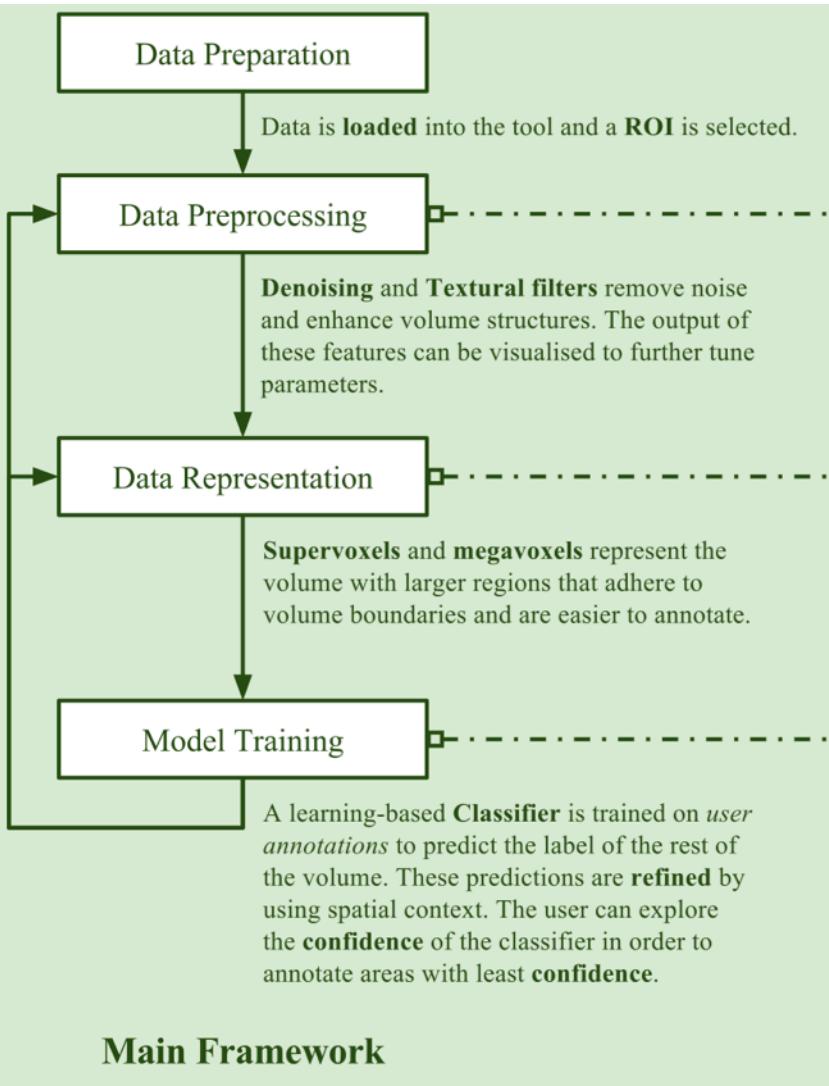


Practical Uses of SuRVoS Workbench

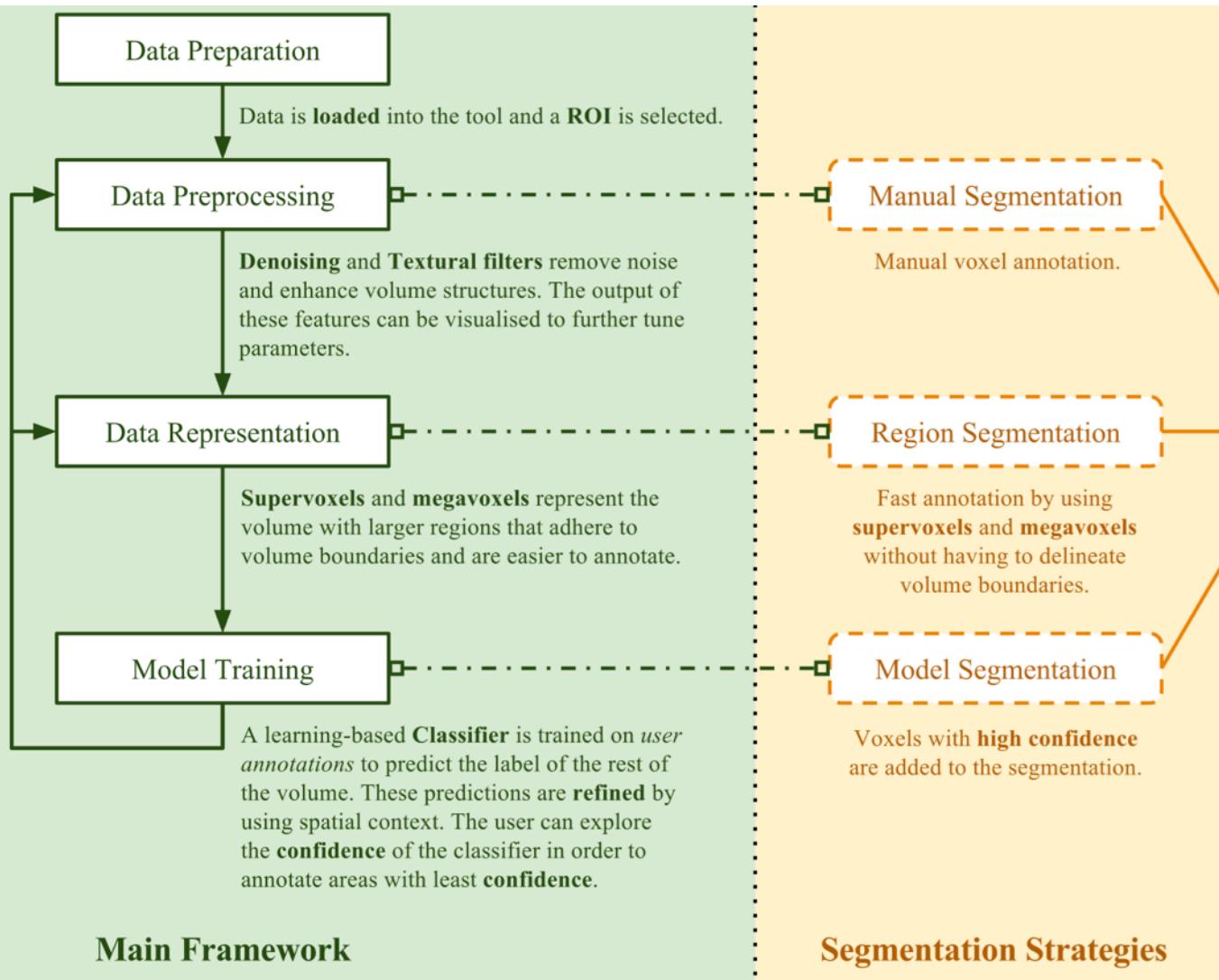
Michele C. Darrow & Matt Spink

Beamline B24
Diamond Light Source

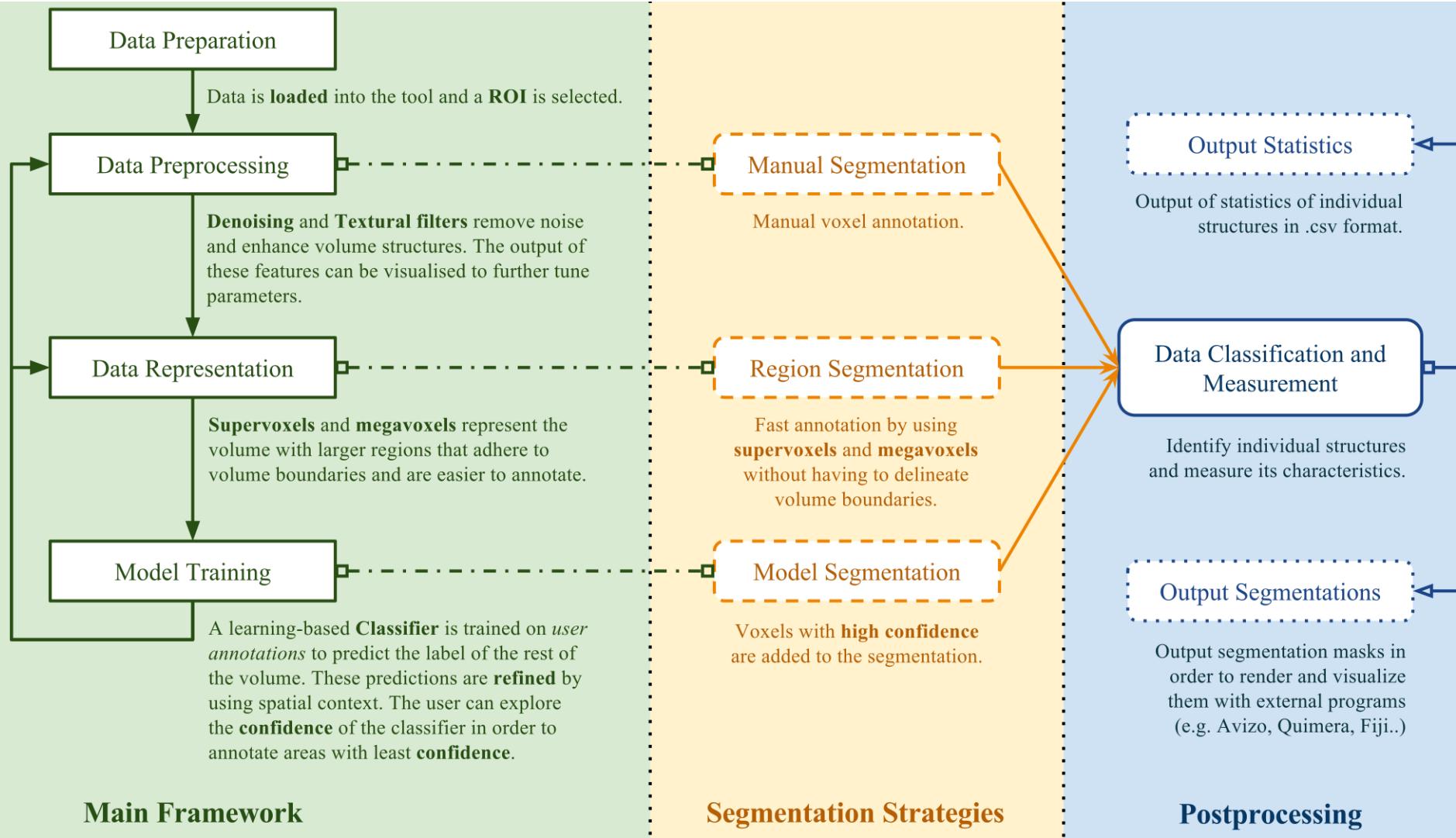
Main Framework



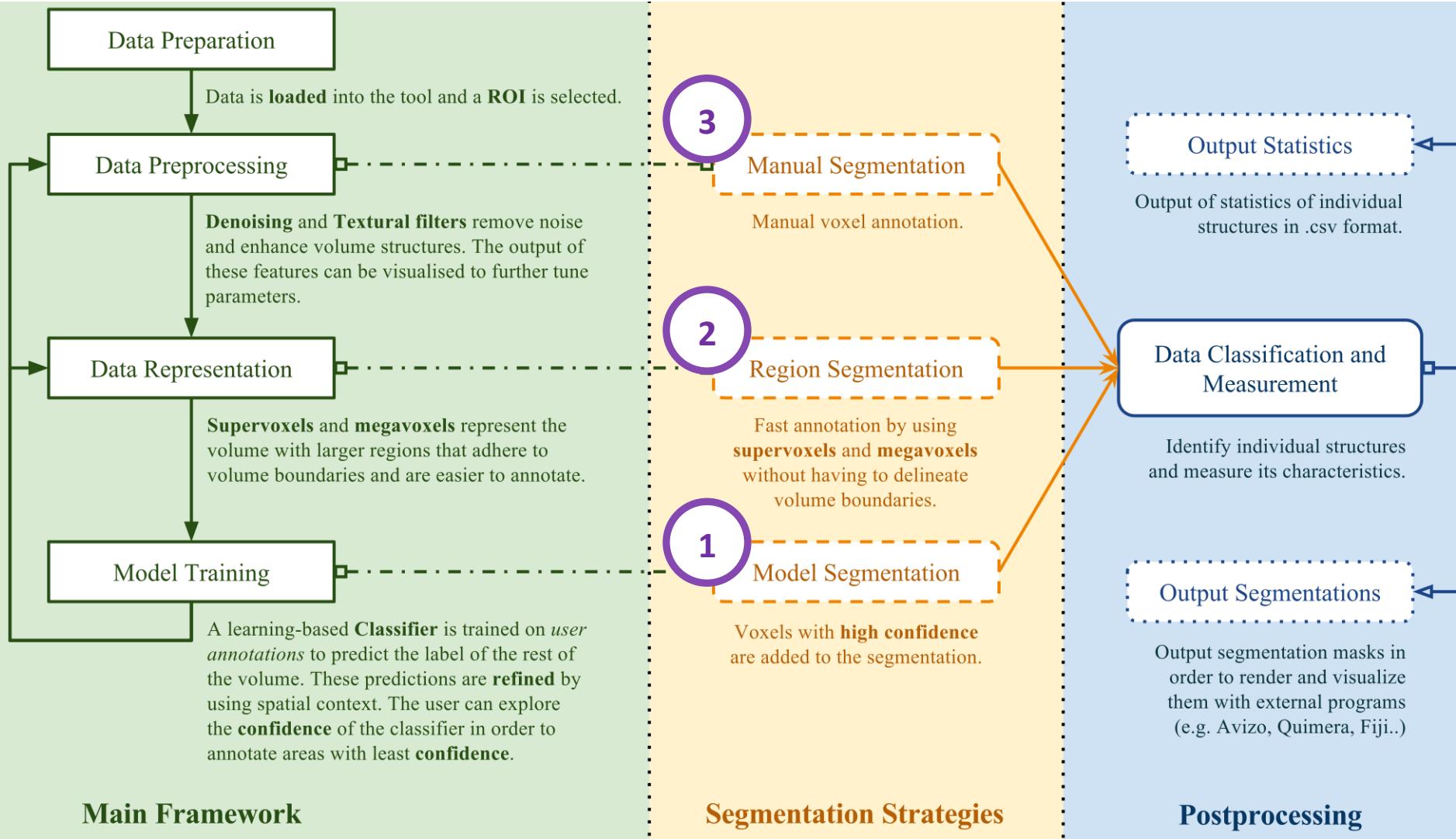
Main Framework & Segmentation Strategies



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.

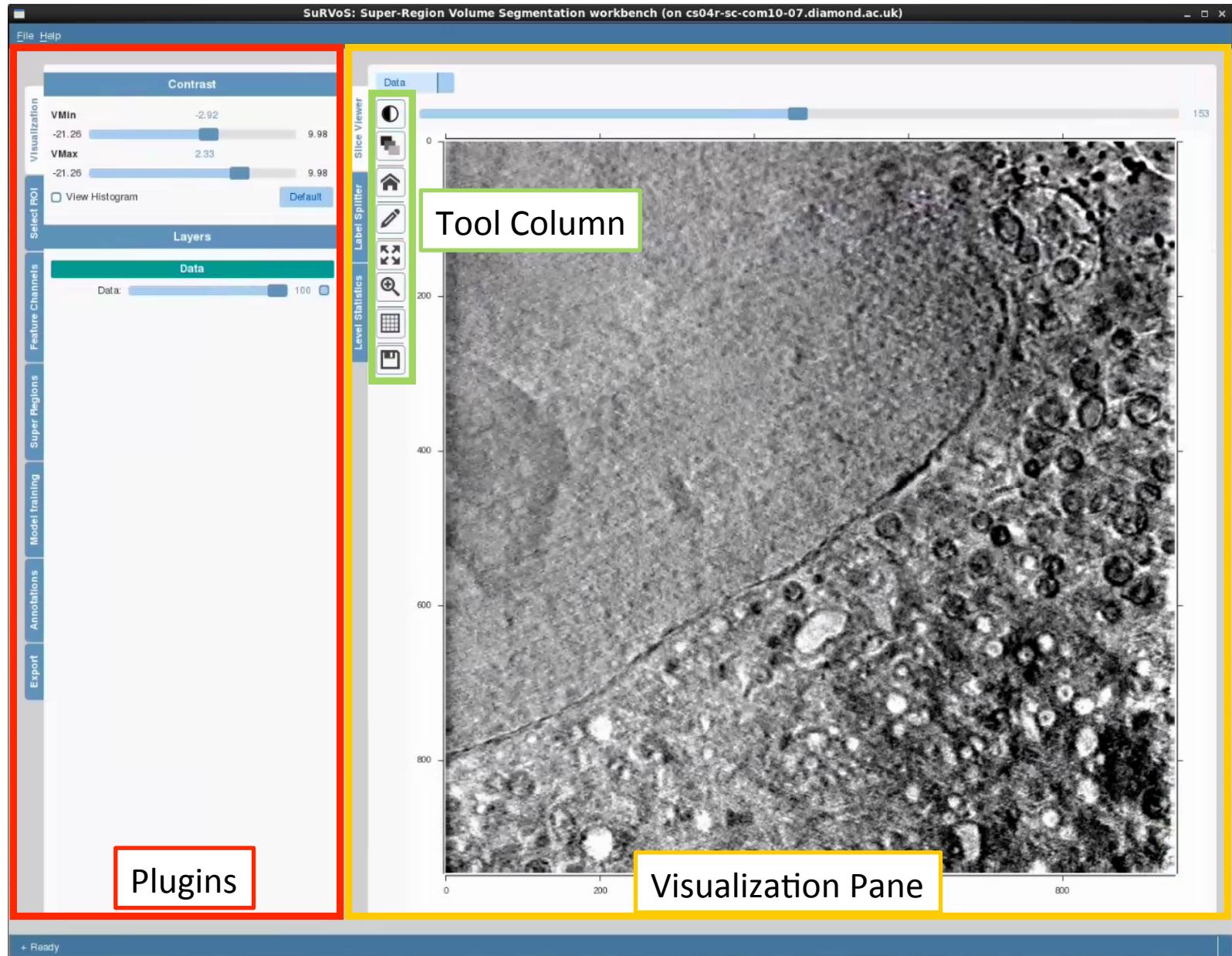
All the feature channels, super-regions and annotations will be recovered.

Starting a New Project

Continuing a Project



Orientation to SuRVoS



Tool Column, Shortcuts



Adjust contrast



Viewer window



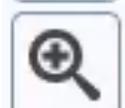
Home (go back to original view)



Annotate



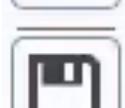
Pan Axis with the left mouse button, zoom with right



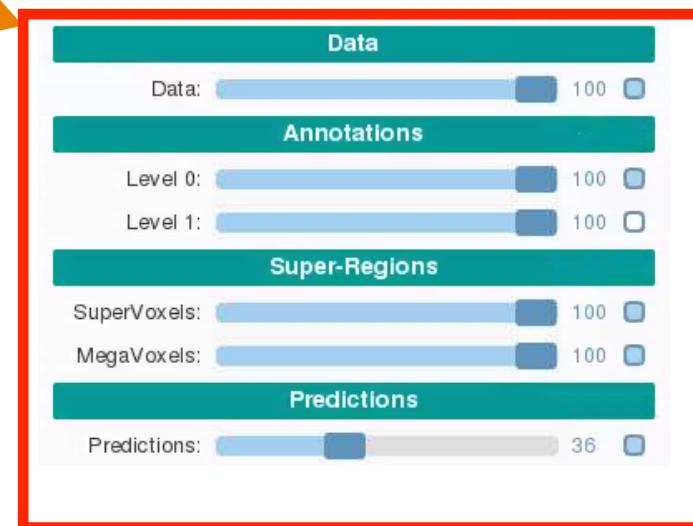
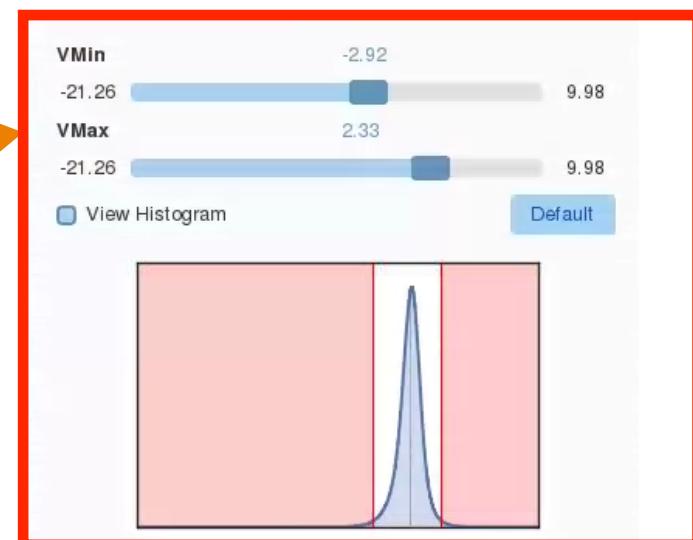
Draw a rectangle and zoom to that area



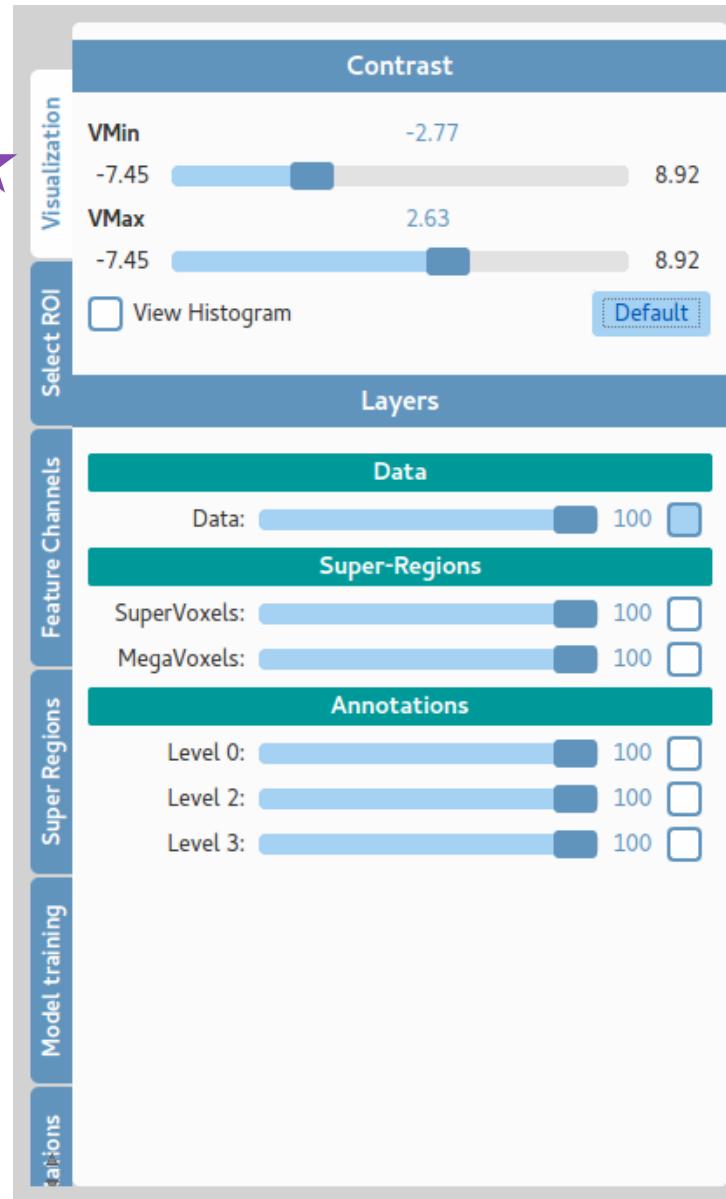
Turn on/ off a grid



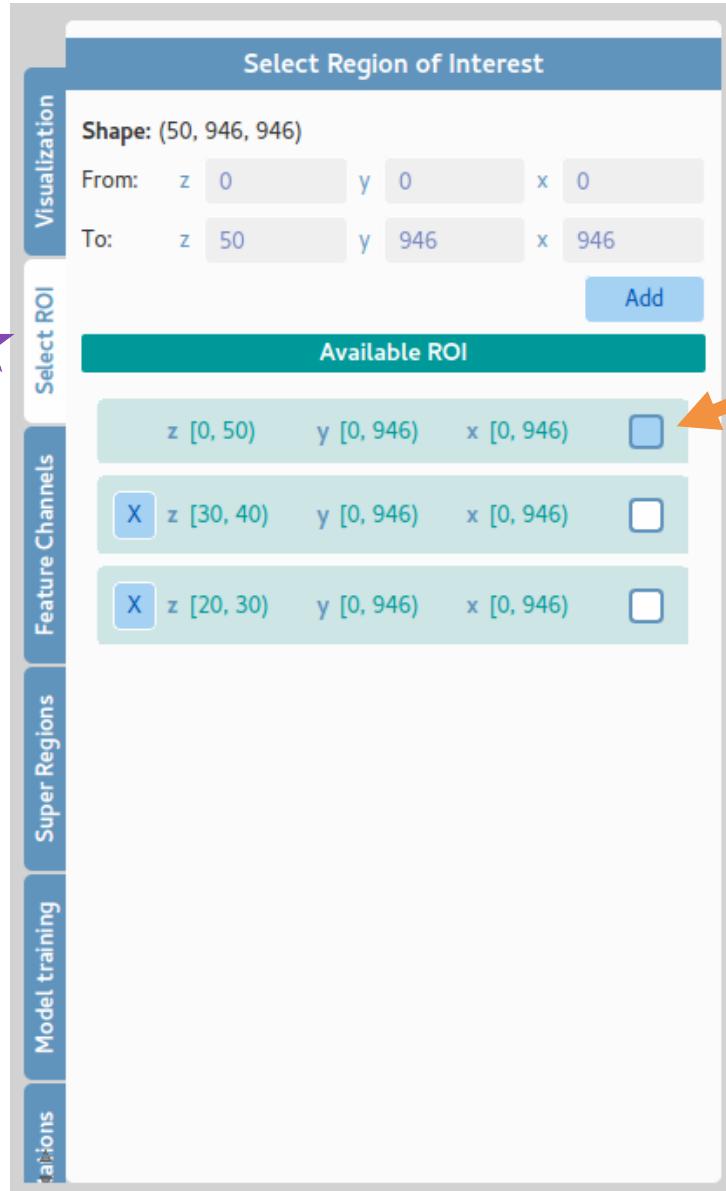
Save current image



Visualization Tab in GUI



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.

- 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

Select Region of Interest

Shape: (306, 946, 946)

From: z 95 y 440 x 250

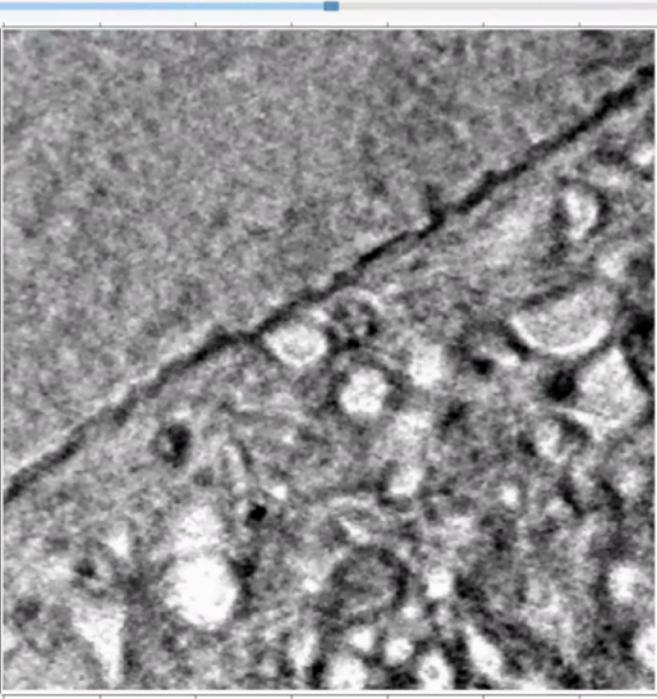
To: z 145 y 780 x 590

ROI

1 Add

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

2



Group B ROI

Select Region of Interest

Shape: (306, 946, 946)

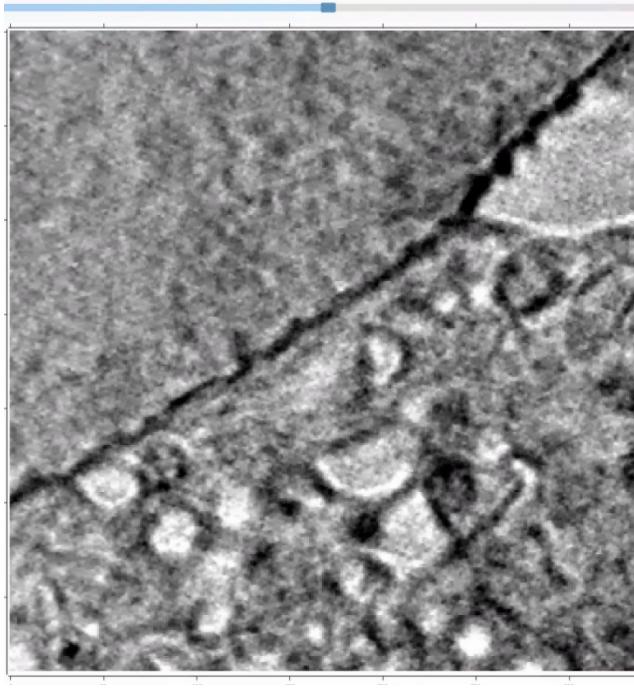
From: z 95 y 360 x 350

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

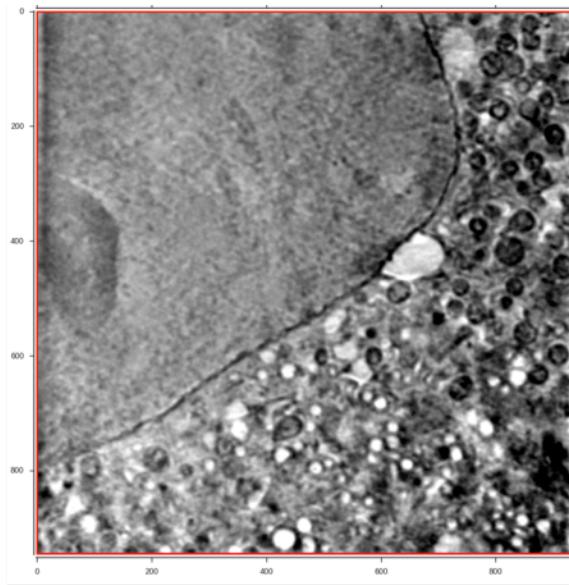
- Raw
 - Binary selection of data
- Denoising
 - Removes noise
- Local Features
 - Uses info from neighboring voxels based on a cube
- Gaussian Features
 - Uses info from neighboring voxels based on a Gaussian neighborhood

- Blob Detection
 - Uses info from neighboring voxels based on a Lapcian 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

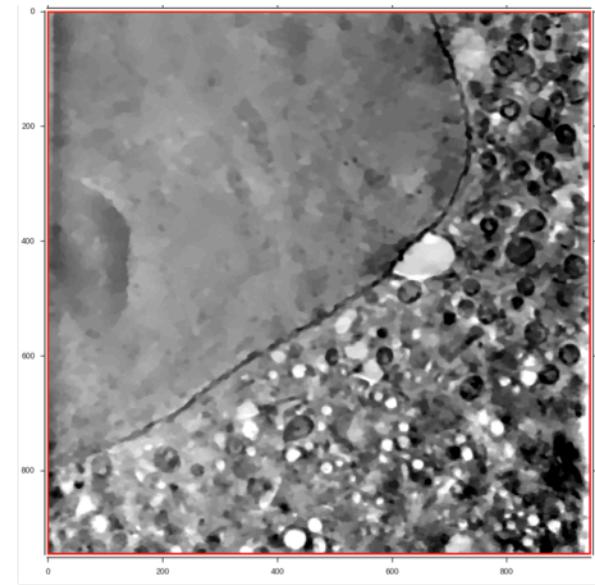
Available Filter Algorithms



(a) Raw SIRT reconstruction



(b) Gaussian Smooth



(c) Total Variation

Recommend

Sigma: 2.0 (range 1-5)

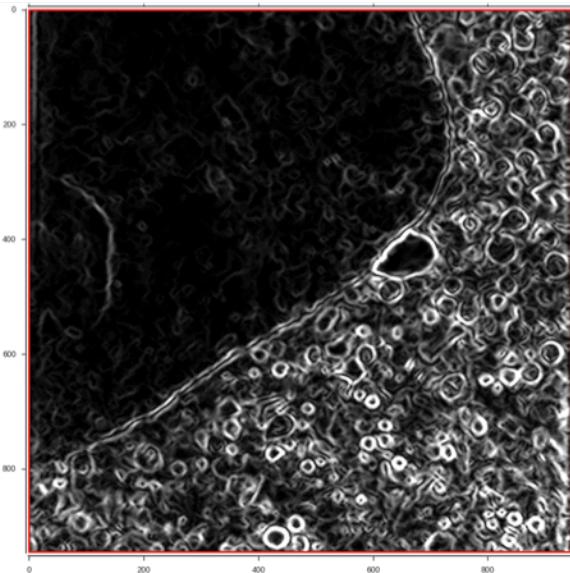
Recommend

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

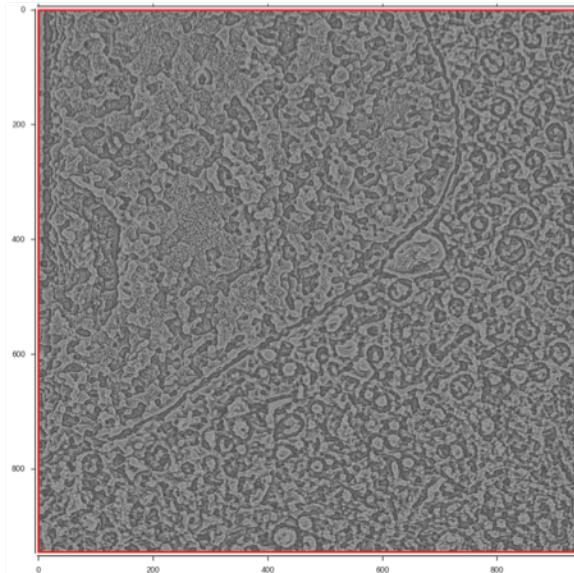
Note: Recommend to use Gaussian filter for supervoxel and megavoxel calculations, total variation for filter and feature calculations.

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

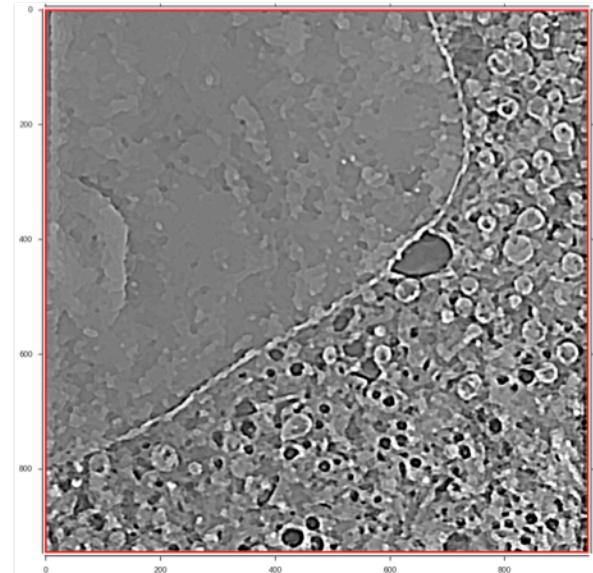
Selected Feature Filters



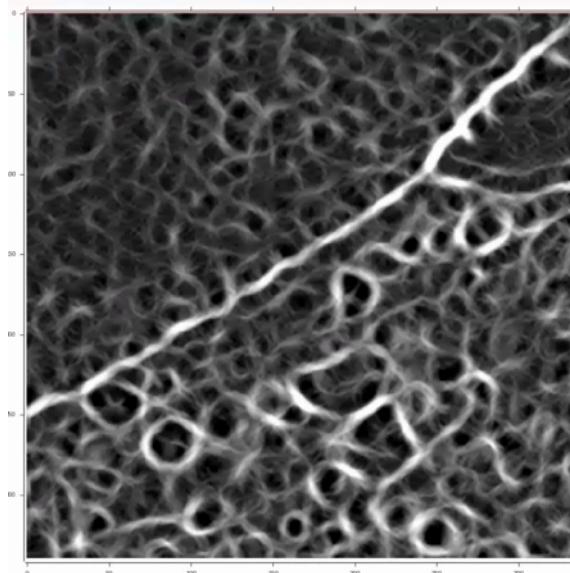
(d) Gradient Magnitude



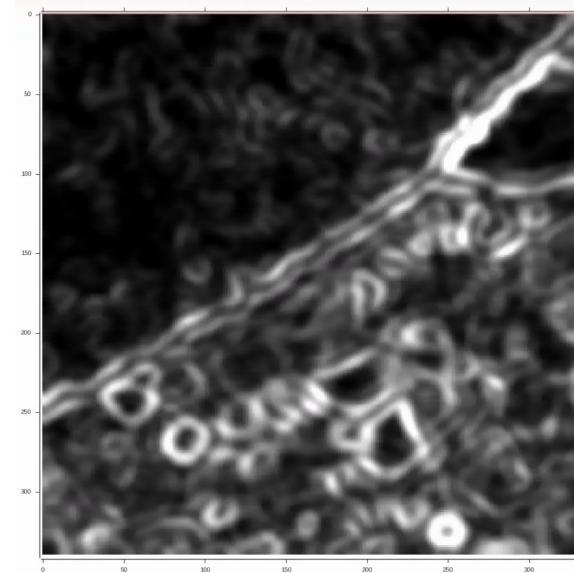
(e) Gaussian Local Normalization



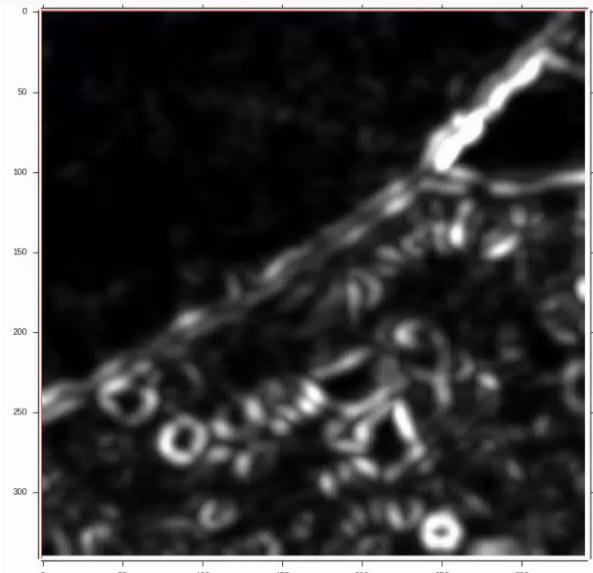
(f) Laplacian of Gaussian



(g) Hessian Eigenvalues

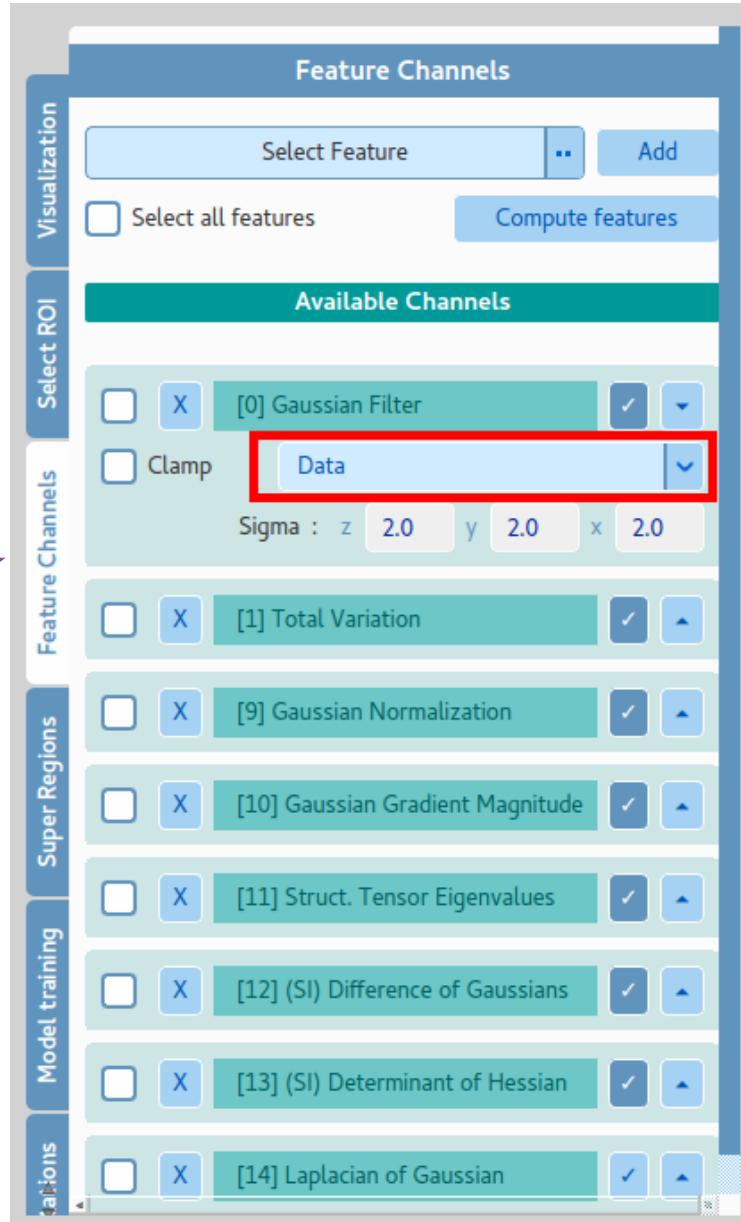


(h) Local Gradient Magnitude



(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

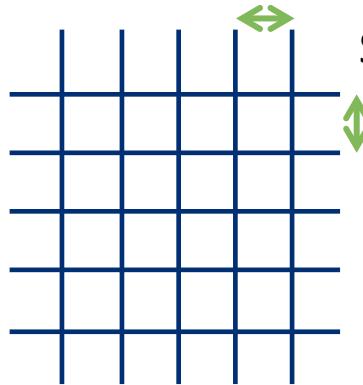


- Supervoxels and megavoxels are **edge preserving**, and **three-dimensional**.
- Segmentations are **less time and labor intensive** and should be **less subjective**.

Super Region Parameters: Supervoxels

SuperVoxels:

- SP shape
- Spacing
- Compactness

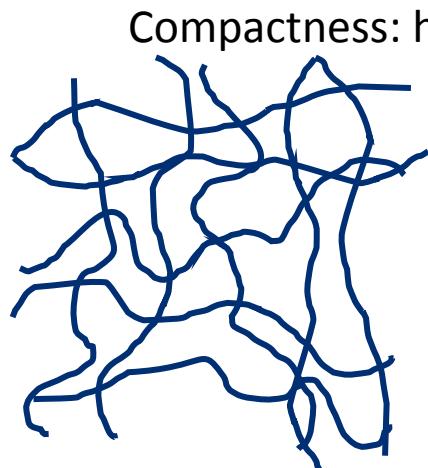


SP Shape: i.e. # of voxels to include in supervoxel grid

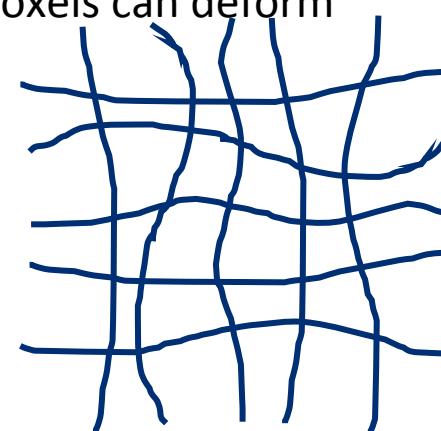
Note: order is Z, Y, X



Spacing: Importance of boundaries in each direction.



Low compactness #



High compactness #

Compactness: how much supervoxels can deform

Super Region Parameters: Megavoxels

Megavoxels:

- Lambda
- NumBins
- Gamma



Gamma: controls the cost of merging two supervoxels together

Super Region Parameters: Recommendations

The screenshot shows a software interface with a vertical sidebar on the left containing tabs: 'Visualizations', 'Select ROI', 'Feature Channels', 'Super Regions', and 'Model training'. The 'Super Regions' tab is currently active.

SuperVoxels:

- Source: [1] Total Variation
- SP shape: z 10 y 10 x 10
- Spacing: z 1 y 1 x 1
- Compactness: 50.0

MegaVoxels:

- Source: [1] Total Variation
- Lamda: 0.1
- NumBins: 10
- Gamma: None

Each section has an 'Apply' button at the bottom right.

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”

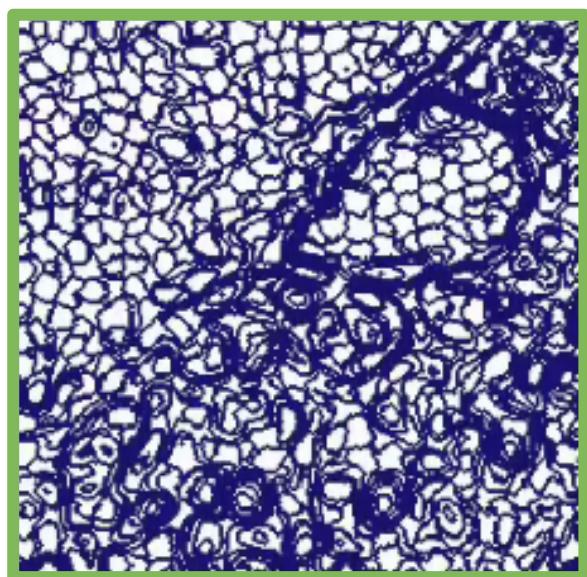
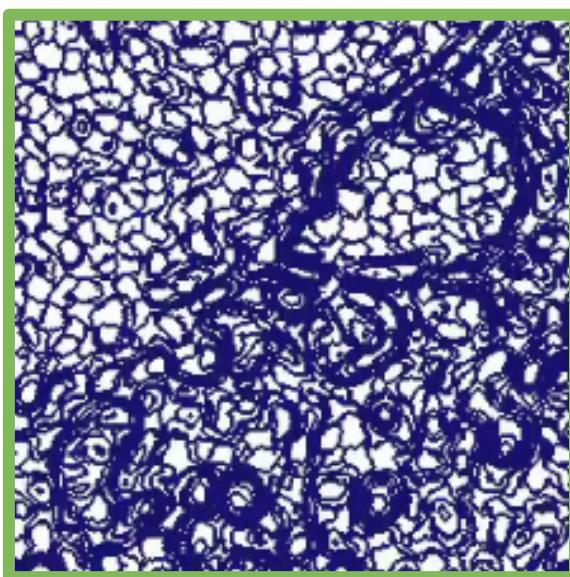
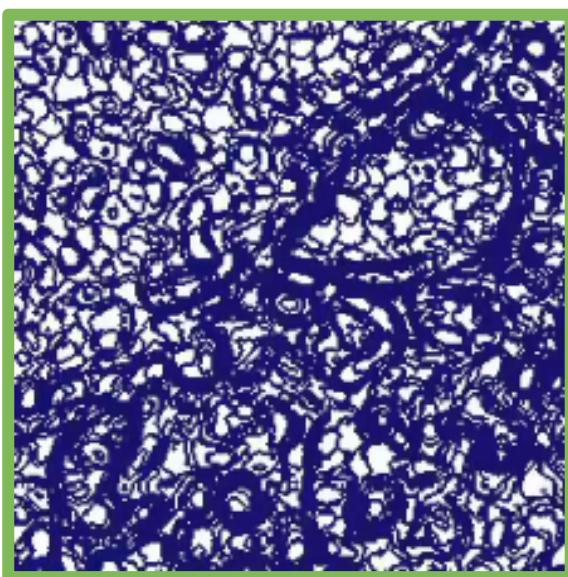
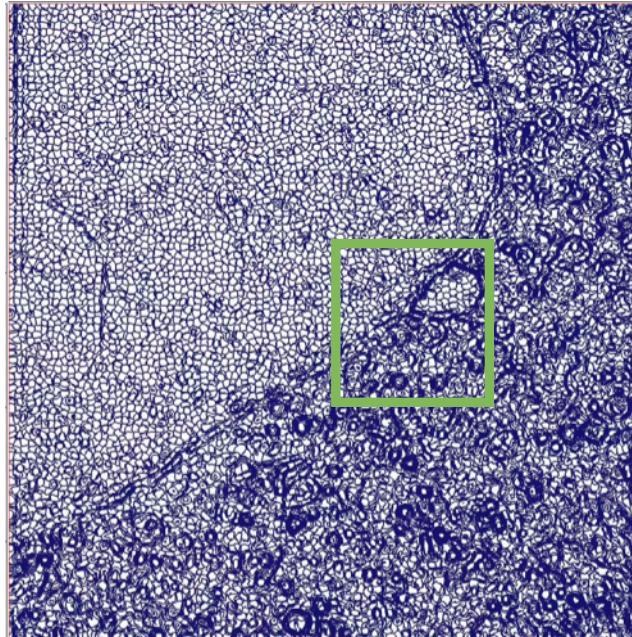
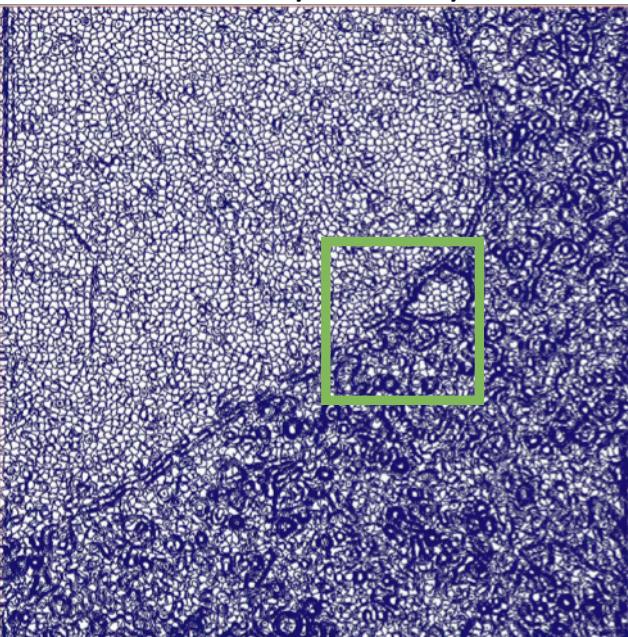
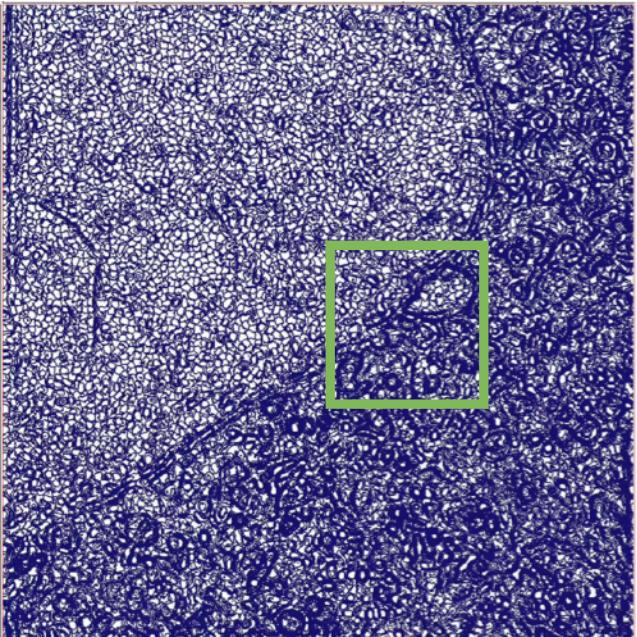
Notes:

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

Default:

10x10x10 / 1x1x1 / 20

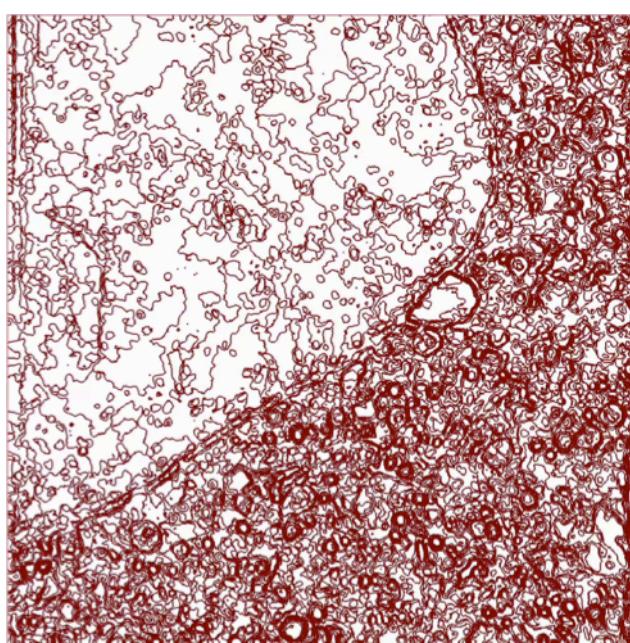
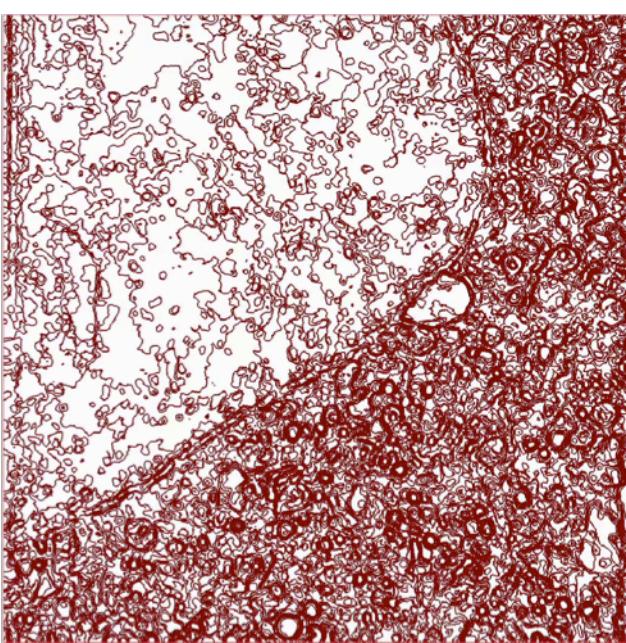
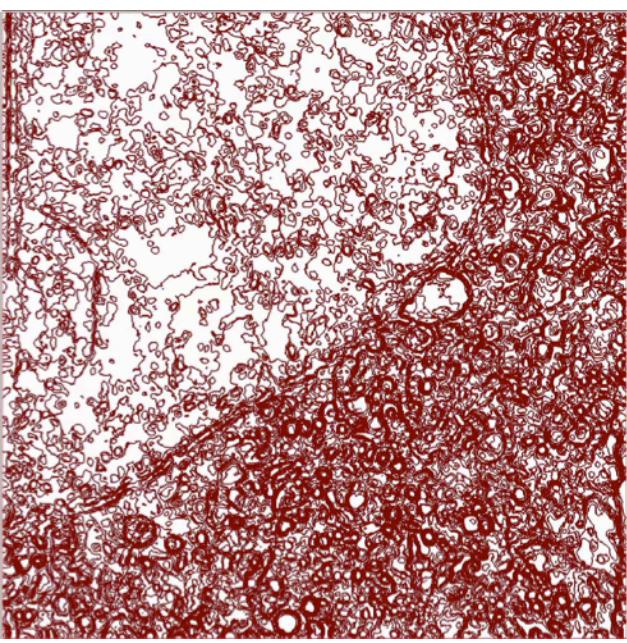
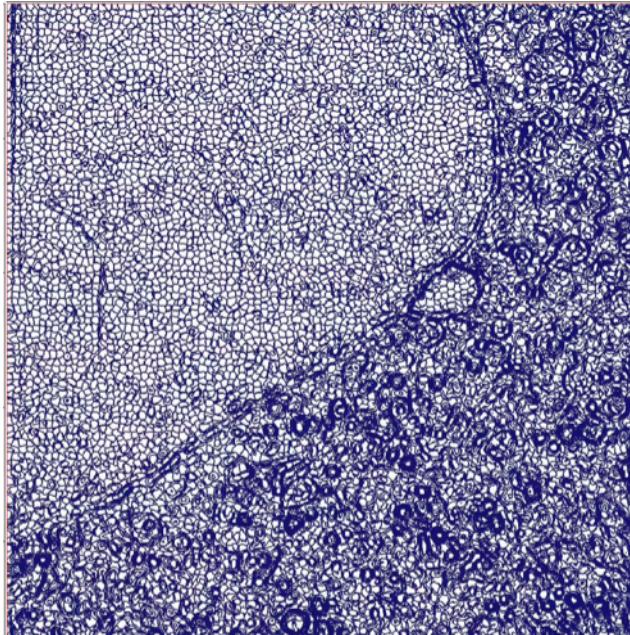
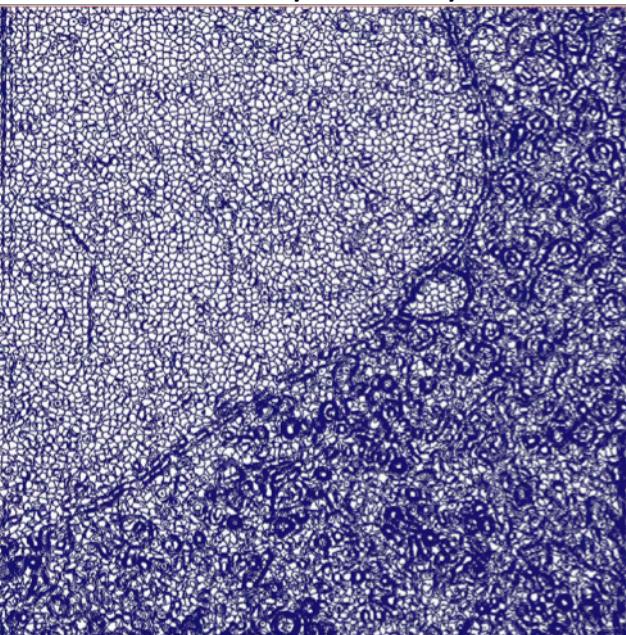
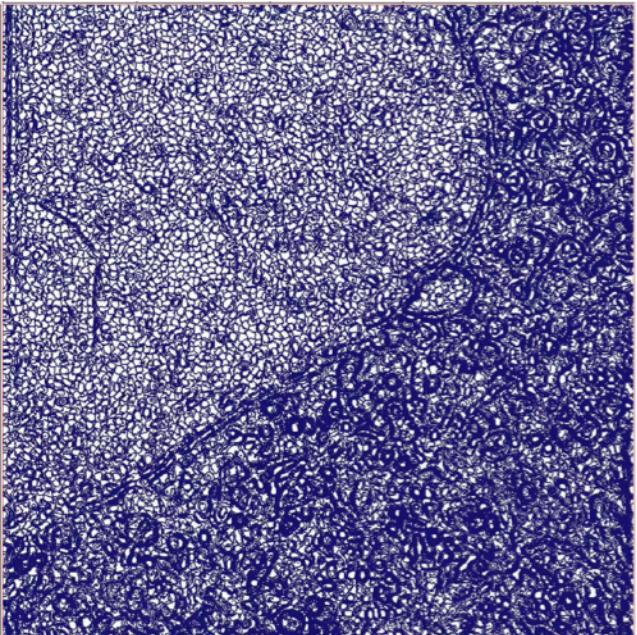
10x10x10 / 1x1x1 / 10



Default:

10x10x10 / 1x1x1 / 20

10x10x10 / 1x1x1 / 10

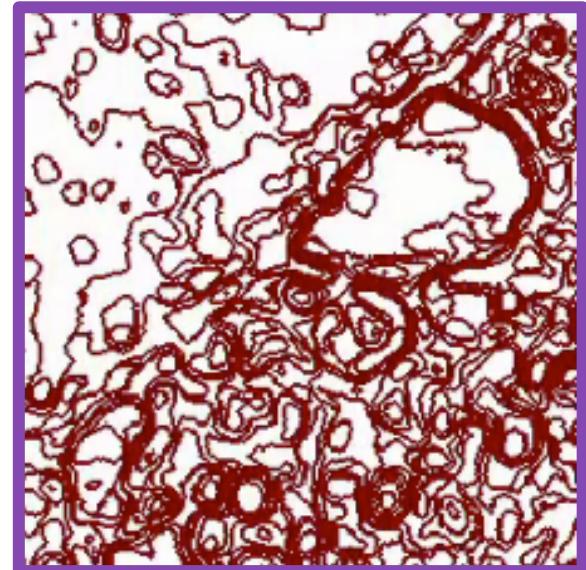
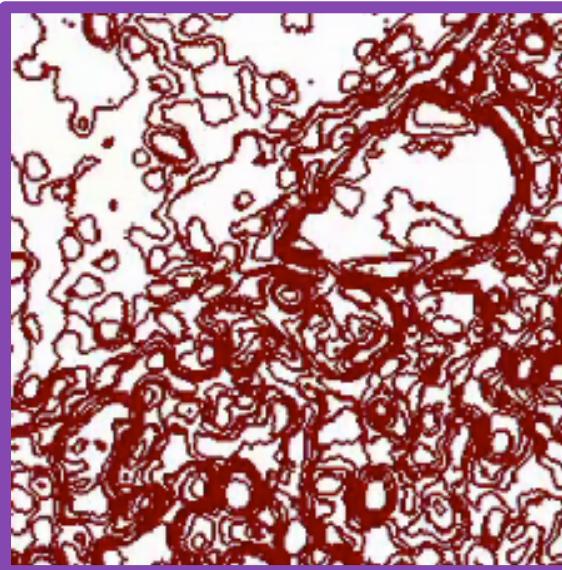
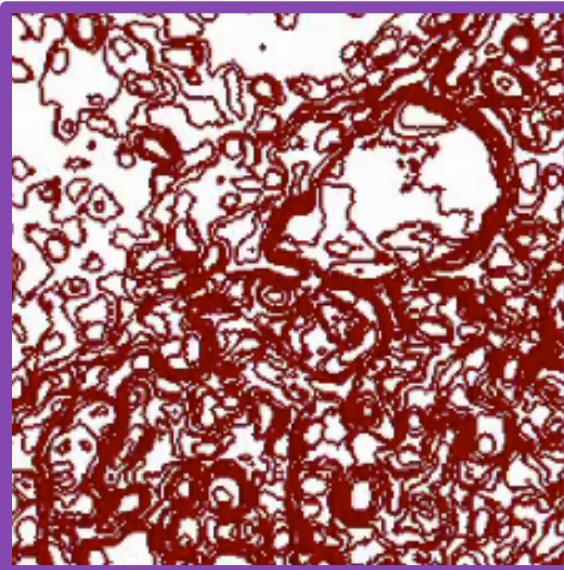
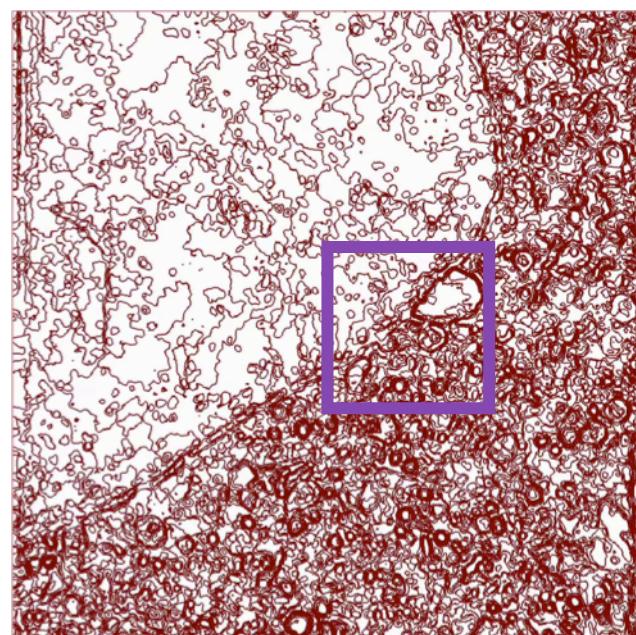
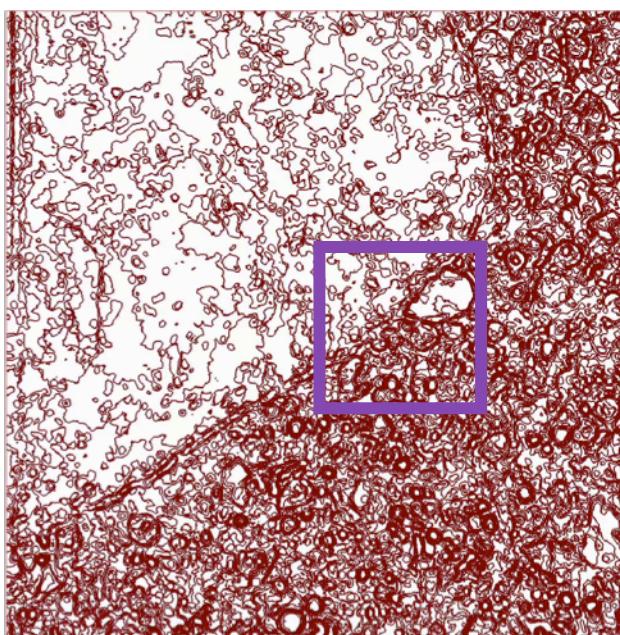
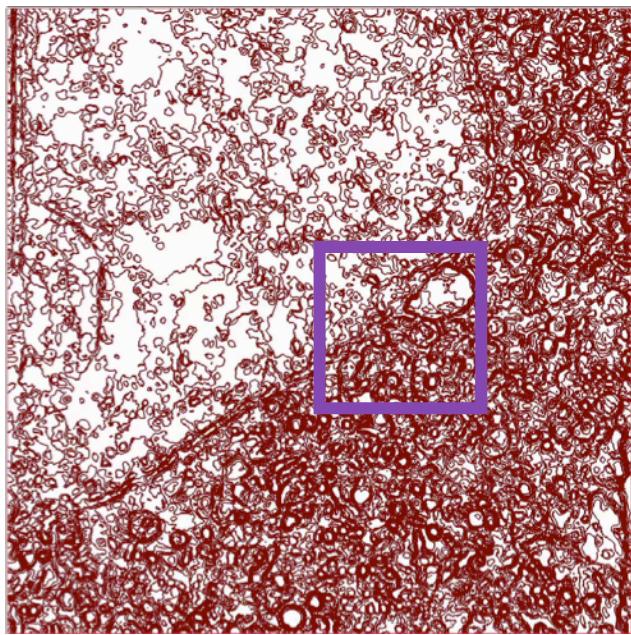


