

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.

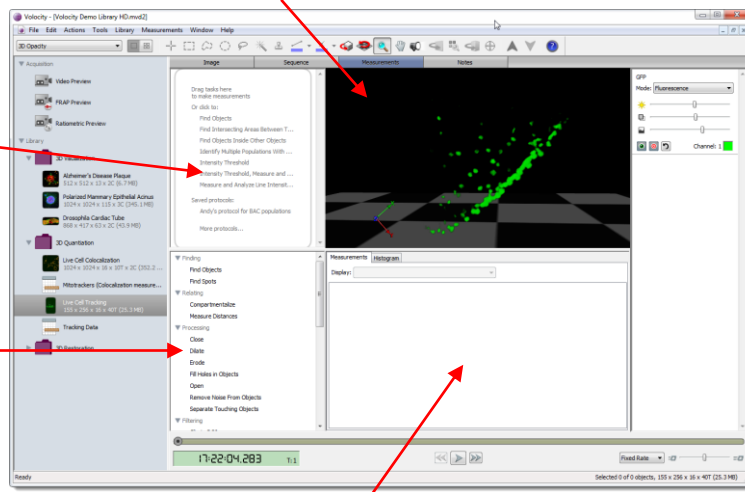
Finding objects

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

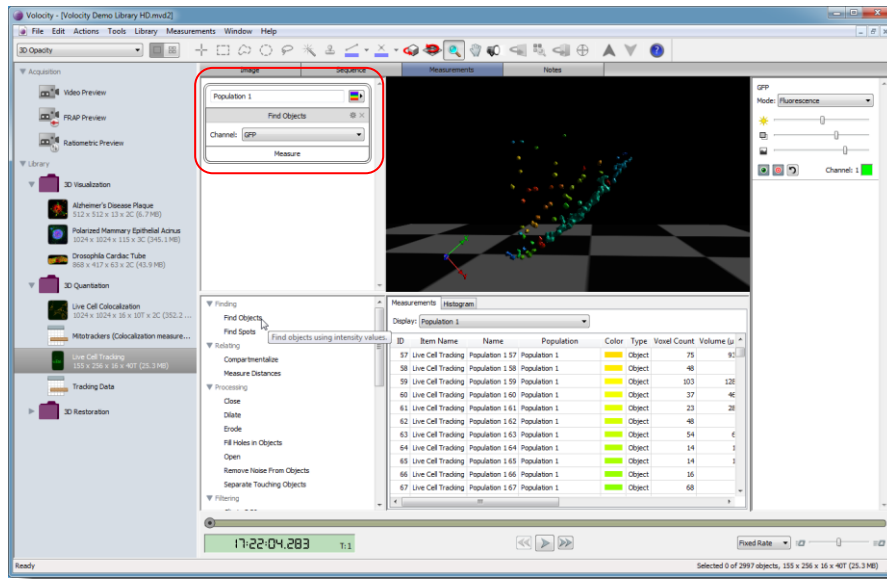


Measurements are shown here as a table or histogram

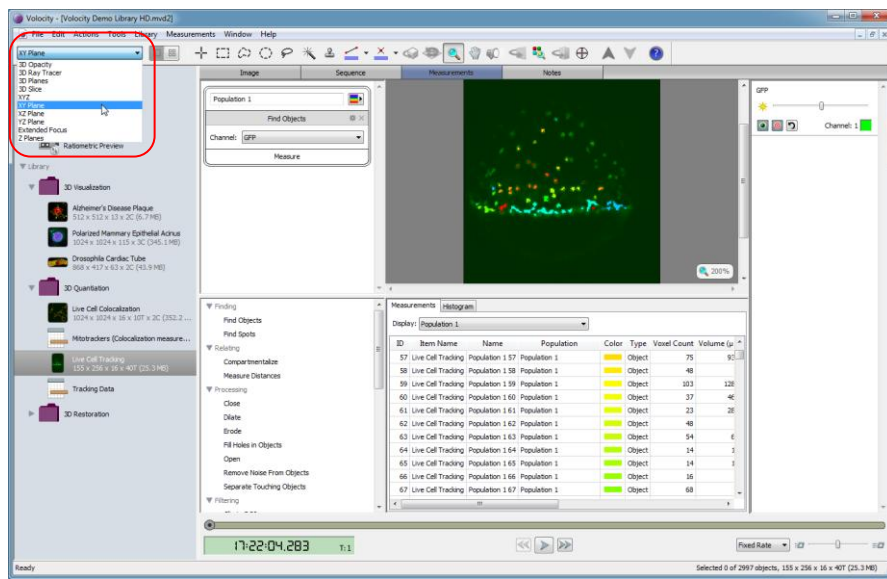
It is important that the Measurement Protocol identifies objects as accurately as possible in each timepoint as this will be essential for the tracking algorithm.

