Installation

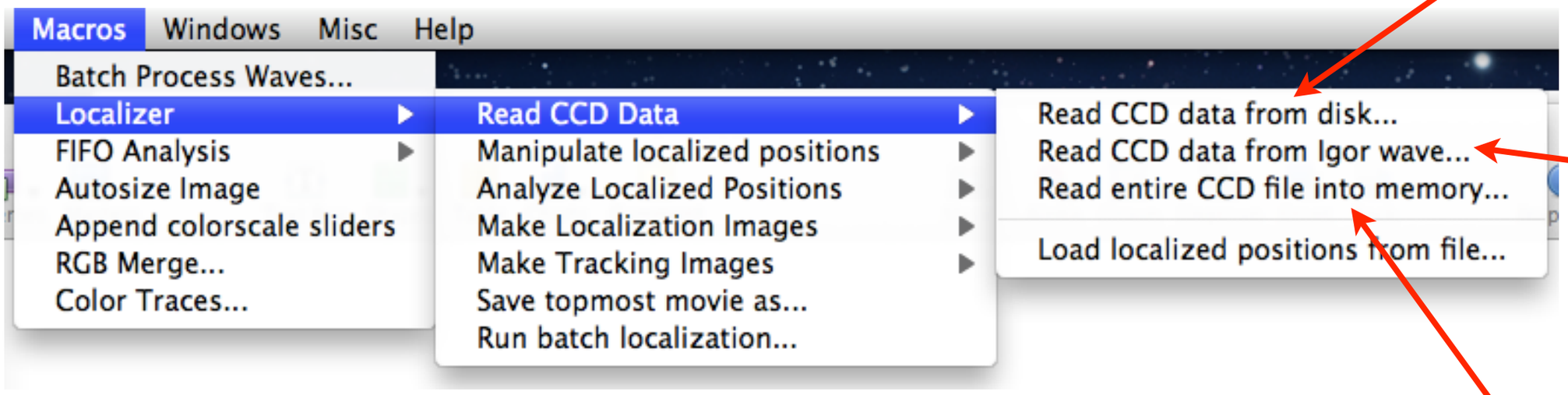
1. Place Localizer.ipf into ~/Documents/WaveMetrics/Igor Pro 6 User Files/Igor Procedures (Mac) or the equivalent folder in My Documents (Windows).

2. Place Localizer.xop into ~/Documents/WaveMetrics/Igor Pro 6 User Files/Igor Extensions (Mac) or the equivalent folder in My Documents (Windows).

3. Restart Igor.

load your data

1. Click the Macros menu in Igor

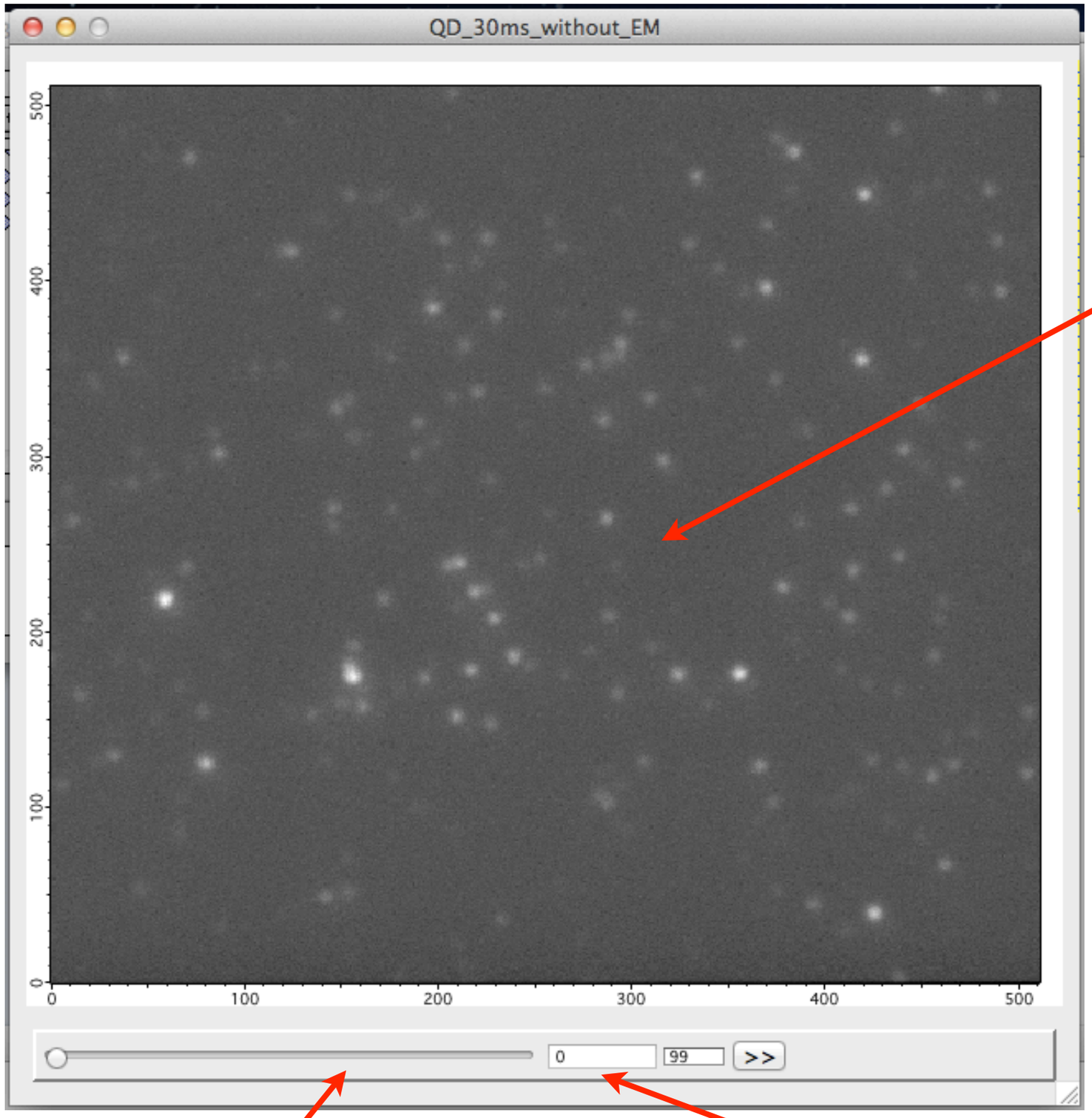


You will usually want this

Use this if the data is already in an Igor wave

Use this if the data is on disk but you want to read all of it into RAM for faster subsequent processing

explore your data



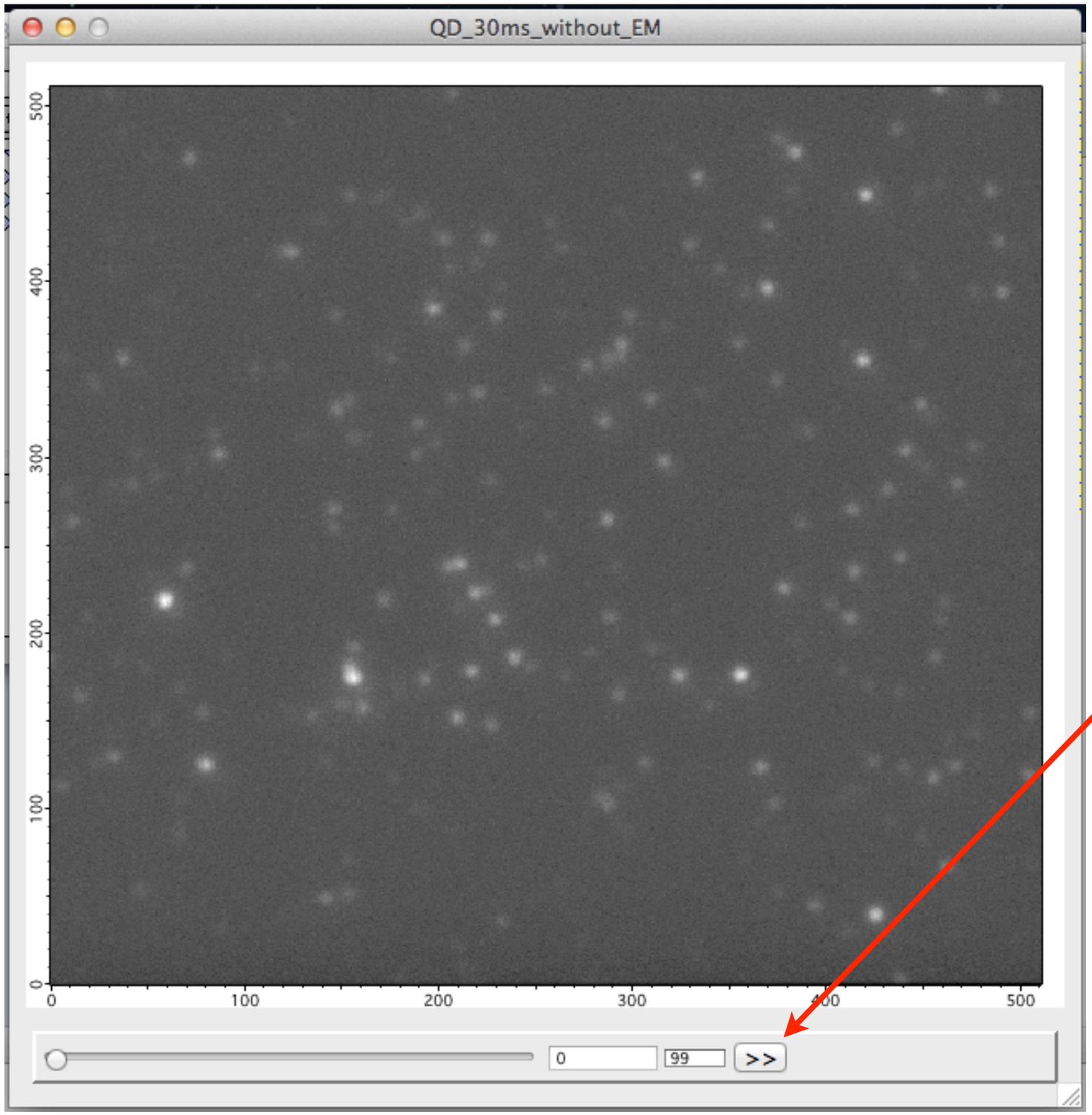
This is a standard Igor image plot. You can customize it as usual (colorscale, range, etc)

