

# Tracking in Imaris (7.6.5)

Basics of Imaris Spots

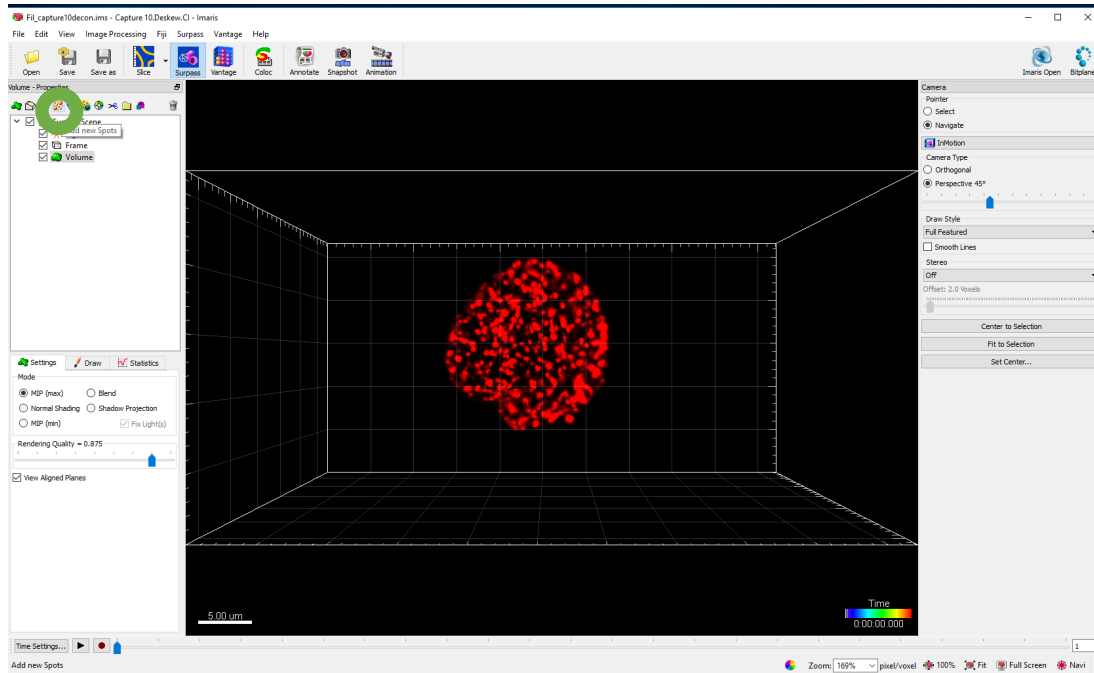
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9<sup>th</sup> November 2018 / CAMDU @ WMS / Helena Coker

## Getting data into Imaris

- Follow the instructions in the presentation '4D rendering of Lattice Light Sheet data' to import your file to Imaris and to check the pixel scaling values in Image Properties.

