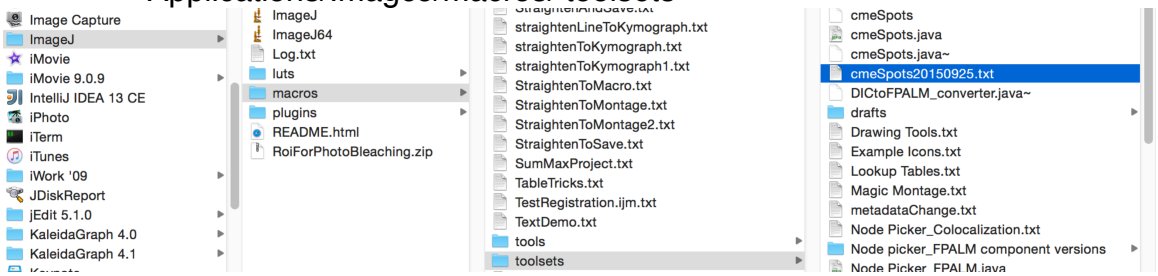
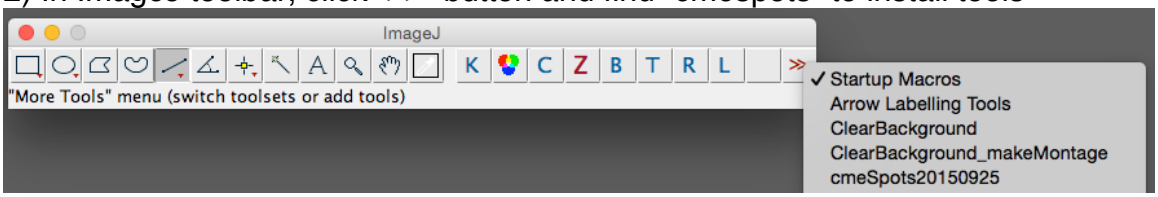
How to use CMEspots.txt

1) Install toolset into your ImageJ or Fiji toolsets folder
   - For Fiji (mac): Go to Applications/ Fiji.app. Right click Fiji and select “show package contents”
     - Drag “cmespots.txt” into Fiji/macros/toolsets folder
   - For ImageJ (mac): Drag “cmespots.txt” into the following folder: Applications/ImageJ/macros/ toolsets



2) In ImageJ toolbar, click “>>” button and find “cmespots” to install tools



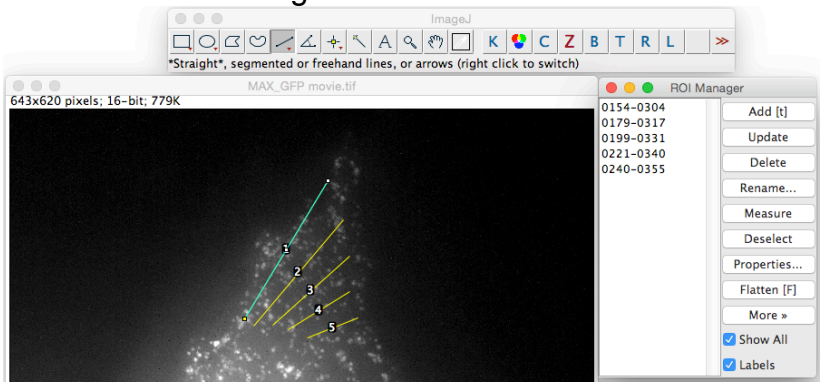
CME spots tools

The first set of tools is used to make kymographs from a single-color movie. The second set of tools is used to make two-color kymographs from two channels of a movie.

[K] Make kymographs

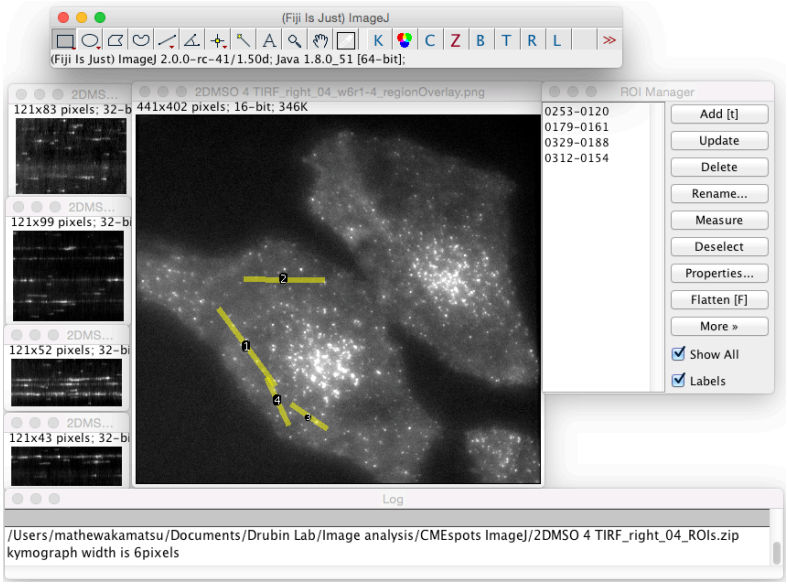
This tool will make kymographs from line regions you create.
Start with a single-color movie.

