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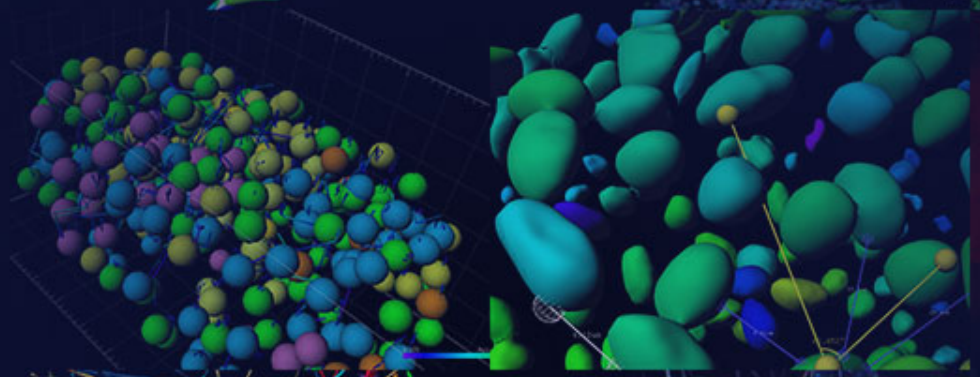

IMARIS CELL

Making Sense out of your Cell's Relationships

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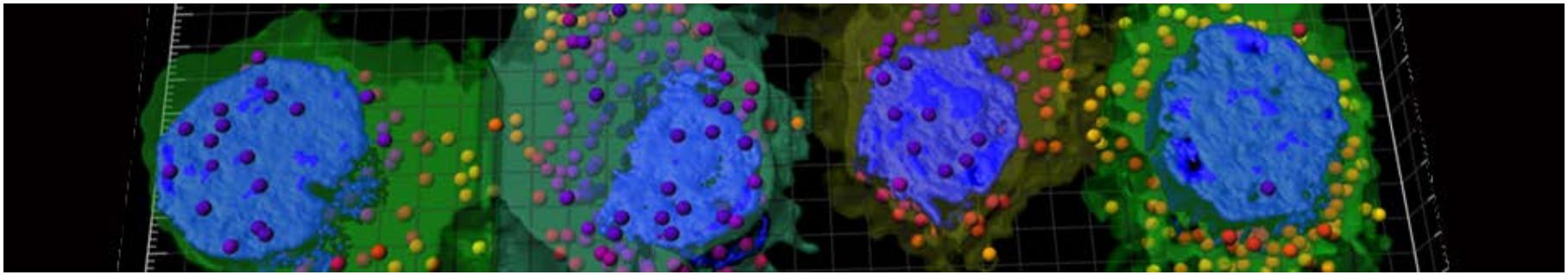
Imaris Cell objectives



- ✓ To report by biologically meaningful unit
- ✓ To segment the biological unit and its organelles simultaneouly
- ✓ To quantify the relationships between the biological unit and its organelles

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Imaris Cell Basics

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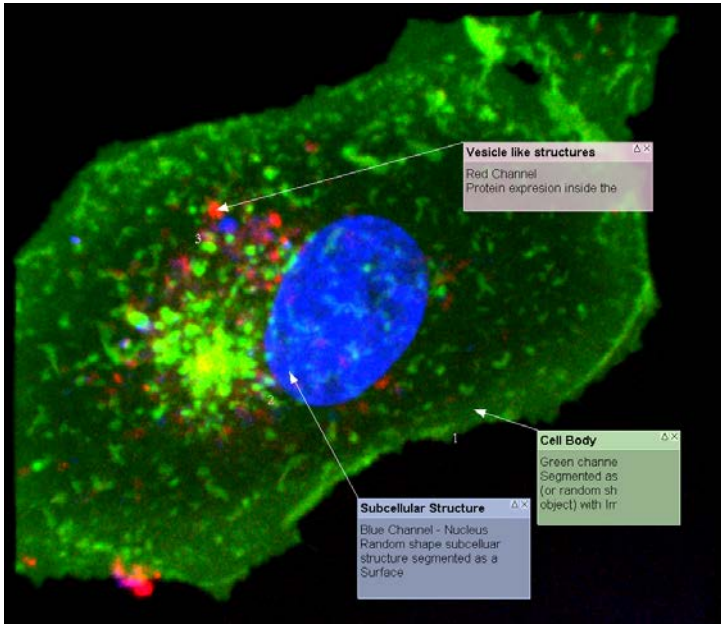
Imaris Cell allows you to segment simultaneously:

- A surface object: which represent the boundaries of your biological unit, for example the cell
- A second surface object: in this case it's an object contained within the biological unit, like for example the nucleus, or another random shaped structure
- As many vesicular like structures contained within the biological unit, such for example viruses, liposomes, proteins….



☞ *TIP: Explore your options*

Your biological unit can be an organ, and the secondary object cells expressed inside.

Or your biological unit can be the Nucleus and the vesicular like structures can be your FISH signal

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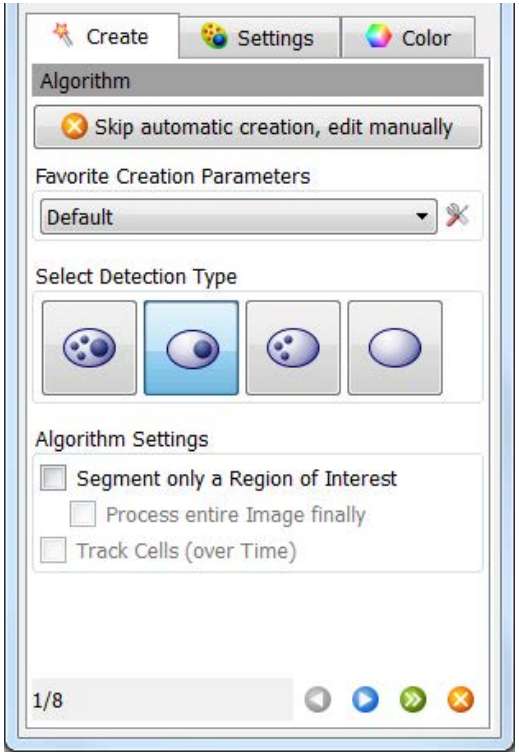
Select Detection Type



Situation in which we have labelled **Nucleus, Cell and Vesicles**
1- The biological unit or “Cell”
2- A subcellular structure like for example the “nucleus”
3- Vesicle like structures

Situation in which we have labelled **Nucleus and Cell**
1- The biological unit or “Cell”
2- A subcellular structure like for example the “nucleus”

Situation in which we have labelled **Cell and Vesicles**
1- The biological unit or “Cell”
2- Vesicle like structures

We only have the biological unit labelled **Cell**

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Segmentation of the Subcellular structures

