Localizer – localization microscopy toolkit for Igor Pro

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Step 0: installation

Mac

1. Quit Igor
2. Put the ".xop" file (or an alias/shortcut) in ~/Documents/WaveMetrics/Igor Pro 6 User Files/Igor Extensions
3. Put the ".ipf" file in ~/Documents/WaveMetrics/Igor Procedures
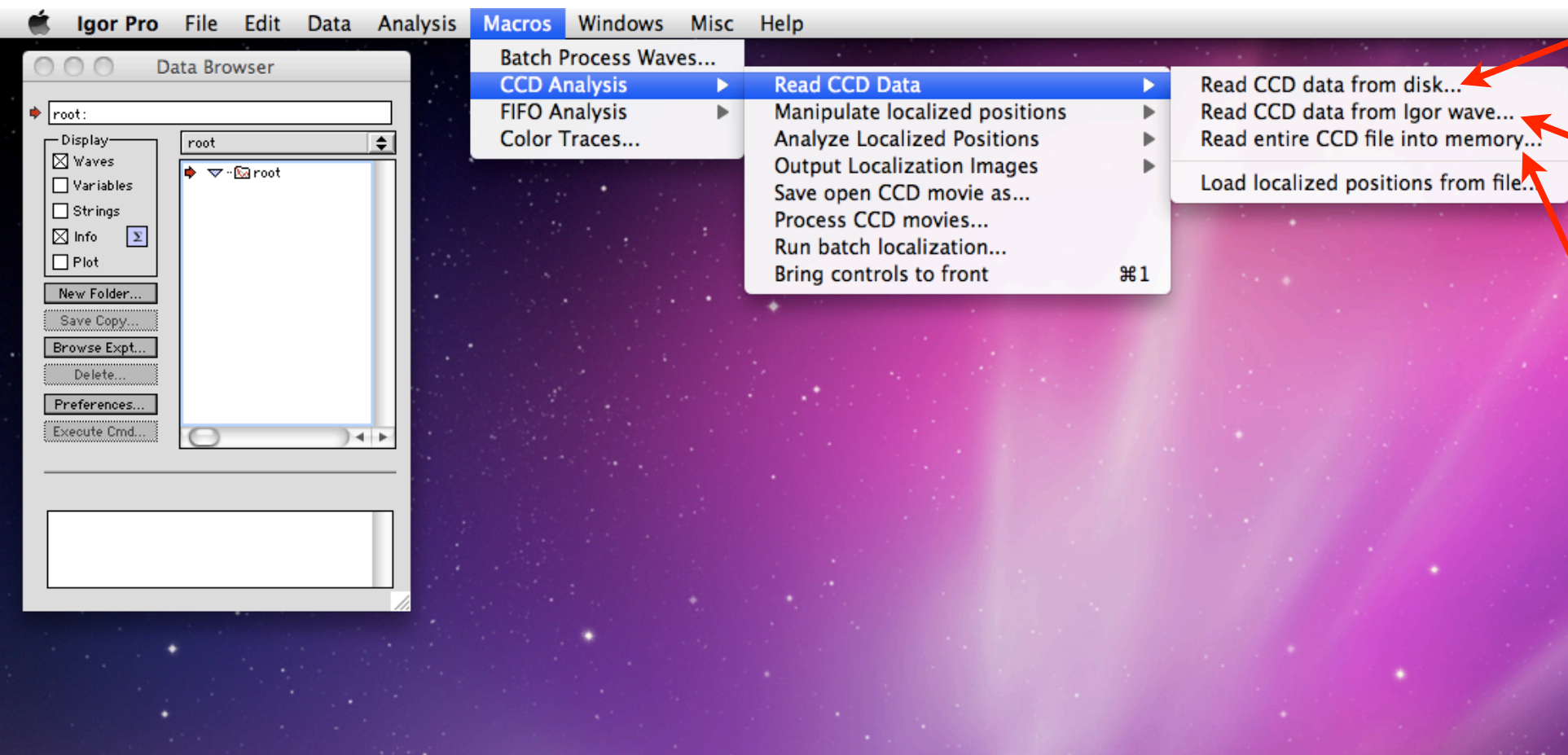4. Launch Igor

Windows

1. Exit Igor
2. Put the ".xop" file (or an alias/shortcut) in My Documents\WaveMetrics\Igor Pro 6 User Files\Igor Extensions
3. Put the ".ipf" file in My Documents\WaveMetrics\Igor Procedures
4. Launch Igor

If Igor reports an error during startup, be sure that you're running the latest version (6.21 at the time of this writing). Update using the free download at www.wavemetrics.net

(Windows only) If you get an "XOP can't initialize itself" message at startup, download and install the Visual Studio 2008 runtime from https://www.microsoft.com/downloads/en/details.aspx?familyid=A5C84275-3B97-4AB7-A40D-3802B2AF5FC2&displaylang=en
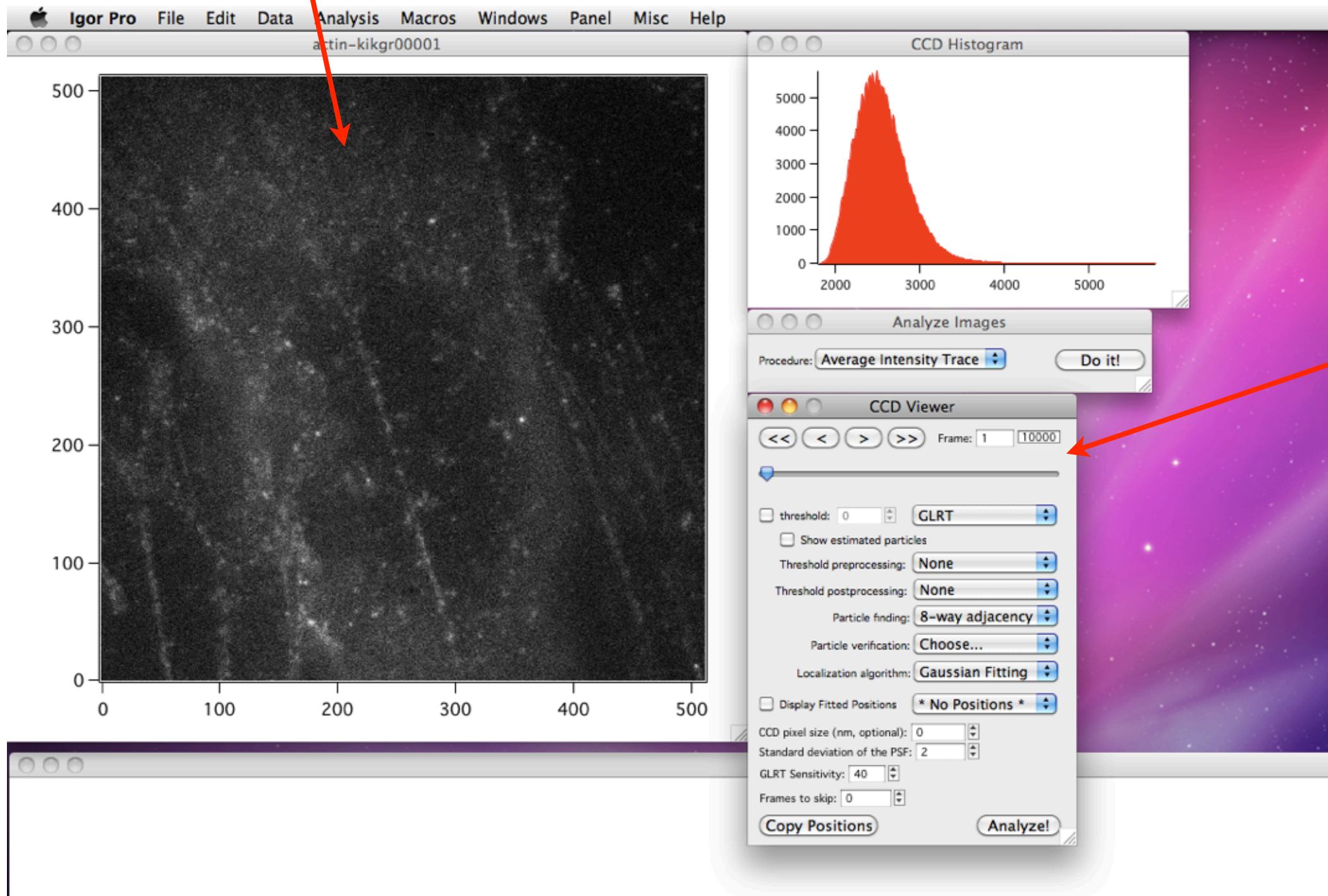
Step 1: open a file containing image data

