

When do you typically use background subtraction for a kymograph

Question

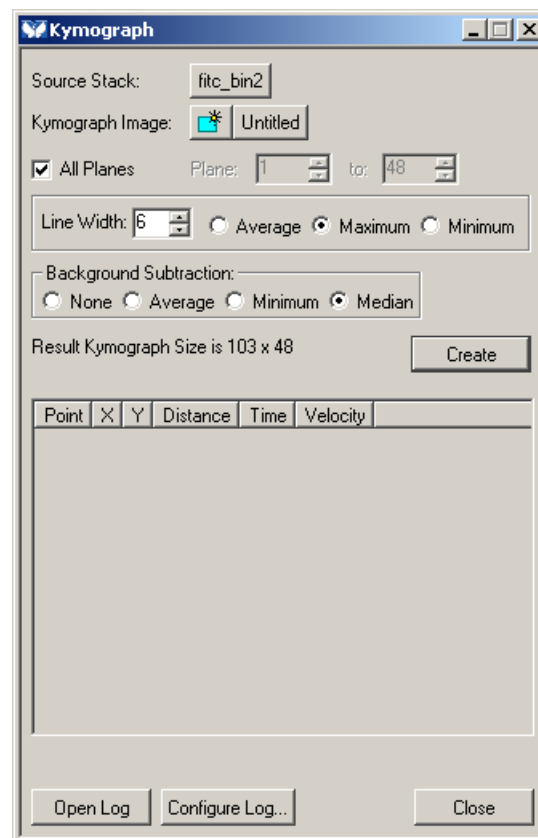
Kymograph allows you to set up background subtraction. When do you typically use the background subtraction? And is that subtracting the value (minimum, average or maximum) of the line from all pixels when it builds the kymograph.

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Answer

You use the background subtraction to deal with un-evenness along the line that does not change. This is most likely due to non-motile objects or shading in the image.

Background subtraction takes the Average, Minimum, or Maximum of each column in the kymograph and subtracts it from that column.

You should not use background subtraction if you see light changes over time due to photobleaching, production of changes in fluorescence, flickering of the light source etc. as an incorrect value will be subtracted.

You should use minimum if your objects of interest are bright and maximum if your objects are dark. If your objects of interest are both bright and dark (DIC for example) you should use average. If you choose to use average make sure that there are enough planes so that you average out bright or dark objects than move along the line.

An example of using a background to eliminate the vertical lines from the kymograph:

