

TUTORIAL NOTE

Cellular Imaging and Analysis

Velocity Tutorial Colocalization

This tutorial demonstrates how to use the Colocalization View in Velocity[®]. The theory of Colocalization analysis is discussed in more detail in the online tutorial: *Thresholded Pearson's Correlation Coefficient (PCC) Analysis*.

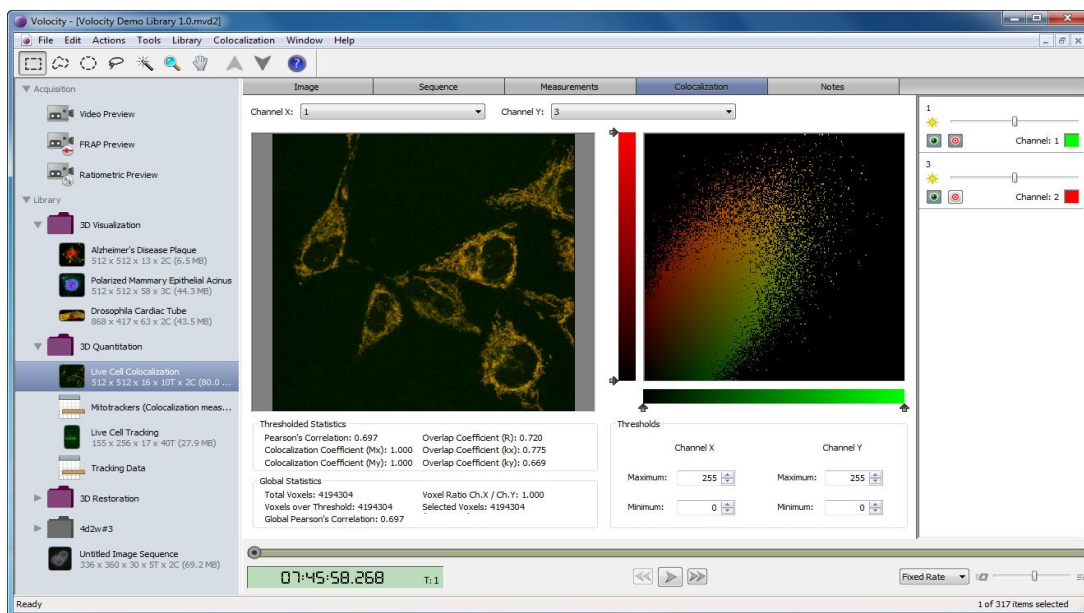
Data

Live Cell Colocalization

Dataset courtesy of Chris Guerin and Sam Knoppen, VIB, University of Gent.