Use this if your data is in a file

Use this if your data is already loaded in an Igor wave in its entirety

Use this if you want to completely copy the data from hard disk into memory for faster processing
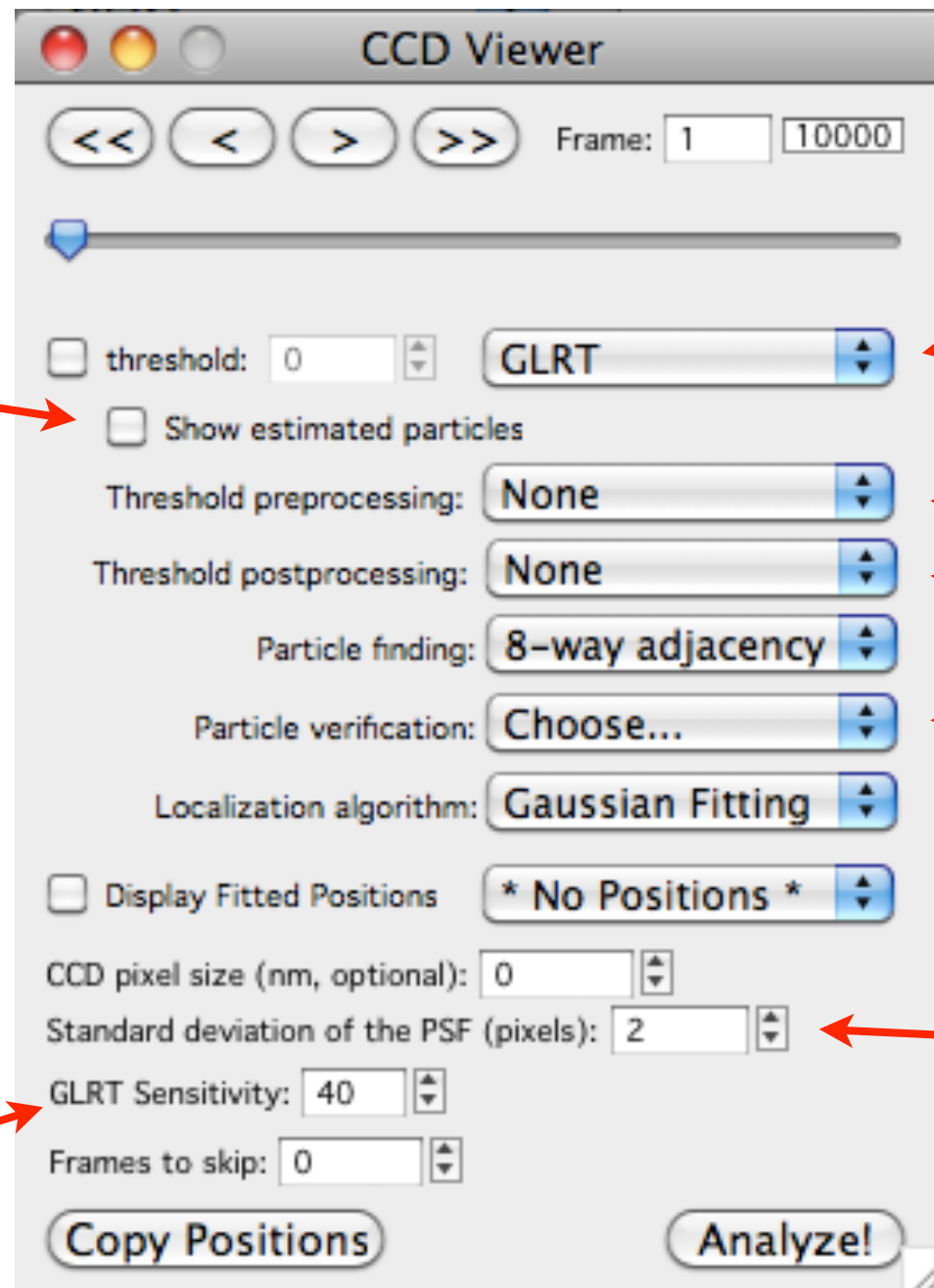
Step 2: explore your data

This is what the currently-selected data looks like

Use these controls or the arrow keys to move back and forward through the movie

Step 3: choose the localization settings

Check this to see how good the software is at recognizing emitters with the current settings (appears as an overlay on the image viewer)
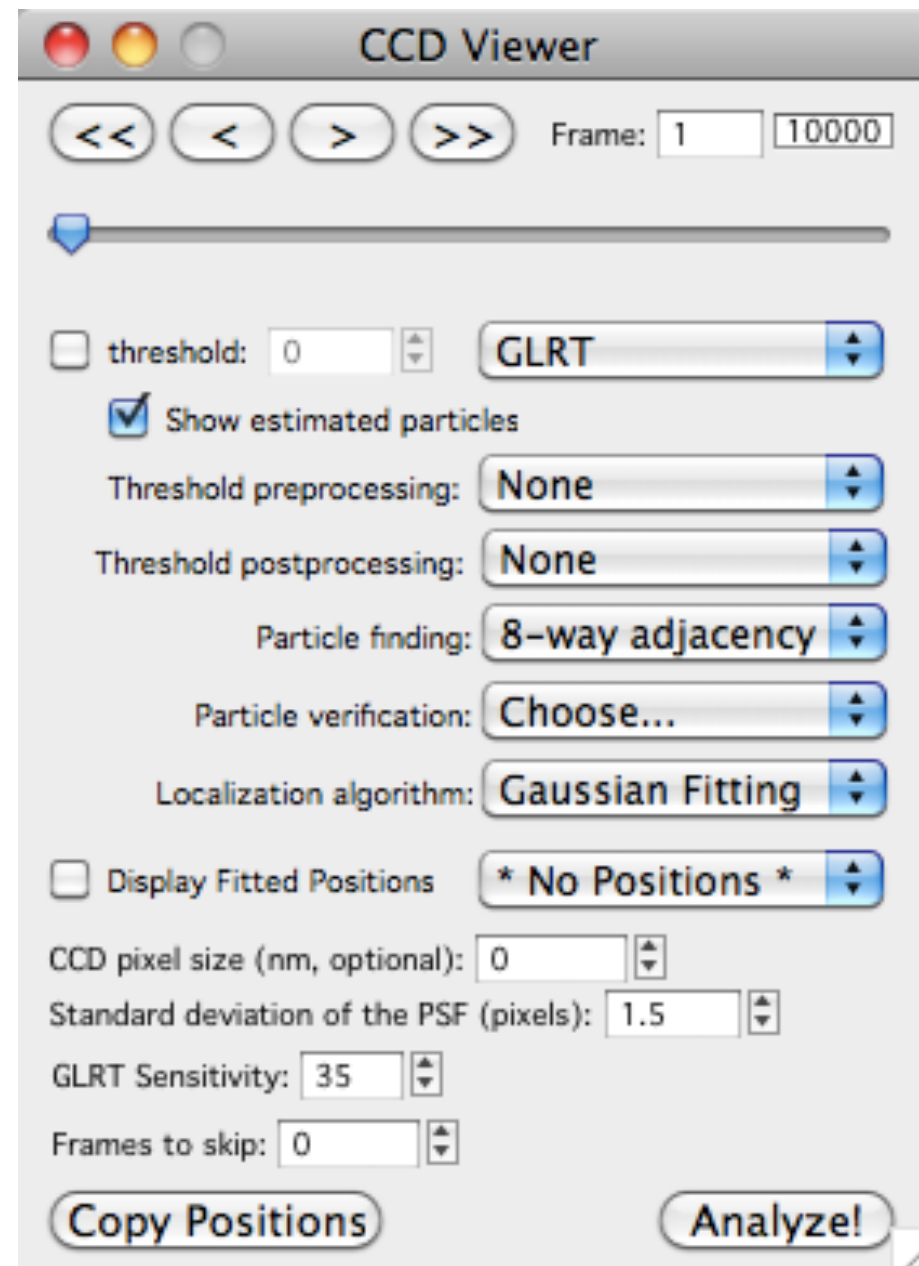
This selects the segmentation algorithm. 'GLRT' is probably the only one you'll need