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.

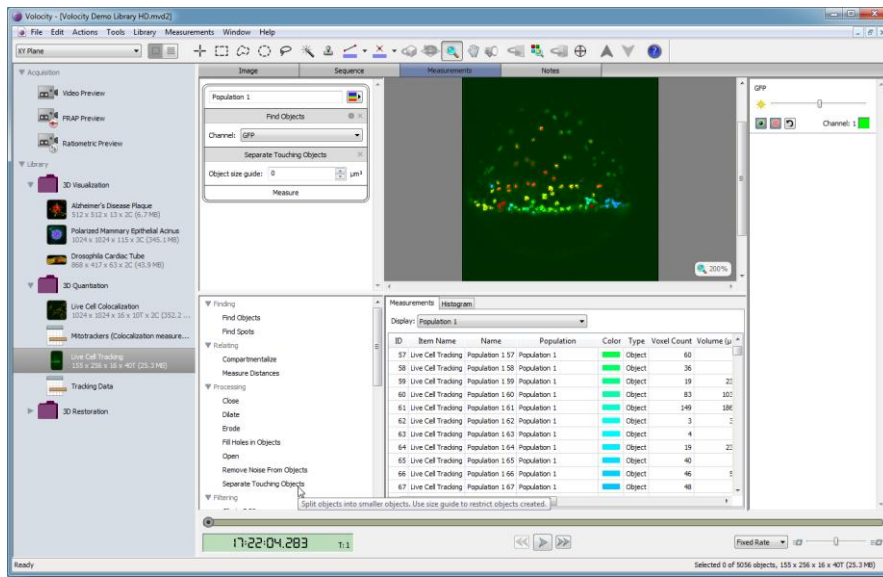
The task **“Find Objects”** is designed to operate even in these challenging conditions. Drag this task to the protocol pane above.



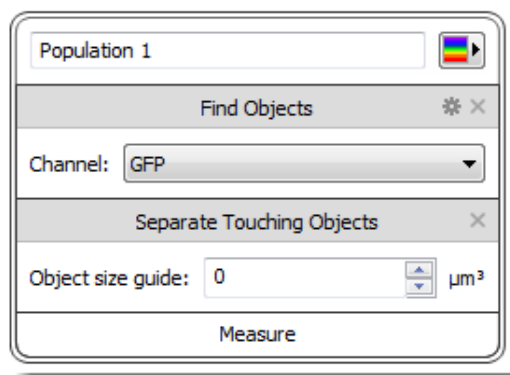
Where intensities are found within range a colored overlay is applied. Groups of selected intensities form objects. View the image, with object overlays, in different ways by changing the mode of view in the top left.



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.