10x10x10 / 1x1x1 / 10

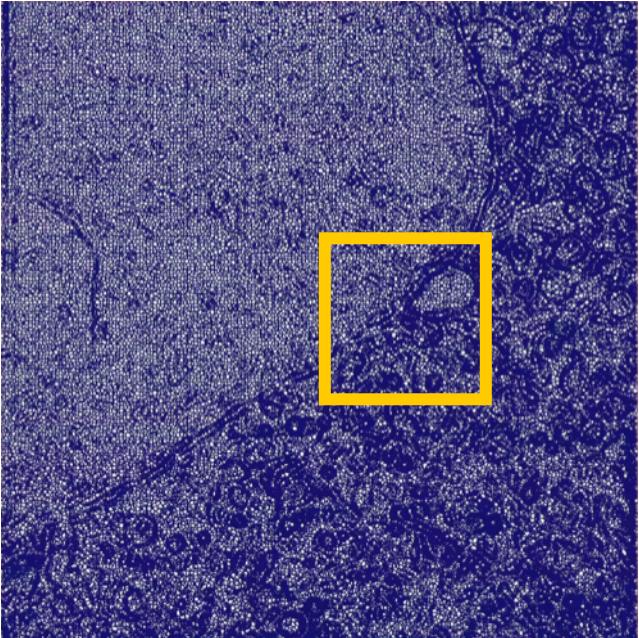
Default:

10x10x10 / 1x1x1 / 20

10x10x10 / 1x1x1 / 30

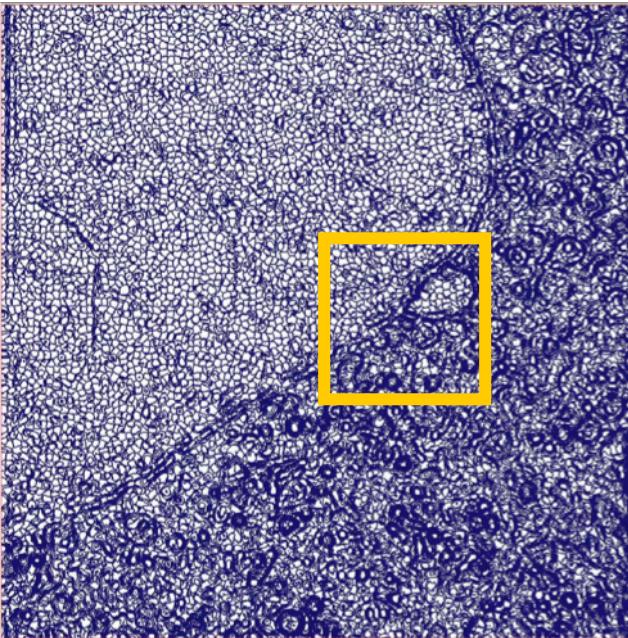


5x5x5 / 1x1x1 / 20

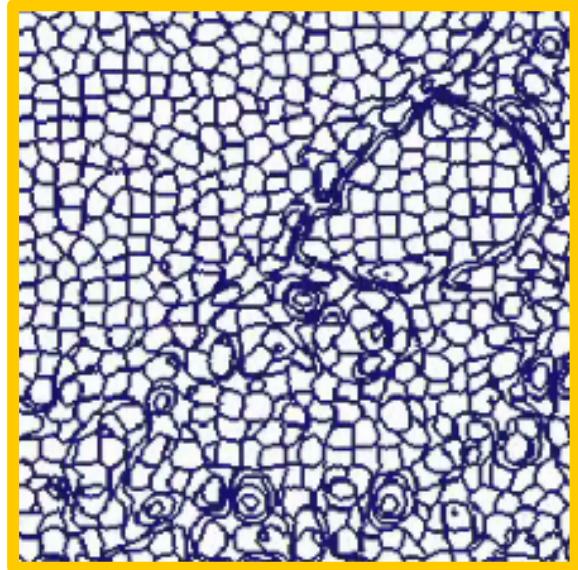
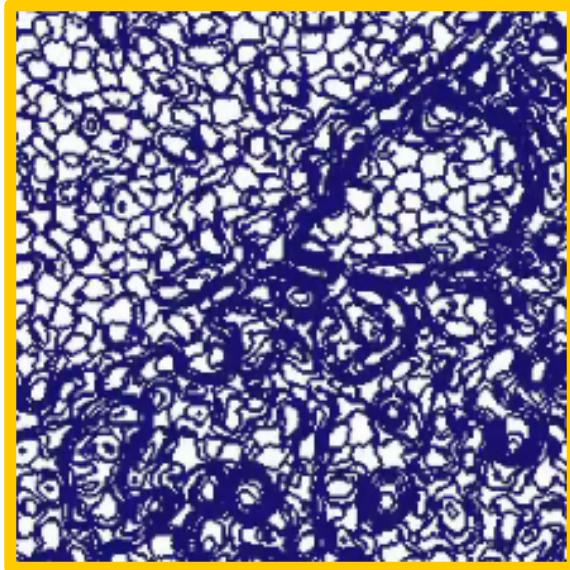
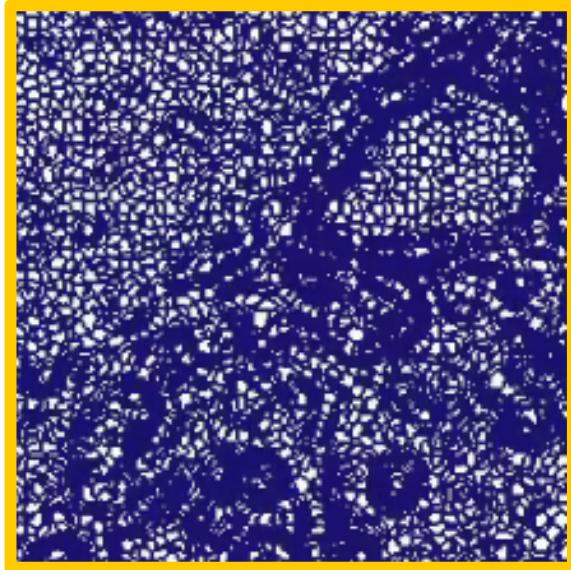
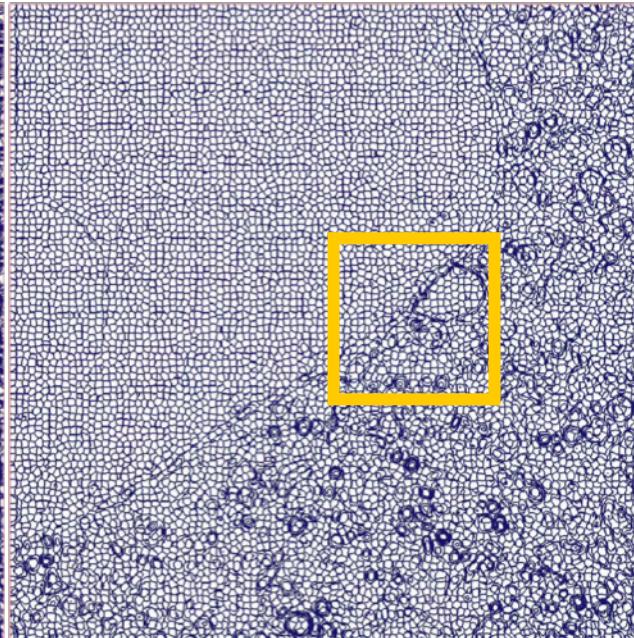


Default:

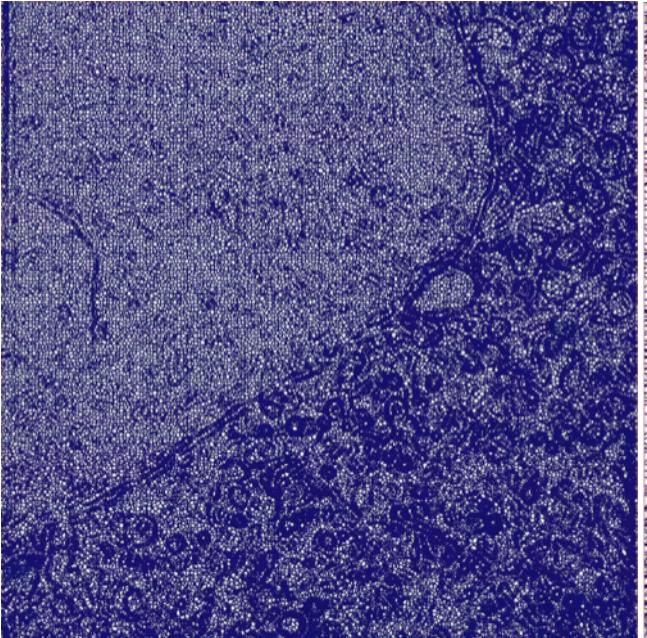
10x10x10 / 1x1x1 / 20



10x10x10 / 3x3x3 / 20

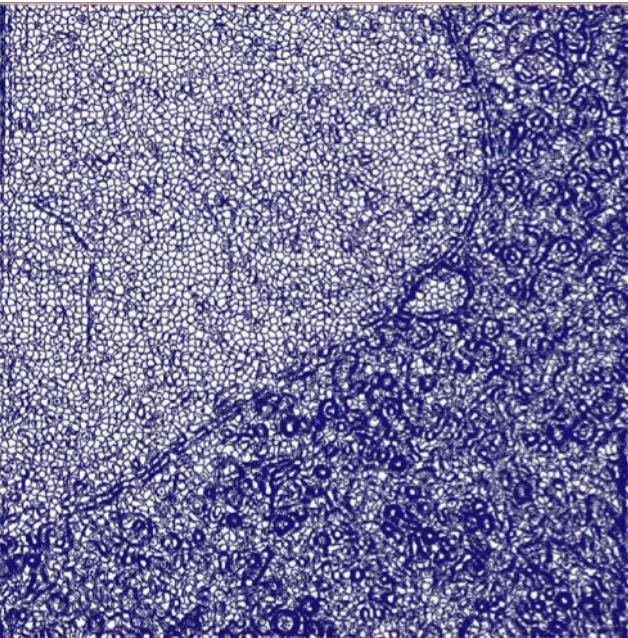


5x5x5 / 1x1x1 / 20

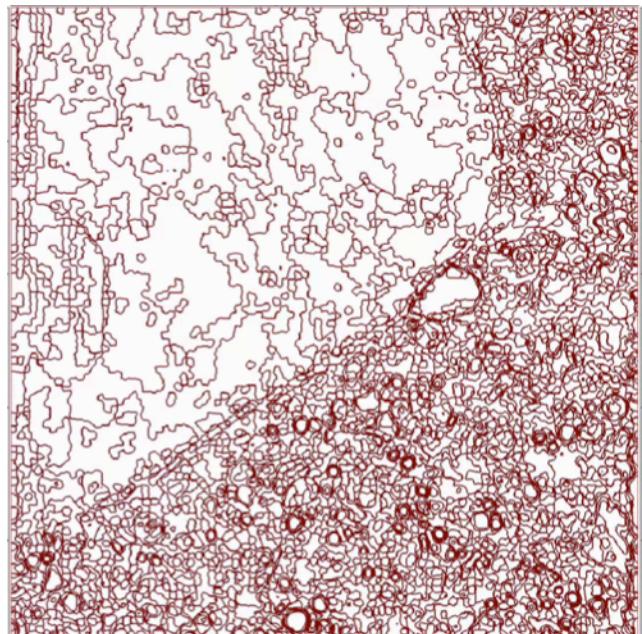
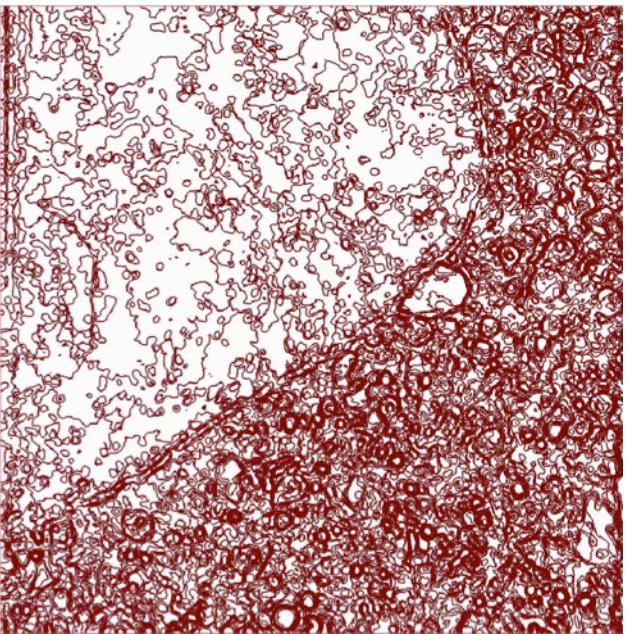
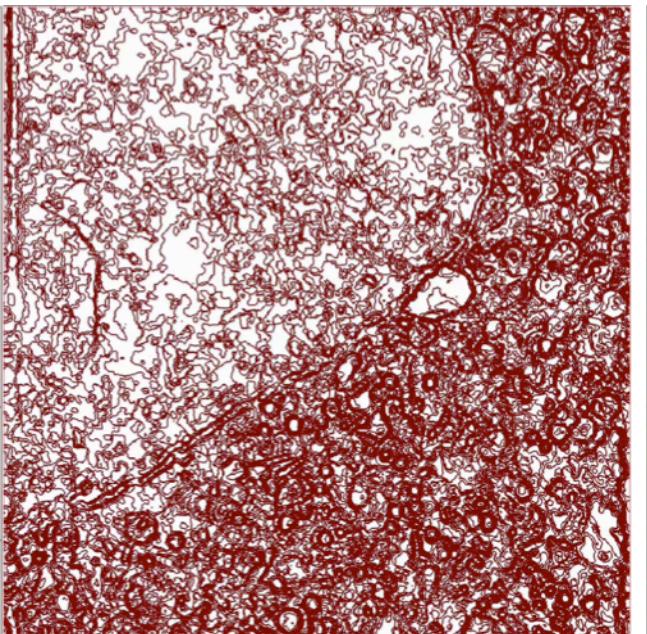
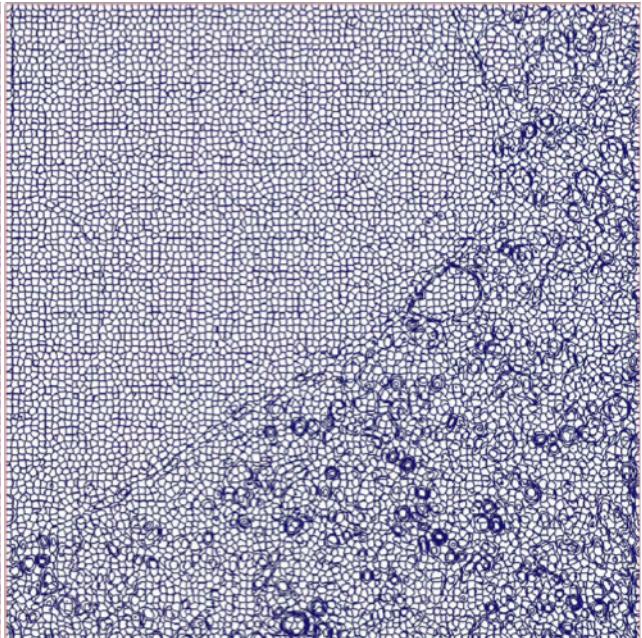


Default:

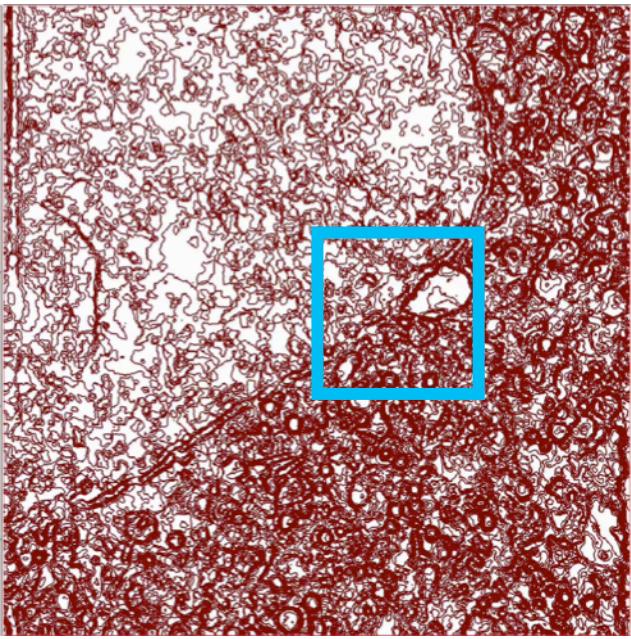
10x10x10 / 1x1x1 / 20



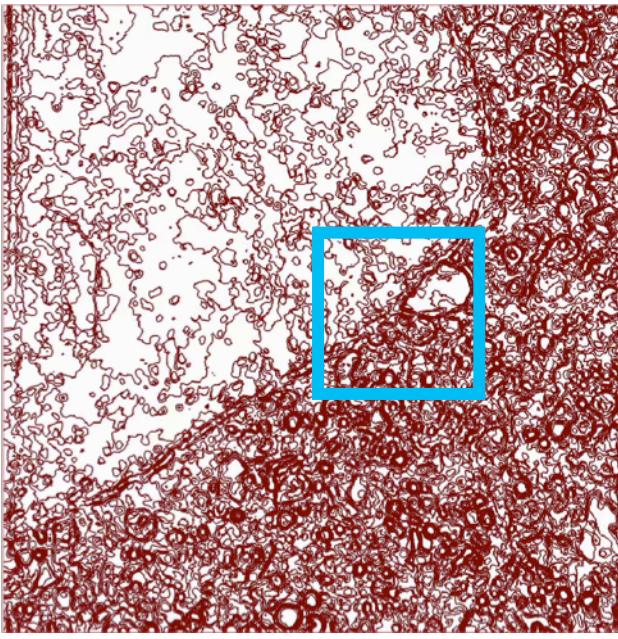
10x10x10 / 3x3x3 / 20



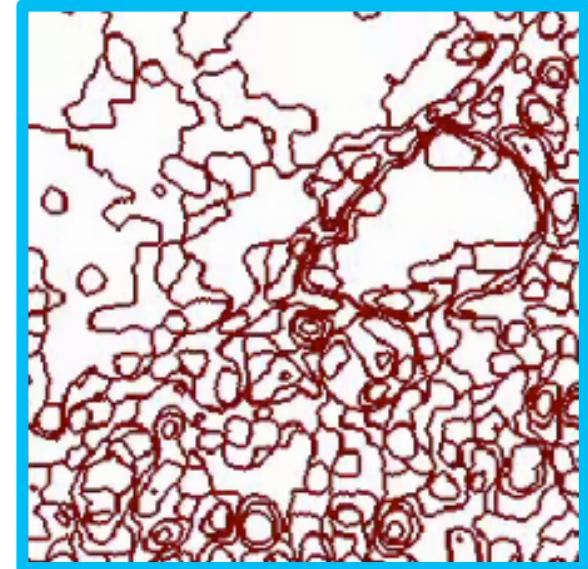
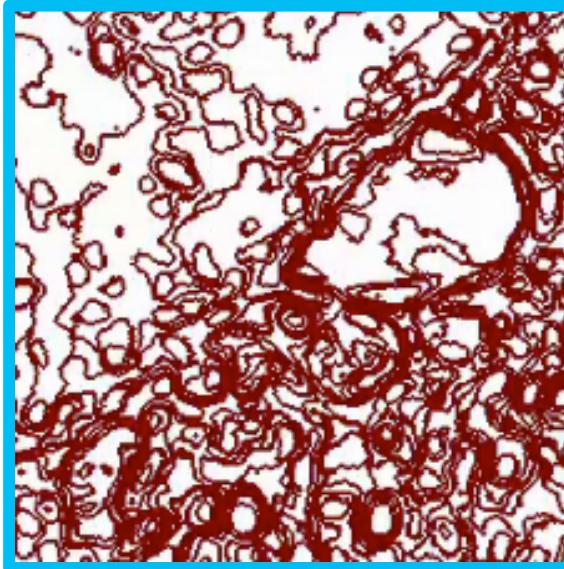
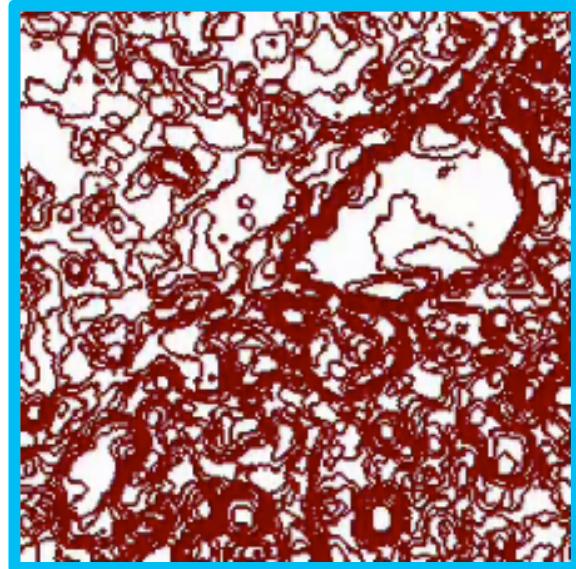
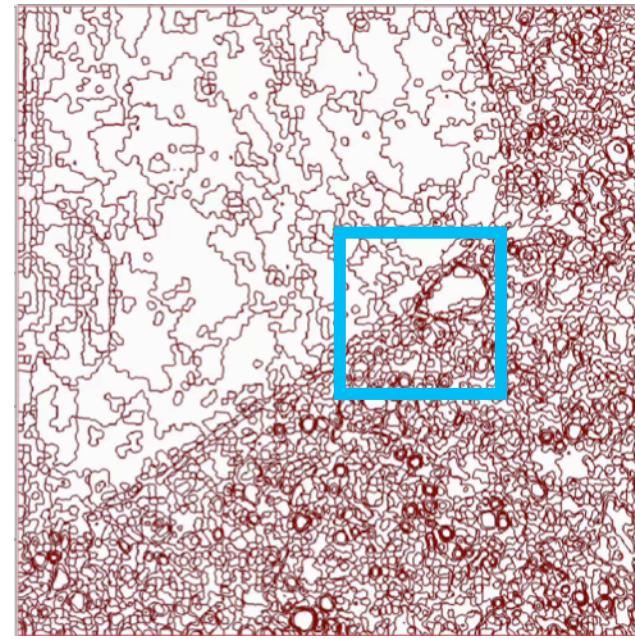
5x5x5 / 1x1x1 / 20



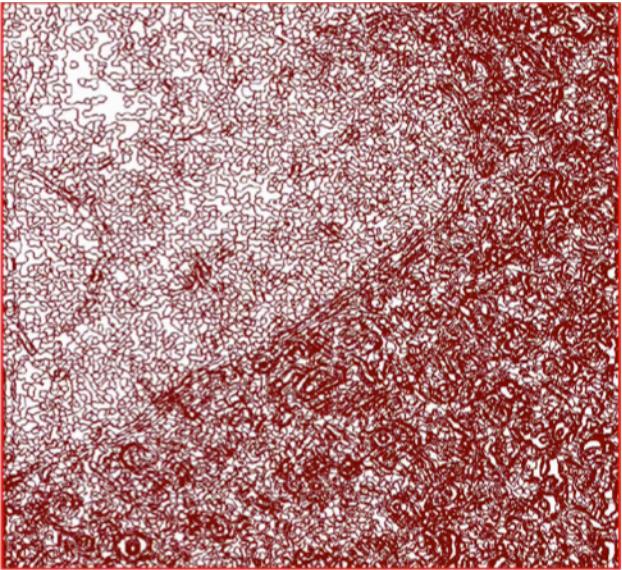
**Default:
10x10x10 / 1x1x1 / 20**



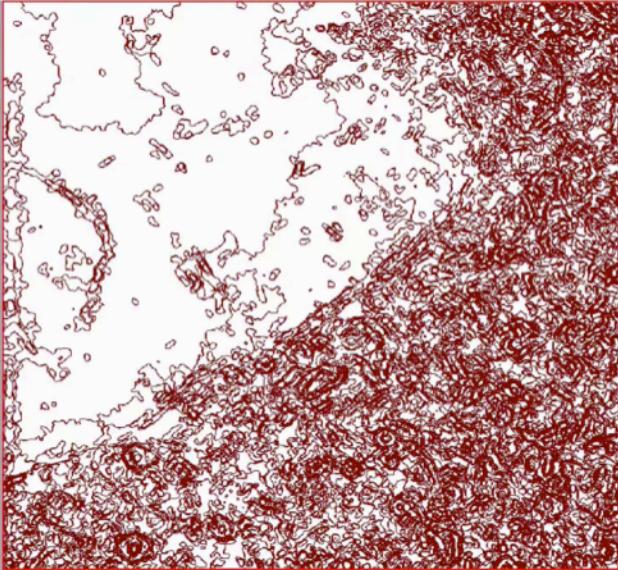
10x10x10 / 3x3x3 / 20



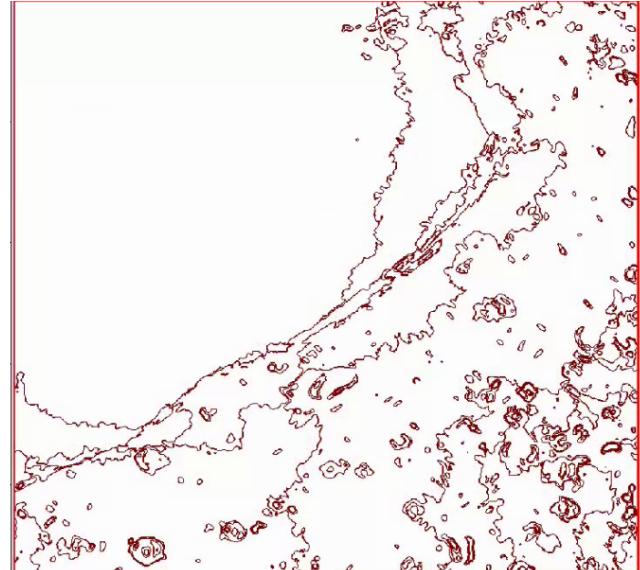
$\lambda=0.01$ /NumBins=20



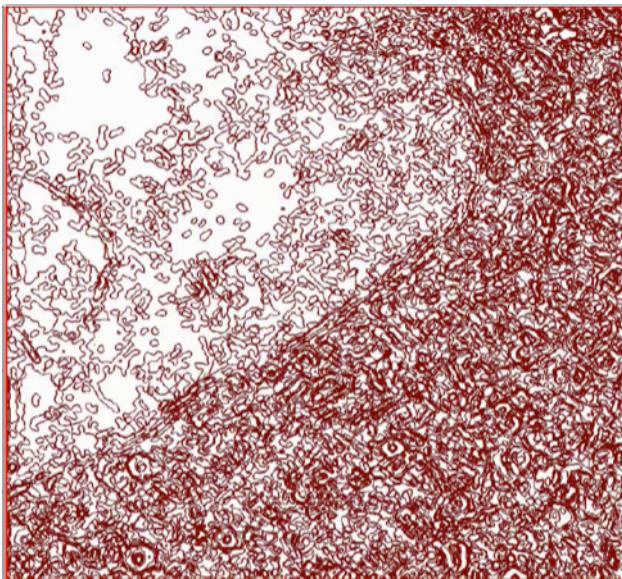
**Default:
 $\lambda=0.1$ /NumBins=20**



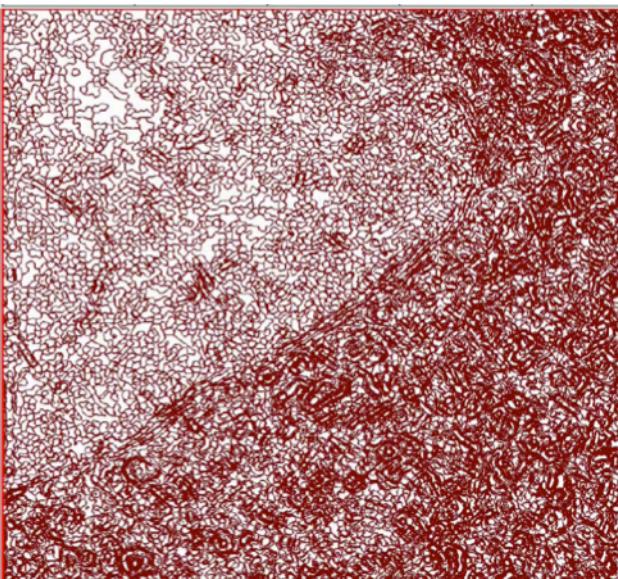
$\lambda=1$ /NumBins=20



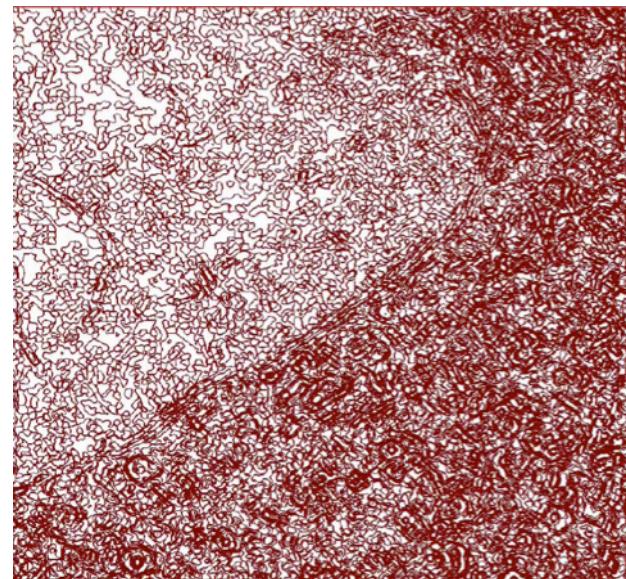
$\lambda=0.1$ /NumBins=10



$\lambda=0.1$ /NumBins=30

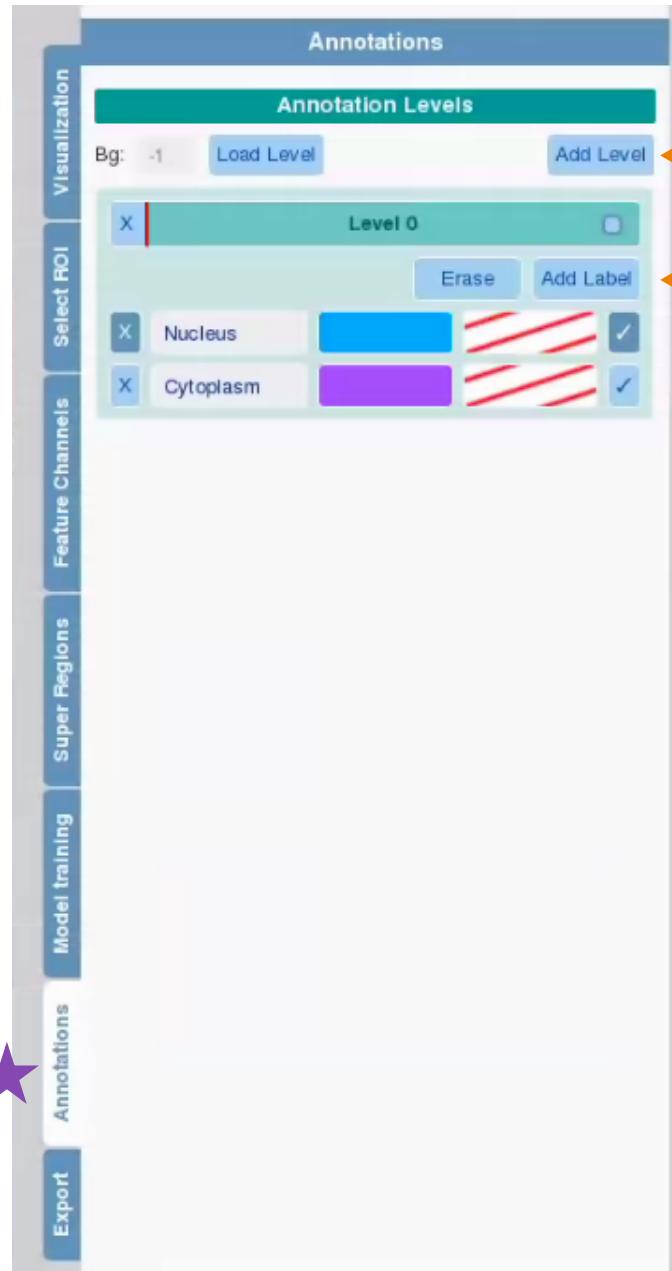


Defaults, gamma=auto



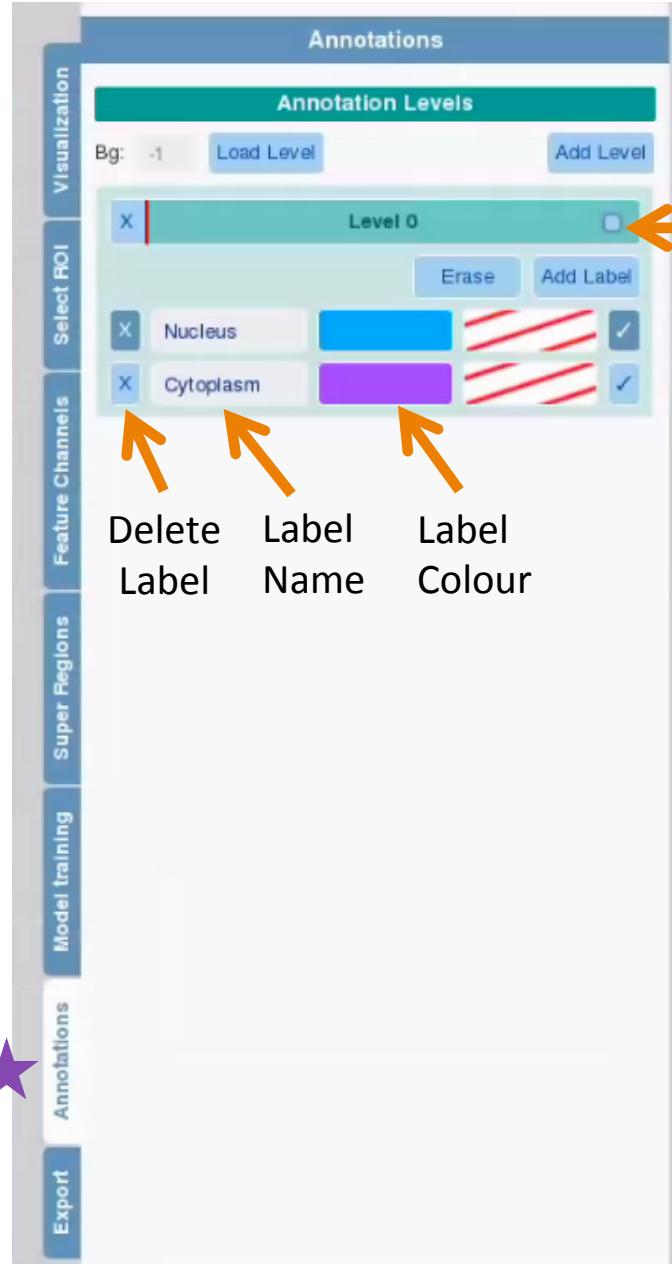
Time – 15 minutes

The Annotation Tab



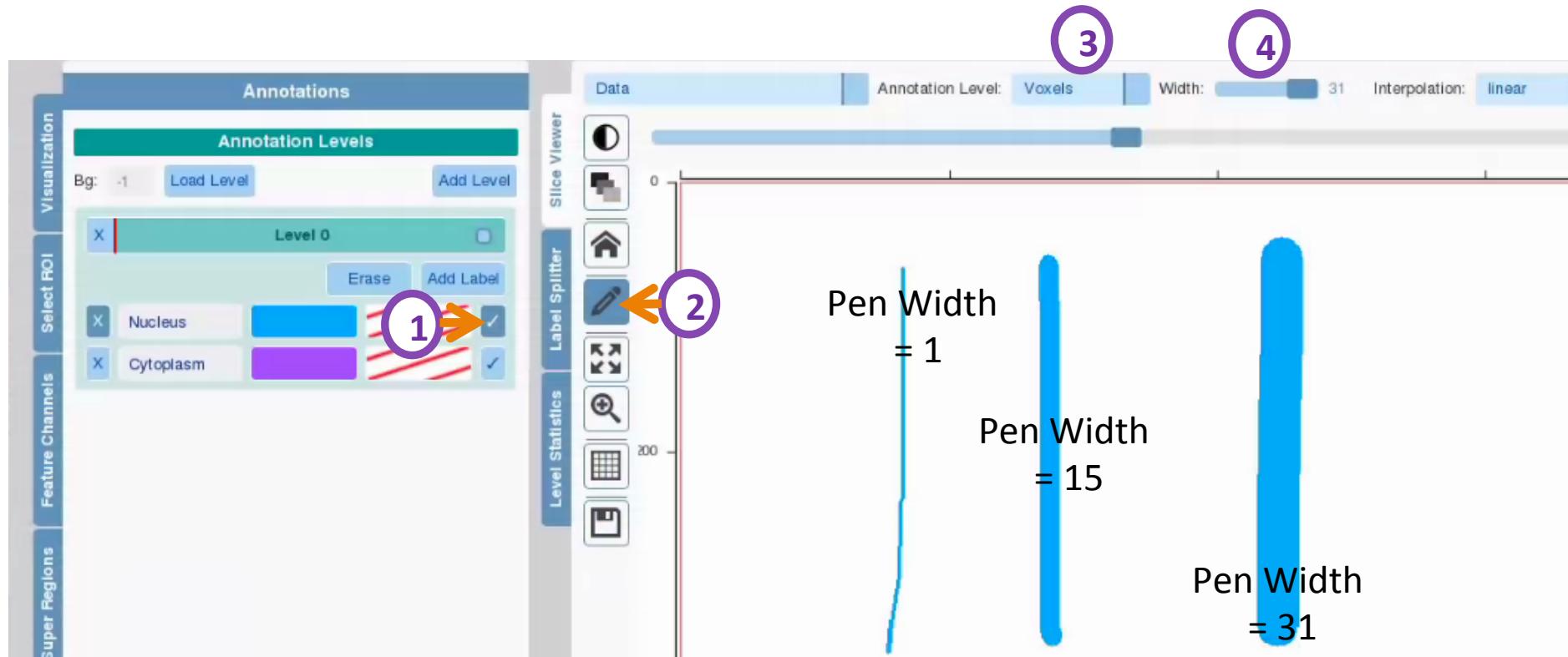
- To add a Level click on Add Level.
- To add Labels to a Level, click Add Label. Multiple labels can be added to any Level.

