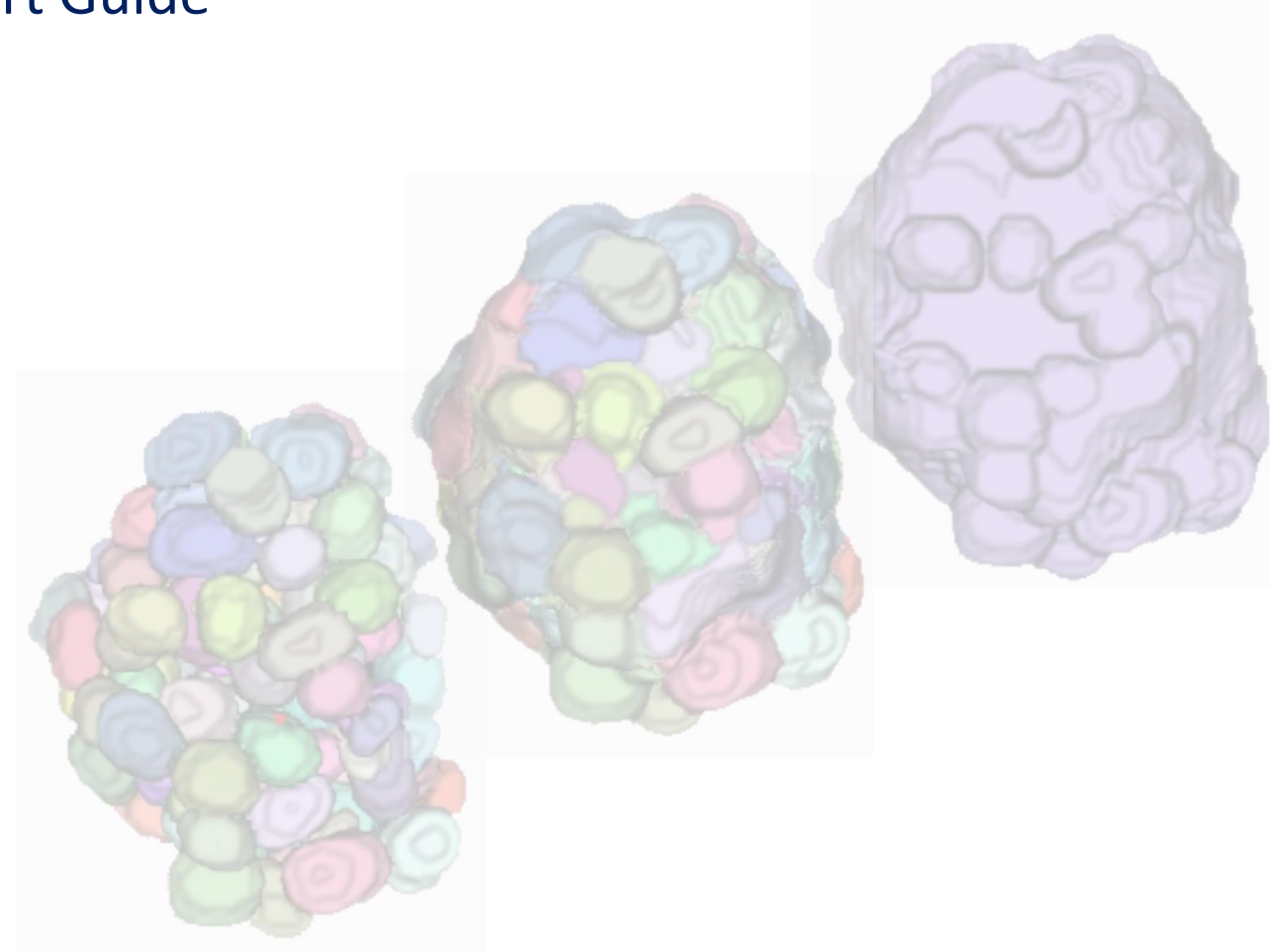
