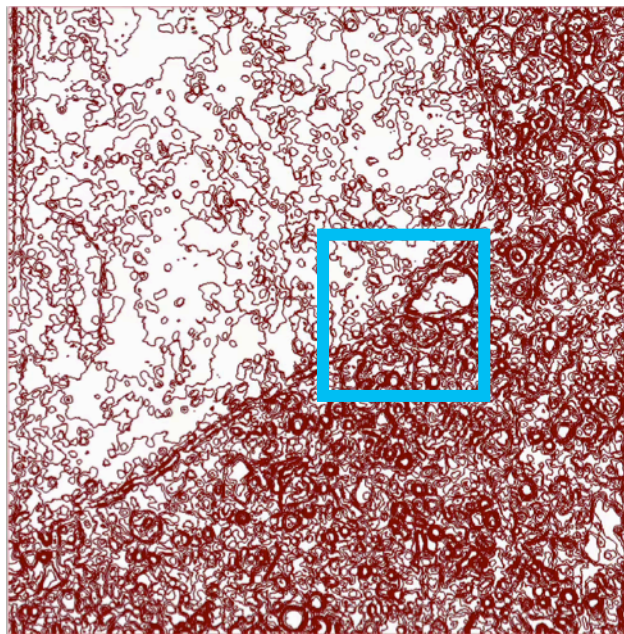


5x5x5 / 1x1x1 / 20

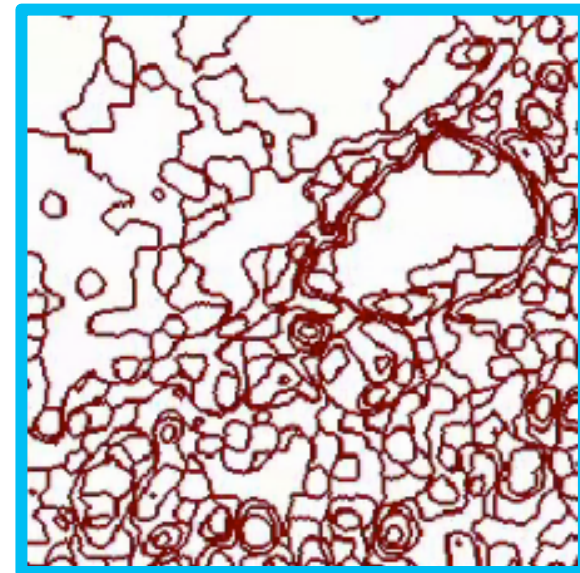
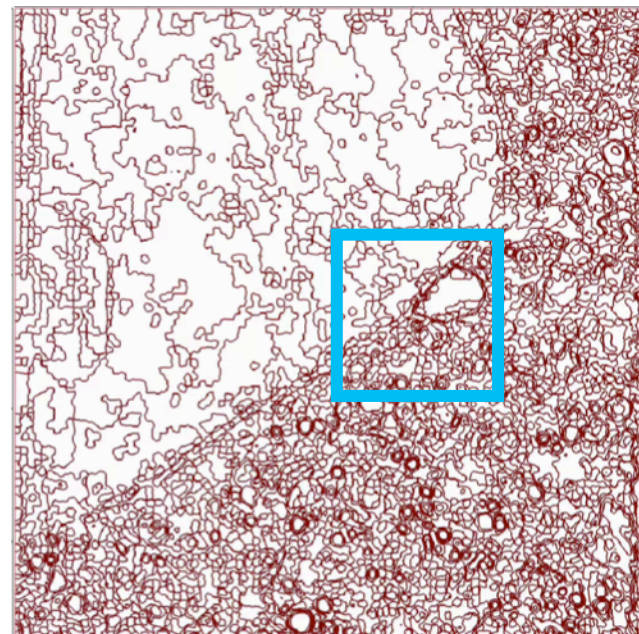


Default:

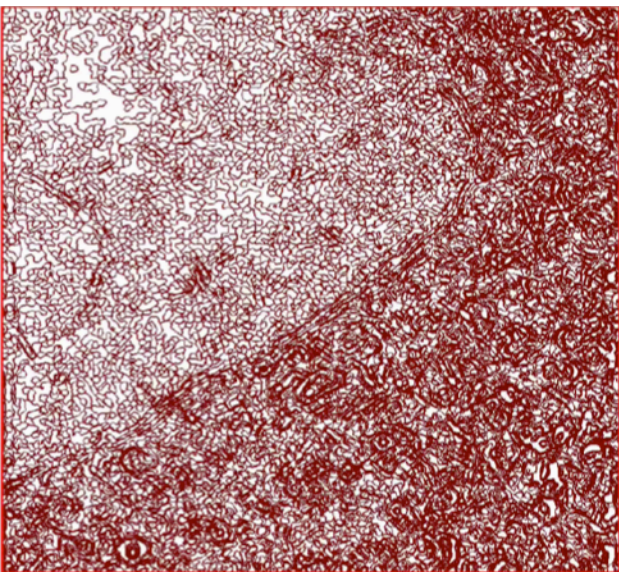
10x10x10 / 1x1x1 / 20



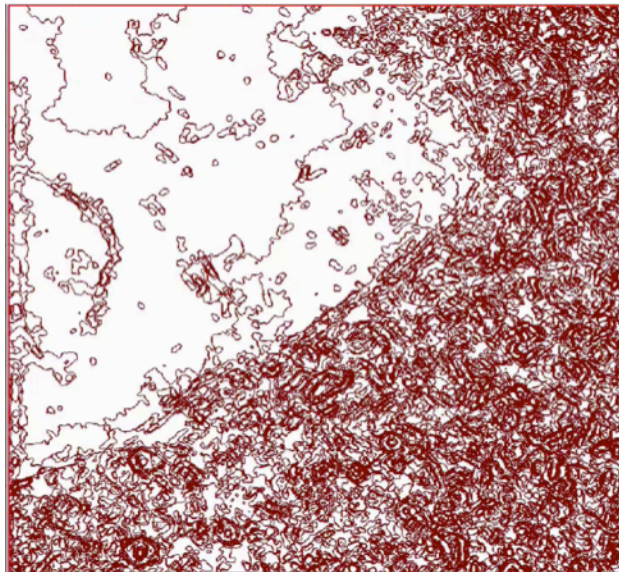
10x10x10 / 3x3x3 / 20



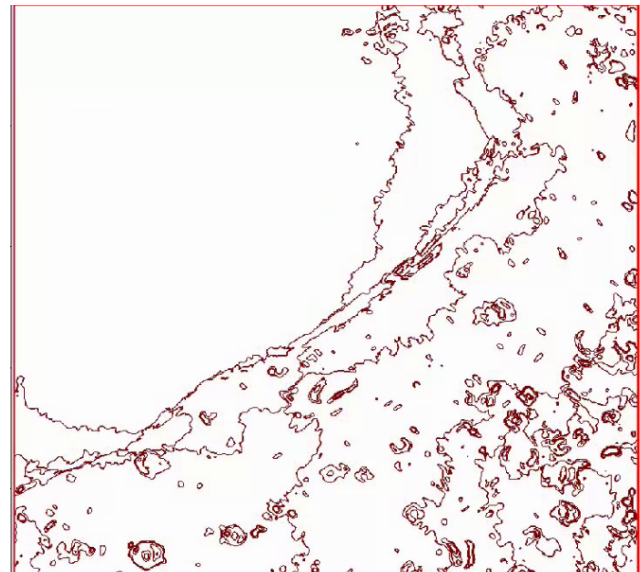
$\lambda=0.01$ /NumBins=20



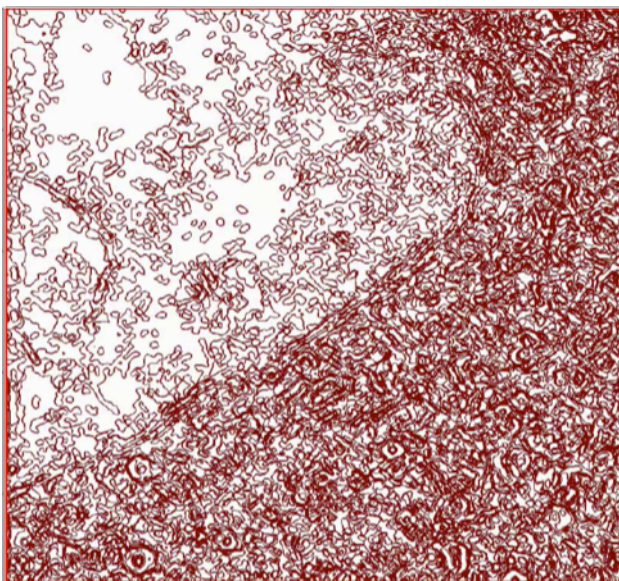
Default:
 $\lambda=0.1$ /NumBins=20



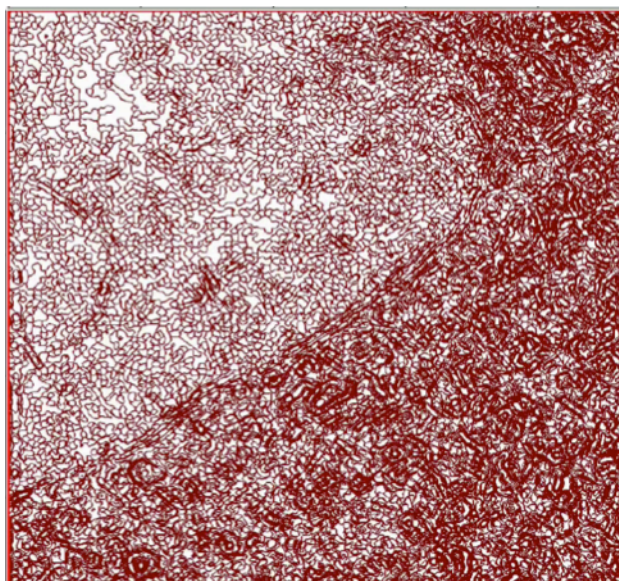
$\lambda=1$ /NumBins=20



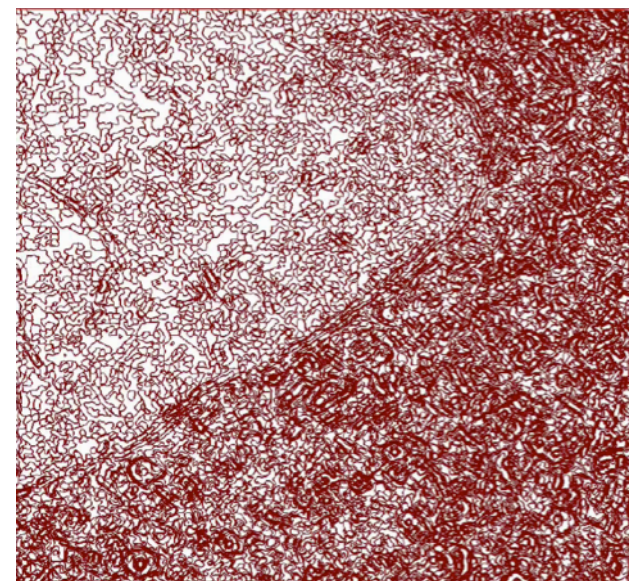
$\lambda=0.1$ /NumBins=10



$\lambda=0.1$ /NumBins=30



Defaults, γ =auto



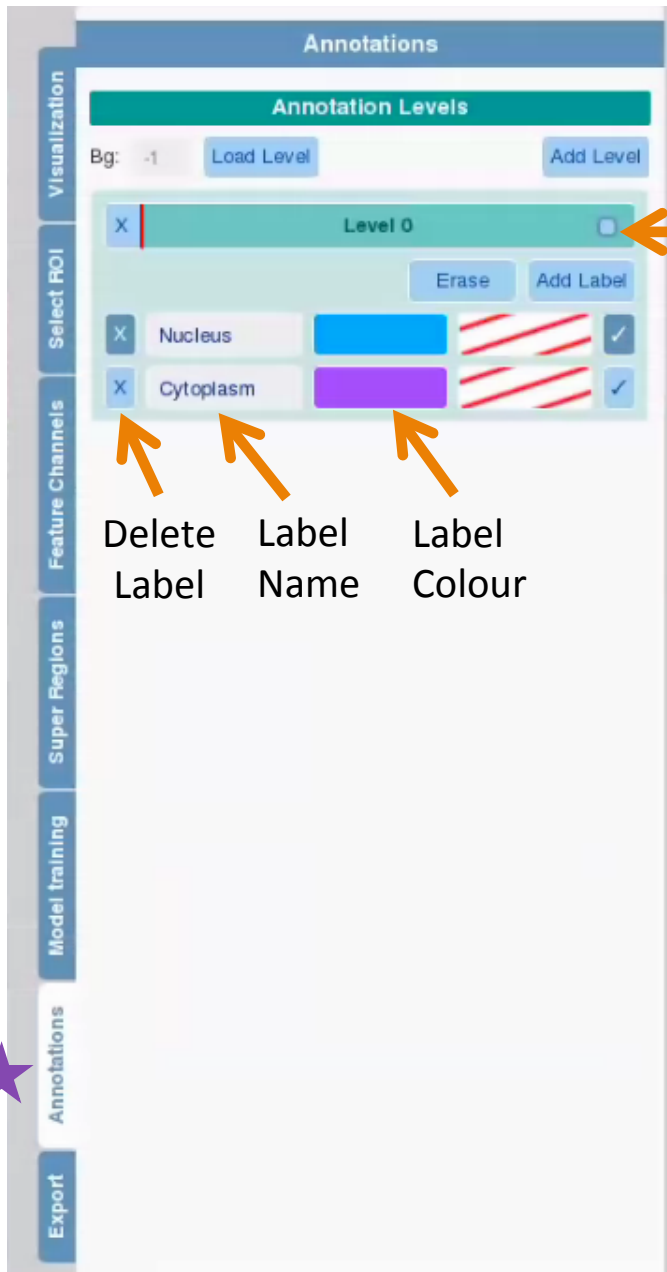
Time – 15 minutes

The Annotation Tab



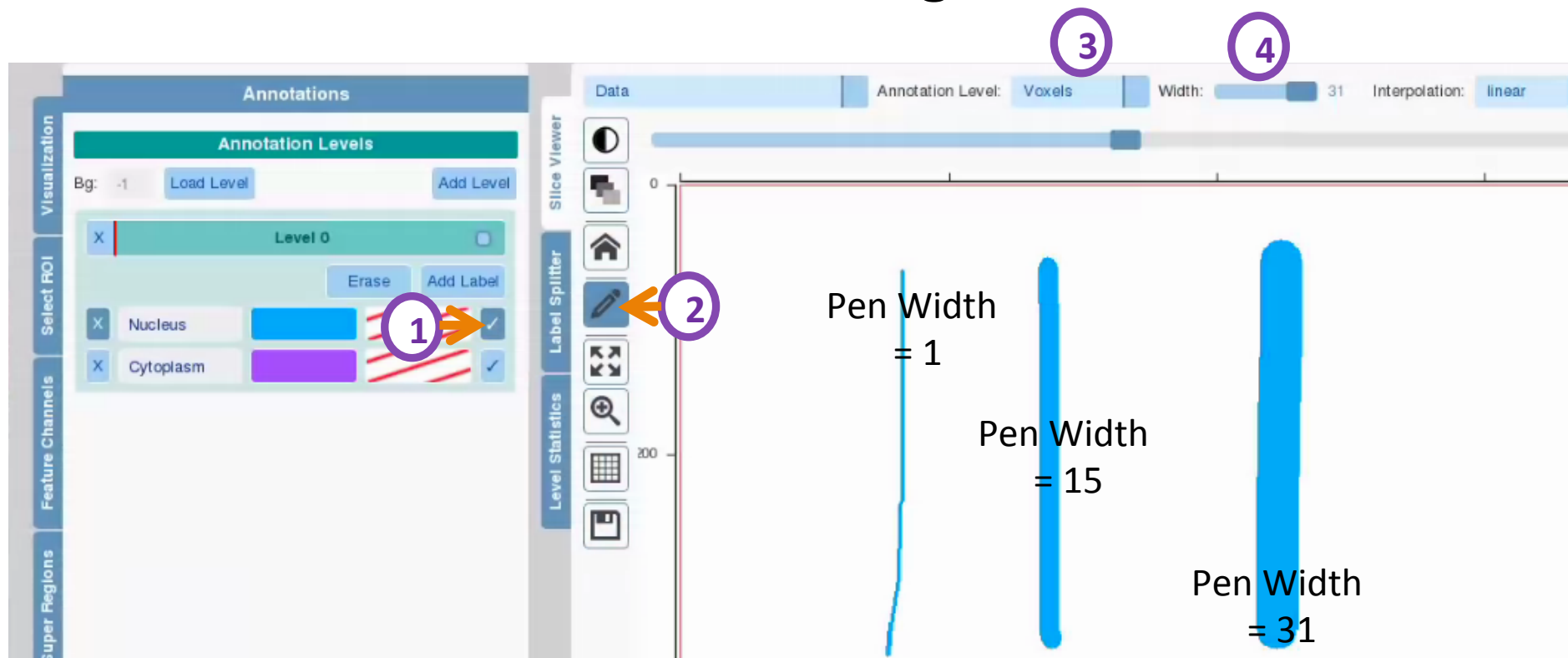
- To add a Level click on Add Level.
- To add Labels to a Level, click Add Label. Multiple labels can be added to any Level.

The Annotation Tab



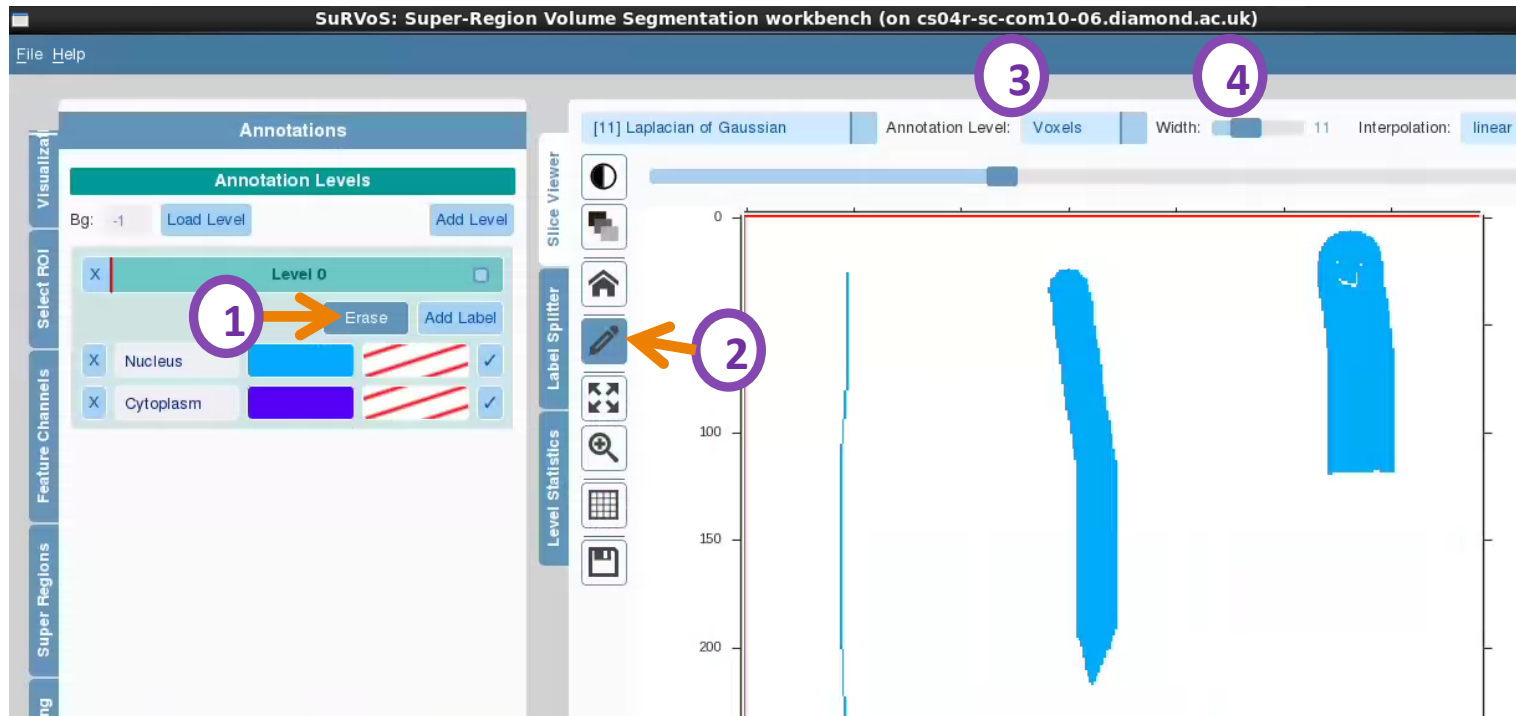
- To add a Level click on Add Level.
- To add Labels to a Level, click Add Label. Multiple labels can be added to any Level.
- Labels can be given names, the colour used to represent them in annotations can be changed. Or if they are no longer needed, labels can be deleted.

