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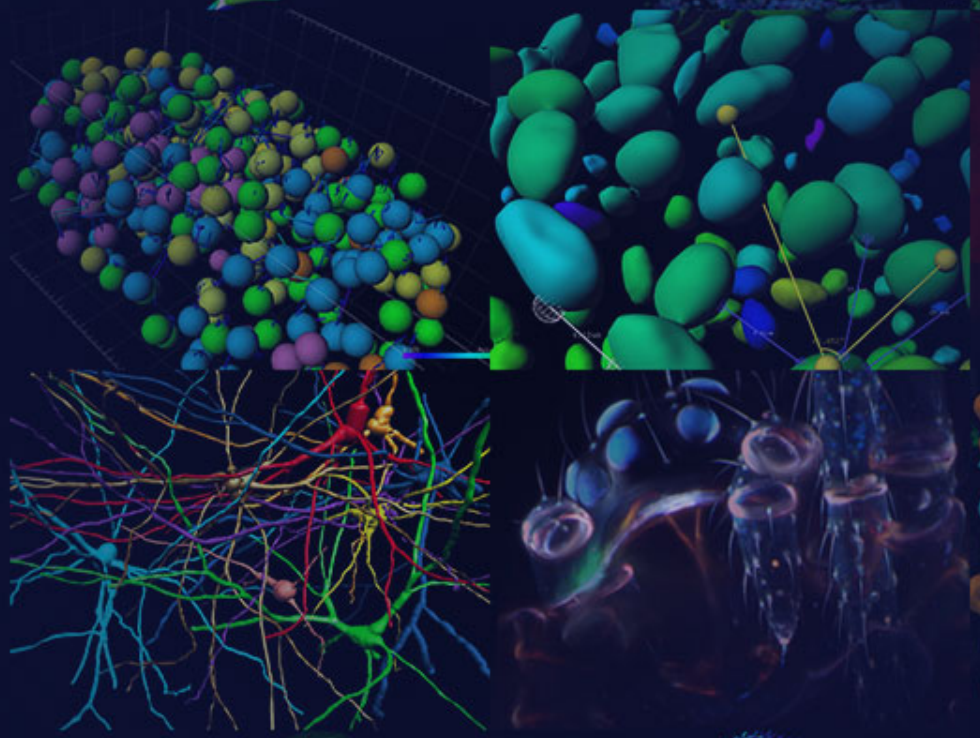

Imaris Track and Lineage

Tracking Analysis Overview

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Step 1- Object Identification

-> A. Spot detection
-> B. Surface detection
-> C. Cell / Nucleus / Vesicle detection
-> D. Filament detection

Step 2 – Tracking

-> Chose Algorithm
-> Set Tracking Parameters

Step 3 – Filter Tracks

-> Only analyse relevant data

Step 4 – Visualize the results

-> Display data in most informative manner

Step 5 – Edit Tracks/Objects

-> Correct mistakes

Step 6 – Quantify, Export and Interpret Results

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Object Detection

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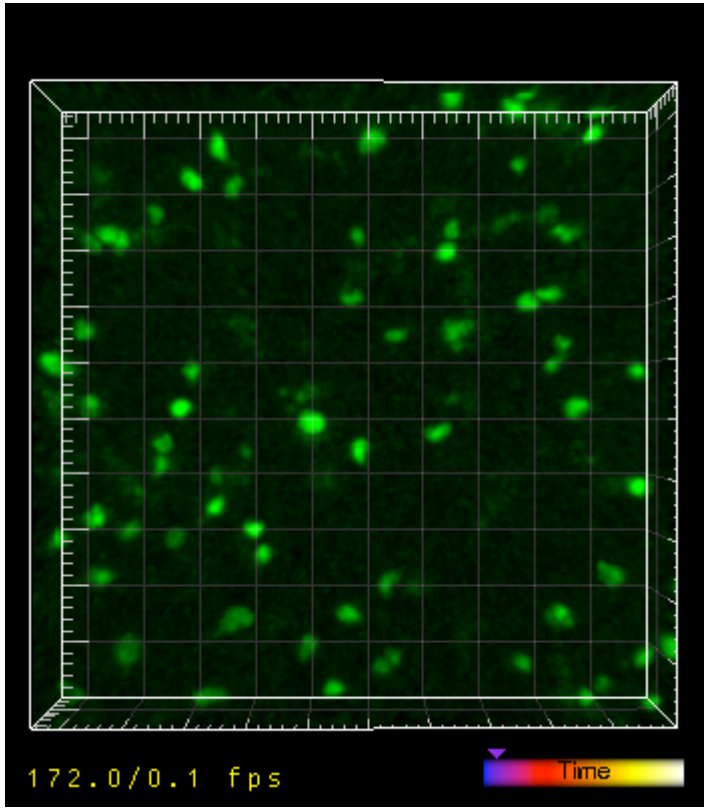

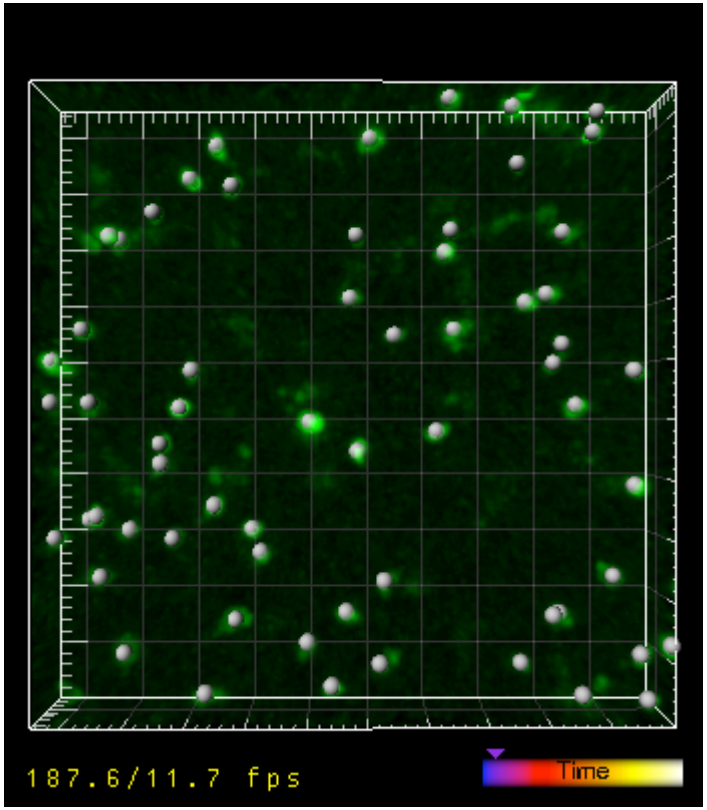

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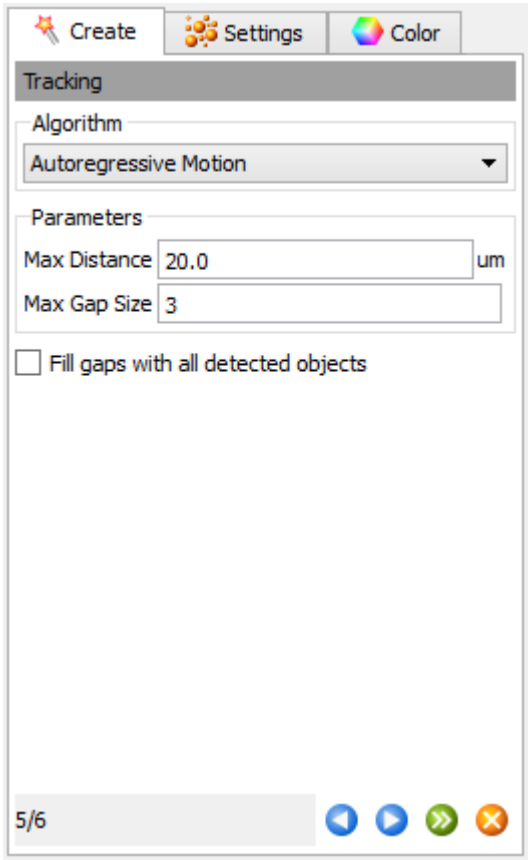
Tracking



1) Select from 5 Algorithms

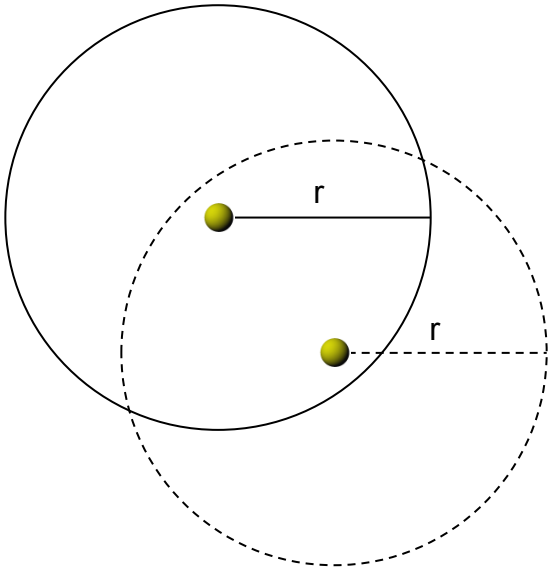
- **Brownian Motion**
- **Autoregressive Motion**
- **Autoregressive Motion Expert**
- **Connected Components**
- **Lineage**

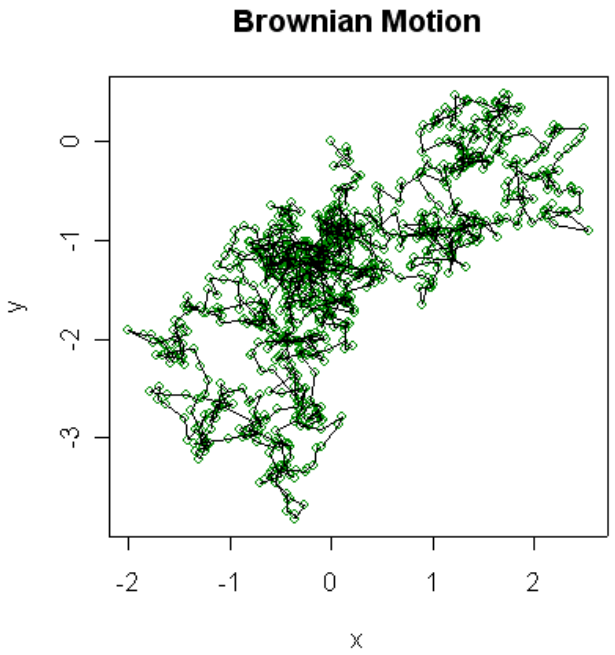
2) Specify Tracking Parameters

- “maximum distance”
- “gap size”