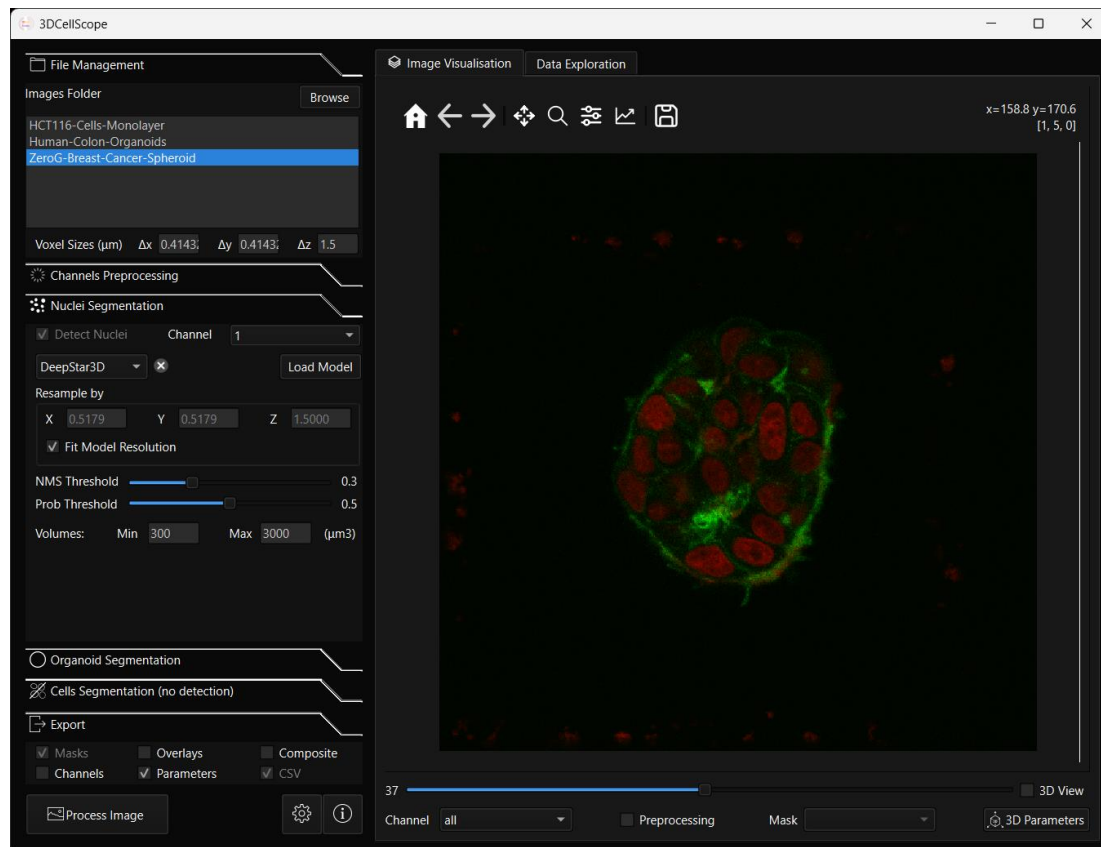


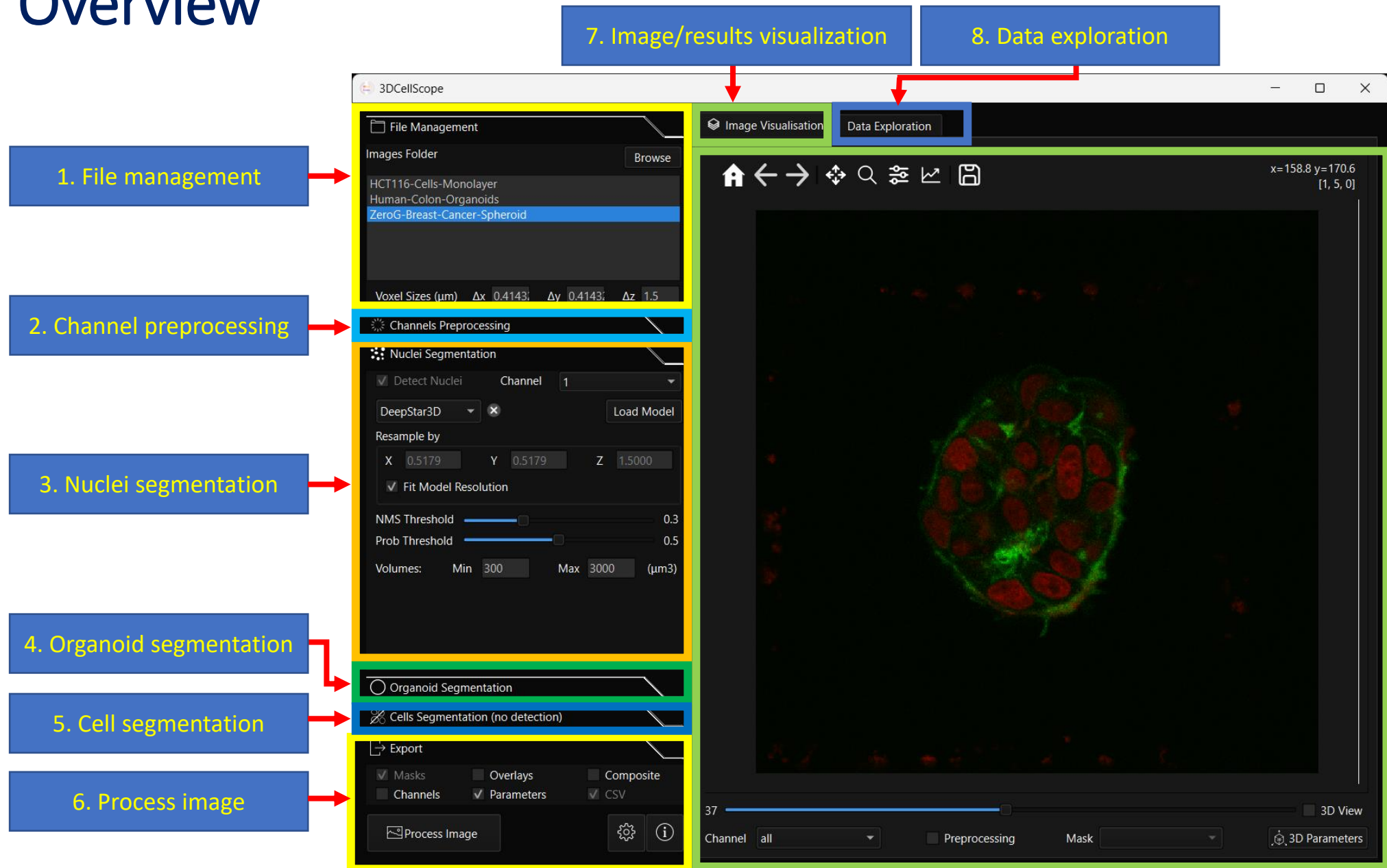
# 3DCellScope



## Quick Start Guide



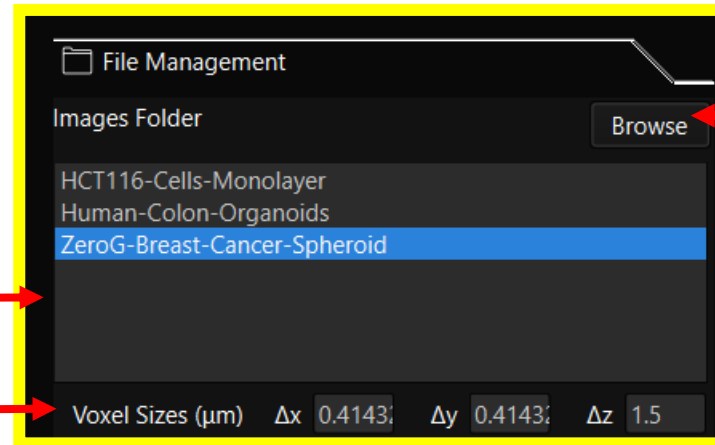
# Overview



# 1. File management

2. Select image for visualization or processing

Voxel sizes of the selected image



1. Select a folder containing .tif images:

- Grayscale ZXY (8 bit, 16 bit, 32 bit)
- RGB ZXY (8 bit)
- Multichannel ZCXY (8 bit, 16 bit, 32 bit)
- Separated channel file images, with multiple ZXY (8 bit, 16 bit, 32 bit) image files containing the C1/C2/C3 suffixes

## 2. Channels preprocessing

The screenshot shows a 'Channels Preprocessing' dialog box with the following elements:

- Channel:** A dropdown menu currently set to '1'.
- Method:** A dropdown menu currently set to 'correct bleaching'.
- Sigma:** An input field.
- Amount:** An input field.
- Processing List:** A list box containing one item, 'C1 correct bleaching', which is highlighted in blue.
- Add:** A button to the right of the Method dropdown.
- Remove:** A button to the right of the Processing List.

