

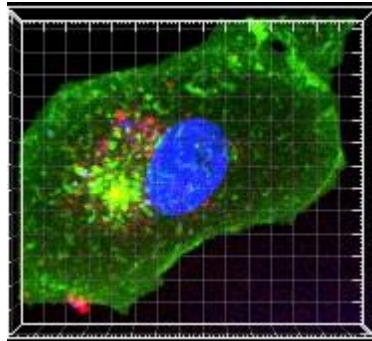
Imaris Cell – Cell feature classification and tracking

ImarisCell is designed specifically to identify, visualize, track, measure and analyze cells in 2D and 3D timeresolved images.

Bear in mind that the parameters of this tutorial are specific to this data set (Cell demo). Your data will require its own specific parameters.

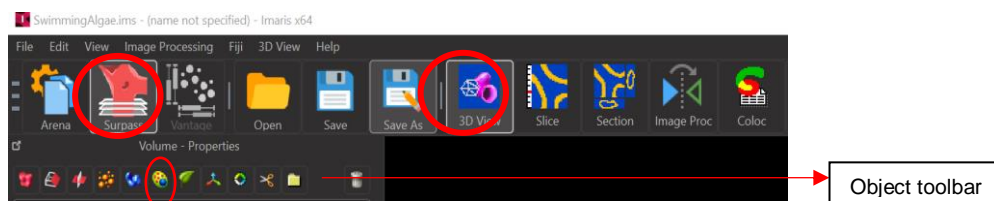
1 Visualization


Double-click on the image thumbnail to open it in the Surpass view.



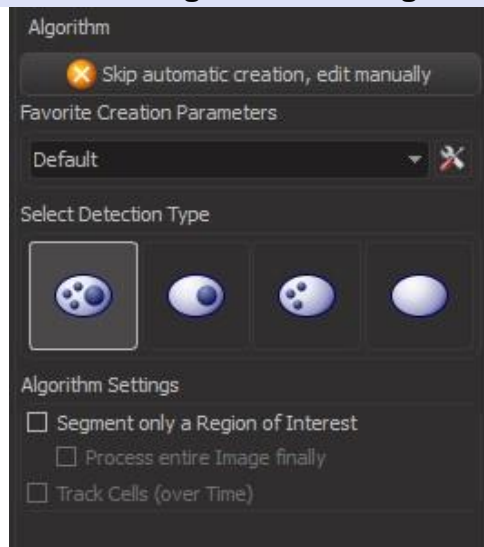
The cell image displays in the viewing area.

For cell feature classification/ tracking ensure you are the Under **3D-view** and can see the **object toolbar**.



In the Objects toolbar of the Surpass view click on the Cell icon  to start the Cell Creation Wizard. In the Object Properties area, the Cells Creation Wizard appears automatically.

Creation Wizard - 1/9 Algorithm Settings



Imaris Cell lets you choose from four different detection types.

You can select to detect Nucleus, Cell and Vesicles, detect Nucleus and Cell, detect Cell and Vesicles or just to detect Cell.

In this example we will select the detect Nucleus, Cell and Vesicles algorithm. We'll process the whole image, so leave the other boxes un-checked. Click on the Next button.

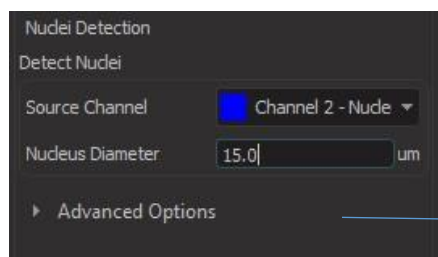
2 Nucleus Detection

Creation Wizard - 2/9 Nuclei Detection

In the next step you define the Nucleus object, its source channel and diameter. For Nuclei detection the **Channel 2** is preselected. If you are not sure of the size of your Nucleus object, click on the **Slicemode** icon




Now you can look at the slices of your image. By two consecutive clicks measure the nucleus diameter. Now reselect the **3D view** icon.



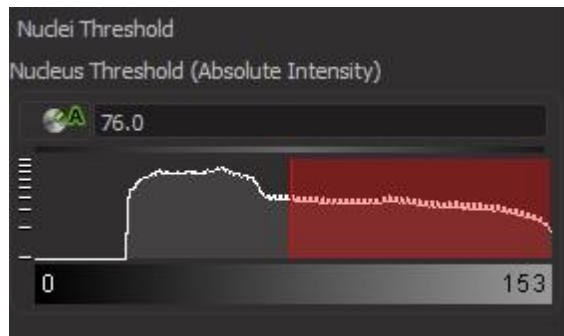
The advanced options lets you adjust the filter settings and seed points algorithm (if you require further thresholding).

Enter Nucleus diameter enter the measured value

Click on the Next .

Creation Wizard - 3/9 Nuclei Threshold

Now we will adjust the threshold value for the nucleus. To change the threshold, click on the histogram and drag the line. Alternatively, you can enter a value directly into the field. In this example, however, accept the automatically calculated value.



Click on the Next button .