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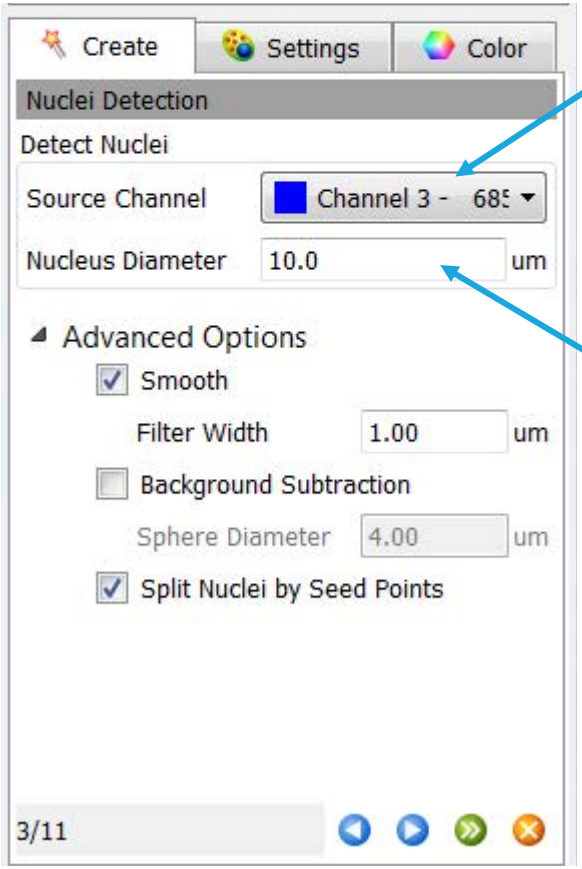

Select the channel that labels your sub-cellular structure.
TIP: the nomenclature "Detect Nuclei" is only an example as this structure does not necessarily has to be the Nuclei, but just a sub-structure inside your biological unit.

Enter the approximate diameter of the sub-cellular structure.

Advanced Options:

The "Smooth" will be set automatically. It represents the detail of the segmented surface that will be created, normally it is set to 10% of the diameter of the object to be segmented.

The "Split Nuclei by Seed Points" option will allow us to separate the objects in case they are touching each other.

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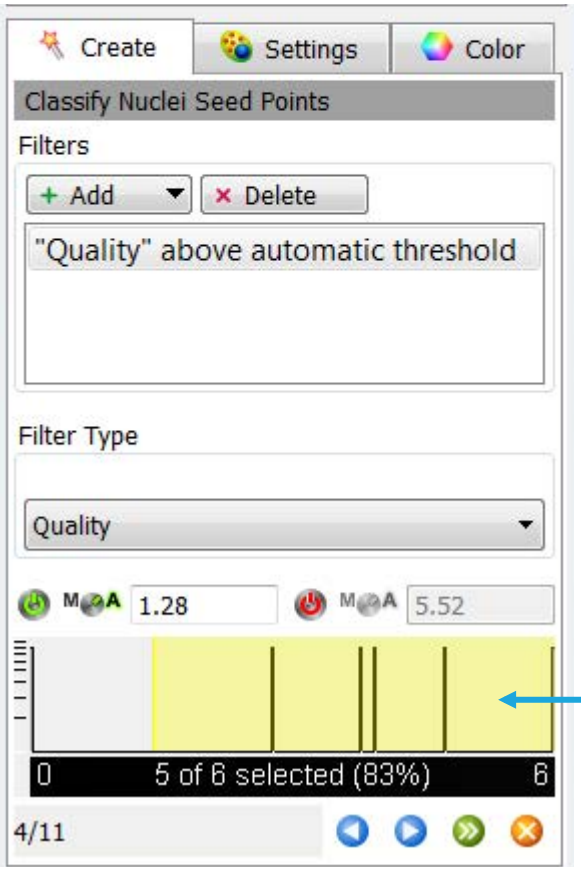
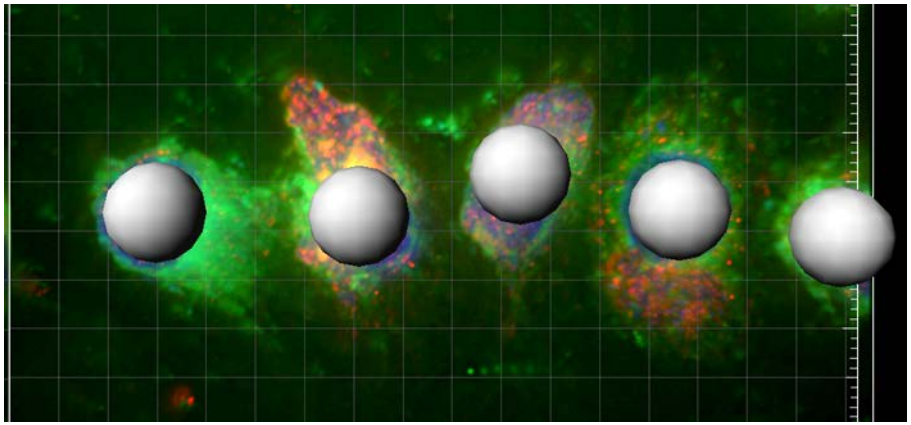
Segmentation of Subcellular structures





If the option “Split Nuclei by seed points” has been selected at the previous step, adjust the Threshold to make sure you have one sphere per object.

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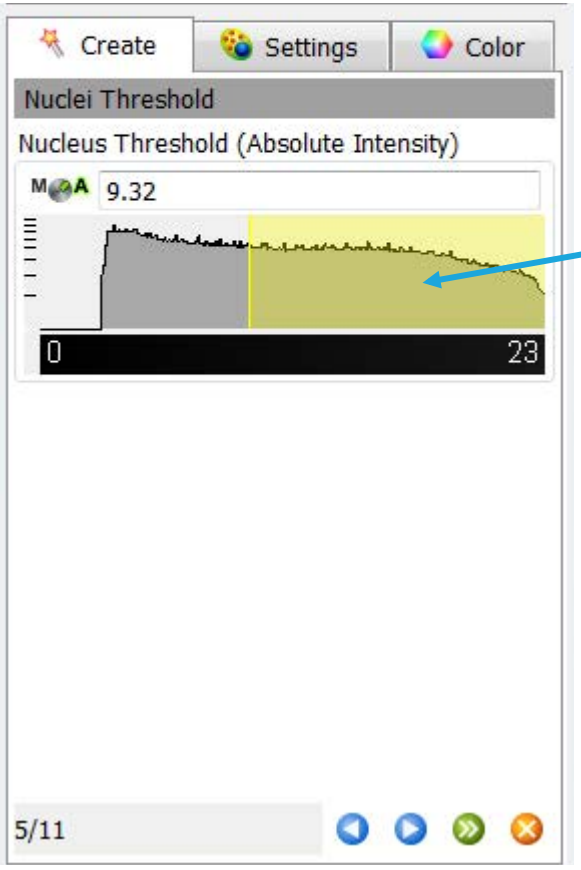
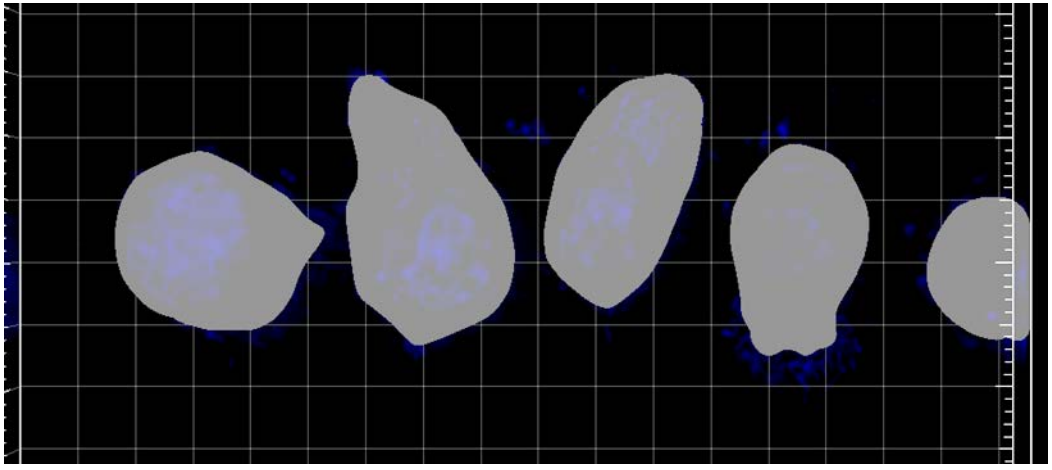
Segmentation of Subcellular structures