3) Create Track

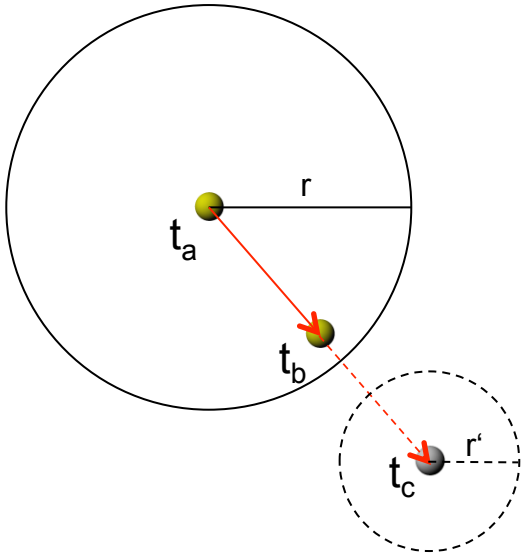
Option I: Brownian Motion

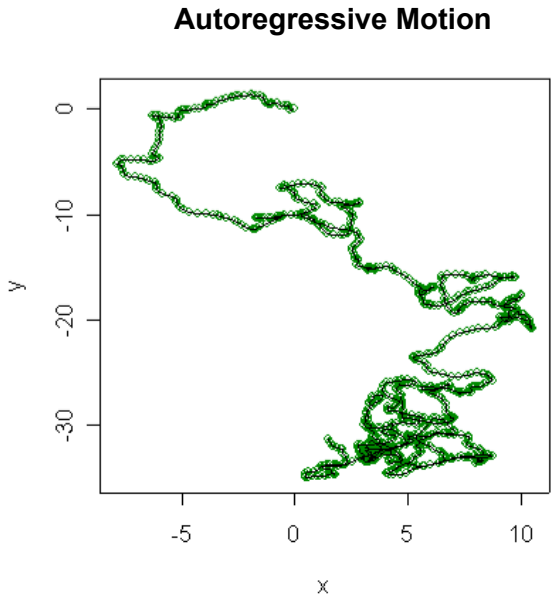




- Used for objects which change their direction very frequently with no discernable pattern
- Only prediction: Maximum distance from one timepoint to the next
- Imaris searches within this radius for the object in the next timepoint

Option II: Autoregressive Motion





- Used for objects with more or less continuous motion
- Prediction: from timepoint B to C, the object will move in the same direction and distance as from A to B. The algorithm is always looking one timepoint back.
- Maximum Distance is here the distance that a spot is allowed to deviate from the predicted position
- This leads to the toleration of some directional changes

Option III: Autoregressive Motion Expert

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- An additional parameter comes into play: Intensity weight
- Now Imaris is not only looking for the expected loci of the object but also takes care of the intensity difference
- You can determine to which extend Imaris will take intensity differences into account

This is very suitable when objects in your sample come very close together but have different intensities.

TIP: The Expert mode is slower than the normal mode because also intensity has to be calculated. Try first the normal mode and go to expert when the normal mode fails

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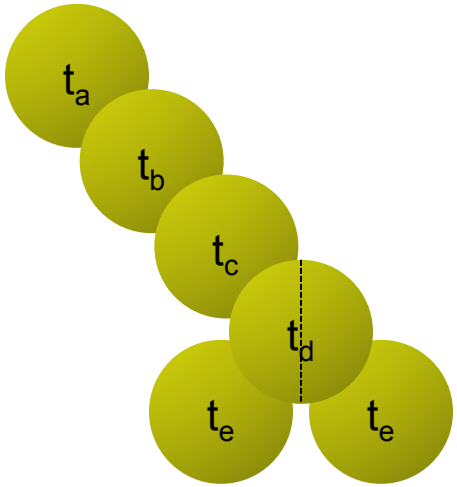
Option IV: Connected Components



- Spots are considered to be connected if their position overlaps in adjacent timepoints.
- This algorithm only works if the time resolution is very high

This tracking algorithm could be used for analysis of cell lineage: In a dividing cell both daughter cells will be located in a position overlapping with their mother cell.

Option V: Lineage

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- The Lineage model is intended for events such as **cell division** in which the splitting or merging of objects is to be analyzed.
- This algorithm models the motion of each object as an **autoregressive AR1 process**.
- The object data is then compared so that only the objects that **undergo splitting or merging 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.

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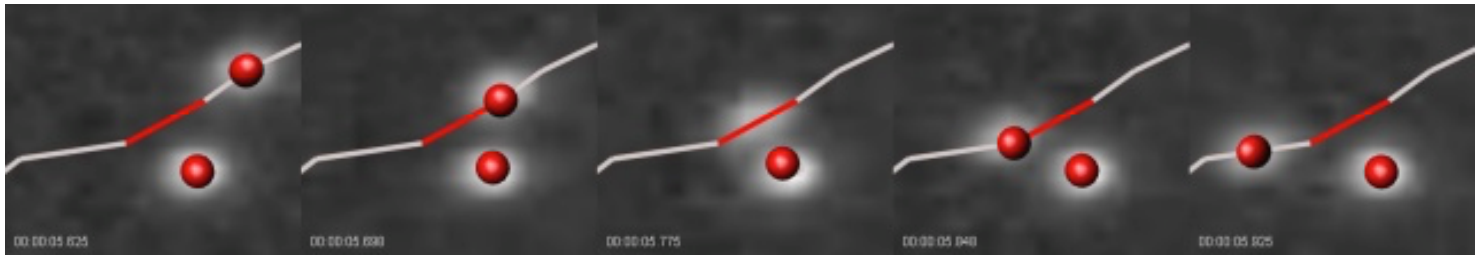
Paremeters – Gap Close



Gap Problem:

- Object detection can fail at some points.
  - Two close objects detected as one
  - Object briefly leaves focal plane / volume
  - Object intensity briefly drops

Resulting Issues with Tracks:

- Single object is separated into two or more tracks which leads to:
  - Invalid Statistics
  - Manual track correction (extra work)

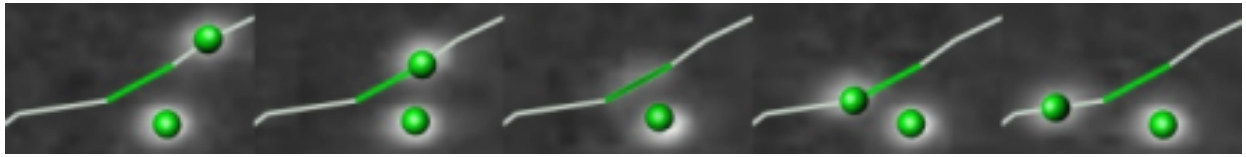
To Correct This Issue:

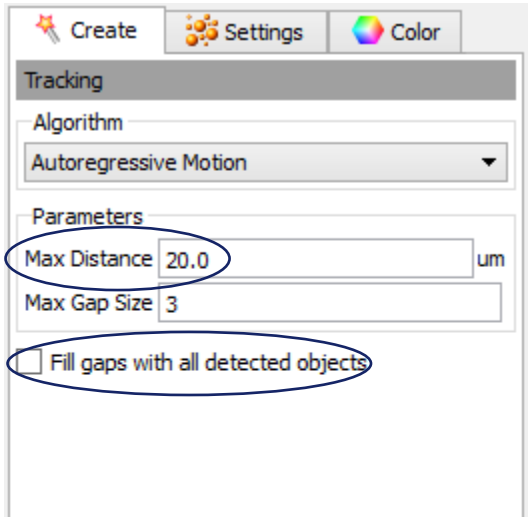
- *Gap Close to the rescue!*

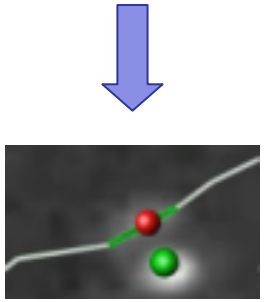
The gap closing algorithm allows the “disappearing” objects to reconnect with their original track, should it reappear at a future time point

- Single-point statistics (Volume, Diameter, Intensity etc.) will not be computed at the gap point (possibly resolved by “fill gaps”, next slide)

Fill gaps with all detected objects







- The **Fill Gaps function** drops the detection threshold *close to the expected position predicted by the tracking algorithm*
- Local segmentation becomes accurate without affecting global segmentation settings
- Will not work if there is “nothing to detect” in the image

MAXIMUM DISTANCE: disallows the connection between an object at t = x and a candidate match at t = x+1 if the distance between the predicted future position of the object and the candidate position exceeds the Max Distance

Parameters – Max Distance and Max Gap Size

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Max Distance

The maximum search distance that a tracked object are looked for is specified with the Max Distance value. This parameter disallows connections between an Object and a candidate match if the distance between the predicted future position of the Object and the candidate position exceeds the maximum distance.

The parameter is only available if you select Brownian Motion or Autoregressive Motion or Autoregressive Motion Expert or Lineage.

Max Gap Size

An object might fail to be segmented from some time points, which could lead to a Track breaking apart and the creation of Tracks fractions.

Instead of creating track fragments, with gaps between undetected objects, the gap-closing algorithm creates tracks by linearly connecting objects associated with the same track. The time period in which the objects connection is automatically established is based on the value defined in the Max Gap Size value.

The **Max Gap Size** defines the maximum number of the consecutive time points, which are allowed to be missing in order to a track to be continues.

The parameter is only available if you select Autoregressive Motion or Autoregressive Motion Expert or Lineage.

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Tracking – Tips for Success

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- **Try to get one Spot (or Surface) per object**
  - Adjust the spot size and threshold values to accomplish this
  - Spots/Surfaces detection will take the longest amount of time.
  - Edit Spots before tracking (if planning on doing any Spot editing)

- **Try different tracking algorithms and distance settings**
  - Tracking is quite quick to calculate
  - Just click the Back button  to try a new algorithm or value
  - Compare results visually and by numbers (e.g. number of tracks)

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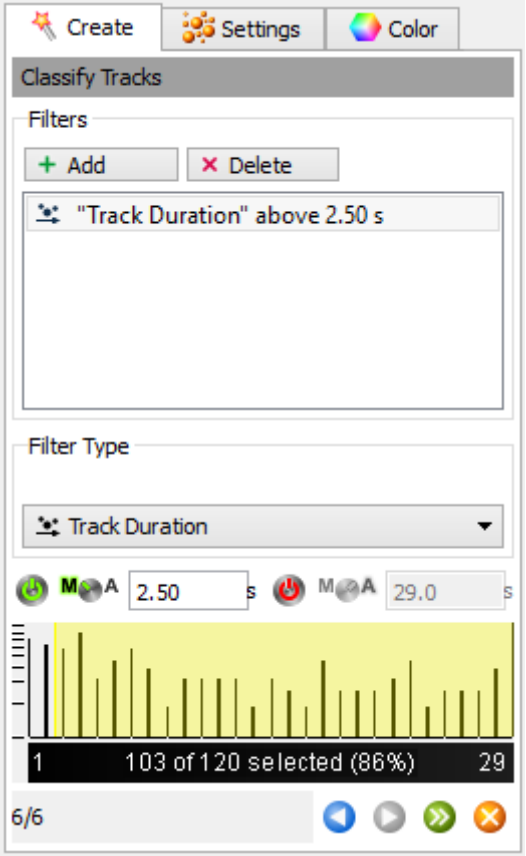
Track Filtering



Select a subset of the detected Tracks:

- *e.g.* Filter Track Duration: Remove brief duration tracks
  - Very brief tracks are often inaccurate or not informative (statistical noise) ... Get rid of them!