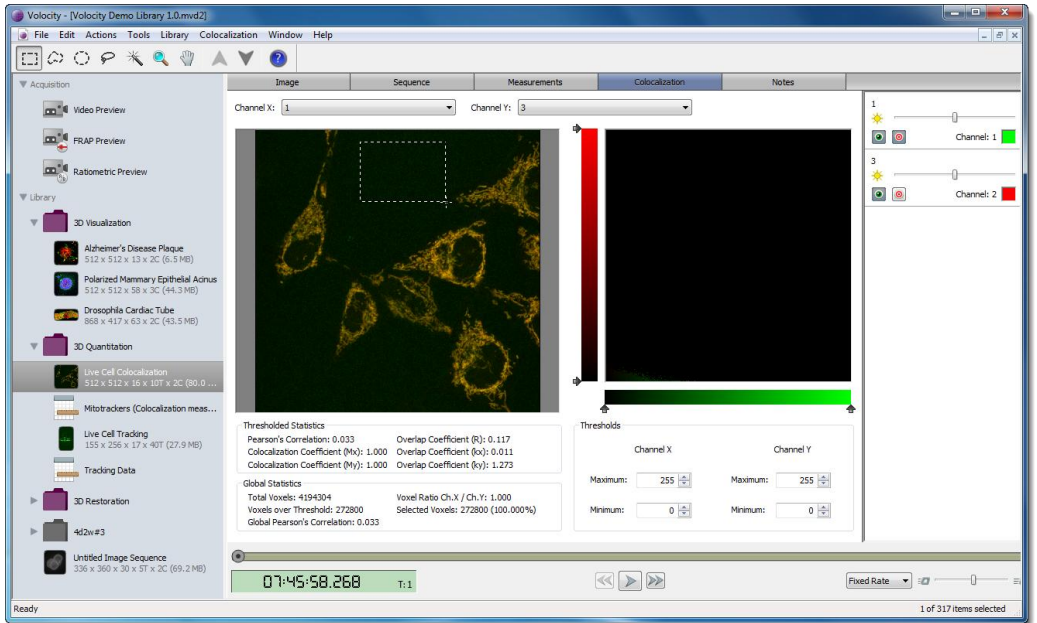
Workflow

Click once on the data in the library list and click on the Colocalization tab to display the Colocalization View. Please note that this example dataset has multiple timepoints. In the Colocalization View, select the channels to be analyzed from the pop-up menus at the top of the screen. A preview of the two channels overlaid is shown on the left. On the right, a scatter plot shows intensities from these two channels. Global and Thresholded statistics are displayed below the image, and on the right is the area for setting threshold values.

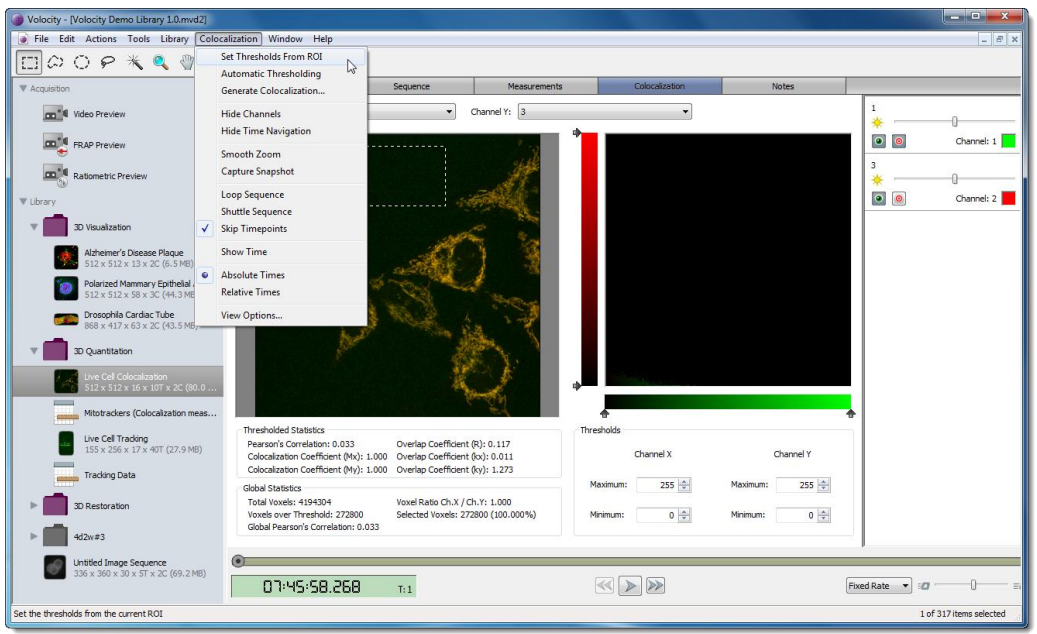


First set thresholds for each channel. There are three methods for setting thresholds in Velocity, one of which involves drawing a Region of Interest (ROI), and this is discussed in more detail below. The other methods are beyond the scope of this tutorial.