Adjust the Threshold for the grey shape to adjust to the object in the best way possible.

TIP: you can turn on and off the channels that you are not working with at this stage.



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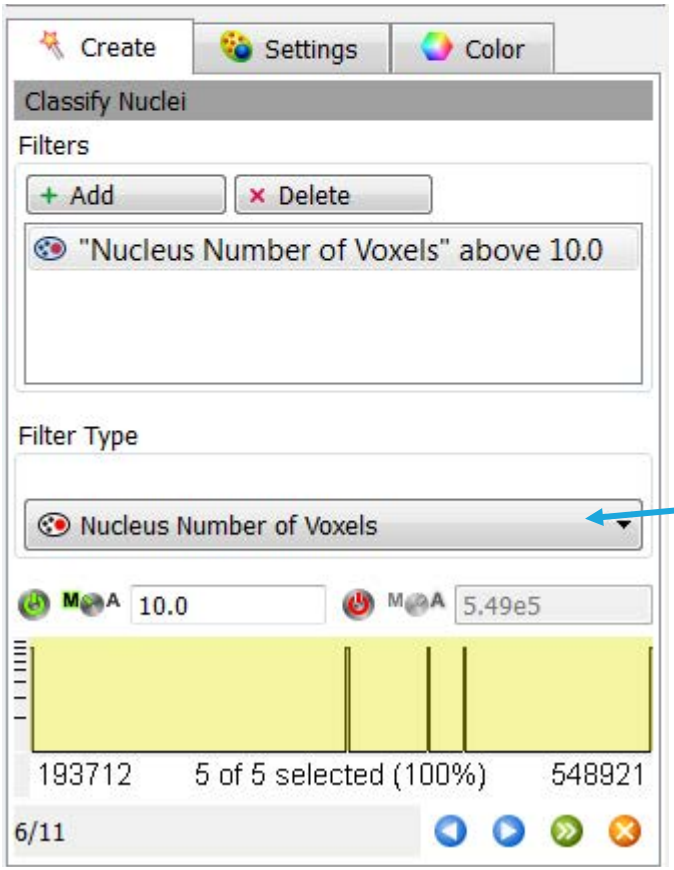
Segmentation of Subcellular structures



Filtering step in which you can add multiple filters in order to select the subcellular structures that are of interest.

You can Add as many Filters as you want.

Click in the drop down menu to display all possible filters and adjust the threshold.

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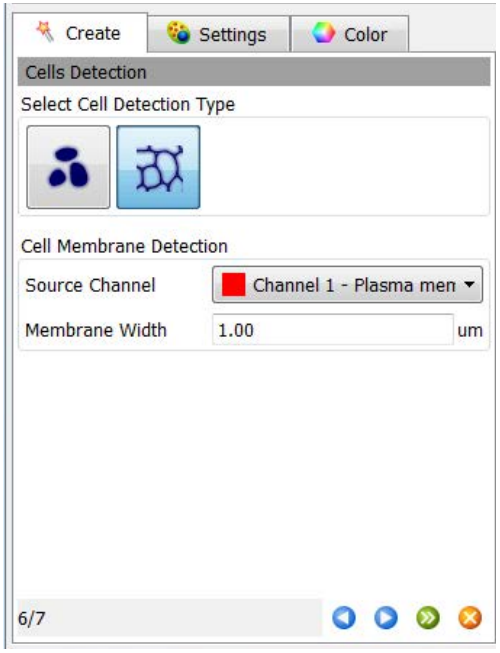
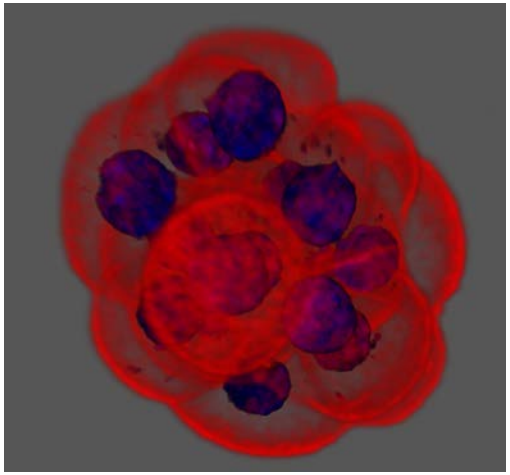
Cells Detection





Select Cell Detection Type:

 Cell body labelling

 Cell membrane labelling

Cell Membrane Detection:

Select the channel that labels the boundaries of the biological unit.

Enter the Width of the membrane.

Note: for this algorithm to work the membrane has to be continues in XYZ and a nucleus staining is required.

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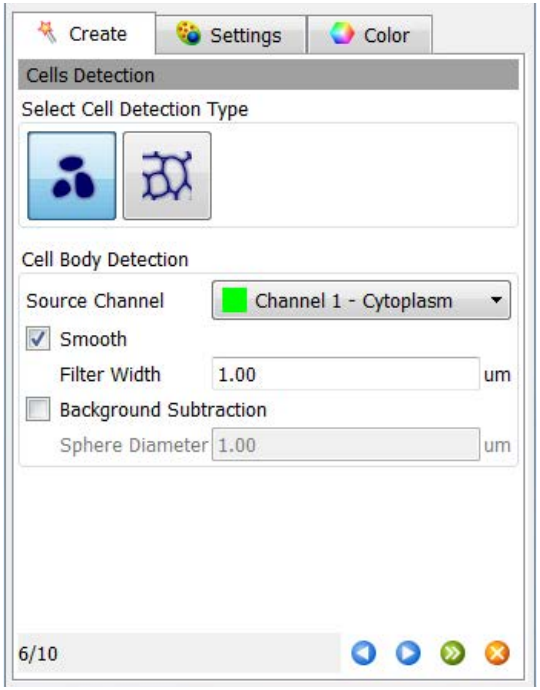
Cells Detection



Select Cell Detection Type:

Cell body labelling

Cell membrane labelling

Cell Body Detection:

Select the channel that labels the boundaries of the biological unit.