hit spacebar to play the movie automatically or to stop

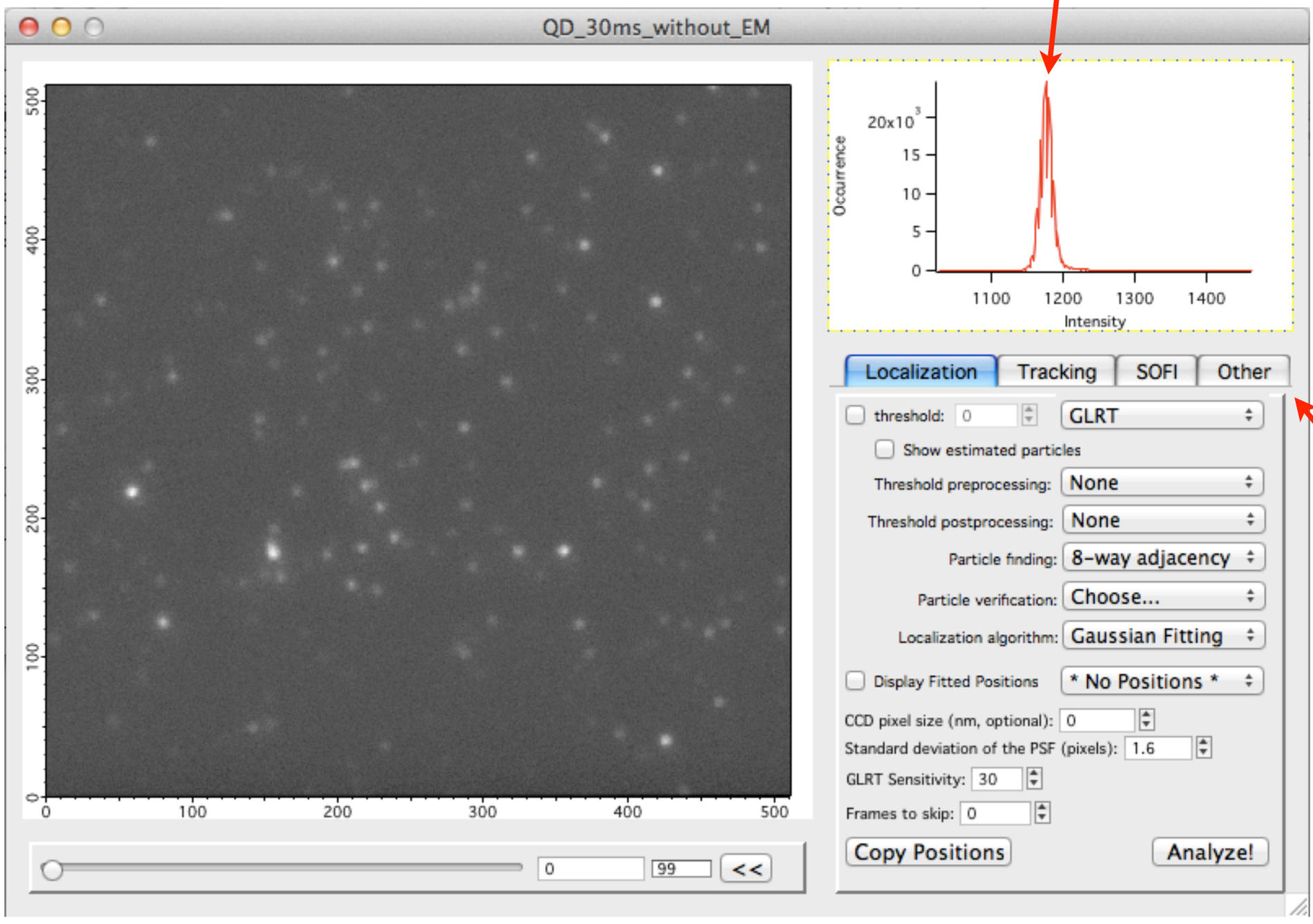
Play with this or use the arrow keys to navigate...