Annotation Using Voxels



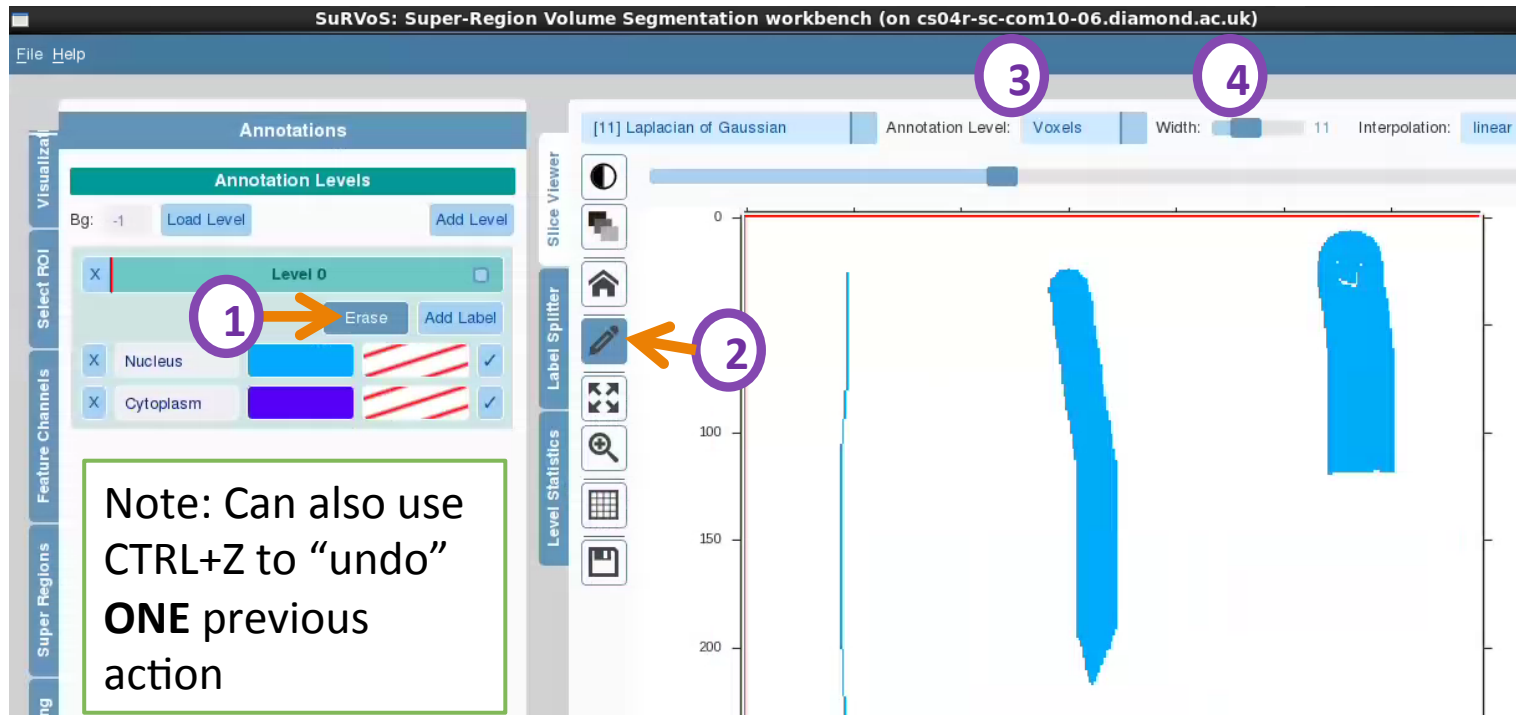
- 1) In the annotation tab select the label you wish to annotate with
- 2) Select the pencil tool
- 3) Select annotation level (**voxel**, supervoxel, or megavoxel)
- 4) Choose an appropriate width for the feature that you are annotating and draw using the left mouse button

Erasing Annotations



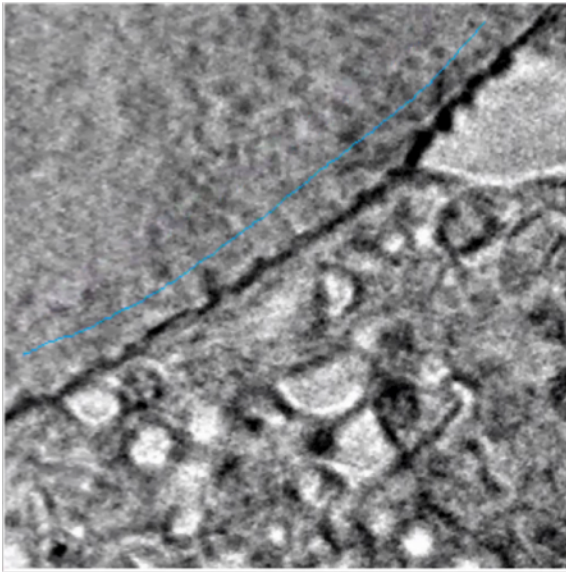
- 1) In the annotation tab select erase on the level you wish to use the use the eraser on
- 2) Select the pencil tool (if not already selected)
- 3) Select annotation level (voxel, supervoxel, or megavoxel)
- 4) Choose an appropriate width for the feature that you are erasing and erase using the left mouse button
- 5) To begin annotating again, select the appropriate label and parameters.

Erasing Annotations

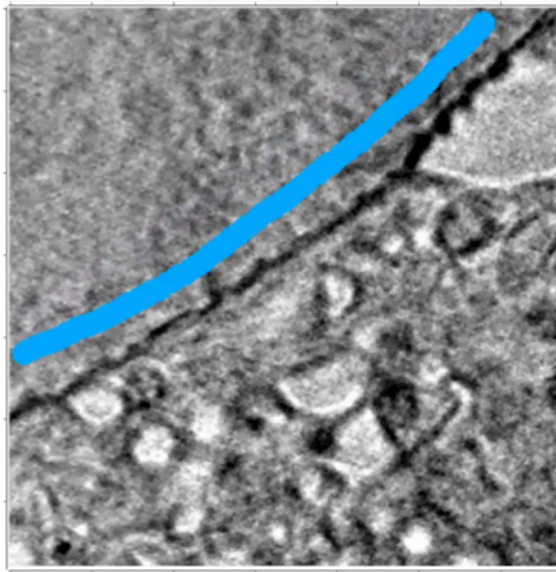


- 1) In the annotation tab select erase on the level you wish to use the use the eraser on
- 2) Select the pencil tool (if not already selected)
- 3) Select annotation level (voxel, supervoxel, or megavoxel)
- 4) Choose an appropriate width for the feature that you are erasing and erase using the left mouse button
- 5) To begin annotating again, select the appropriate label and parameters.

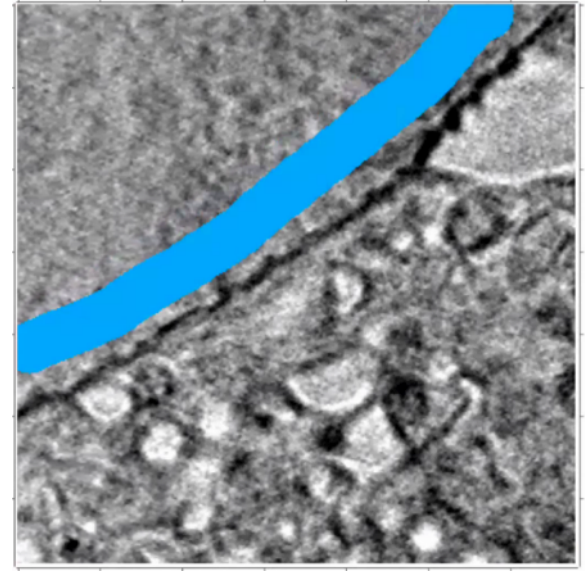
Annotation Using Voxels



Pen Width = 1



Pen Width = 15

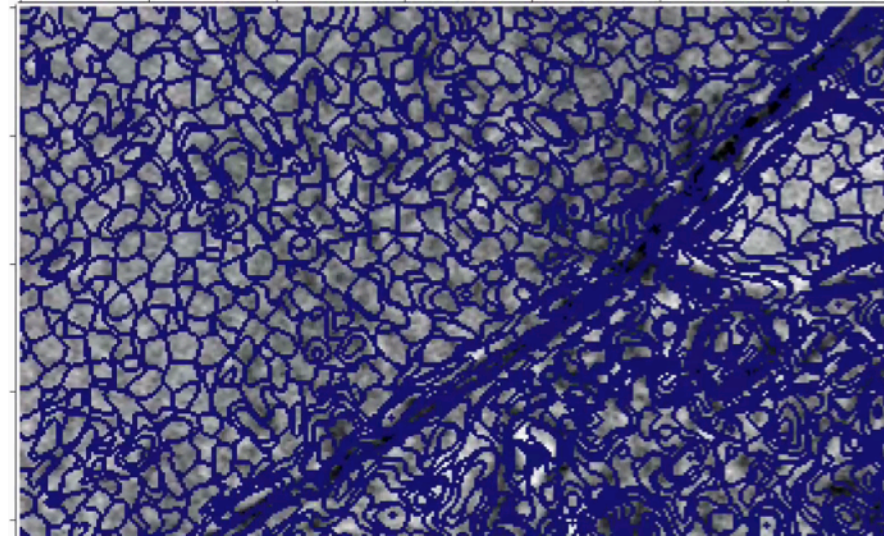
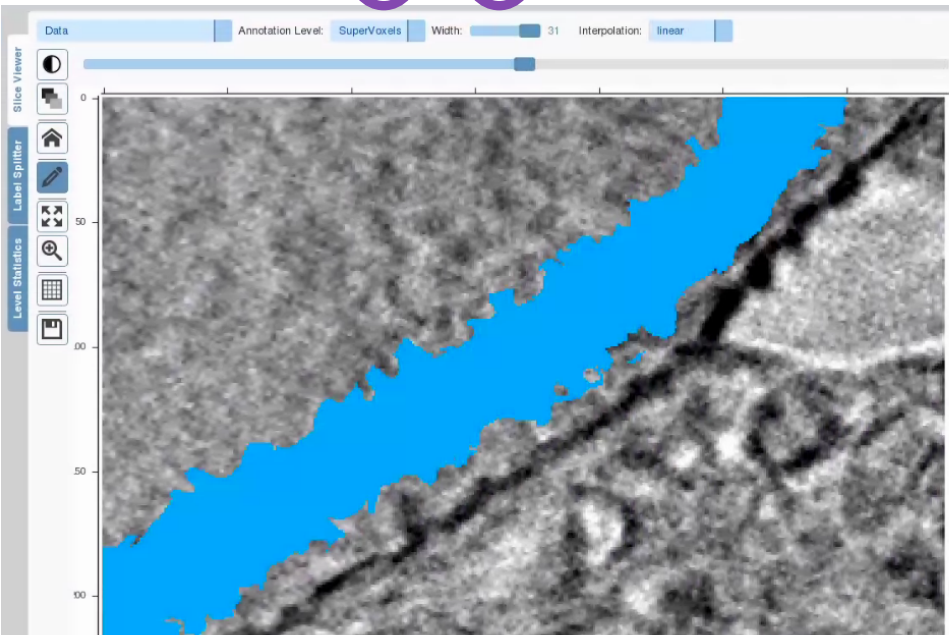


Pen Width = 31

- Annotation using voxels can be done with different pen widths.
- Whilst annotating in voxels annotations do not penetrate the volume in Z

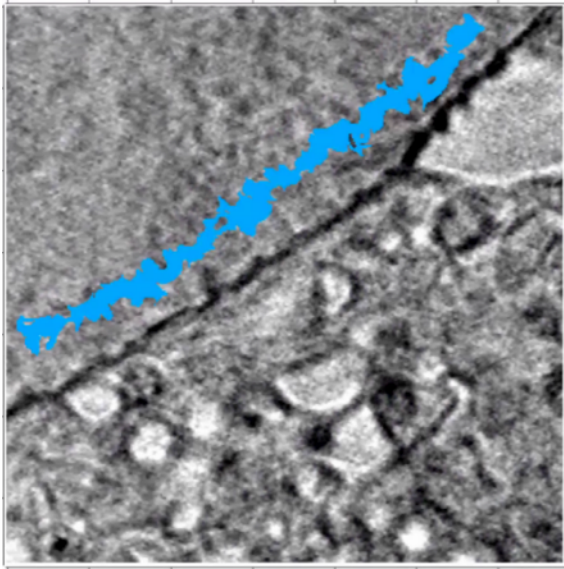
Annotation Using Supervoxels

① ②

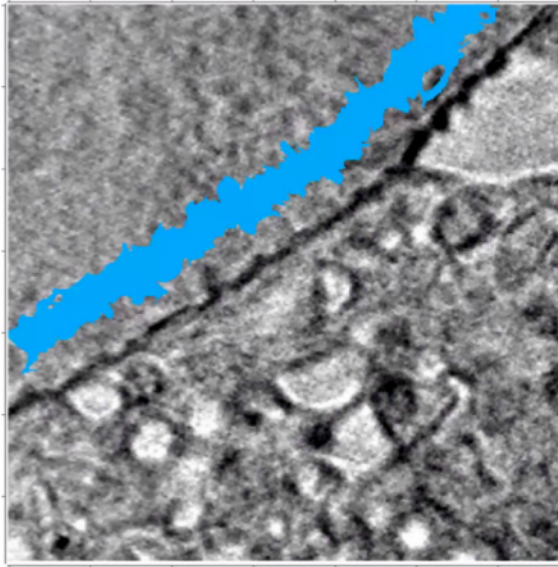


- 1) Select annotation level (voxel, **supervoxel**, or megavoxel)
- 2) Choose a width appropriate for the feature that you are annotating and draw using the left mouse button.

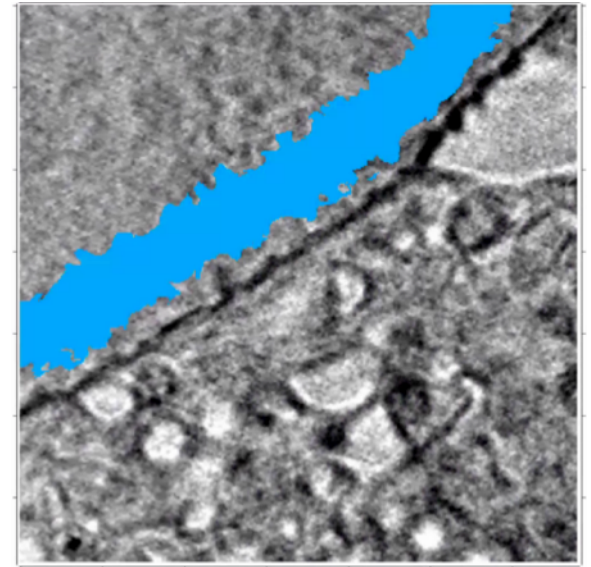
Annotation Using Supervoxels



Pen Width = 1

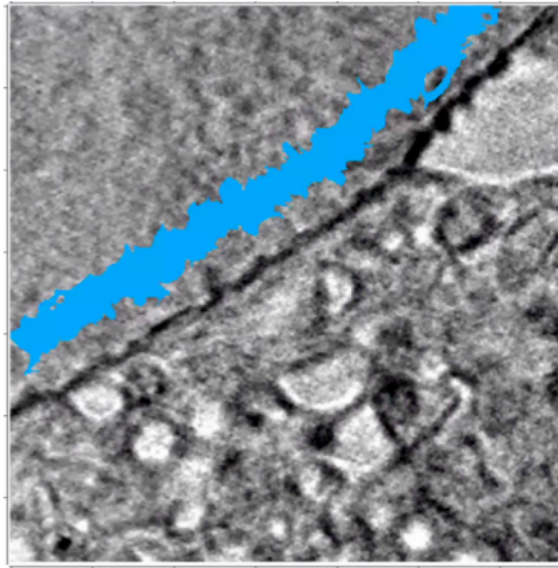


Pen Width = 15



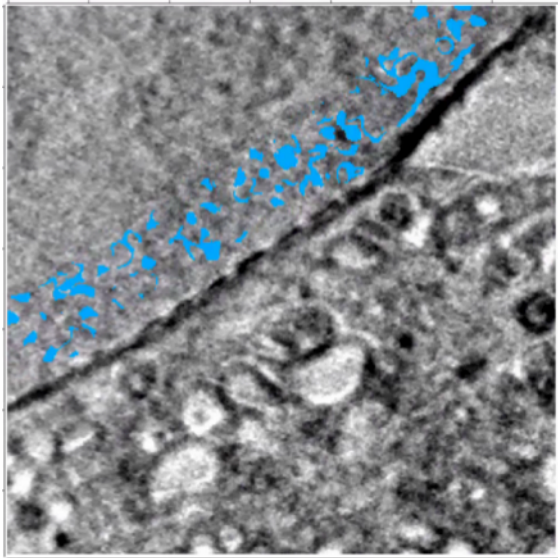
Pen Width = 31

Annotation Using Supervoxels

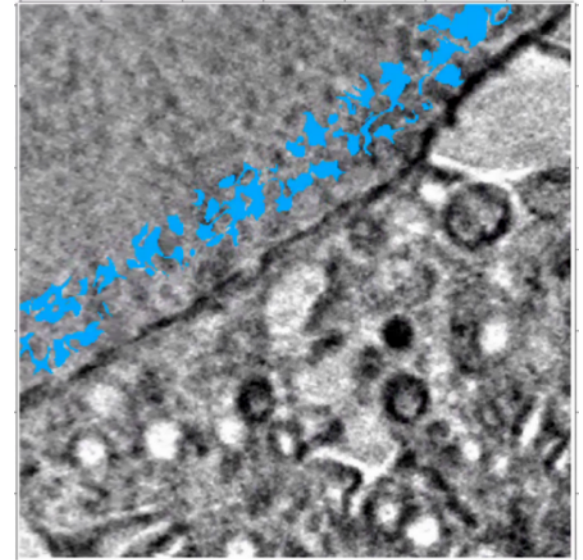


Pen Width = 15
Center Slice

10 slices below



10 slices above

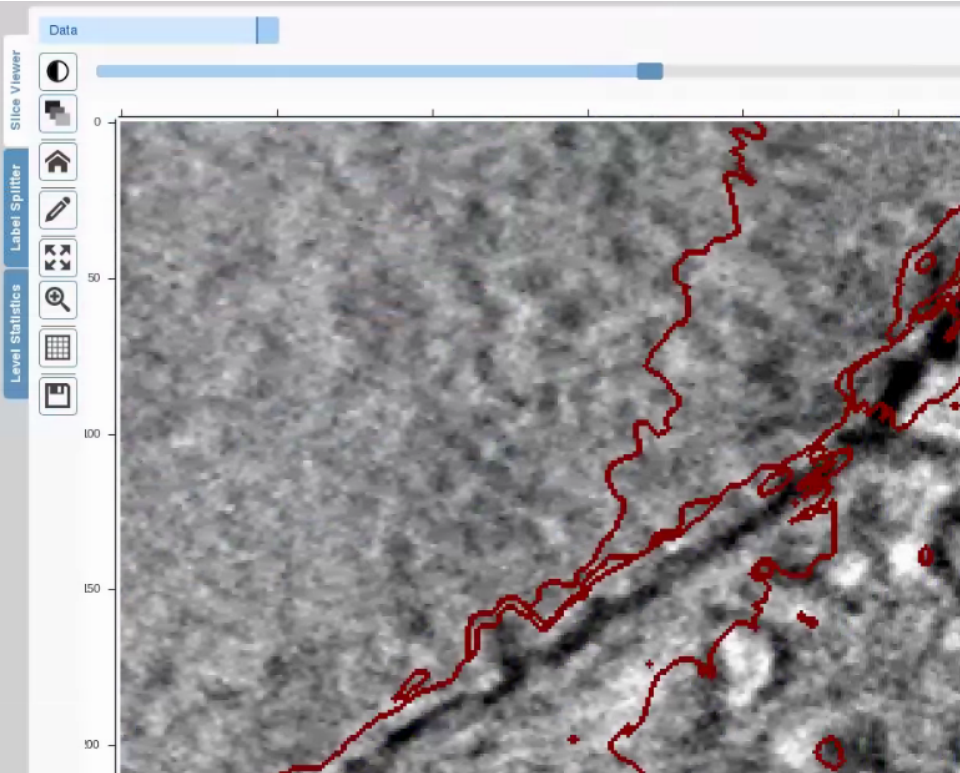
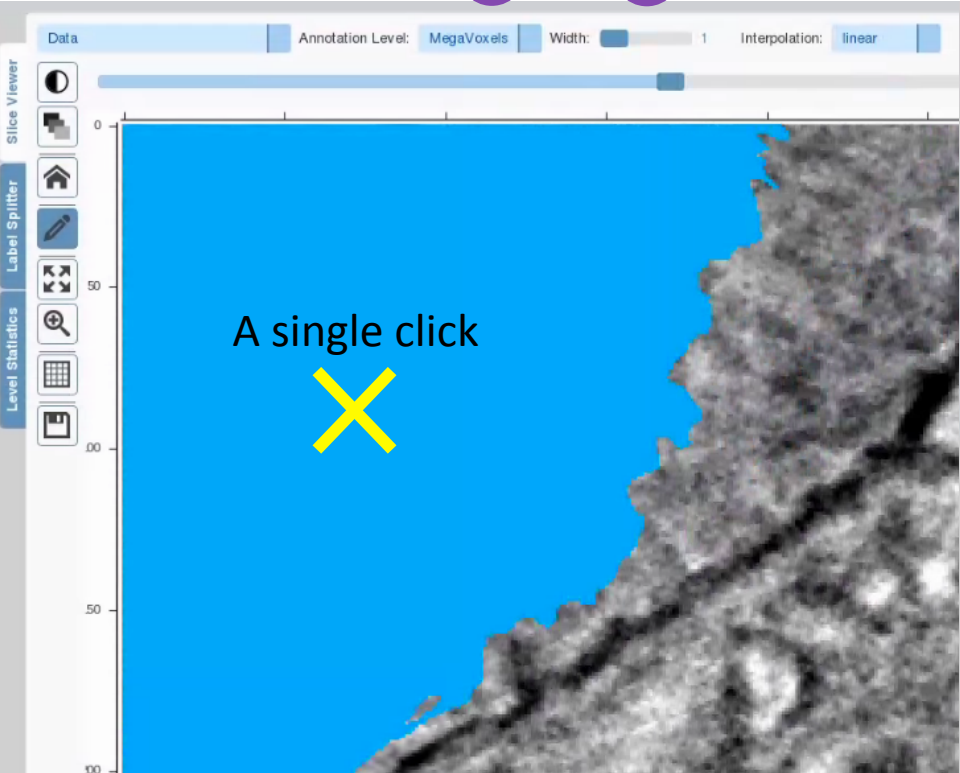


Annotating using
supervoxels penetrates
multiple Z-slices

Annotation using Megavoxels

①

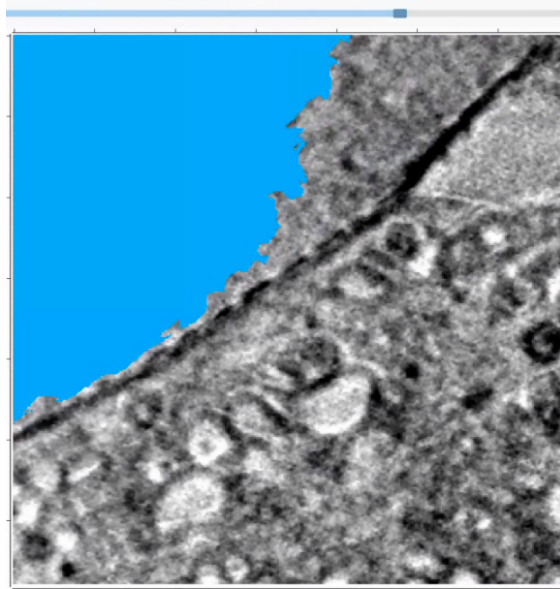
②



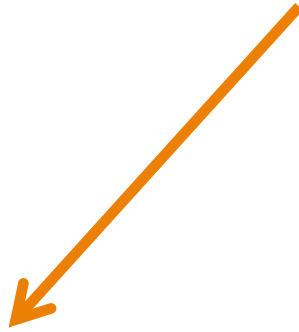
1) Select annotation level (voxel, supervoxel, or **megavoxel**)

2) Choose a width appropriate for the feature that you are annotating and draw using the left mouse button.

