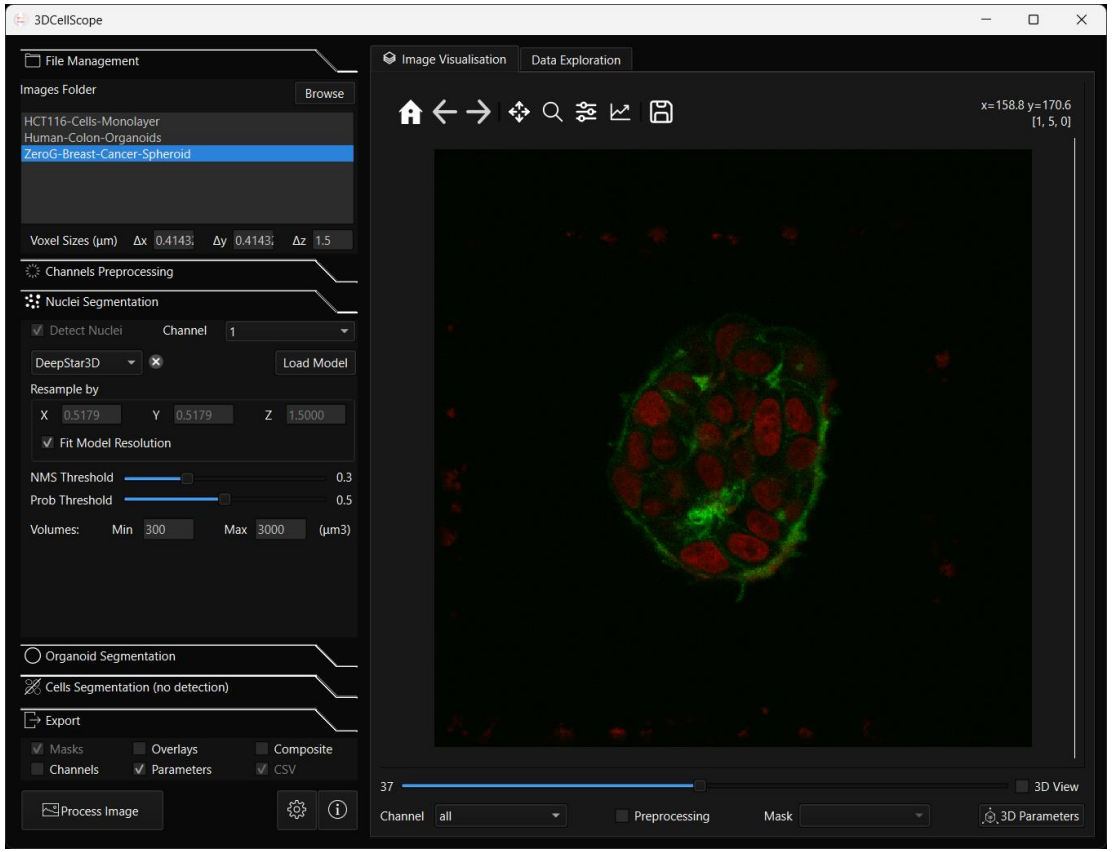