- Find tracks of interest by choosing any statistics value to filter tracks
  - Filter high or low values, or combine both high and low filters
  - You can combine a series of different statistical filters

TIP: when filtering options are used within the creation wizard tracks are automatically excluded from the statistics results. When Filtering options are applied after running the creation wizard the filter tab helps you to select a specific set of tracks and create new Spots ‘containers’

Results Visualization

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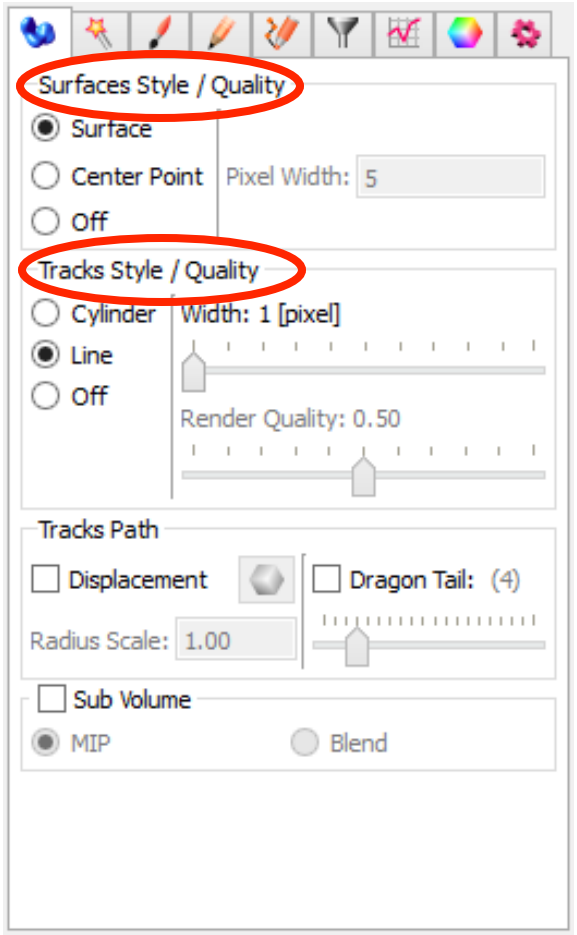
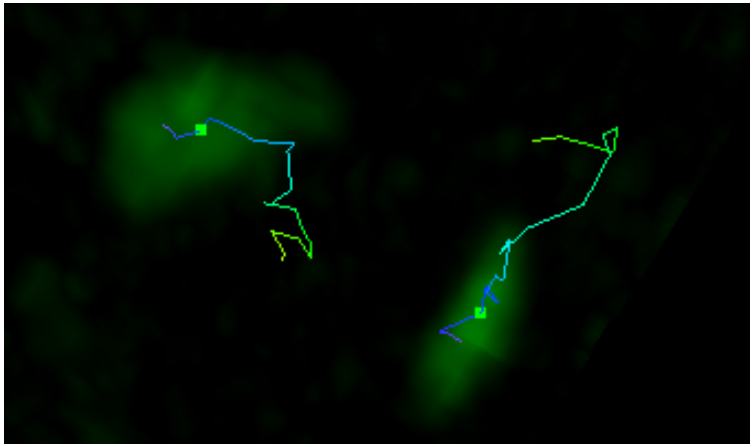
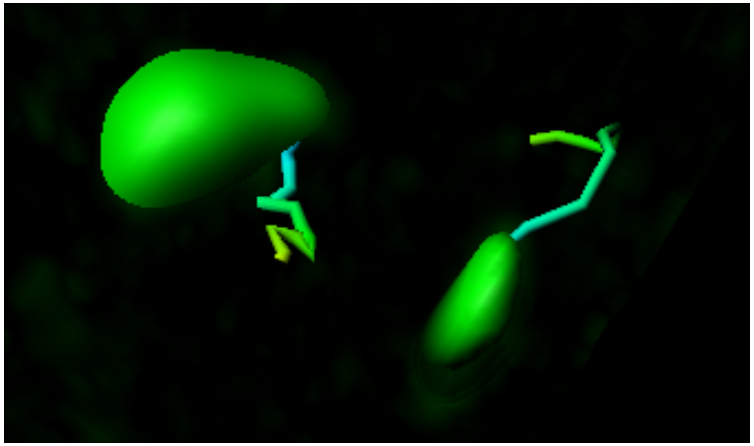
Settings Tab (Visualization Settings)







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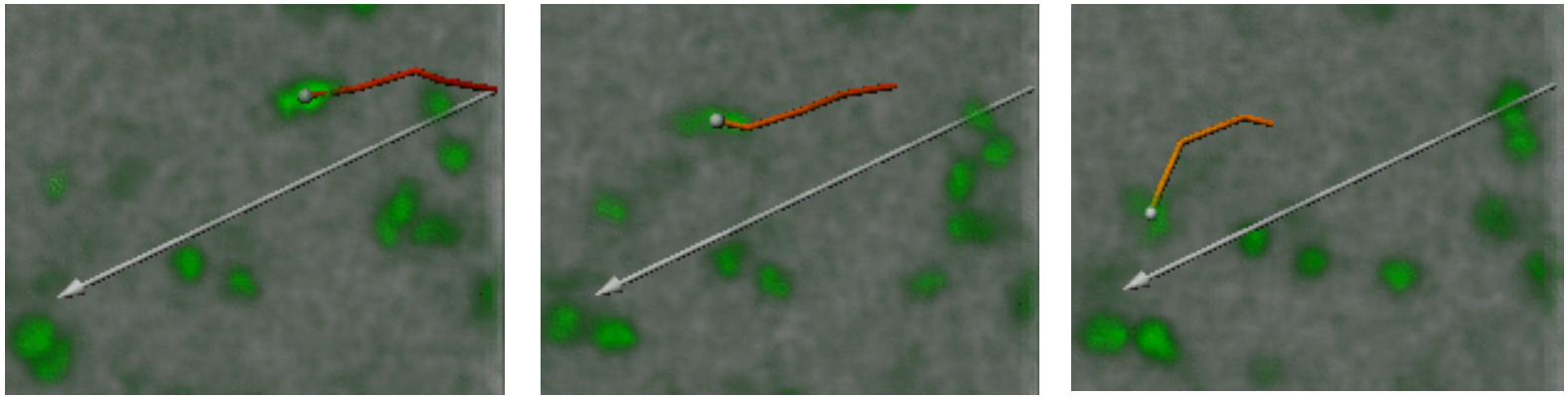
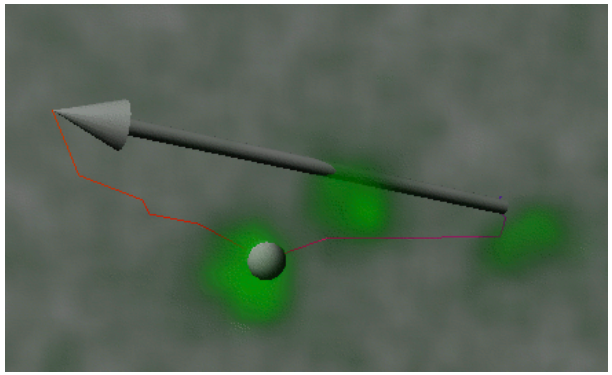
Results Visualization



Track as Dragon Tail Cylinder, w/ Displacement as Arrow



Track as Line, w/ Displacement as Arrow

Results Visualization

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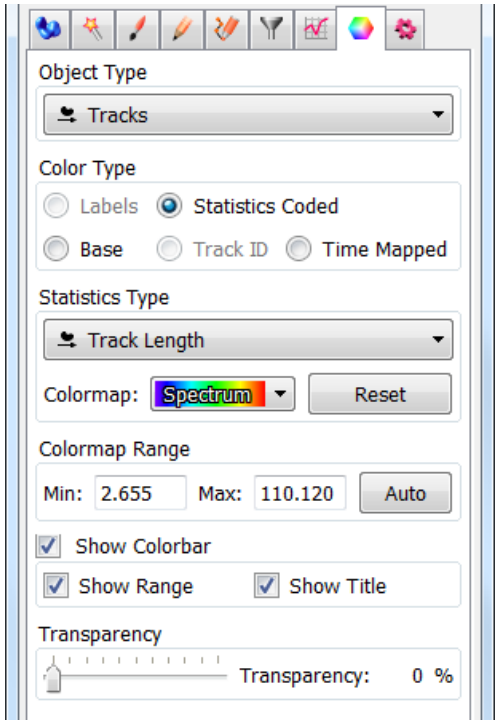
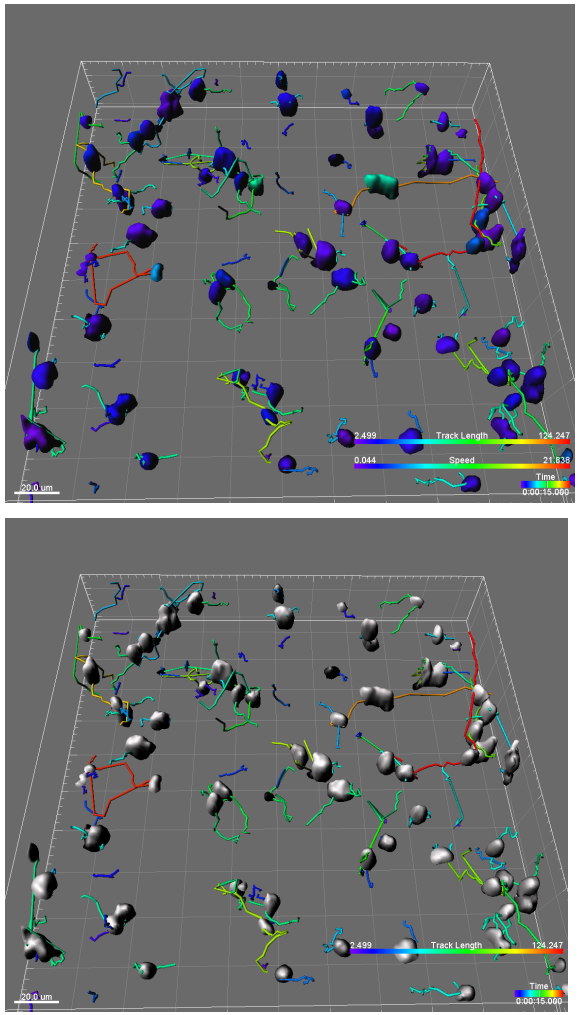
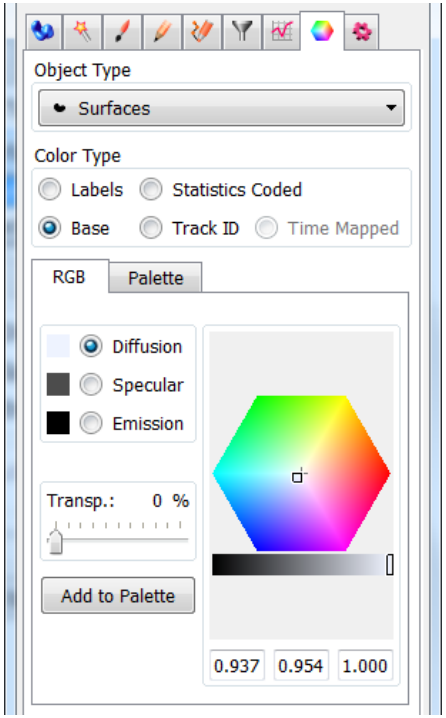
Statistics Coded Track Colors





"Base" Color for Tracks



Note: these changes not only affect the tracks in Surpass, but also in the Track editor view.