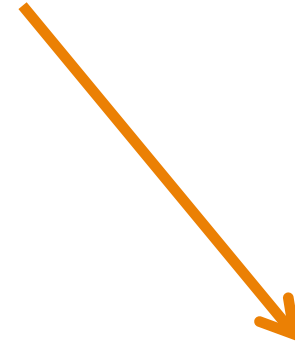
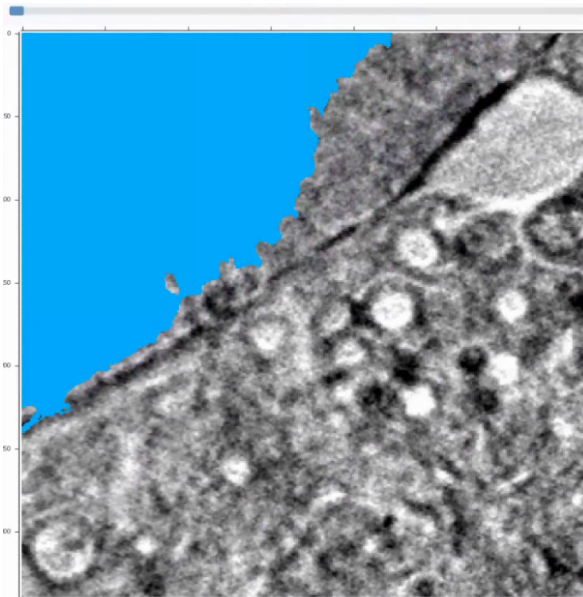
Annotation using Megavoxels



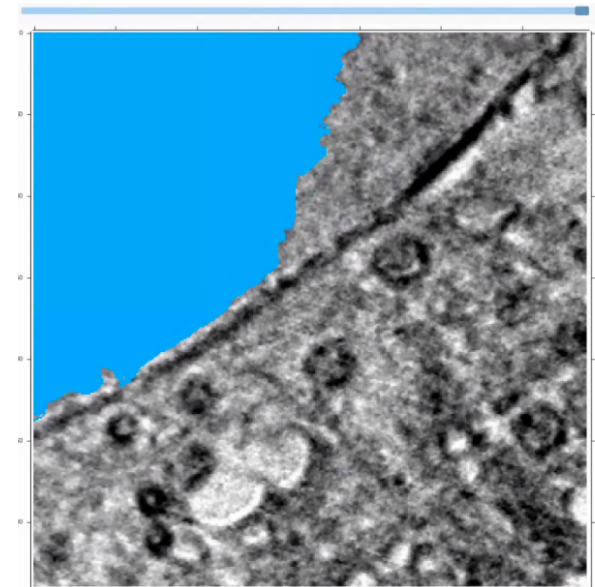
Center Slice



First Slice in Stack



Last Slice in Stack



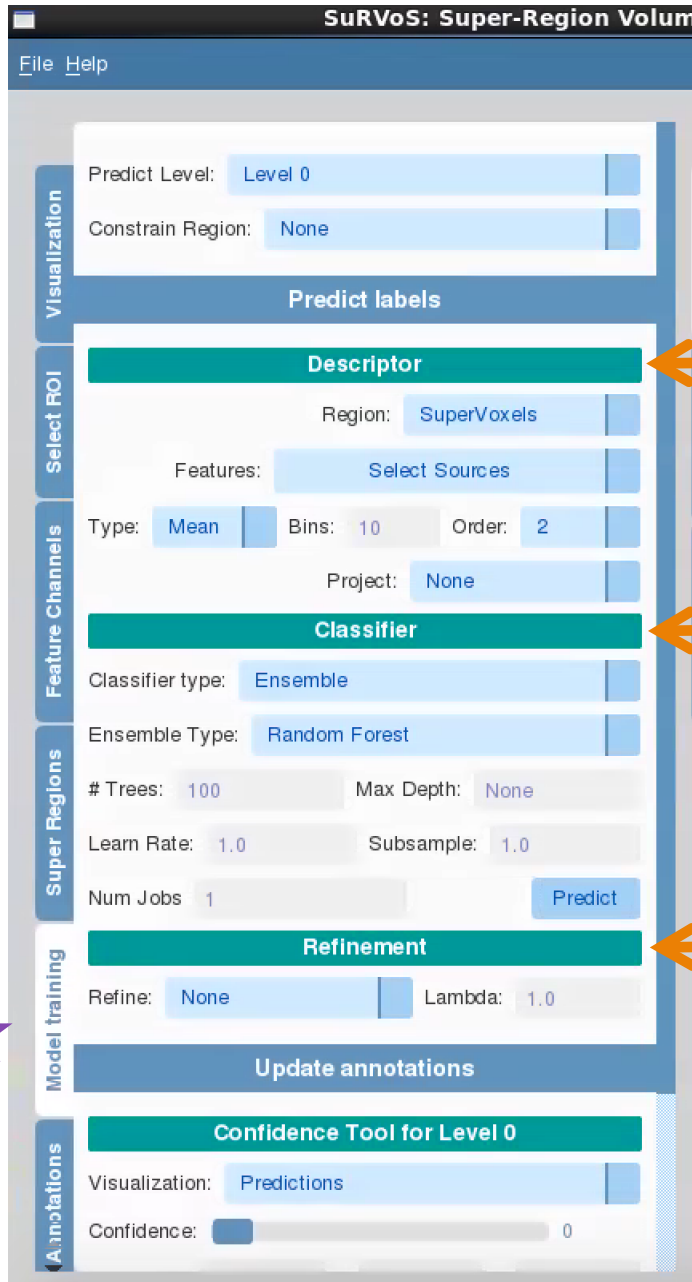
Annotating using
megavoxels penetrates
multiple Z-slices

Reminder: Visualization Shortcut



Time – 10 minutes

Model Training Introduction



SuRVoS: Super-Region Volume

File Help

Visualization

Predict Level: Level 0

Constrain Region: None

Predict labels

Descriptor

Region: SuperVoxels

Features: Select Sources

Type: Mean Bins: 10 Order: 2

Project: None

Classifier

Classifier type: Ensemble

Ensemble Type: Random Forest

Trees: 100 Max Depth: None

Learn Rate: 1.0 Subsample: 1.0

Num Jobs: 1 Predict

Refinement

Refine: None Lambda: 1.0

Update annotations

Confidence Tool for Level 0

Visualization: Predictions

Confidence: 0

Describes the data at the region level you choose.

Learns a model from the descriptor and classifies the unlabeled data based on this model.

Improves the prediction of the resultant model.

Model Training: How to Use Features

Small
amount of
annotations

Large
amount of
annotations



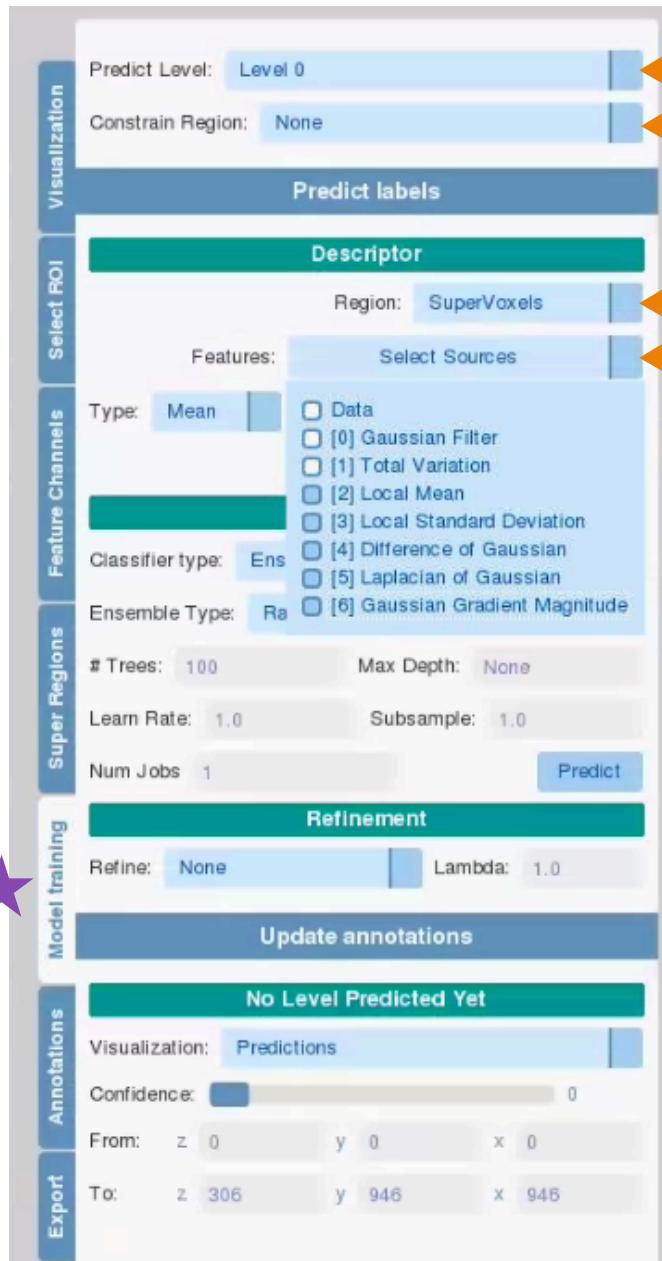
A few robust features

- Blob Detection
- Texture and Structure
- Robust Features

More, varied features

- Any available, as long as you use more of them

Model Training Introduction



The screenshot shows a web-based interface for model training. On the left is a vertical sidebar with tabs: Visualization, Select ROI, Feature Channels, Super Regions, Model training (marked with a purple star), Annotations, and Export. The main panel is divided into sections: Visualization (Predict Level: Level 0, Constrain Region: None), Predictor (Descriptor, Region: SuperVoxels, Features: Select Sources), Feature Channels (Type: Mean, Classifier type: Ens, Ensemble Type: Ra, a list of feature filters), Super Regions (# Trees: 100, Max Depth: None, Learn Rate: 1.0, Subsample: 1.0, Num Jobs: 1, Predict button), Refinement (Refine: None, Lambda: 1.0), Update annotations, No Level Predicted Yet, Visualization: Predictions, Confidence: 0, and From/To coordinates.

Choose which level to train with

Optional (can constrain model training region based on annotation levels)

Choose to train with either voxels or supervoxels

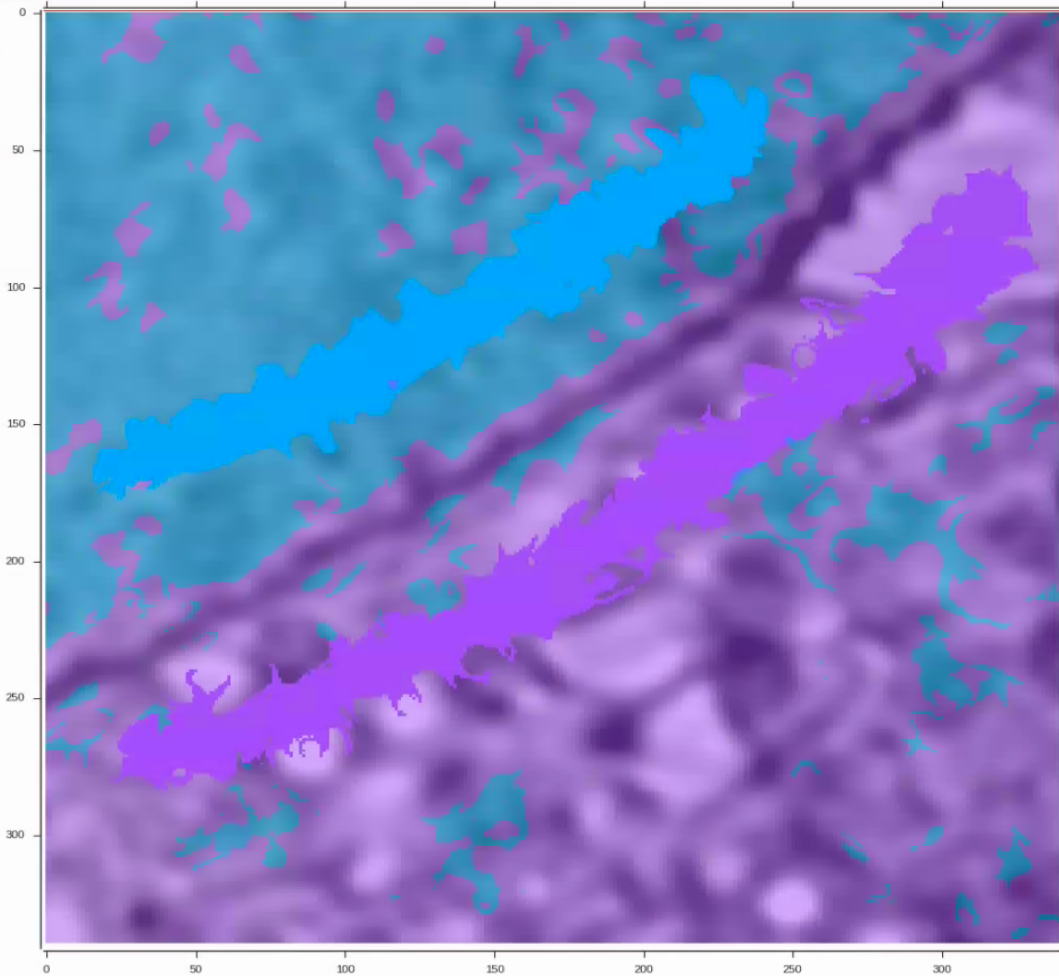
Choose sources to train the descriptor

- Generally want to choose as many sources as possible
- Generally do not choose raw data
- For large areas try: Total Variation, Blob Detection, Texture and Structure, and Robust Feature Filters
- For small areas try: the above, plus Local Feature and Gaussian Feature Filters

Note:

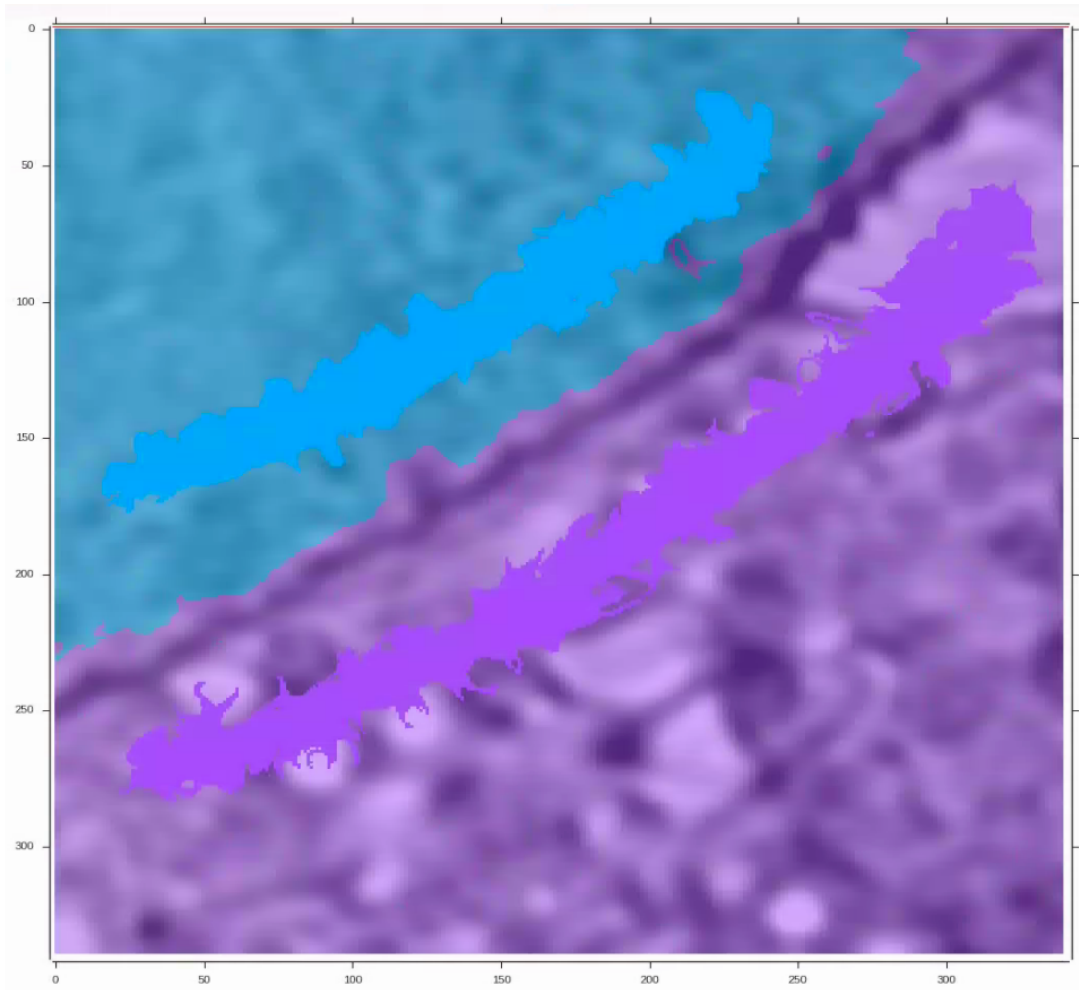
- Smaller objects will require more input annotations to accurately train the model.
- Model training with voxels will be quite slow for large region of interest.

Model Training Options



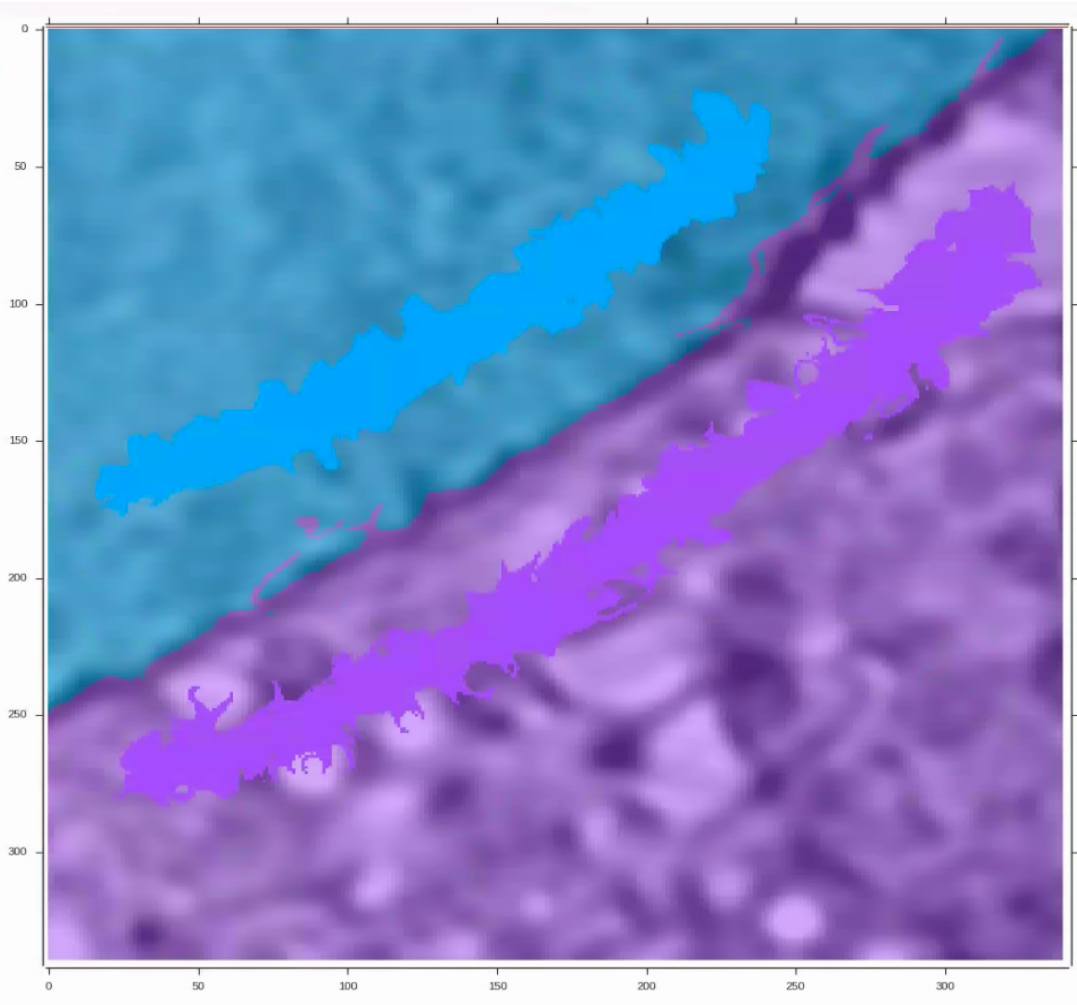
Classifier: Ensemble
Ensemble Type: Random Forest
(with defaults)
Refine: None