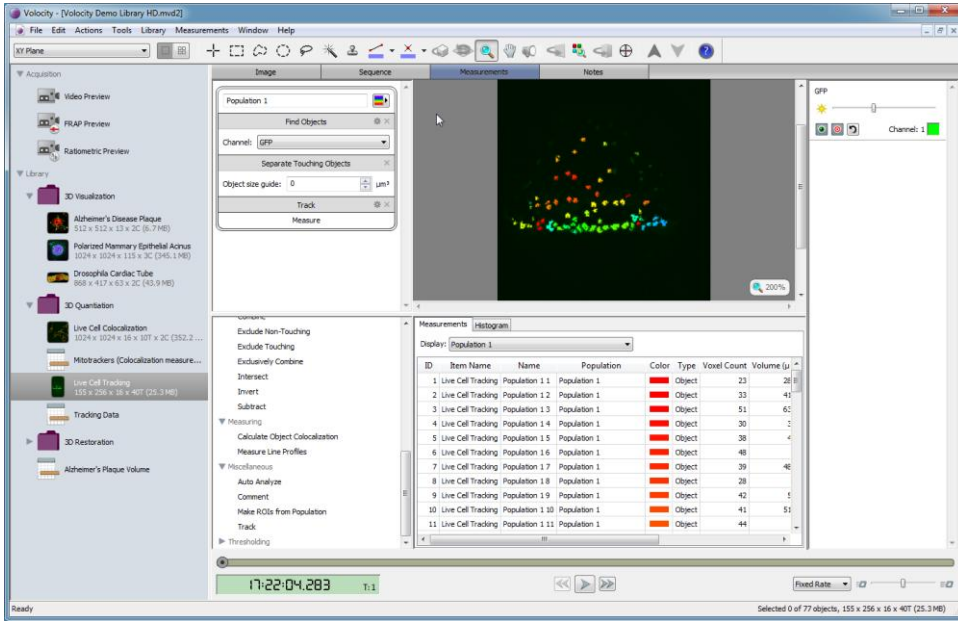
The Object size guide, shown in the **“Separate Touching Objects”** task box, can be set to the approximate size of the smallest object that can be created by the separating step. In this example the Object size guide is 0 μm^3 , the best separation is achieved by not restricting the size of objects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