Don't bother with these settings while you're still starting out

After checking the box above, play with this number. Look for a value that gives you the best segmentation (no false positives and few missed emitters)
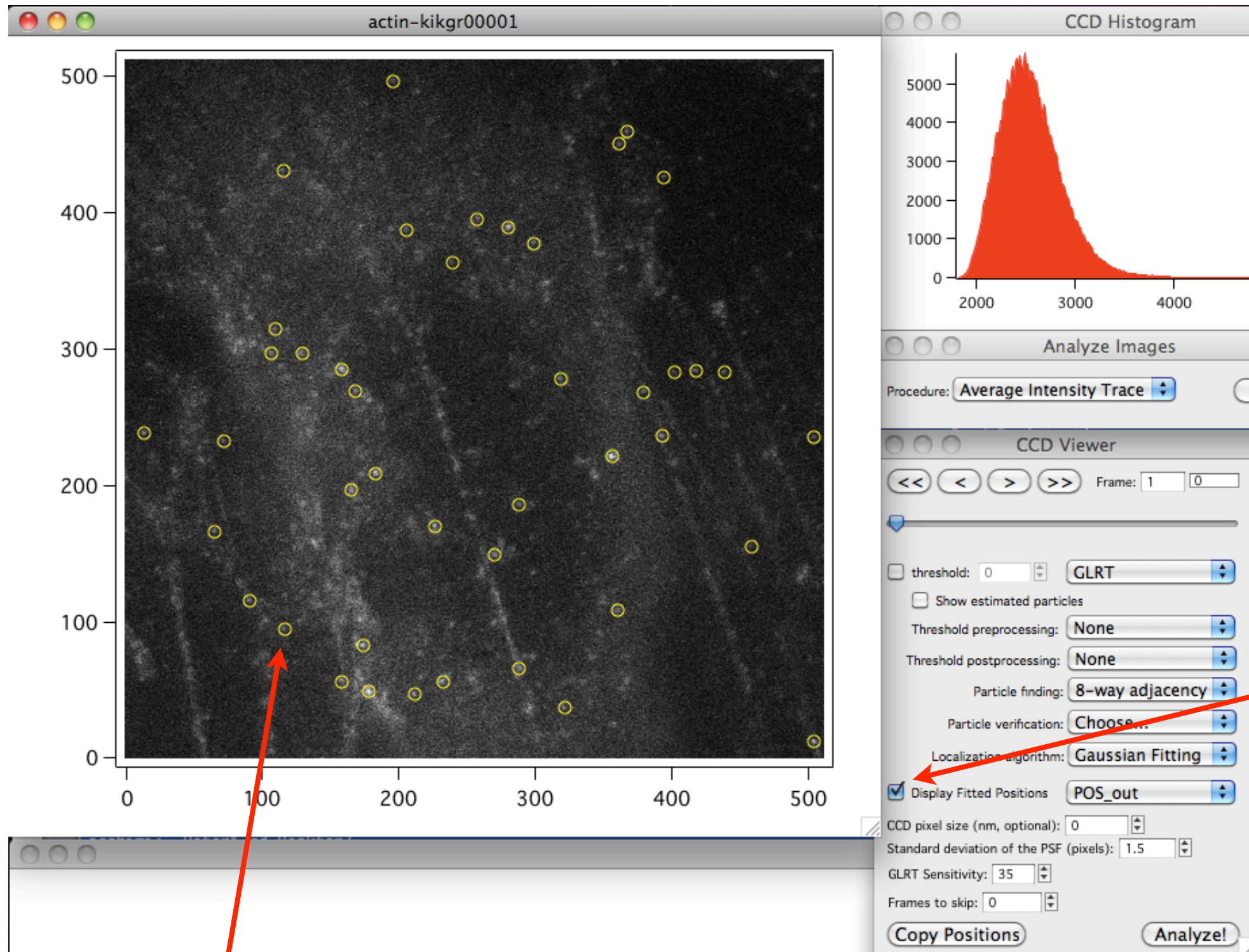
Set this to estimated standard deviation of the point-spread-function (the width of an emission spot). If unsure, choose 1.5 or 2.

CCD Viewer

<< < > >>   Frame: 1   10000

☐ threshold: 0   GLRT
☐ Show estimated particles
Threshold preprocessing: None
Threshold postprocessing: None
Particle finding: 8-way adjacency
Particle verification: Choose...
Localization algorithm: Gaussian Fitting
☐ Display Fitted Positions   * No Positions *
CCD pixel size (nm, optional): 0
Standard deviation of the PSF (pixels): 2
GLRT Sensitivity: 40
Frames to skip: 0

Copy Positions   Analyze!