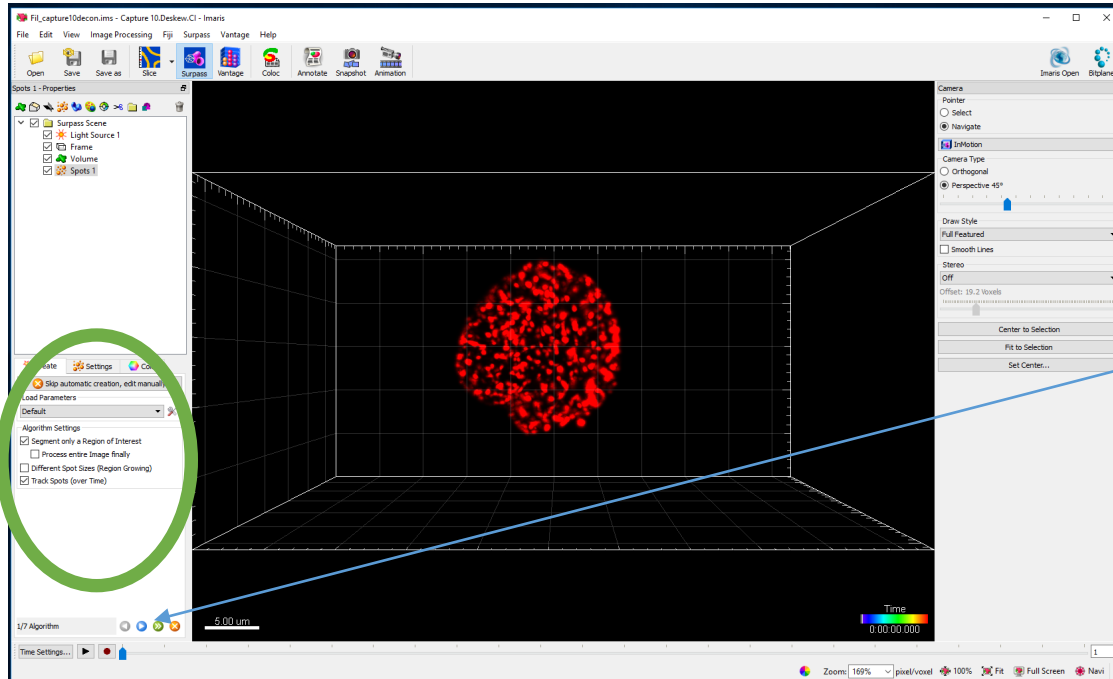
# Spots



- Click on Add New Spots icon

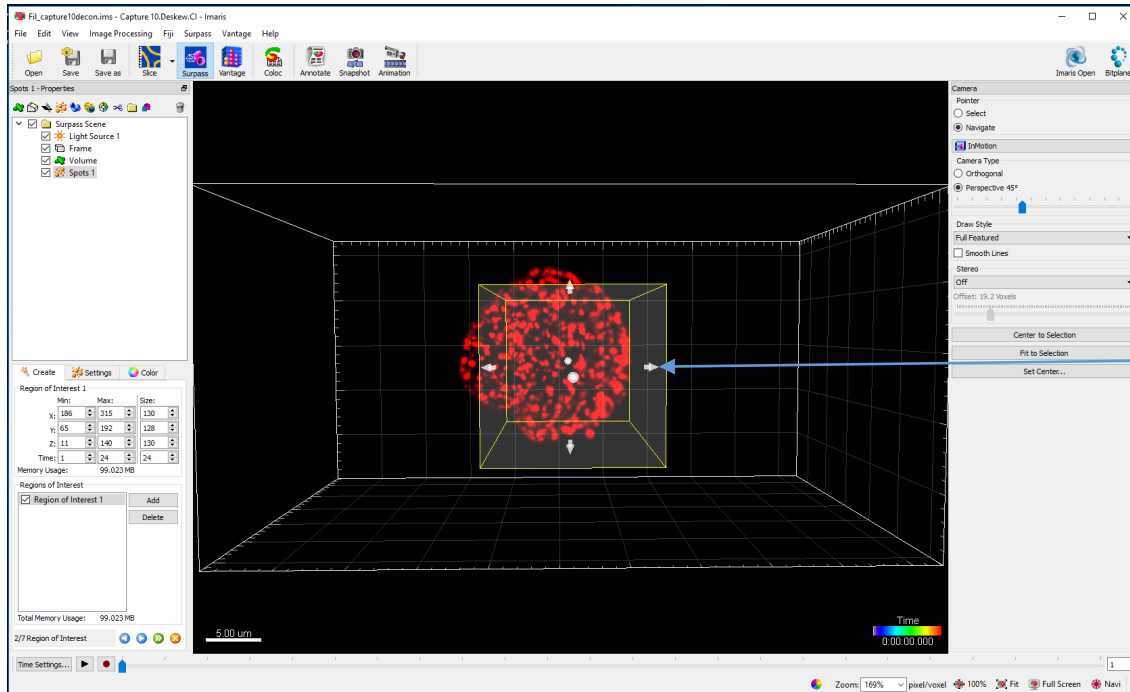


## Spots



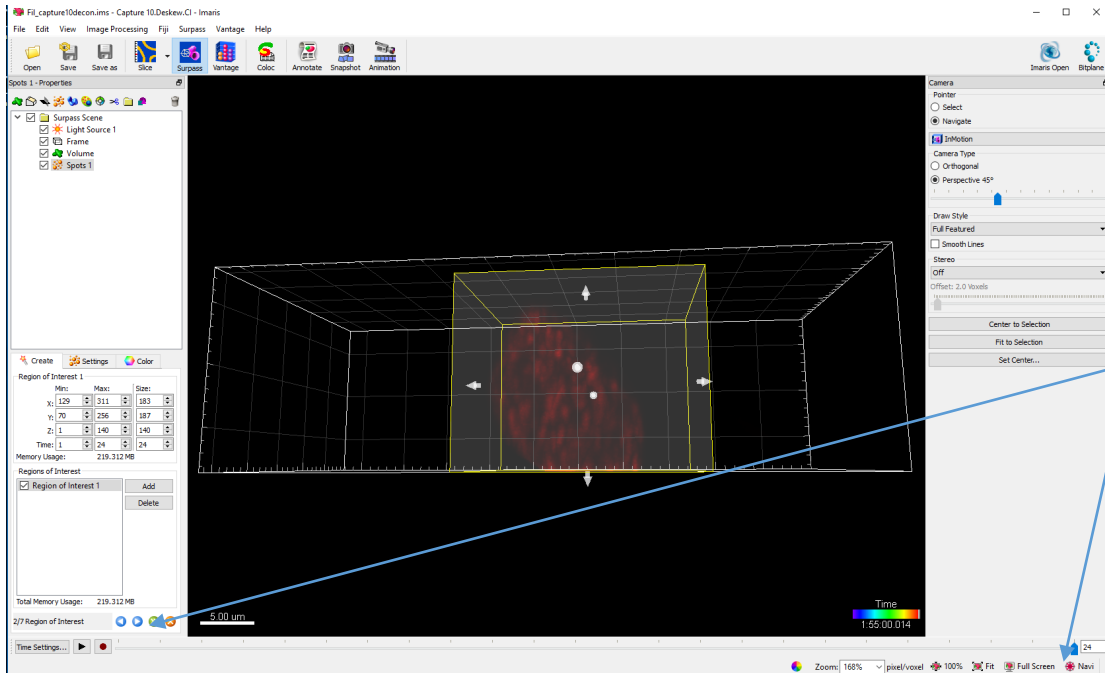
- The spots panel will open in the bottom left
- Selecting a region is less computationally taxing and will increase the probability of successful tracking (without crashing)