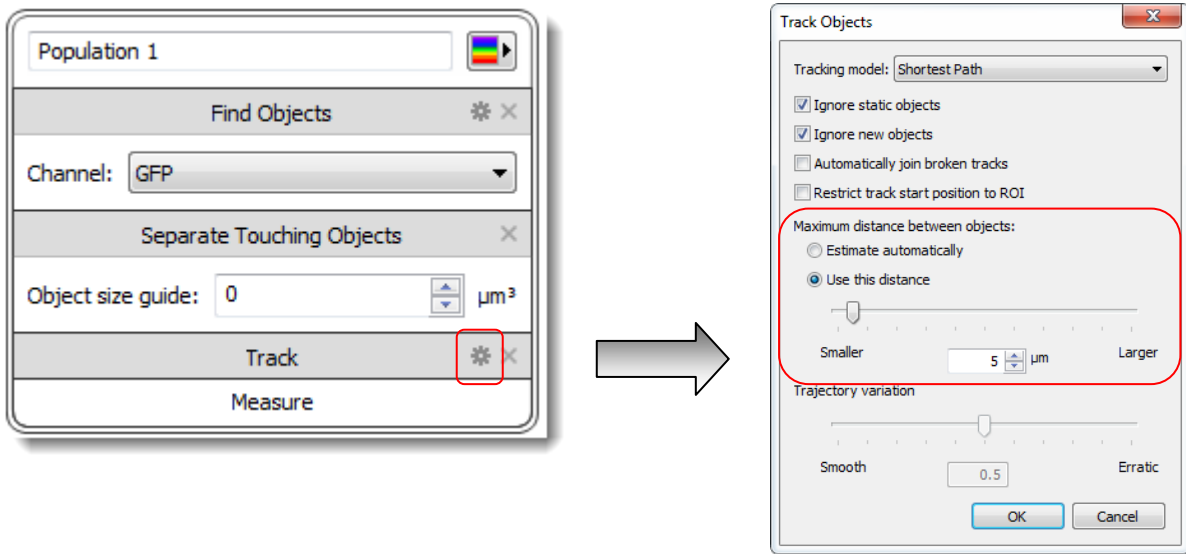
Tracking

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

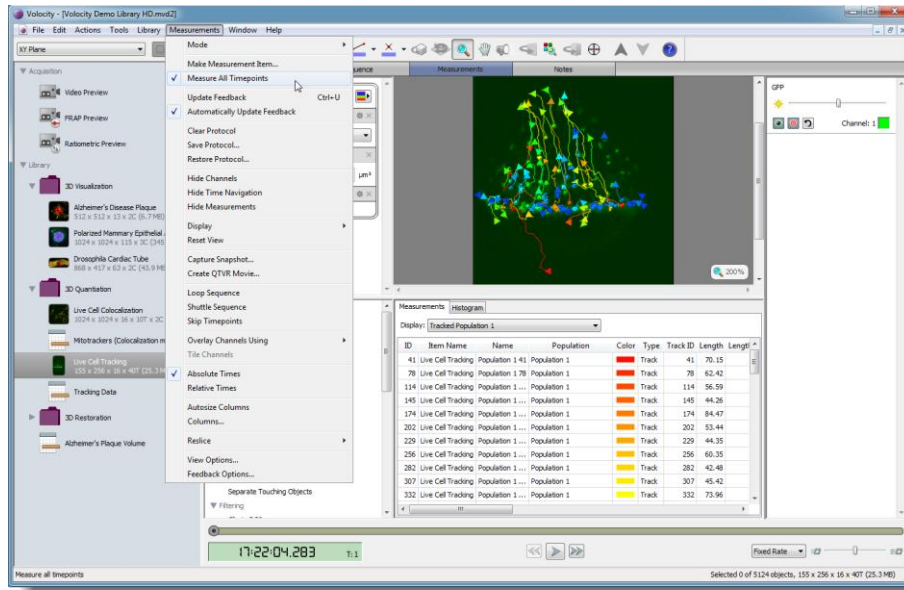
The tracking algorithm uses the centroid measurement for each previously identified object to determine whether there is any movement of objects over time. Tracks are generated by connecting the centroids so as to trace the path of a moving object. The track objects task will always place itself at the bottom of the list of tasks in the protocol since objects must be found before they can be tracked.



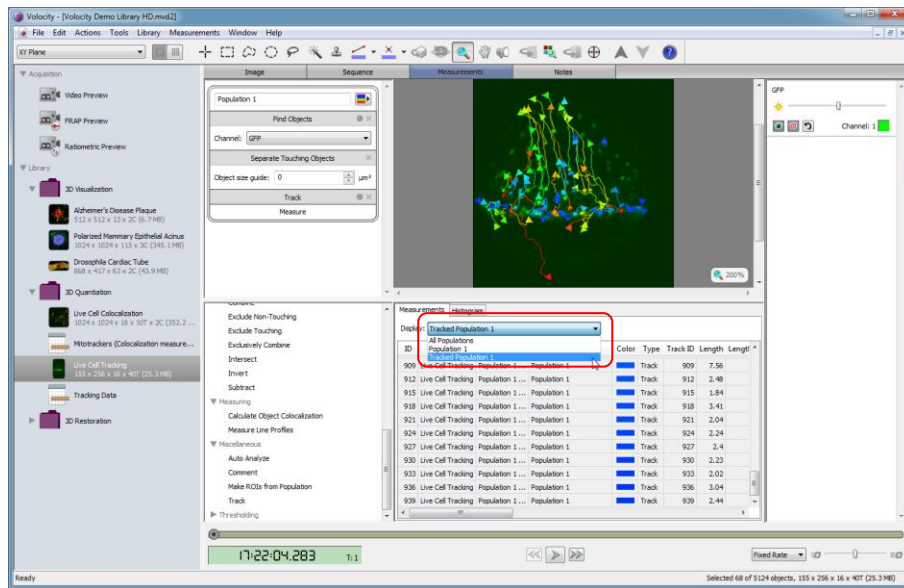
To alter how the tracks are measured, click on the cog icon on the “Track” task to access the secondary dialog for this task. For example, it may be necessary to set a maximum distance between objects, in this example 5 μm.



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.