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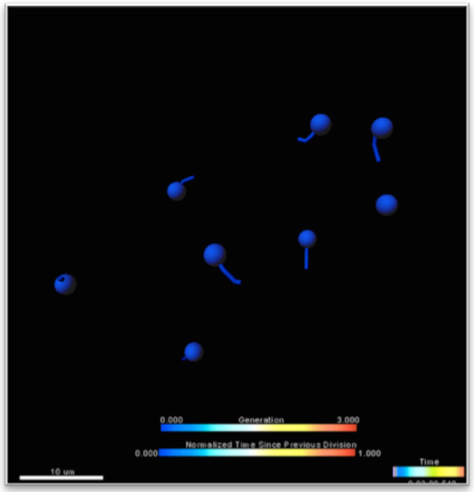
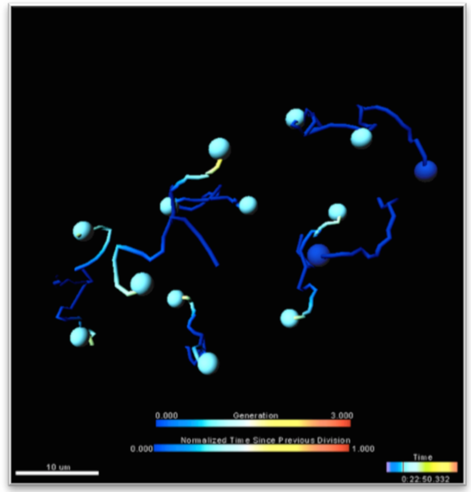
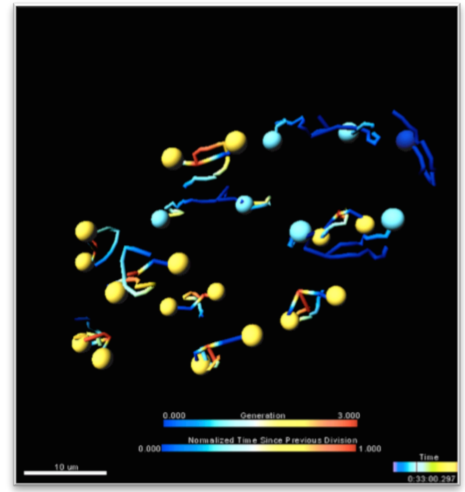
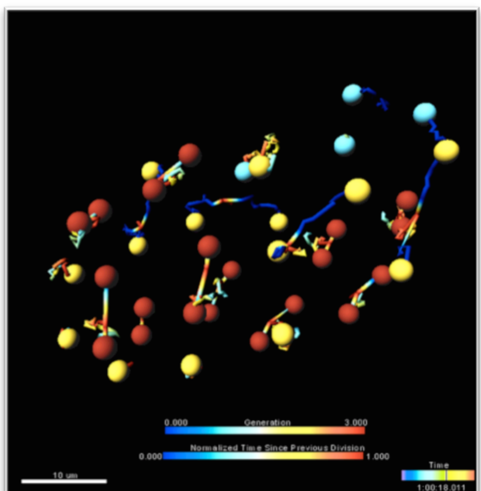
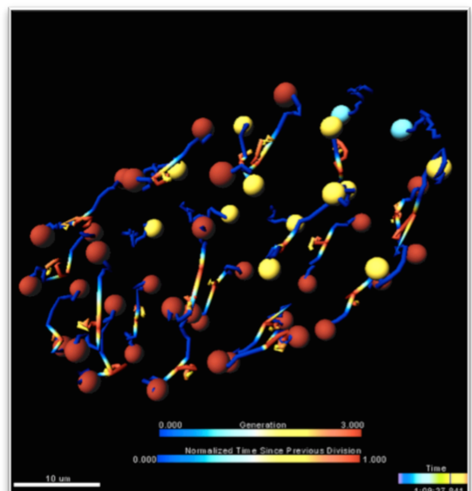
Color coding tracks and spots with lineage measurements

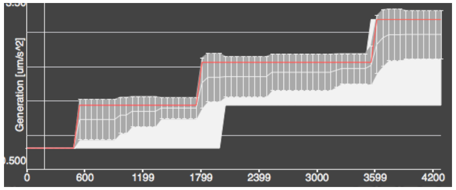






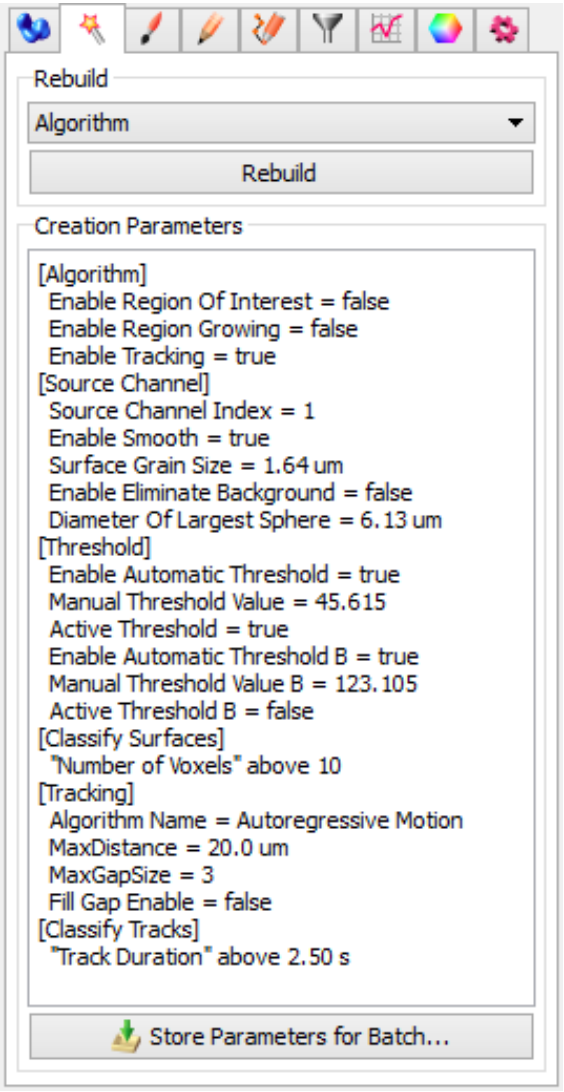




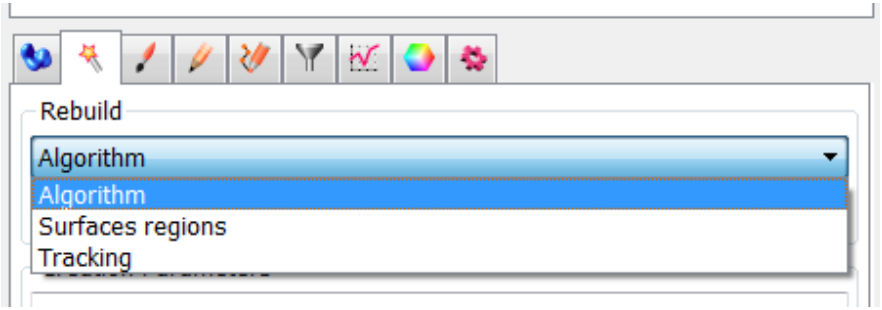


- Spots color coded for Generation
- Tracks color coded for Time since previous division

Track Rebuild



- On the  Creation Tab, review a list of all the settings
- "Rebuild": go back and change one or more settings
  - Drop-down list lets you choose where Rebuild starts
  - Rebuild from very beginning ("Algorithm")
  - Rebuild the tracks only, and skip object detection ("Tracking")



Track Editor

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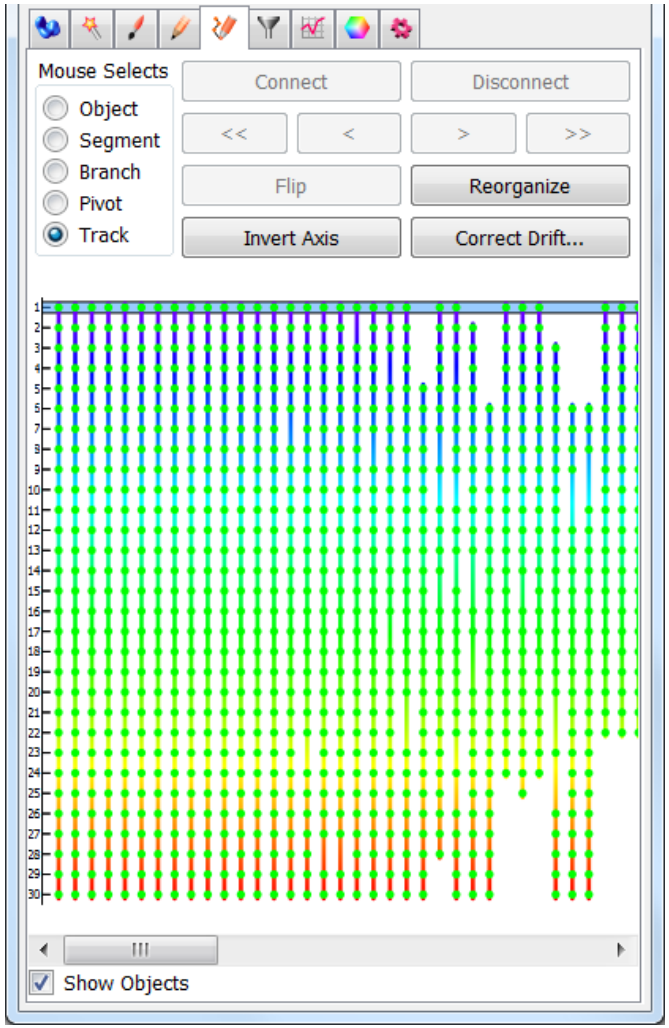
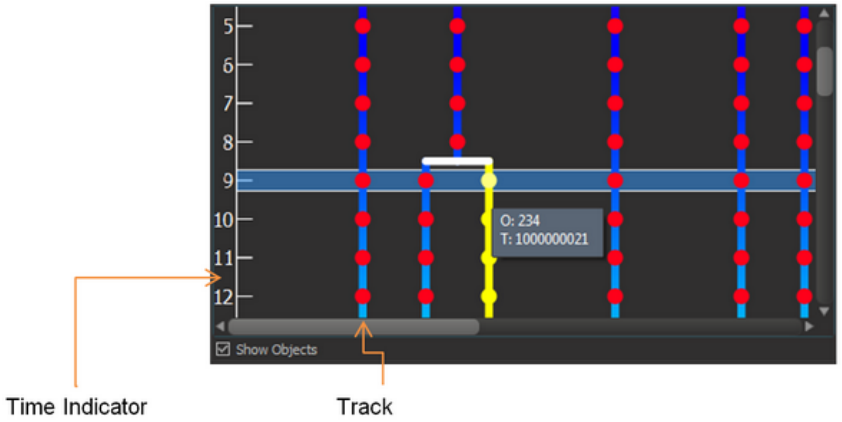
Interactive tool for manual track editing