- Click blue next arrow button

## Select region



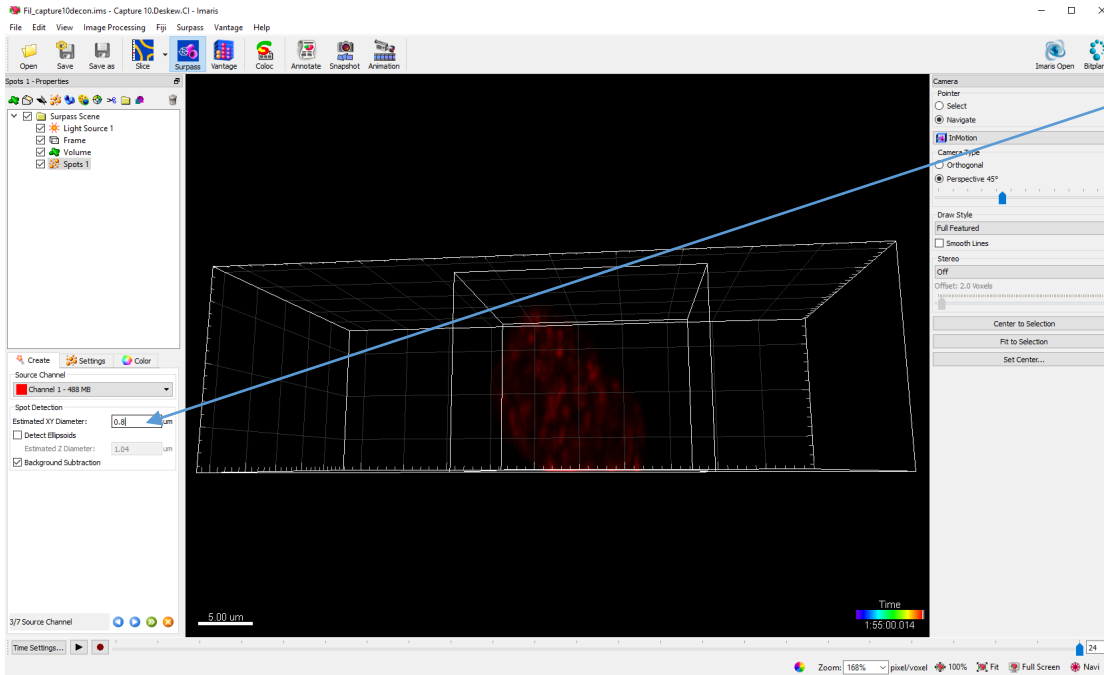
- Select your region of interest in the volume viewer
- Use the Esc key on the keyboard to toggle between turning the image volume and moving the region of interest (ROI)
- Use the white arrows on the ROI to change its size