Define the Smooth factor: 10% of the diameter of the object that you want to segment.

Select Background Subtraction option if the background signal is very similar to the signal of your object (poor signal to noise ratio).

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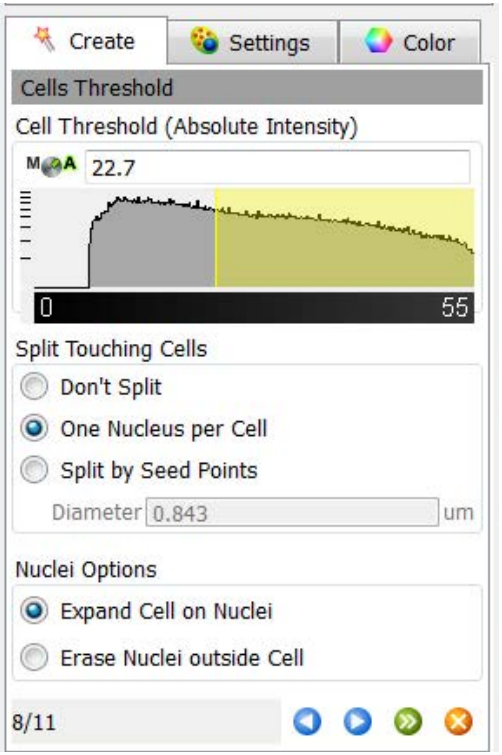
Segmentation of the Biological Unit, Cell



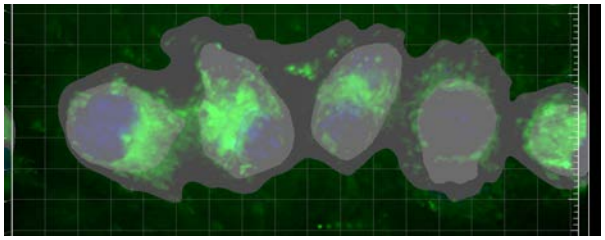
Adjust the Cell Threshold for the grey shape to adjust to the object in the best way possible.

TIP: you can click in the Settings tab to uncheck the visualization of the Nuclei objects to help you set the Cell threshold.

Split Touching Objects:

Select the option that suits best to separate the individual cells in case that they are touching each other.

One Nucleus per Cell is optimal if we have a nuclear staining and the nuclei have been segmented in the previous step.



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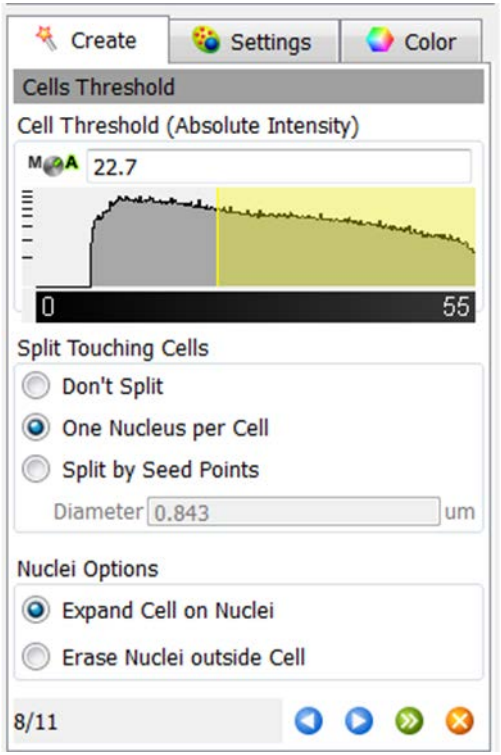
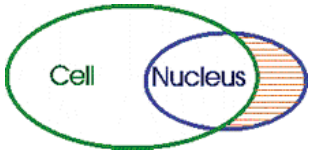
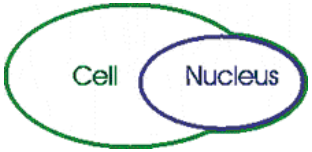
Segmentation of the Biological Unit, Cell



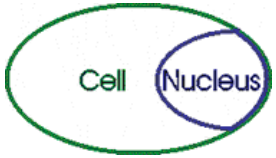
Nuclei Options:



- Simultaneous segmentation of cell and nucleus allows us to define the relationship between them



- *Expand Cell on Nucleus*: cell is expanded outside the original borders to include voxels which are defined by the nucleus segmentation step



- *Erase Nucleus outside Cell*: any voxels of nucleus segmented outside the cell object are removed.

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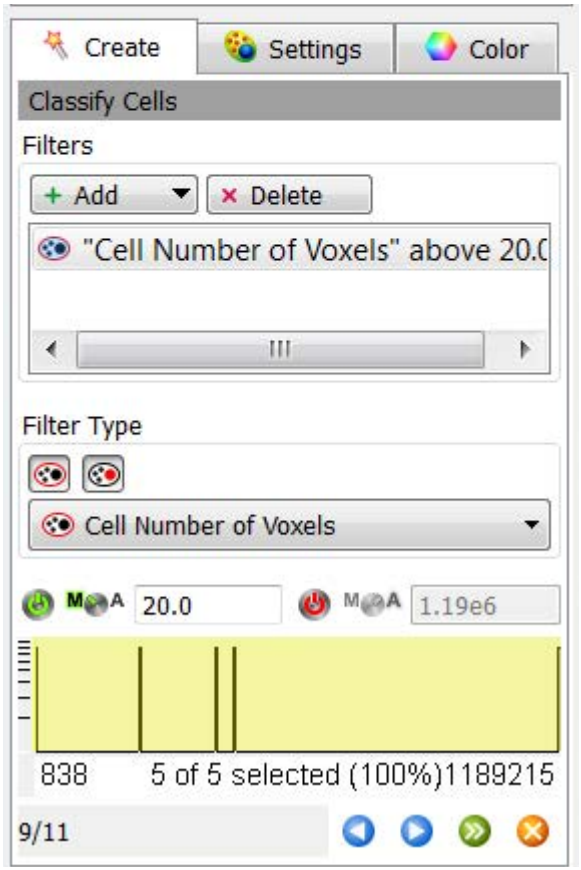
Segmentation of the Biological Unit, Cell



Filtering Step:
Equivalent to the one explained for the subcellular structures but in this case for the Biological units or cells.

TIP: you can click in the Settings tab to uncheck the visualization of the Nuclei objects to help you set the Cell threshold.