Model Training Options



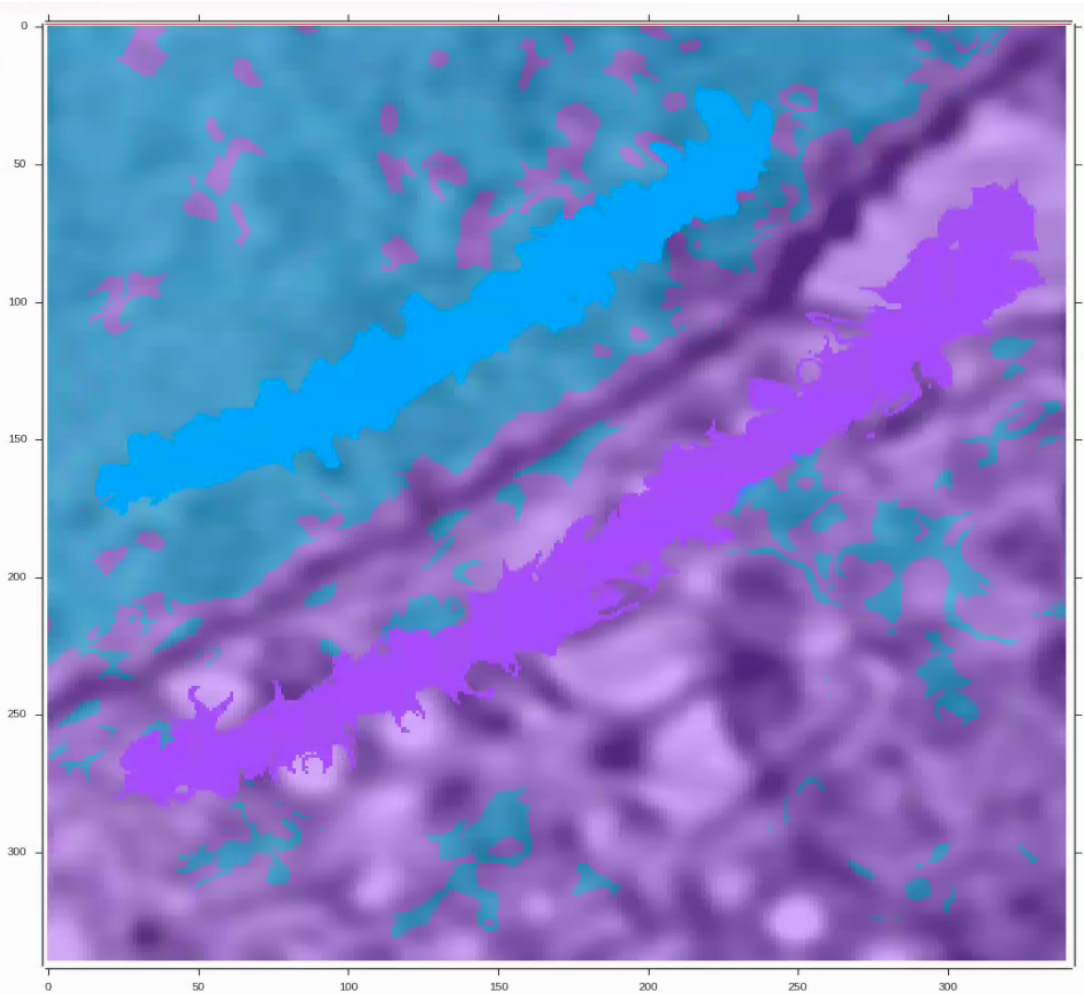
Classifier: Ensemble
Ensemble Type: Random Forest (with defaults)
Refine: Potts (50)

Model Training Options



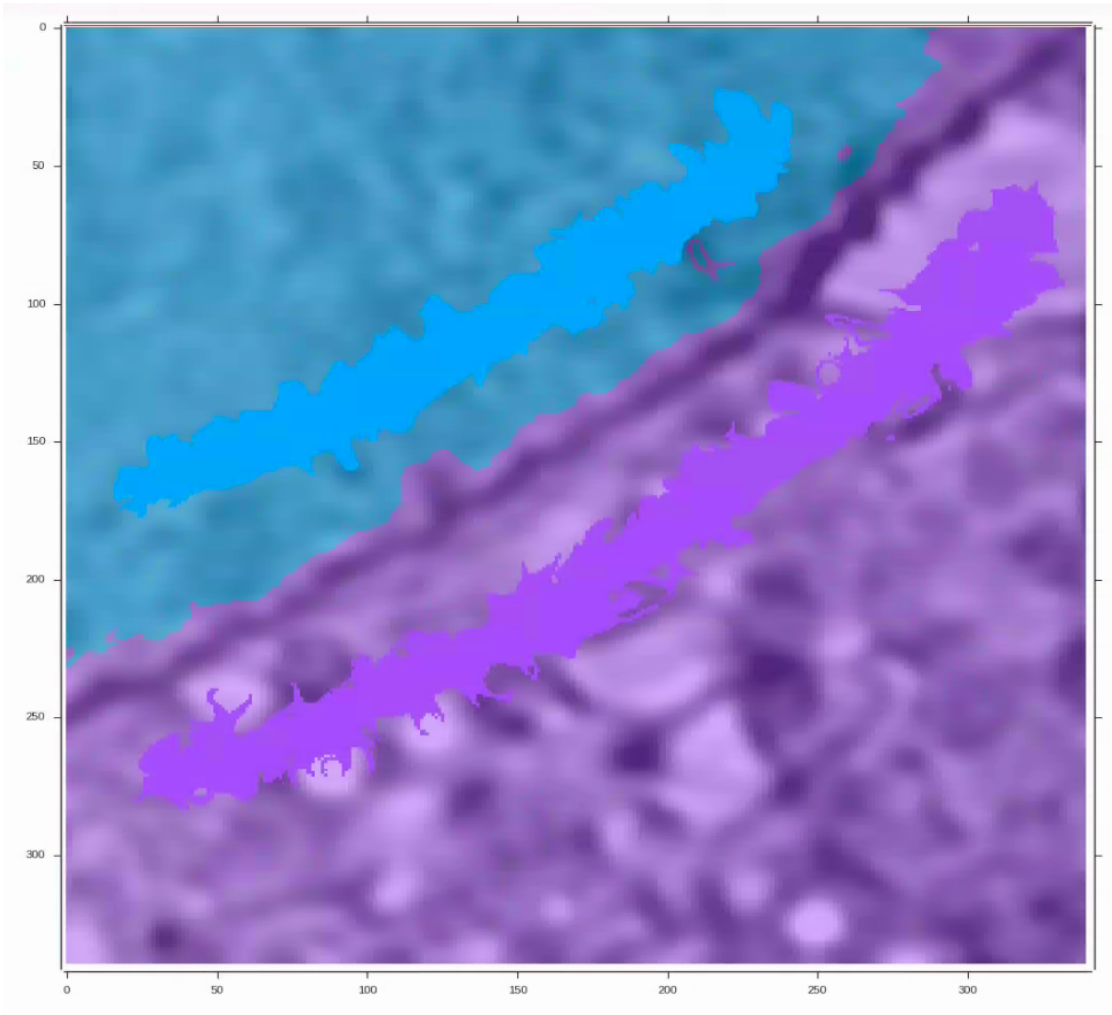
Classifier: Ensemble
Ensemble Type: Random Forest (with defaults)
Refine: Appearance (50)

Model Training Options



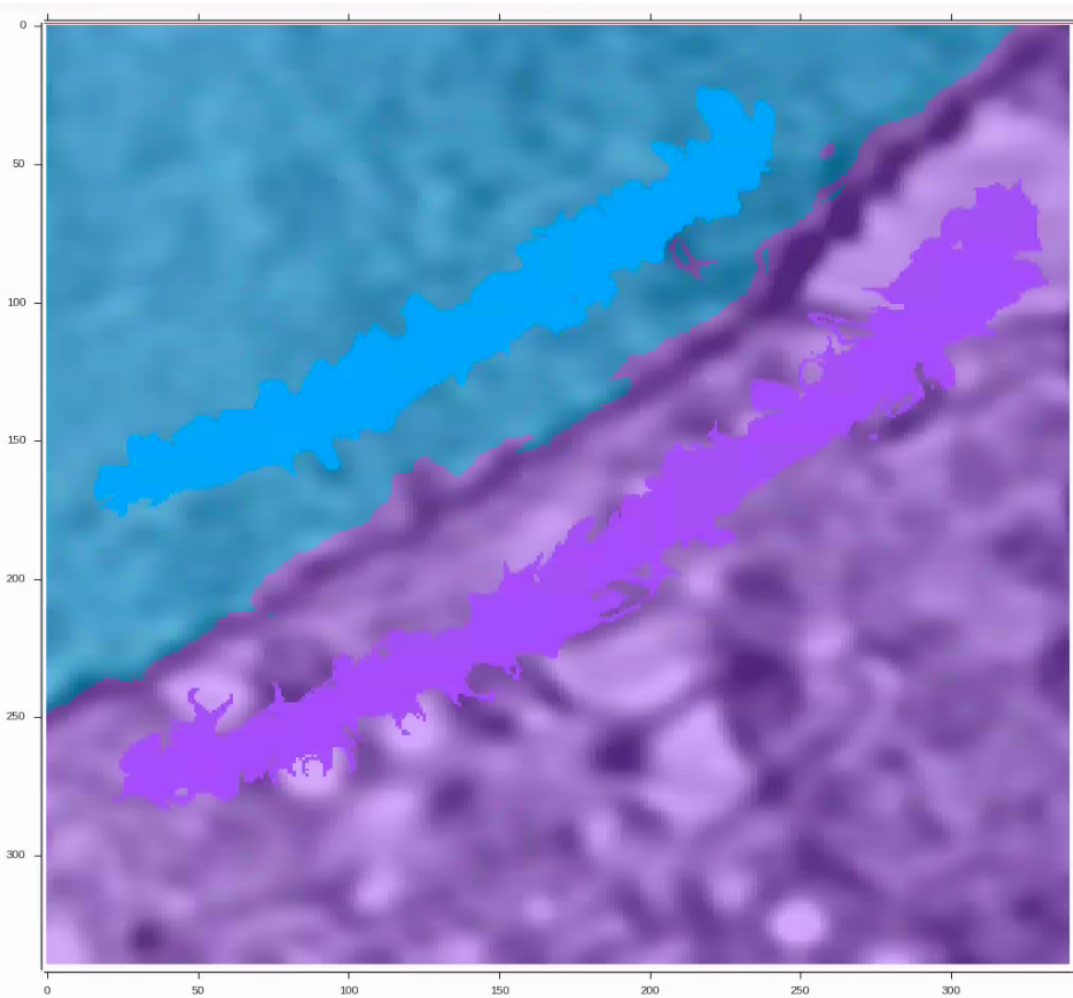
Classifier: Ensemble
Ensemble Type: Extra Random Forest
(with defaults)
Refine: None

Model Training Options



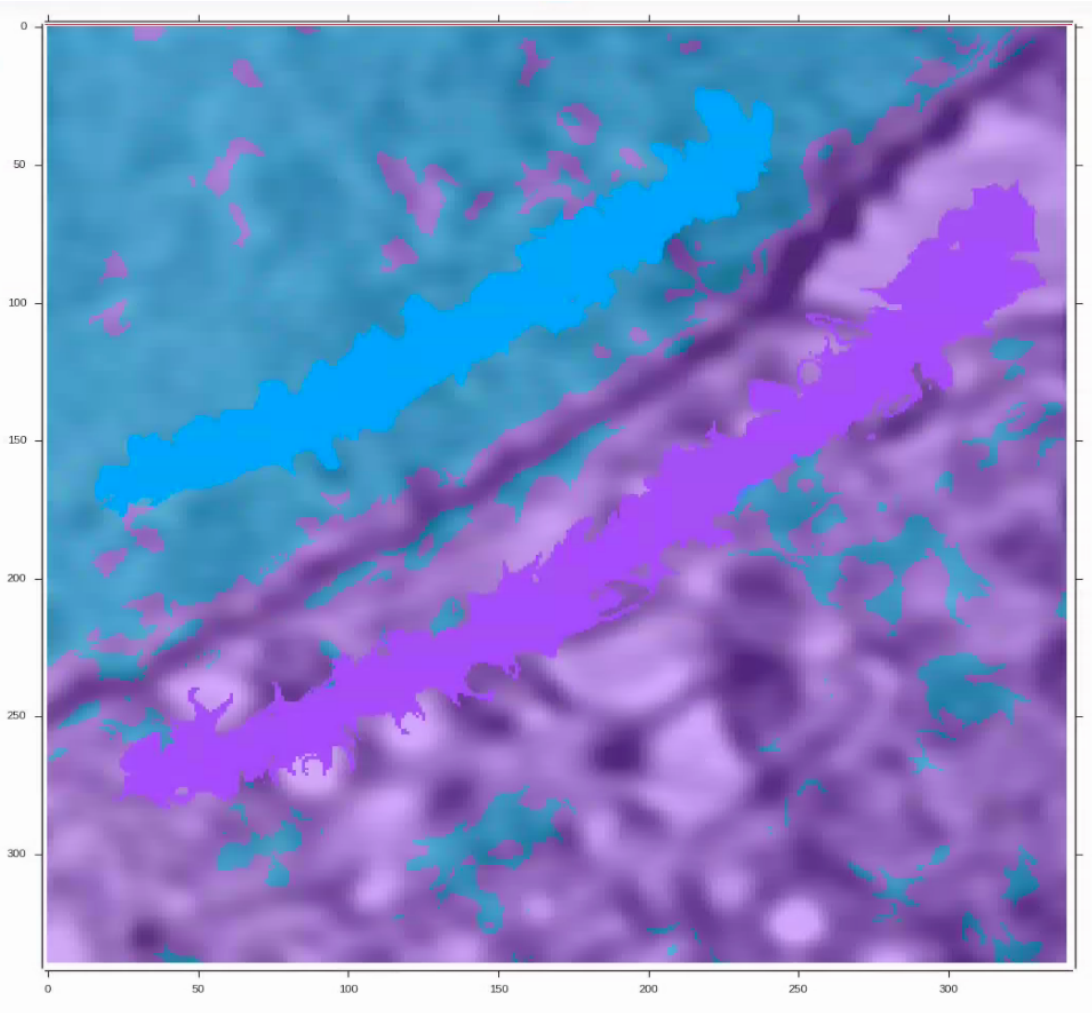
Classifier: Ensemble
Ensemble Type: Extra Random Forest
(with defaults)
Refine: Potts (50)

Model Training Options



Classifier: Ensemble
Ensemble Type: Extra Random Forest
(with defaults)
Refine: Appearance (50)

Model Training Options

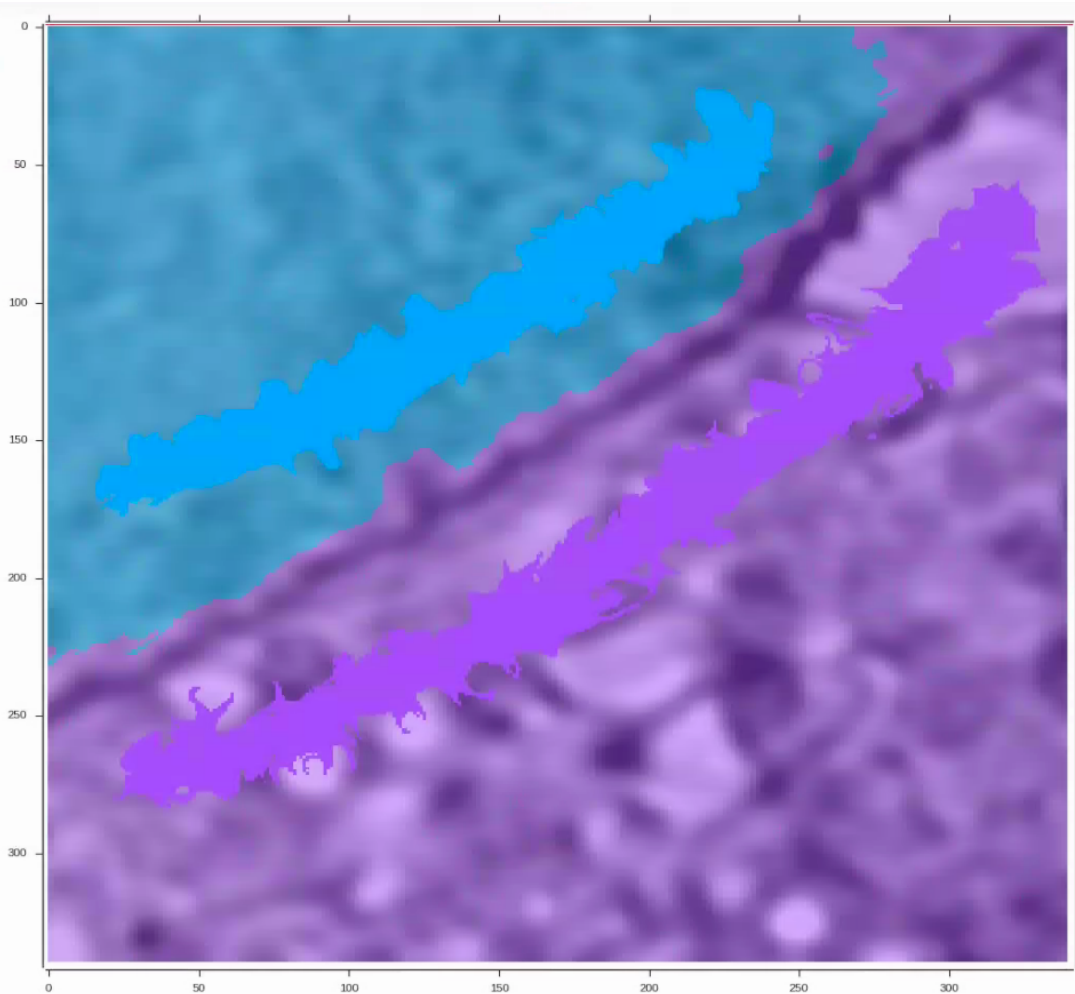


Classifier: SVM

Kernel Type: rbf (with defaults)

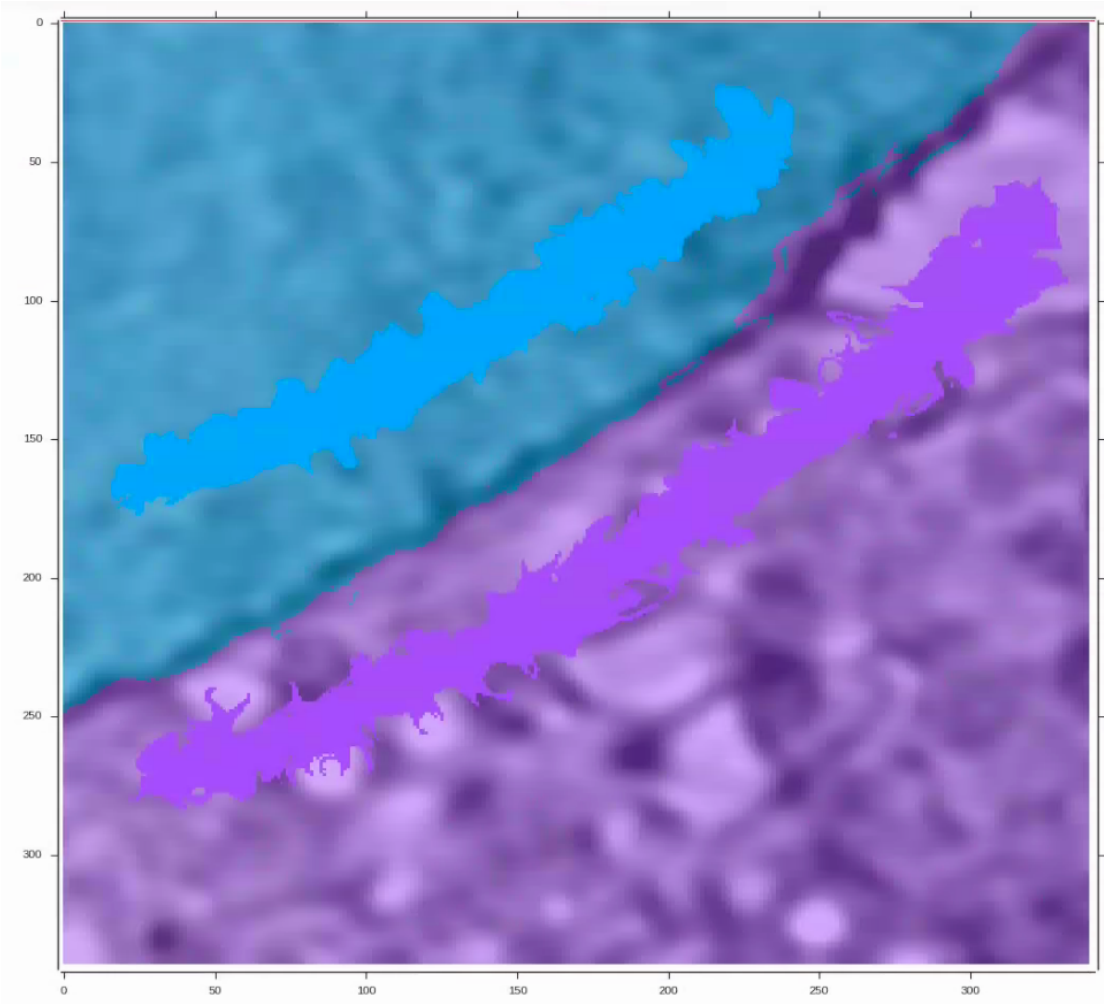
Refine: None

Model Training Options



Classifier: SVM
Kernel Type: rbf (with defaults)
Refine: Potts (50)