The Annotation Tab



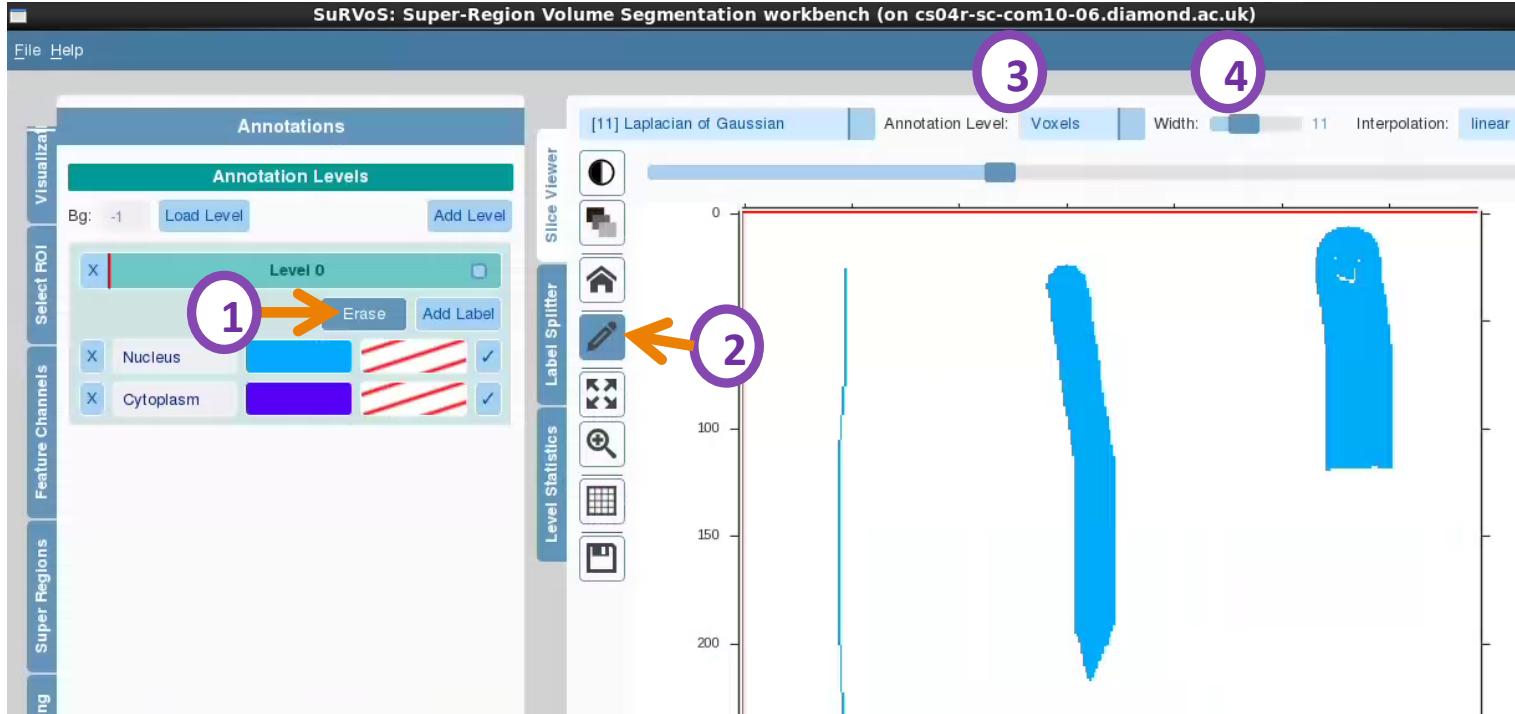
- To add a Level click on Add Level.
- To add Labels to a Level, click Add Label. Multiple labels can be added to any Level.
- Labels can be given names, the colour used to represent them in annotations can be changed. Or if they are no longer needed, labels can be deleted.

Annotation Using Voxels



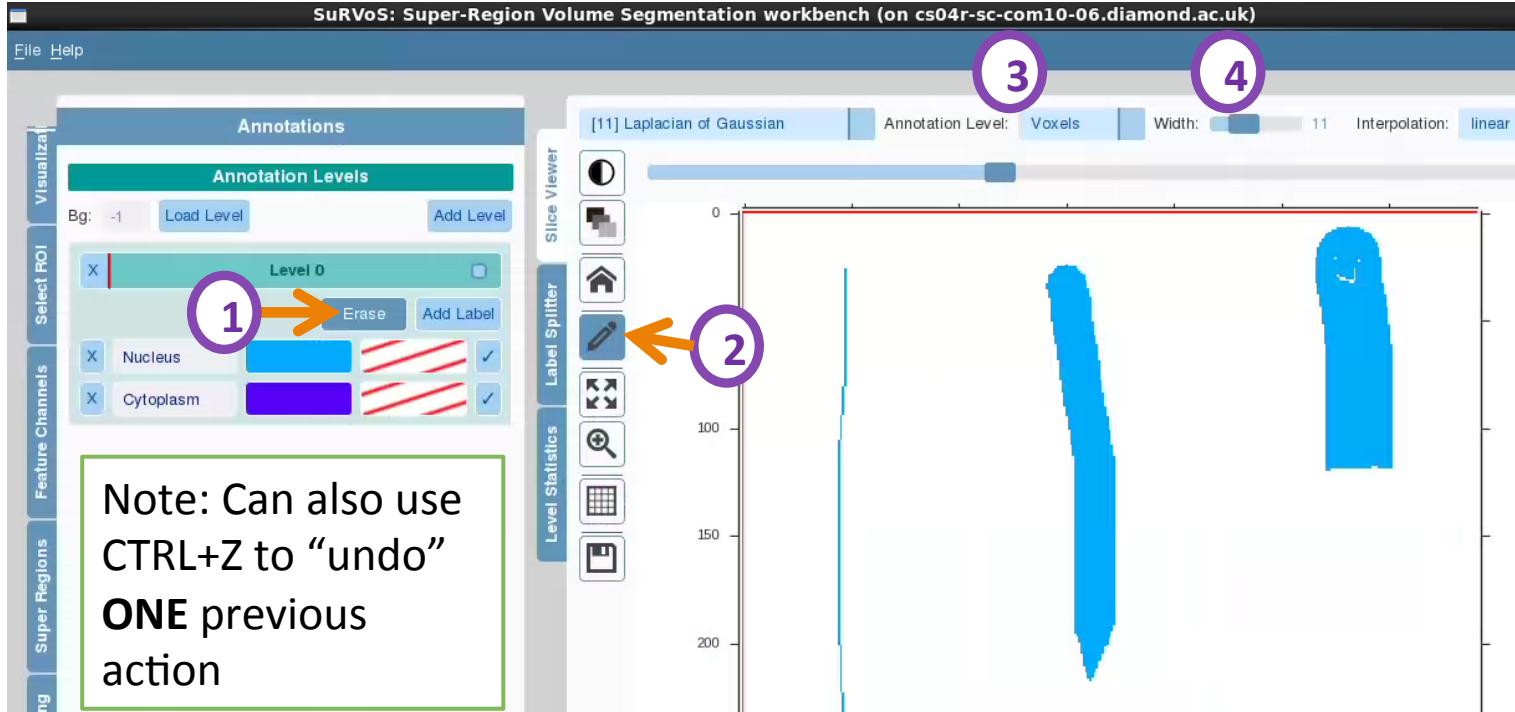
- 1) In the annotation tab select the label you wish to annotate with
- 2) Select the pencil tool
- 3) Select annotation level (**voxel**, supervoxel, or megavoxel)
- 4) Choose an appropriate width for the feature that you are annotating and draw using the left mouse button

Erasing Annotations



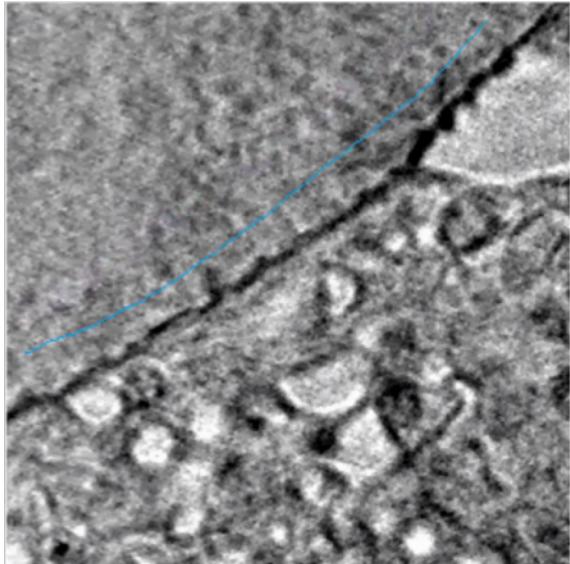
- 1) In the annotation tab select erase on the level you wish to use the use the eraser on
- 2) Select the pencil tool (if not already selected)
- 3) Select annotation level (voxel, supervoxel, or megavoxel)
- 4) Choose an appropriate width for the feature that you are erasing and erase using the left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

Erasing Annotations

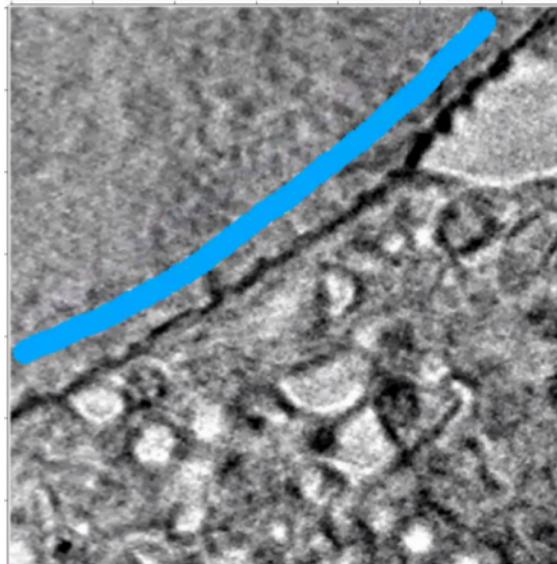


- 1) In the annotation tab select erase on the level you wish to use the use the eraser on
- 2) Select the pencil tool (if not already selected)
- 3) Select annotation level (voxel, supervoxel, or megavoxel)
- 4) Choose an appropriate width for the feature that you are erasing and erase using the left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

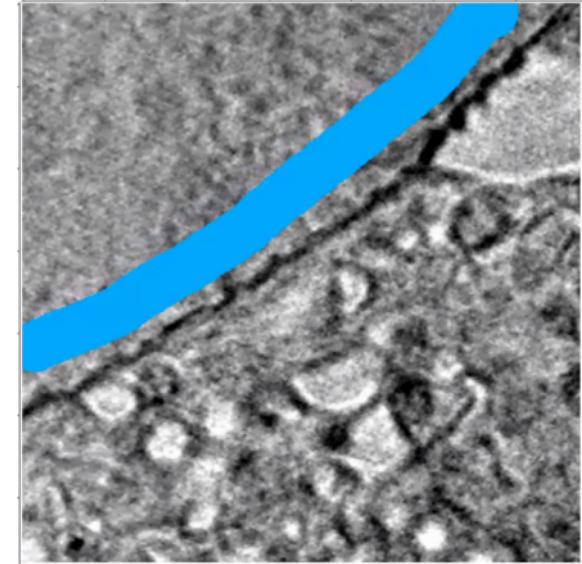
Annotation Using Voxels



Pen Width = 1



Pen Width = 15

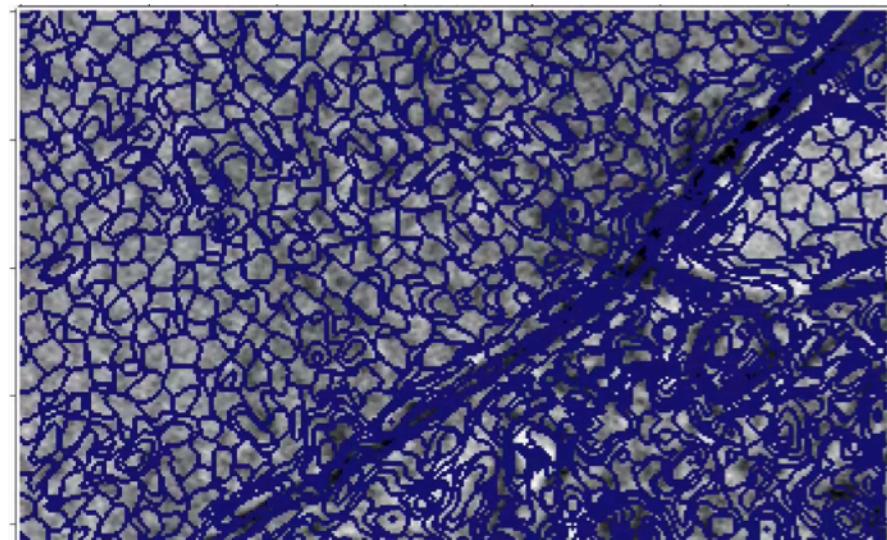
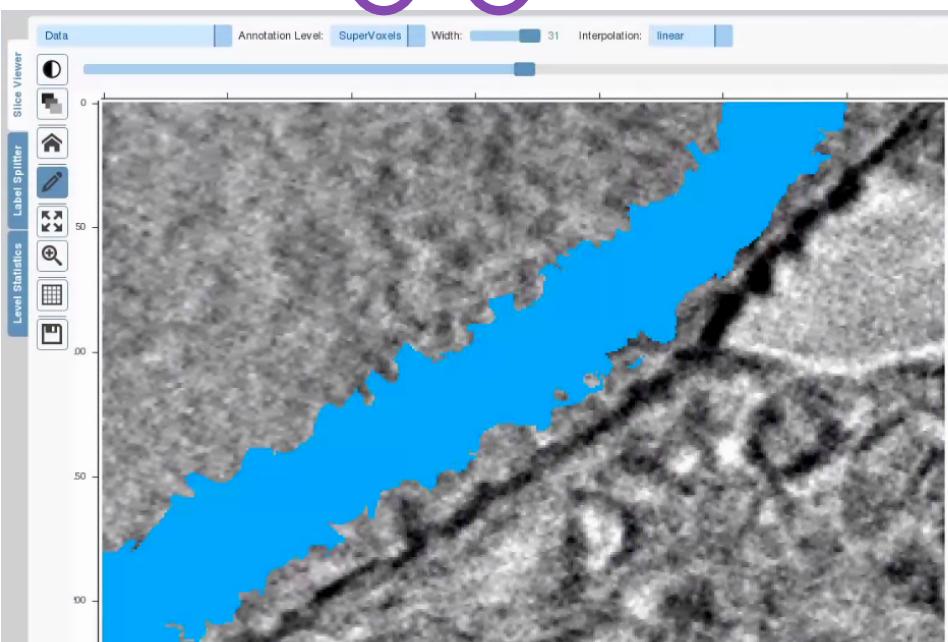


Pen Width = 31

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

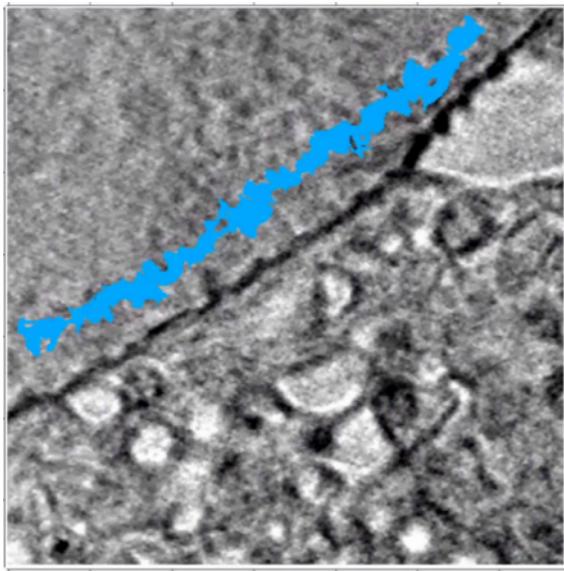
Annotation Using Supervoxels

1 2

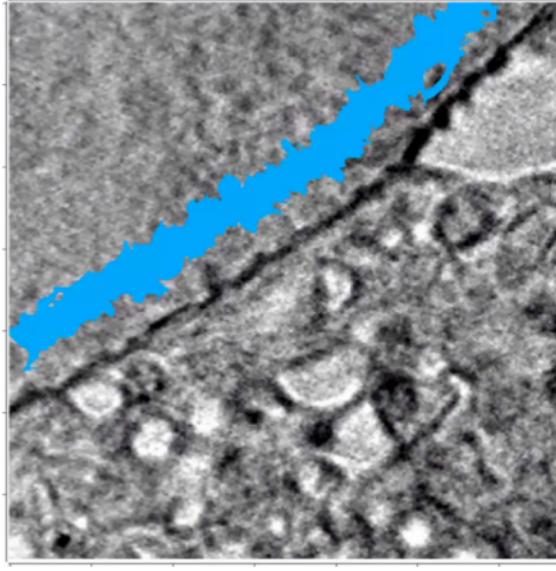


- 1) Select annotation level (voxel, **supervoxel**, or megavoxel)
- 2) Choose a width appropriate for the feature that you are annotating and draw using the left mouse button.

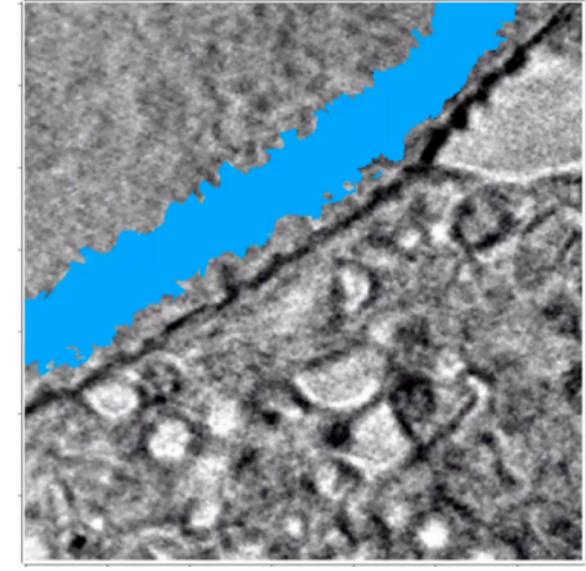
Annotation Using Supervoxels



Pen Width = 1

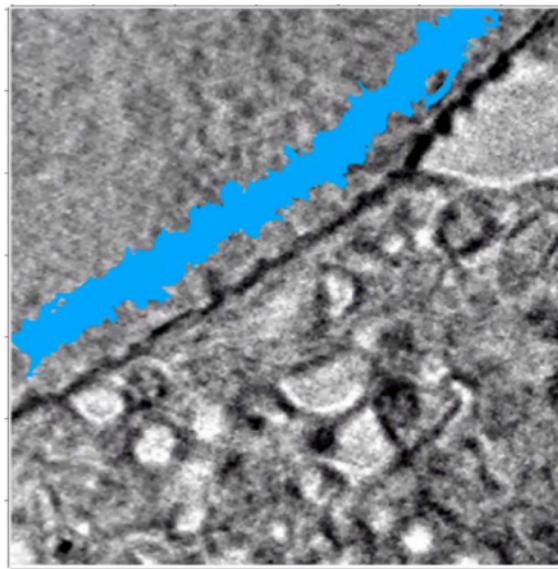


Pen Width = 15

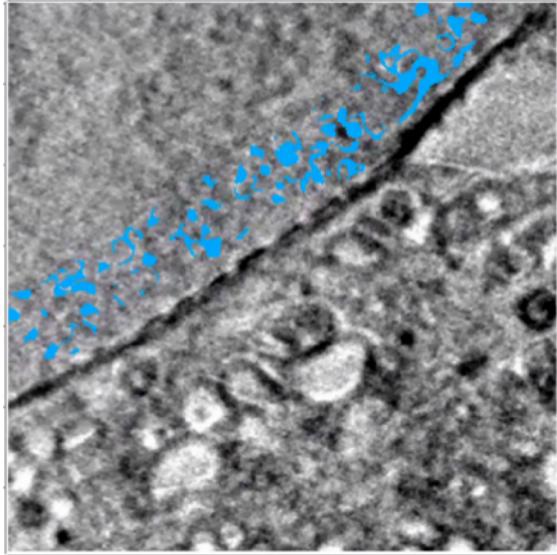


Pen Width = 31

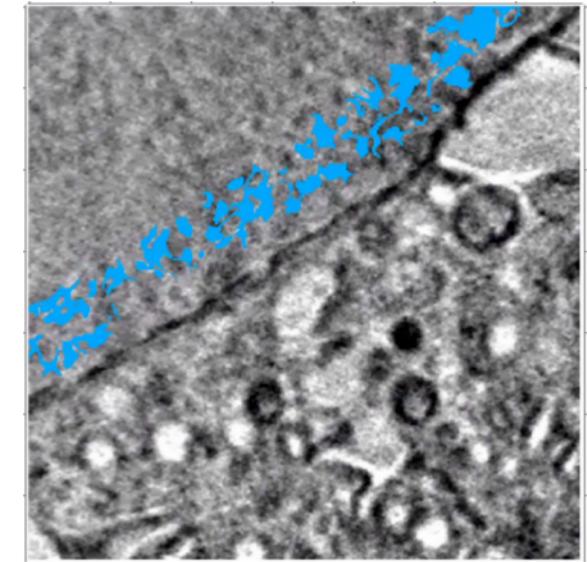
Annotation Using Supervoxels



10 slices below



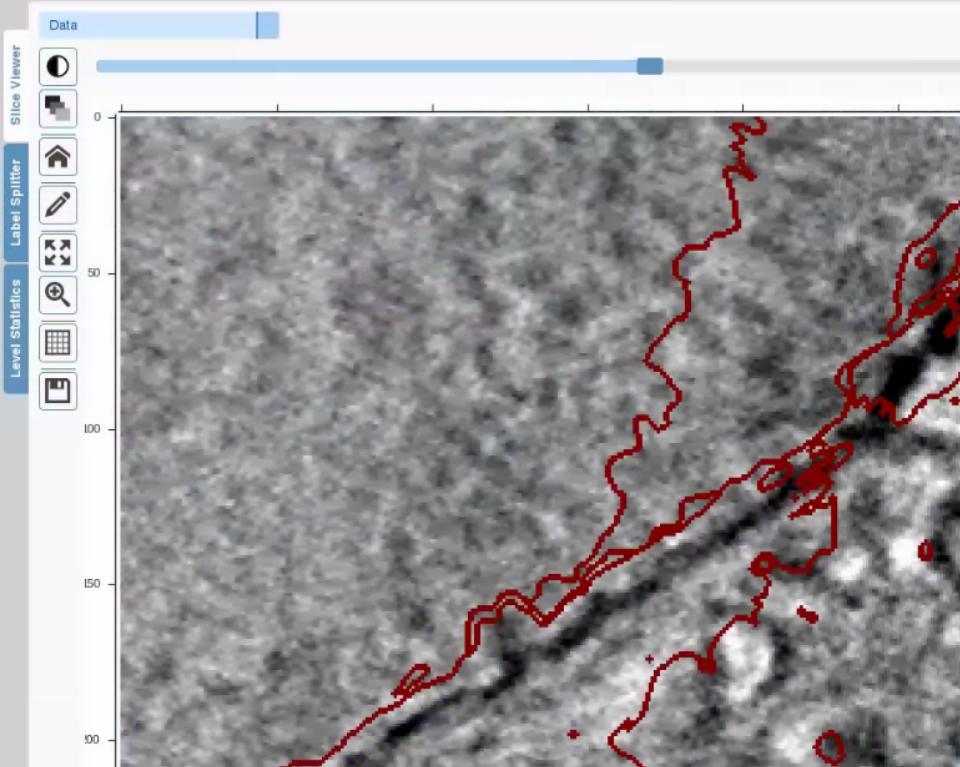
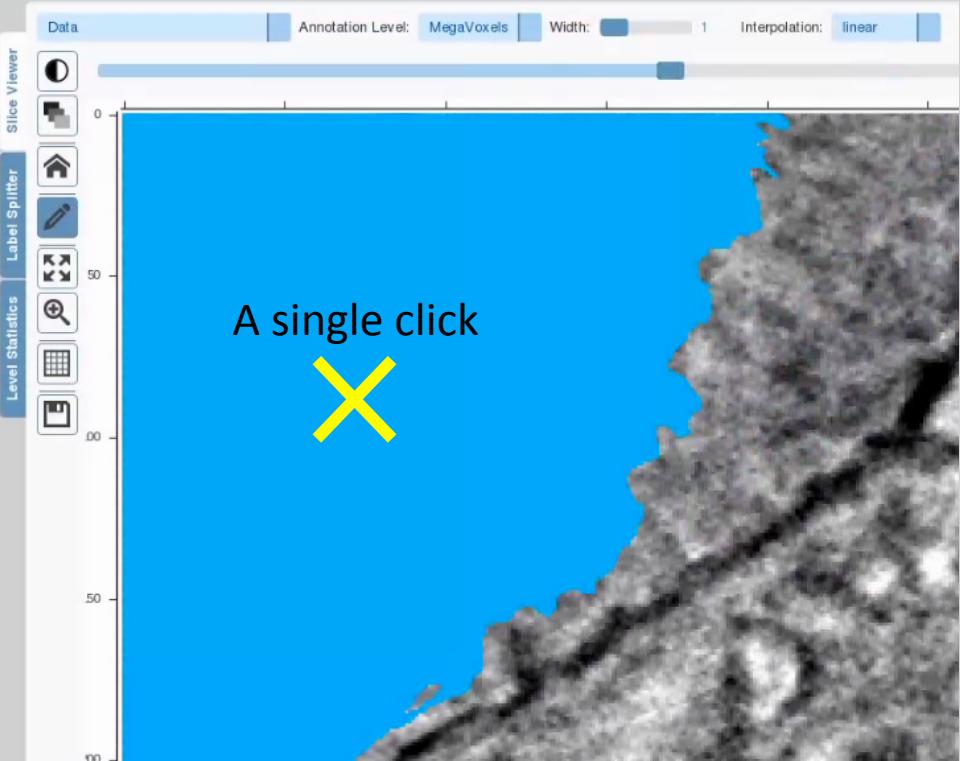
10 slices above



Annotating using
supervoxels penetrates
multiple Z-slices

Annotation using Megavoxels

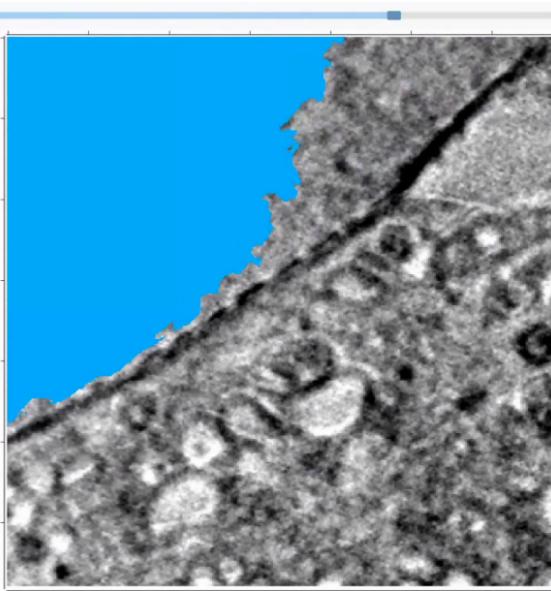
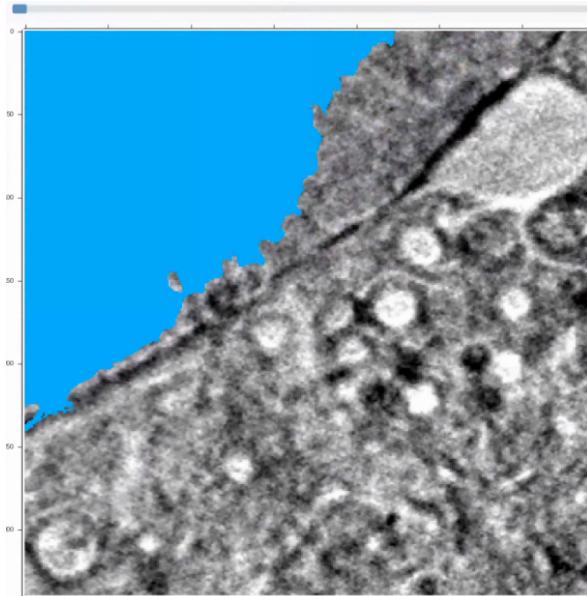
① ②



- 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.