Four instructional callouts with red arrows point to these elements:

- 1. Select channel for preprocessing** points to the Channel dropdown.
- 2. Select method** points to the Method dropdown. Below this title is a list:
  - correct bleaching
  - to correct for signal decay due to depth penetration
- 3. Add preprocessing step** points to the Add button.
- Remove selected preprocessing step** points to the Remove button.

# 3. Nuclei segmentation

## 3. Choose one of the pre-loaded models

- DeepStar3D
- Cellos
- Iso-Basic
- AnyStar

## 4. Fit model resolution

- Fit model resolution
  - to match the voxel size of the training data automatically
- Or
- Manually input the resampling factor
  - Value < 1 means downsampling while value > 1 means upsampling.

## 7. Nuclei size filtering

- Enter minimum and maximum nuclei volume

The screenshot shows the 'Nuclei Segmentation' window. It has a dark theme. At the top, there's a title bar with a logo and the text 'Nuclei Segmentation'. Below the title bar, there's a checkbox labeled 'Detect Nuclei' which is checked. To its right is a dropdown menu labeled 'Channel' with '1' selected. Below these, there's a dropdown menu showing 'DeepStar3D' with a close button 'x' to its right. To the right of this is a button labeled 'Load Model'. Below the model dropdown, there's a section labeled 'Resample by' with three input fields: 'X' with value '0.5179', 'Y' with value '0.5179', and 'Z' with value '1.5000'. Below this is a checkbox labeled 'Fit Model Resolution' which is checked. Below that are two sliders: 'NMS Threshold' with a value of '0.3' and 'Prob Threshold' with a value of '0.5'. At the bottom, there's a section labeled 'Volumes:' with 'Min' set to '300' and 'Max' set to '3000' (with a unit 'μm3' to the right).

## 1. Select channel for nuclei segmentation

## 2. Load model

- By choosing a folder that contains the .h5 file

## 5. Non-Maximum Suppression Threshold

- To select one entity out of many overlapping entities. Higher NMS threshold gives more overlapping nuclei and vice versa. (Range from 0 to 1)

## 6. Probability Threshold

- Higher probability threshold gives less nuclei and vice versa. (Range from 0 to 1)

## 4. Organoid segmentation

### 1. Enable Detect Organoid

### 4. Adjust parameter

- Otsu Scale (for Otsu Threshold method)
  - Default = 0.8. Increase the threshold to get smaller region and vice versa.
- Or
- Dynamic Range Threshold for (Dynamic Range Threshold method)
  - Default = 0.1. Increase the threshold to get smaller region and vice versa.

The screenshot shows the 'Organoid Segmentation' window. At the top, there is a radio button labeled 'Organoid Segmentation'. Below it, a checkbox labeled 'Detect Organoid' is checked. Underneath, there are two dropdown menus: 'Channel' set to 'all' and 'Method' set to 'Otsu Threshold'. Below these is a slider for 'Otsu Scale' with a value of 0.8. At the bottom, there are two radio buttons: 'Keep Largest Organoid' (selected) and 'Keep Organoids Larger Than' (with an empty input field and a unit of  $\mu\text{m}^3$ ). Red arrows point from the instruction boxes to the 'Detect Organoid' checkbox, the 'Channel' dropdown, the 'Otsu Scale' slider, the 'Method' dropdown, and the 'Keep Largest Organoid' radio button.

### 2. Select channel for organoid segmentation

- all
  - mean intensity of all channels will be used

### 3. Choose method

- Otsu Threshold
- Dynamic Range Threshold

### 5. Organoid size filtering

- Keep largest organoid
- Or
- Keep organoids larger than a minimum volume

# 5. Cell segmentation

## 1. Enable Detect Cells

## 4. Adjust parameter

- Dist max (um)
  - Default = 14. The region growing is limited to a distance, **dist max**, that sets the maximum distance of the cell edge from the edge of the nucleus.

