Volocity Tutorial
Tracking in Volocity Software

This tutorial will demonstrate how to perform tracking using Volocity® Software.

Data

Live Cell Tracking

Workflow

Tracking objects may be appropriate if you are interested in characterizing the movement of objects (i.e. their speed, direction), or monitoring properties of objects as they move over time. In Volocity, tracking is a two stage process:

1) The identification of the objects.
2) The analysis of the positions of those objects and the building of tracks.

Finding objects

Click once on the data in the library list and click on the Measurements tab to display the Measurement View. The image is shown in the mode that best shows the objects to be measured, and below it is an area where all measurements made will be displayed. At the top left of the screen is an area where a measurement protocol will be built, to find objects of interest in the dataset and track them, using the list of tasks in the area below.

Image preview shows feedback as measurements are made

Drag measurement protocol tasks to this pane to make protocols

Measurement protocol tasks
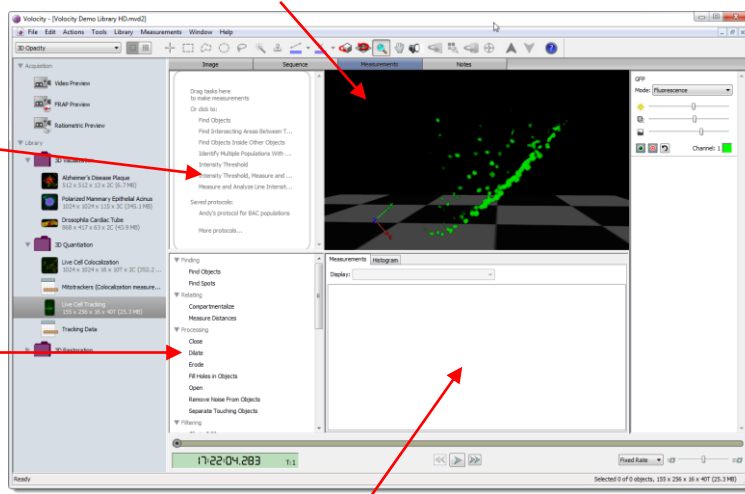
Measurements are shown here as a table or histogram

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It is important that the Measurement Protocol identifies objects as accurately as possible in each timepoint as this will be essential for the tracking algorithm.

The objects within this dataset do not exhibit the same intensity values throughout the time-course; therefore thresholding on the same intensity values in each timepoint is unlikely to be successful.

The task "**Find Objects**" is designed to operate even in these challenging conditions. Drag this task to the protocol pane above.
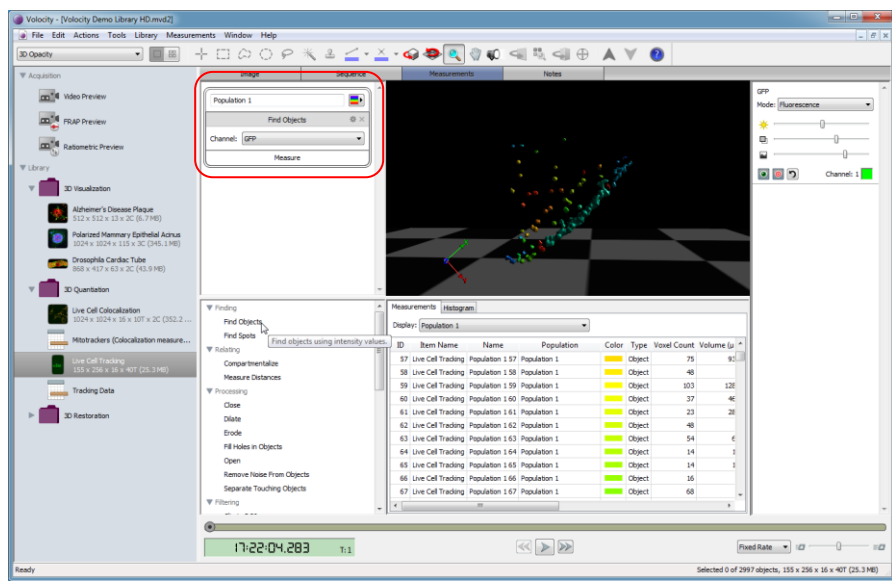