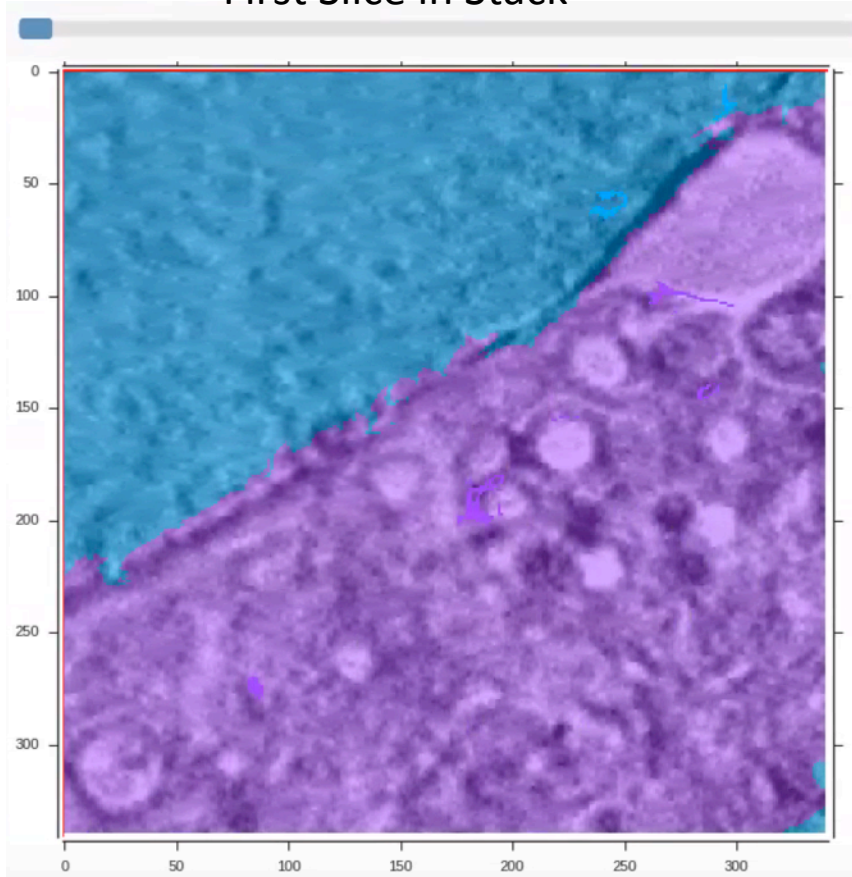
Model Training Options



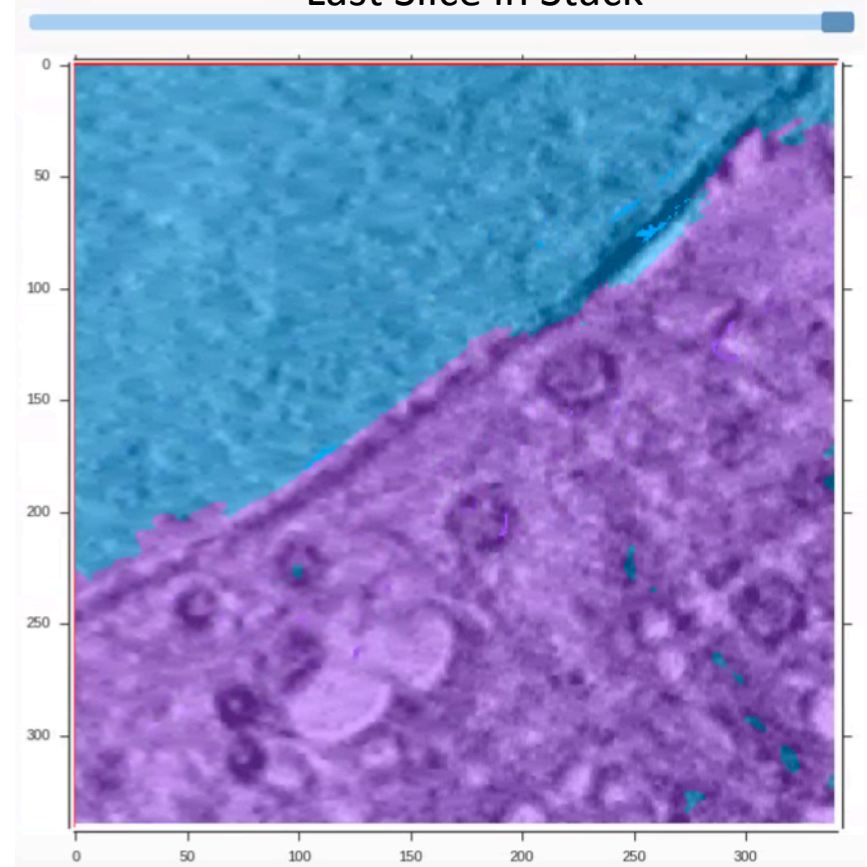
Classifier: SVM
Kernel Type: rbf (with defaults)
Refine: Appearance (50)

Model Training is in 3D

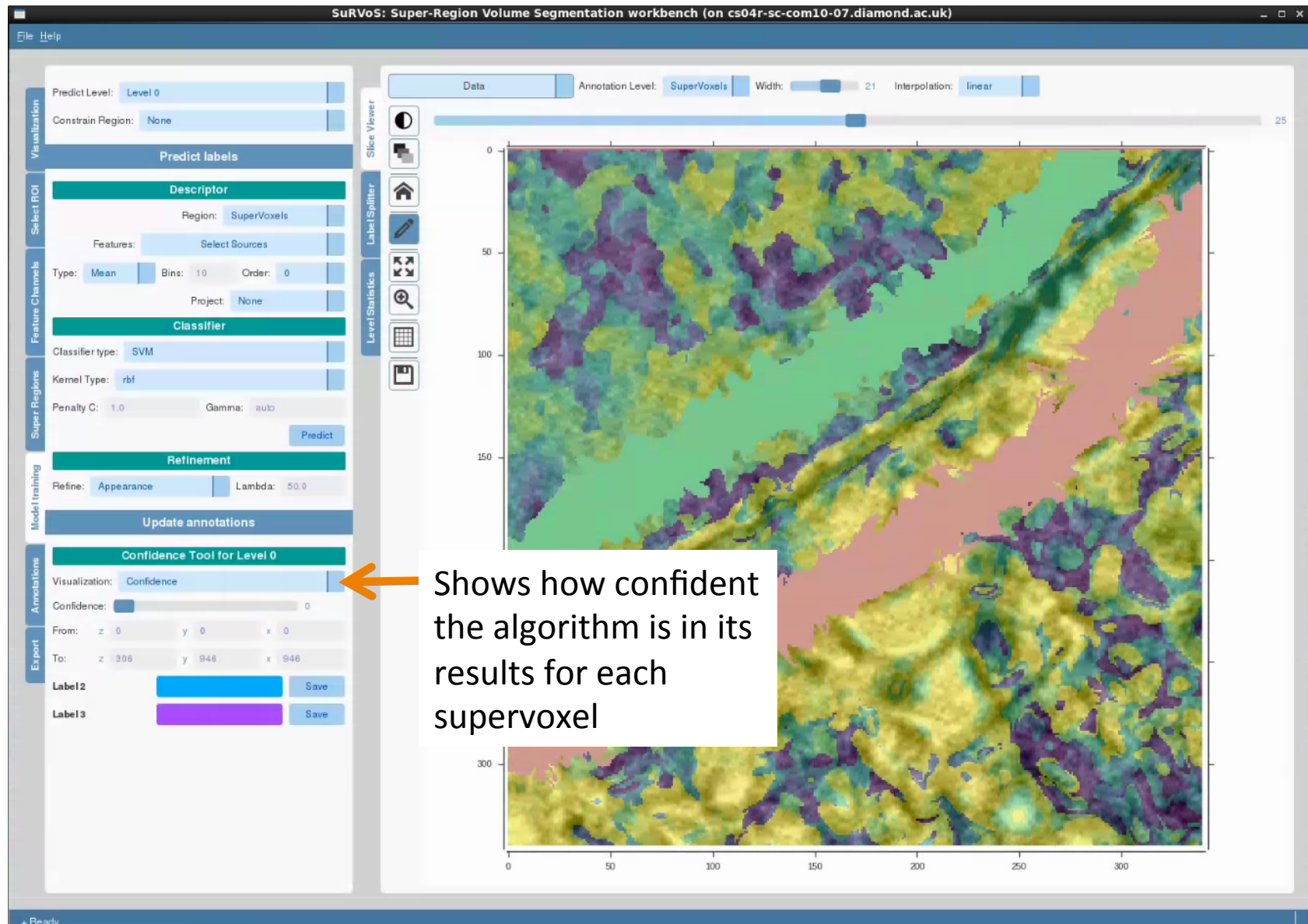
First Slice in Stack



Last Slice in Stack



Model Training: Confidence and Update Annotations



Model Training: Confidence and Update Annotations

SuRVoS: Super-Region Volume Segmentation workbench (on cs04r-sc-com10-07.diamond.ac.uk)

File Help

Predict Level: Level 0
Constrain Region: None

Predict labels

Descriptor
Region: SuperVoxels
Features: Select Sources
Type: Mean Bins: 10 Order: 0
Project: None

Classifier
Classifier type: SVM
Kernel Type: rbf
Penalty C: 1.0 Gamma: auto
Predict

Refinement
Refine: Appearance Lambda: 50.0

Update annotations

Confidence Tool for Level 0
Visualization: Predictions
Confidence: 50
From: z 0 y 0 x 0
To: z 306 y 946 x 946
Label 2 Save
Label 3 Save

Annotation Level: SuperVoxels Width: 21 Interpolation: linear

25

0 50 100 150 200

0 50 100 150 200 250 300

Can use to control which supervoxels are shown based on confidence

+ Ready

Model Training: Confidence and Update Annotations

SuRVoS: Super-Region Volume Segmentation workbench (on cs04r-sc-com10-07.diamond.ac.uk)

File Help

Predict Level: Level 0
Constrain Region: None

Predict labels

Descriptor

Region: SuperVoxels

Features: Select Sources

Type: Mean Bins: 10 Order: 0

Project: None

Classifier

Classifier type: SVM

Kernel Type: rbf

Penalty C: 1.0 Gamma: auto

Predict

Refinement

Refine: Appearance Lambda: 50.0

Update annotations

Confidence Tool for Level 0

Visualization: Predictions

Confidence: 50

From: z 25 y 0 x 0

To: z 49 y 946 x 946

Label2 Save

Label3 Save

Data Annotation Level: SuperVoxels Width: 21 Interpolation: linear

25

0 50 100 150 200

0 50 100 150 200 250 300

300

+ Ready

Can restrict area to save annotations from

Add each label individually

Model Training: Confidence and Update Annotations

SuRVoS: Super-Region Volume Segmentation workbench (on cs04r-sc-com10-07.diamond.ac.uk)

File Help

Predict Level: Level 0
Constrain Region: None

Predict labels

Descriptor
Region: SuperVoxels
Features: Select Sources
Type: Mean Bins: 10 Order: 0
Project: None

Classifier
Classifier type: SVM
Kernel Type: rbf
Penalty C: 1.0 Gamma: auto

Refinement
Refine: Appearance Lambda: 50.0

Update annotations

Confidence Tool for Level 0
Visualization: Predictions
Confidence: 50
From: z 0 y 250 x 0
To: z 306 y 350 x 946

Label2 Save
Label3 Save

Data Annotation Level: SuperVoxels Width: 21 Interpolation: linear

38

0 50 100 150 200 250 300

0 50 100 150 200 250 300

0 50 100 150 200 250 300

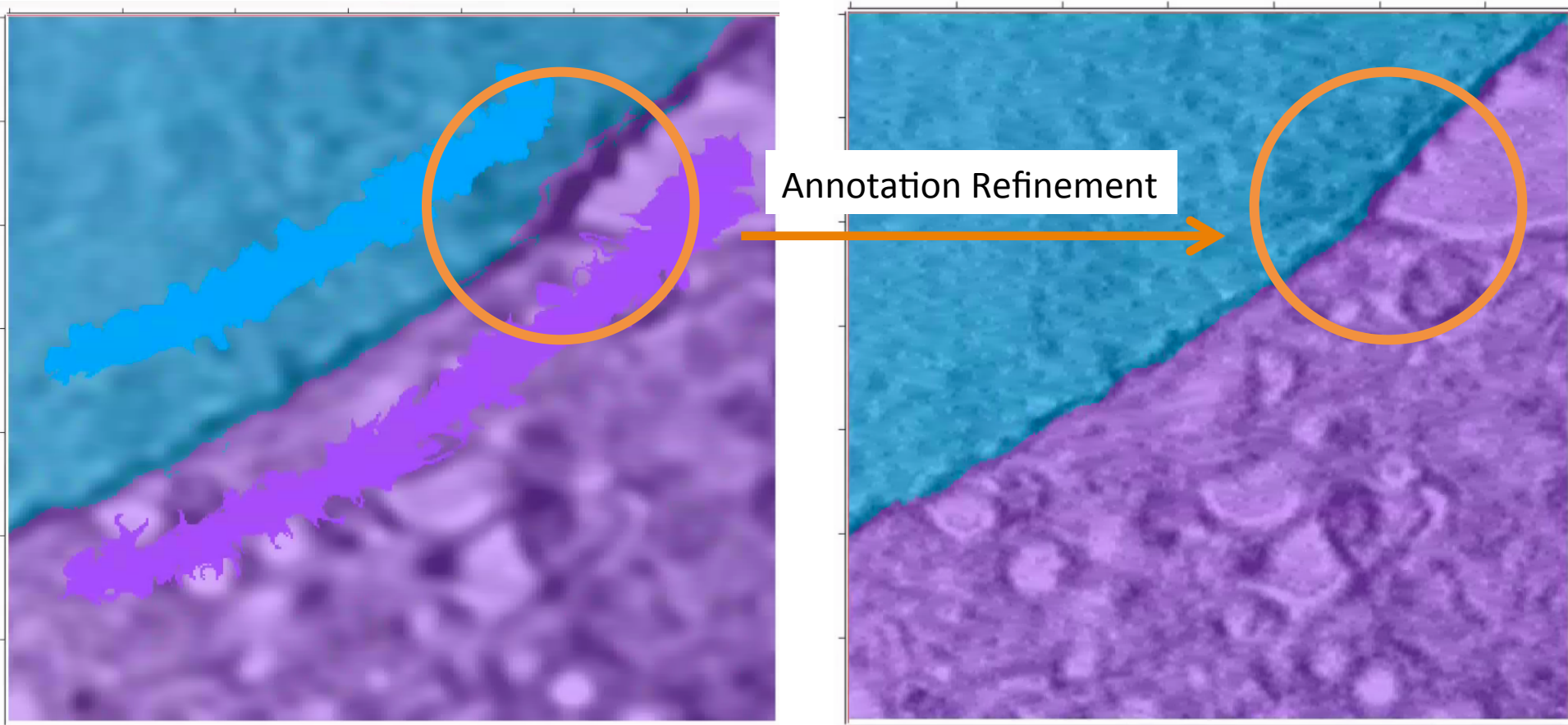
← Ready

New restricted area

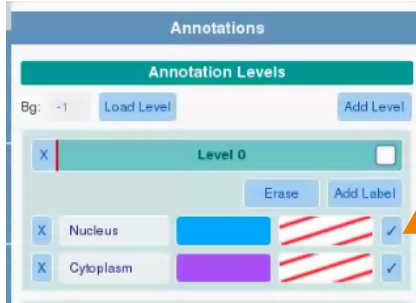
Add label of interest

Model Training: Refining Annotations

Don't worry about getting the model training perfect.
The annotations can be refined.



Model Training: Refining Annotations



In the annotation tab select a label to refine.



We can refine a label on

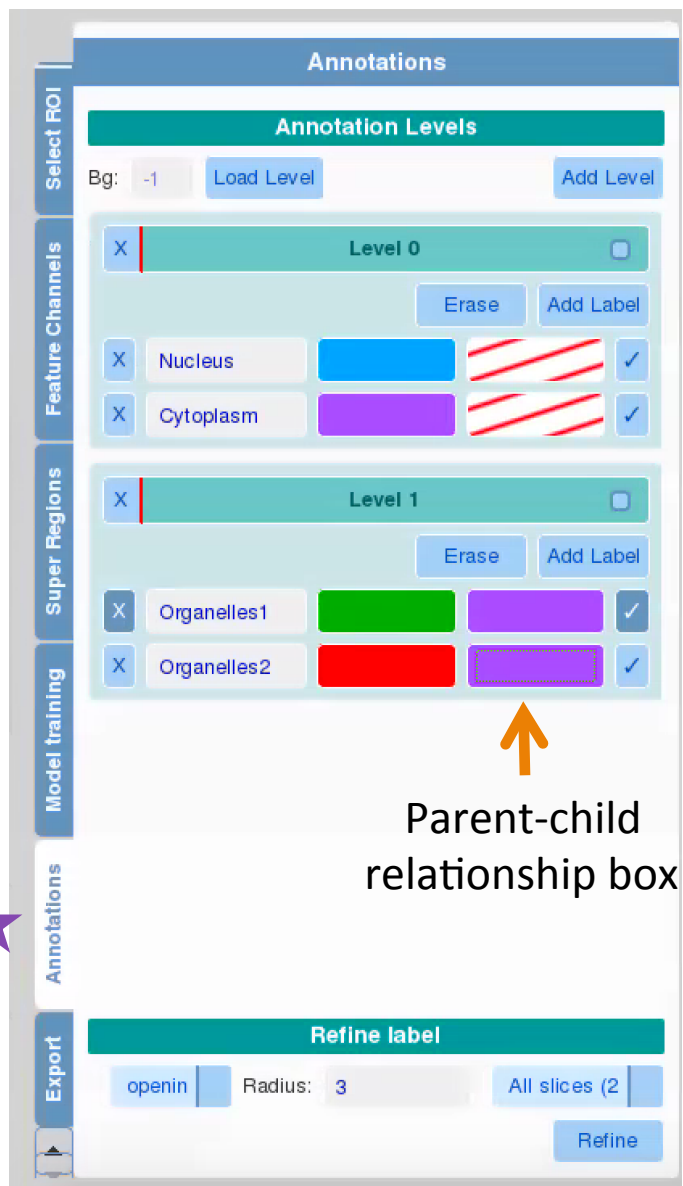
- This slice
- **All slices 2D (recommended)**
- Whole volume 3D

Refinement methods:

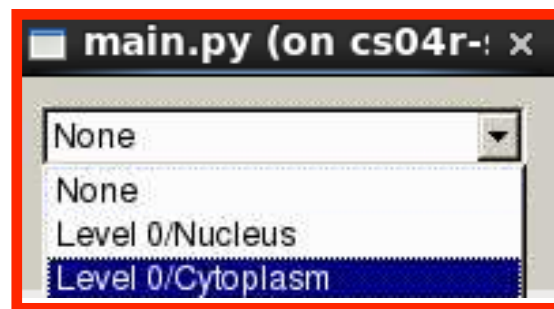
- **Dilation** - This adds pixels to the inner and outer boundary of the annotation.
- **Erosion** - This strips away layers of pixels from the inner and outer boundary of the annotation. Small objects can be deleted easily with erosion.
- **Opening** - Erosion followed by dilation.
- **Closing** - Dilation followed by erosion.
- **Fill holes** - Fills holes when a label surrounds them entirely.

Time – 15 minutes

Parent-child Relationship Between Levels

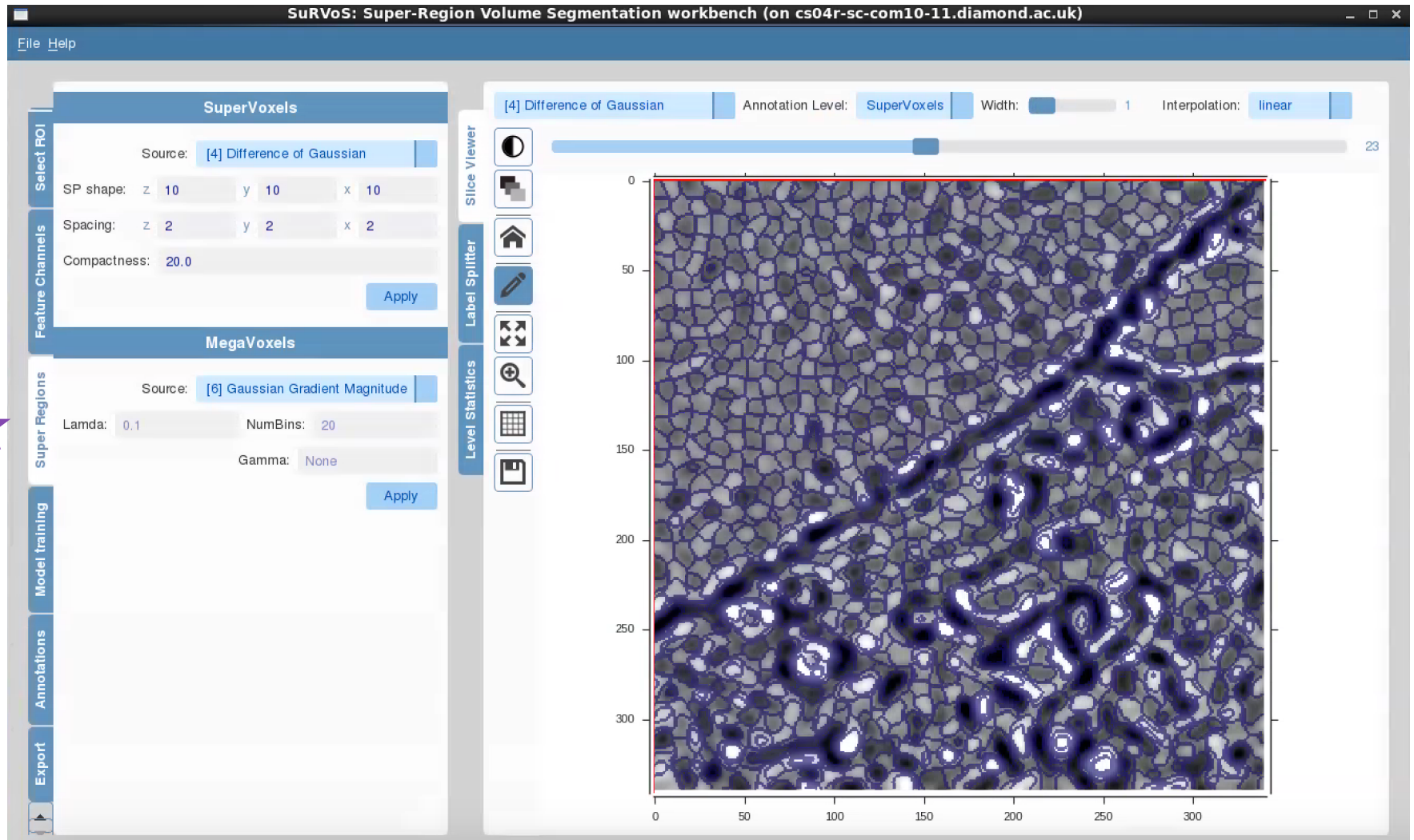


- Add a new level
- Add labels to this level
- A parent of the label can be specified by clicking on the parent-child relationship box for each label
- In the example shown (left) we are wanting to segment organelles which are located in the cytoplasm, so we choose the parent label: Level 0/Cytoplasm



Note: Objects next to each other must be different labels to be considered different objects!