... or fill in an image number here an press enter

plan an analysis



This is a histogram of the intensity in the currently display image



Click these tabs to choose the type of analysis

Localization = PALM/STORM/ GSDIM/....

Tracking = particle tracking (localization needs to be done first)

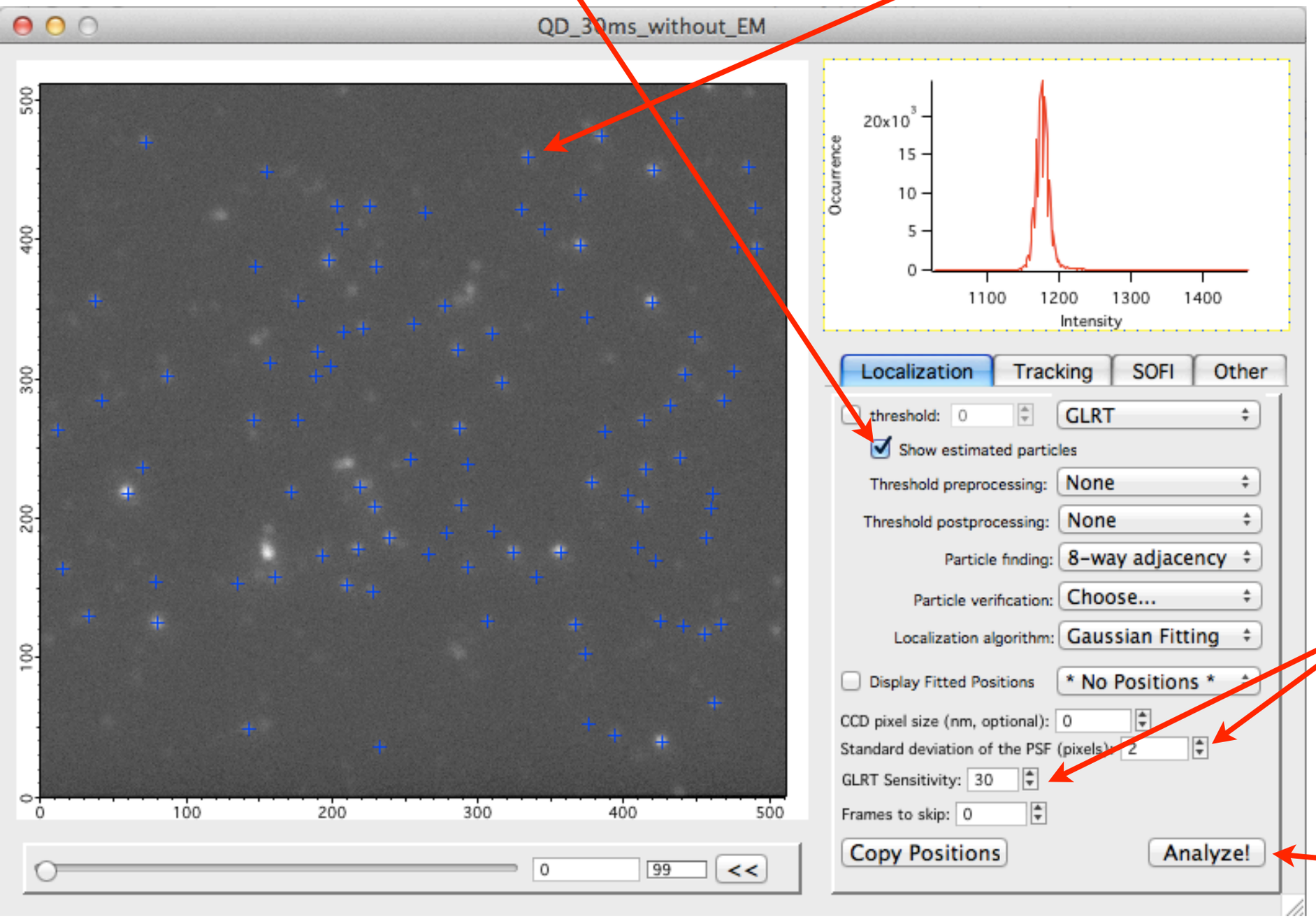
SOFI = superresolution optical fluctuation imaging