Where intensities are found within range a colored overlay is applied. Groups of selected intensities form objects. View the image, with object overlays, in different ways by changing the mode of view in the top left.
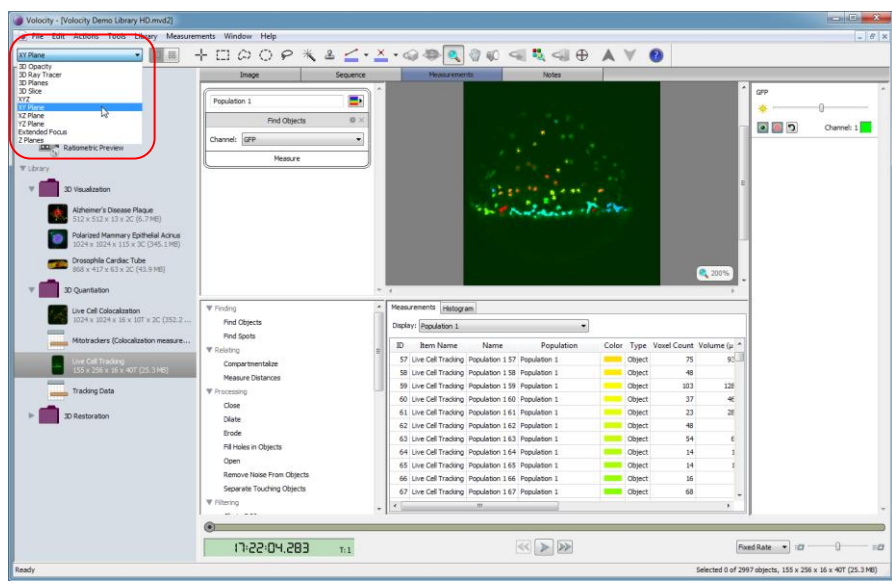
In this example, some of the objects formed are too large, they are actually two or more objects currently identified as one. Use the task "**Separate Touching Objects**" to improve this situation. This task will be applied to the previously selected population, so make sure that it is dragged into the series and contained within the same colored bubble.
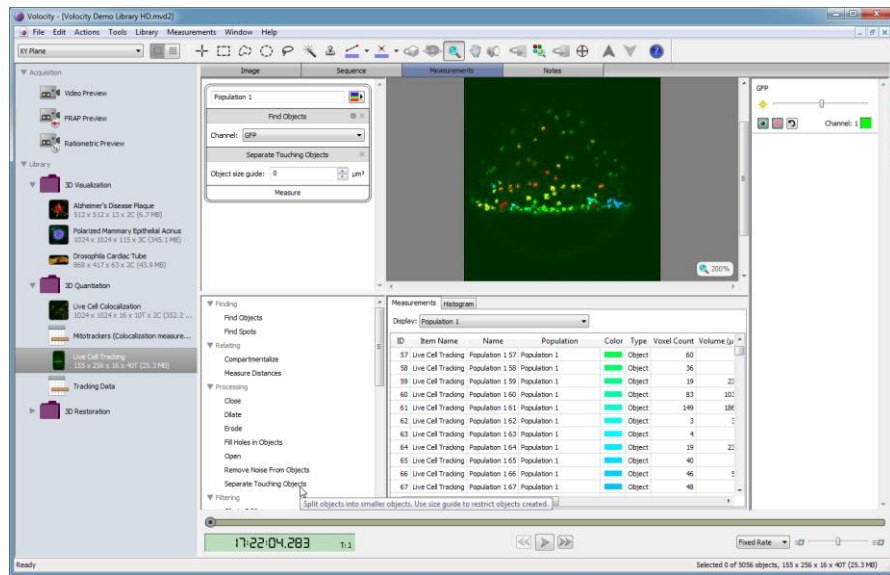

The Object size guide, shown in the "**Separate Touching Objects**" task box, can be set to the approximate size of the smallest object that can be created by the separating step. In this example the Object size guide is 0 μm^3, the best separation is achieved by not restricting the size of objects that will be made.
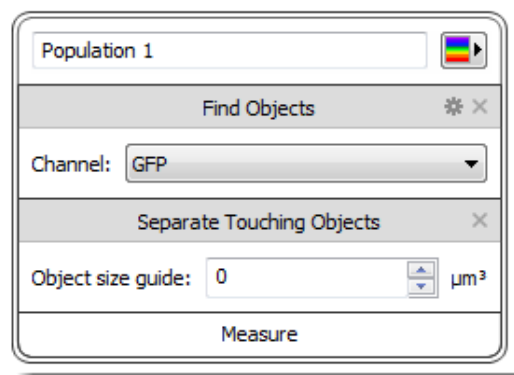

Tracking

Once all objects have been identified in all timepoints they can be connected together by tracks. Add a "**Track**" task to the measurement protocol.