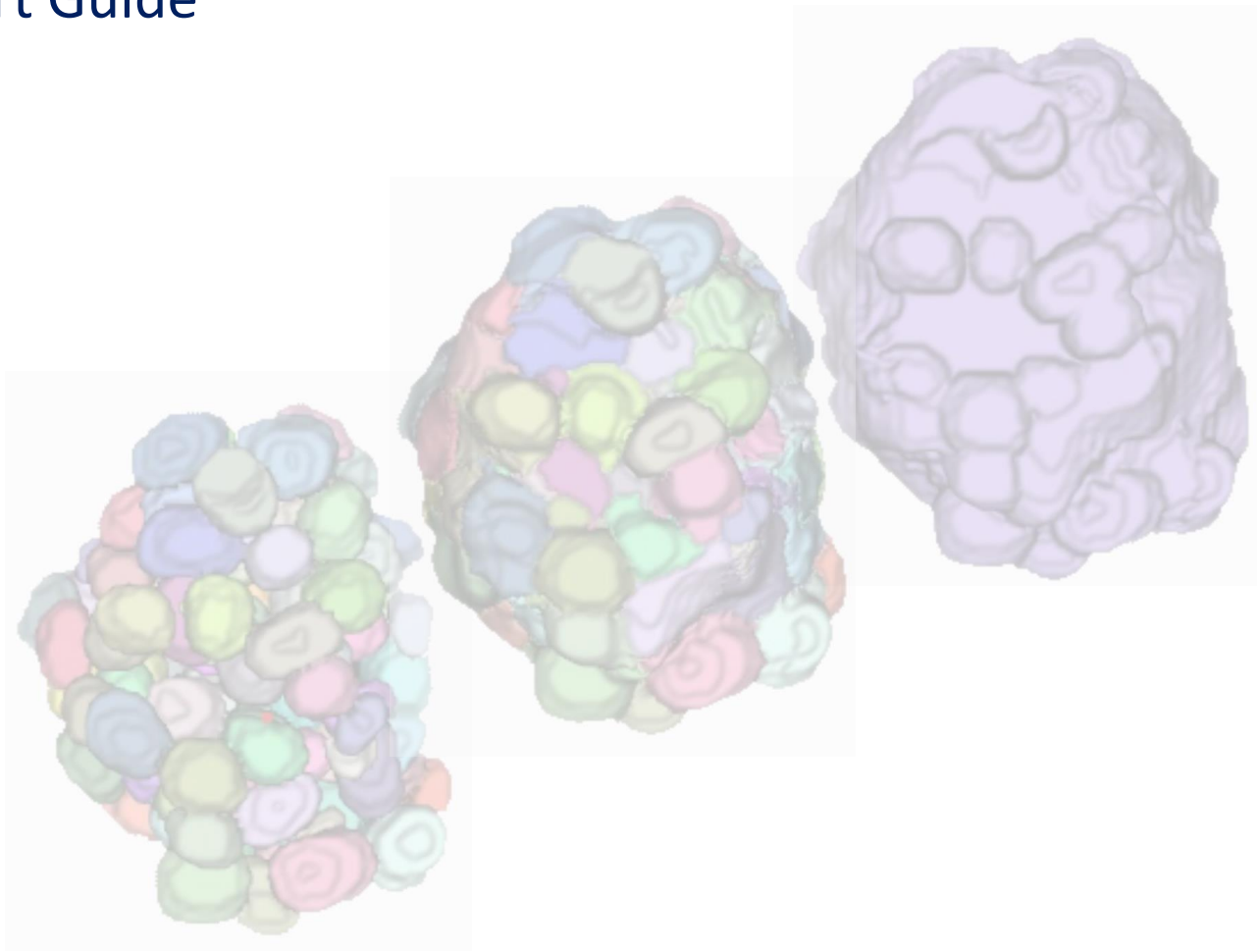
3DCellScope