Time indicator
The axis on the left displays the frame number.

Track
Each track is displayed in a vertical format by default. If connected components or Lineage algorithms are used there may be a number of branch points. The branch points indicate where objects merge or split, e.g. cells undergoing division.

Object
Each data-point is displayed as an object in the track with a unique Object ID. The object will retain its unique ID number- even if, for example, it is connected manually to another track.
If the mouse pointer is on an object, a tooltip appears that identifies the Object ID number (O) and track ID number (T). You can untick the Show Objects checkbox to hide objects from the display.

Track Editor with Lineage Tree visualization

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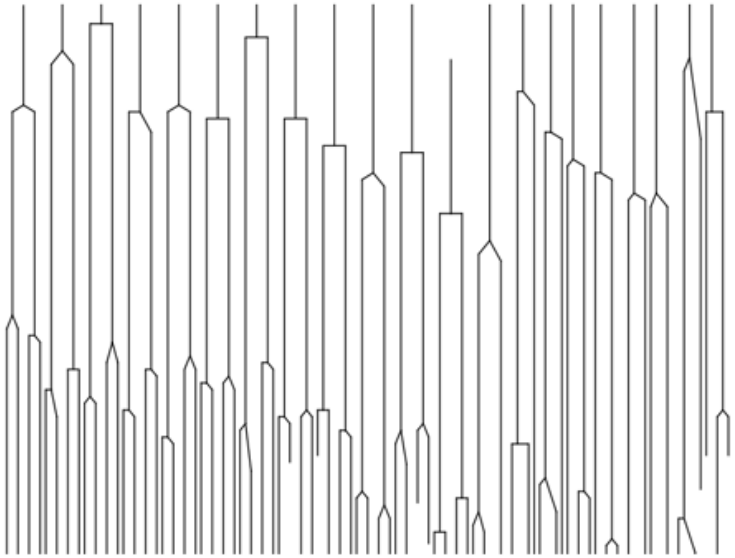
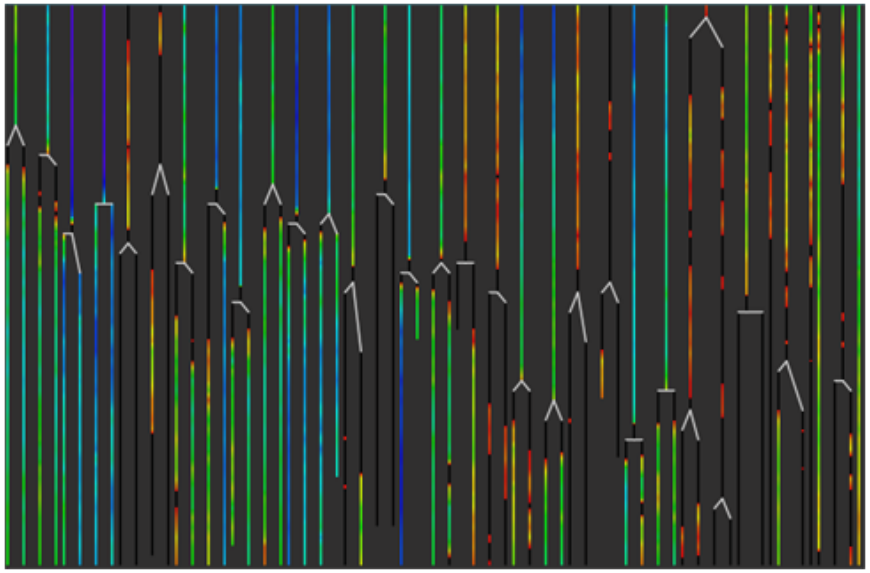


To complement the lineage tracking functionality Imaris8.2 has a new track editor that displays tracks as “lineage tree” in such a way that the number of intersections in the display is minimal (often zero).

The track editor has the functionality to **overlay colors on the tracks**. This can be used to display any statistical measurement value as a color on the tracks. It enables for example a display of the intensity of a second channel for each tracked object.



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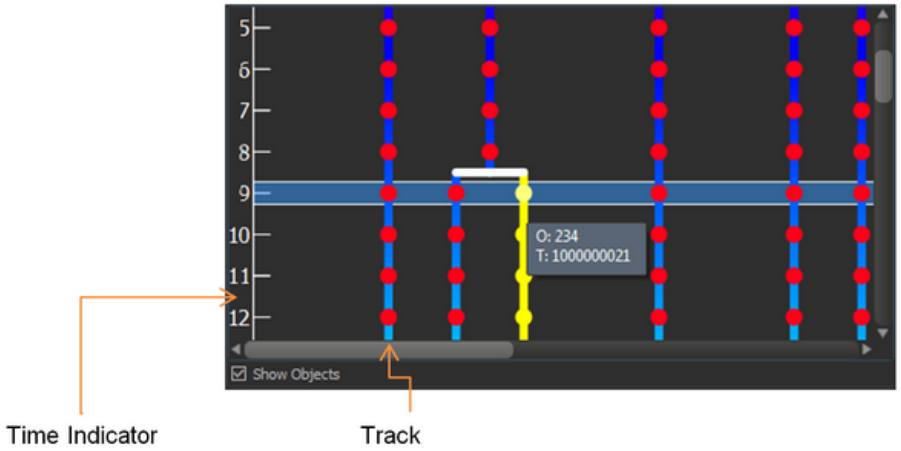
Track Editor – Interactive Selection



- When you click on an individual column, the editing table highlights the corresponding object in the image. Similarly, by clicking on an object within the image (while the pointer is in Select mode) the corresponding track (column in the table) is highlighted.
- In addition, the Statistical Tab and Filter Tab can be used to select tracks for editing. By clicking on the value in the statistical table, the corresponding object in the viewing area as well as the track in the track-editing table is selected.
- For multiple selections press Ctrl+click (Mac: Command+click).

Track Editor – Mouse Selects

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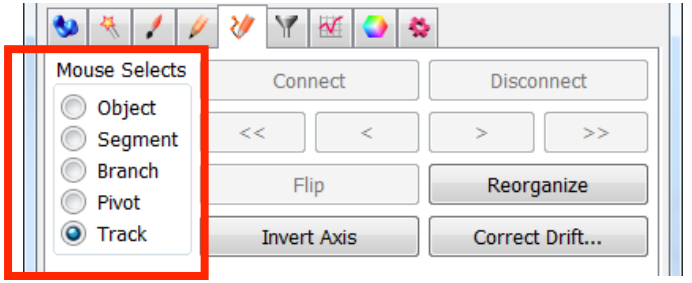
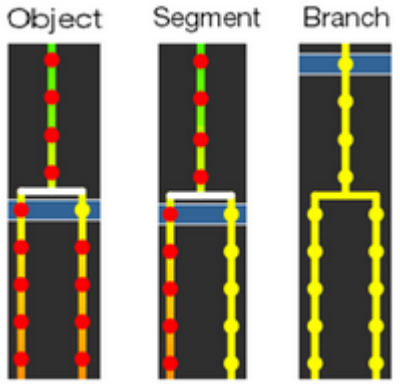
- **Object**

Click Object to select an individual object, or number of specific objects. To connect two unconnected objects, simply select both objects and click the Connect button. To disconnect two connected objects, select both objects and click the Disconnect button.



- **Segment**

Click Segment to select a specific segment (between branch points).



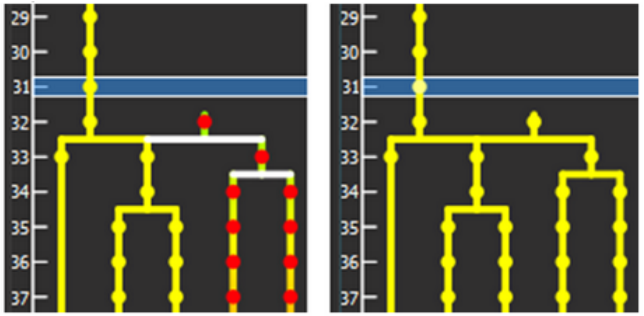
- **Branch**

Select Branch to select the segment and the branched segments and objects that appear later in time only.

- **Pivot**

Pivot enables selection of a segment and all segments and objects that are linked afterwards. This includes objects later in time, and also backwards in time.

For example, selecting branch (left) selects only the connected segments and objects that are later in time. Selecting Pivot (right) all connected segments and objects to be selected.



- **Track**

Select track to select a complete track.

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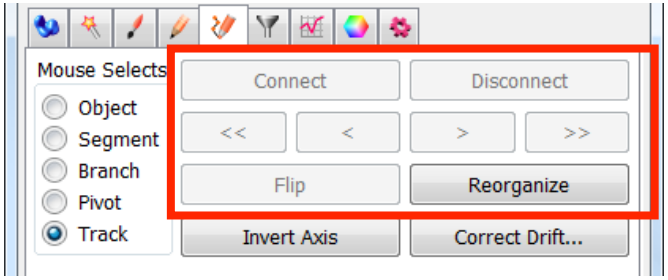
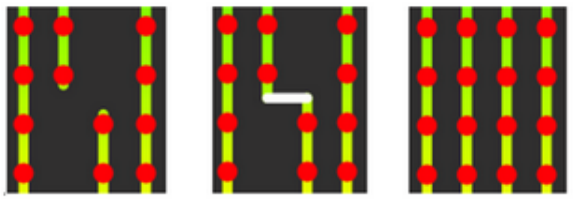
Track Editor – Edit





Editing tracks. Connection added. Tracks reorganized.