Other = analysis and processing that does not fit in the above

localization analysis

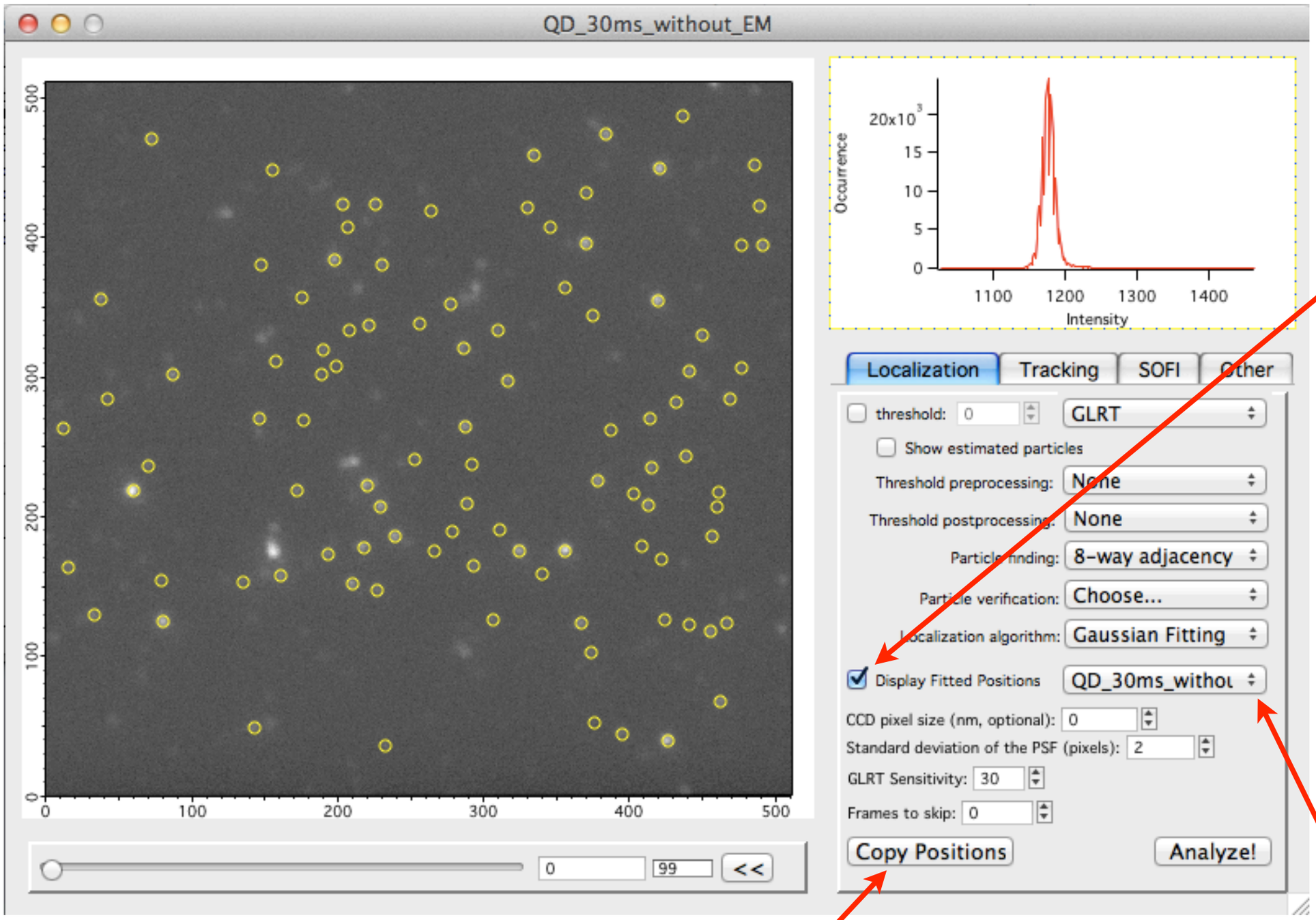
Check this box to see what Localizer thinks are emission spots in your data

The spots are shown with blue markers



Play with these settings until it does a good job (this is very important!)