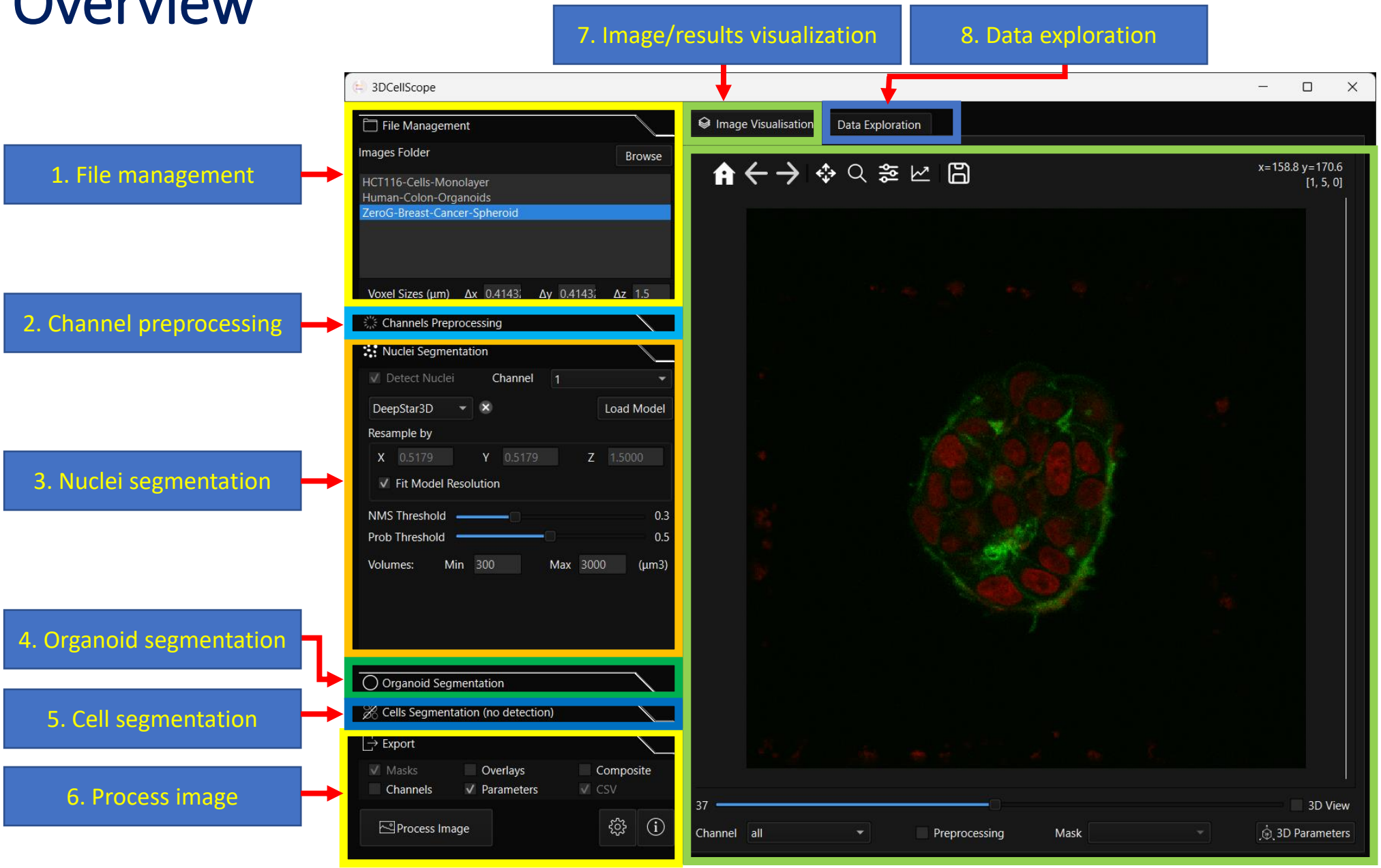
Quick Start Guide



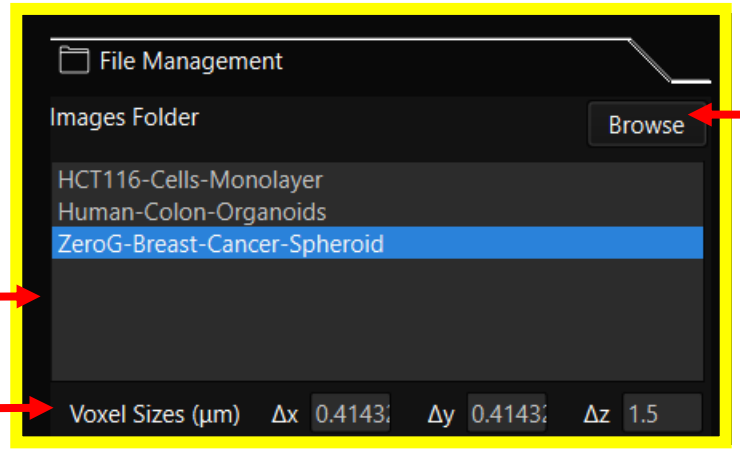


QUANTACELL SIMBA

Overview


7. Image/results visualization
8. Data exploration
1. File management
2. Channel preprocessing
3. Nuclei segmentation
4. Organoid segmentation
5. Cell segmentation
6. Process image
3DCellScope
File Management
Images Folder
Browse
HCT116-Cells-Monolayer
Human-Colon-Organoids
ZeroG-Breast-Cancer-Spheroid
Voxel Sizes (µm)
Channels Preprocessing
Nuclei Segmentation
Detect Nuclei
Channel
DeepStar3D
Load Model
Resample by
X 0.5179
Y 0.5179
Z 1.5000
Fit Model Resolution
NMS Threshold 0.3
Prob Threshold 0.5
Volumes: Min 300 Max 3000 (µm3)
Organoid Segmentation
Cells Segmentation (no detection)
Export
Masks
Overlays
Composite
Channels
Parameters
CSV
Process Image
Image Visualisation
Data Exploration
x=158.8 y=170.6
[1, 5, 0]
37
3D View
Channel all
Preprocessing
Mask
3D Parameters