The tracking algorithm uses the centroid measurement for each previously identified object to determine whether there is any movement of objects over time. Tracks are generated by connecting the centroids so as to trace the path of a moving object. The track objects task will always place itself at the bottom of the list of tasks in the protocol since objects must be found before they can be tracked.
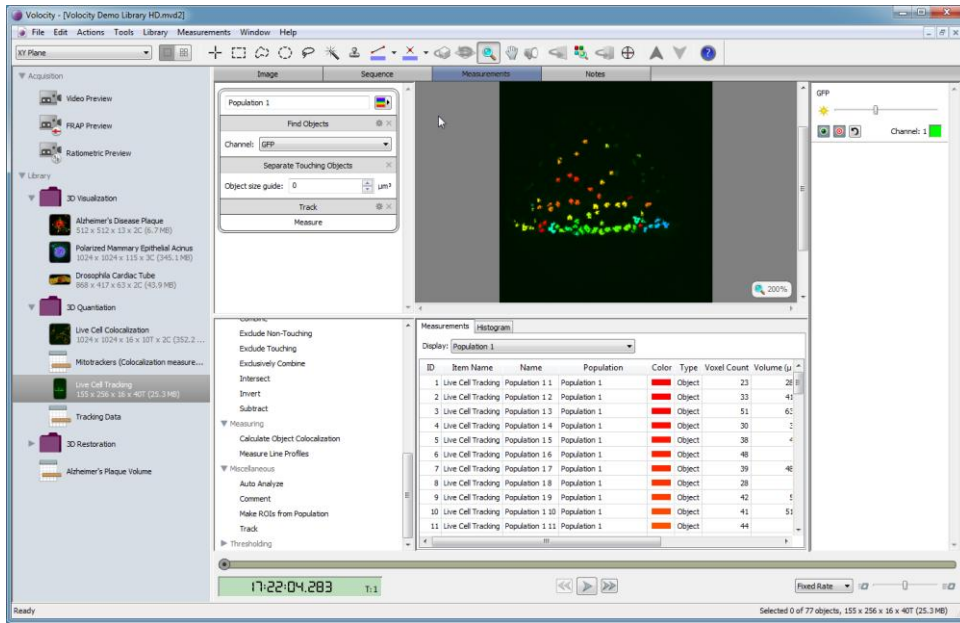
To alter how the tracks are measured, click on the cog icon on the "**Track**" task to access the secondary