## Select region



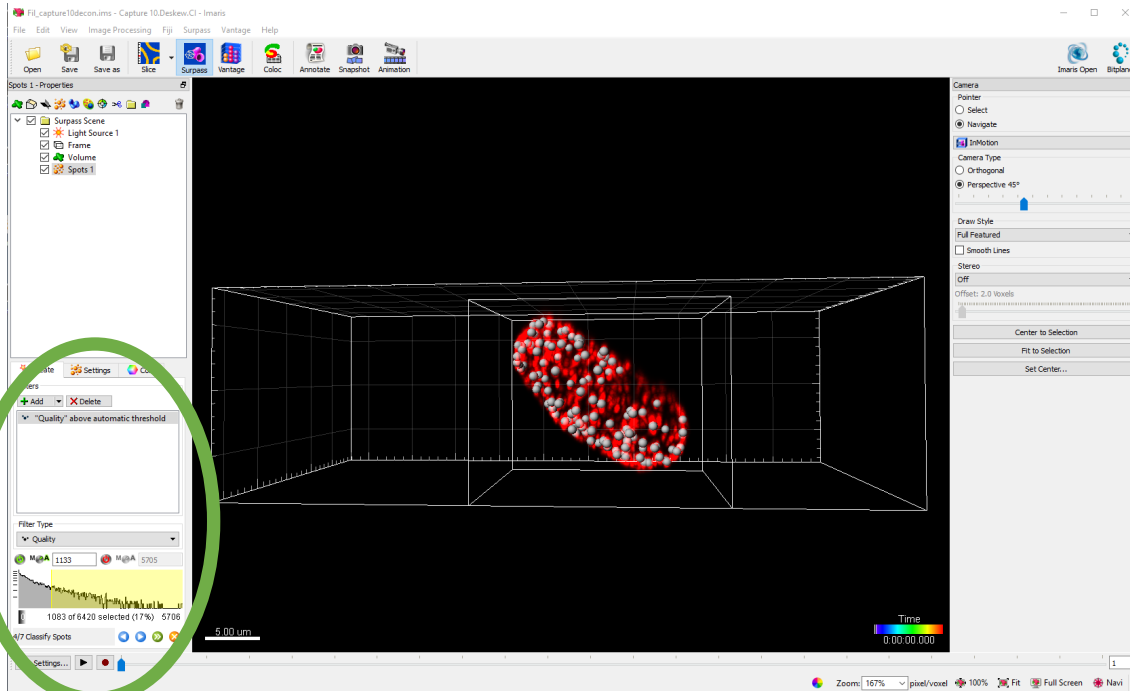
- Make sure that the ROI covers the whole of your cell in all three dimensions AND from start to finish in time (time is adjusted by the blue slider)
- When you are happy with your ROI, click the blue arrow to proceed

## Select spot parameters



- Insert the estimated diameter of your spots
- Click the blue arrow to proceed
- Wait for the spots to be detected, a progress bar can be found in the bottom right