1. File management



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

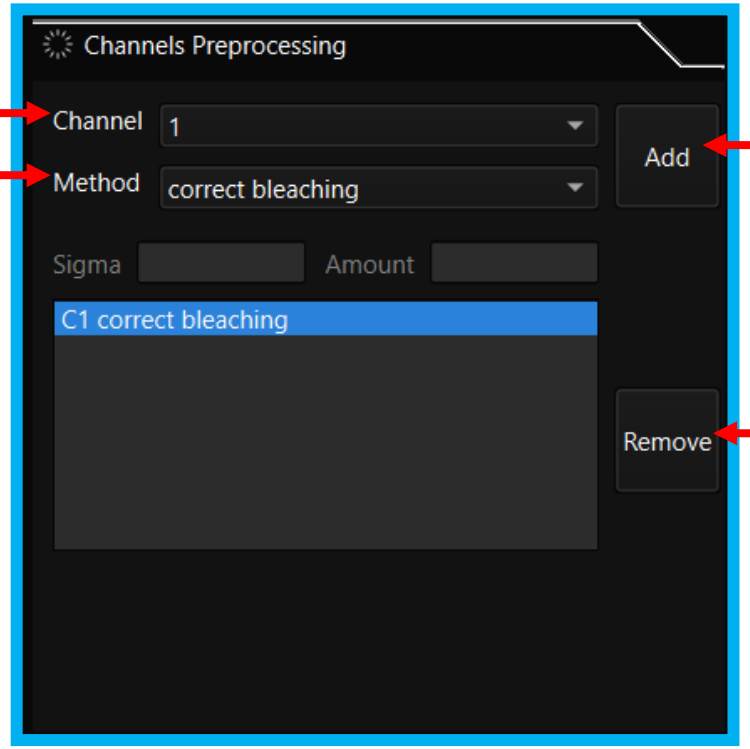
2. Select image for visualization or processing

Voxel sizes of the selected image

2. Channels preprocessing

1. Select channel for preprocessing

2. Select method
- correct bleaching
  - to correct for signal decay due to depth penetration



3. Add preprocessing step

Remove selected preprocessing step

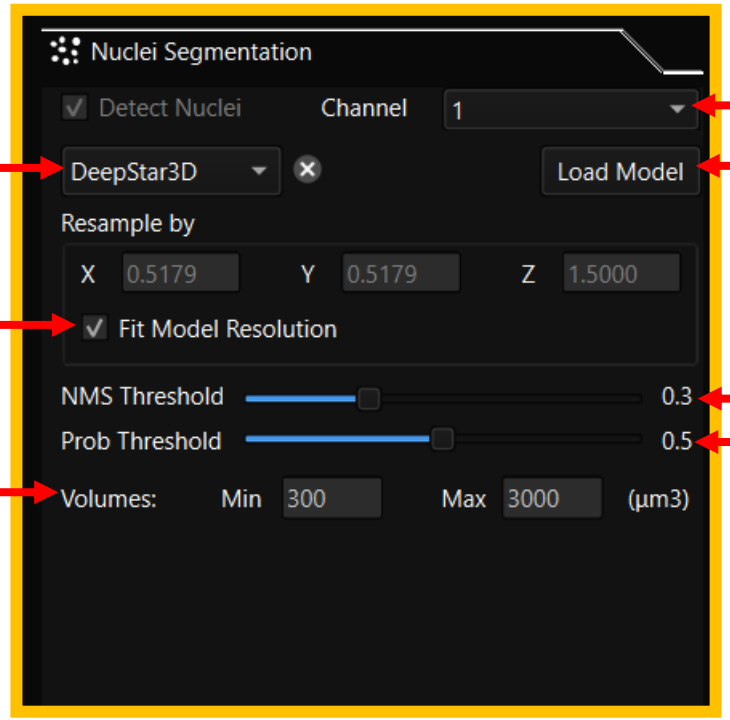
3. Nuclei segmentation

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



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)