Click here to start calculating



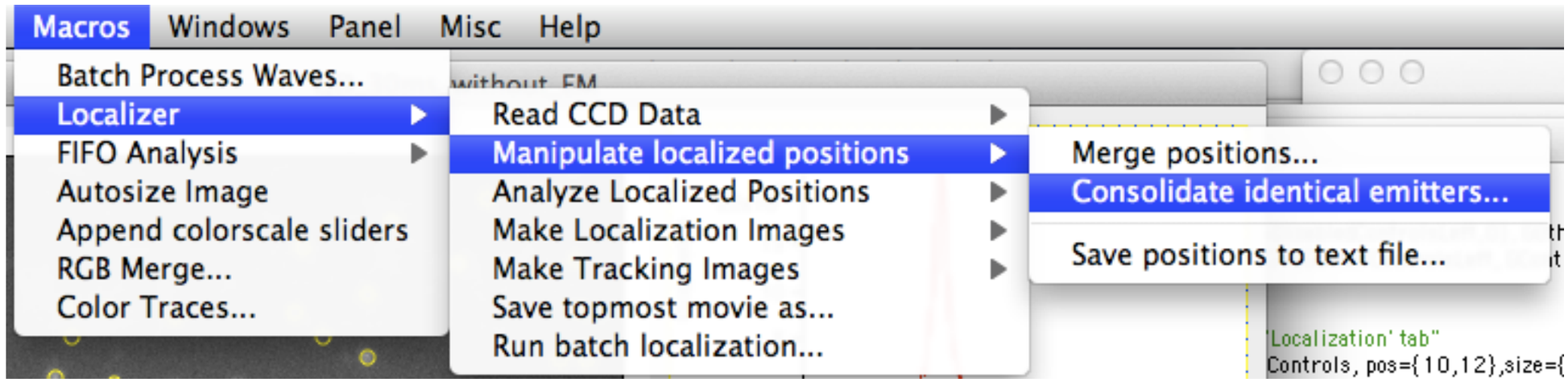
click here to show the result on the image (the yellow markers)

click here to save the positions for later use

If you did more than one analysis, and saved the positions, click here to show different ones

Once the localization has been performed, Localizer is effectively ‘done’ with the data (exception: drift correction). All the information is now contained in the set of localized positions.

Postprocess the result

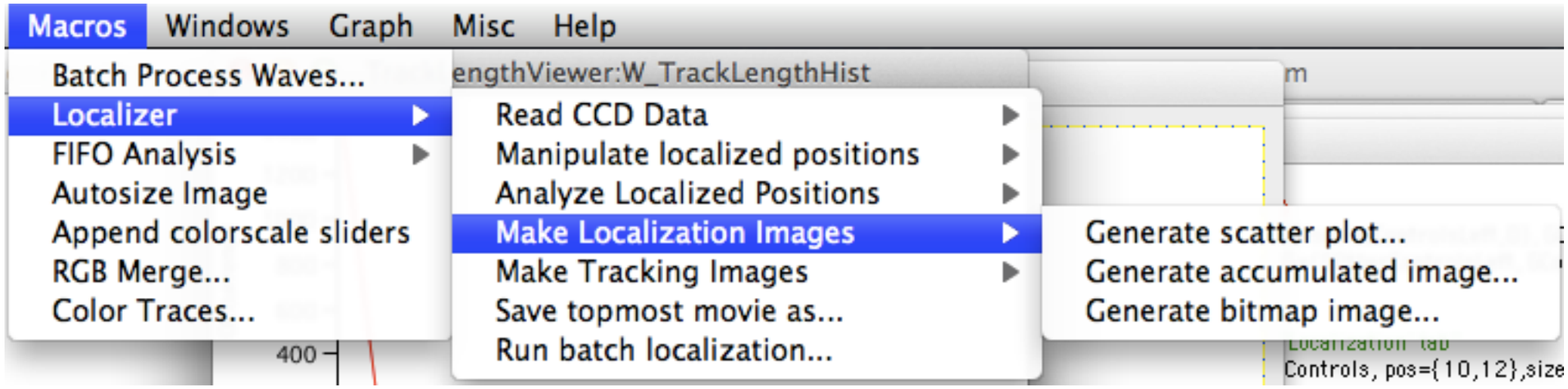


If the molecules in your sample blink, or stay ‘on’ longer than a single exposure time, some or all of them will show as more than one localization.

To fix this you can ‘consolidate’ the localizations. When you do this, Localizer loops over all the fitted positions, and combines those that are close together and occur within a few subsequent frames (customizable).

Localizer will also try to use this to estimate the localization error in the calculation, and report it to you, but it is only an estimate