Step 4: run the analysis

Click this button. Wait a bit while the analysis is running (the calculation progress will be printed in the command window)
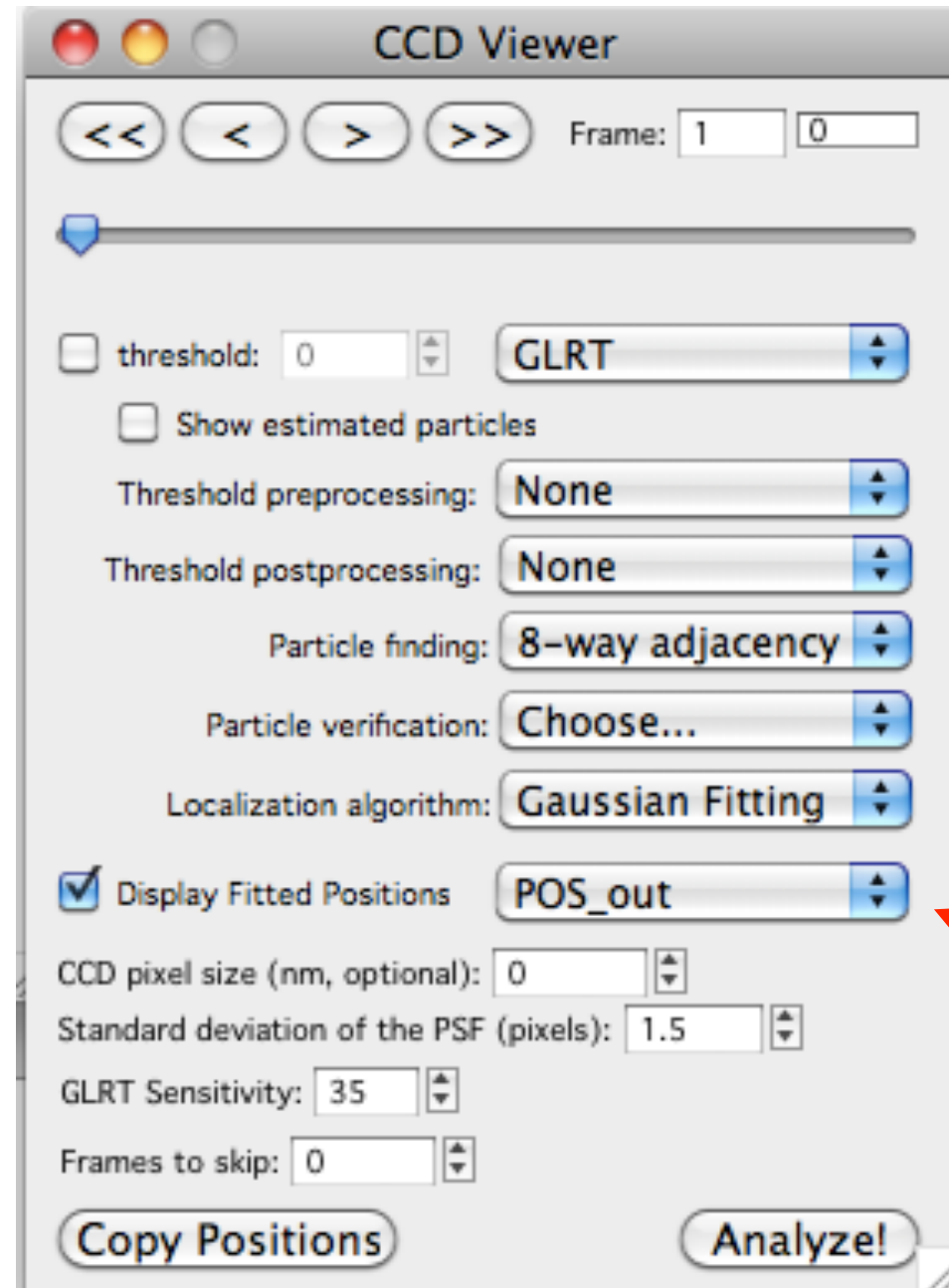
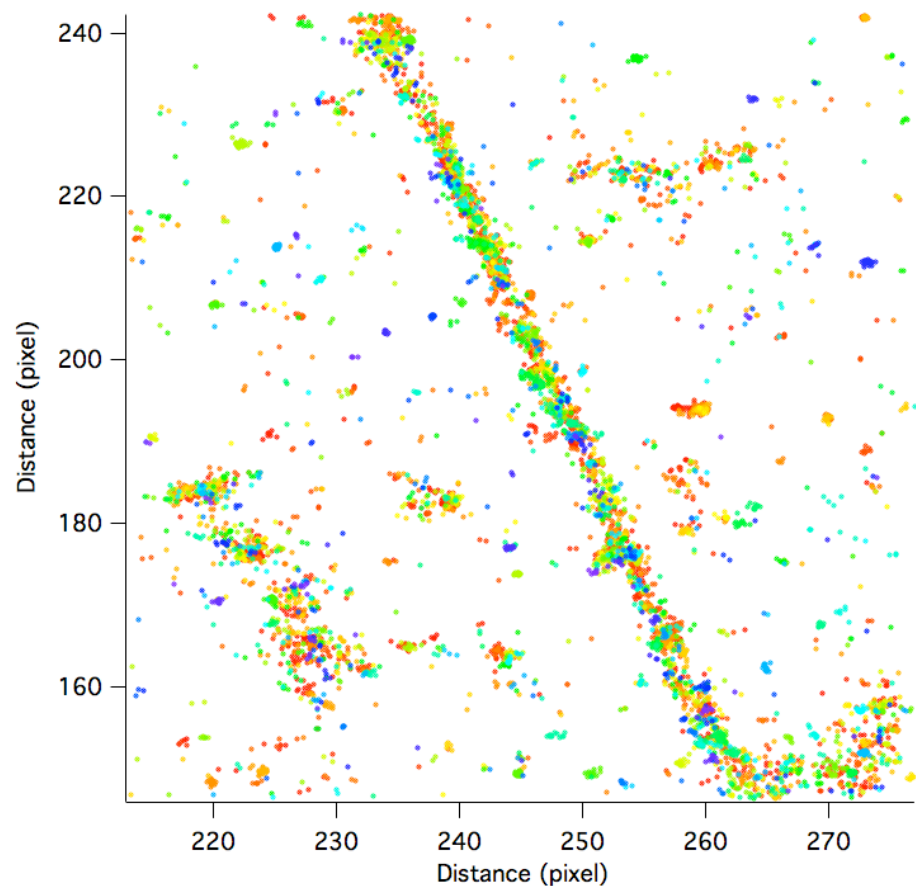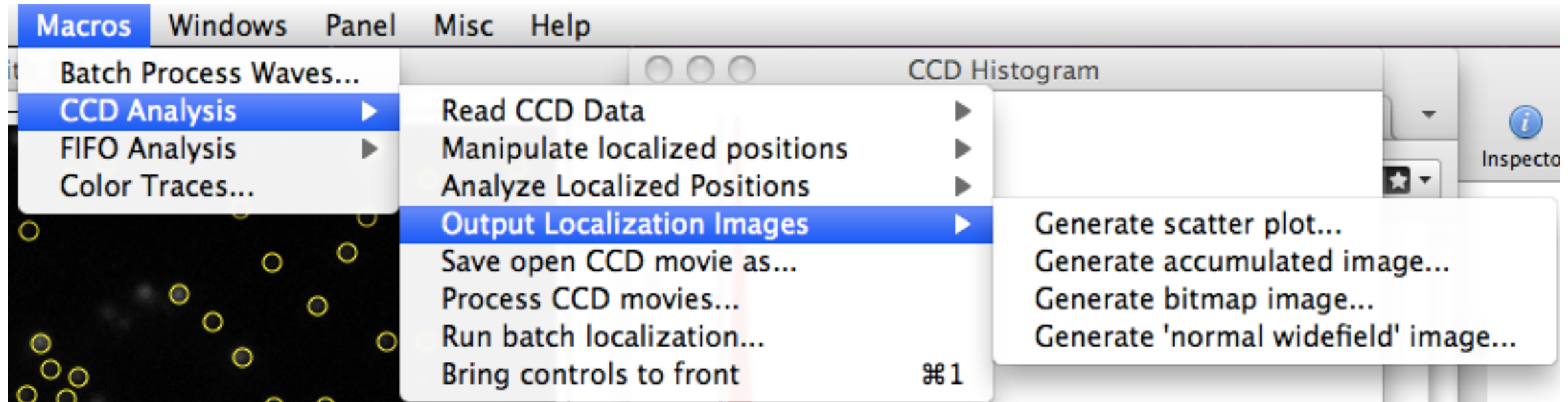# Step 5: inspect the quality of the localization
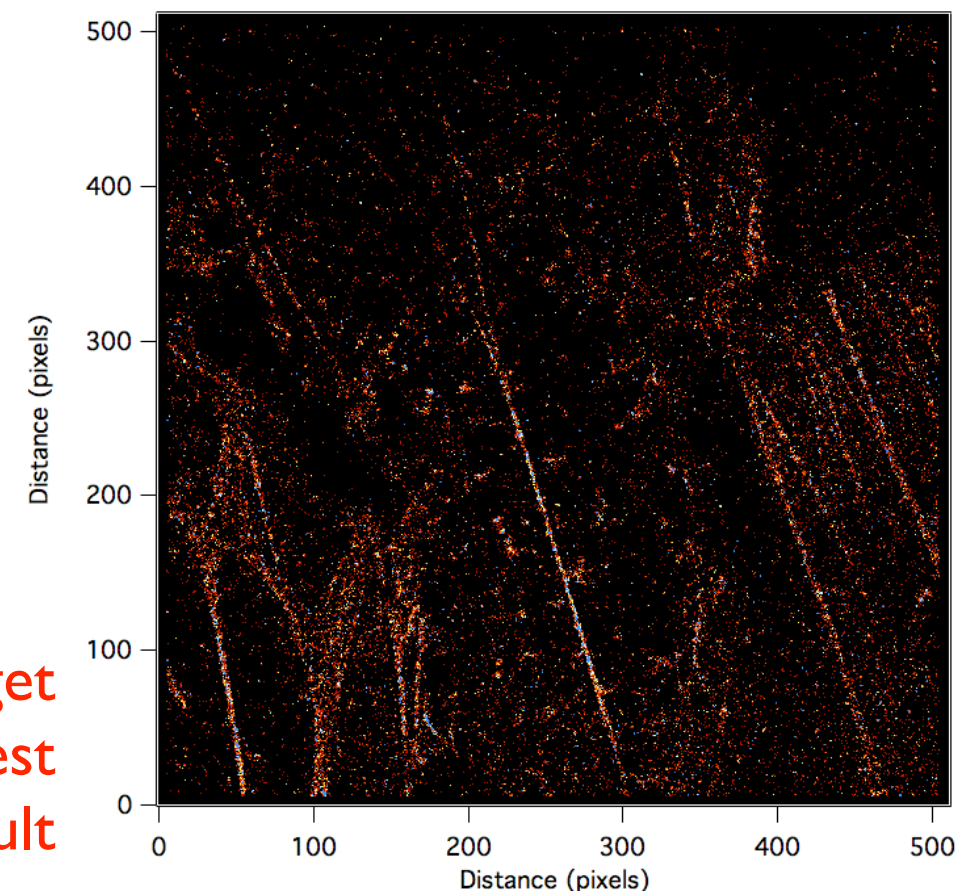
Step 6: copy the positions for safekeeping