The screenshot shows the 'Cells Segmentation' window with the following settings:

- Detect Cells:** A checkbox that is checked.
- Channel:** A dropdown menu set to '2'.
- Method:** A dropdown menu set to 'Watershed intensity channel'.
- Dist max (um):** A text input field containing the value '14'.
- Stain Type:** Two radio buttons are present: 'Cytoplasmic membrane stain' (which is selected) and 'Cytoplasmic stain'.

Red arrows point from the numbered callout boxes to these specific settings in the interface.

## 2. Select channel for cell segmentation

## 3. Choose method

- Watershed intensity channel
  - Uses the cytoplasmic channel
- Watershed distance map
  - Uses the distance map between nuclei centroid

## 5. Choose the type of cell channel

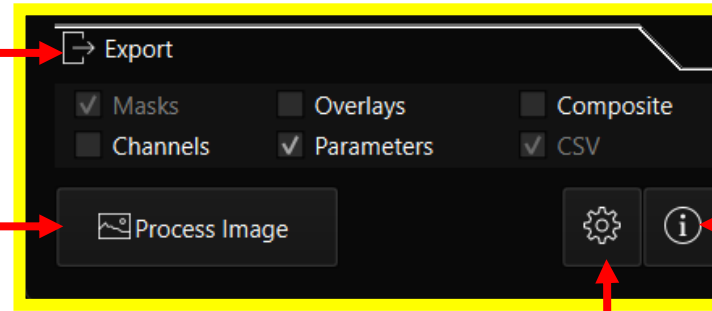
# 6. Process image

## 1. Export

- Select type of outputs to be exported

## 2. Process image

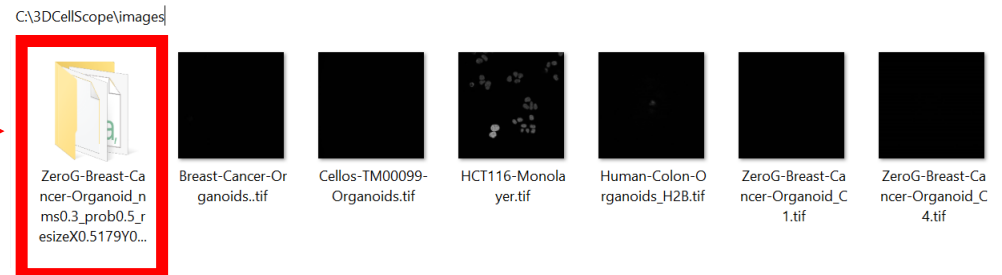
- Process the selected image



Help

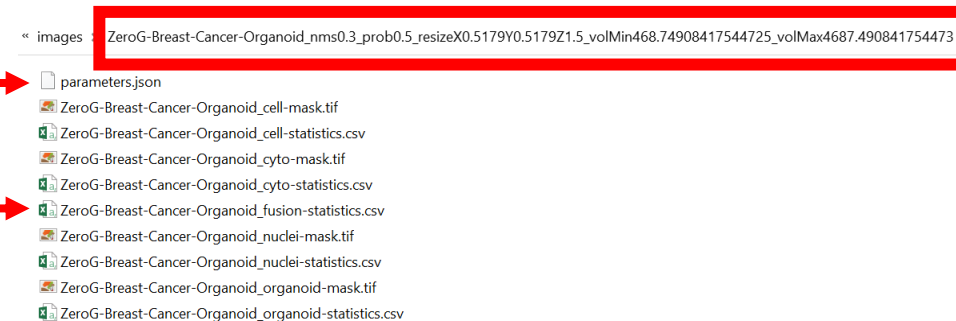
Load a json parameter file (optional)