Voxel Count	Volume (μm^3)	Min	Max	Mean	Sum	Standard Deviation	Track ID	Length
137	171.25	101	255	180.2	24687	58.87	882	N/A
150	187.5	96	255	173	25950	58.61	882	N/A
130	162.5	95	255	181.08	23541	58.86	882	N/A
16	20	95	255	195.88	3134	59.68	882	N/A
N/A	N/A	N/A	N/A	N/A	N/A	N/A	882	6.22
20	25	102	188	131.1	2622	27.23	877	N/A
16	20	96	215	140.38	2246	42.01	877	N/A
10	12.5	95	158	114	1140	19.91	877	N/A
18	22.5	95	178	125.61	2261	24.21	877	N/A
23	28.75	93	200	127	2921	28.62	877	N/A
N/A	N/A	N/A	N/A	N/A	N/A	N/A	877	7.73

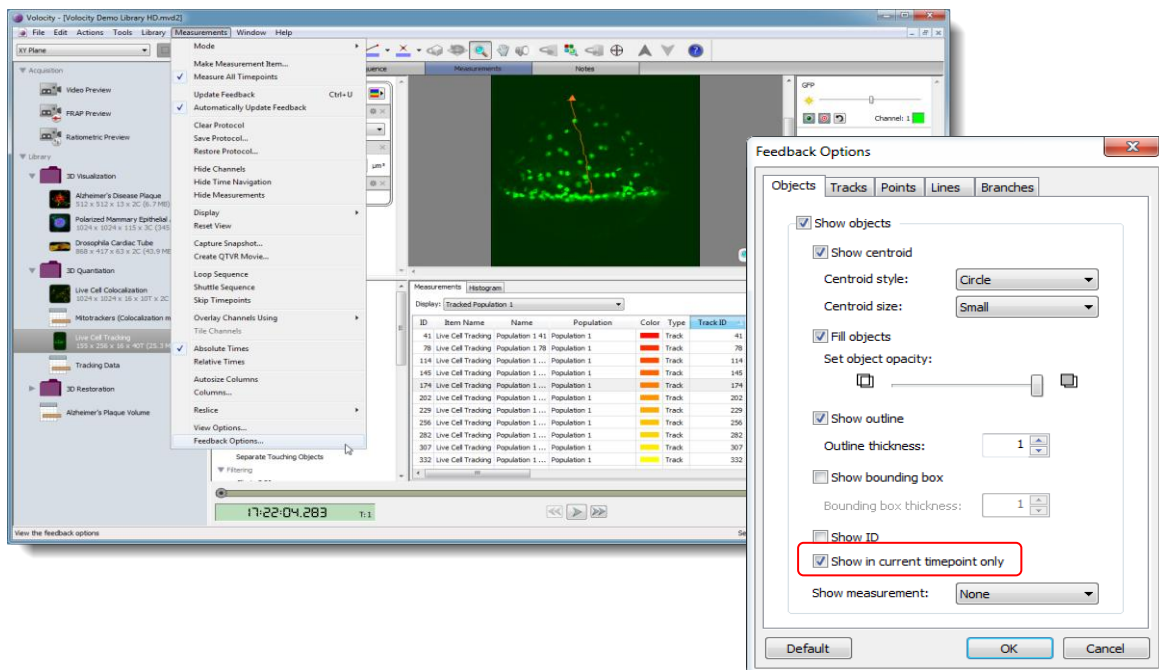
Now that the objects have been measured and tracked, we should confirm these tracking results by examining individual tracks. Select a row (shift-click to select multiple rows) in the table, representing a track, to show the individual overlay of that track on the image.