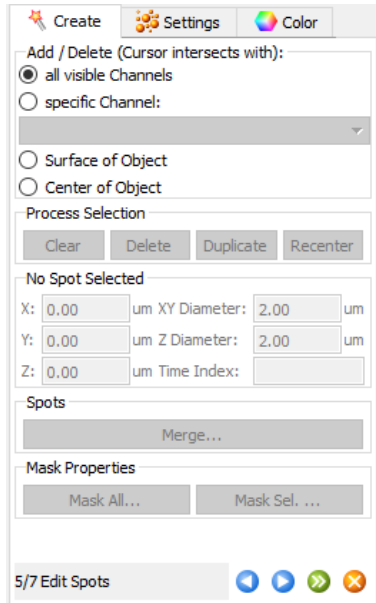
## Filter Spots



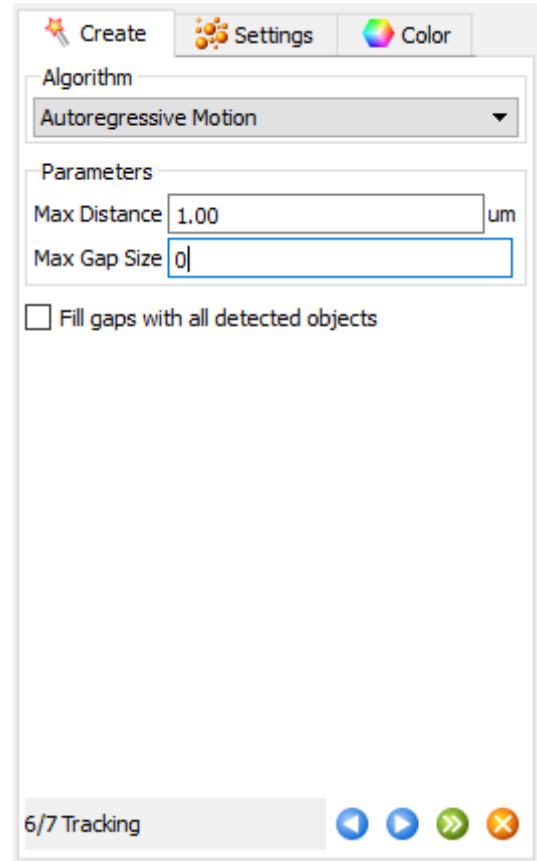
- Use a combination of filters to choose the spots to track
- Make sure spots in the last frame are detected if they are still present. This may be difficult if your sample has photobleached significantly
- Quality, Intensity standard deviation and position are good filters to start with
- Try to minimise detections of things you don't trust are spots
- When you are happy, click the blue arrow to advance



# Track



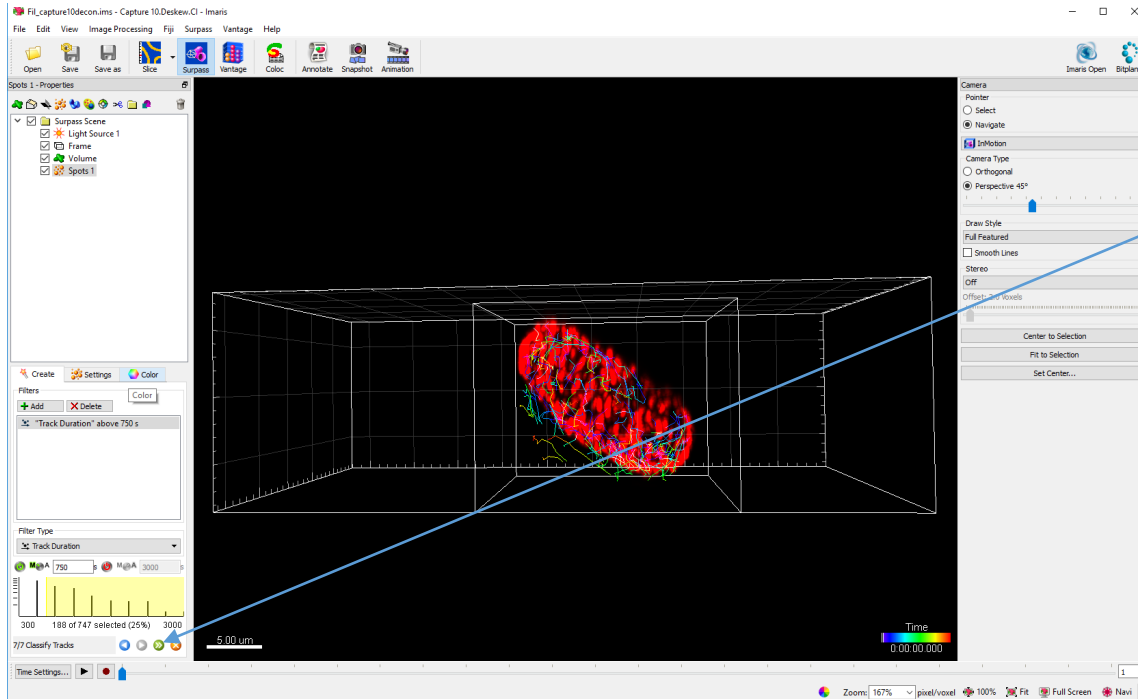
Create Settings Color  
 Add / Delete (Cursor intersects with):  
 all visible Channels  
 specific Channel:  
 Surface of Object  
 Center of Object  
 Process Selection  
 Clear Delete Duplicate Recenter  
 No Spot Selected  
 X: 0.00 um XY Diameter: 2.00 um  
 Y: 0.00 um Z Diameter: 2.00 um  
 Z: 0.00 um Time Index:  
 Spots  
 Merge...  
 Mask Properties  
 Mask All... Mask Sel. ...  
 5/7 Edit Spots



Create Settings Color  
 Algorithm  
 Autoregressive Motion  
 Parameters  
 Max Distance 1.00 um  
 Max Gap Size 0  
 Fill gaps with all detected objects  
 6/7 Tracking