This popup determines what positions are displayed and copied.

Click this button. When prompted, choose a name. This will make a copy of the localized positions so you don't accidentally overwrite it with your next fit
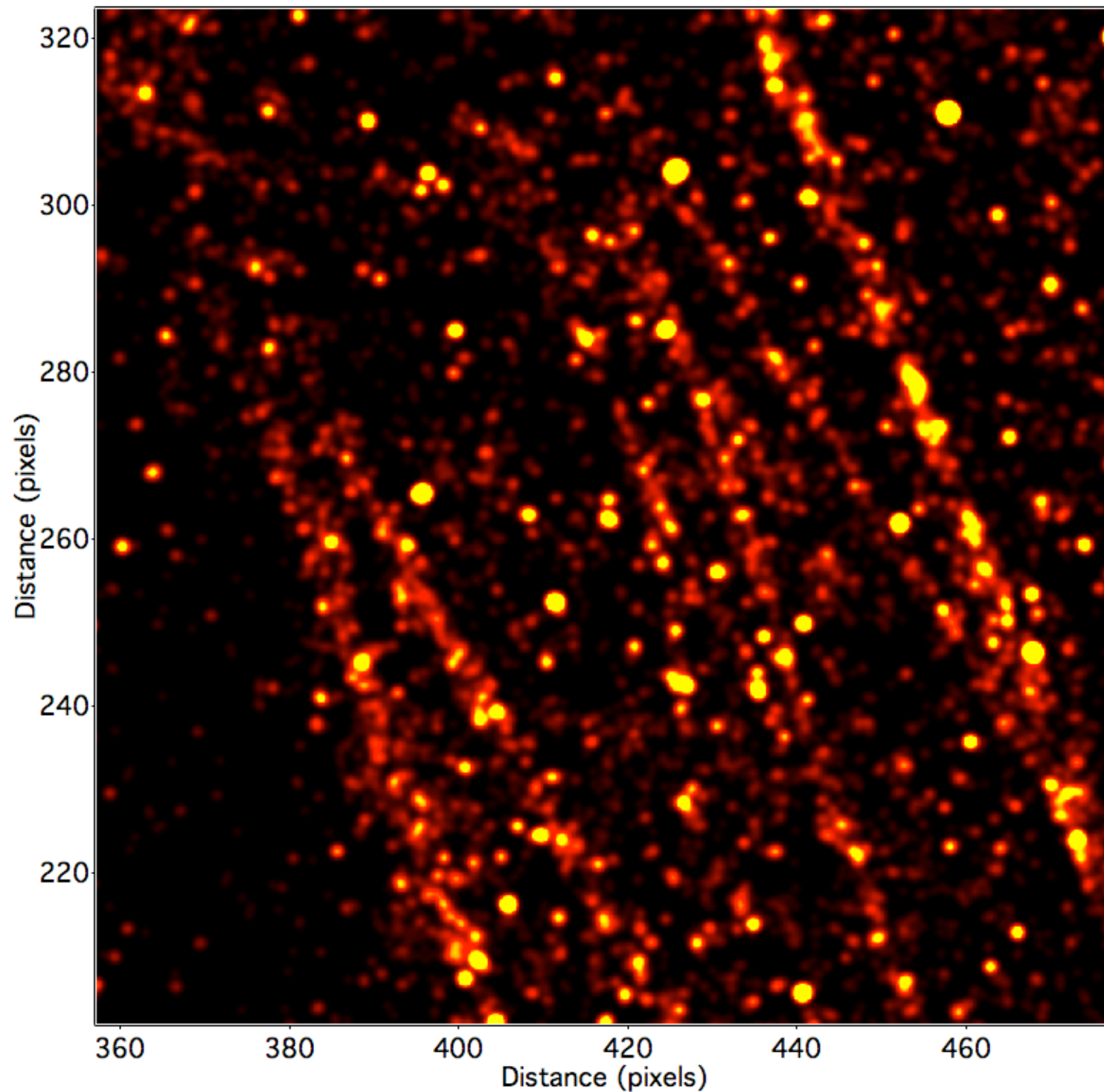
Step 7a: visualize the results

A scatter plot with a colorscale is great to detect sample drift and check the localization density, but rather difficult for everything else. The colors correspond to the time in the sequence when the emitter was localized