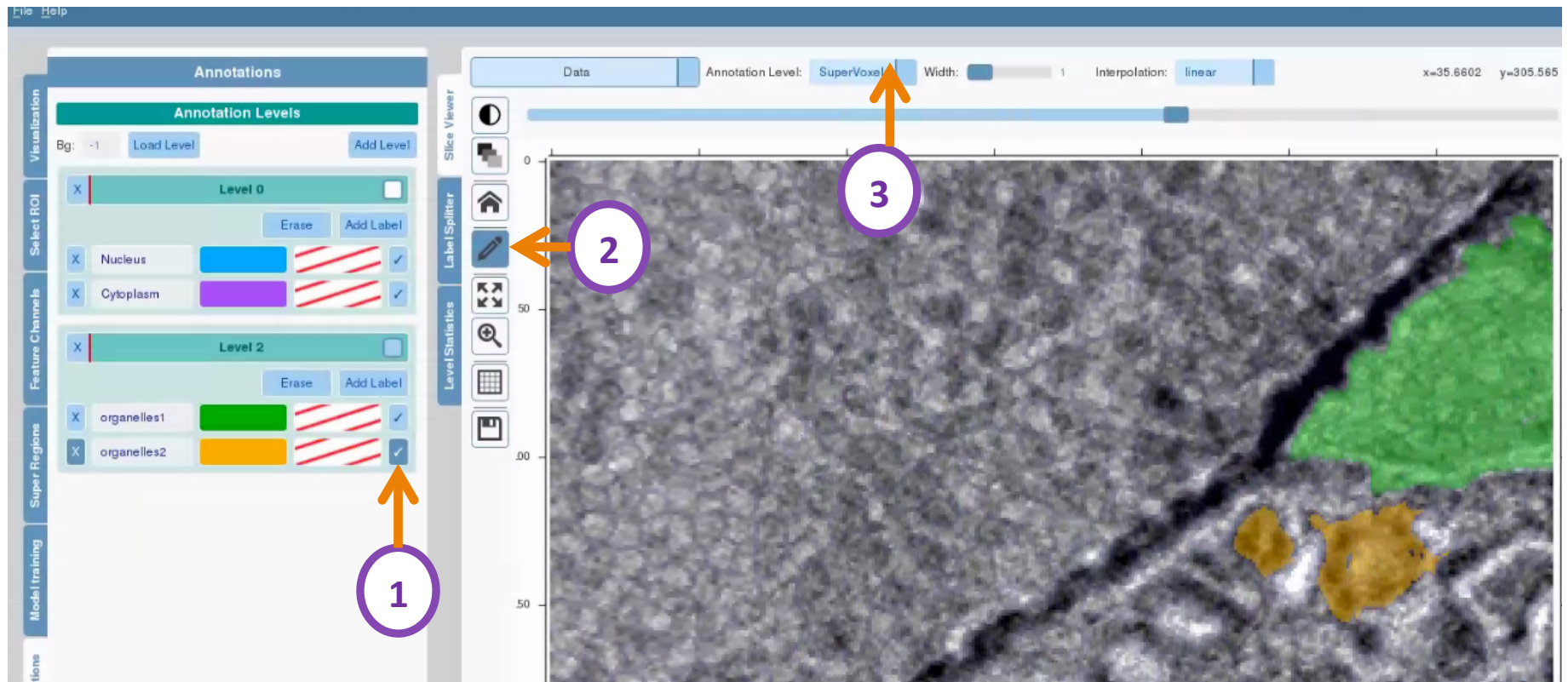
Choosing New Supervoxels for Organelle Segmentation



- **Supervoxels can be recalculated at anytime, using any source.**
- This is useful for generating supervoxels that represent specific organelles.

Time – 5 minutes

Segmenting Organelles Using Supervoxels

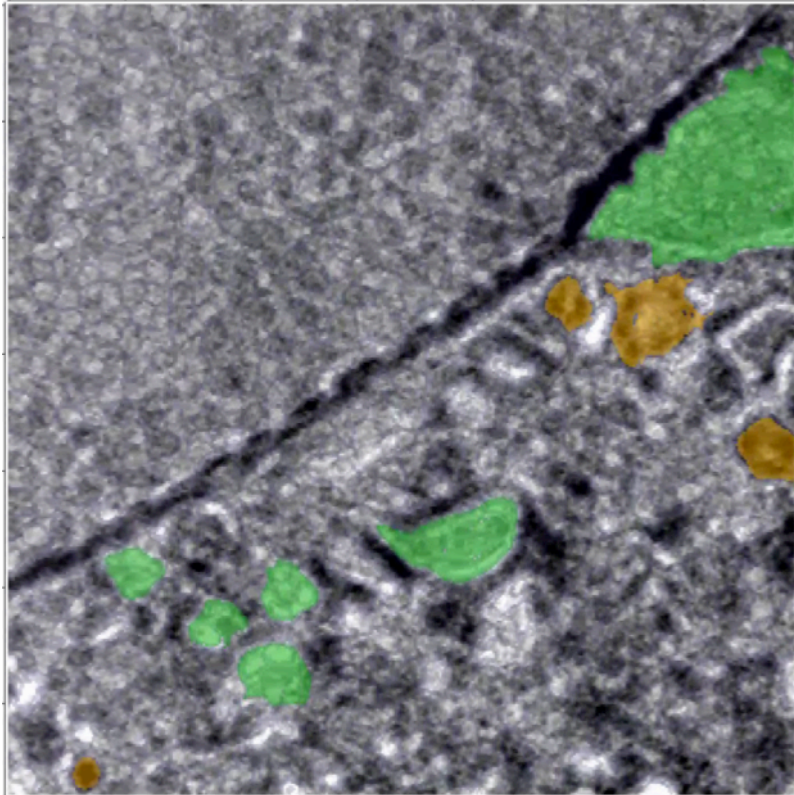


Reminder:

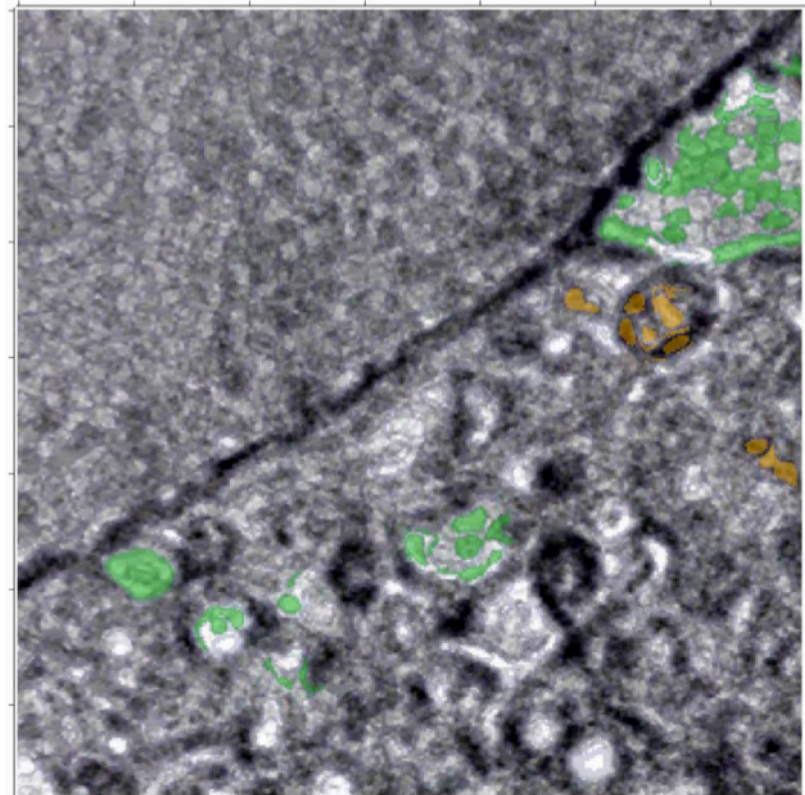
- 1) Select the label to annotate with
- 2) Select the pen tool
- 3) Select annotation level to supervoxels and left click to annotate

Segmenting Organelles Using Supervoxels

Segmented Z-slice

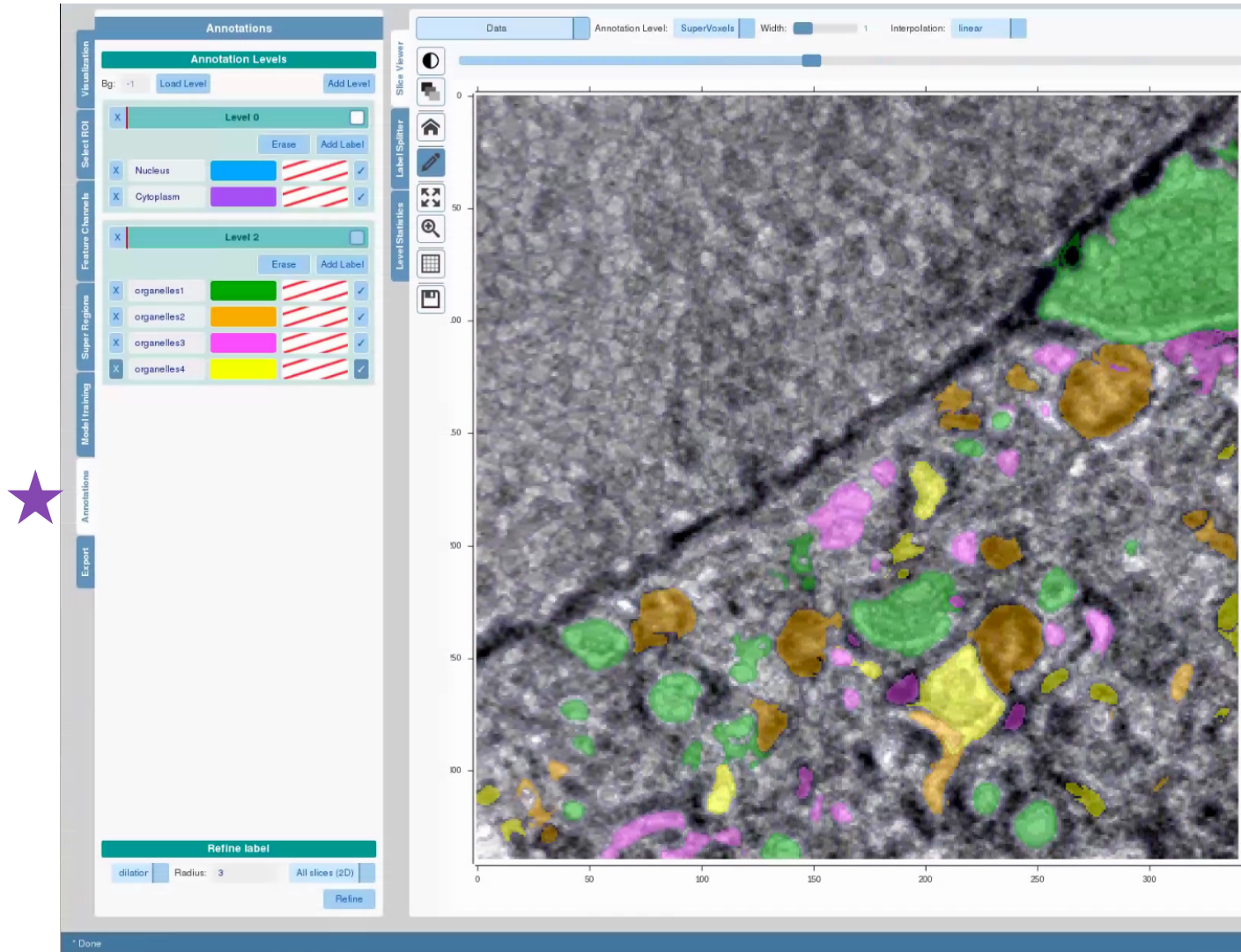


10 slices below



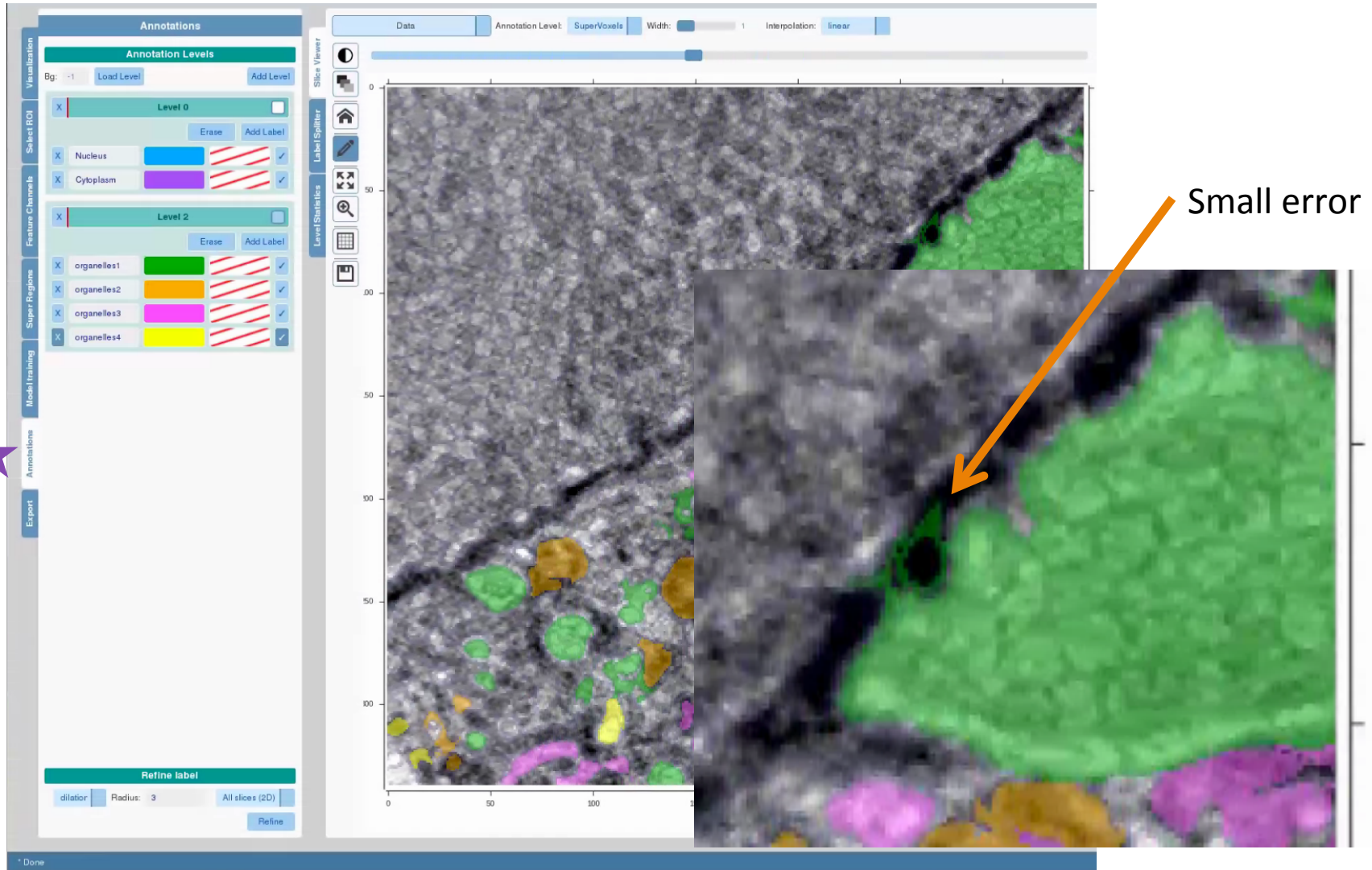
- Segment organelles in one slice
- Move through a few slices in Z
- Re-annotate keeping organelles the same colour

Segmenting Organelles Using Supervoxels



Once happy that the organelles have been segmented apart from some small errors then the annotation can be refined.

Segmenting Organelles Using Supervoxels



Once happy that the organelles have been segmented apart from some small errors then the annotation can be refined.

Segmenting Organelles: Refinement



In the annotation tab
select a label to refine.



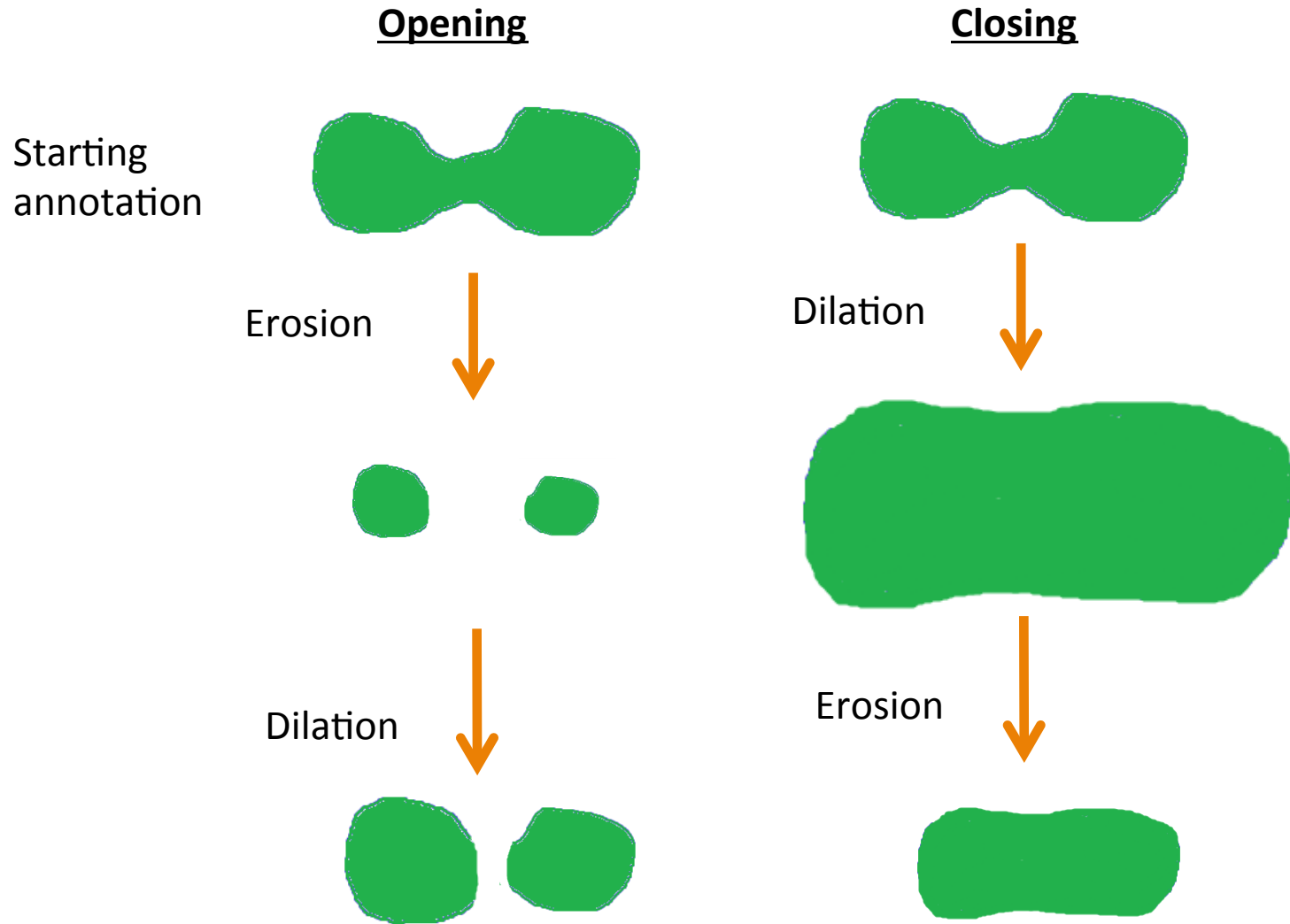
We can refine a label on

- This slice
- **All slices 2D**
- Whole volume 3D

Refinement methods:

- **Dilation** - This adds pixels to the inner and outer boundary of the annotation.
- **Erosion** - This strips away layers of pixels from the inner and outer boundary of the annotation. Small objects can be deleted easily with erosion.
- **Opening** - Erosion followed by dilation.
- **Closing** - Dilation followed by erosion.
- **Fill holes** - Fills holes when a label surrounds them entirely.