dialog for this task. For example, it may be necessary to set a maximum distance between objects, in this example 5 µm.
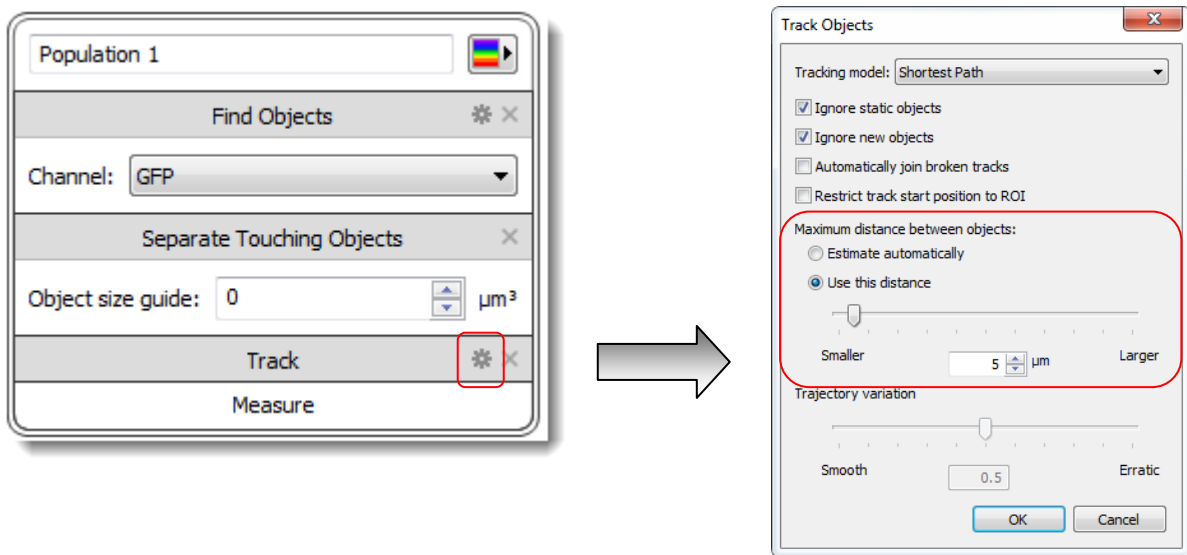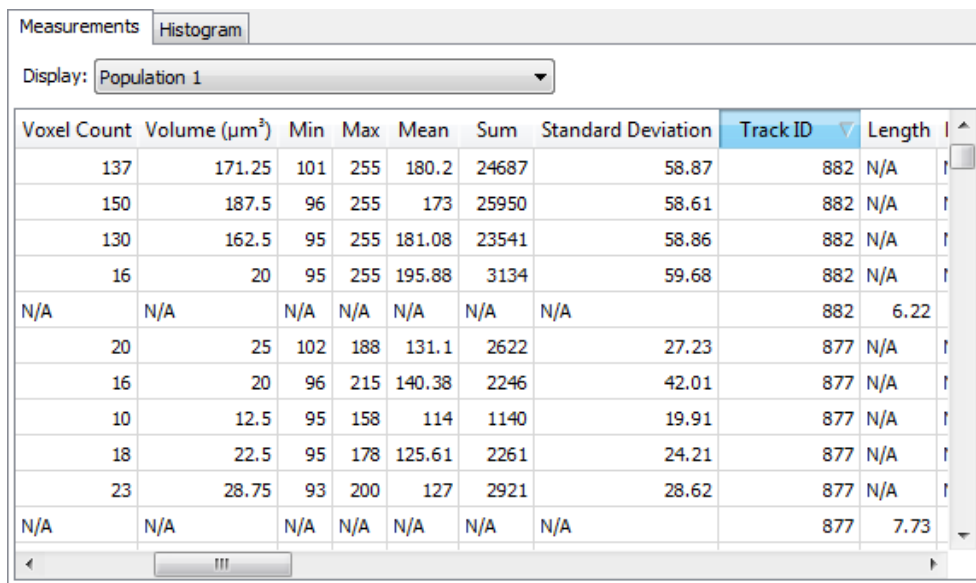
To see the results of the "**Track**" task, all timepoints must be measured. Select **Measure all timepoints** from the Measurements menu.
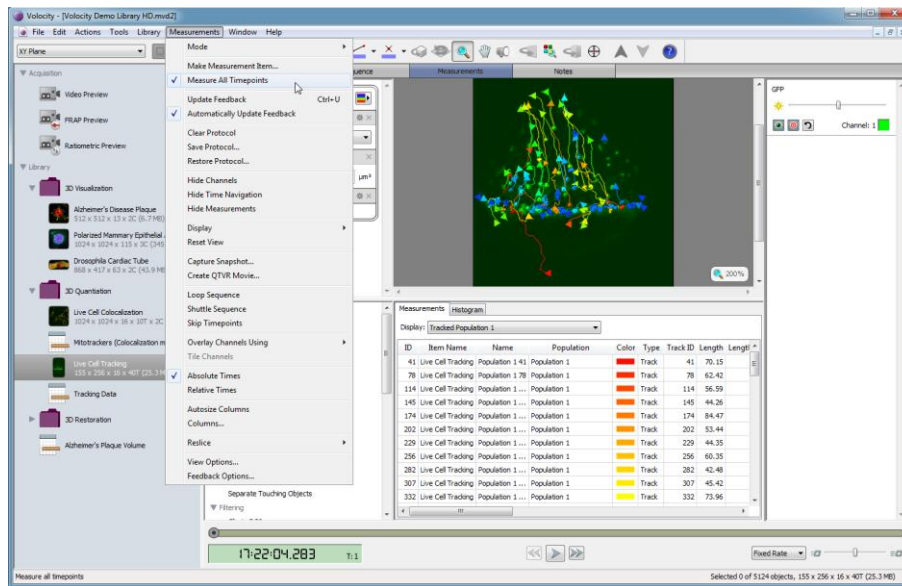
Objects that have been tracked, and are therefore determined to be the same object in different locations are collected in a separate population for tracks. Display