An accumulated image is like a 2D histogram. Don't forget to modify the color scale and color range to get the best result
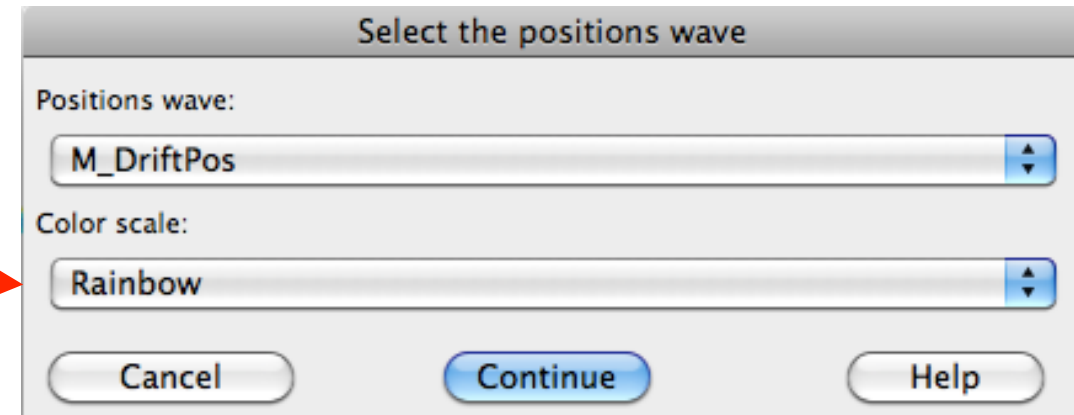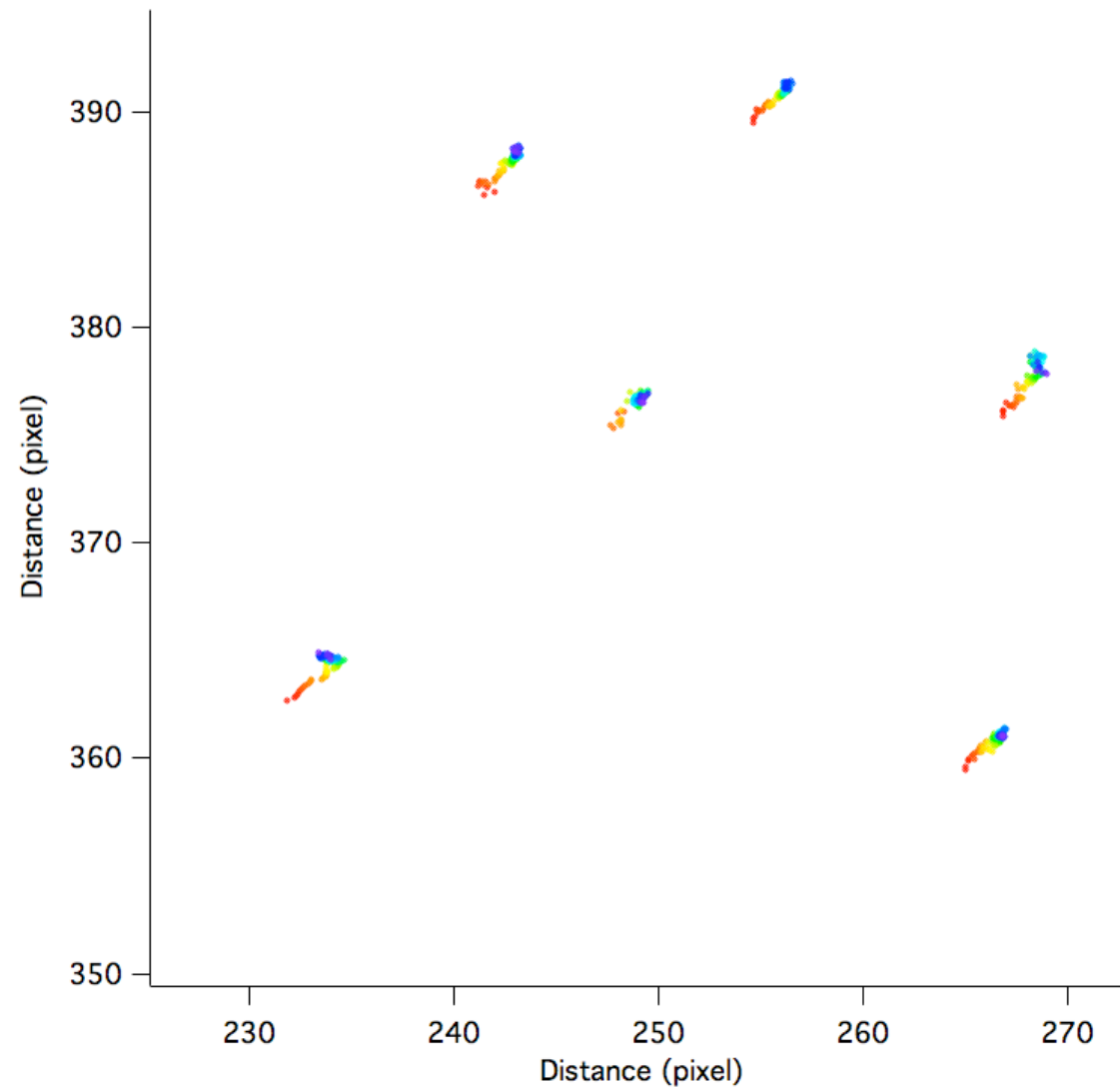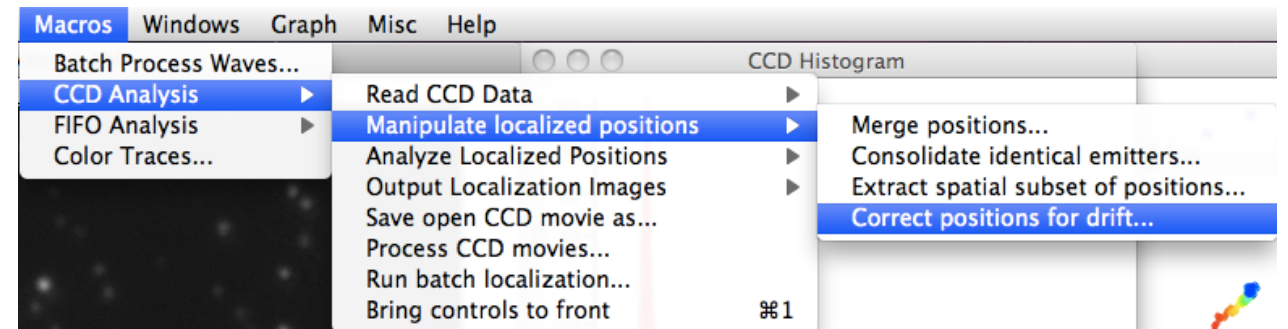
Step 7b: visualize the results

A bitmap image shows you a nice and smooth image where the contribution of and localization precision each emitter is directly visible. Be sure to take a nice color scale, play with the color range, and to choose large dimensions for the output image (4000 pixels or more if possible).

Step 8a: Perform drift correction

Long measurement duration can be affected by sample drift. A great way to pick this up is by making scatter plots with a color scale

Select the positions wave

Positions wave:

M_DriftPos
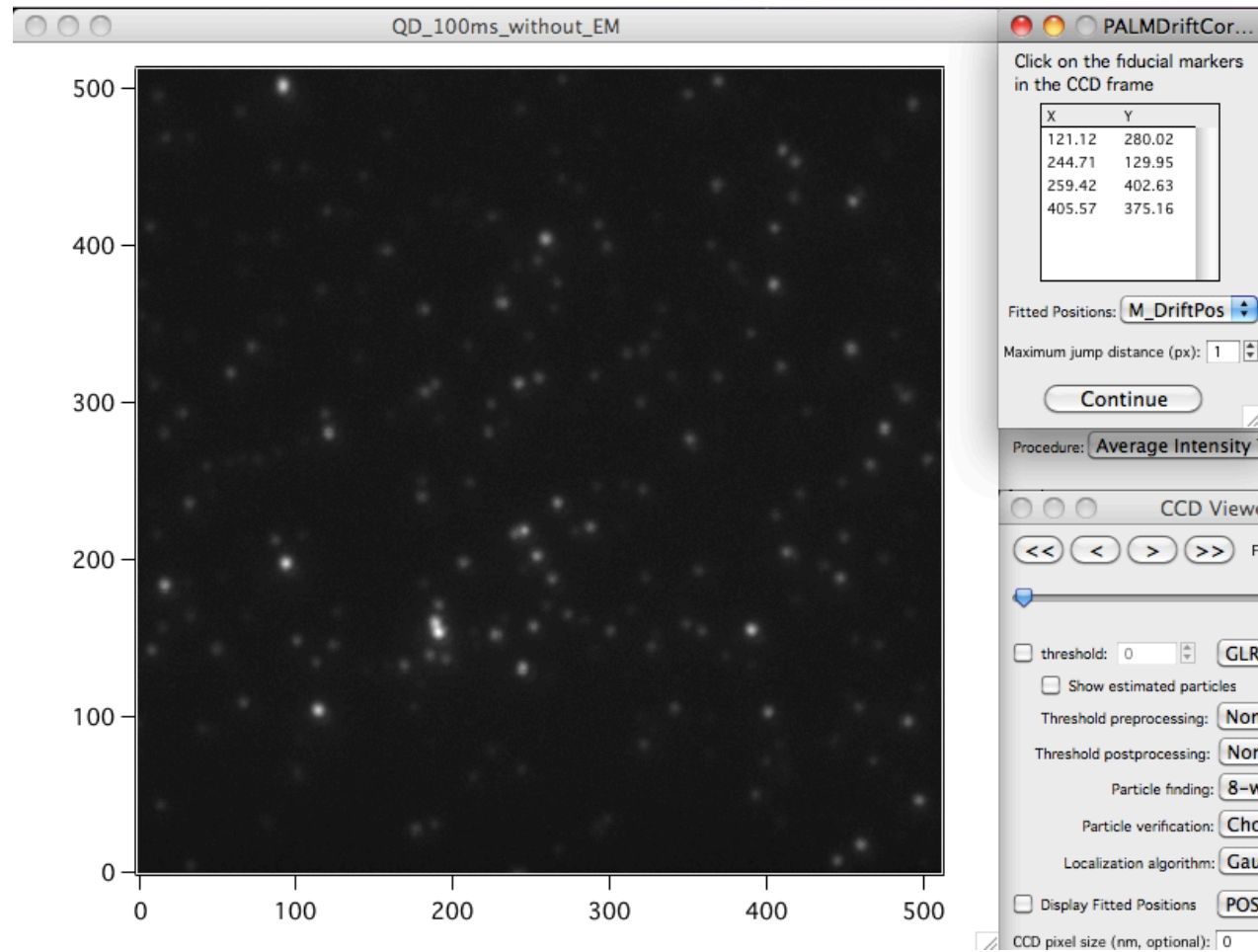
Color scale:

Rainbow

Cancel   Continue   Help

The example on the left clearly shows drift, evident from the nice, systematic way in which the colors appear
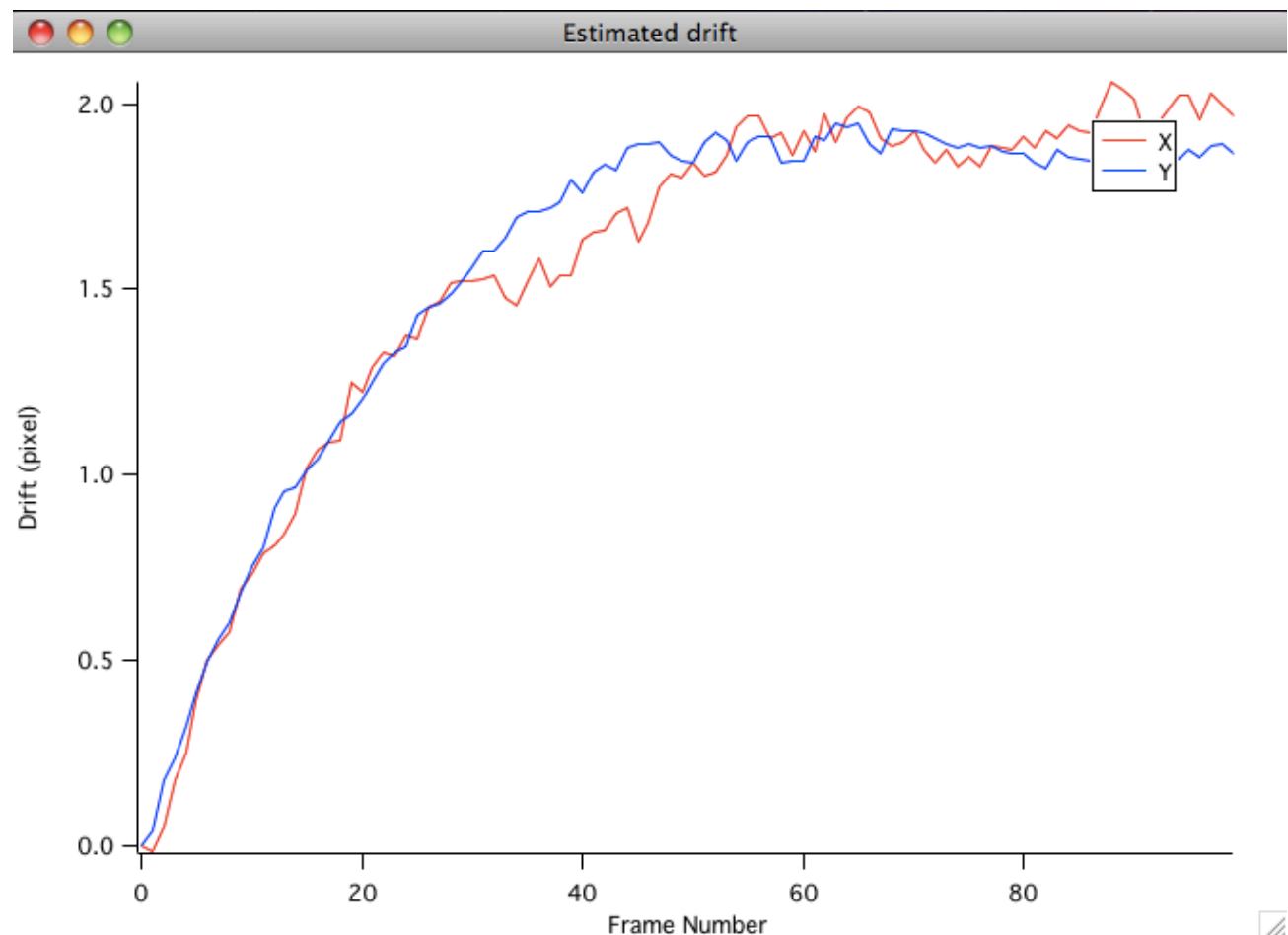

To correct the drift, you need to have a sample with fiducial markers (strong emitters that are always fluorescent), that are fitted in the localization analysis. To use these markers, open the raw data and select the corresponding menu option
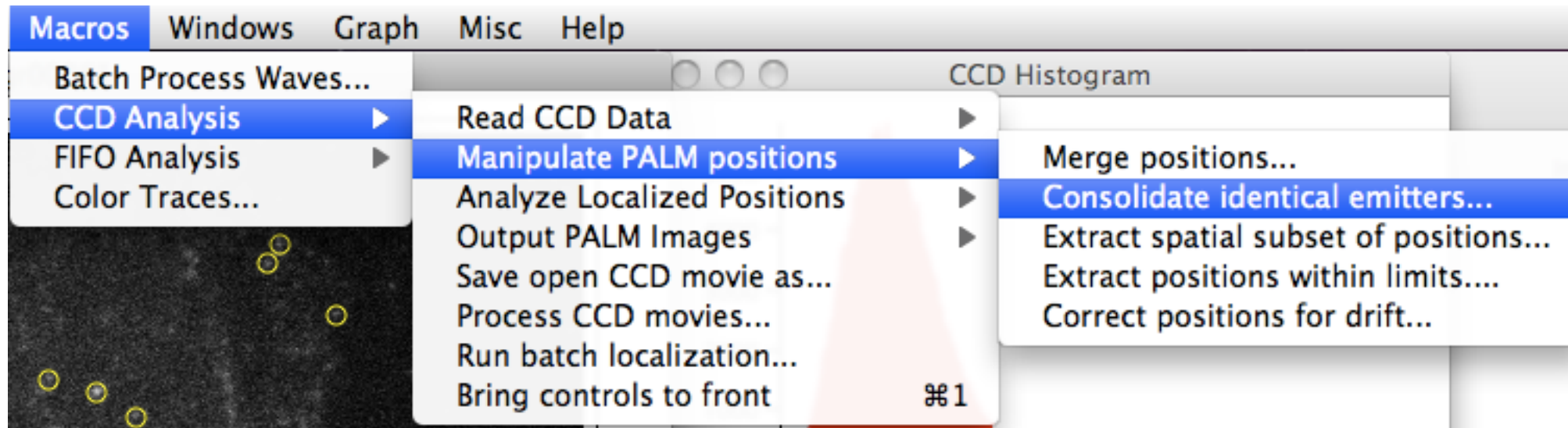
Step 8b: Perform drift correction