Results folder created inside images folder



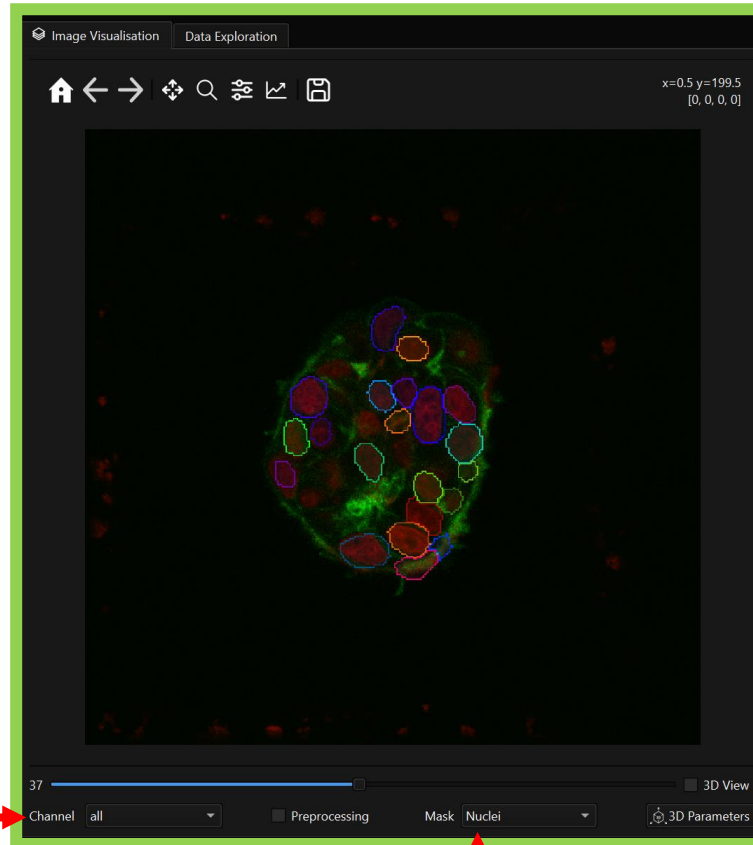
The parameters chosen for segmentation

fusion-statistics of cell, nuclei and organoid



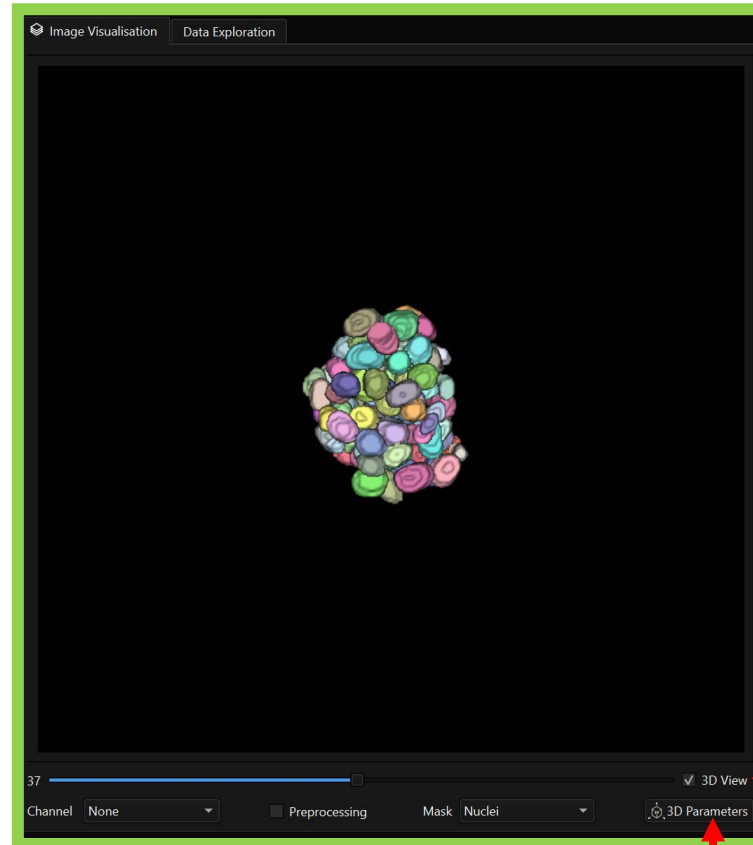


# 7. Image/results visualization



Choose the channel to be displayed

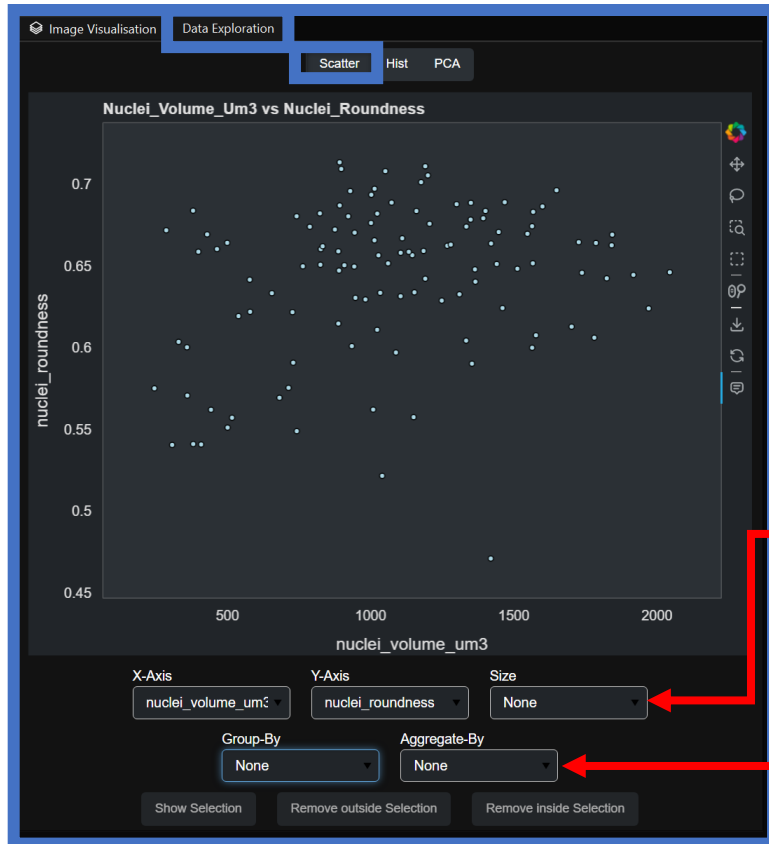