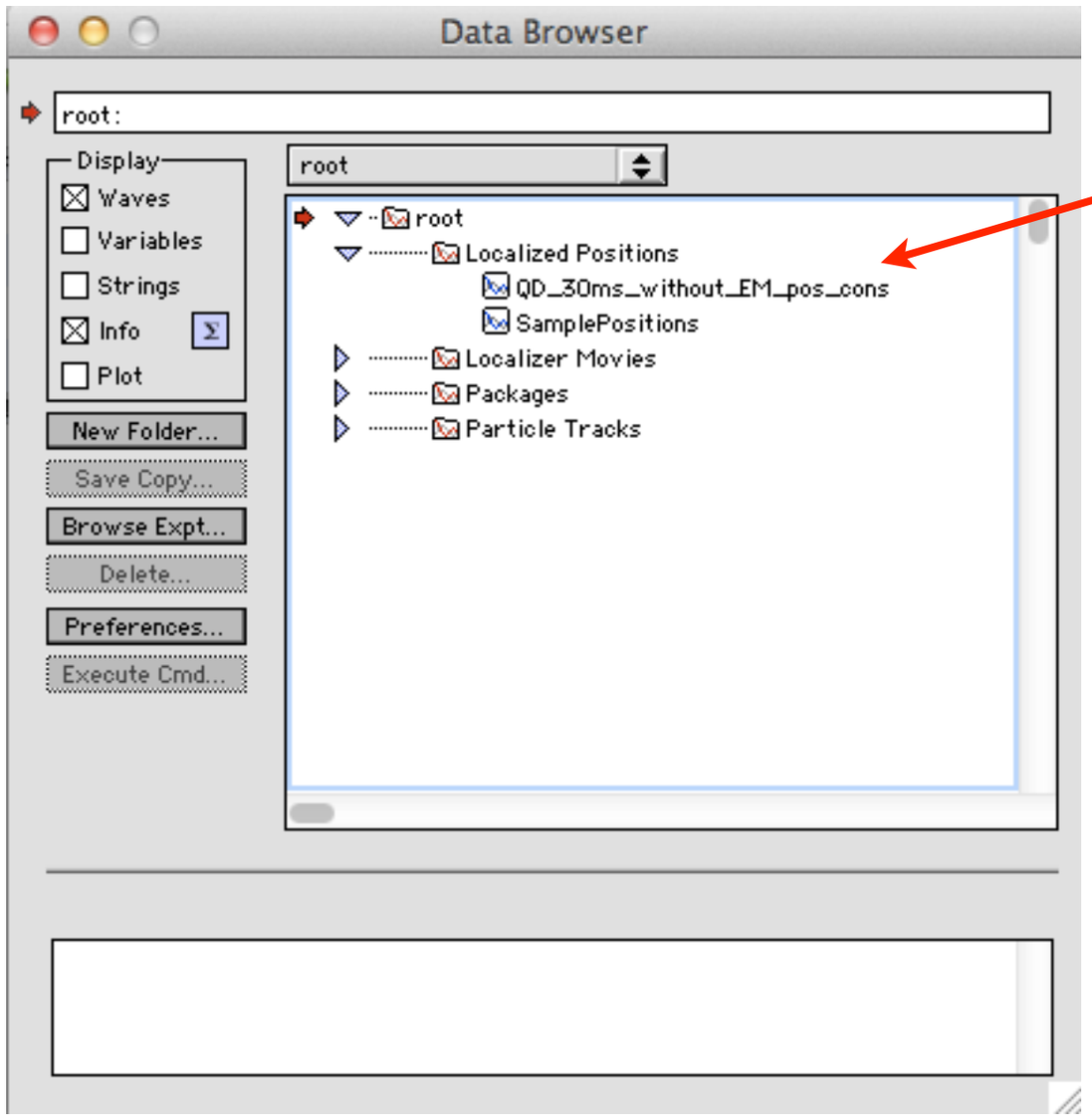
Visualize the result



The different visualization options each have their own strengths and weaknesses. Play around with them and see what makes the most sense for your application.

Remember that they are all standard Igor graphs – you can modify them to your heart's content.

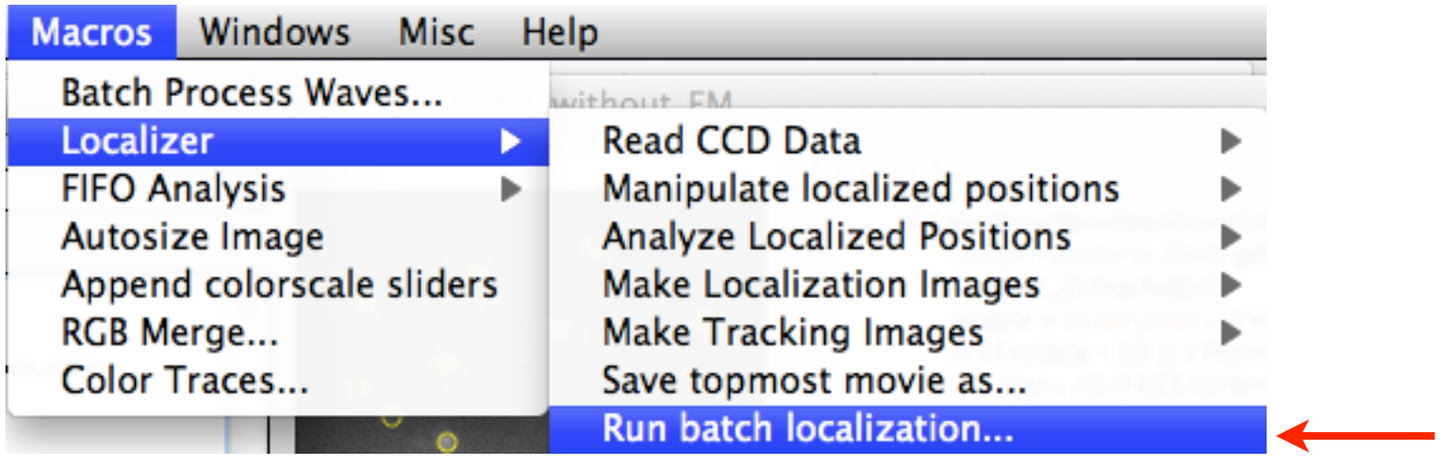
Analyse the data yourself or export to another program



The localized positions are created in the ‘Localized Positions’ folder (after you have save a copy).