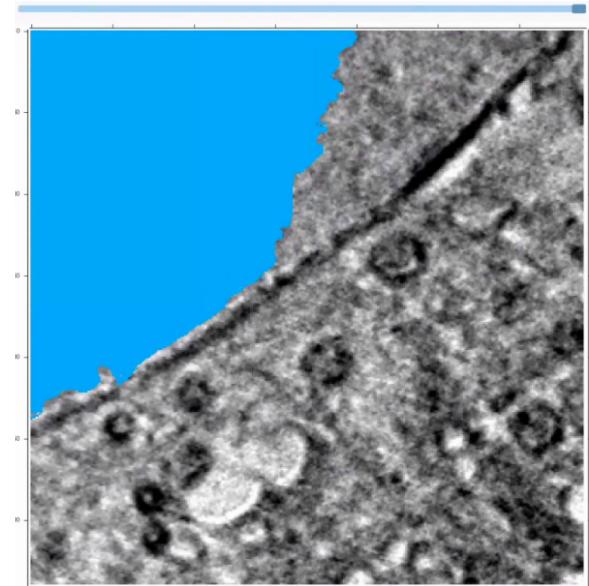
Annotation using Megavoxels

First Slice in Stack



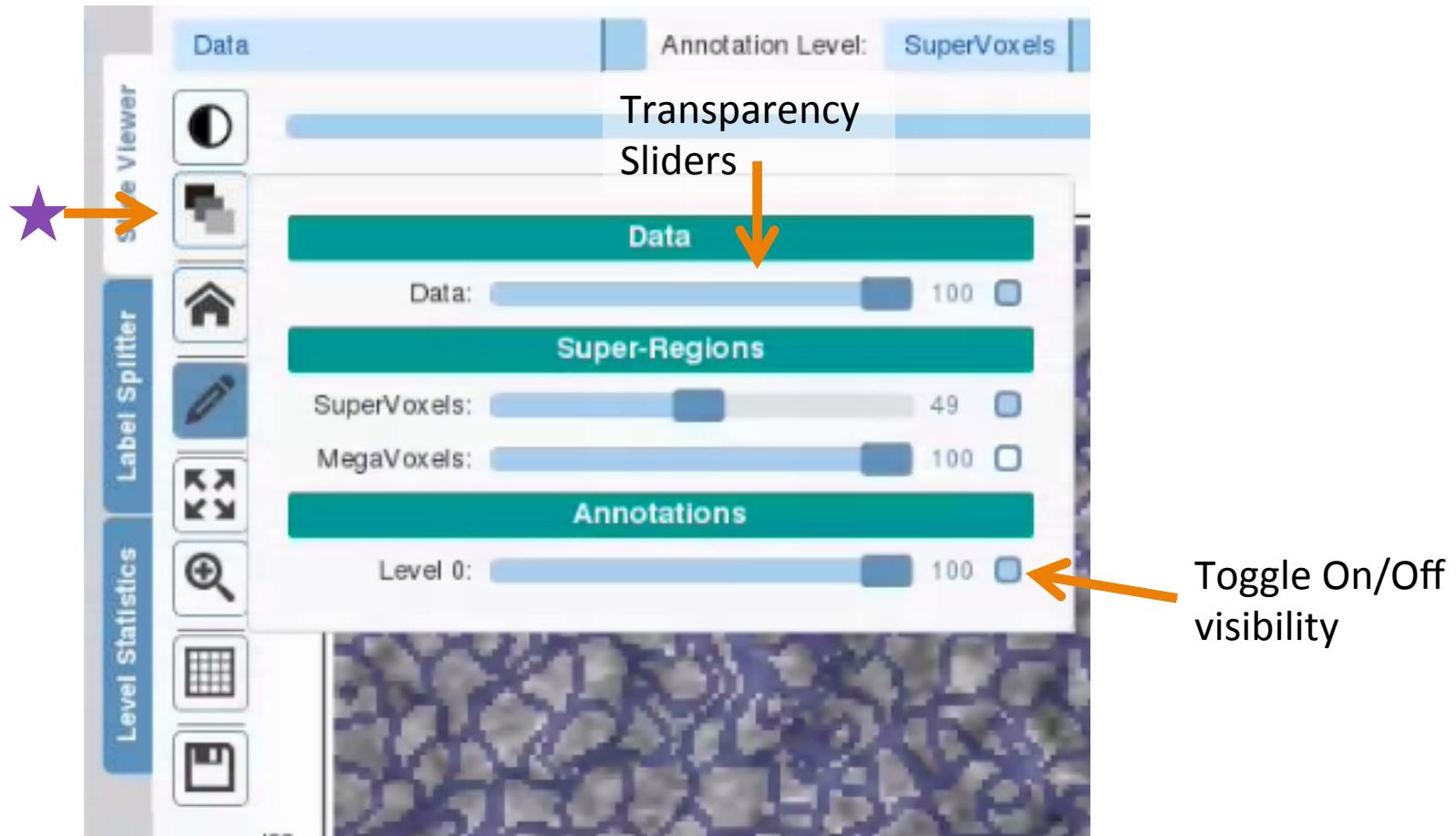
Center Slice

Last Slice in Stack



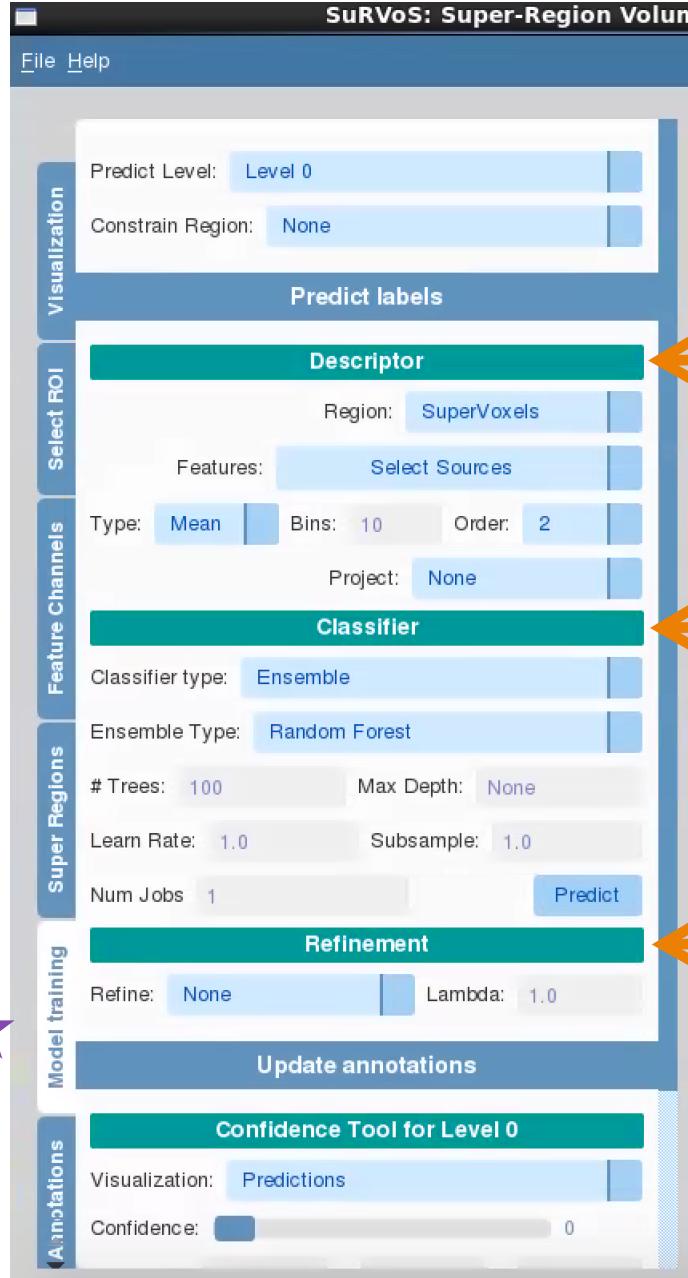
Annotating using
megavoxels penetrates
multiple Z-slices

Reminder: Visualization Shortcut



Time – 10 minutes

Model Training Introduction

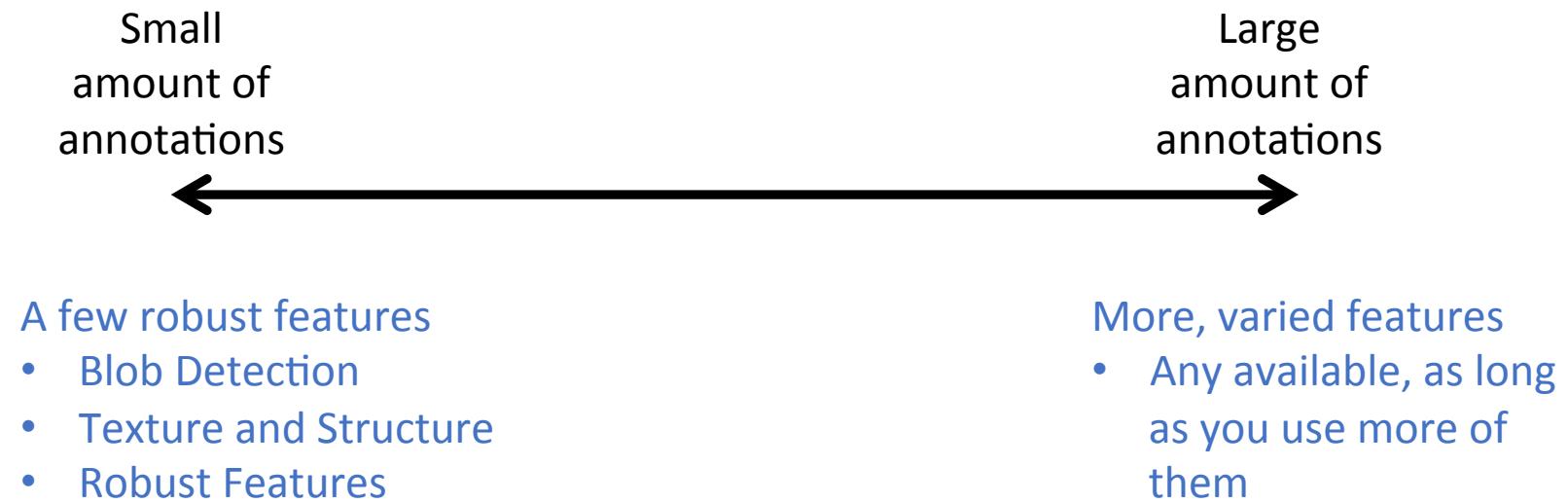


Describes the data at the region level you choose.

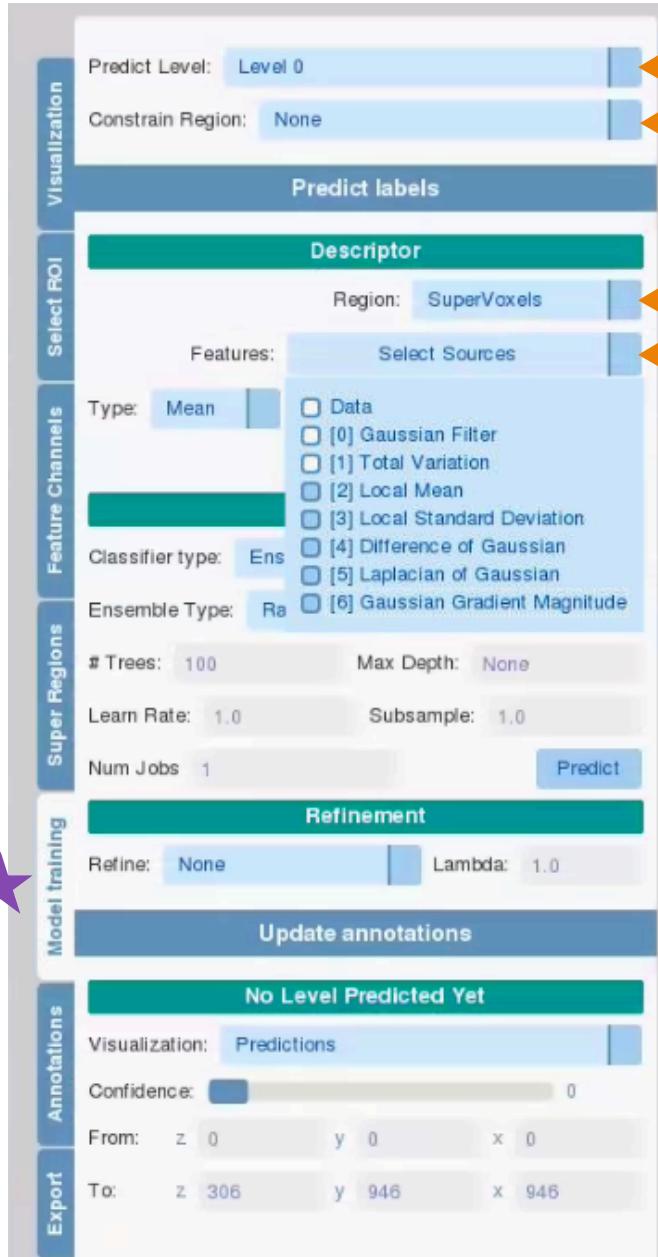
Learns a model from the descriptor and classifies the unlabeled data based on this model.

Improves the prediction of the resultant model.

Model Training: How to Use Features



Model Training Introduction



Choose which level to train with
Optional (can constrain model training region based on annotation levels)

Choose to train with either voxels or supervoxels

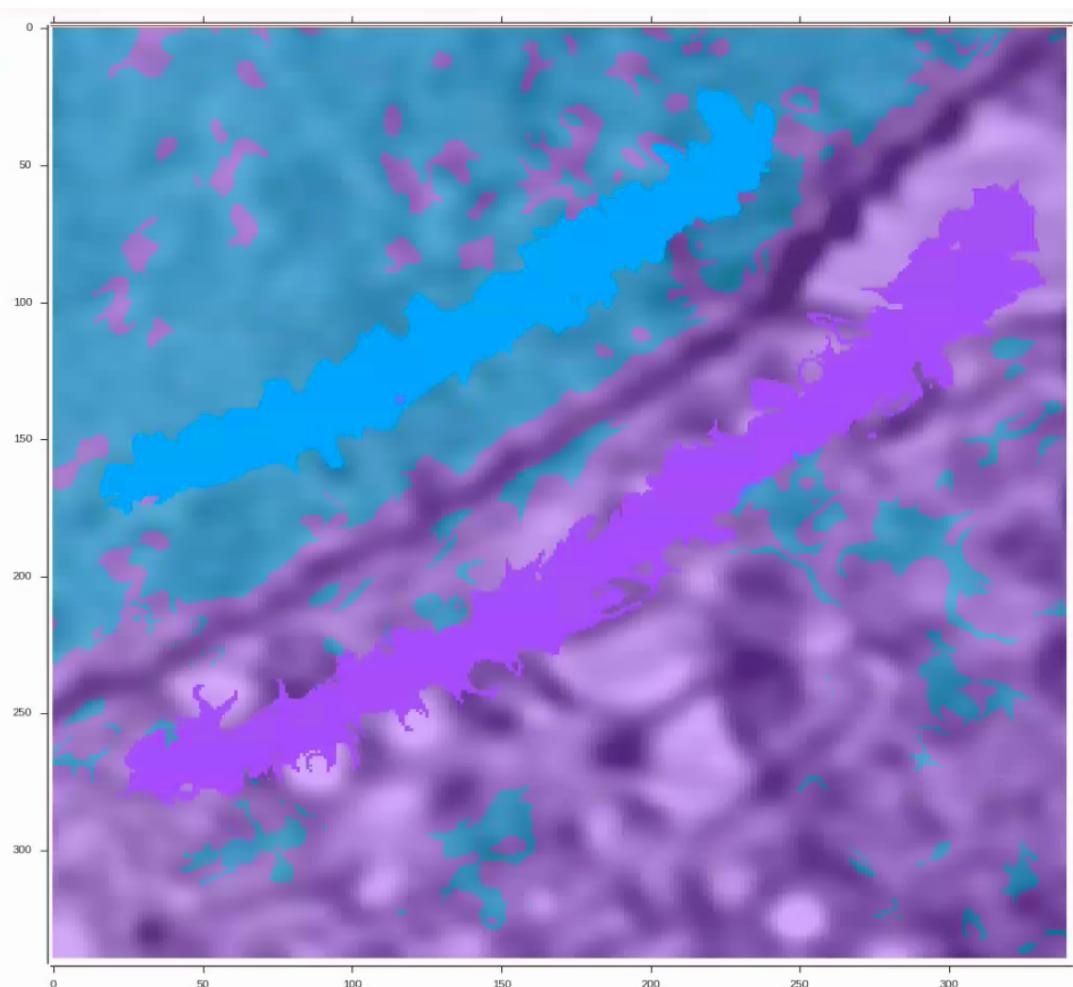
Choose sources to train the descriptor

- 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:

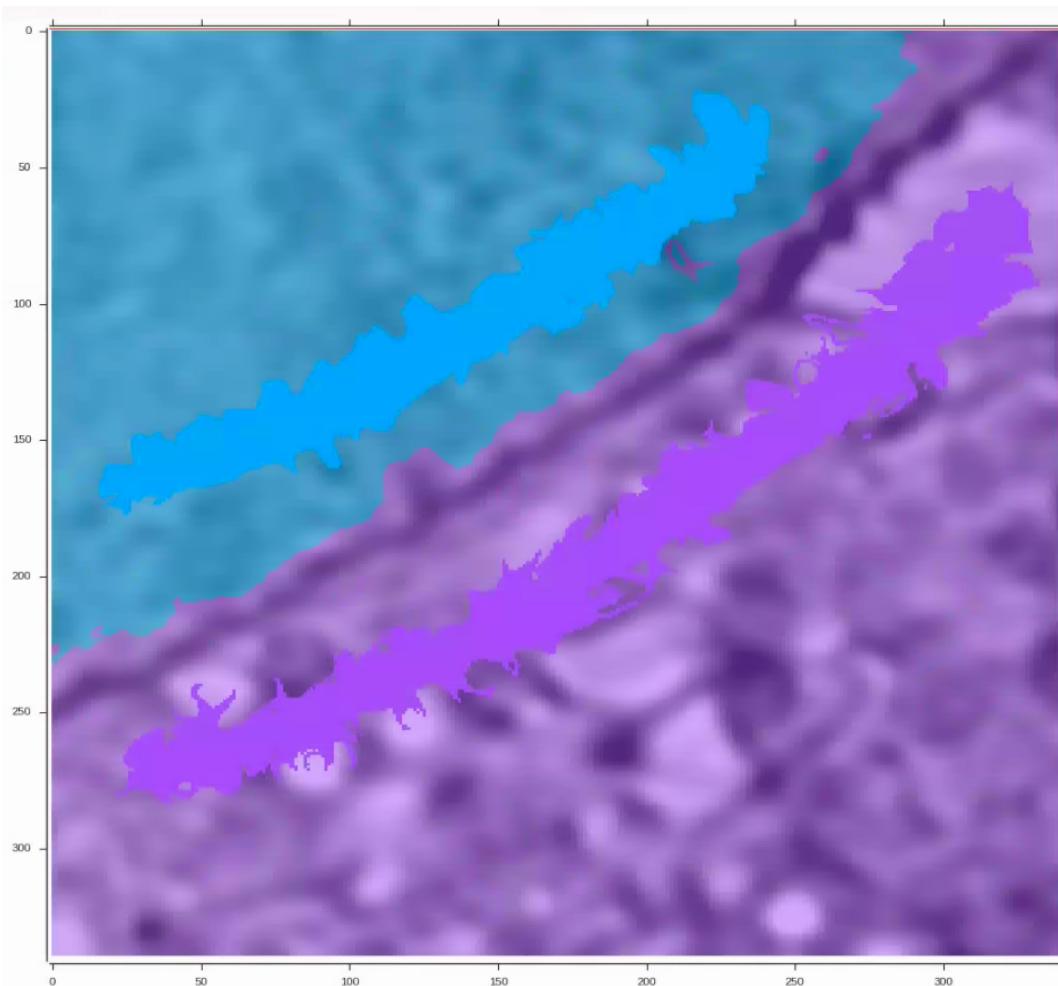
- Smaller objects will require more input annotations to accurately train the model.
- Model training with voxels will be quite slow for large region of interest.

Model Training Options



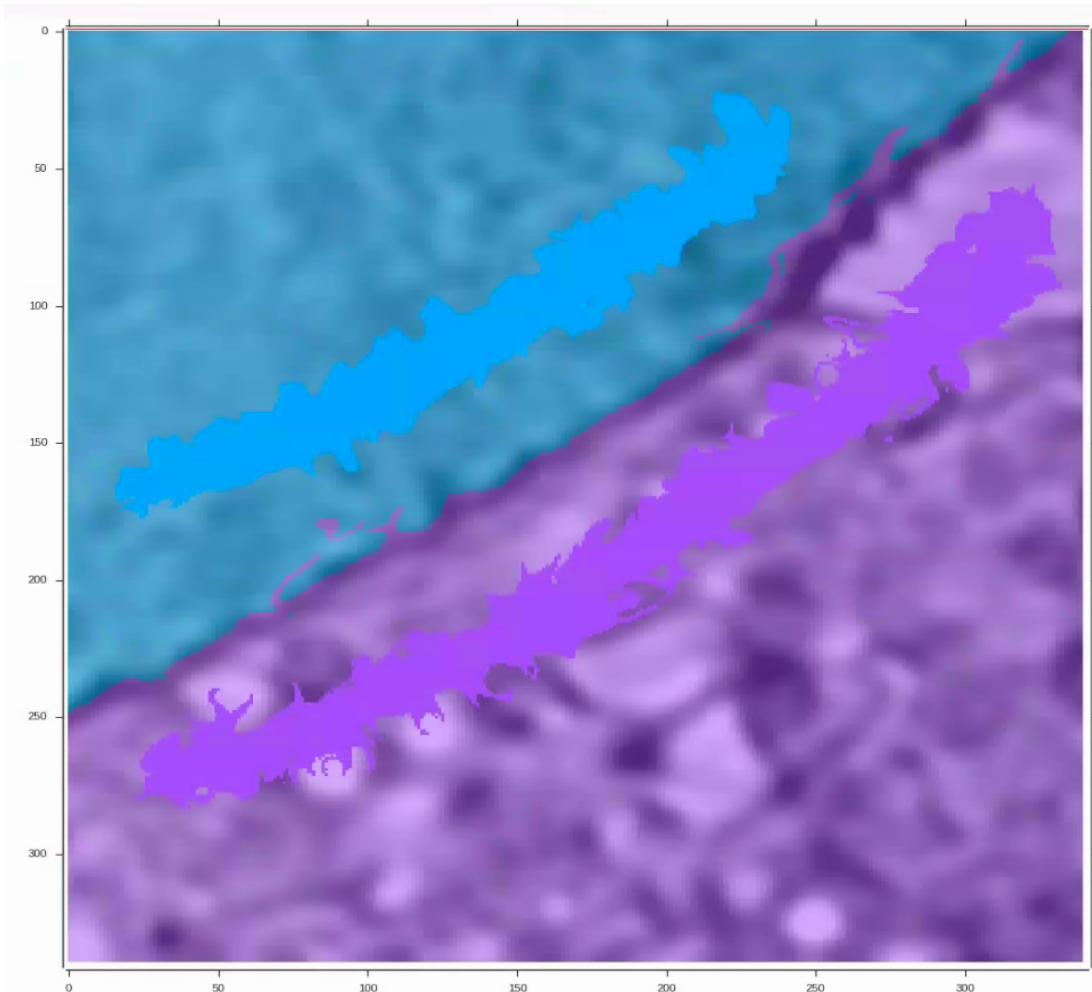
Classifier: Ensemble
Ensemble Type: Random Forest
(with defaults)
Refine: None

Model Training Options



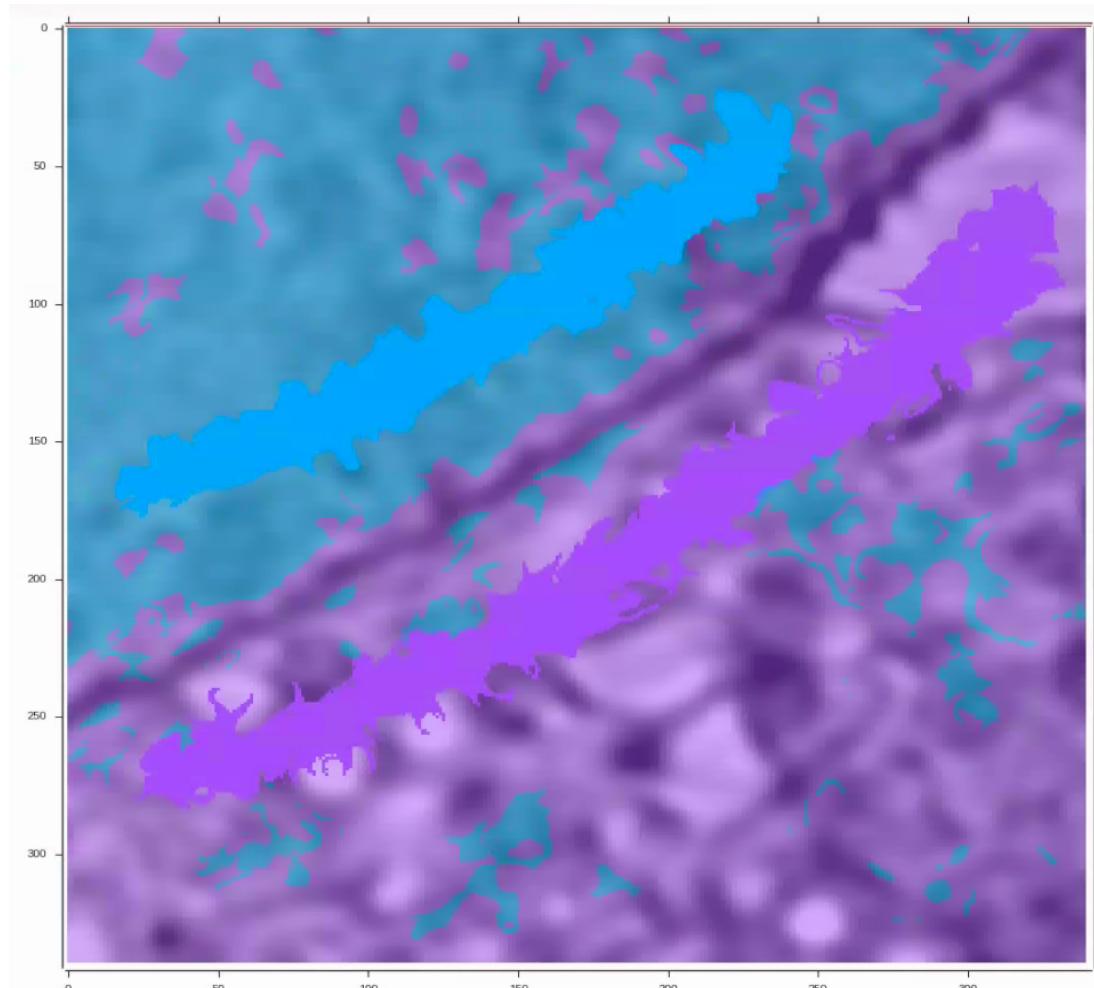
Classifier: Ensemble
Ensemble Type: Random Forest (with defaults)
Refine: Potts (50)

Model Training Options



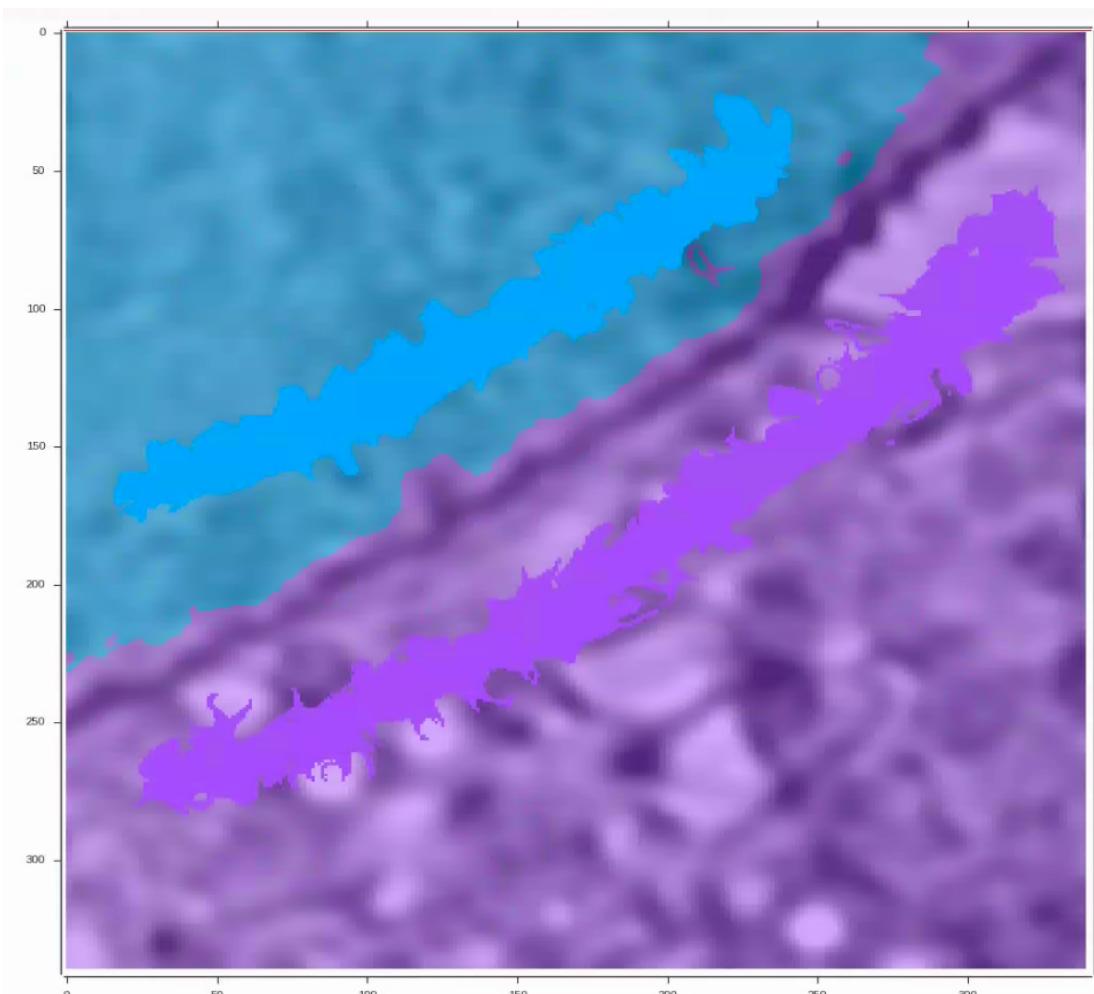
Classifier: Ensemble
Ensemble Type: Random Forest (with defaults)
Refine: Appearance (50)

Model Training Options



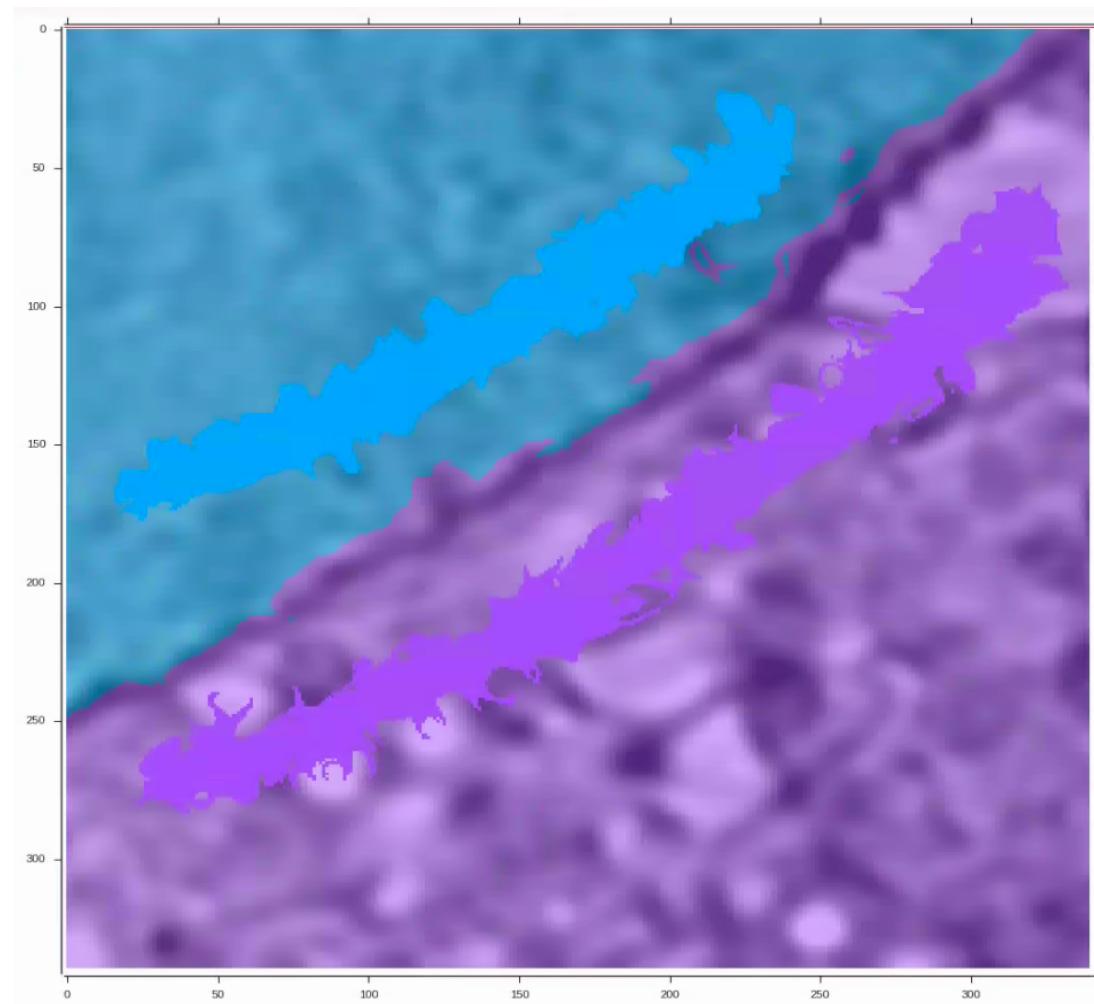
Classifier: Ensemble
Ensemble Type: Extra Random Forest
(with defaults)
Refine: None