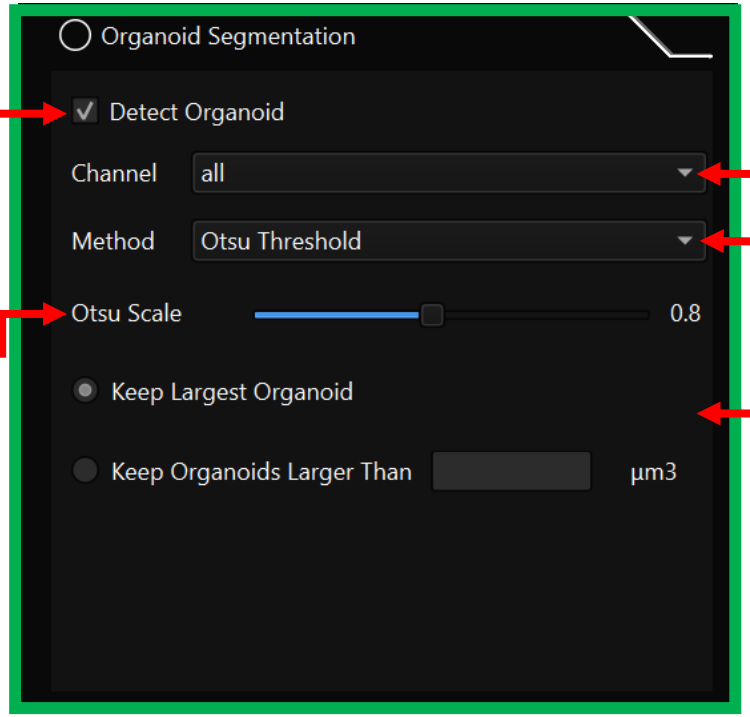
6. Probability Threshold
- Higher probability threshold gives less nuclei and vice versa. (Range from 0 to 1)

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.



2. Select channel for organoid segmentation
- all
  - mean intensity of all channels will be used

3. Choose method
- Otsu Threshold
- Dynamic Range Threshold

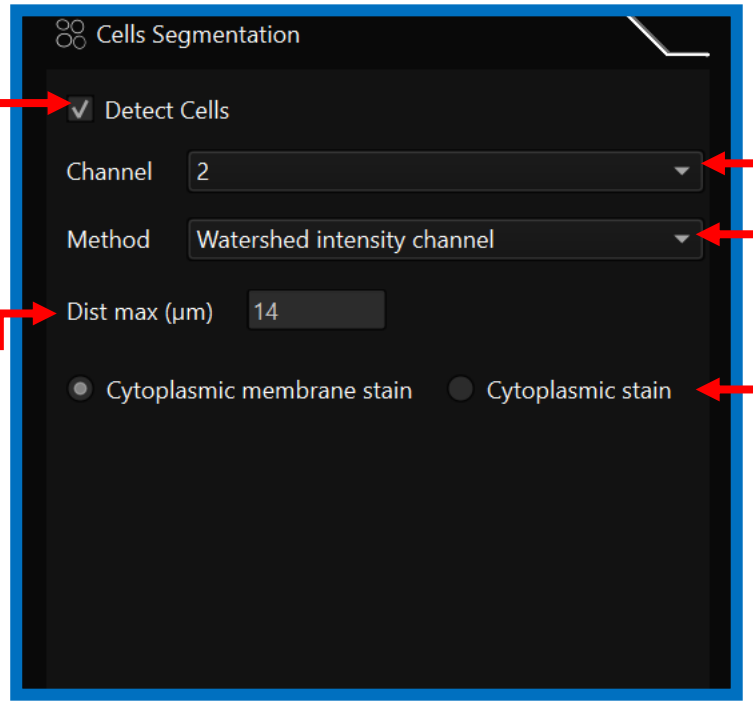
5. Organoid size filtering
- Keep largest organoid

  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.



2. Select channel for cell segmentation

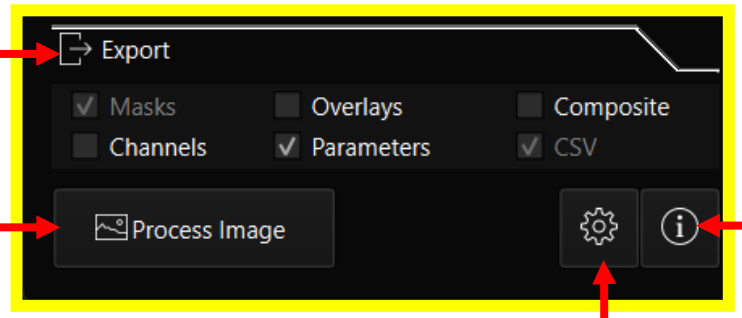
3. Choose method
- Watershed intensity channel
  - Uses the cytoplasmic channel
- Watershed distance map
  - Uses the distance map between nuclei centroid