Connect Button: Click this button to connect two existing objects. Select two objects in two different tracks at the subsequent time points (just one object per time point) and click on the button Connect. The connection is automatically created between all selected objects.

Disconnect Button: Select an object and click on the button Disconnect. The connection between the two selected objects in the time sequence is broken.

< Button: Click this button to move a selected track left. When axis is inverted (horizontal) the selected track moves up.

> Button: Click this button to move a selected track right. When axis is inverted (horizontal) the selected track moves down.

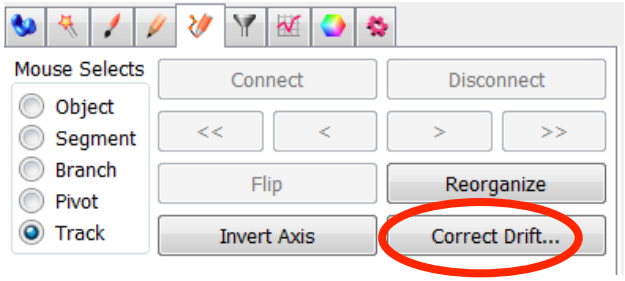
<< Button: Click this button to move a selected track all the way to the left. When axis is inverted (horizontal) the selected track moves to the top.

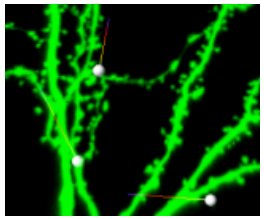
>> Button: Click this button to move a selected track all the way to the right. When axis is inverted (horizontal) the selected track moves to the bottom.

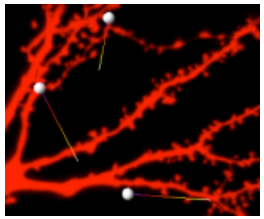
Flip: The Flip button may be used (with Pivot mode selected) to flip the order that the connected branched segments and objects are displayed in about the pivot point.

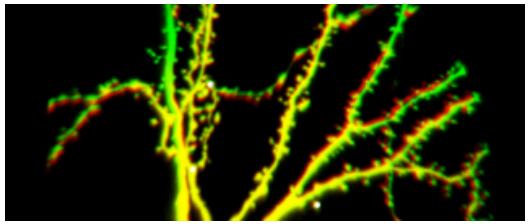
Reorganize: The Reorganize button can be used after editing of tracks is complete so that tracks are displayed in a clear and logical format without affecting the track arrangement during the editing process.

Drift Correction

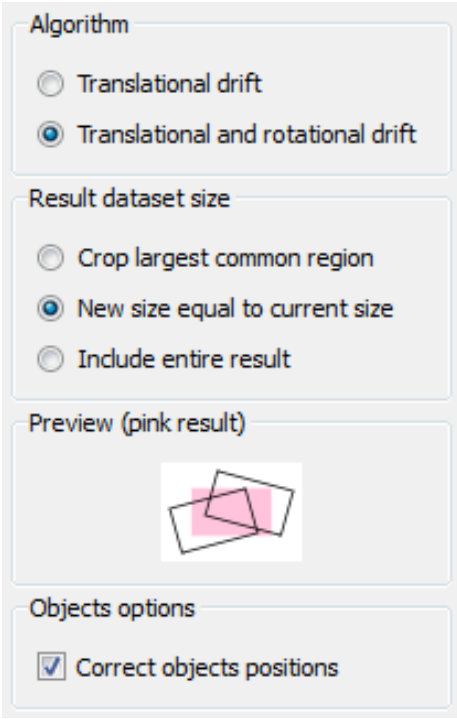


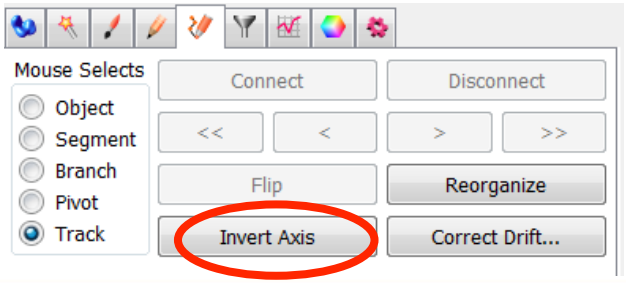






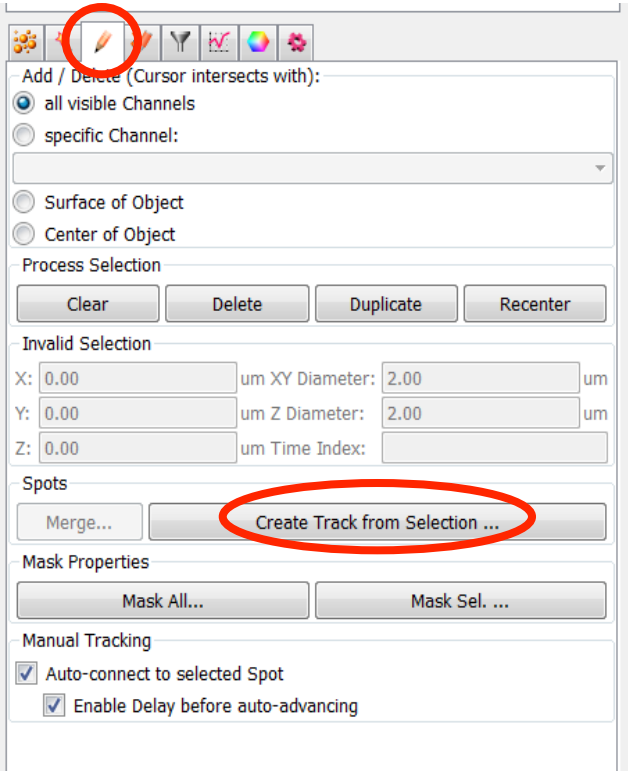
- Stationary object track(s) used as a reference to subtract overall motion
- Select track(s) of object(s) that should not be moving.
- Press the drift correction button
- Image is realigned
- For 3D rotational correction, select at least 3 tracks



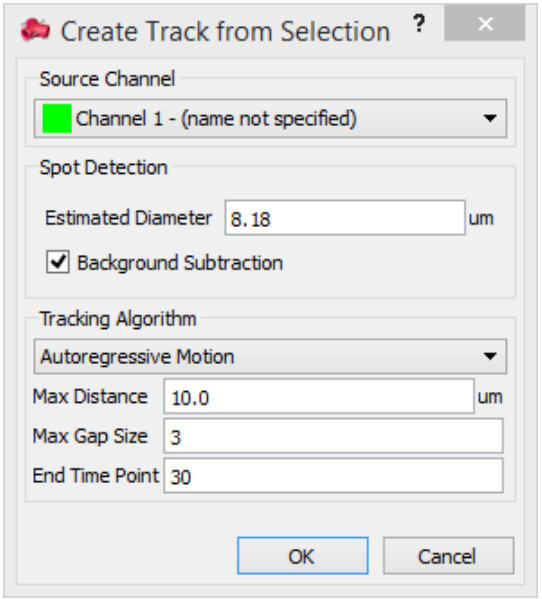


Click the Invert Axis button to switch between displaying the tracks vertically (default) and horizontally.

Isolated Spot Tracking



- Select or create a Spot that you want to track (skip automatic creation, edit manually: if you do not want to track all objects)
- Click on Create Track From Selection
- Enter tracking parameters in pop-up window
- Tries to detect object only near next predicted position
- Fast, efficient tracking approach for isolated object(s), but is unable to use other nearby tracks (e.g. linear assignment) to optimize the tracking result.



Fast and Easy Manual Tracking

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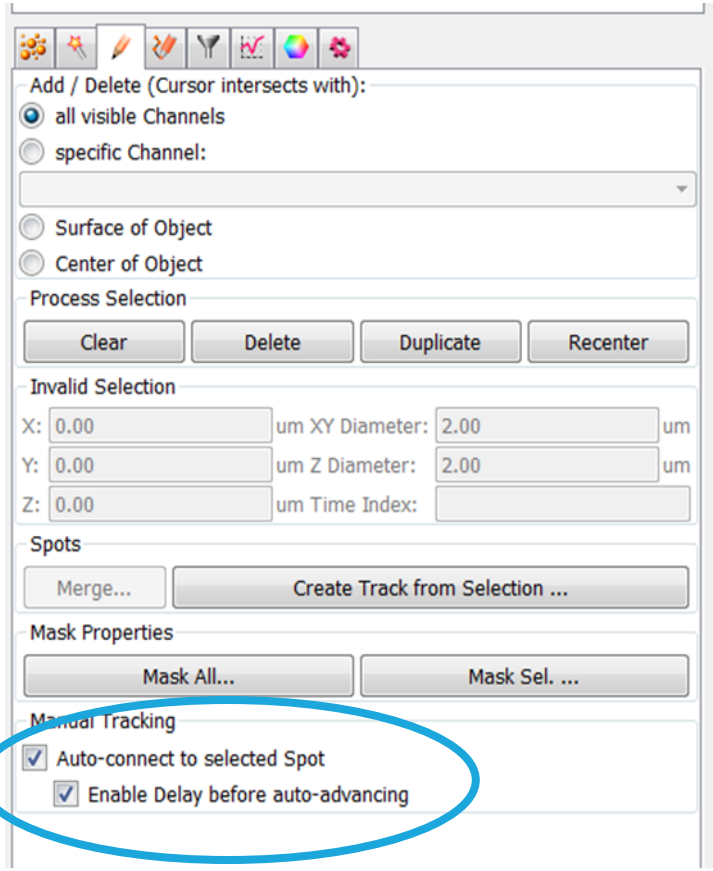

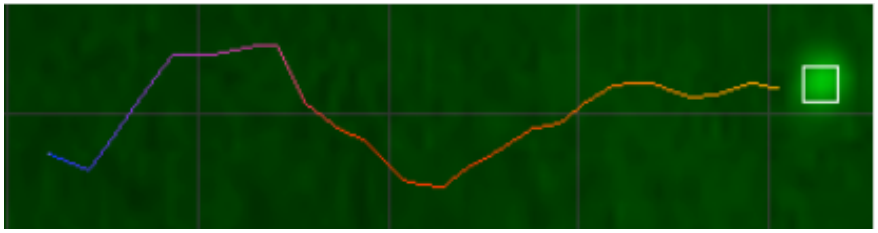

- Single click places a new spot and advances the time by one frame
- Full manual control at a reduced number of mouse clicks.
- Use “Recenter” afterward on entire track(s) to align the positions in 3D to the intensity peaks