Click on the fiducial markers in the image, and select the positions that you would like to correct. You can click as many markers as you like
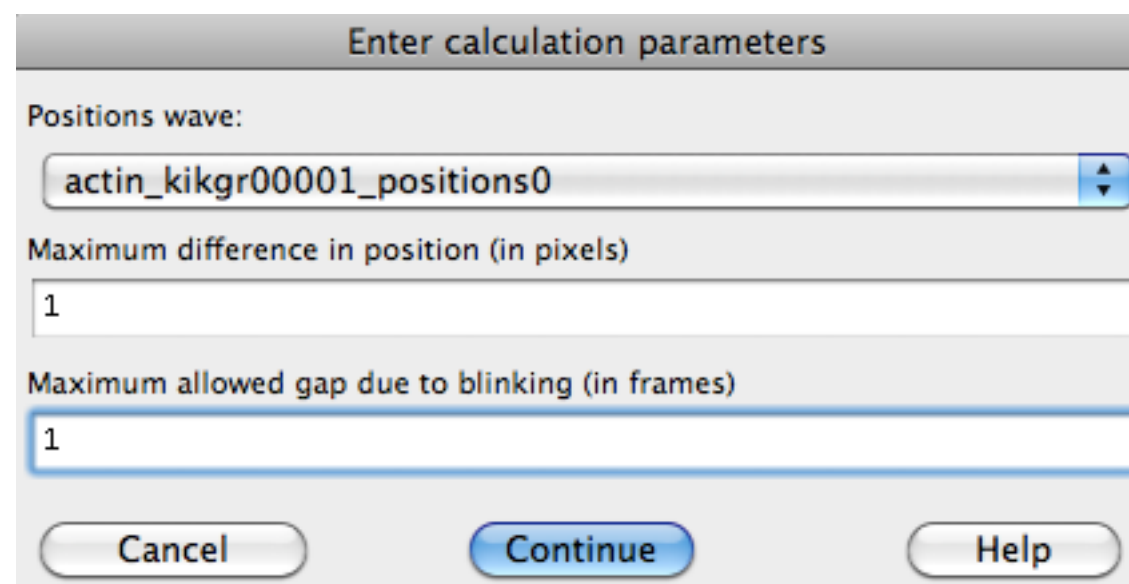

The corrected positions will be saved with the name you specify. A plot will also appear, showing you the estimated drift that occurred.
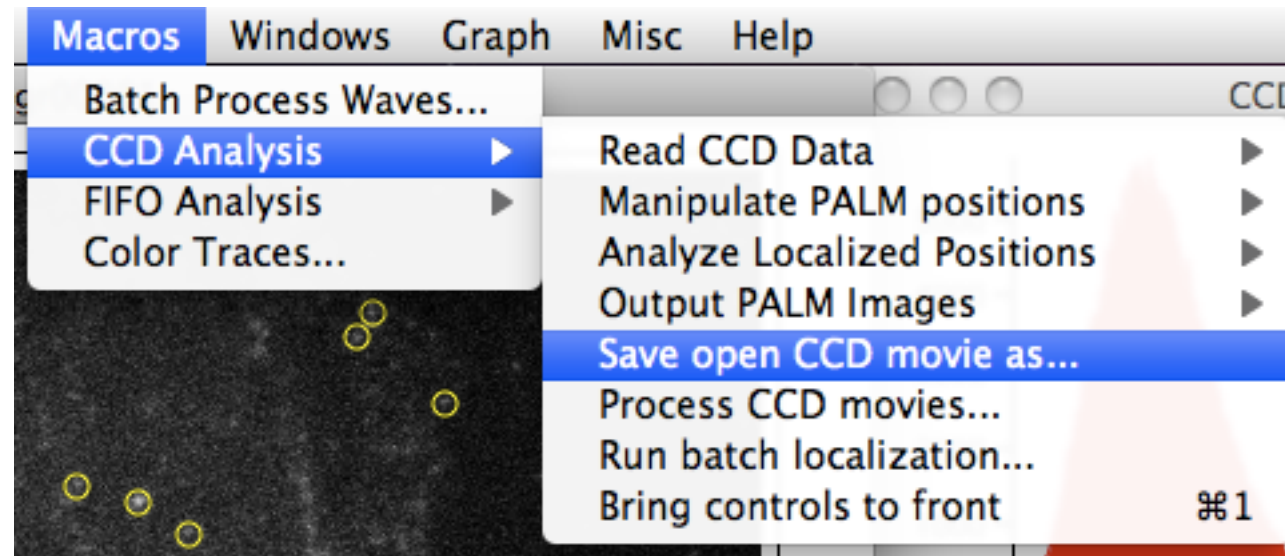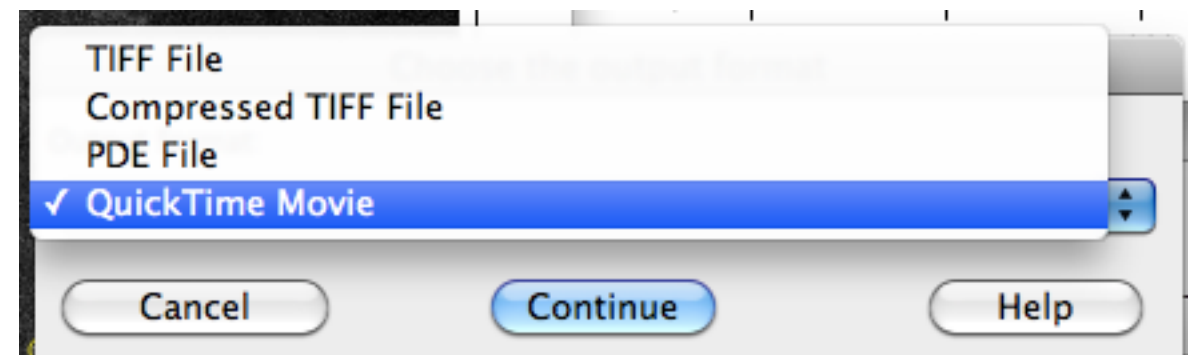
Step 9: postprocess the localized positions

Emitters that are 'on' for more than a single frame present a problem because they will have more weight than those that turn off quickly. To compensate this, use the 'emitter consolidation' function. This will look for events where the same emitter is present in subsequent frames (judged by closeness), and replace those with a single emitter containing averaged values. In general I'd recommend to always perform this calculation, and to only use data treated in this way for visualization and analysis.

Step 10: save the raw data in a different format

TIFF files are good for archiving and importing the raw data into a different program. The conversion is lossless, so no information is lost (except metadata related to the acquisition). These files comply with the TIFF standard.

The PDE format is a custom, specialty format. It's only useful if you want to process or save data for use in this program. The file format is completely specified in the reference help file.

The Quicktime movie format is excellent for making a movie to be shown at presentations or as supplementary info. It will use the settings, colors, etc., you've selected for the viewer window.