# Object Tracking with IMARIS

Summary: The first step is the Visualization of the data set and the automatic Spot Detection (Segmentation) over time. With the automatic Tracking you link consecutive time points and the result is a colored Track - the motion path of a single object over time. You can show the Track Displacement. Using the Filter function, you can group and analyze the Tracks depending on various criteria.
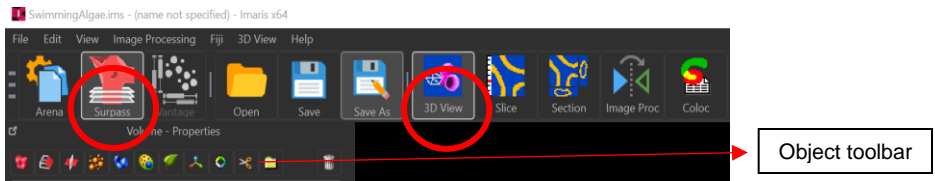
The demo image shows the movement of the algae C*hlamydomonas reinhardtii zoospores*.
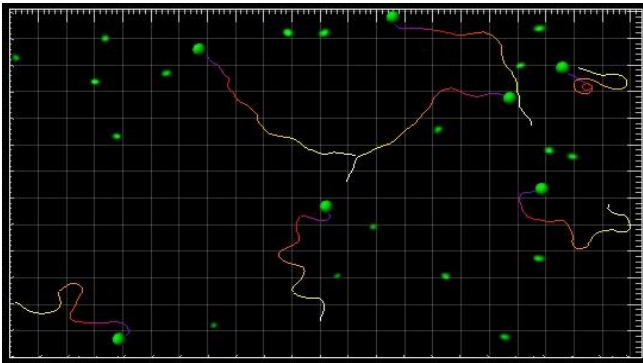
Spots & Tracks, Swimming Algae

1   Visualization

Double-click on the **image** thumbnail to open it in the Surpass view. For object tracking ensure you are the Under **3D-view** and can see the **object toolbar.**

2 Time Concept

Time Window and Time Slider

On the left hand side you find the button for the **Time Settings...**, the **Play** button, and the **Movie** button. Each bar in the time slider represents one time point.

	Go to previous/next time point
Click slider (select) + arrow to the right on keyboard	Go to next time point
Click slider (select) + arrow to the left on keyboard	Go to previous time point
	Play/Pause through all time points
	Record movie of all time points

3 Segmentation

First you will identify and create spots.

Then you will establish a time dependant relationship between spots (track them over time) and create Tracks.

Finally, you will sort the Tracks based on the Track duration and visualize tracking results.

In the **Objects** toolbar of the Surpass view click on the icon to add new Spots.
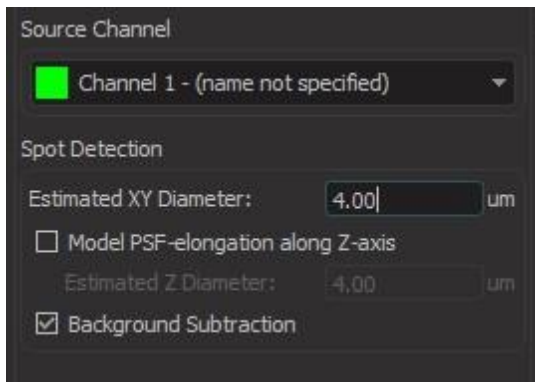
Creation Wizard - 1/7 Algorithm

In the Algorithm Setting check the **Track Spots (over Time). Click classify   spots**
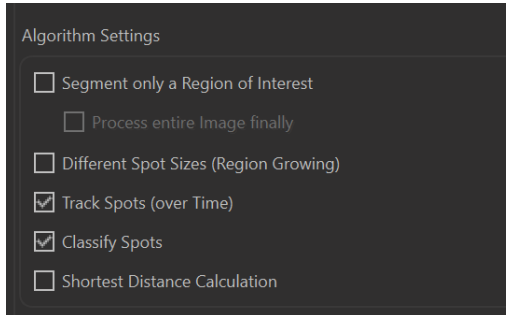
Click on

Creation Wizard - 2/7 Source Channel

Select as **Source Channel** the Channel you would like to track objects in and set the **Estimated Diameter.** (note: To estimate the diameter, go to **slice view**, click on the edges of one typical object, that gives the measure of the estimated diameter)