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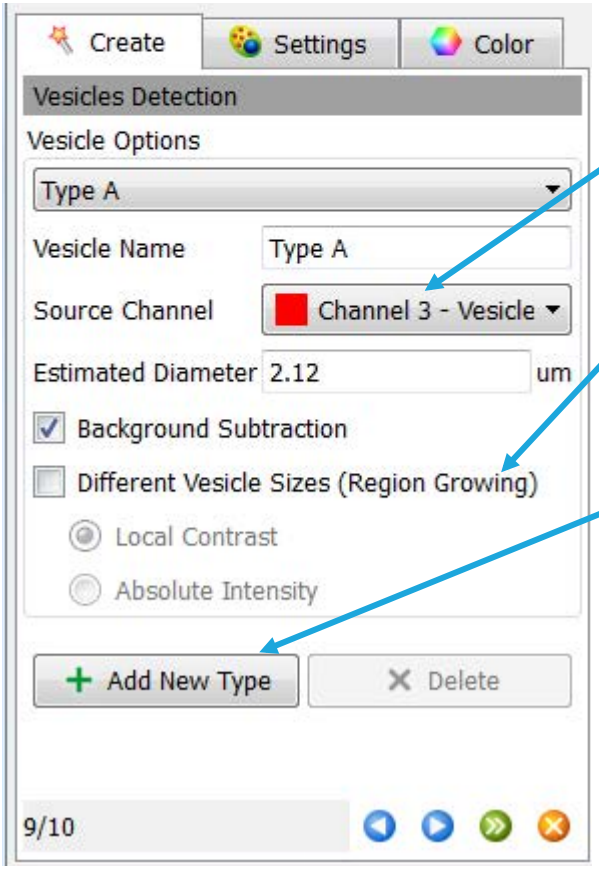
Detection of Vesicle-like structures



Select the Source Channel that labels the vesicles
Enter the “Estimated Diameter” for such vesicles

If the vesicles vary in size select the option “Different Vesicle Sizes”

Click in the Add New Type option if there are other channels that labels other vesicle type structures

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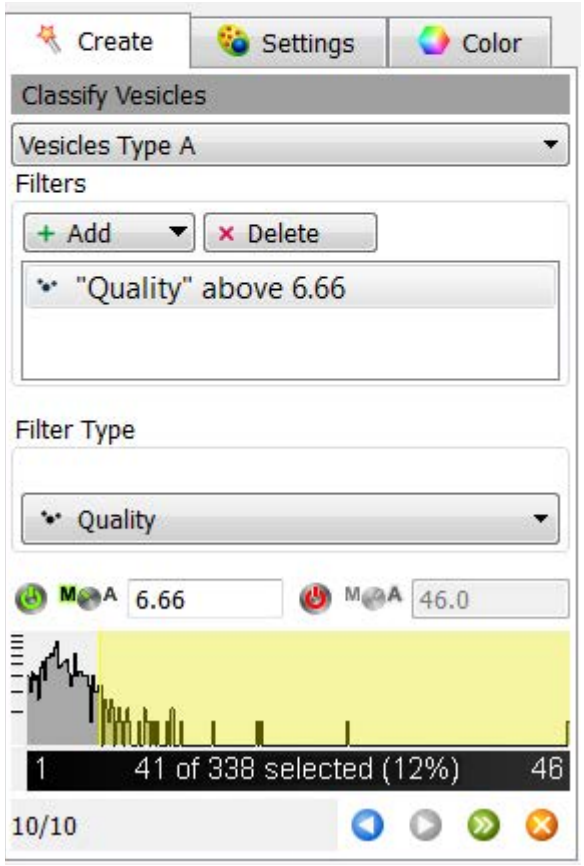
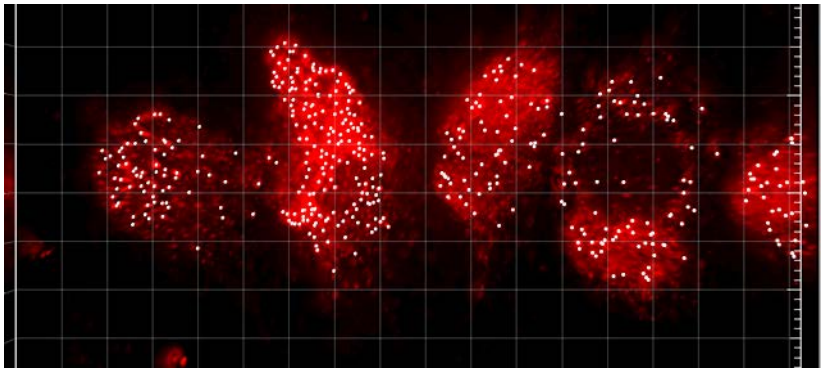
Detection of Vesicle like structures

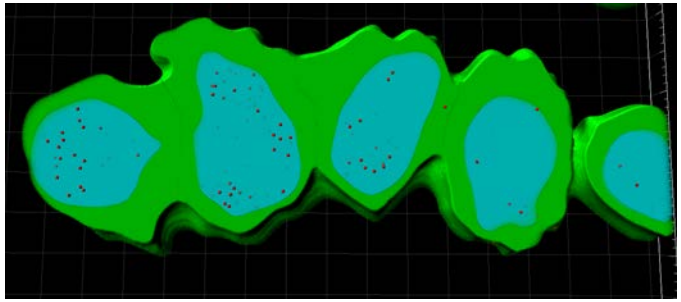




Adjust the Threshold to detect the vesicular like structures.

TIP: you can turn off the visualization of the Cell and Nuclei in the "Settings" tab to help the visualization of the vesicles.

Click the double green arrow to finish the creation wizard.



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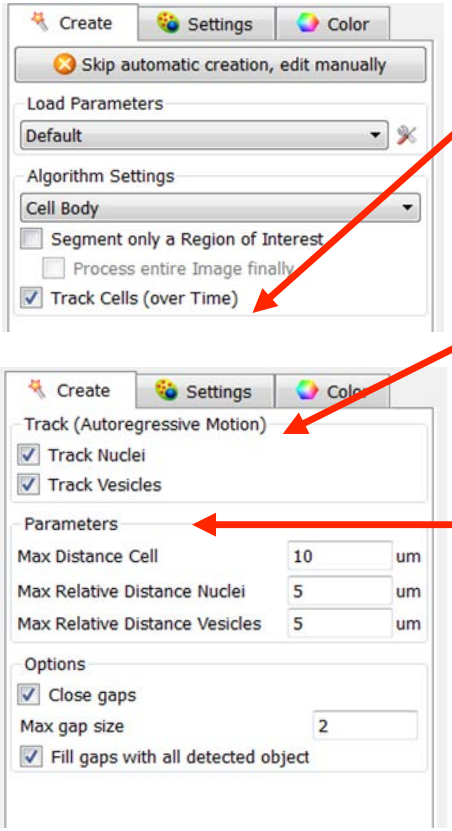
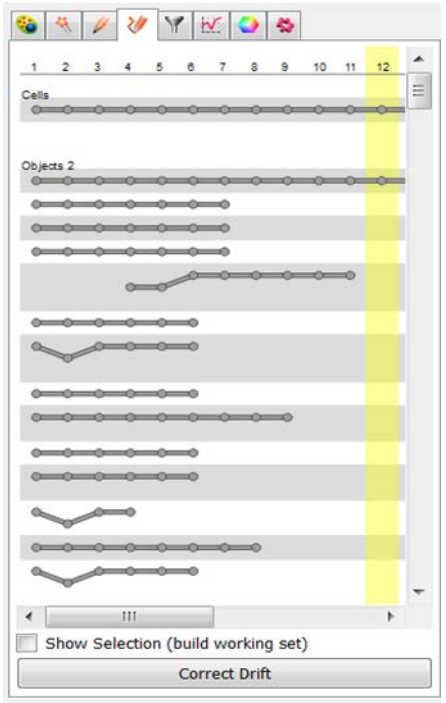
Tracking of Cells components