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



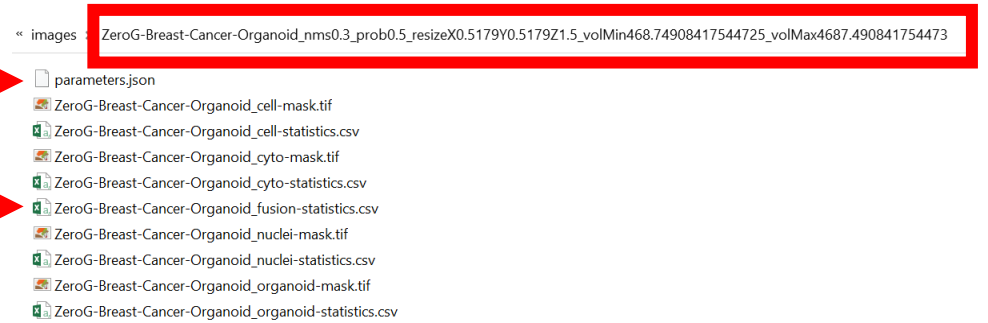
Help

Load a json parameter file (optional)

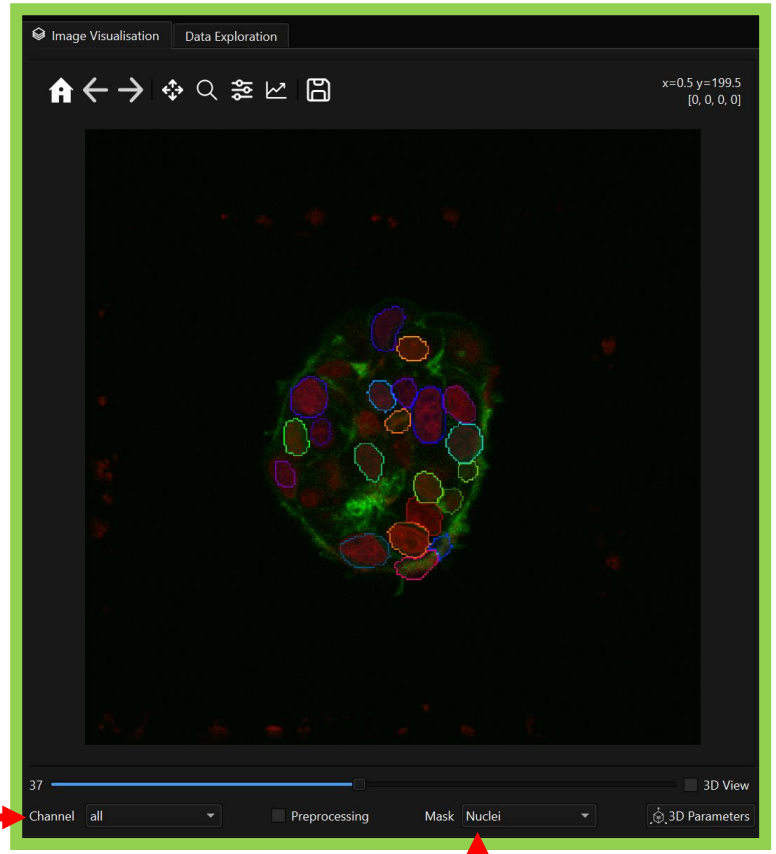
Results folder created inside images folder