Leave the **Detect Ellipsoids** option unselected and select the **Background Subtraction** options. Click on 🔵 (Next).
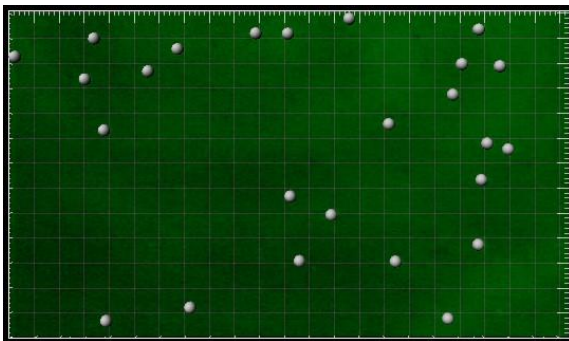
Creation Wizard - 3/7 Filter Spots

Select the appropriate **filter type** and **threshold** that can best segment your objects of interest.

In the example, **Filter Type** has been selected as **Quality**, selected the **Manual option** and set Lower threshold to **10.000.**

You will see the gray Spots and the Volume rendered channel at the same time.


Click on ⏵ (Next).

Creation Wizard - 4/7 Edit Spots

At this point of the algorithm you can manually **add or delete spots**- you will not do so in this example, so click on Next ⏵ .

5   Tracking

Creation Wizard - 5/7 Tracking

A Track is a component within the Surpass view that describes the behavior of an object over time. (To automatically create all Tracks you did check the box **Track Spots (over Time)** on the first window of the Creation Wizard.)
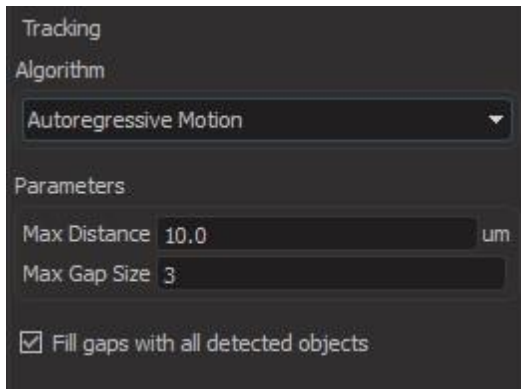
Each object, based on its property will have a defined motion. Set the appropriate one that matches your object: Brownian/Autoregressive/ Connected components/ Lineage/ Maximum overlapping.

Details on the algorithm on which type you should choose, is as below:

Brownian Motion

This algorithm models the motion of each object (Nuclei or Vesicles) as a Brownian motion. This model is appropriate if the object performs similar to a Brownian motion (the random movement of objects with very frequent changes in direction and no discernible pattern).

The only prediction applied is that the object does not move further than the specified speed allows, so the maximum speed constrains the distance from one time-point to the next.

Autoregressive Motion

This algorithm models the motion of each cell object (Nuclei or Vesicles) as an autoregressive AR1 process. This model is appropriate if the object performs any kind of continuous motion. The AR1 model looks back to one time-point, and predicts that the object will move again the same distance and in the same direction. The user-specified **Maximum Distance** value is the distance in which the object is allowed to deviate from the predicted position. Some changes in direction are tolerated because of the maximum distance parameter, and because AR1 does not remember farther back than one time-point.

Autoregressive Motion Expert

This algorithm models the motion of each cell object (Nuclei or Vesicles) as an autoregressive AR1 process (see above). It is suitable for tracing of multiple objects with adjacent or crossing paths, especially if the neighboring objects have a stable (but different) intensity. In Autoregressive Motion Expert the algorithm called Total Cost determines the most appropriate connections for tracks by seeking the lowest Total Cost. In this case, the Total Cost algorithm combines Intensity Cost and Distance Cost functions (see Intensity Weight below).

Connected Components

For this algorithm, objects in adjacent time-points are considered connected if the object spheres occupy some of the same space (the spheres would overlap if two time-points were fused into one). It compares the amount of overlaps between identified objects in the previous frame with those in the current frame. All the object with the overlap will be assigned the same track ID.

Connected Components and Lineage are the only algorithms that automatically handle lineage (objects that fuse or fragment). It will only work with images that have excellent time resolution, because the object cannot ever jump a distance larger than its own size.

Lineage

The Lineage model is intended for events such as cell division in which the splitting of objects is to be analyzed. This algorithm models the motion of each object (Nuclei or Vesicles) as an autoregressive AR1 process (refer to Autoregressive Motion above). The object data is then compared so that only the objects that undergo splitting are connected, and assigned the same track ID. Objects that pass in close proximity, or overlap, are not associated. This is in contrast to connected components, where any objects that overlap are determined and considered connected.