- If Tracking is enabled, the overall Cell position is always tracked; additional components are optional
- An Autoregressive motion algorithm is used to track the Cell and its components
- Organelle components are tracked relative to center of the Cell (the Cell movement is subtracted from movement of the organelles, as if the Cell was stationary)



The track editor is available for the Cell and all its components

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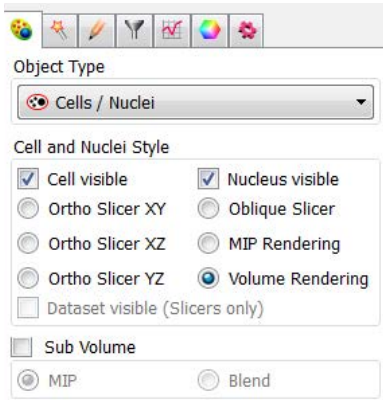
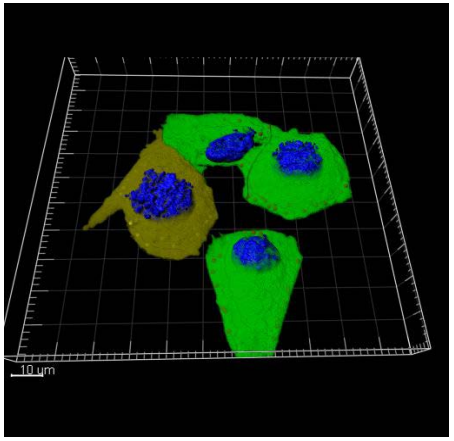
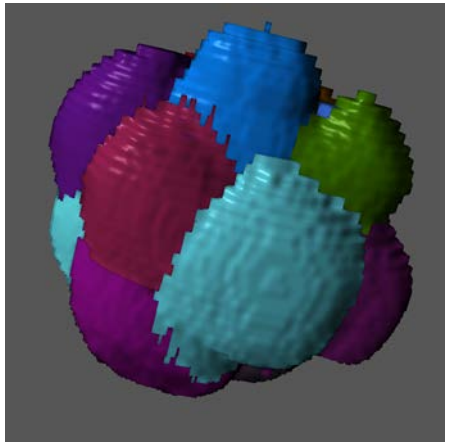
Visualization and Export Function



Imaris Cell comes with a build-in MIP, Volume, etc… rendering options.



The **Export function** allows you to export all the individual elements as individual surfaces (for cell and nuclei) or spots (vesicles).



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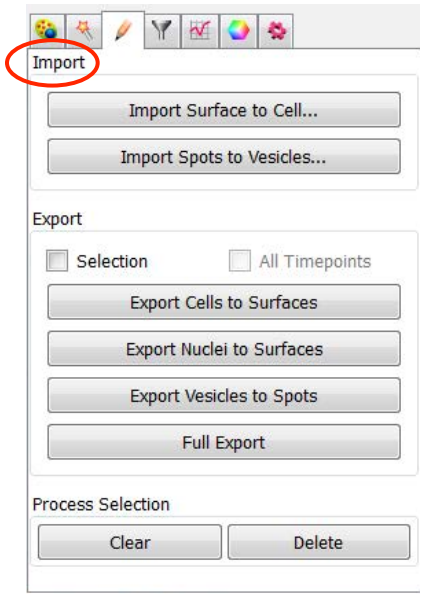
Manual Cell Creation: Import Function

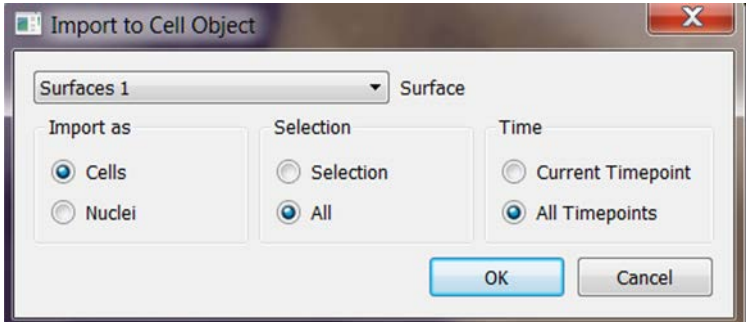


Instead of using ImarisCell creation wizard, surfaces can be created and imported as Cells or Nuclei.

Spots can also be created and imported as Vesicles.

These options are important for when the automatic creation wizard fails .

The import function also gives you the flexibility of segmenting Cell and Nuclei using the creation wizard and import spots.



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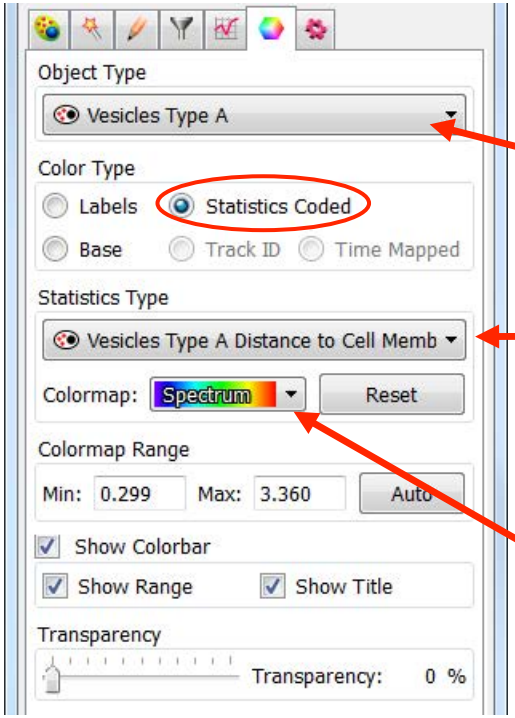
Imaris Cell Color coding

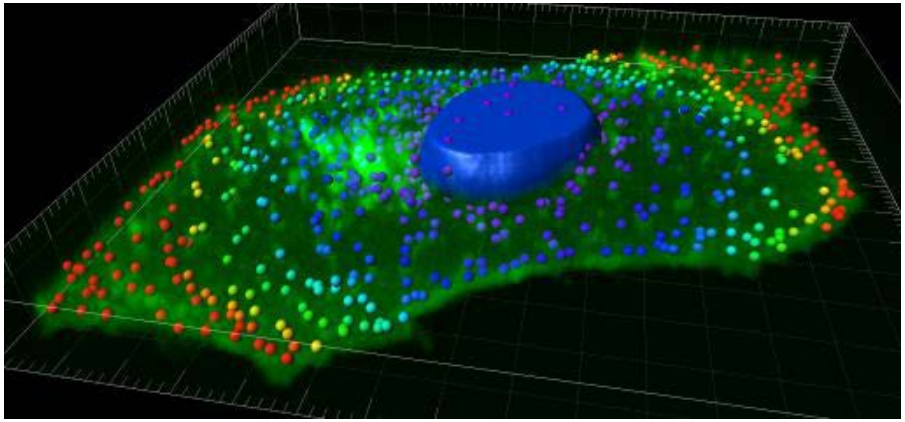


Drop down menu from where a statistical value can be chosen

Option available for Cell, Nuclei and Vesicle features.