Choose the masks to be displayed



Visualize channels and masks in 3D

Set 3D visualization parameters

## 8. Data exploration – Scatter and Hist

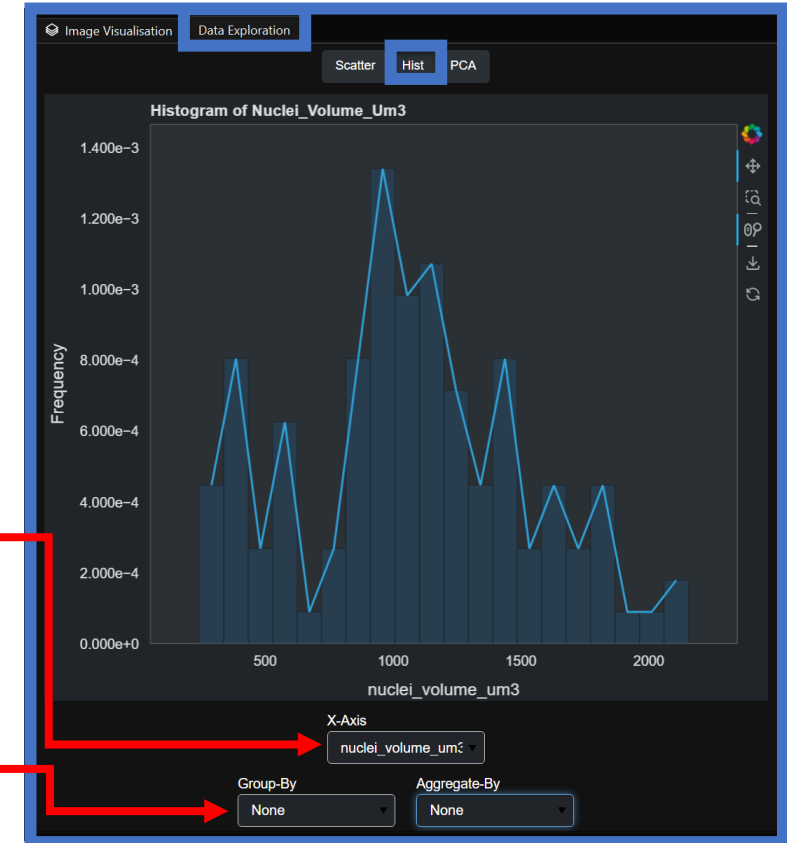


### Choose variables

- from list of morphological and topological descriptors for nuclei, cell and organoid.