this population to see only measurements made on track information. This contains summary information such as track length, the average velocity for the duration of the track, the trajectory and the meandering index.
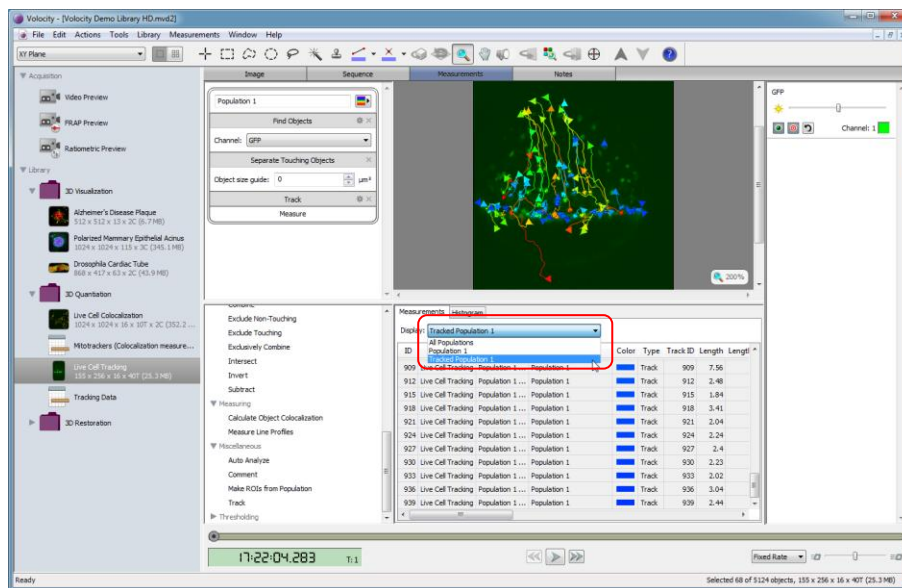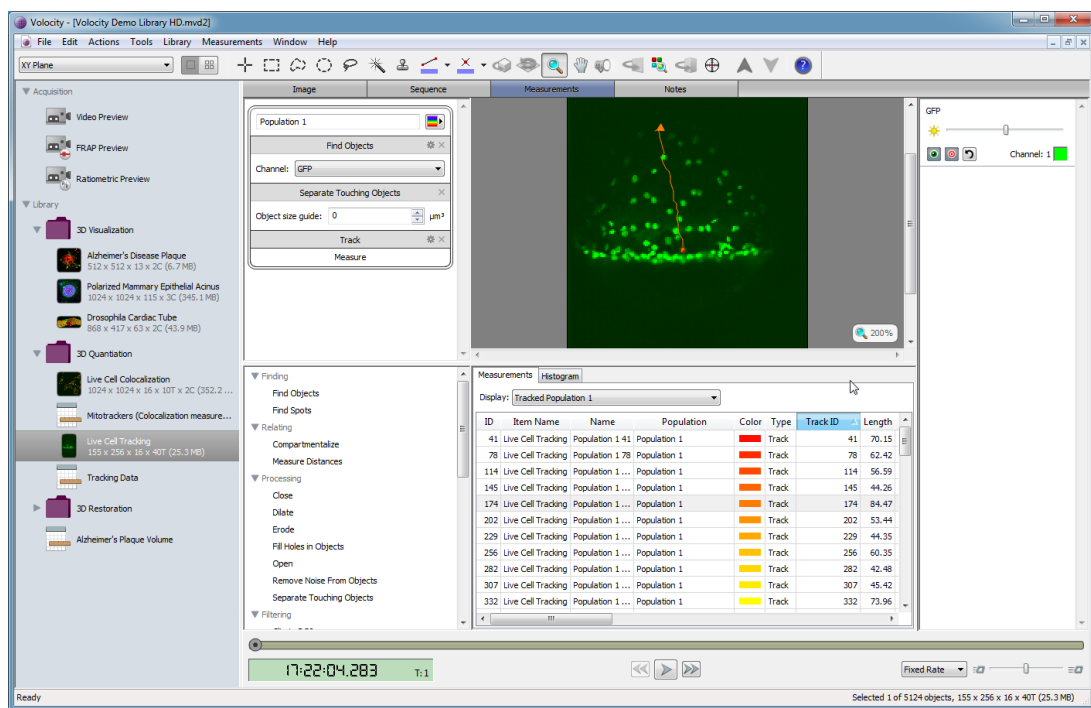
To see individual properties of each cell, and how those properties change over time, display the population and sort by track ID. This will sort the table so that the measurements for each cell in each timepoint, and the track, are listed in order.