Model Training Options



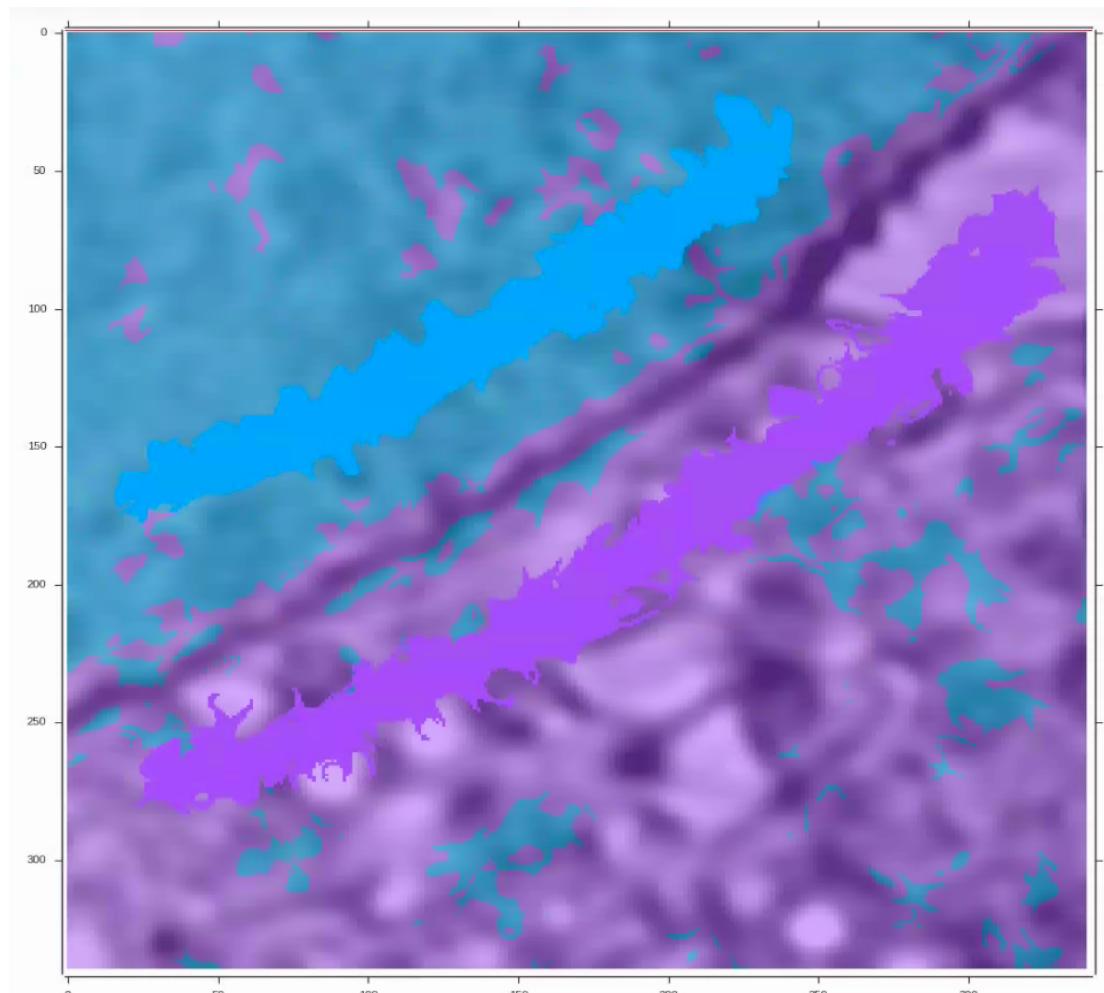
Classifier: Ensemble
Ensemble Type: Extra Random Forest
(with defaults)
Refine: Potts (50)

Model Training Options



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

Model Training Options

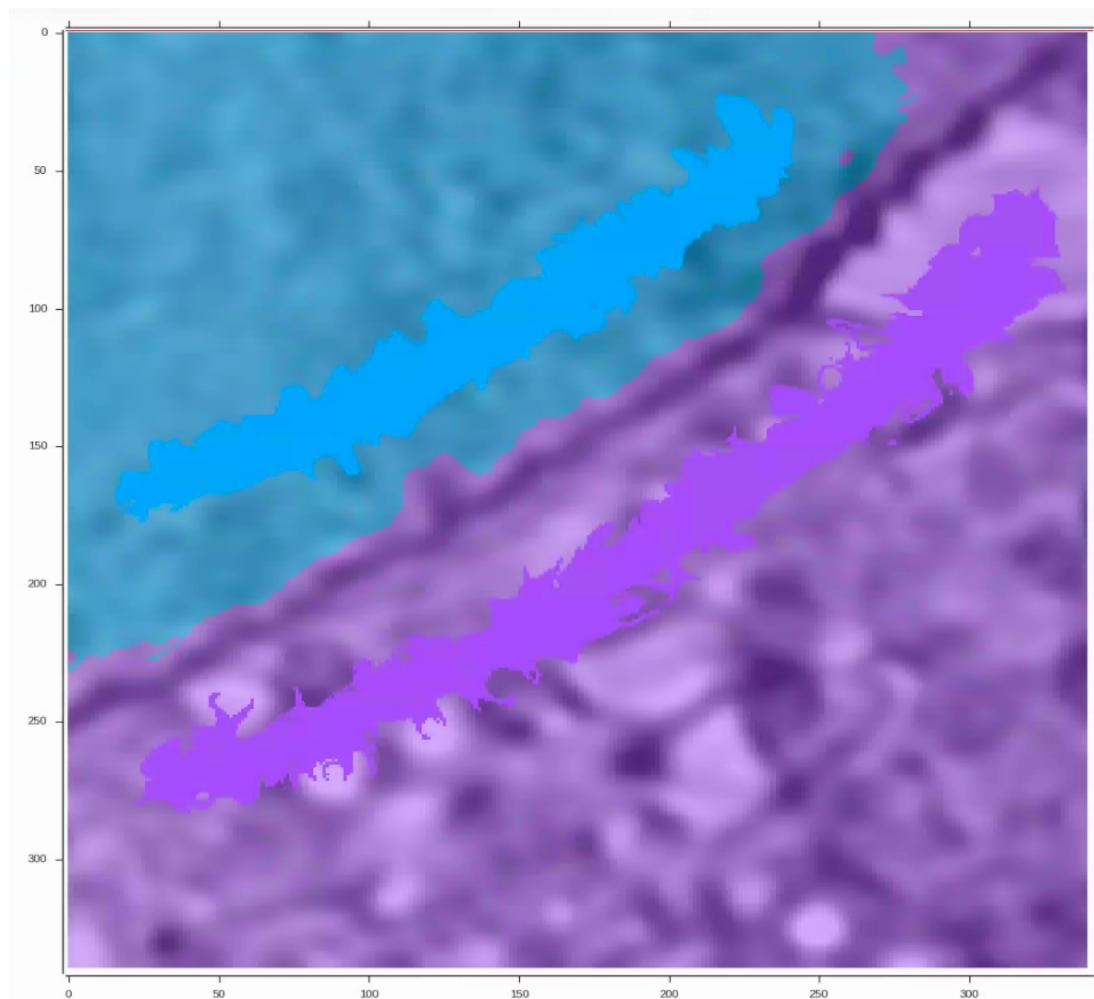


Classifier: SVM

Kernel Type: rbf (with defaults)

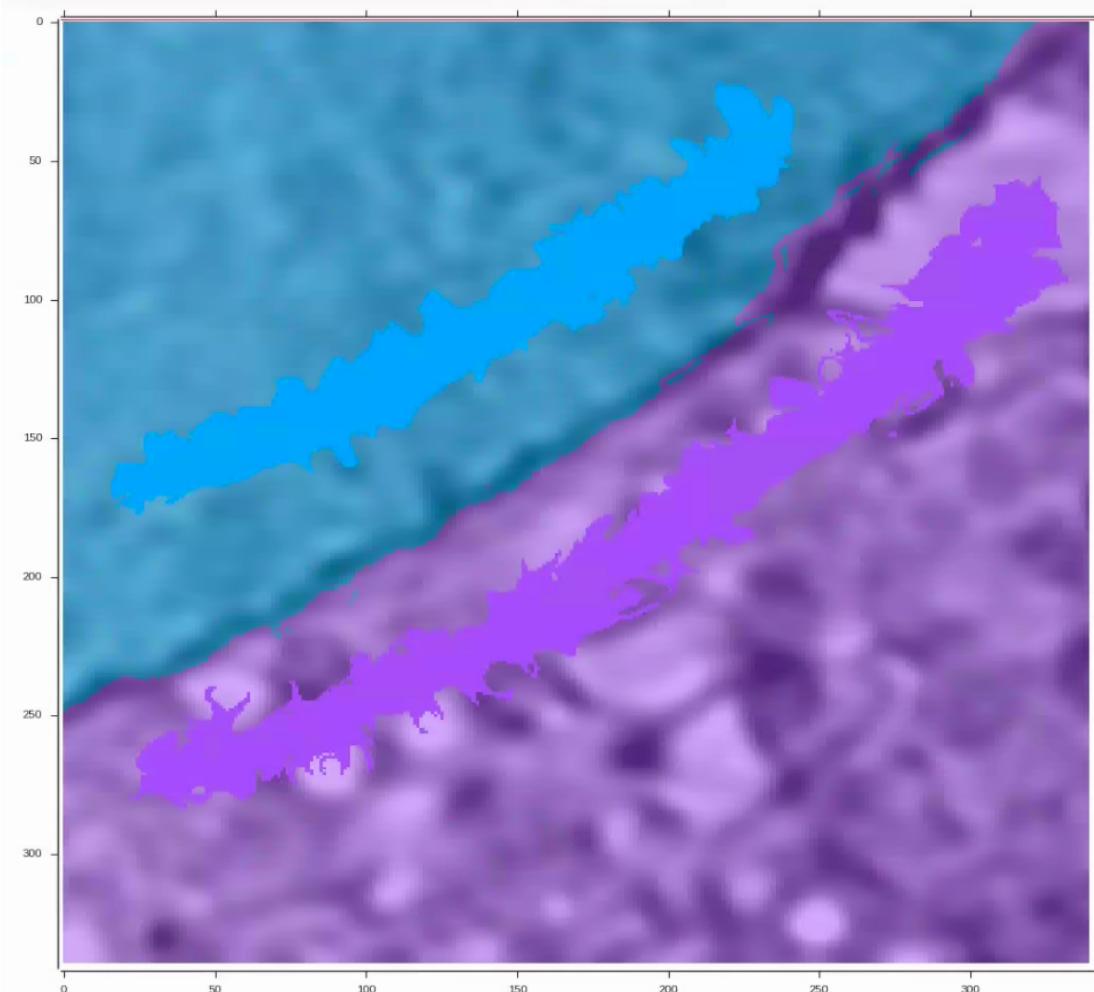
Refine: None

Model Training Options



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

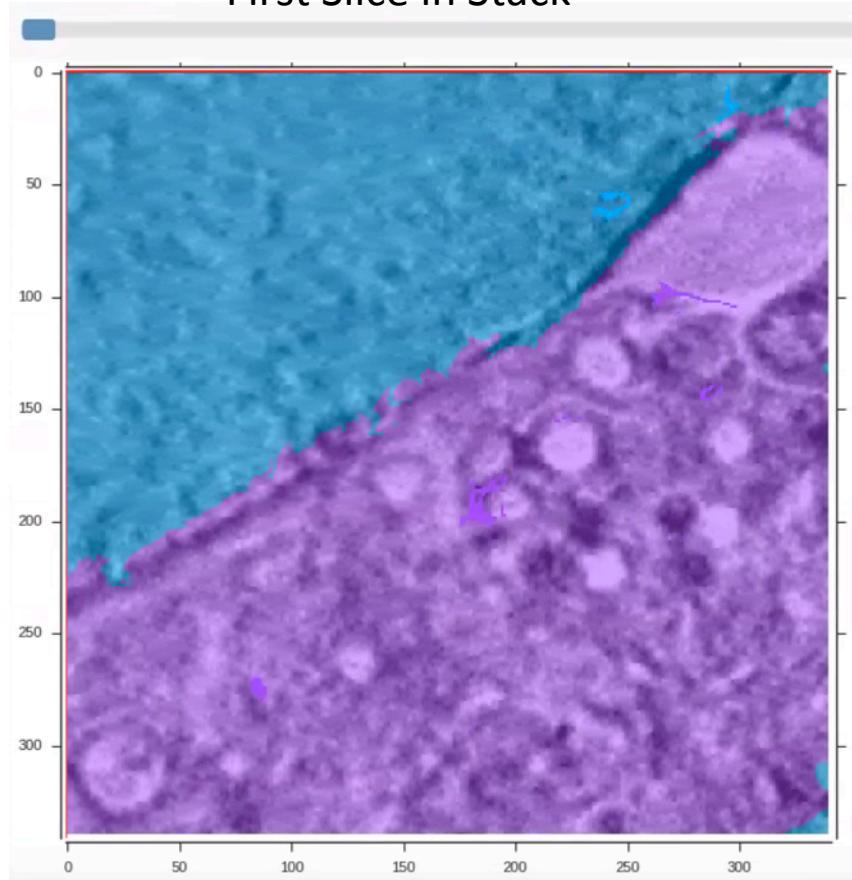
Model Training Options



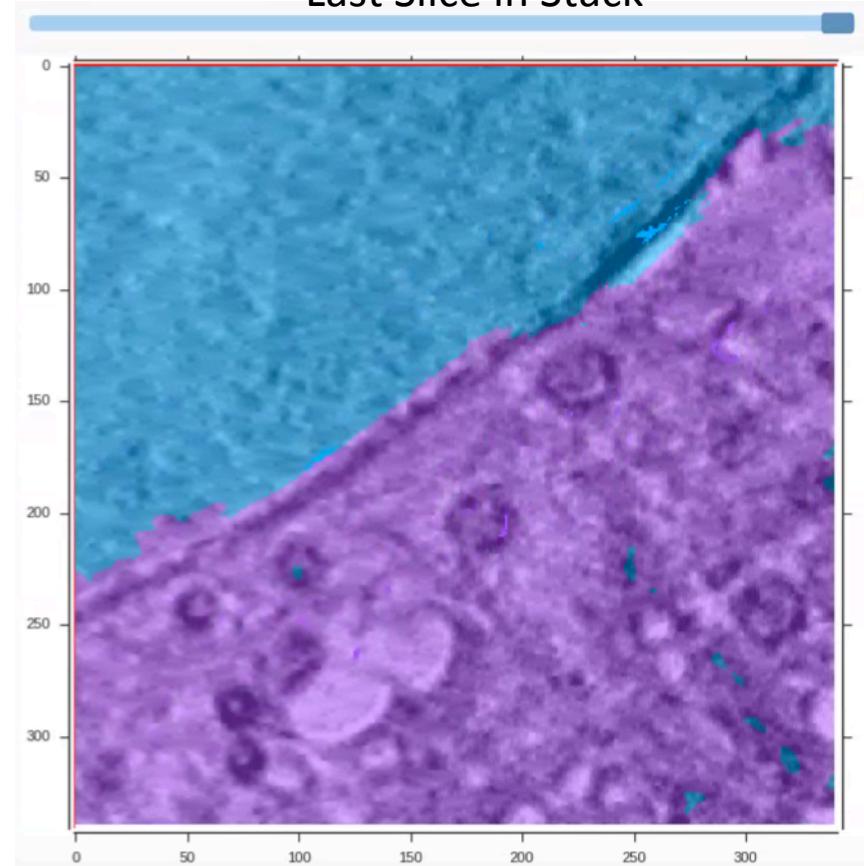
Classifier: SVM
Kernel Type: rbf (with defaults)
Refine: Appearance (50)

Model Training is in 3D

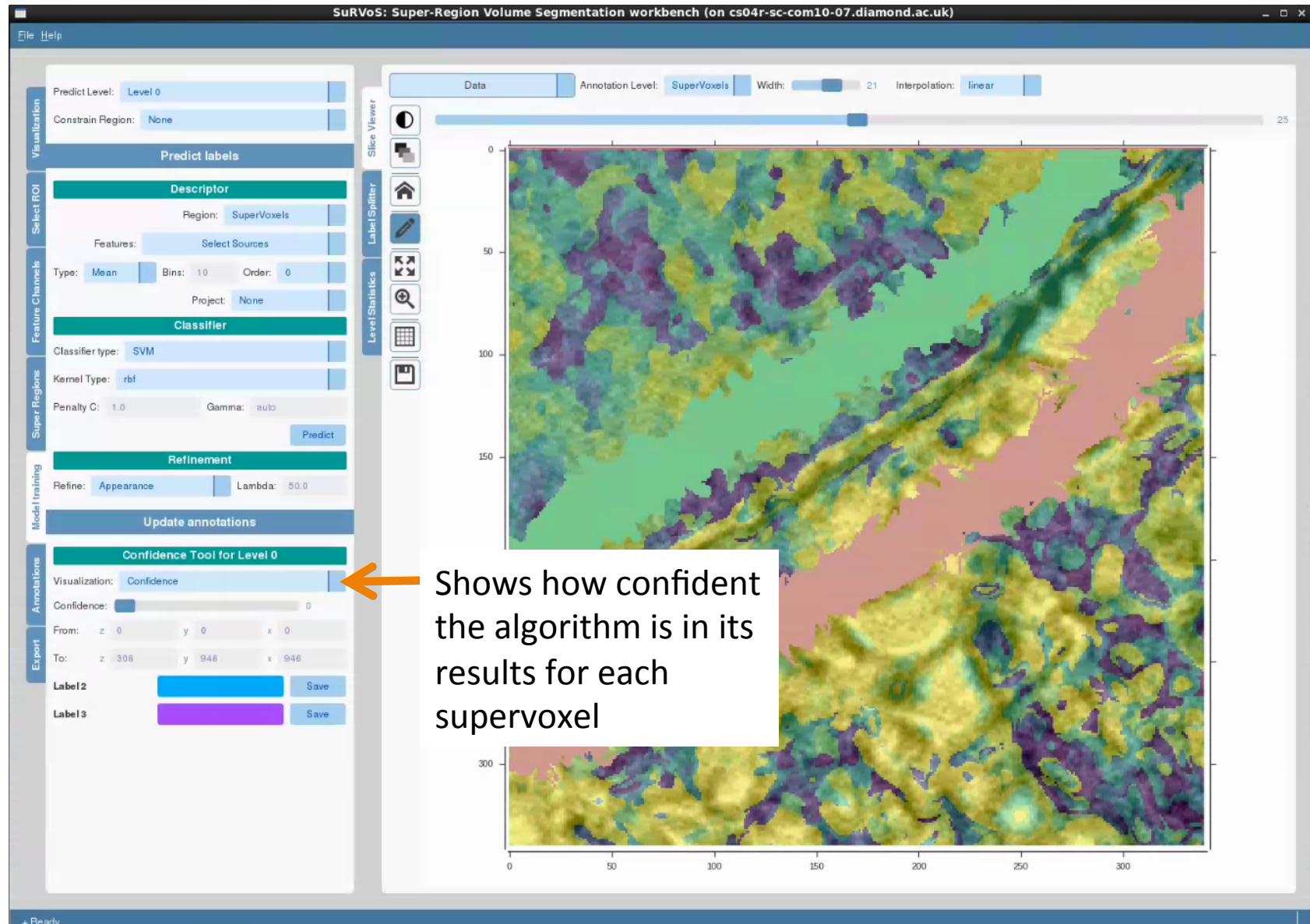
First Slice in Stack



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

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
Label2 Save
Label3 Save

Data Annotation Level: SuperVoxels Width: 21 Interpolation: linear

Slice Viewer

Label Splitter

Level Statistics

Model training

Annotations

Export

Ready

Can use to control which supervoxels are shown based on confidence

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 25 y 0 x 0 To: z 49 y 946 x 946

Label2 Save Label3 Save

Annotation Level: SuperVoxels Width: 21 Interpolation: linear

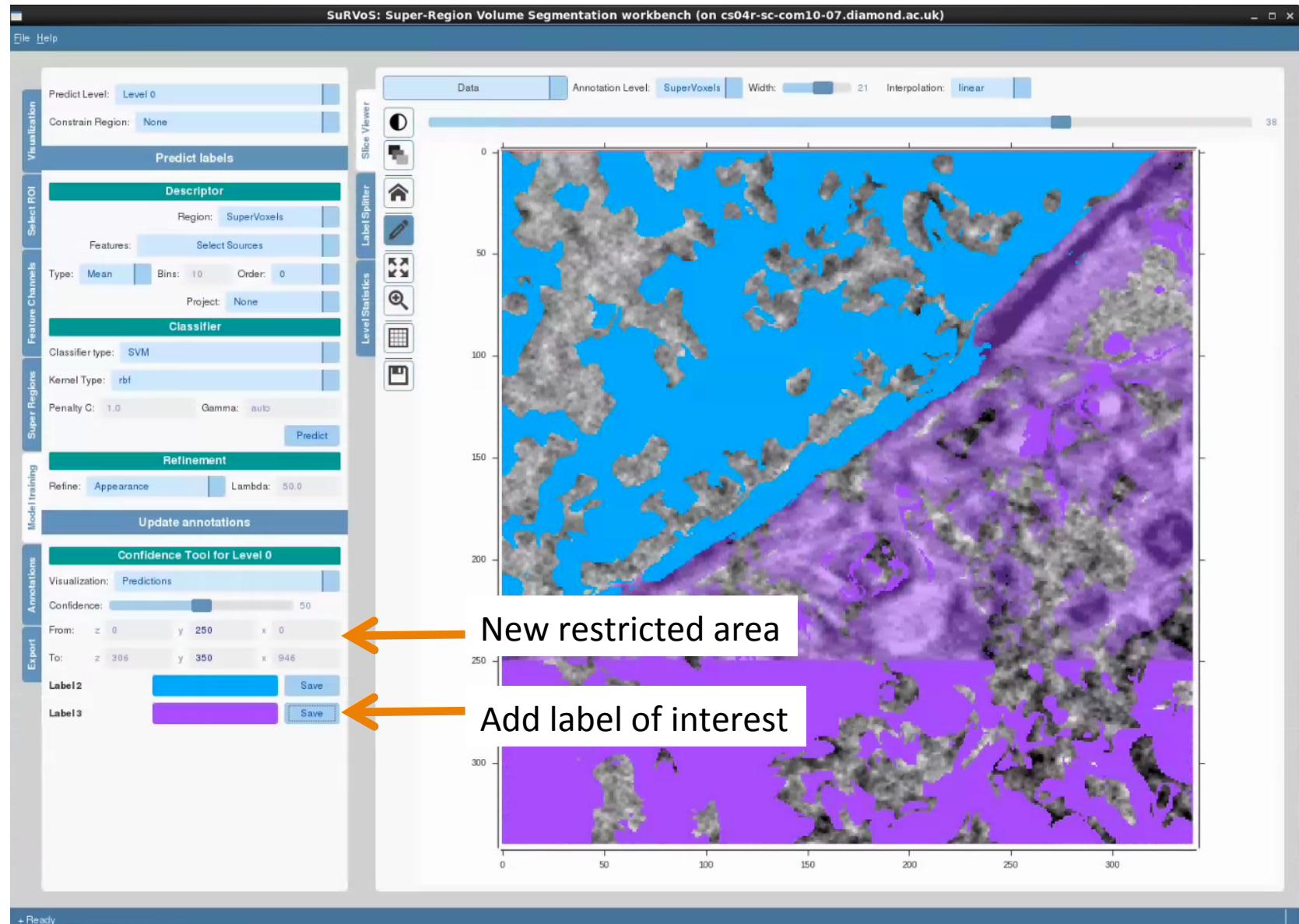
Slice Viewer
Label Splitter
Level Statistics

Can restrict area to save annotations from

Add each label individually

+ Ready

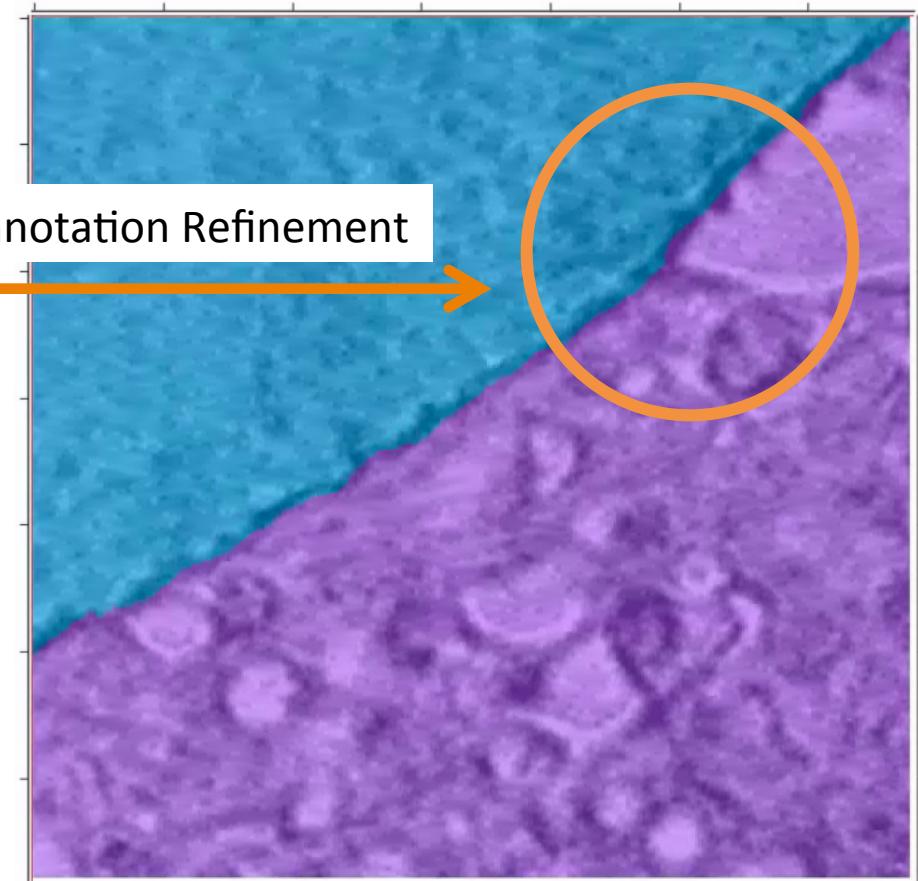
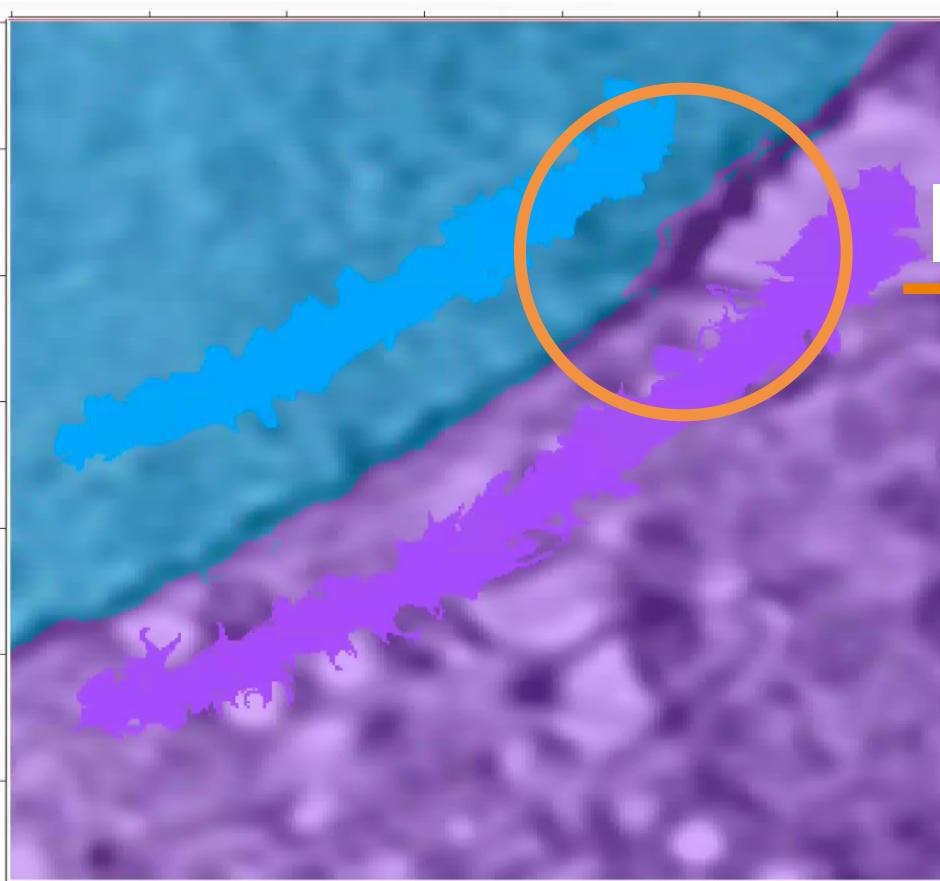
Model Training: Confidence and Update Annotations



Model Training: Refining Annotations

Don't worry about getting the model training perfect.

The annotations can be refined.



Annotation Refinement

Model Training: Refining Annotations



In the annotation tab select a label to refine.



We can refine a label on

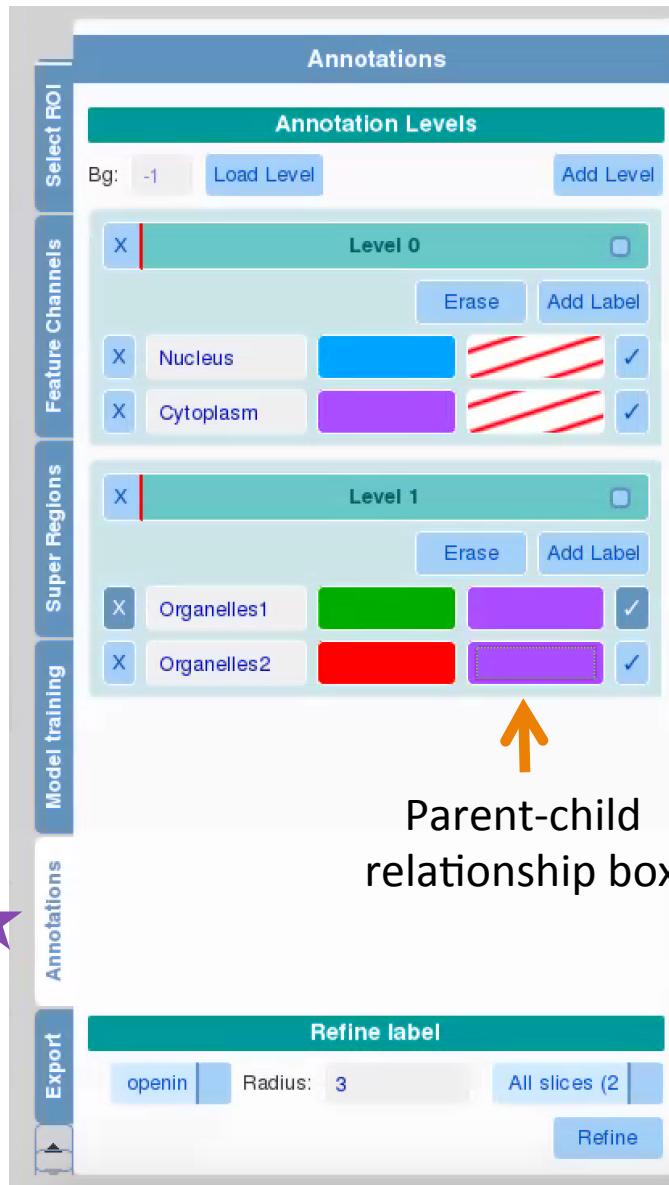
- This slice
- **All slices 2D (recommended)**
- Whole volume 3D

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

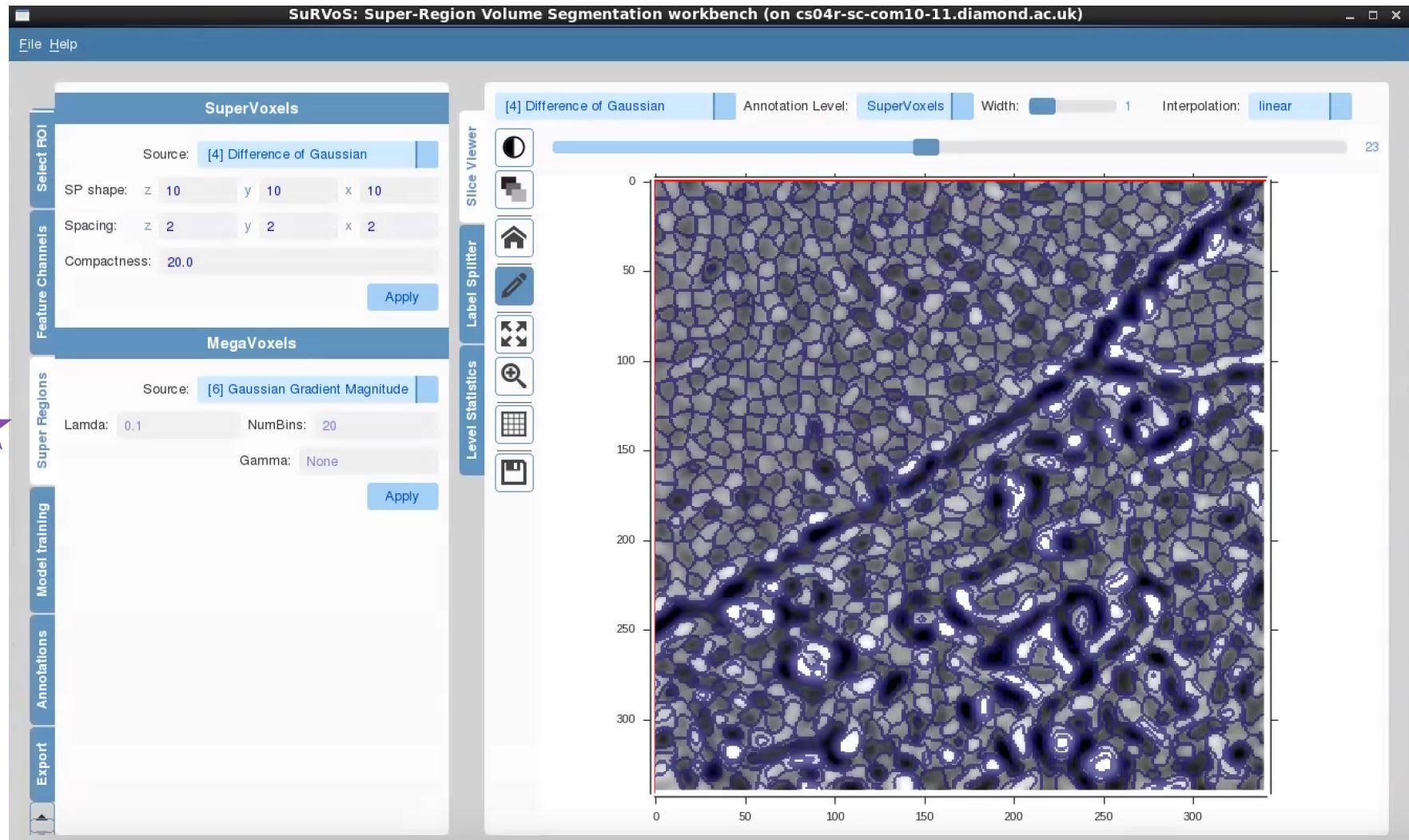


- Add a new level
- Add labels to this level
- A parent of the label can be specified by clicking on the parent-child relationship box for each label
- In the example shown (left) we are wanting to segment organelles which are located in the cytoplasm, so we choose the parent label: Level 0/Cytoplasm

A terminal window titled 'main.py (on cs04r-: x)' is shown. A dropdown menu is open, listing four options: 'None', 'None', 'Level 0/Nucleus', and 'Level 0/Cytoplasm'. The 'Level 0/Cytoplasm' option is highlighted with a blue selection bar at the bottom of the list.