Any of the statistical values calculated by ImarisCell can be used as a color driver.

Use the Color map drop down menu to chose your color scheme.



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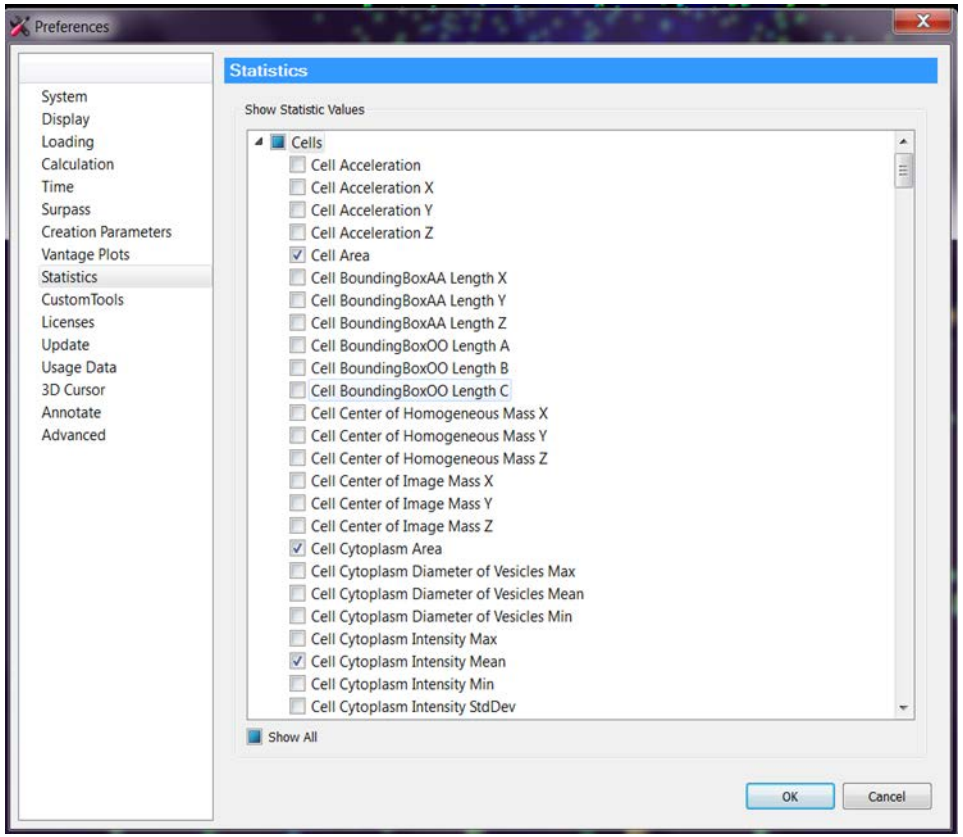
Imaris Cell Statistics



ImarisCell is able to report over 300 statistical values for full-featured cells.

Some of these are very expensive to compute: make sure to only select the ones you need.

ImarisCell is integrated with Batch – a huge time saver!!

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ImarisCell Exercise 1

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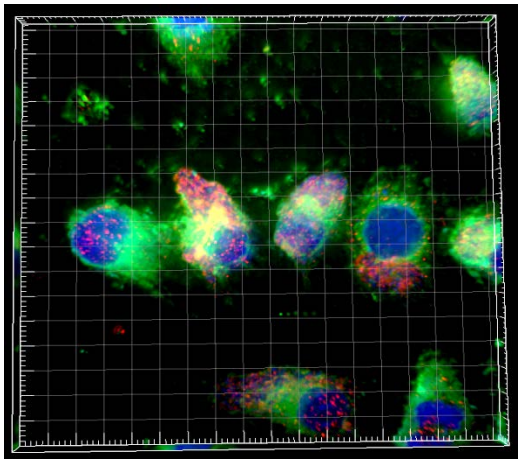
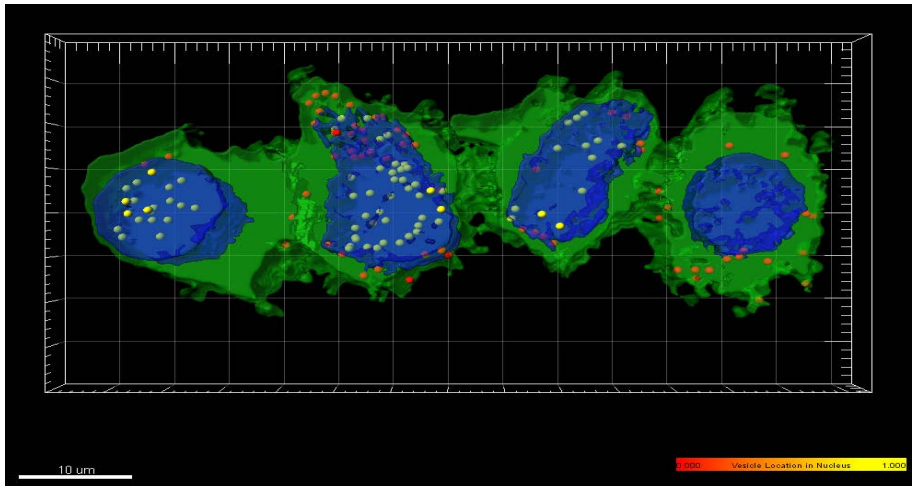


- Dataset: kg01_STE.May05.ims
- Only analyze the cells that aren't touching the edges of the dataset.

TIPS: use a ROI.

A large smooth factor can help cell and nuclei segmentation.

- Use the nuclei to split the cells
- Adjust the transparency of the Cells & Nuclei to show the Vesicles more clearly.
- Color-code the "vesicles" for whether they are located in the Nucleus or the Cytoplasm.



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ImarisCell Exercise 2

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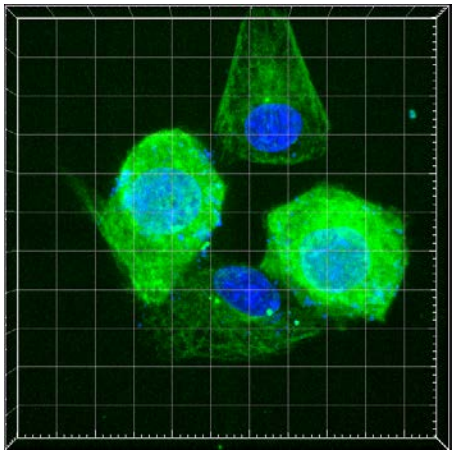
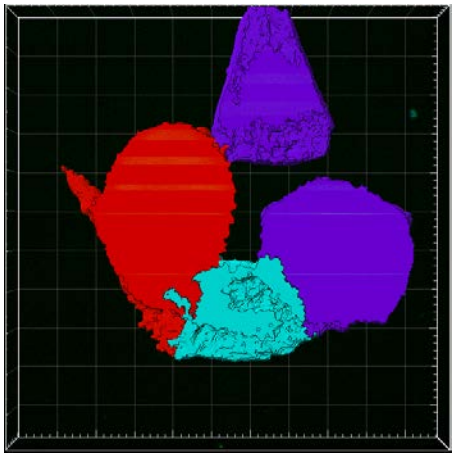
- Dataset: 4-cells.ims
- Challenge: segment all 4 cells, 2 bright, 2 dim

TIP: Filter nucleus number of voxels above 3e4!