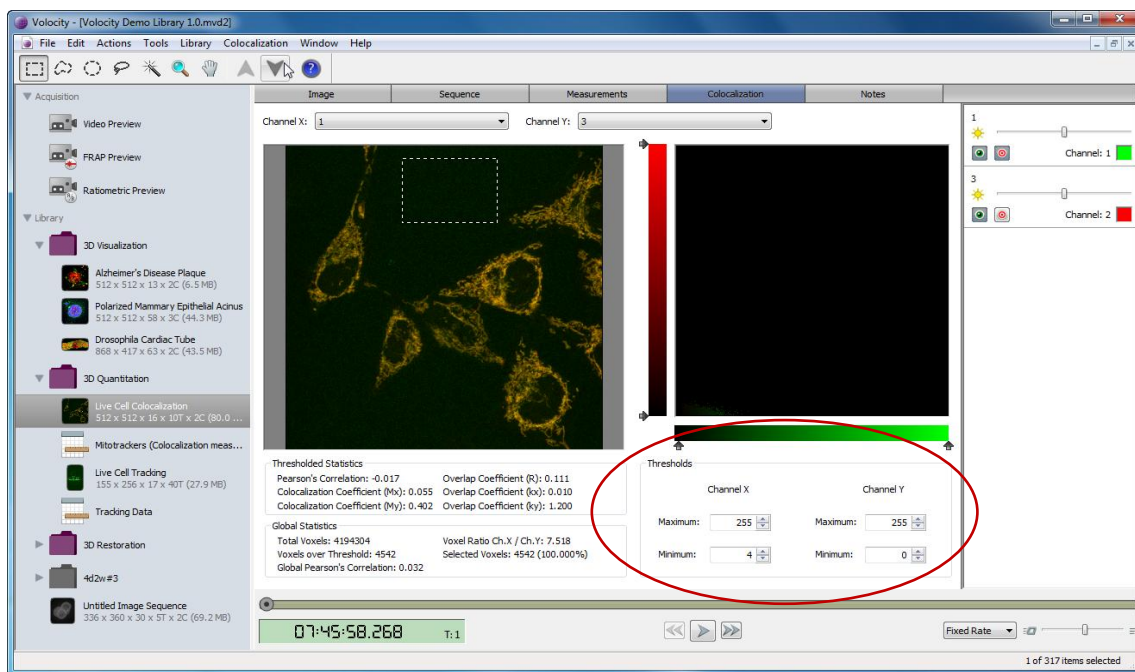
Select one of the ROI tools from the toolbar at the top of the screen and draw a ROI in an area of the image considered background.



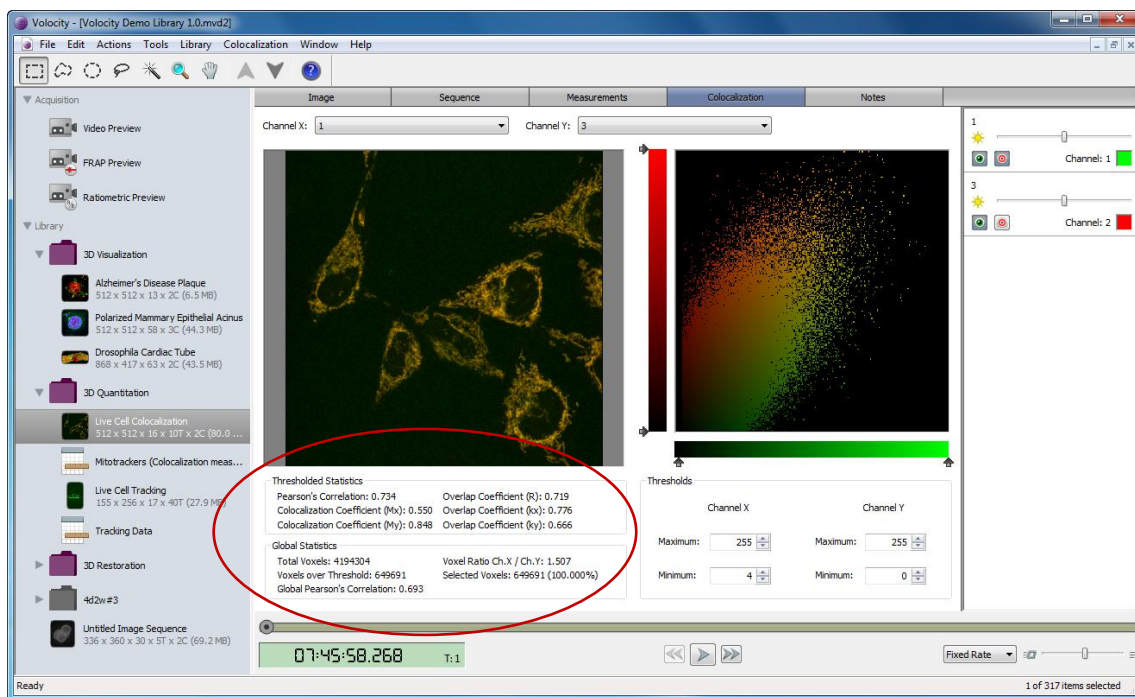
In the Colocalization menu select **Set Thresholds From ROI**.



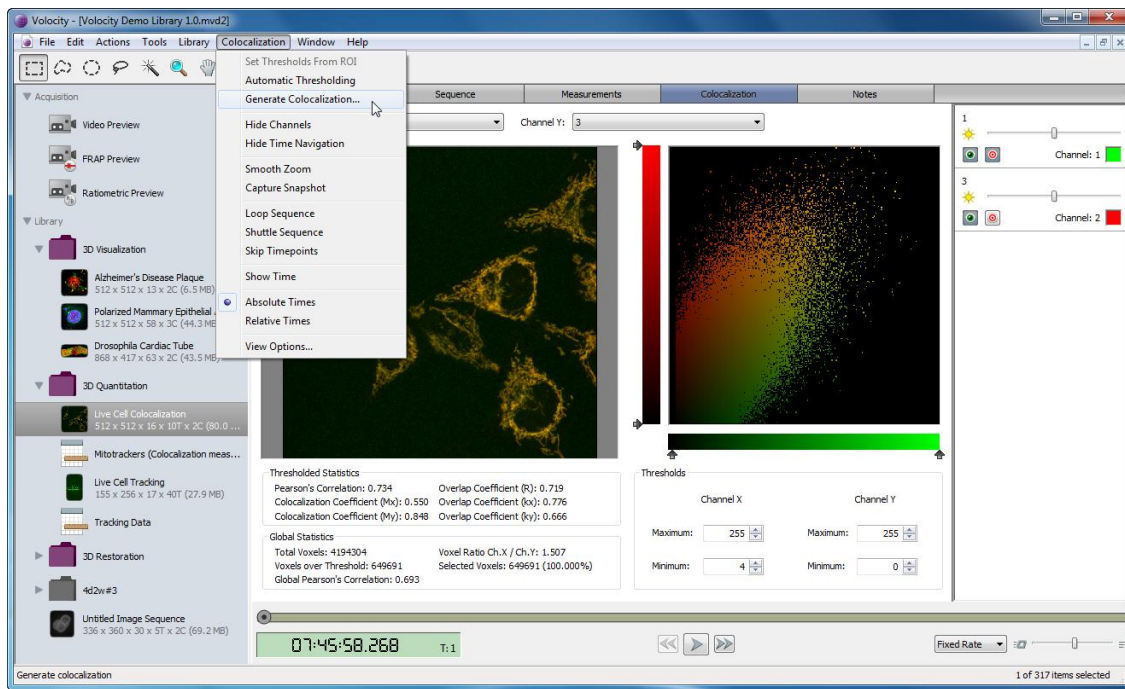
Threshold values for each channel are set as the mean intensity plus three standard deviations for that ROI.