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

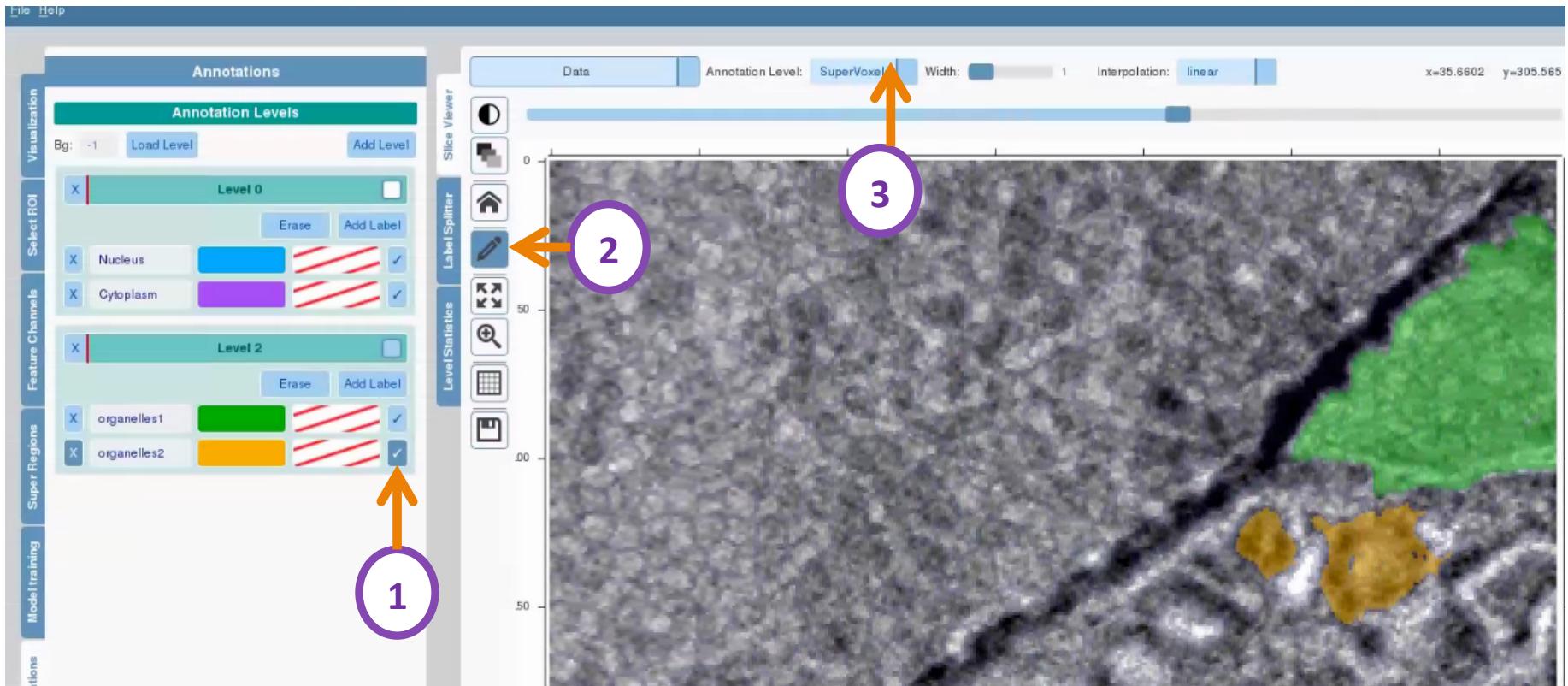
Choosing New Supervoxels for Organelle Segmentation



- **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

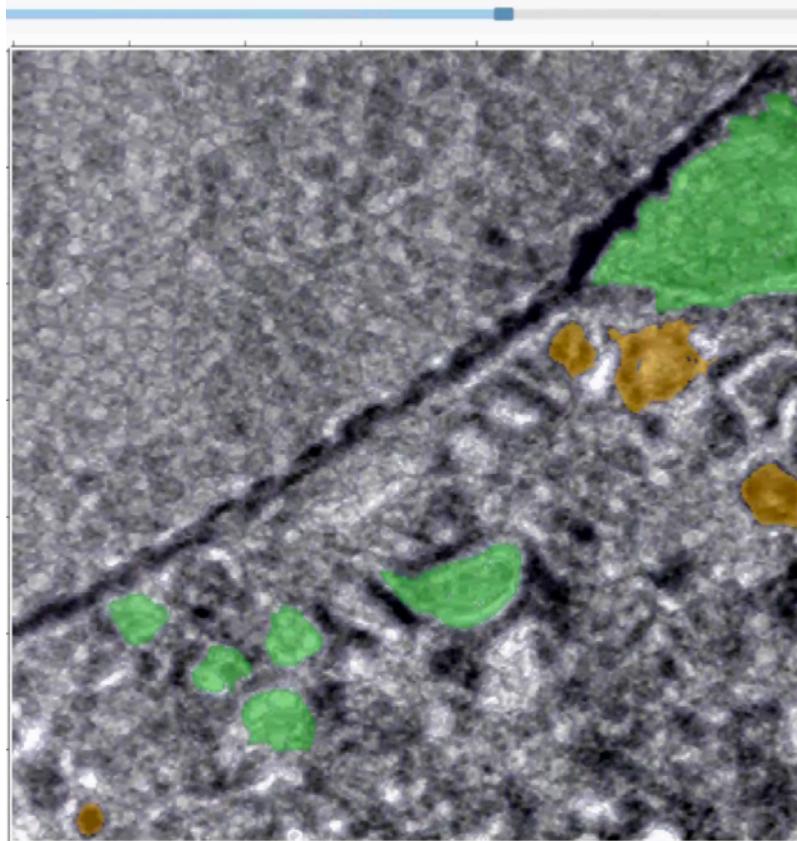


Reminder:

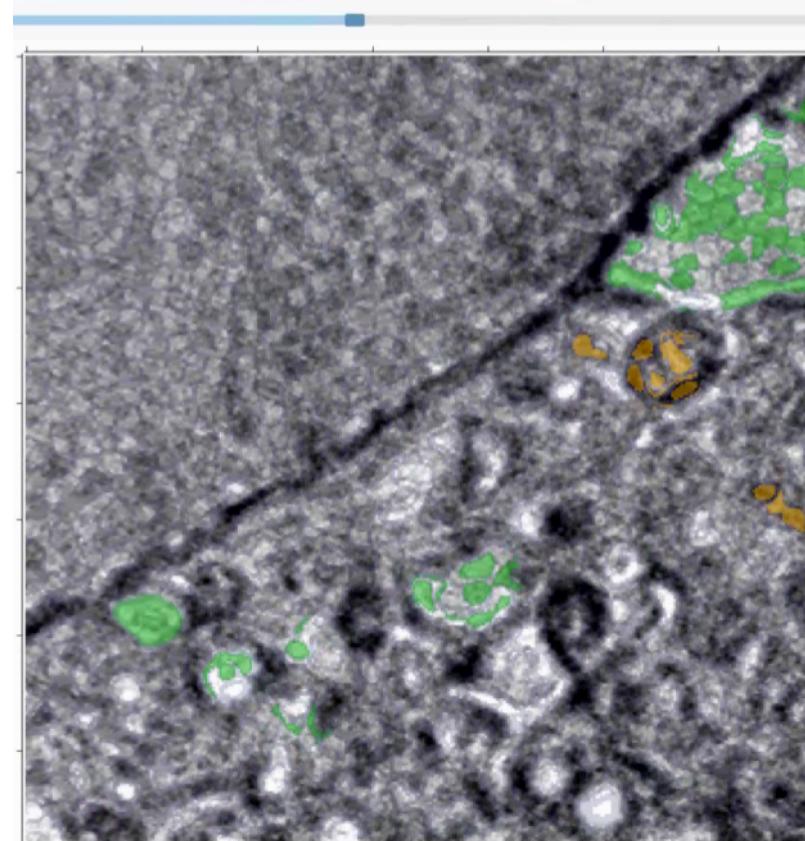
- 1) Select the label to annotate with
- 2) Select the pen tool
- 3) Select annotation level to supervoxels and left click to annotate

Segmenting Organelles Using Supervoxels

Segmented Z-slice

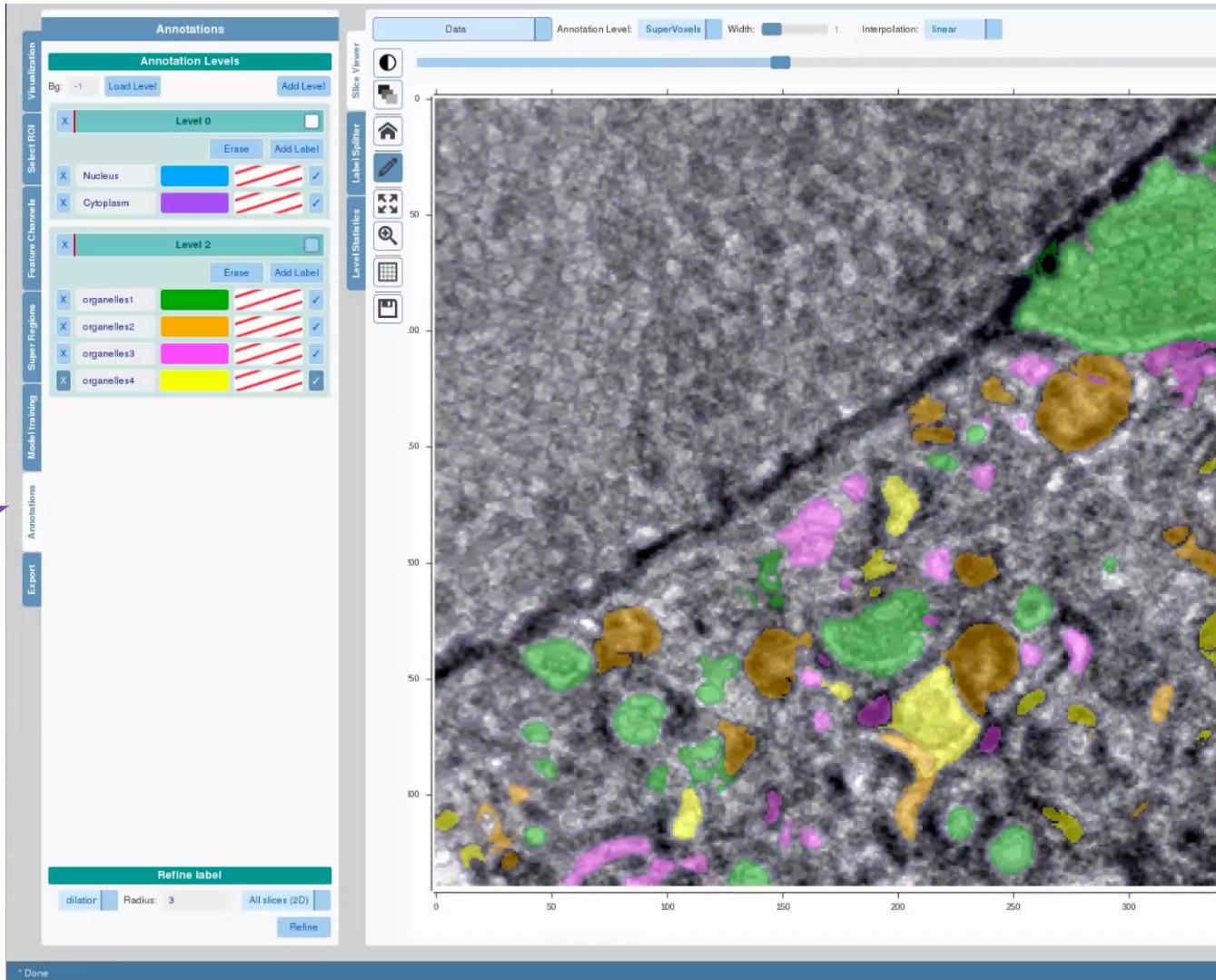


10 slices below



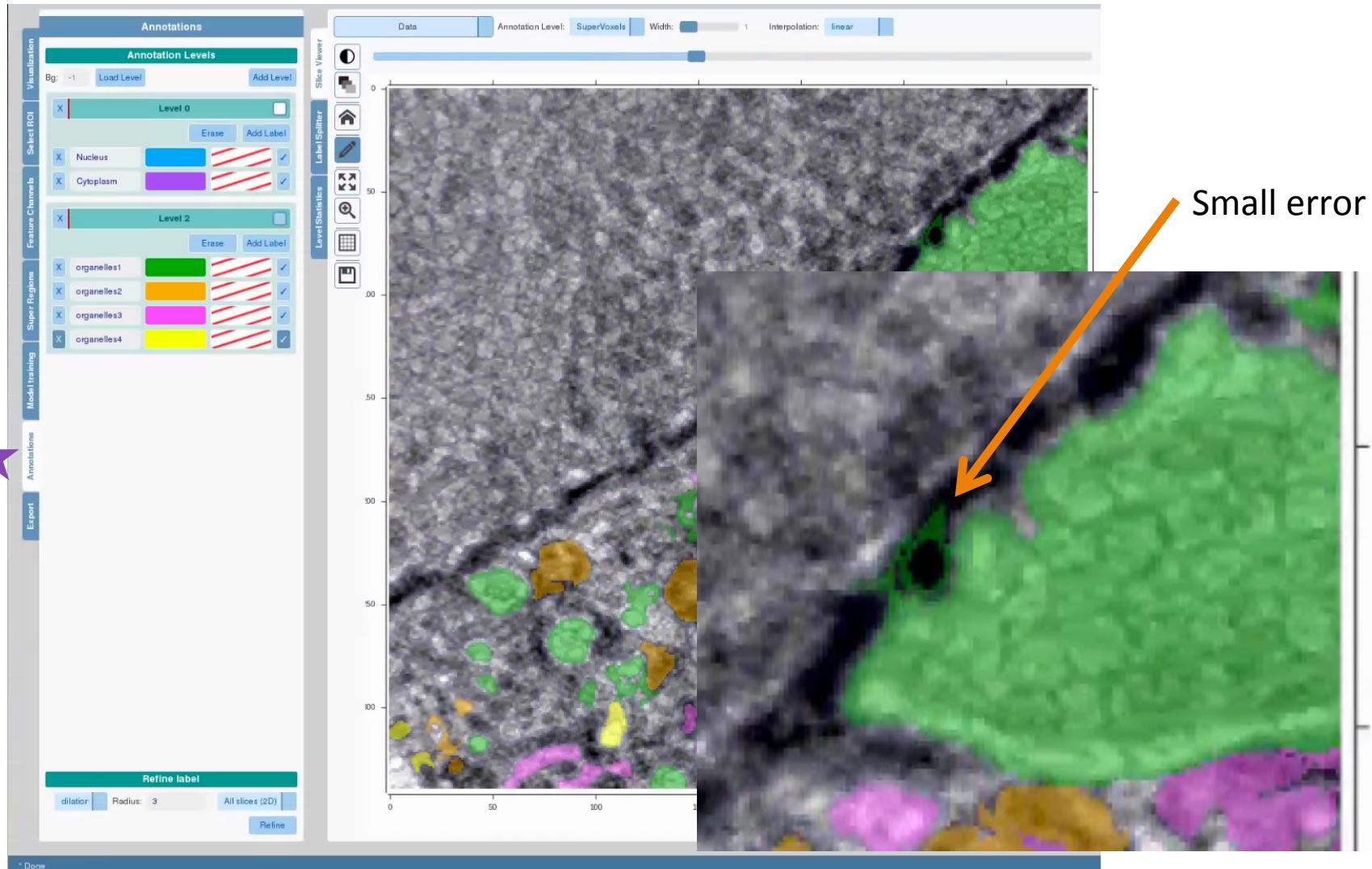
- Segment organelles in one slice
- Move through a few slices in Z
- Re-annotate keeping organelles the same colour

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.

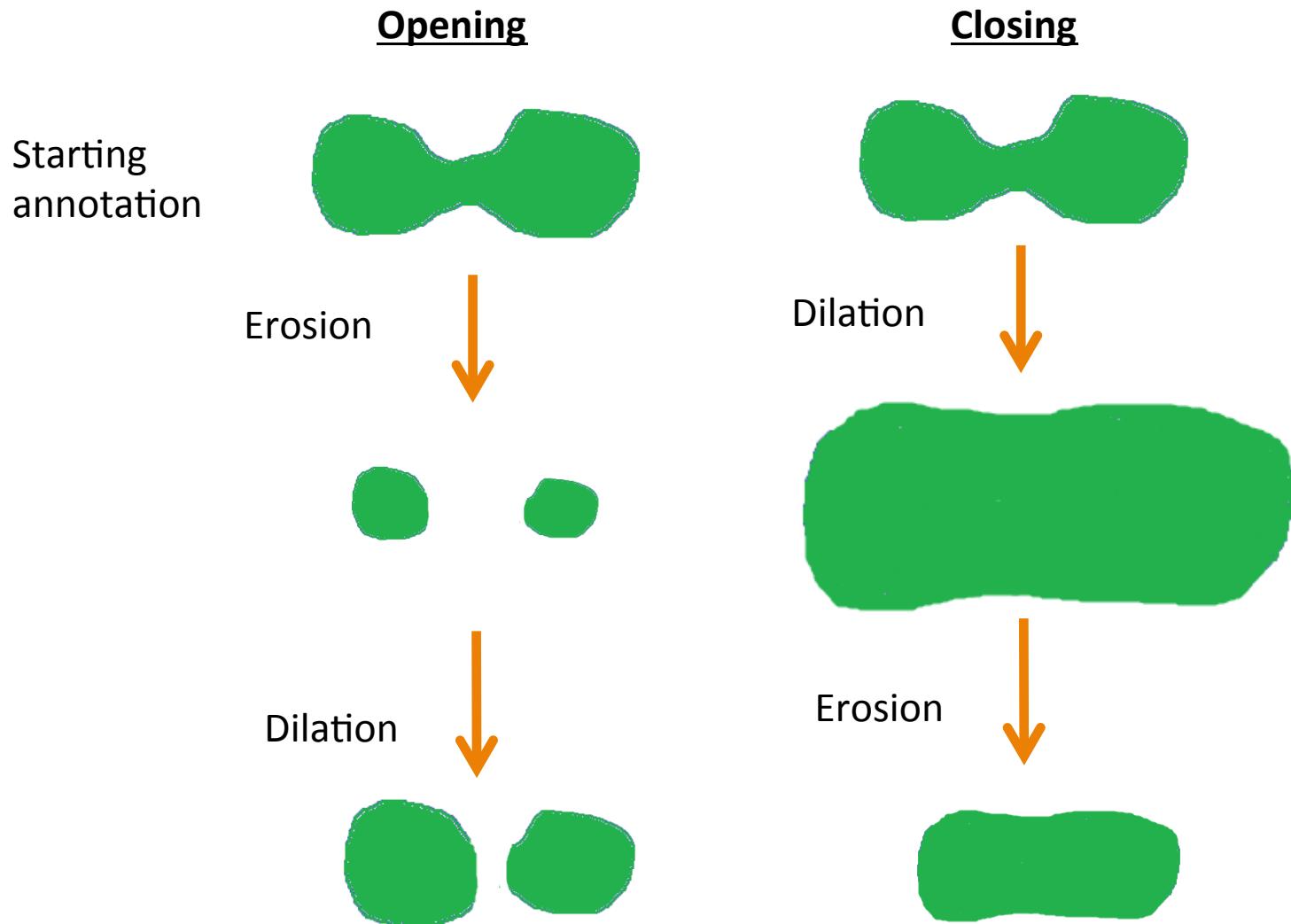


We can refine a label on
- This slice
- **All slices 2D**
- Whole volume 3D

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

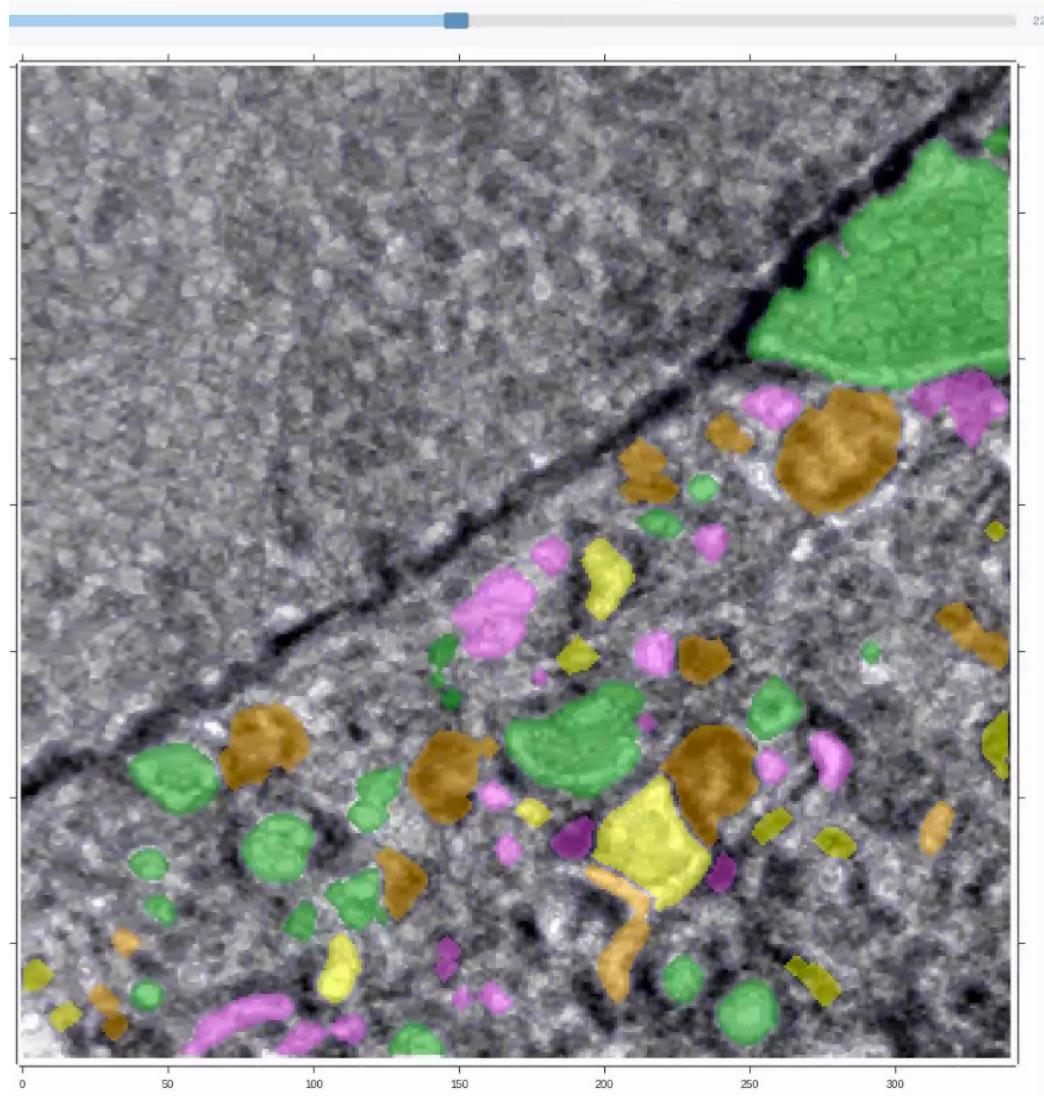
2) Opening - Radius 5

3) Dilation - Radius 2-3

4) Erosion - Radius 1-2

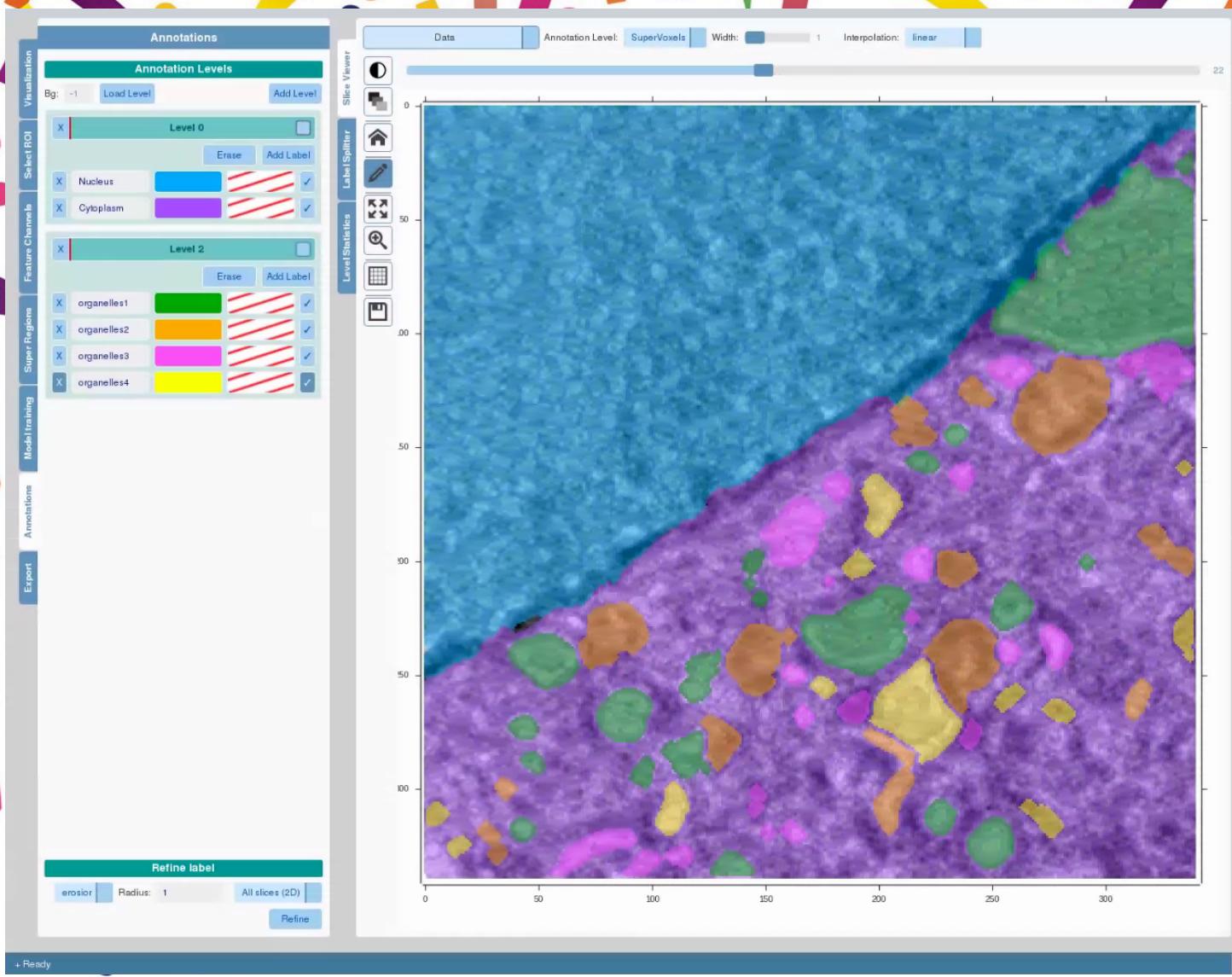
Do each step on each similar label before moving on to next step.