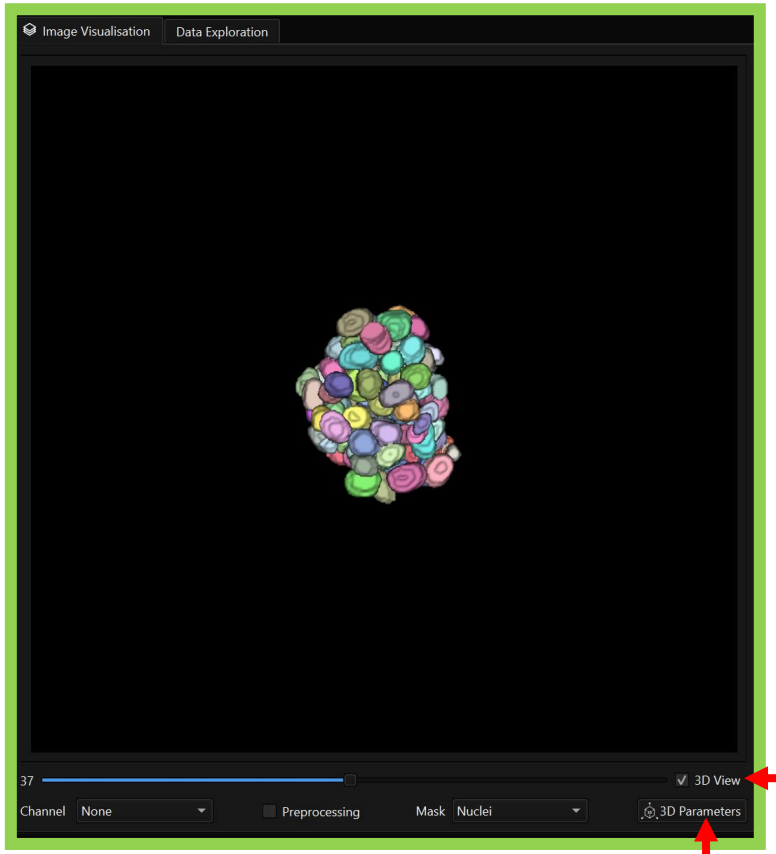
The parameters chosen for segmentation

fusion-statistics of cell, nuclei and organoid



7. Image/results visualization





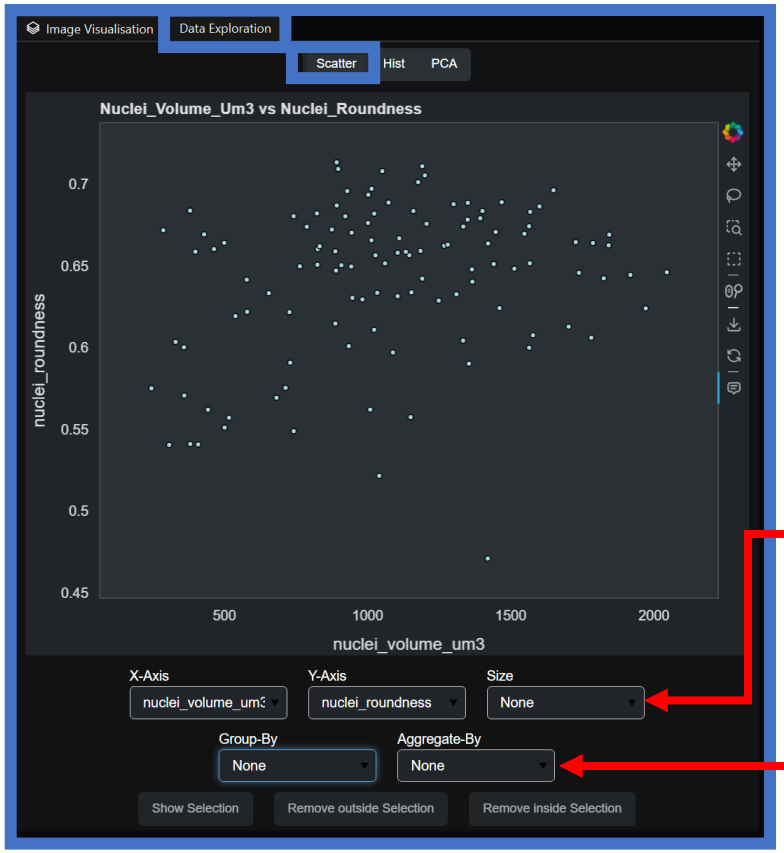
Choose the channel to be displayed

Choose the masks to be displayed

Set 3D visualization parameters

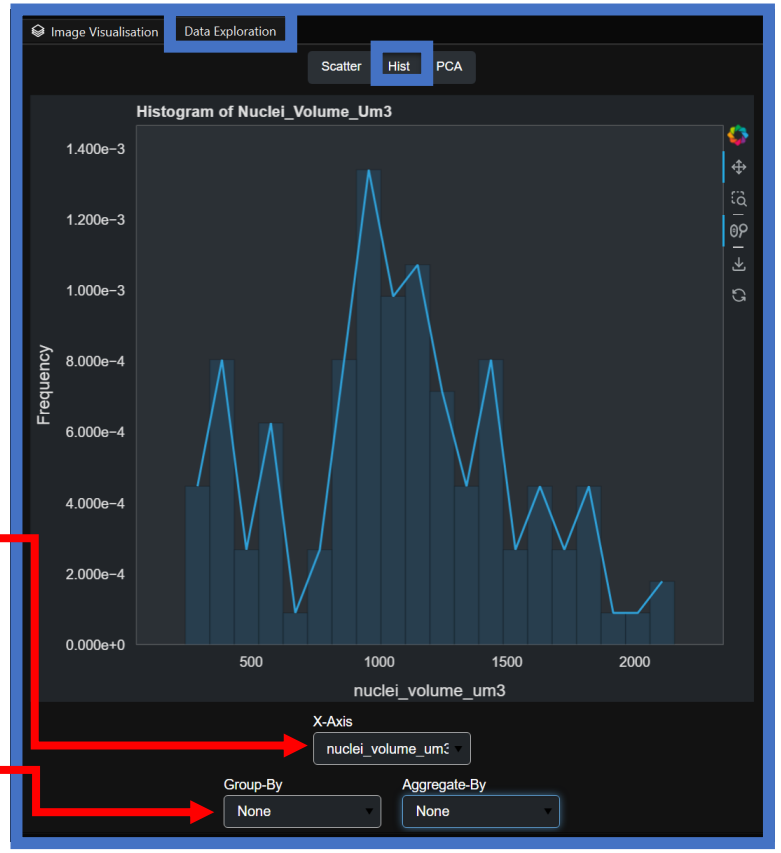
Visualize channels and masks in 3D

8. Data exploration – Scatter and Hist



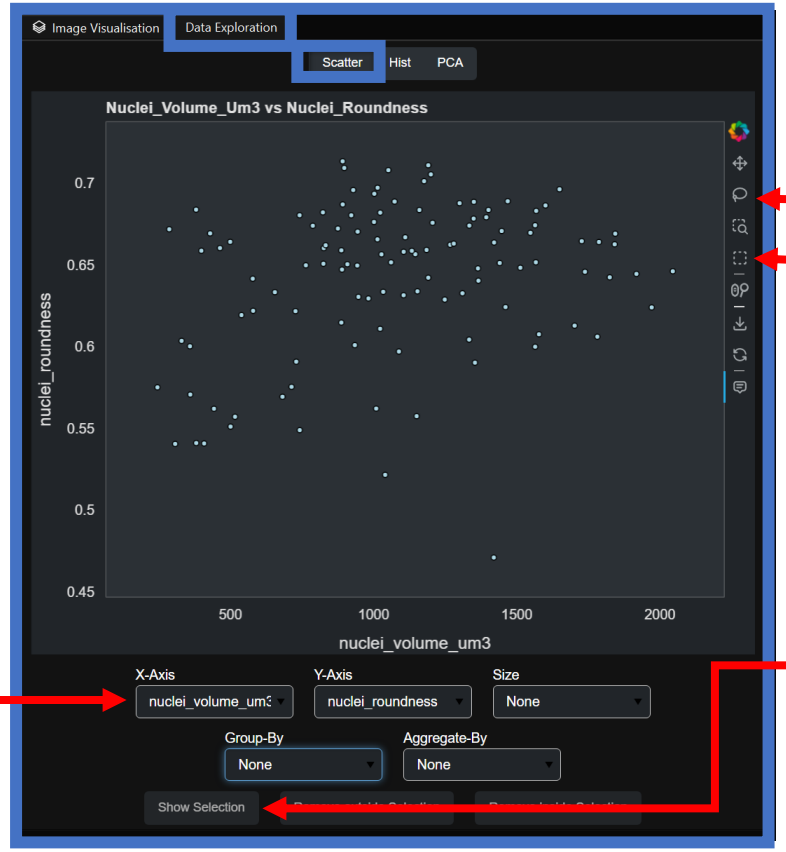