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Tracking Statistics

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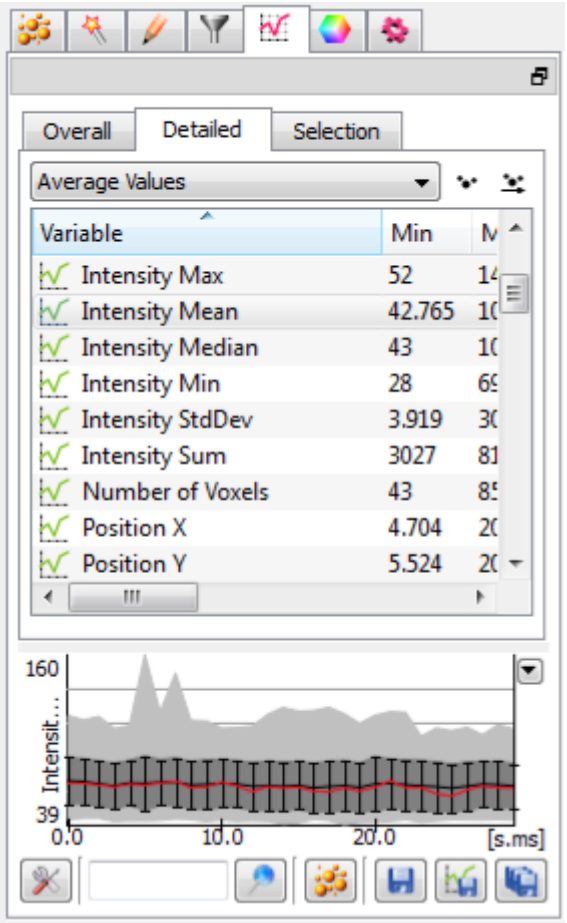

Examples of Tracking Statistics:

- **Temporal Measurements:** Track duration (sec), Instantaneous/Average speed, Speed variability, Velocity (x,y,z)
- **Distance & Morphological Measurements:** Track straightness, Track length, Object displacement, Object volume/area/shape changes over time
- **Intensity Measurements:** Object/Track intensity Mean/Max/Min/StdDev over time
- **Number of branches and fusions** (only 'Connected Components' will generate these)
- **Many additional component relationship changes** (ImarisCell)

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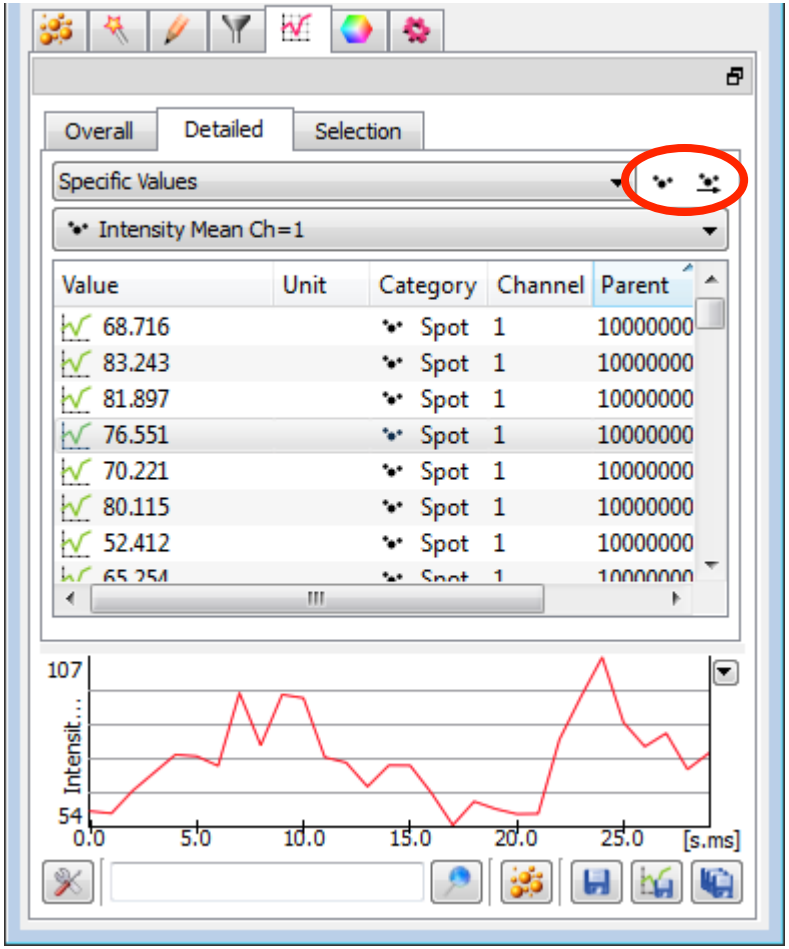
Managing Track Statistics Selection List



- Show/hide Spot related statistics in the list
- Show/hide Track related statistics in the list

Time Plot of Track Statistics

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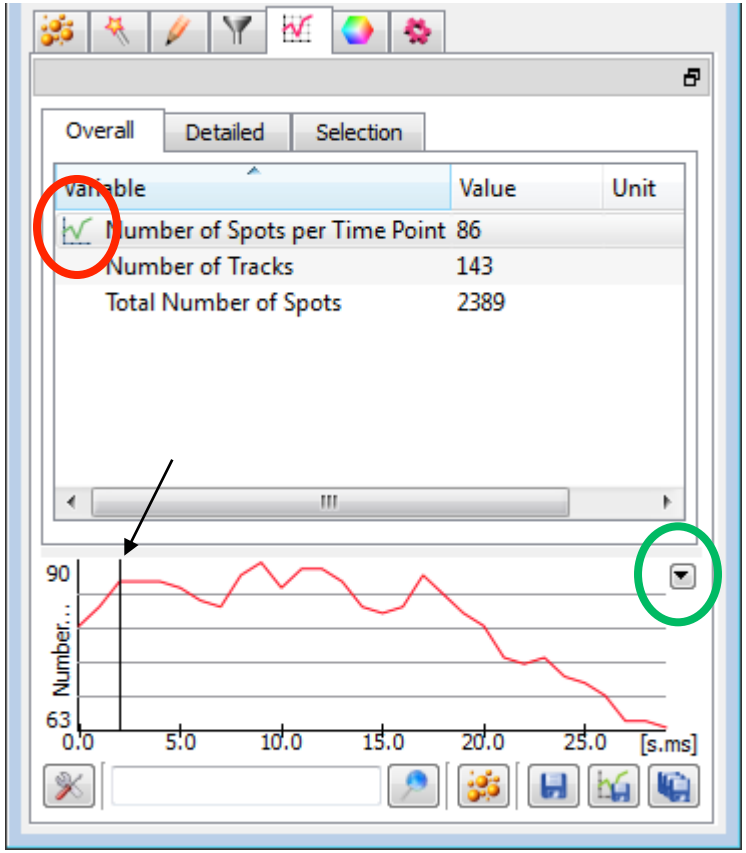
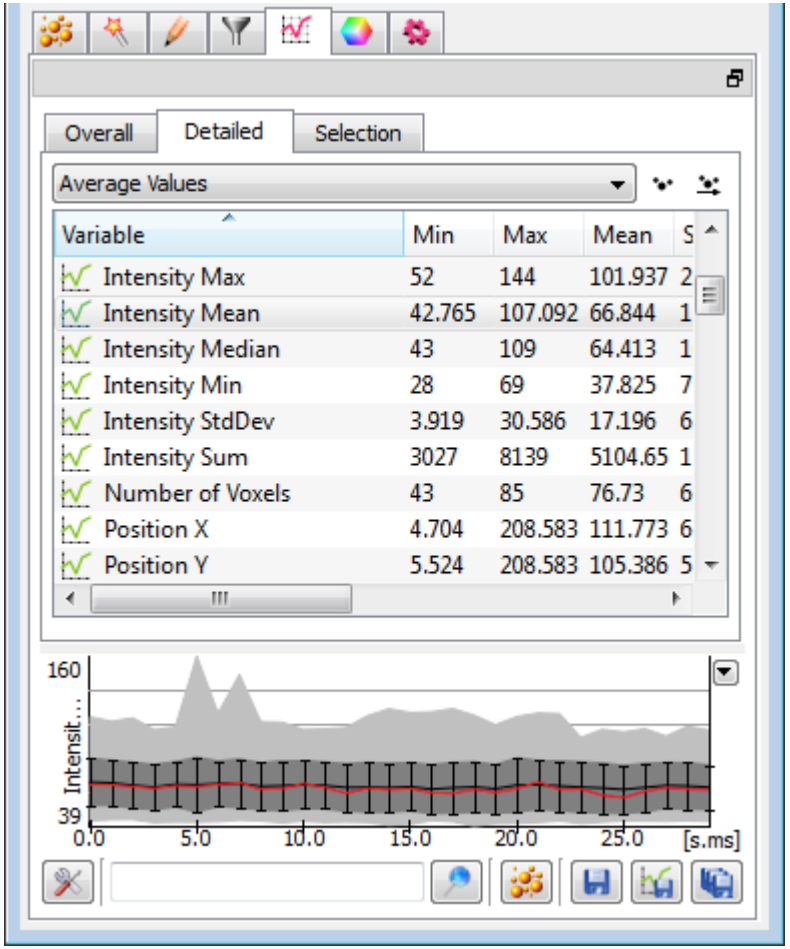


- Any statistic which could change over time will have a graph icon displayed in front of it
- By default the plot is not shown
  - Click arrow button to show plot
- The black line in the graph indicates the present time point.
- Moving the black line in the graph changes which time point is visualized in Surpass.
- Plot is so the user can quickly find interesting time points.
-  Export the statistics used to create the plot.

Track Statistics Average Values Plot



- Average Values have a special time plot
- The **black** line on the time plot indicates where the **mean** value lies
- The **red** indicates the **median**
- The **light gray** area shows the **range** between the **min** and **max** values
- The **dark gray** region labels the **standard deviation range** with error bars.

Lineage specific statistics

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There are a number of useful statistics that are specific to the Lineage tracking algorithm:

- **Generations**

This shows the generation that the selected object belongs to. This is displayed in the format: 0.00 for the initial object, e.g. mother cell and 1.00 for the following 1st generation e.g. daughter cells. Tracked objects without divisions and untracked objects have a generation value of 0.

- **Track Number of Generations**

This statistic shows the number of generations present in the selected track.

- **Time since first Division**

This shows the time elapsed since the first division of the selected object(s).

- **Time since previous Division**

This shows the time between each generation. e.g. the lifespan of an object

- **Normalized Time since previous Division**

Displays the normalized division time. This value is only calculated for objects that have a previous division. Only defined for objects that have a previous division and a following division and no merges.

- **Cell Cycle Duration**

Time since the previous division for objects that divide again. This is only defined for objects that divide and have a previous division.