The screenshot shows the Velocity software interface with the following components:

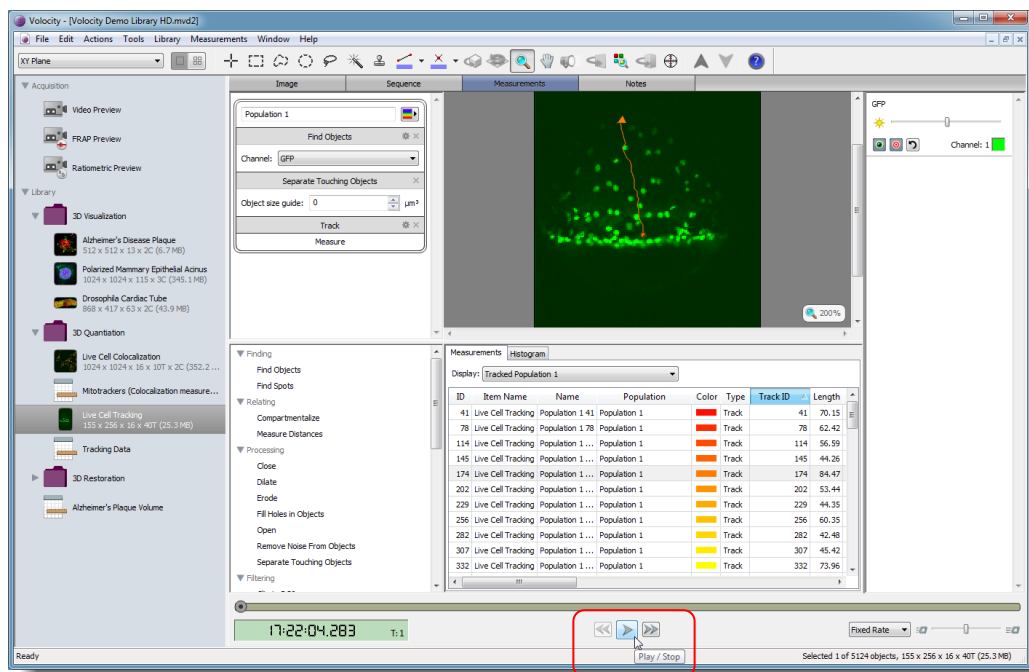
- Left Panel:** Library of 3D models including Alzheimer's Disease Plaque, Polarized Mammary Epithelial Acinus, Drosophila Cardiac Tube, Live Cell Colocalization, Mitotracker, Live Cell Tracking, and 3D Restoration.
- Top Panel:** Acquisition, Image, Sequence, Measurements, and Notes tabs. The Measurements tab is active, showing 'Population 1' with 'Channel: GFP' and 'Object size guide: 0 μm^3 '.
- Center Panel:** A 3D visualization of green fluorescent cells with an orange track overlaid on one of them.
- Bottom Panel:** A detailed table of track data. The table is sorted by Track ID.

ID	Item Name	Name	Population	Color	Type	Track ID	Length
41	Live Cell Tracking	Population 1 41	Population 1	Red	Track	41	70.15
78	Live Cell Tracking	Population 1 78	Population 1	Red	Track	78	62.42
114	Live Cell Tracking	Population 1 ...	Population 1	Orange	Track	114	56.59
145	Live Cell Tracking	Population 1 ...	Population 1	Orange	Track	145	44.26
174	Live Cell Tracking	Population 1 ...	Population 1	Orange	Track	174	84.47
202	Live Cell Tracking	Population 1 ...	Population 1	Orange	Track	202	53.44
229	Live Cell Tracking	Population 1 ...	Population 1	Orange	Track	229	44.35
256	Live Cell Tracking	Population 1 ...	Population 1	Yellow	Track	256	60.35
282	Live Cell Tracking	Population 1 ...	Population 1	Yellow	Track	282	42.48
307	Live Cell Tracking	Population 1 ...	Population 1	Yellow	Track	307	45.42
332	Live Cell Tracking	Population 1 ...	Population 1	Yellow	Track	332	73.96
- Status Bar:** Shows '17:22:04.283 T:1' and 'Selected 1 of 5124 objects, 155 x 256 x 16 x 40T (25.3 MB)'.

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.