Segmenting Organelles: Refinement



Segmenting Organelles: Refinement

General Suggested Procedure:

1) Fill holes - typically use a large radius

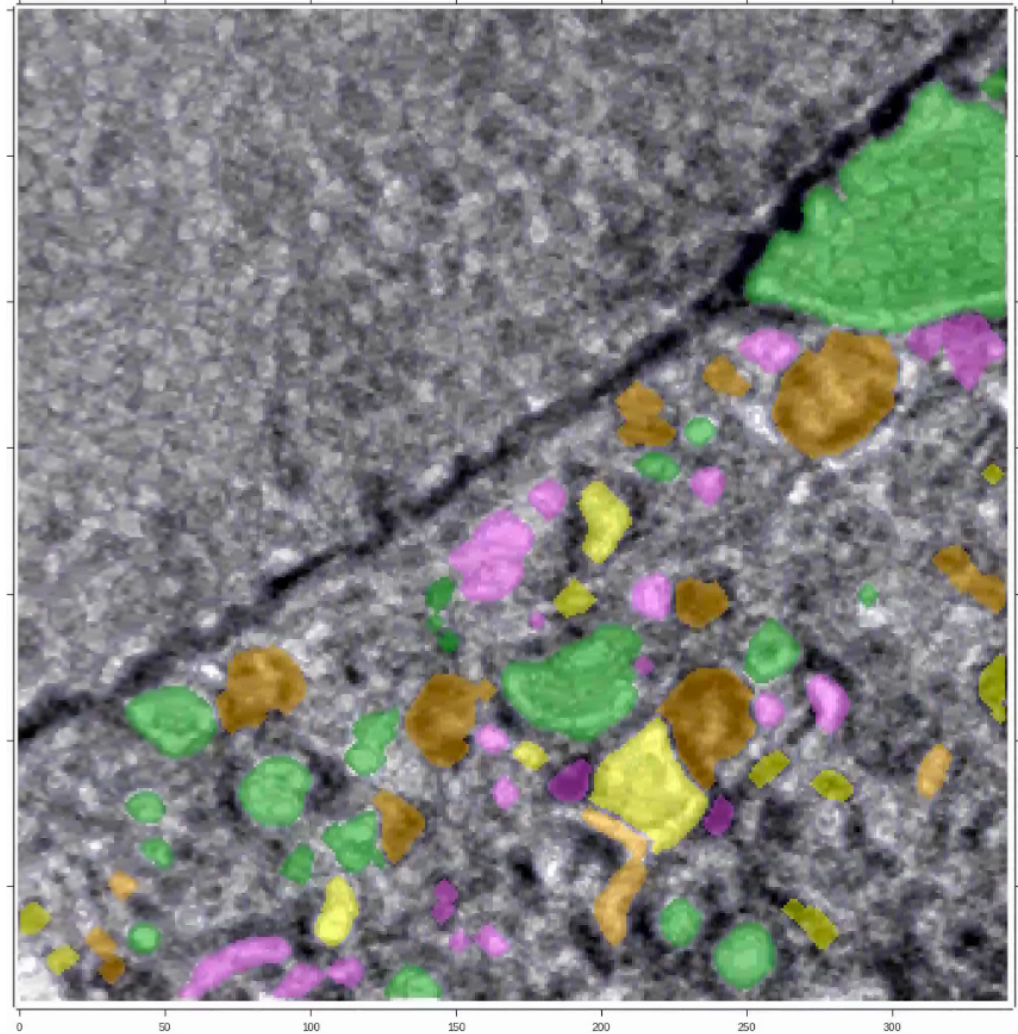
2) Opening - Radius 5

3) Dilation - Radius 2-3

4) Erosion - Radius 1-2

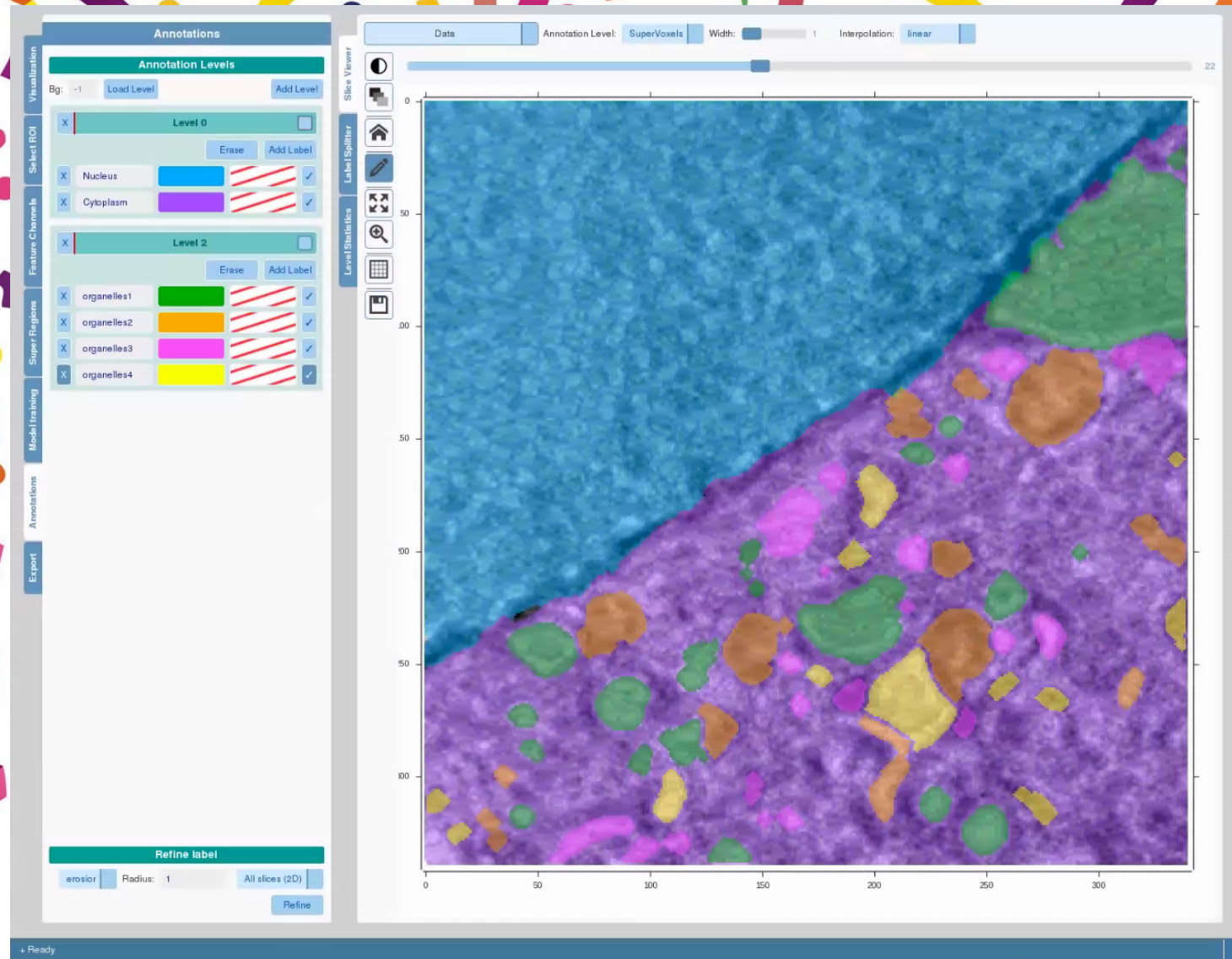
Do each step on each similar label before moving on to next step.

Can check the effects of each step on an individual slice to optimize parameters



Time – 25 minutes

Finished Segmentation



Label Splitter

SuRVoS: Super-Region Volume Segmentation workbench (on cs04r-sc-com10-08.diamond.ac.uk)

File Help

Annotations

Annotation Levels

Bg: -1 Load Level Add Level

Level 0

X Nucleus Erase Add Label

X Cytoplasm Erase Add Label

Level 2

X organelles1 Erase Add Label

X organelles2 Erase Add Label

X organelles3 Erase Add Label

X organelles4 Erase Add Label

Refine label

erosior Radius: 1 All slices (2D) Refine

Visualization Pane

Rule Creation Pane

Level 2 Select Labels Data Label

Select measure: Average Intensity

Fit kernel: biw Export plot Export Stats

organelles

organelles

organelles

organelles

0.06

0.04

0.02

0.00

-0.02

-0.04

-0.06

-0.06 -0.04 -0.02 0.00 0.02 0.04 0.06

Save labels Add new label

+ Done

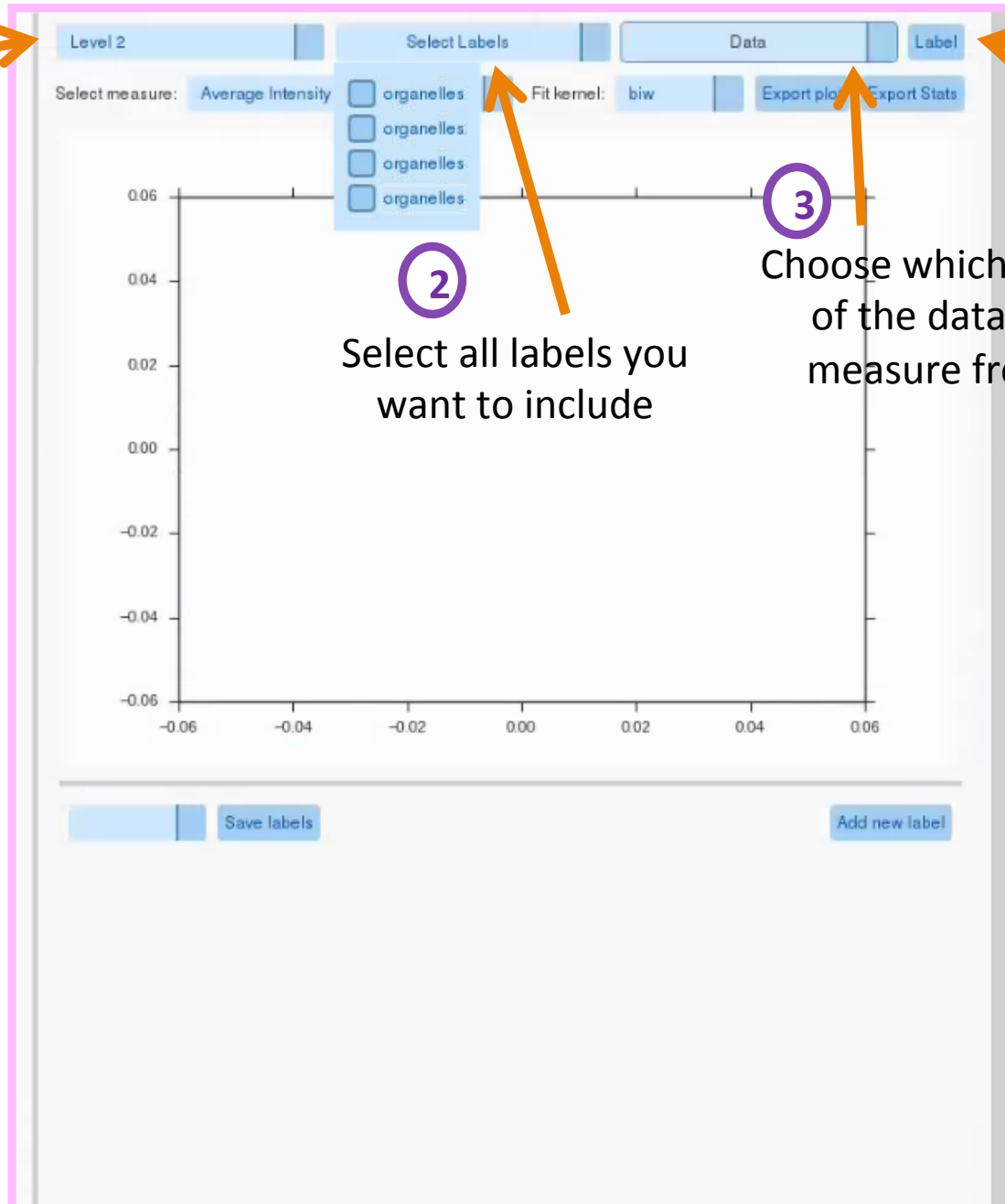
Label Splitter: Rule Creation

1

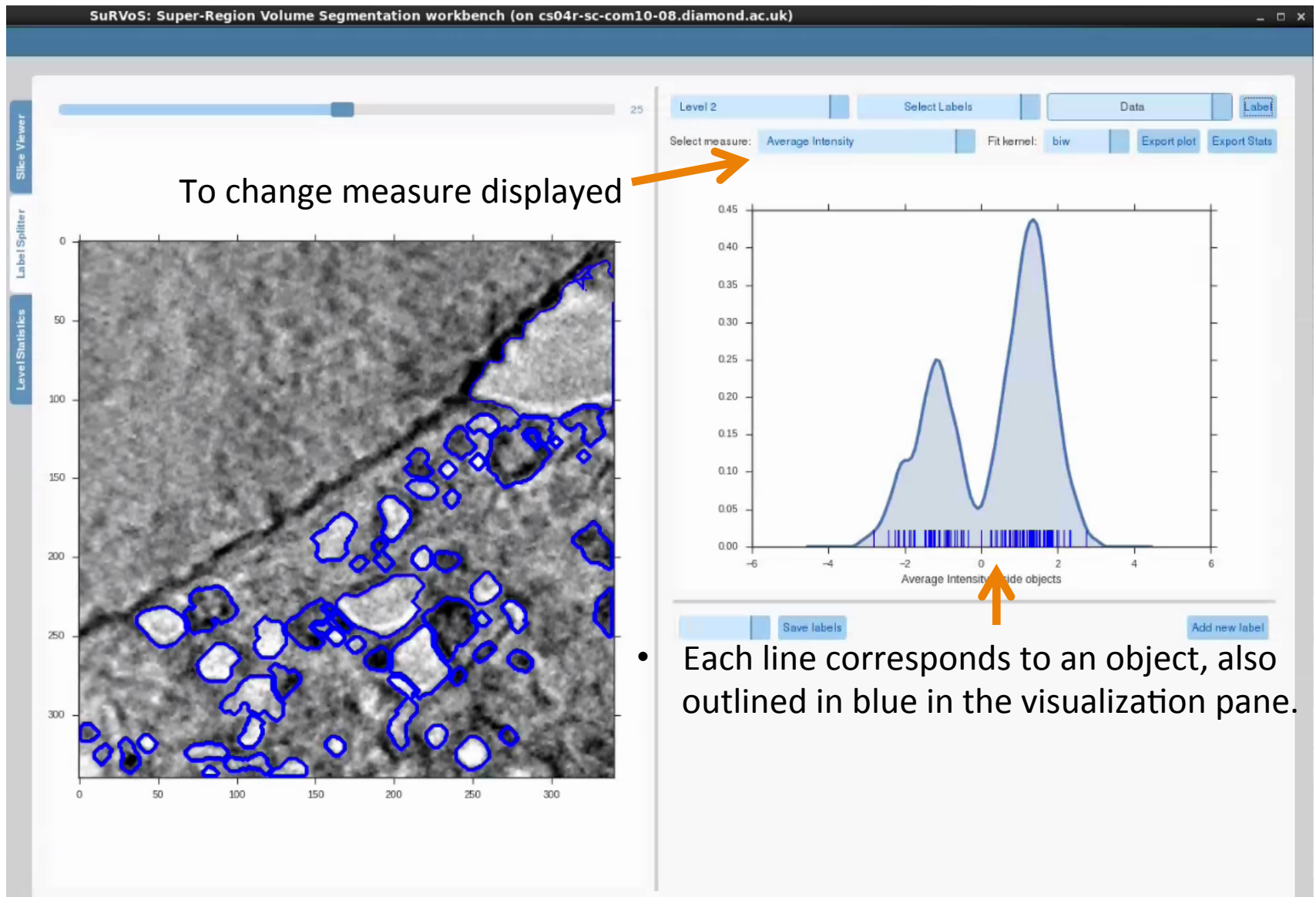
Choose level you will work with

4

Click Label



Label Splitter: Rule Creation



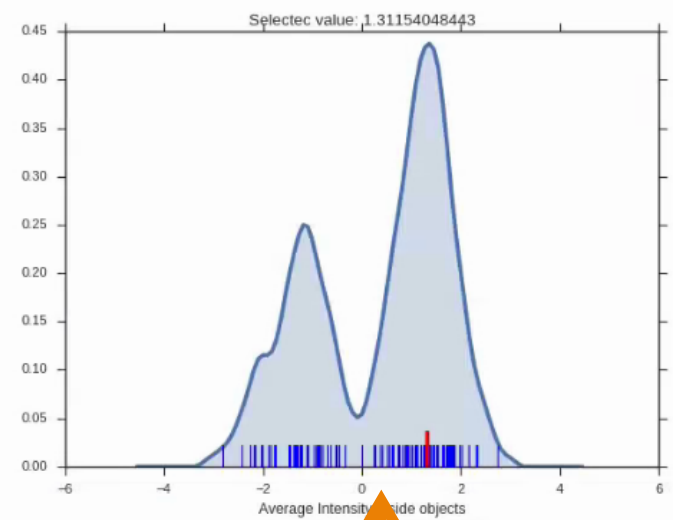
Label Splitter: Rule Creation



To change measure displayed



Level 2 | Select Labels | Data | Label
Select measure: Average Intensity | Fit kernel: biw | Export plot | Export Stats



Save labels | Add new label

- Each line corresponds to an object, also outlined in blue in the visualization pane.
- You can select an individual object by clicking on it in the visualization pane, or on a line in the graph. It will change to a red colour.

Label Splitter: Rule Creation

SuRVoS: Super-Region Volume Segmentation workbench (on cs04r-sc-com10-08.diamond.ac.uk)

Star icon

Label Splitter

Level 2

Select Labels

Data

Label

Select measure: Average Intensity

Fit kernel: biw

Export plot

Export Stats

Selected value: 1.31154048443

Can change the name and colour

1

2

3 Define the rule

4

Save labels

Add new label

X Empty Vesicles

Add new rule

Apply

Select Others

X Rule 1 Feature: Average Intensity

>

0

Label colour is applied here and in plot to right

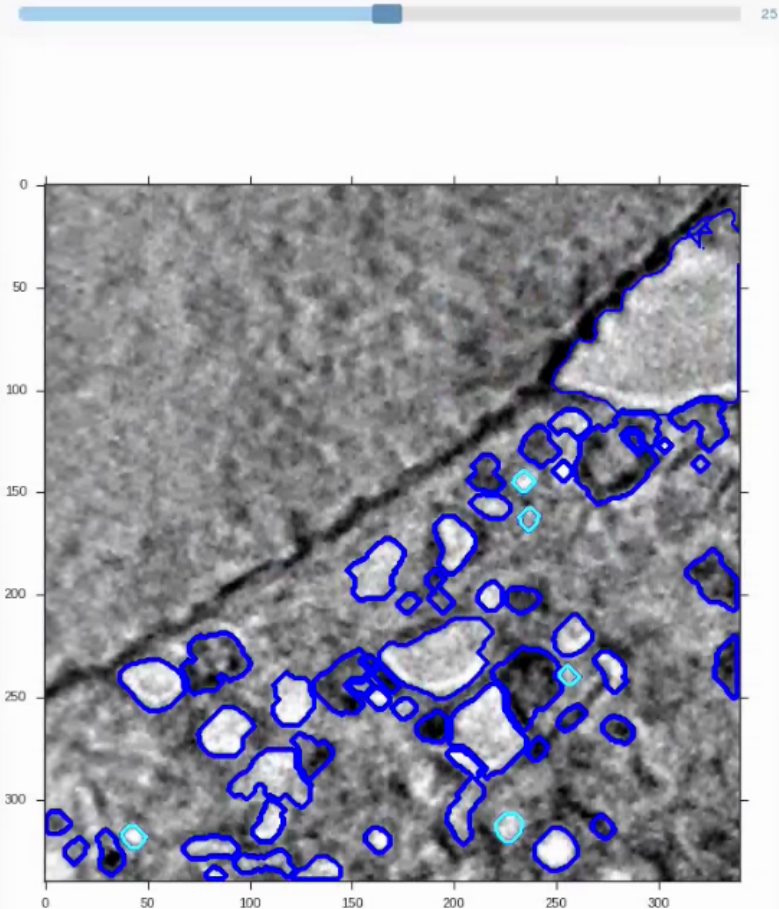
Label Splitter: Rule Creation

SuRVoS: Super-Region Volume Segmentation workbench (on cs04r-sc-com10-08.diamond.ac.uk)

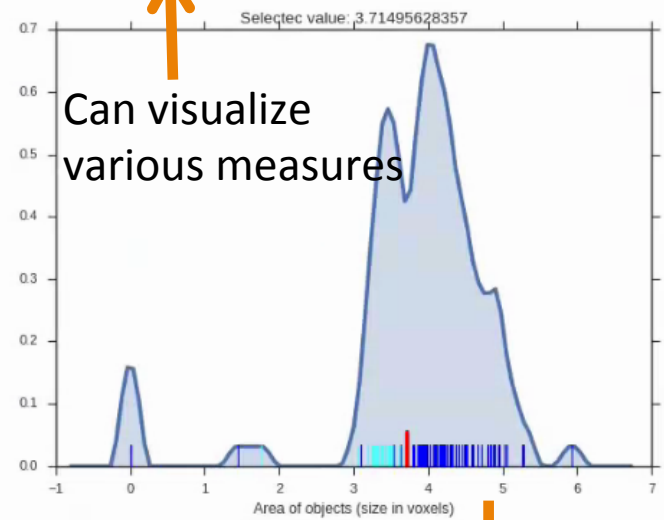
Slice Viewer

Label Splitter

Level Statistics



Level 2 Select Labels Data Label
Select measure: Log10(Size) (Oriented Bounding Bo Fit kernel: biw Export plot Export Stats



Save labels Add new label

X	Empty Vesicles	Add new rule	Apply	Select Others
X	Rule 1 Feature: Average Intensity	>	0	
X	Rule 2 Feature: Log10(Size) (Oriented Bounding Bo	<	3.72	