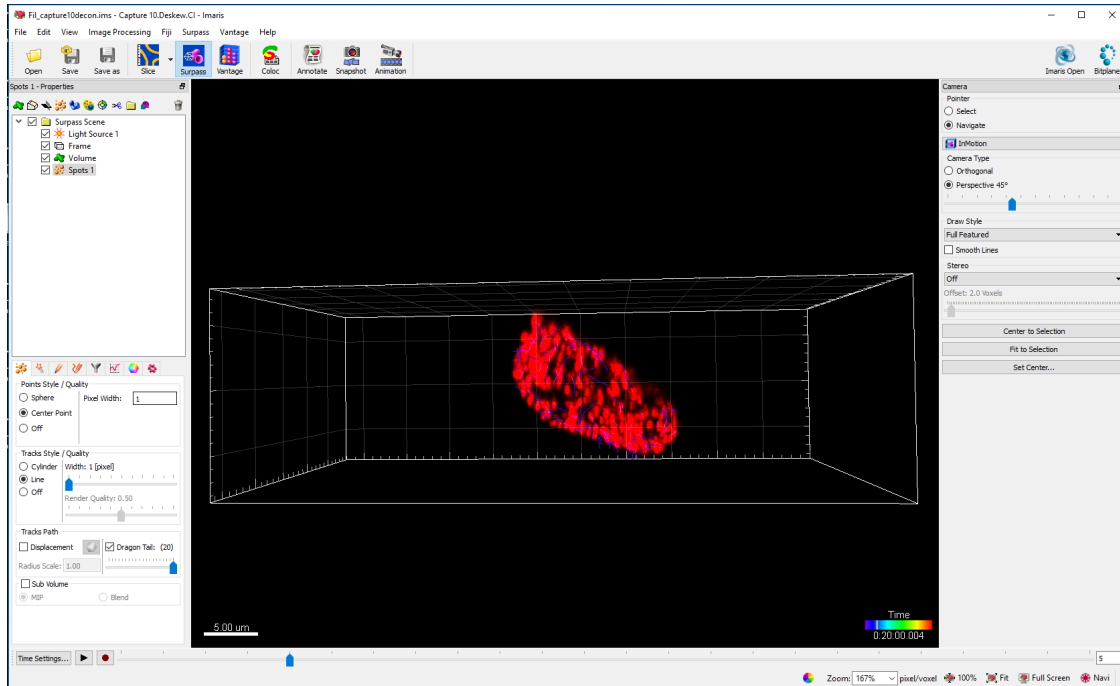
- Click the blue arrow again to advance
- Choose a Maximum Distance which is the limit the spot can move between frames and still be considered the same spot. This will depend on the time between frames and how fast your spots are moving/diffusing.
- Max Gap Size is the maximum number of frames your spot can be missing and then be added to the same track. Usually 0, i.e. no gap closing
- Click the blue arrow again to advance

## Filter tracks



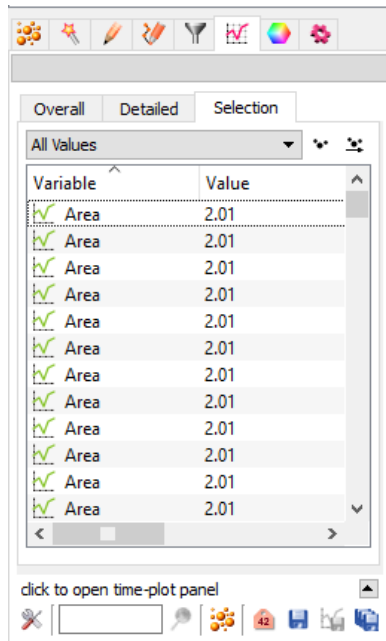
- Filter tracks, Track Duration is usually a good filter
- Click the green arrow to finish




# Visualise



- Fiddle with the visualisation settings to get the desired result
- Follow the instructions in the presentation '4D rendering of Lattice Light Sheet data' to create and save a movie which includes your tracks

## Exporting the tracks output



- Select the Statistics button to review the data 
- Click the Save All Data to File button to create a folder of individual CSVs for each output type (intensity, duration position etc. etc.) 
- Click the Export Statistics on Tab Display to File button to save a folder containing a single CSV with all of these parameters saved in a very long list 

## Notes

- Please make use of the many resources on the internet available from other sources
- Although not for the exact release we have licensed, the Imaris 9 manual has some relevant information

**Any questions? Find me in *in silico* or  
drop me a line at  
[helena.coker@warwick.ac.uk](mailto:helena.coker@warwick.ac.uk)**

Slidebook and Imaris

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