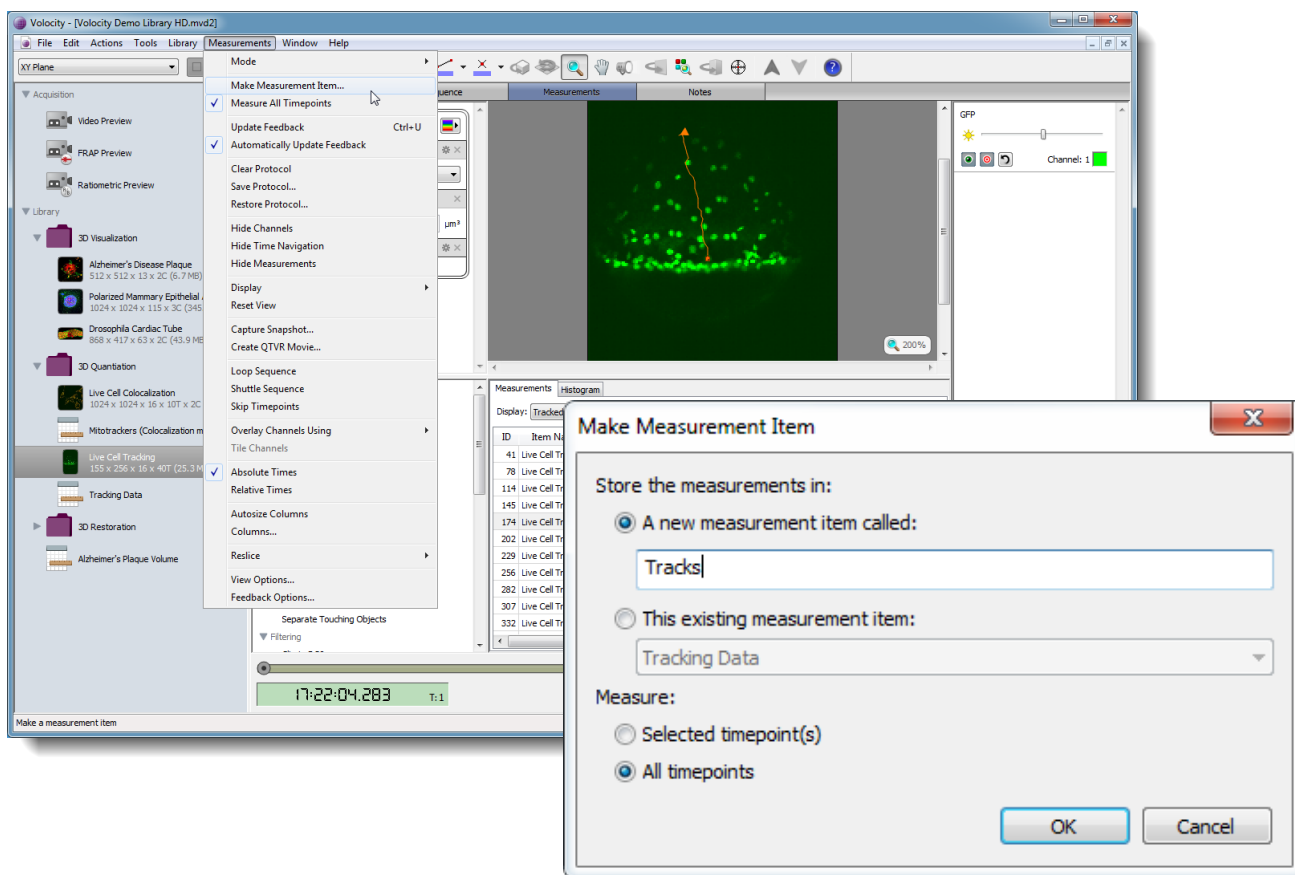
The most likely problem with tracks is caused by setting the wrong maximum distance between objects in the secondary dialogue of the "Track" task (as discussed previously). If tracks are incorrect because they switch

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.