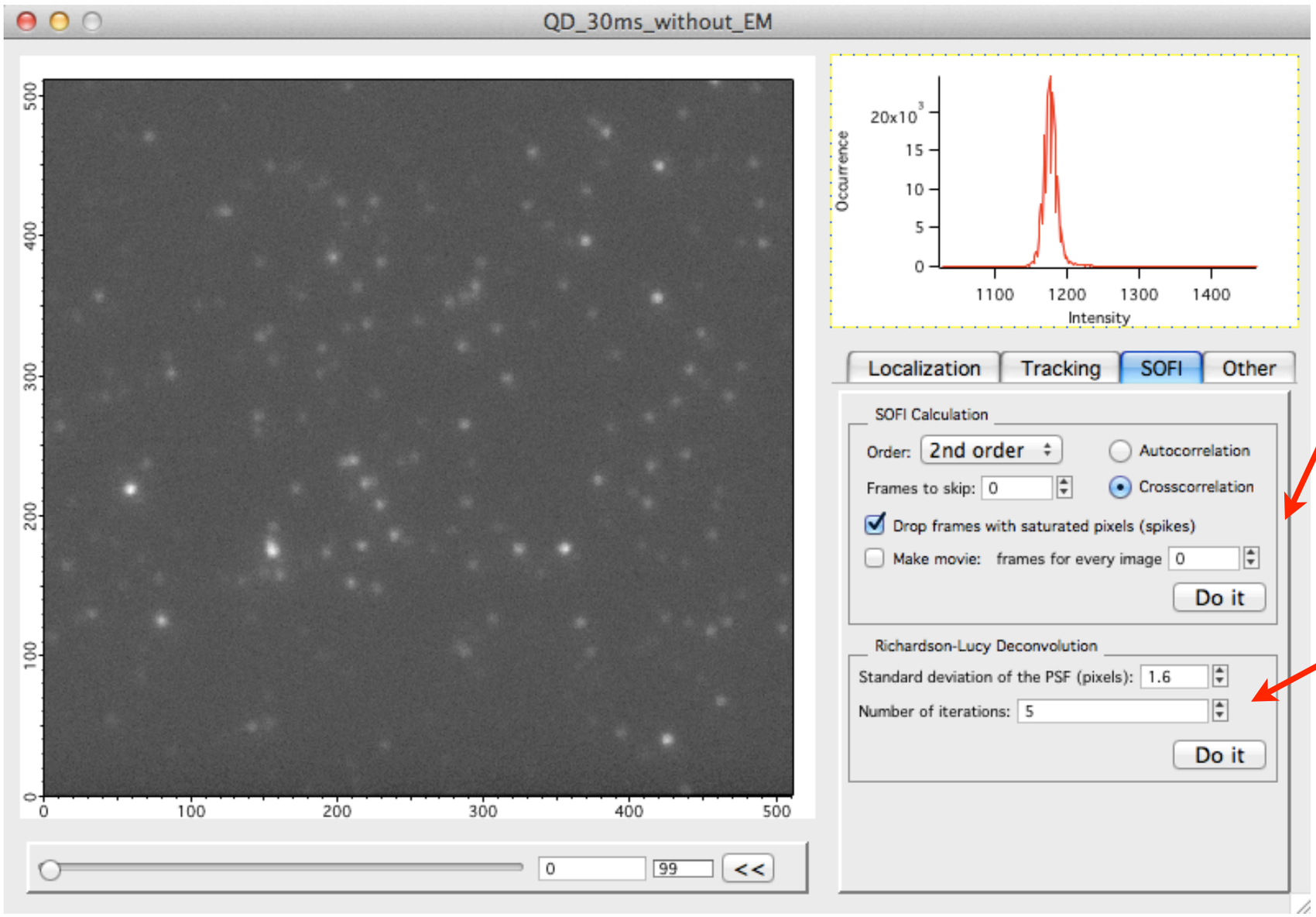
The format of the data depends on the selected localization algorithm. It is described in the included reference file.

Automatic analysis of more than one data file



All files will be analyzed with the same settings,
taken from the topmost analysis window.

SOFI/SCIFI



The actual calculation is here

After the calculation you can deconvolve the image for a better result. But don't overdo it, a couple of iterations is typically fine.