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

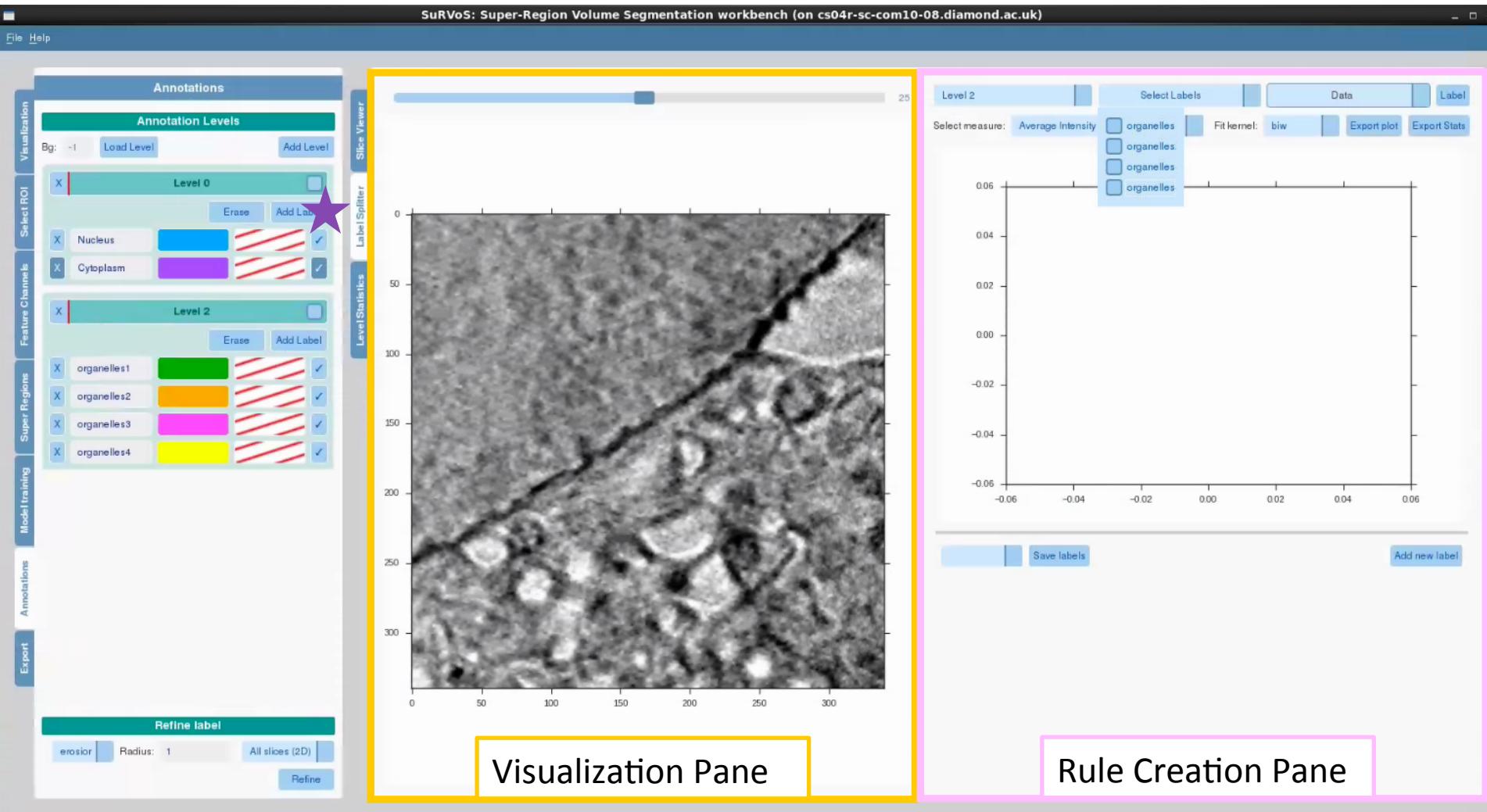


Time – 25 minutes

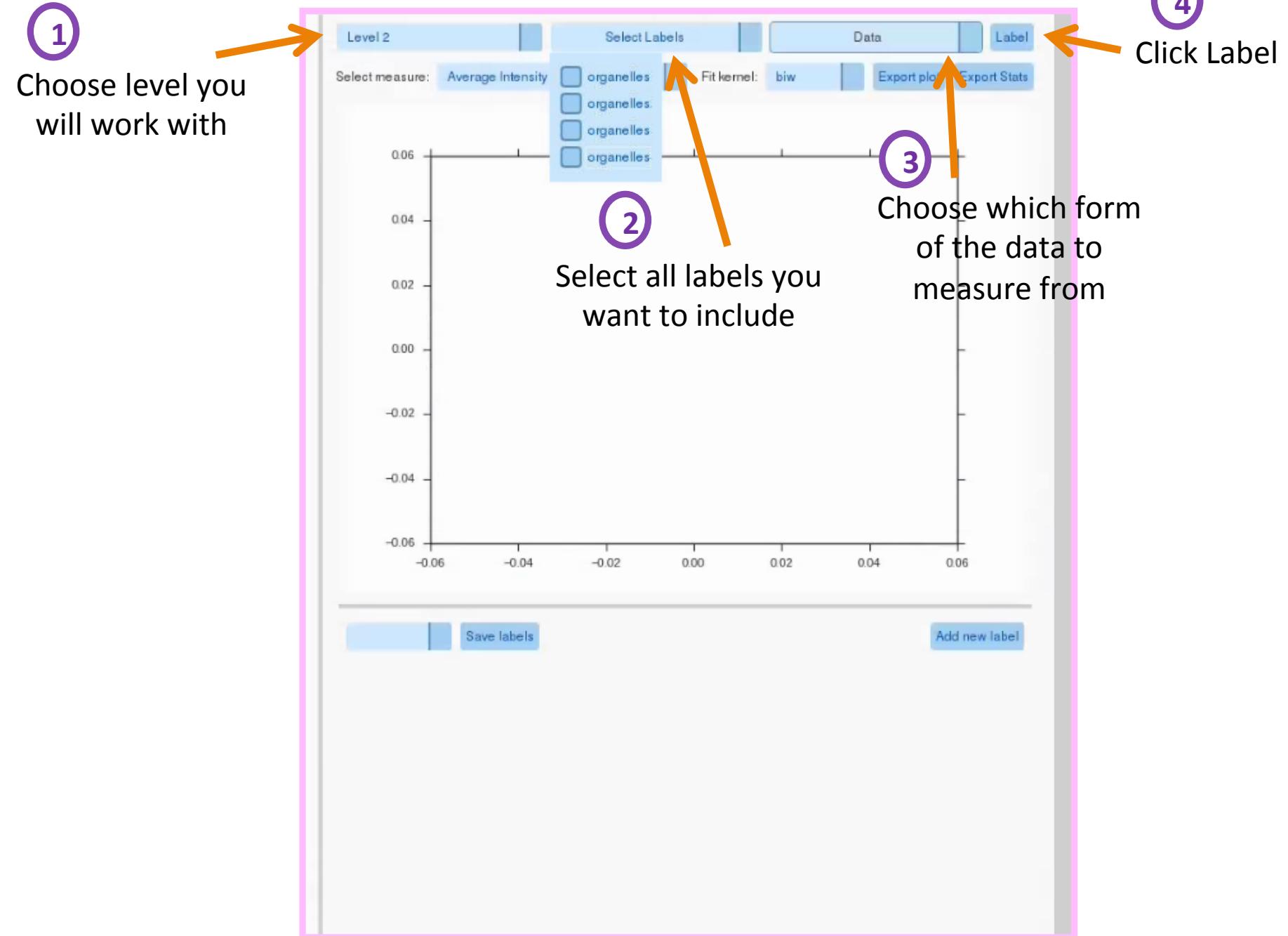
Finished Segmentation



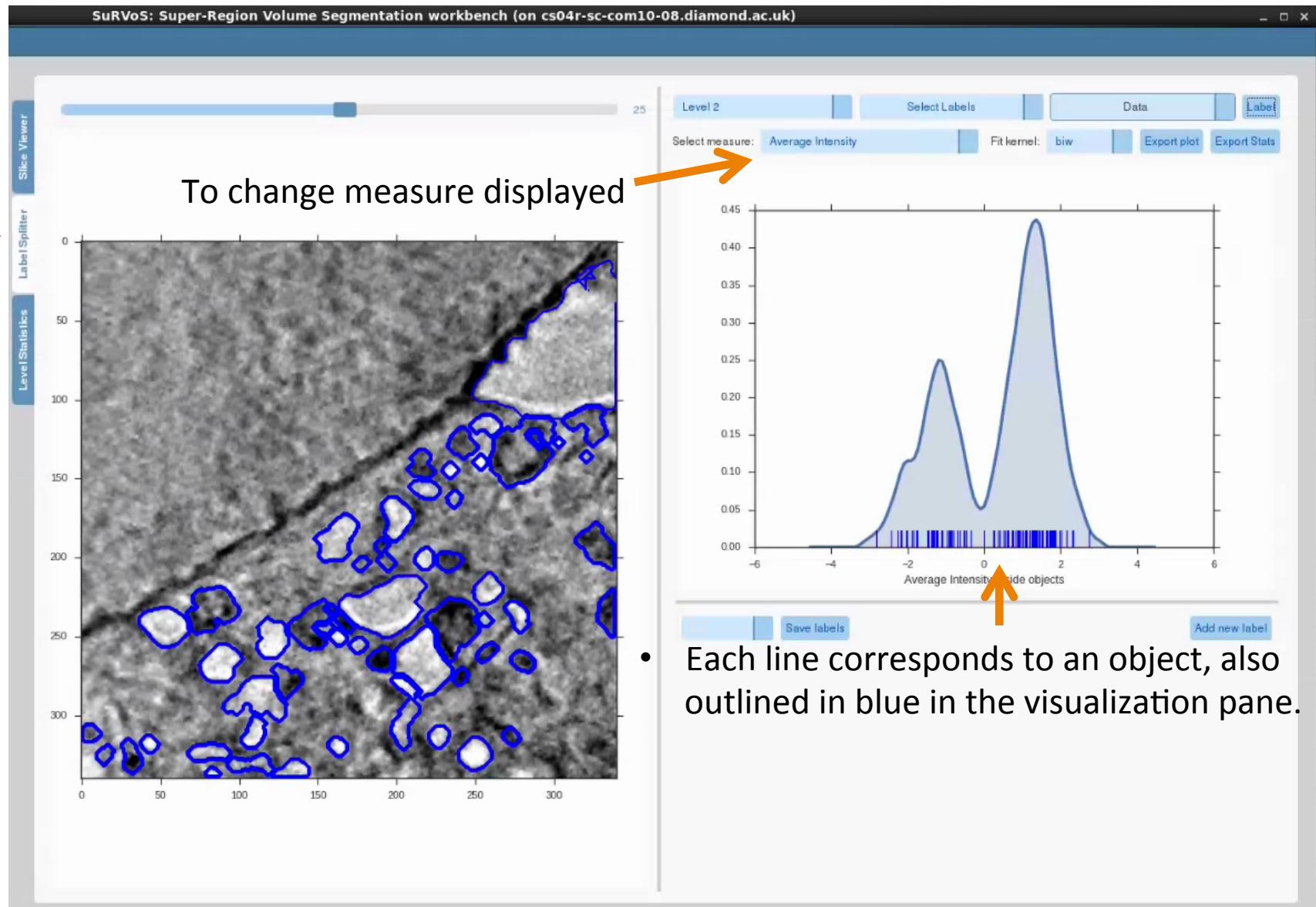
Label Splitter



Label Splitter: Rule Creation



Label Splitter: Rule Creation



Label Splitter: Rule Creation

To change measure displayed

The screenshot shows the SuRVoS software interface. On the left, there is a visualization pane titled 'Label Splitter' showing a grayscale image with numerous objects outlined in blue. A red box highlights one specific object. An orange arrow points to the 'Select measure:' dropdown menu at the top, which is currently set to 'Average Intensity'. On the right, there is a histogram titled 'Selected value: 1.31154048443' with the x-axis labeled 'Average Intensity' and the y-axis ranging from 0.00 to 0.45. The histogram shows two peaks: a smaller one around -2.5 and a larger one around 1.5. A red vertical line marks the selected average intensity value of approximately 1.31. Another orange arrow points to this red line on the histogram. At the bottom of the histogram, there is a button labeled '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)

Level Viewer
Slice Viewer
Label Statistics
Label Splitter

25

Select Labels Data Label

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

0 50 100 150 200 250 300

0 50 100 150 200 250 300

0.45 0.40 0.35 0.30 0.25 0.20 0.15 0.10 0.05 0.00

Selected value: 1.31154048443

Average Intensity inside objects

Can change the name and colour

Save labels Add new label

Empty Vesicles Add new rule Apply Select Others

X Rule 1 Feature: Average Intensity > 0

1 2 3 4

Label colour is applied here and in plot to right

The screenshot shows the SuRVoS software interface. On the left, the 'Slice Viewer' displays a grayscale image of a sample with various regions outlined in different colors (red, cyan, blue). A purple star icon is positioned above the slice viewer. Below it, the 'Label Statistics' panel shows numerical values from 0 to 300. On the right, a histogram titled 'Average Intensity inside objects' plots intensity against average intensity, with a selected peak at 1.31154048443. The 'Label Splitter' panel at the bottom contains a rule definition for 'Empty Vesicles': 'Rule 1 Feature: Average Intensity > 0'. Four numbered circles (1-4) with arrows point to specific controls: circle 1 points to the 'Add new label' button, circle 2 points to the color bar, circle 3 points to the rule definition, and circle 4 points to the 'Select Others' button.

Label Splitter: Rule Creation

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

Level Viewer

Label Splitter

Level Statistics

25

Select Labels Data Label

Select measure: Log10(Size) (Oriented Bounding Box) Fit kernel: biw Export plot Export Stats

Selected value: 3.71495628357

Can visualize various measures

Area of objects (size in voxels)

Save labels Add new label

X Empty Vesicles Add new rule Apply Select Others

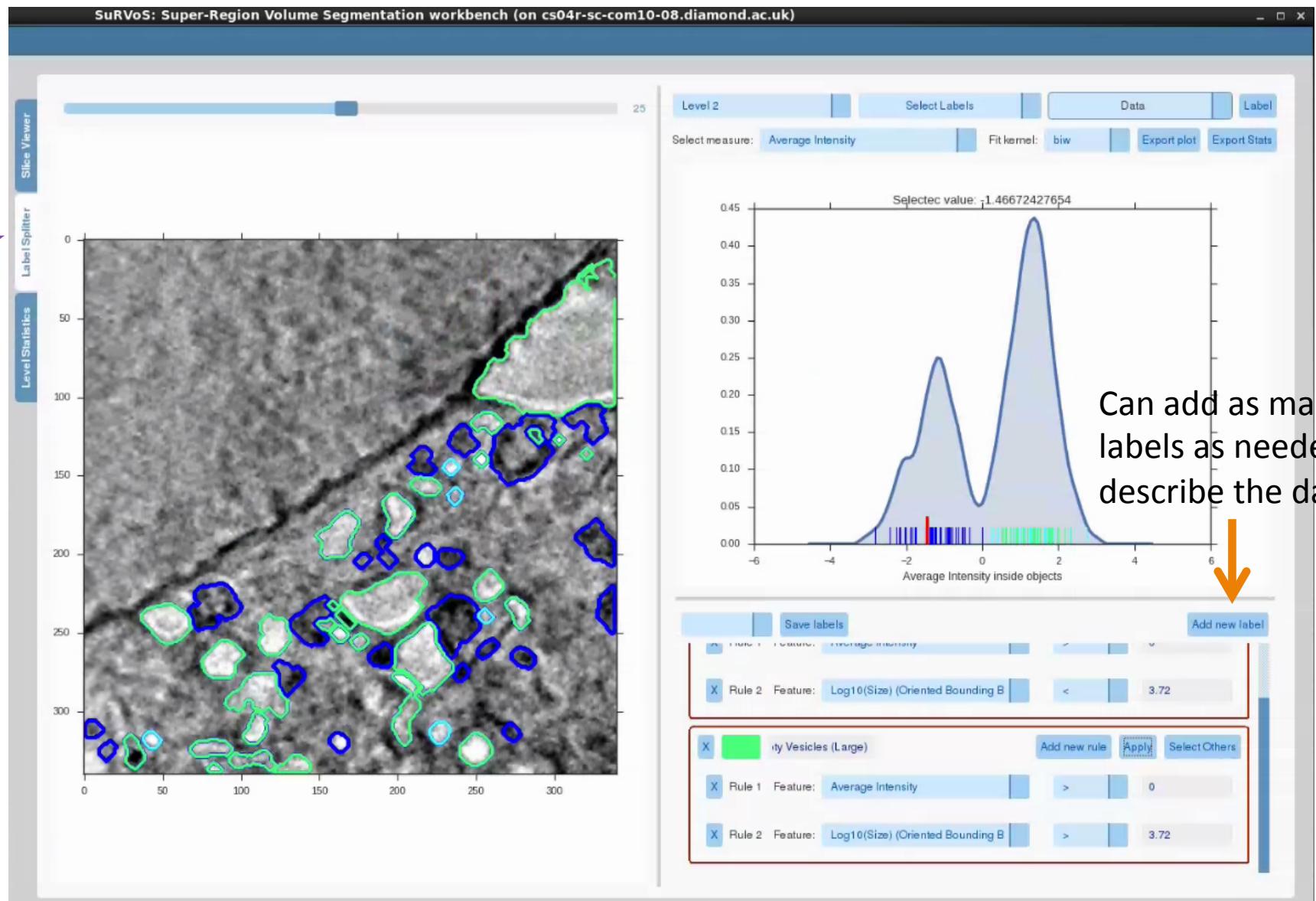
X Rule 1 Feature: Average Intensity > 0

X Rule 2 Feature: Log10(Size) (Oriented Bounding Box) < 3.72

Can add as many rules to define the label as needed

The screenshot shows the SuRVoS Label Splitter interface. On the left, there's a grayscale image with several regions outlined in blue. The right side features a histogram titled 'Area of objects (size in voxels)' with a selected value of 3.71495628357. Below the histogram, a section titled 'Can visualize various measures' is highlighted with an orange arrow. At the bottom, a 'Rule Creation' panel is shown with two rules defined: 'Empty Vesicles' (unchecked) and 'Rule 1' and 'Rule 2'. Rule 1 checks for an average intensity greater than 0, and Rule 2 checks for a Log10(Size) (Oriented Bounding Box) less than 3.72. A red box surrounds the rule creation area, and an orange arrow points to it from the text 'Can add as many rules to define the label as needed' at the bottom.

Label Splitter: Rule Creation



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

Selected value: 1.31154048443

Average Intensity inside objects

Use to gather remaining objects into one class

Add new rule Apply Select Others

The screenshot shows the SuRVoS Label Splitter interface. On the left, there is a 'Slice Viewer' showing a grayscale image with various objects outlined in different colors (green, purple, yellow, blue). A red circle highlights one specific object. On the right, there is a histogram titled 'Average Intensity inside objects' with a selected value of 1.31154048443. The x-axis ranges from -6 to 6, and the y-axis ranges from 0.00 to 0.45. Below the histogram, there is a 'Rule Creation' panel with four rules listed:

- Rule 1: Feature: Log10(Size) (Oriented Boundir) > 0.5
- Rule 2: Feature: Variance > 0.5
- Rule 3: Feature: Variance < 1
- Rule 4: Feature: Variance < 1

Buttons at the bottom include 'Save labels', 'Add new label', 'Add new rule', 'Apply', and 'Select Others'. An orange arrow points to the 'Select Others' button.

Label Splitter: Rule Creation

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

Slice Viewer

Label Splitter

Level Statistics

25

Select measure: Average Intensity

Select Labels

Data

Label

Fit kernel: biw

Export plot

Export Stats

Selected value: 1.31154048443

Average Intensity inside objects

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

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

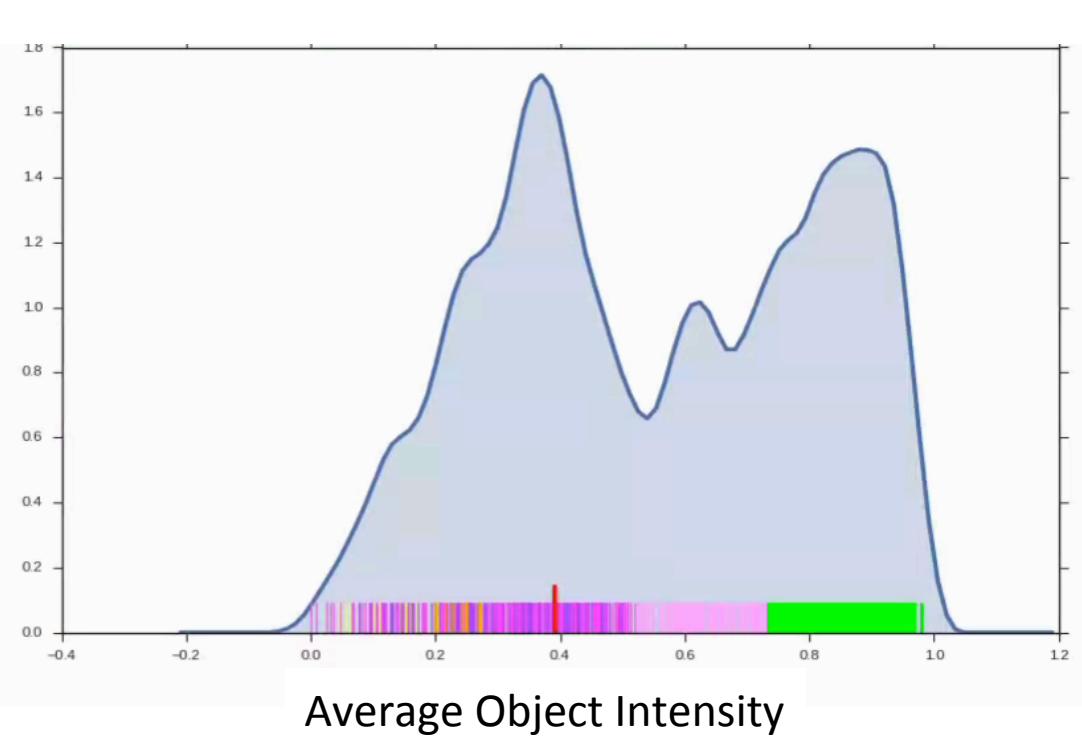
Add new rule Apply Select Others

The screenshot shows the SuRVoS Label Splitter interface. On the left, there's a 'Slice Viewer' showing a grayscale image with various regions outlined in different colors (red, green, yellow, purple). Below it is a 'Level Statistics' panel. In the center, there's a histogram titled 'Average Intensity inside objects' with a peak at approximately 1.31. A red arrow points from the text 'When happy with rules, make new, empty level (in annotation tab of GUI). Then select that level here and click Save Labels' to the 'Save labels' button in the 'Level 4' panel. The 'Level 4' panel contains four rules:

- X Rule 2 Feature: Log10(Size) (Oriented Boundir) > 4.2
- X Rule 3 Feature: Variance > 0.5
- X Rule 4 Feature: Variance < 1

At the bottom, there are buttons for 'Add new rule', 'Apply', and 'Select Others'. A purple star icon is located in the top-left corner of the slide.

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

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

The screenshot shows the SuRVoS Label Statistics interface. On the left, there's a vertical toolbar with buttons for 'Slice Viewer', 'Level Statistics' (which is selected), and 'Label Statistics'. The main area has tabs for 'Select Labels', 'Data', and 'Label'. A legend indicates 'Level 4' and 'Fit kernel: gau'. On the right, there's a list of measurement options under 'Label'.

- Choose level you will work with
- Select all labels you want to include
- Choose which form of the data to measure from
- Click Label

Select Labels:

- Label 1
- Label 2
- Label 3
- Label 4

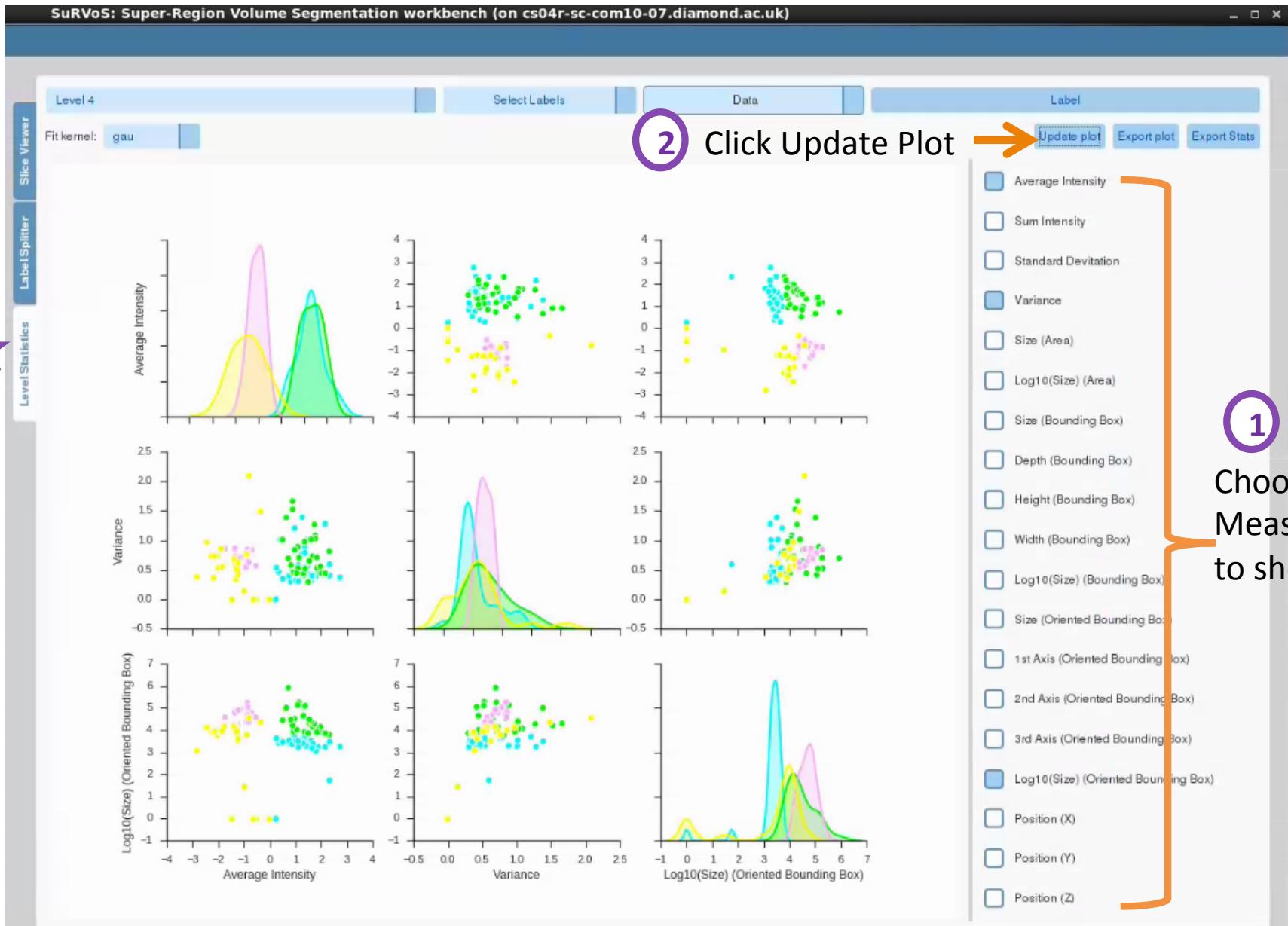
Data:

- Average Intensity
- Sum Intensity
- Standard Deviation
- Distance
- Size (Area)
- Log10(Size) (Area)
- Size (Bounding Box)
- Depth (Bounding Box)
- Height (Bounding Box)
- Width (Bounding Box)
- Log10(Size) (Bounding Box)
- Size (Oriented Bounding Box)
- 1st Axis (Oriented Bounding Box)
- 2nd Axis (Oriented Bounding Box)
- 3rd Axis (Oriented Bounding Box)
- Log10(Size) (Oriented Bounding Box)
- Position (X)
- Position (Y)
- Position (Z)

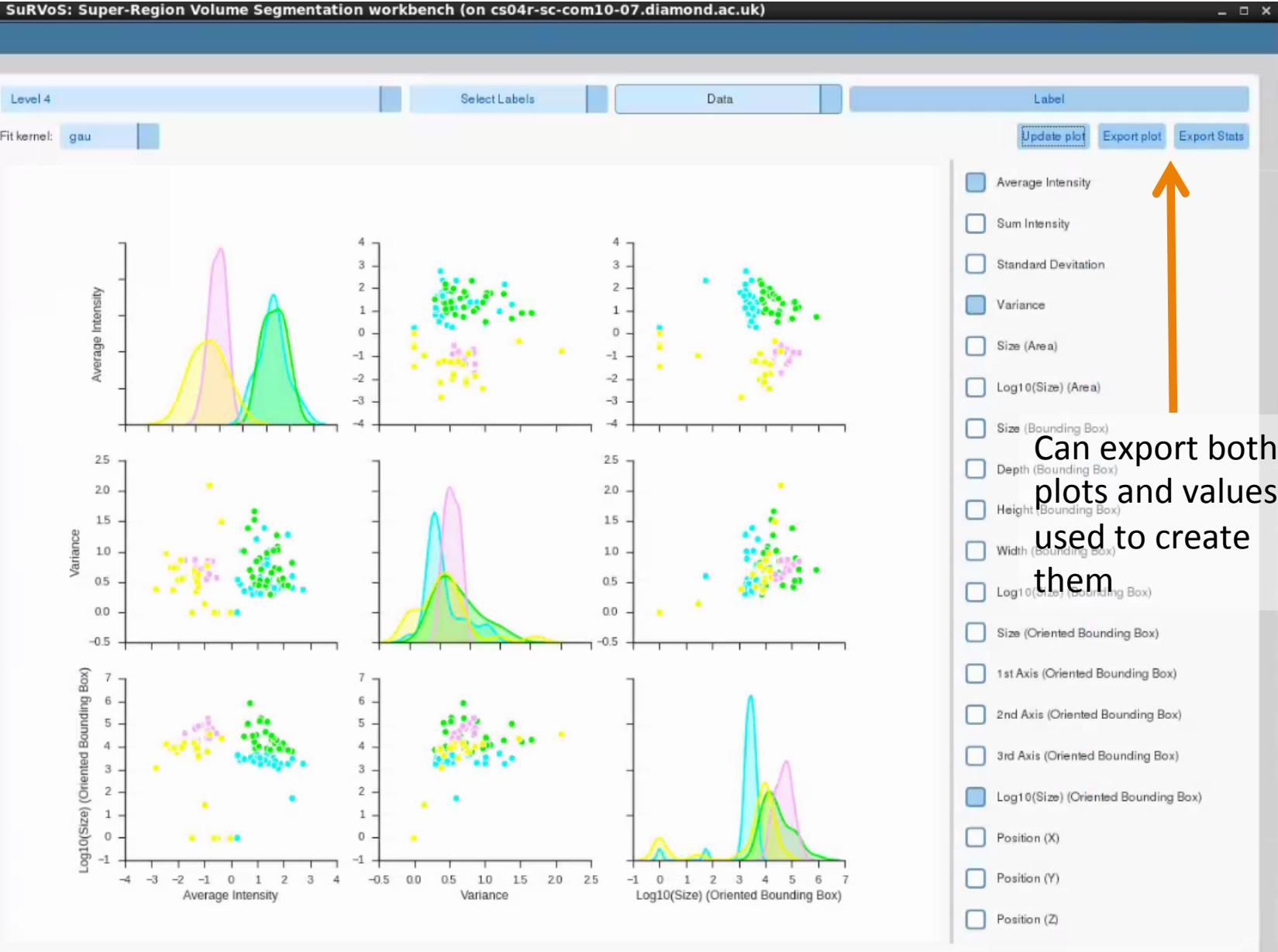
Label:

- Update plot
- Export plot
- Export Stats

Label Statistics

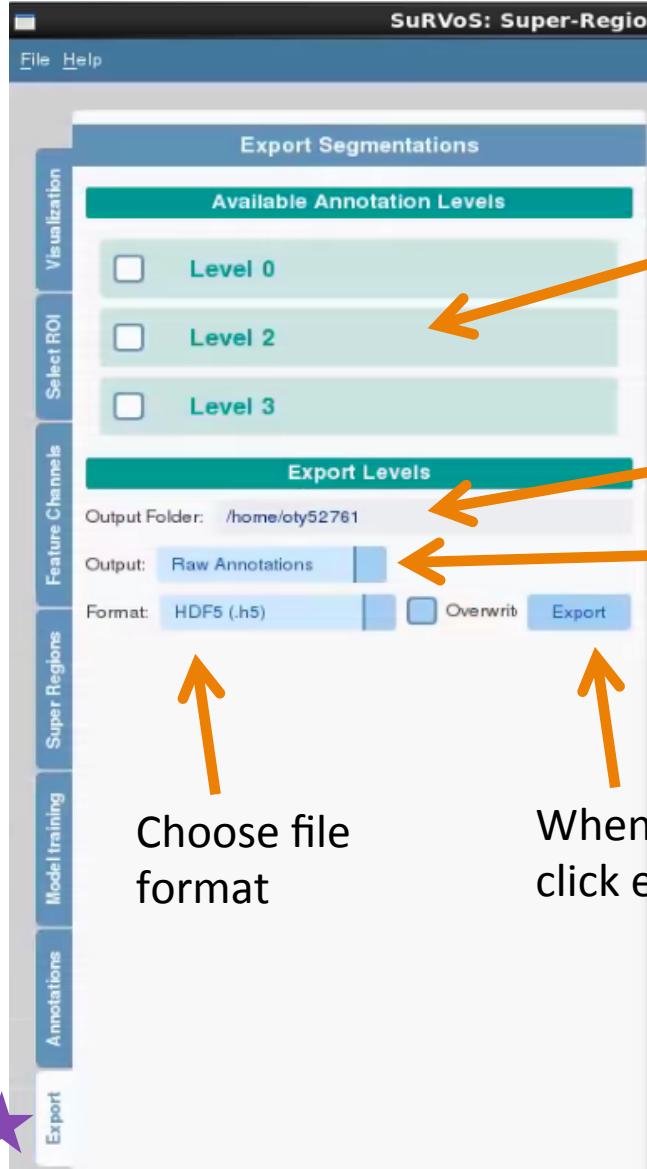


Label Statistics



Time – 5 minutes

Exporting Data



Choose file format

When ready,
click export

Choose levels
to export

Choose location
to export data to

Choose way of
exporting data

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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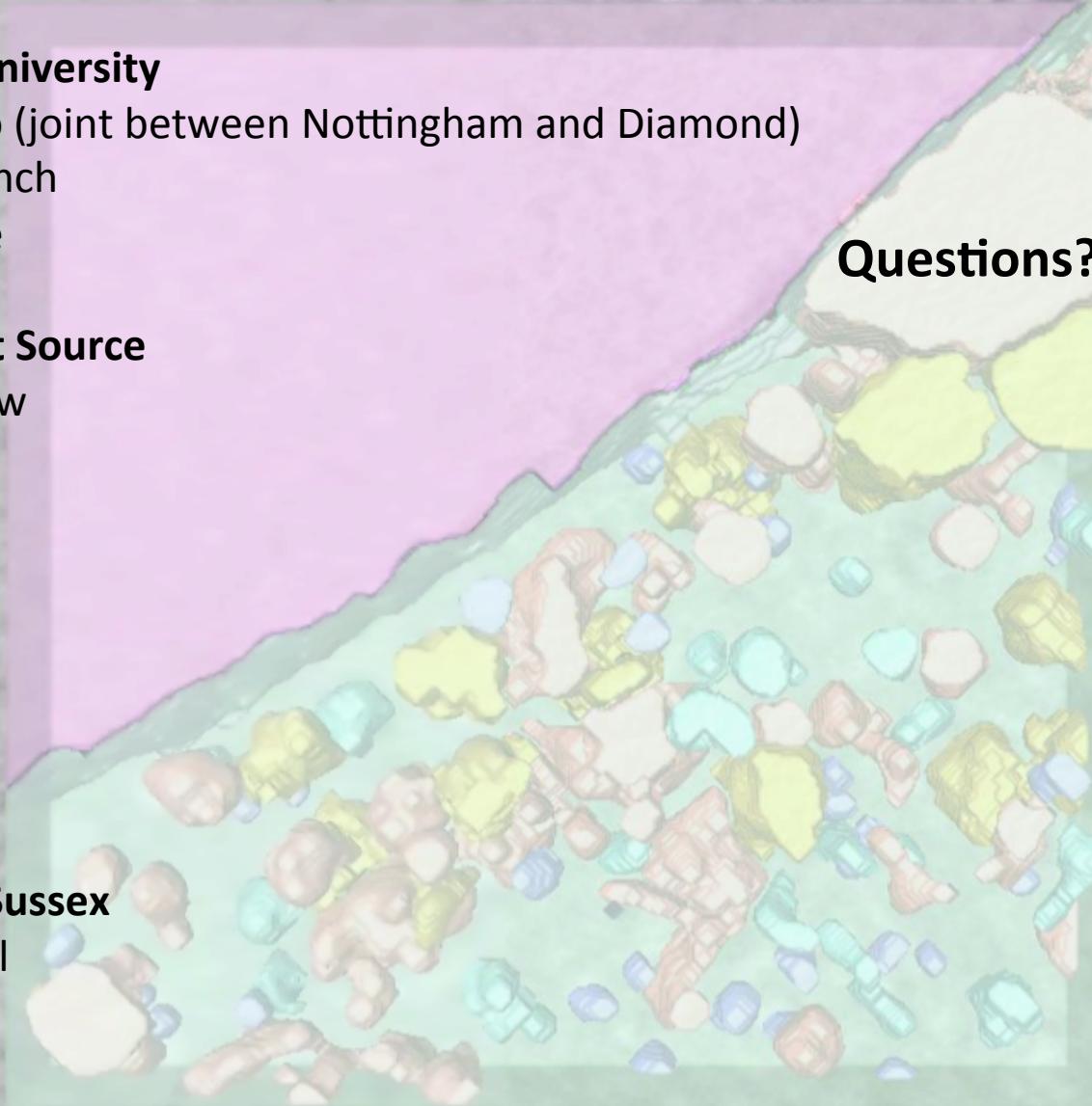
Kyle Dent

University of Sussex

Karen Marshall

Also thanks to

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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 sci
```

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
```