### Group/aggregate by

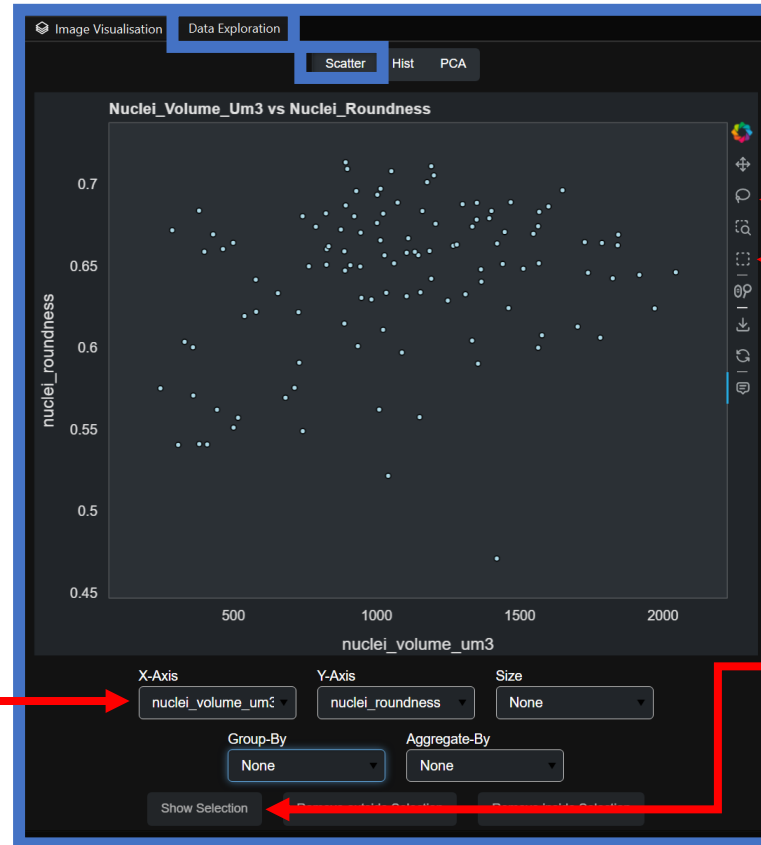
- organoid, file etc.



## 8. Data exploration - Selection

### 1. Choose variables for

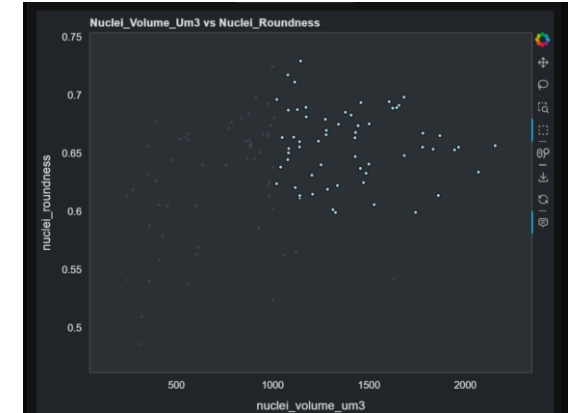
- x-axis
- y-axis
- size



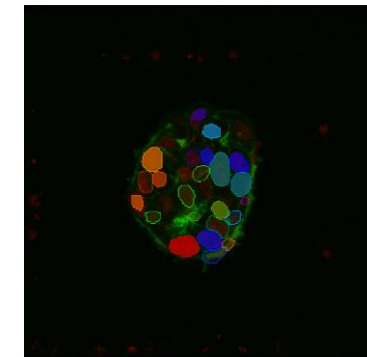
### 2. Select points of interest

Lasso selection tool

Box selection tool



### 3. Show selection



## 8. Data exploration – PCA

The screenshot shows the 'Data Exploration' window with the 'PCA' tab selected. The interface is divided into two main panels: 'Filter available options' on the left and 'Filter selected options' on the right. The left panel contains a list of features, with a blue box highlighting the first 10 items. The right panel contains a list of selected features. A red arrow points from the first callout to the left panel. A second red arrow points from the second callout to the right panel. A third red arrow points from the third callout to the 'Axis to generate' dropdown menu. A fourth red arrow points from the fourth callout to the 'Create PCA Features' button.

1. Select features

2. Add selected features

3. Specify axis to generate

4. Create PCA features