Now that the objects have been measured and tracked, we should confirm these tracking results by examining individual tracks. Select a row (shift-click to select multiple rows) in the table, representing a track, to show the individual overlay of that track on the image.
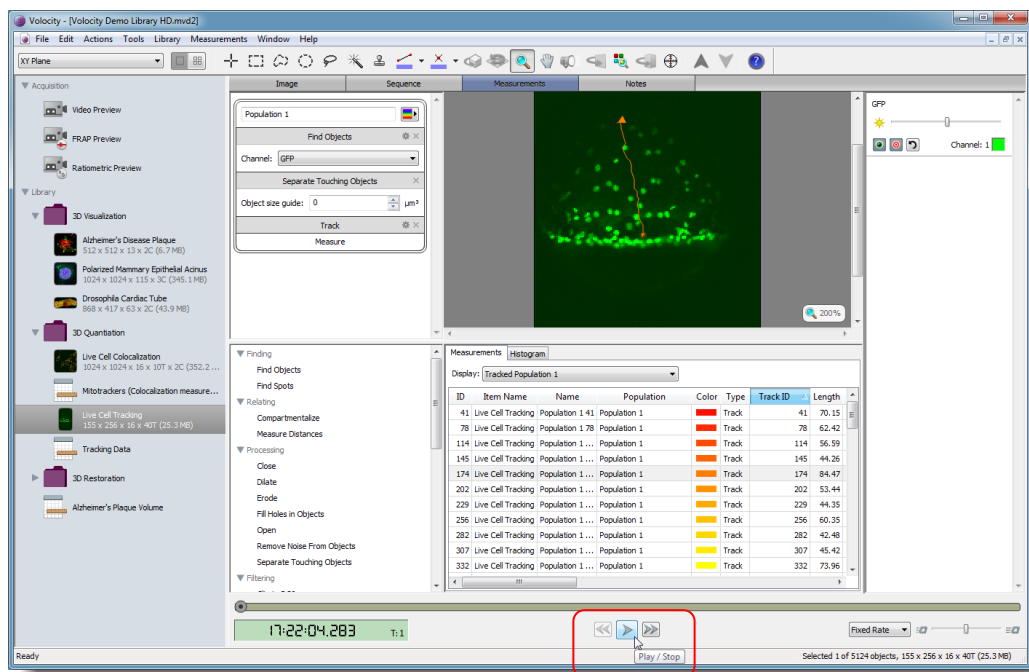
Showing object feedback for the *current* timepoint only can assist in understanding what is being shown. To adjust the feedback that is displayed on the image, select **Feedback Options...** from the Measurements menu.
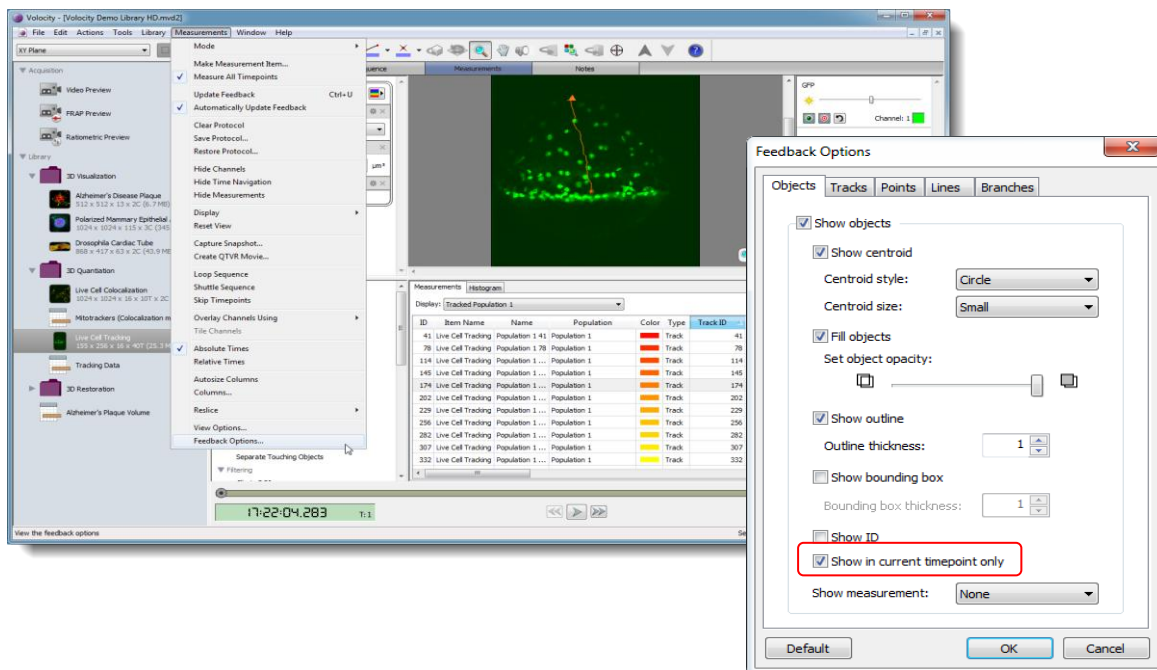
Use the time navigation controls to compare the feedback with the underlying image data.