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Exercise 1

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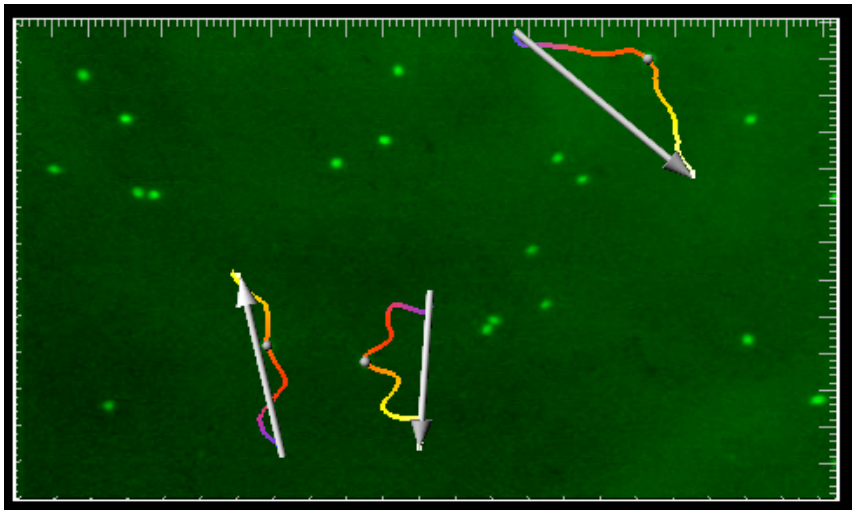
1. Open file SwimmingAlgae.ims

2. Try to track individual algae rather than the whole data set

TIP: skip automatic creation, edit manually option

3. Add & delete spots/tracks

4. Track whole data set



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Exercise 2

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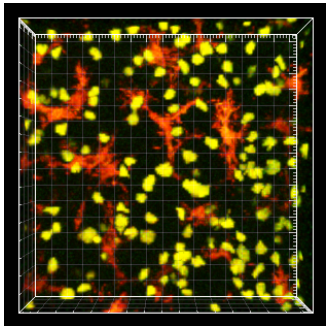
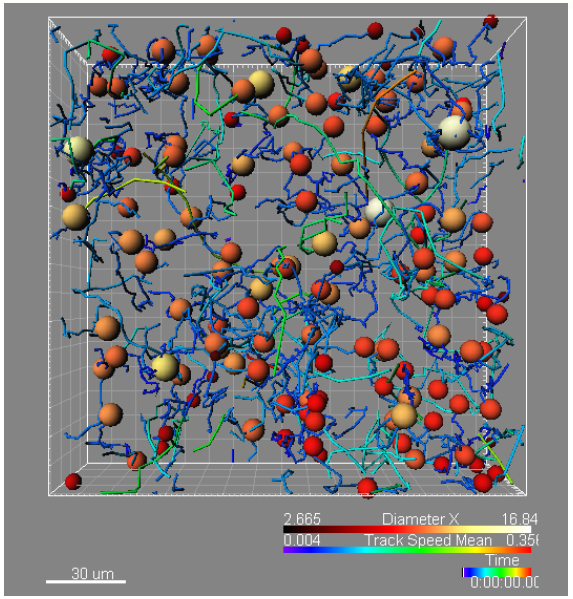
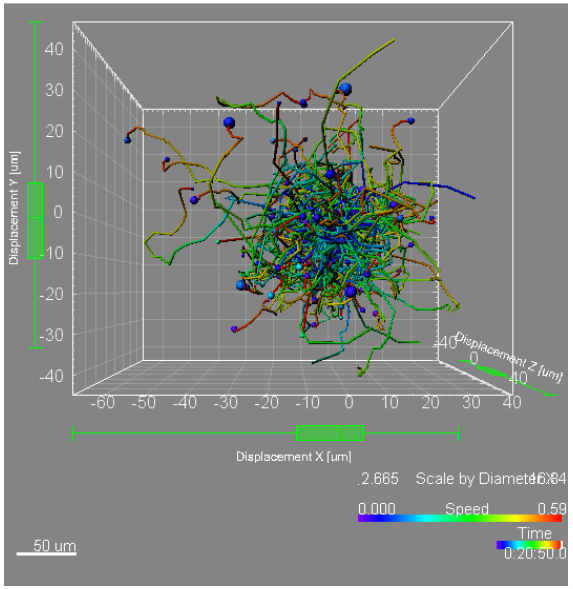
Dataset:
movie3c-resamXY.ims



1. Detect Multiple Spot Sizes
2. Track the green channel and colour-code points for diameter
3. Color-code tracks for Mean Track Speed
4. Build a Vantage 5D scatter plot of x,y and z displacement. The scale parameter can code for Spot diameter and the colour for object speed.
5. If time allows. Track the red channel and build a Vantage plot of your choice





Exercise 3

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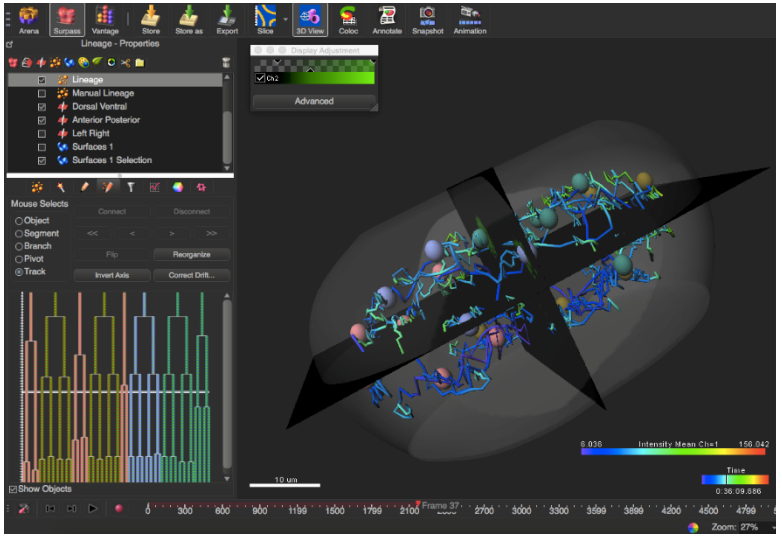
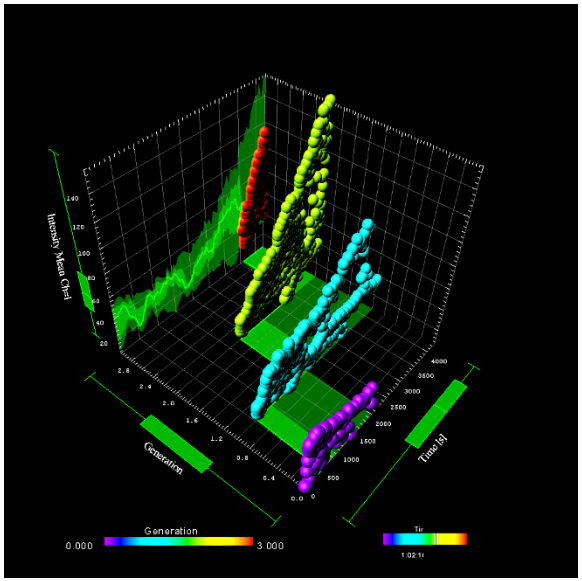
Dataset: C.elegans embryo.ims

1. Lineage Track on ROI for 3 generations (1-69 frame)
2. Check on the Track editor if your lineage trees are connected
3. Rebuild if necessary the Linage Tracking parameters until you obtain successive divisions in a single lineage tree
4. Display the lineage tree in terms of generations
5. Make a Vantage plot displaying generations over time with any other statistical value.





Exercise 3 (cont)

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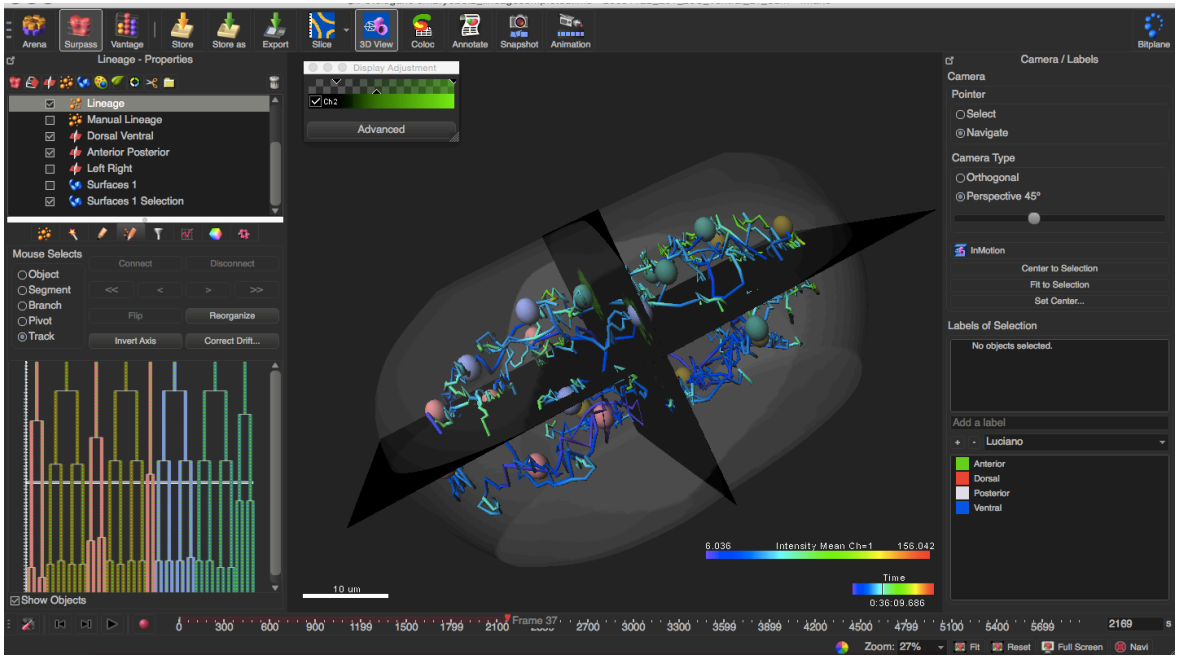
6. Create 4 labels for anterior, dorsal, posterior and ventral
7. Color-code the lineage tracks according to their region.
8. Make use of the ortho slicers



Hint

[Algorithm]
  Enable Region Of Interest = true
  Process Entire Image = false
  Enable Region Growing = false
  Enable Tracking = true
[Region of Interest]
  Region1: XYZT from [1 1 1 1] to [512 275 35 67]
[Source Channel]
  Source Channel Index = 1
  Estimated Diameter = 5.00 um
  Background Subtraction = true
[Classify Spots]
  "Quality" above 3.48
[Tracking]
  Algorithm Name = Lineage
  MaxDistance = 5.00 um
  MaxGapSize = 1
  Fill Gap Enable = false
[Classify Tracks]
  "Track Duration" above 151 s

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