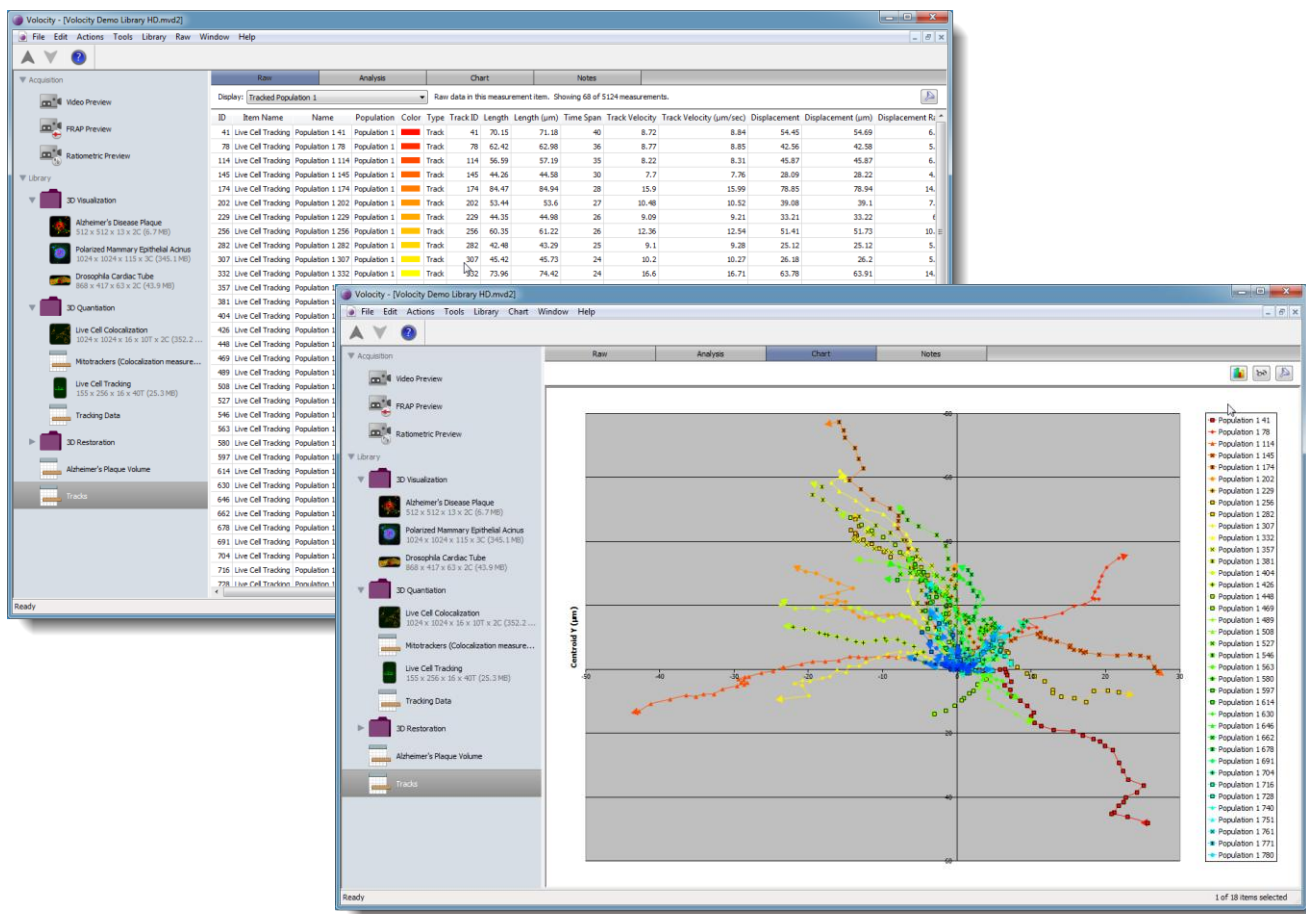
Analysis of tracking data

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 Velocity or exported as text or image files.

PerkinElmer, Inc.
 940 Winter Street
 Waltham, MA 02451 USA
 P: (800) 762-4000 or
 (+1) 203-925-4602
www.perkinelmer.com



For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

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