Creation Wizard - 4/9 Classify Nuclei

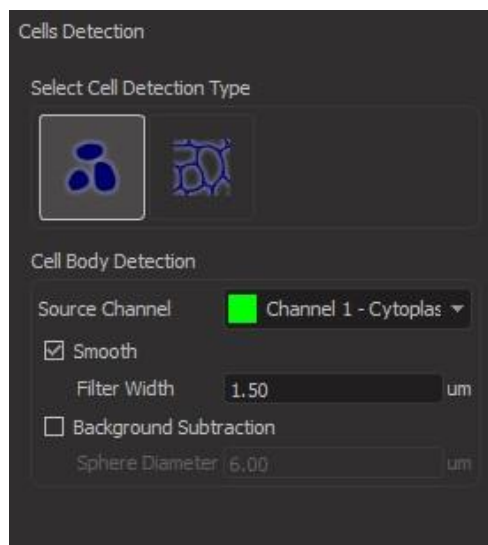
In the nuclei classification step, one Nucleus object is automatically segmented, so click on Next .



3 Cells Detection

Creation Wizard - 5/9 Cells Detection

Now we will identify Cell objects. Here you can choose from two different detection algorithms. Depending on the cell staining techniques and sample preparation used, you can select either to detect the Cell Boundary from Cytoplasm  or to  detect the Cell Boundary from Cell Membrane. Select the Cell Boundary from Cytoplasm  option. Then accept the automatically selected options for Source channel, Smooth and Filter Width. Click on Next .

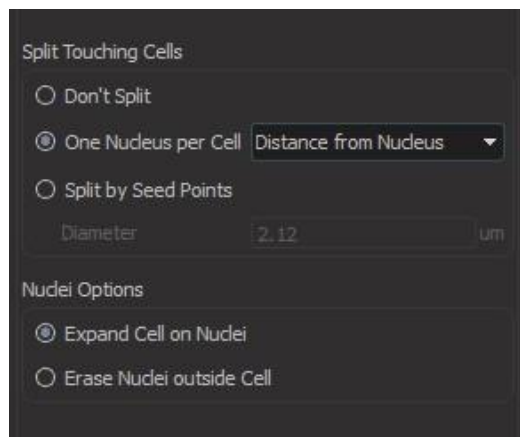


Creation Wizard - 6/9 Cells Threshold

Next you can adjust the Cell threshold value. In this example, accept the automatically calculated value.



In this step further adjustments are available. Here you can also select several options that correspond to particular biological specification. Select the **One Nucleus per Cell** option and leave the automatically selected **Expand Cell on Nucleus** option unchanged.

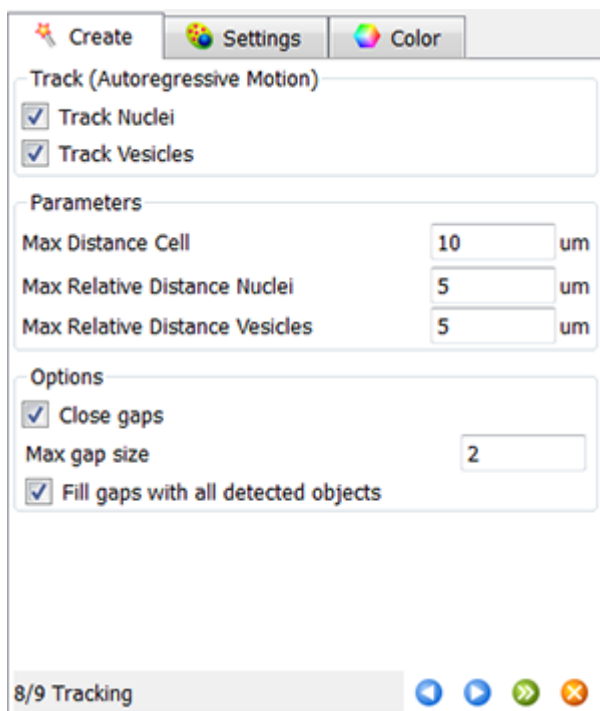


Click on Next .

4 Tracking and Classification

Creation Wizard - 8/9 Tracking

Tracking options within the creation wizard let you set all required parameters including distances and gap sizes.



The tracking of the Nucleus and Vesicle objects in Imaparis is based on **the Autoregressive Motion** algorithm. The concept of relative tracking is used to track Nuclei and Vesicles objects within the Cell

In the Parameters sub-window the user can specify the value of the **Max Distance** parameter.

(Note: This parameter defines the maximum distance that a tracked Cell object could possibly move between two consecutive time points. This option prohibits connections between a tracked object and a candidate if the distance between the predicted future position of the object and the candidate

position exceeds the maximum allowed distance. To establish a maximum distance value for the selected Cell object type, choose two time points between the greatest movement of the objects is visible.)

In the Options sub-window you can further adjust the tracking settings by selecting the gap closing algorithm to define the maximum gap size or by selecting the option to fill gaps with detected objects.



When the Close gap option is selected, the gap closing algorithm tried to connect the objects associated with the same track. It joins track segments beyond the missing section based on the value entered in the Max Gap Size field. The Max Gap Size defines the maximum number of the consecutive time points that are allowed to be missing in order for a track to be continuous.

If the Fill gaps with all detected objects option is selected, the algorithm performs the optimization of the objects expected position for the particular time point. Then the track is generated by connecting objects assuming their optimized positions.

Once the tracking is complete, the Classify tracks step allows you to exclude tracks that would interfere with the interpretation of results. Here you can filter Cells, Nuclei and Vesicle tracks based on a variety of relevant statistical variables.

Creation Wizard – 8/8 Classify Cells

At this point of the algorithm you can classify your Cell object based on the parameter of interest, and save your results.