Maximum Overlapping

For a cell at time point t the overlap with all cells at the previous time point t-1 is computed and the cell is linked to that cell from the previous time point with which it has the largest overlap. The link is only created if the ratio of the volume of the smaller of the two linked cells to the larger is greater than the "maximum overlapping ratio".

Also define the maximum range of distance that your objects travels

In this example, As **Algorithm** selected **Autoregressive Motion** and as **MaxDistance** set **10.000** um, and for the **MaxGapSize** set **3** and selected the **Fill gaps with all detected objects** option.
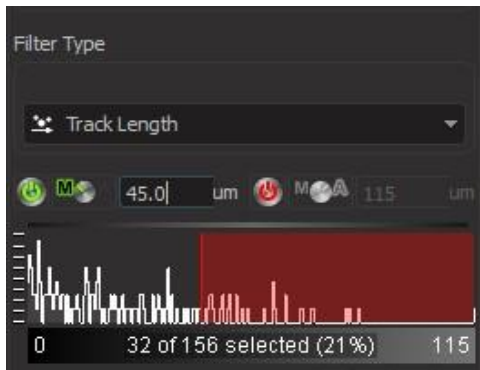
Click on (Next). This is the result in the viewing area.
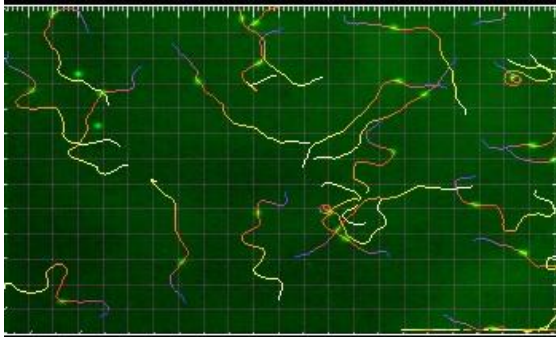
# 6   Filter Tracks

Creation Wizard - 6/7 Classify Tracks

Sorting the Tracks for the further analysis. You may sort the Tracks based on their length/ area/ duration/ Intensity/ speed/ position/ volume/branching

In this example, Chosen **Filter Type: Track Length**. Selected the **Manual** option and You may enter a manual value (here e.g. **45.000** um.) Alternatively click on the yellow line and drag it. Moving the line changes, the filter setting, thus removing or adding some
of the tracks. The effect is directly visible in the viewing area.

Click on (Finish).
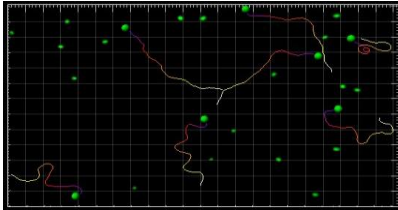
This is the Spots and Tracks display in the viewing area.

7   Classification

Creation Wizard - 6/7 Classify Tracks
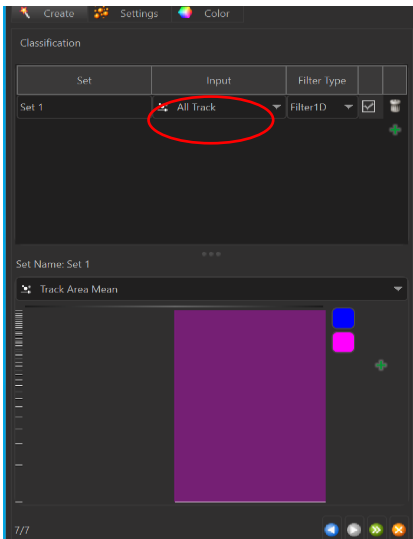
In the Input click "All track" so that a unique ID is assigned to each track.

Create sets based on which the track feature you would want to classify the spots/tracks.

Click Finish

Displacement

This visualization mode shows the displacement of a Track as an arrow.
Select the tab **Settings** .
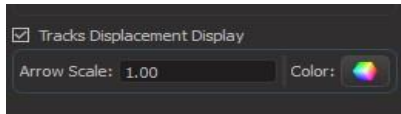
In the **Tracks Style** adjustment window check the respective check box **Tracks Displacement Display**.

The displacement arrows display in the viewing area.

Filtering and saving results

In the next screen, you may edit the spots/ tracks for display and save the views,

The **statistical table** tab shows you either the

Overall / detailed / selected track information and parameters.

You may select the appropriate tracks and measurements and save them