- Use the nuclei to split the cells
- Color-code cells based on number of vesicles (green foci)





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Exercise 2 Hints:

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[Algorithm]
Cell Type = Cell Body
Enable Region of Interest = false
Track Cells (over time) = false
[Detection]
Detect Nuclei = true
Cell Source Channel = 1
Cell Smooth Enable = true
Cell Smooth Filter Width = 0.423 um
Cell Background Subtraction = false
Cell Background Subtraction Sphere Diameter = 1.59 um
Nucleus Source Channel = 2
Nucleus Smooth Enable = true
Nucleus Smooth Filter Width = 0.212 um
Nucleus Background Subtraction = false
Nucleus Background Subtraction Sphere Diameter = 1.06 um
[Threshold]
Cell Automatic Threshold = false
Cell Manual Threshold = 1434.890
Nucleus Automatic Threshold = false
Nucleus Manual Threshold = 6016.930

[Options]
Cell Split Option = Enforce One Nucleus Per Cell
Cell Option = Expand Cell on Nucleus
Fill Holes in Cell = true
Fill Holes in Nucleus = true
Split Nuclei by Seed Points = false
[Classify]
"Cell Number of Voxels" above 3.68e5
"Nucleus Number of Voxels" above 3.14e4
[Detect Vesicles]
Type A
Vesicle Channel Index = 1
Vesicle Estimated Diameter = 1
Vesicle Estimated Diameter = 1.00 um
Vesicle Background Subtraction = true
Enable Region Growing = false
[Classify Vesicles]
Type A Filters
"Quality" above 661

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ImarisCell Exercise 3-Bonus!

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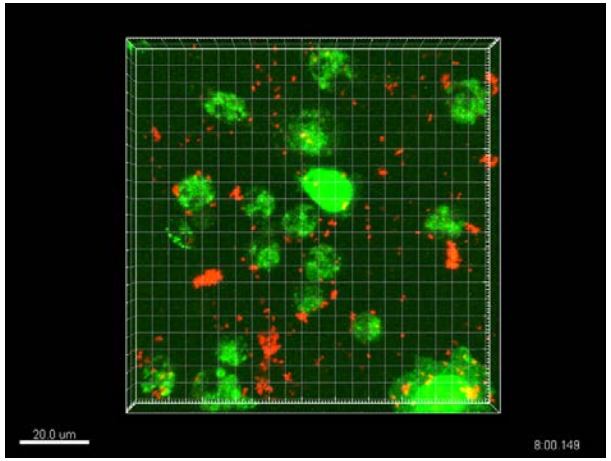
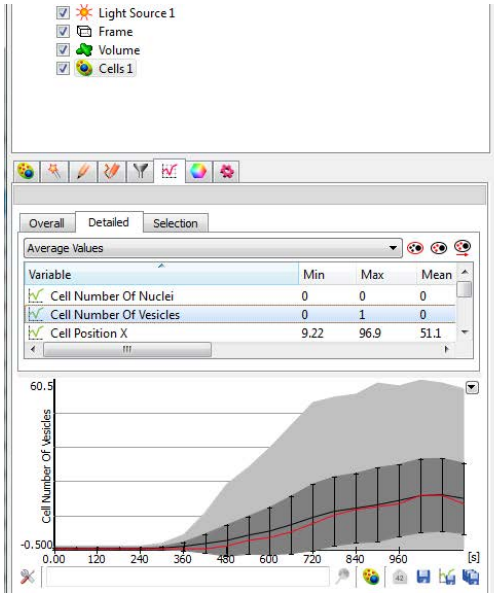
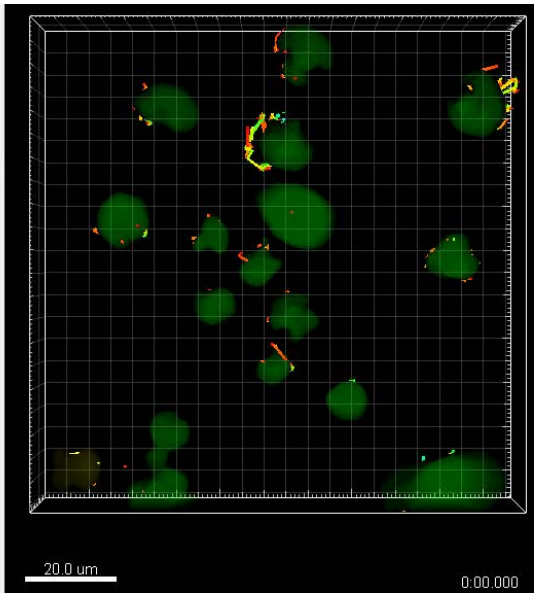


- Dataset: g119cellseatbugs.ims
- How many red bacteria do our cells ingest over time?

TIPS: background subtraction to segment cells + very little smoothing + very low threshold to find cells,

- Use Seed points to split cells
- Look in Stats->Detailed-> average values..





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Exercise 3 hints!

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[Algorithm]
 Cell Type = Cell Body
 Enable Region of Interest = false
 Track Cells (over time) = true
[Detection]
 Detect Nuclei = false
 Cell Source Channel = 1
 Cell Smooth Enable = true
 Cell Smooth Filter Width = 1.00 um
 Cell Background Subtraction = true
 Cell Background Subtraction Sphere Diameter = 10.0 um
[Threshold]
 Cell Automatic Threshold = false
 Cell Manual Threshold = 3.000
[Options]
 Cell Split Option = Split Cells by Seed Points
  Cell Seeds Estimated Diameter = 8.00 um
 Fill Holes in Cell = true
[Classify Cells Seed Points]
  "Quality" above 1.00
[Classify]
  "Cell Number of Voxels" above 3406

[Detect Vesicles]
 Type A
  Vesicle Channel Index = 2
  Vesicle Estimated Diameter = 1
  Vesicle Estimated Diameter = 1.00 um
  Vesicle Background Subtraction = true
  Enable Region Growing = false
[Classify Vesicles]
Type A Filters
  "Quality" above 6.14
[Tracking]
 Close Gaps = true
 Max Gap Size = 2
 Fill Gaps = true
 Track Vesicles = true
 Max Distance Cell = 5.00 um
 Max Relative Distance Nuclei = 5.00 um
 Max Relative Distance Vesicles = 5.00 um
[Classify Tracks]

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