Can add as many rules to define the label as needed

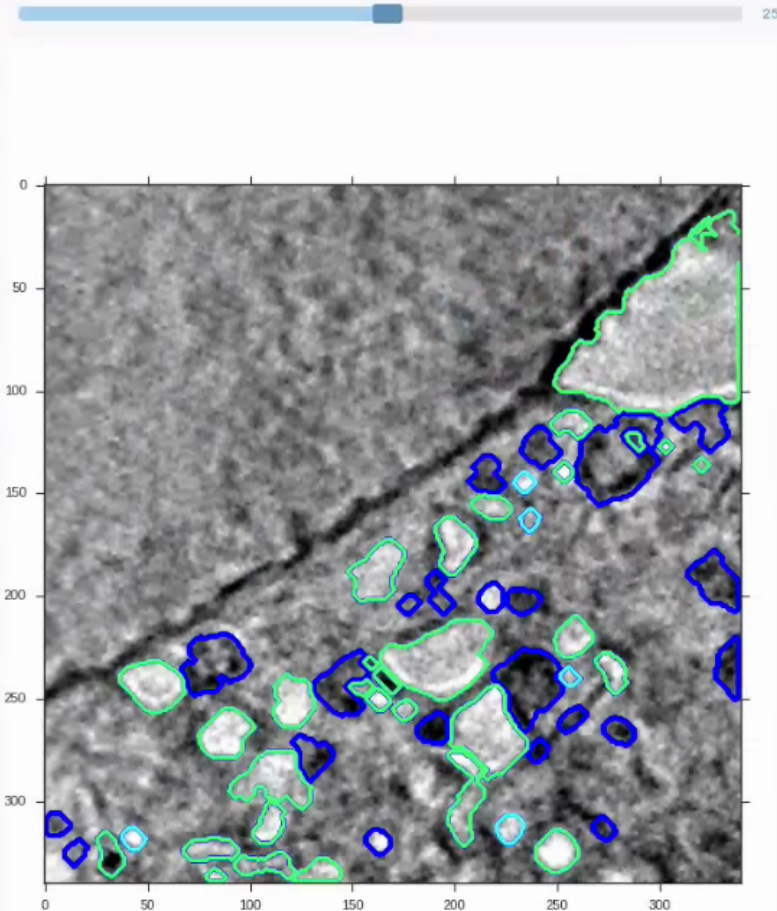
Label Splitter: Rule Creation

SuRVoS: Super-Region Volume Segmentation workbench (on cs04r-sc-com10-08.diamond.ac.uk)

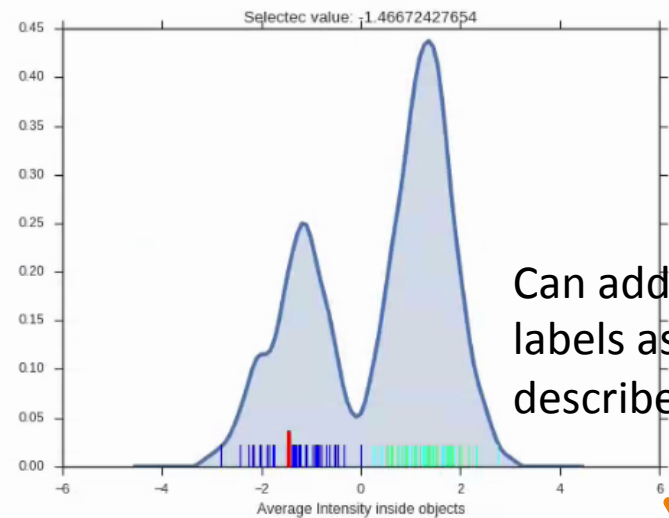
Slice Viewer

Label Splitter

Level Statistics



Level 2 Select Labels Data Label
Select measure: Average Intensity Fit kernel: biw Export plot Export Stats



Can add as many labels as needed to describe the data



Save labels Add new label

Rule	Feature	Operator	Value
X Rule 1	Feature: Average Intensity	<	3.72
X Rule 2	Feature: Log10(Size) (Oriented Bounding B	<	3.72
X	ity Vesicles (Large)		
X Rule 1	Feature: Average Intensity	>	0
X Rule 2	Feature: Log10(Size) (Oriented Bounding B	>	3.72

Add new rule Apply Select Others

Label Splitter: Rule Creation

SuRVoS: Super-Region Volume Segmentation workbench (on cs04r-sc-com10-07.diamond.ac.uk)

Level 2 | Select Labels | Data | Label

Select measure: Average Intensity | Fit kernel: biw | Export plot | Export Stats

Selectec value: 1.31154048443

Average Intensity inside objects

Use to gather remaining objects into one class

Save labels

Rule 1 Feature: Average Intensity

X Rule 2 Feature: Log10(Size) (Oriented Boundir

X Rule 3 Feature: Variance

X Rule 4 Feature: Variance

X Label 4

Add new rule | Apply | Select Others

Label Splitter: Rule Creation

SURVoS: Super-Region Volume Segmentation workbench (on cs04r-sc-com10-07.diamond.ac.uk)

Level 2 | Select Labels | Data | Label

Select measure: Average Intensity | Fit kernel: biw | Export plot | Export Stats

Selectec value: 1.31154048443

When happy with rules, make new, empty level (in annotation tab of GUI). Then select that level here and click Save Labels

Level 4 | Save labels | Add new label

Rule 1 Feature: Average Intensity

X Rule 2 Feature: Log10(Size) (Oriented Boundir) > 4.2

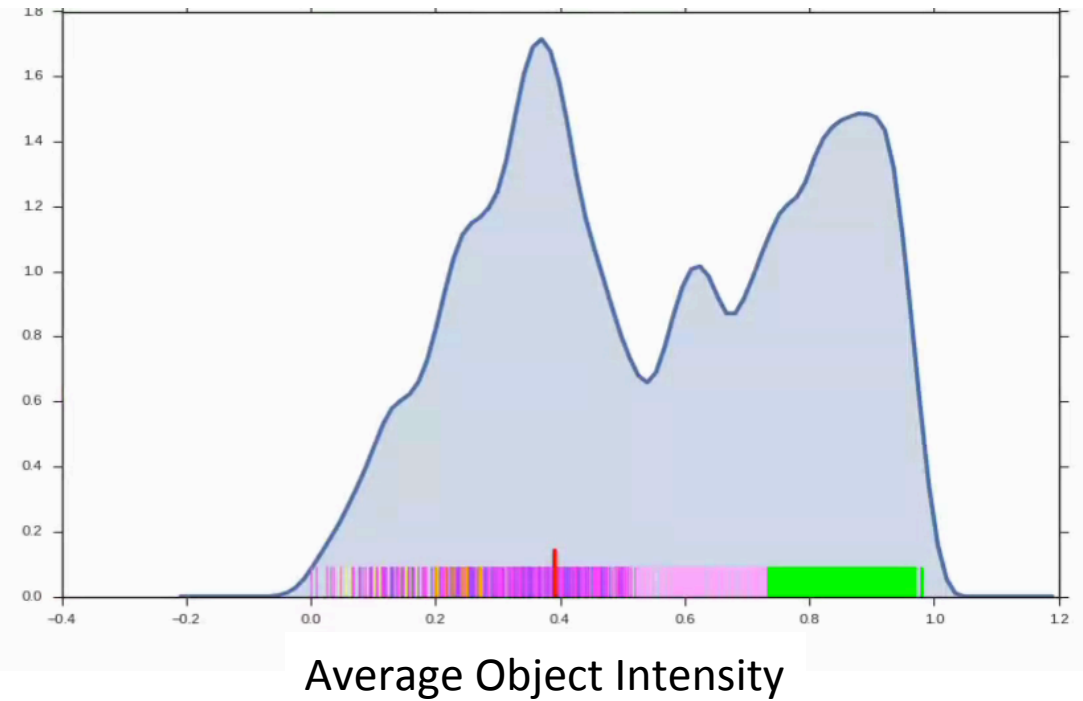
X Rule 3 Feature: Variance > 0.5

X Rule 4 Feature: Variance < 1

X Label 4 Add new rule Apply Select Others

The screenshot displays the SURVoS Label Splitter interface. On the left, a grayscale image shows segmented regions with various colored outlines (green, purple, yellow, red, cyan). A vertical sidebar on the left contains buttons for 'Slice Viewer', 'Label Splitter' (highlighted with a purple star), and 'Level Statistics'. A horizontal slider at the top of the image view is set to 25. On the right, a control panel includes buttons for 'Level 2', 'Select Labels', 'Data', and 'Label'. Below these are options for 'Select measure' (Average Intensity), 'Fit kernel' (biw), and buttons for 'Export plot' and 'Export Stats'. A histogram plot shows a distribution of 'Average Intensity inside objects' with a peak around 1.3. An orange arrow points from the text 'Then select that level here' to the 'Level 4' button. Below the plot, a 'Save labels' button is visible. At the bottom, a rule creation section shows four rules: Rule 1 (Average Intensity), Rule 2 (Log10(Size) (Oriented Boundir) > 4.2), Rule 3 (Variance > 0.5), and Rule 4 (Variance < 1). A new label 'Label 4' is being created, represented by a yellow square.

Label Splitter: Rule Creation



Measures:

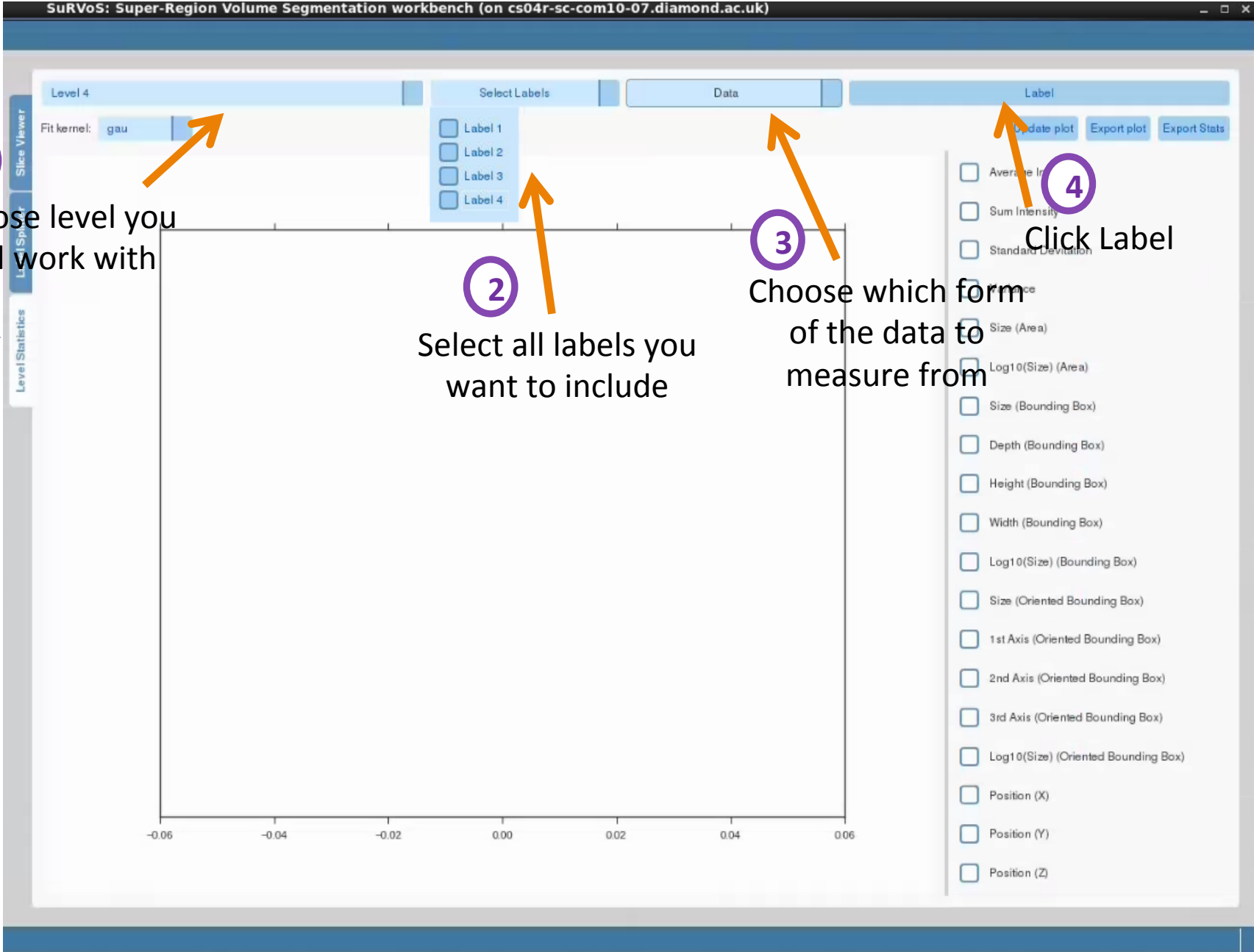
- Average Object Intensity
- Sum of Object Intensity
- Standard Deviation of Object Intensity
- Variance of Object Intensity
- Area of Object
- Bounding Box of Object
 - Depth, width, height
- Oriented Bounding Box of Object
 - Depth, width, height
- X, Y, Z positions of Object

Two Ways to Use:

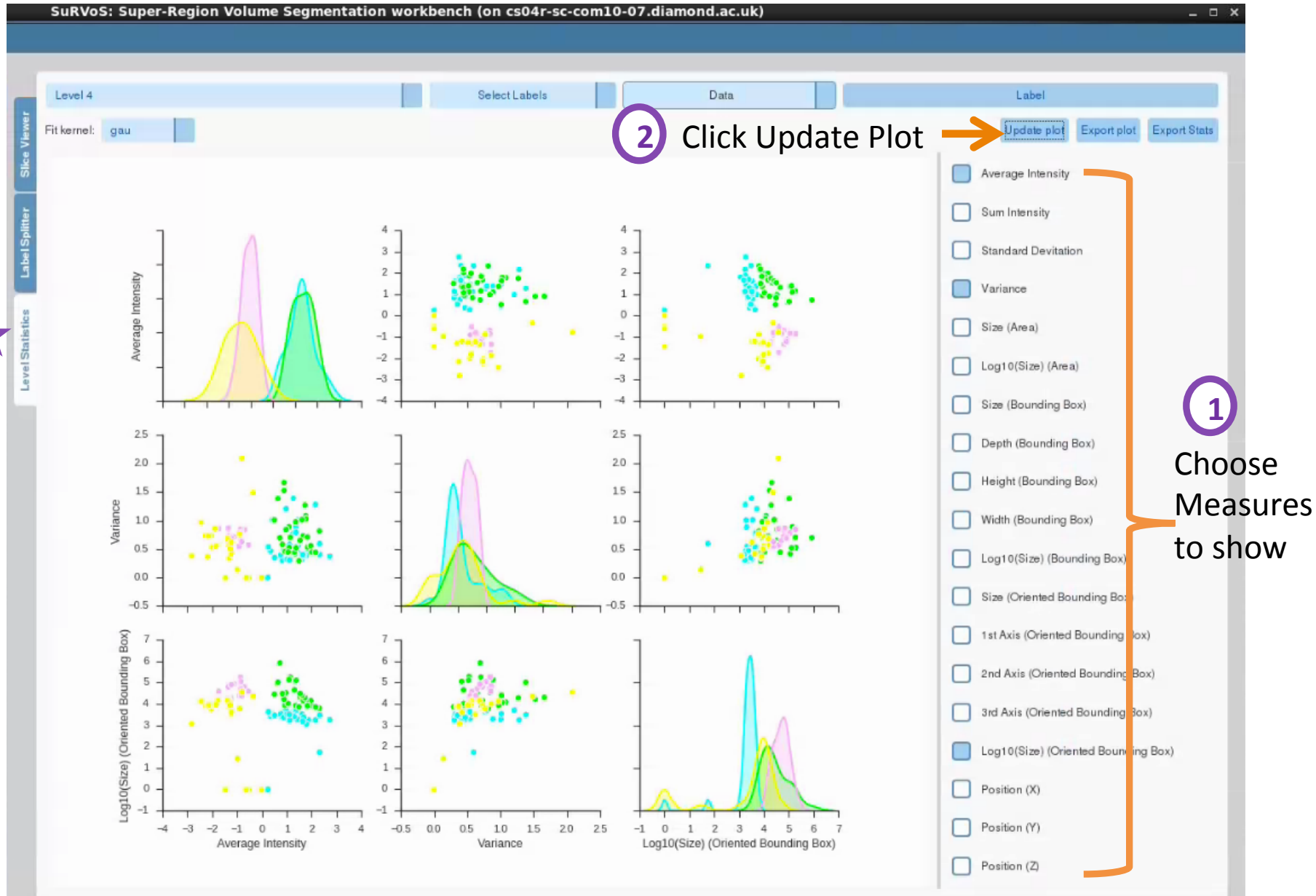
1. Use these characteristics to create rules to separate features into classes.
2. Use other methods to separate features into classes and then use characteristics to describe classes.

Time – 15 minutes

Label Statistics



Label Statistics



Label Statistics



Time – 5 minutes

Exporting Data

SuRVoS: Super-Region

File Help

Visualization

Export Segmentations

Available Annotation Levels

☐ Level 0

☐ Level 2

☐ Level 3

Export Levels

Output Folder: /home/oty52761

Output: Raw Annotations

Format: HDF5 (.h5) ☐ Overwrite

Export

Choose levels to export

Choose location to export data to

Choose way of exporting data

Choose file format

When ready, click export

Output options:

- Raw Data
- Raw Annotations
- Segmentation Masks
- Masked Data

Format:

- HDF5
- MRC
- Tiff stack

Note: If you choose Masked Data, you can choose which dataset to pull data from (i.e. Gaussian, Total Variation, etc). We recommend using the raw data. You can also choose whether to scale the data (0 to 1) or to invert. This will depend on your downstream application

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Also thanks to

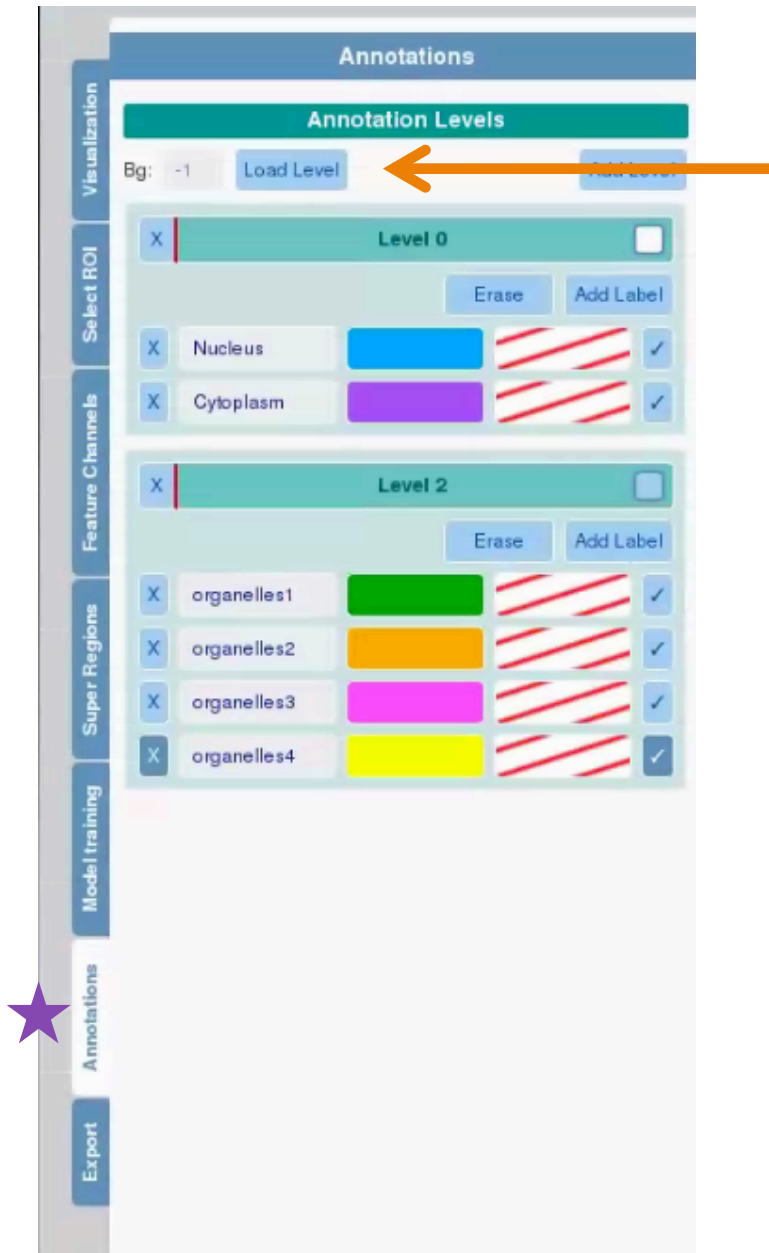
Nvidia Corporation for the donation of a Tesla K40.

Questions?



Time – 5 minutes

Troubleshooting



Can use Load Level to load just the annotation information from a previously segmented project.

If you do this, you will need to load in data as if it is a new project. And if you want to continue segmenting, you will need to recalculate appropriate filters, supervoxels, megavoxels, etc.

Can be used to re-split data or to output plots or object measures.

Installation and Documentation

Website: <https://diamondlightsource.github.io/SuRVoS/docs/installation/>

Installation

Requirements

- Python Distribution
- NVIDIA GPU with at least 1024 threads
- CUDA SDK already installed and configured.

If CUDA is not yet installed in the system, follow instructions in:

<https://developer.nvidia.com/cuda-downloads>

1. Installing a Python distribution

If there is no Python distribution installed on your system, we recommend installing [Anaconda](#). Otherwise, skip to **step 2**.

1.1 Download Anaconda:

<https://www.continuum.io/downloads>

1.2 Install Anaconda:

(Windows) Just double click on the installer and follow instructions.

(Linux) Open a terminal and type the following commands:

```
$> cd /path/to/anaconda/  
$> chmod a+x Anaconda2-4.0.0-Linux-x86_64.sh  
$> ./Anaconda2-4.0.0-Linux-x86_64.sh  
    1. press ENTER  
    2. press Q  
    3. enter "yes"  
    4. pres ENTER  
    5. enter "yes"  
$> source ~/.bashrc
```

NOTE: Replace 2-4.0.0 with your version of Anaconda.

Installation and Documentation Cont.

2. Installing Dependencies

With Anaconda:

```
$> conda update conda pip
$> conda install numpy scipy matplotlib scikit-learn scikit-image cython seaborn networkx pyside
$> pip install scikit-tensor
```

With another python distribution:

```
$> pip install --upgrade pip
$> pip install --upgrade numpy scipy matplotlib scikit-learn scikit-image cython seaborn networkx scil
```

3. Install SuRVoS

3.1 Download SuRVoS

Navigate in a terminal (using `cd`) to a folder where you want to save SuRVoS and type the following commands

```
$> git clone https://github.com/DiamondLightSource/SuRVoS.git
$> cd SuRVoS
```

3.2 Compile SuRVoS features

This step requires CUDA already installed and NVCC compiler in the path (type `which nvcc` to verify it).

```
$> python setup.py build_ext -i
```

4. Run SuRVoS

From the SuRVoS folder:

```
$> ./SuRVoS
```