The most likely problem with tracks is caused by setting the wrong maximum distance between objects in the secondary dialogue of the "**Track**" task (as discussed previously). If tracks are incorrect because they switch

to different objects part way through the time series, the maximum distance assigned is too great. If tracks are incorrect because they do not follow an object far enough in the time course, the maximum distance may be too small. Adjust as appropriate.

Analysis of tracking data

To easily extract what is of particular interest from this wealth of information, you may store all the measurements, in table format, as a separate Measurement Item within the library, and then perform further analysis.

Select **Make Measurement Item…** from the Measurements menu, remembering to select **Measure All Timepoints** when prompted.
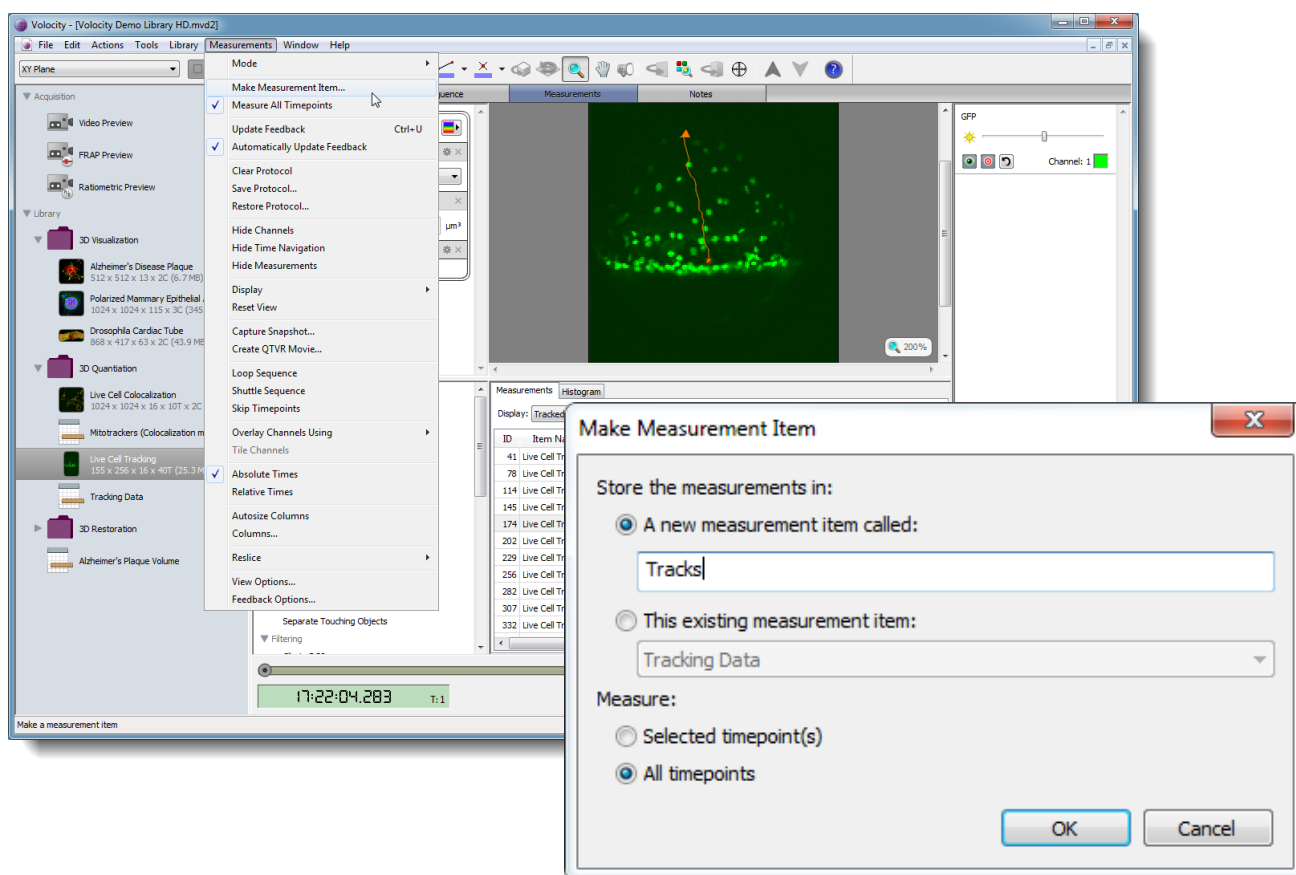
The measurement item has different tabs, such as **Raw**, **Analysis** and **Chart**. The raw view shows the same information for objects and tracks as was visible in the measurements view. Click on the Chart tab to immediately show the default representation of track information.
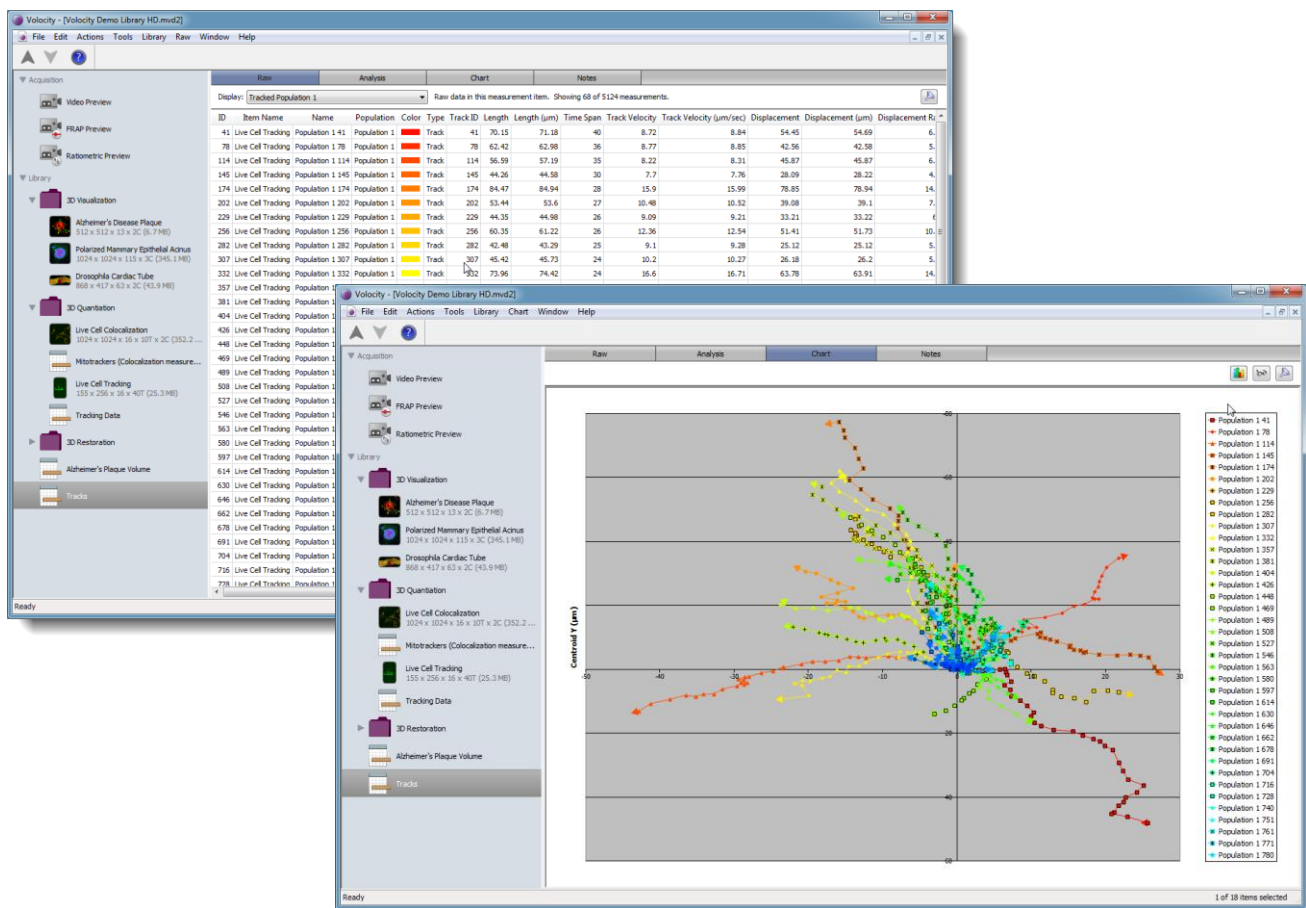
Raw tables, analysis tables and charts, created within the resulting Measurement Item, can all be viewed in Volocity or exported as text or image files.

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