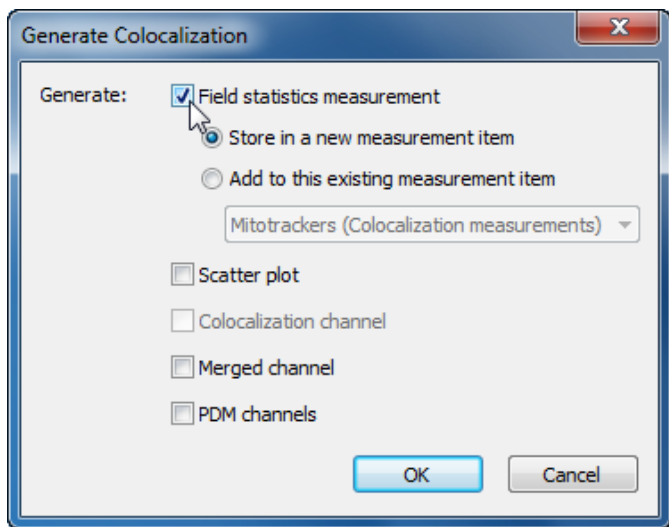
Click outside the ROI to view the statistics and the scatter plot for the whole image. Thresholded and Global statistics are displayed in the panel below the image. These statistics are discussed in more detail in the video tutorial: *Thresholded Pearson's Correlation Coefficient (PCC) Analysis*.



A table of these statistics can be created in the library, called a Measurement Item, by selecting **Generate Colocalization...** from the Colocalization menu.



Generate "Field statistics measurements" to add the statistics from the Colocalization view to a new or existing measurement item.



The Measurement Item includes field statistics for every timepoint in the dataset. The threshold values used are also recorded in the table for reference.

Name	Type	ID	Item Name	Timepoint	Abs. Time	Rel. Time (s)	Color	Pearsons Correlation	Colocalization Coefficient Mx	Colocalization Coeff
Colocalization Object 1	Object	1	Live Cell Colocalization	1 07:45:58.268 on 10/08/2009		0		0.733718	0.549588	
Colocalization Object 2	Object	2	Live Cell Colocalization	2 07:48:44.116 on 10/08/2009	165.847375	165.847375		0.777518	0.602623	
Colocalization Object 3	Object	3	Live Cell Colocalization	3 07:51:29.959 on 10/08/2009	331.690375	331.690375		0.793072	0.636438	
Colocalization Object 4	Object	4	Live Cell Colocalization	4 07:54:15.804 on 10/08/2009	497.535375	497.535375		0.787772	0.620945	
Colocalization Object 5	Object	5	Live Cell Colocalization	5 07:57:01.649 on 10/08/2009	663.380375	663.380375		0.809115	0.658704	
Colocalization Object 6	Object	6	Live Cell Colocalization	6 07:59:47.492 on 10/08/2009	829.223375	829.223375		0.828083	0.699376	
Colocalization Object 7	Object	7	Live Cell Colocalization	7 08:02:33.337 on 10/08/2009	995.068375	995.068375		0.818271	0.683103	
Colocalization Object 8	Object	8	Live Cell Colocalization	8 08:05:19.182 on 10/08/2009	1160.913375	1160.913375		0.825589	0.698174	
Colocalization Object 9	Object	9	Live Cell Colocalization	9 08:08:05.036 on 10/08/2009	1326.767562	1326.767562		0.833133	0.737802	
Colocalization Object 10	Object	10	Live Cell Colocalization	10 08:10:50.885 on 10/08/2009	1492.616375	1492.616375		0.814789	0.723542	

If you are interested in finding out more information on the statistics used for Colocalization in Velocity, please see the following papers:

Manders MM, Verbeek PJ and Aten JA (1993). Measurement of colocalization of objects in dual color confocal images. *Journal of Microscopy*. 169: 375-382.

Costes SV, Daelemans D, Cho EH, Dobbin Z, Pavlakis G and Lockett S (2004). Automatic and Quantitative Measurement of Protein-Protein Colocalization in Live Cells. *Biophysical Journal*. 86: 3339-4003.

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