Choose variables
- from list of morphological and topological descriptors for nuclei, cell and organoid.

Group/aggregate by
- organoid, file etc.



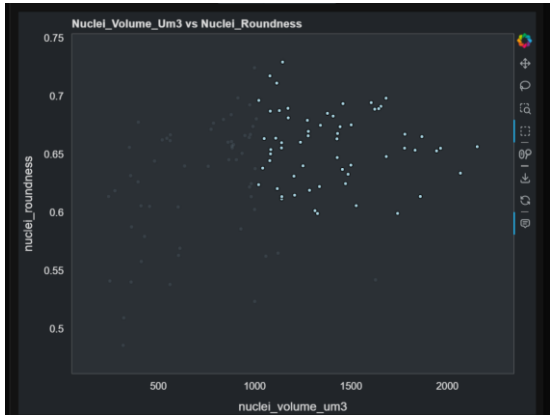
8. Data exploration - Selection

1. Choose variables for
   - x-axis
   - y-axis
   - size

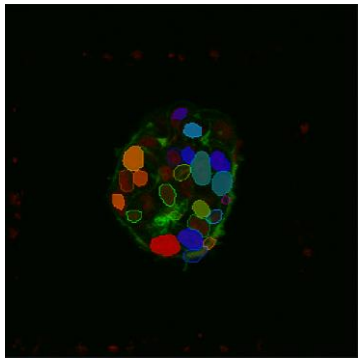


2. Select points of interest

- Lasso selection tool
- Box selection tool



3. Show selection



8. Data exploration – PCA