1. Create line regions



   a. Open your movie
   b. Optional: Create a maximum projection using the “Z” button on the toolbar
   c. Use the Line Tool to draw lines on the regions of the cells you want to make kymographs for. Save each region by pressing **“t”** on the keyboard or clicking “Add [t]” in the ROI manager.

2. Make kymographs
   a. Click **“K”** on the toolbar.
   b. The program will automatically make kymographs for each line and save them as .tif and .png files in the same folder as your movies. It will also save the regions as a .zip file and an image of the regions overlaid on the cell.



Options:

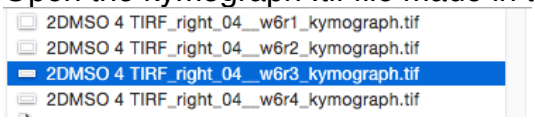
- * To make this run much faster, hold **shift** while you click the “K” button. In this “batch” mode, the program will close all open images, and then open a dialog for you to find your movie on your hard drive. The program will make the kymographs in 1-2 seconds and save them in the same folder as your movie. Be sure to save any open images before you run this version of the program.
- If you already have line regions saved in a file, then leave the ROI manager empty and the program will ask for the .zip file of line regions.
- In the file name of the kymographs, “w” stands for width of the region used to make the kymographs. You can change the width used in the text file “cmespots.txt.” In the first few lines you should see “var kymographWidth = 6.” You can change “6” to a different value. (This is currently line 7 of the code.) Then save the file and restart ImageJ. (The program makes maximum intensity projections along that width.)

[L] Measure line from kymograph

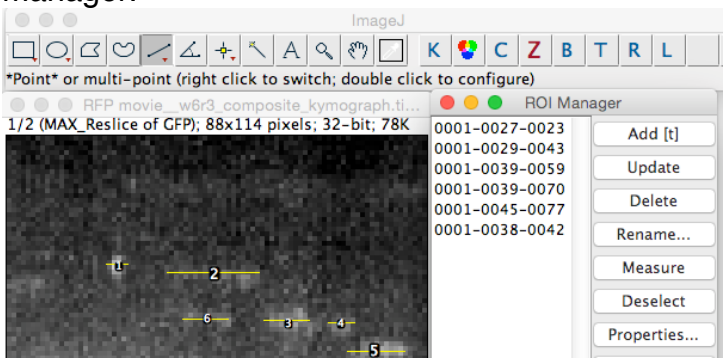
This tool measures lines you have drawn on kymographs and saves them with the same name as the kymograph.

<u>If you made a single color kymograph (using [K])</u>

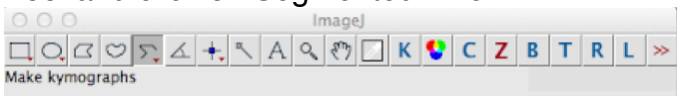
1. Open the kymograph .tif file made in the previous step



2. Use the line tool to draw lines across the fluorescence you see in the kymograph. Save each line by pressing **"t"** on the keyboard or clicking "Add [t]" in the ROI manager.



- **Yeast people:** if you want to make circular regions, right-click the Line Tool and click on Segmented Line.

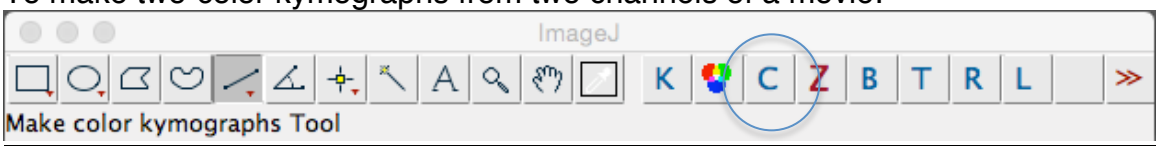


3. Once you have made selections for each line, then click **[L]** on the toolbar. The program will make measurements and save them as .xls and .txt files. The regions will be saved in a .zip file.
4. Open the measurement files. The "Length" measurement, multiplied by the time step of the movie, gives you the approximate lifetime of the event. You can plot these in Origin or Matlab. I have a Matlab program that will plot histograms of the lifetimes if you input the file name.

Options:

- If you want a measure of the frequency of events, open cmespots.txt and make "var measureInitiations = 1" instead of 0. This measures number of events (number of line ROIs) and divides by measurement area (kymograph height and thickness) and total time (kymograph width). The final units are in events/μm^2/s. For the measurement to have the right units, the "timeinterval" and "micronsperpixel" variables need to be correct for your movie (in cmespots.txt).

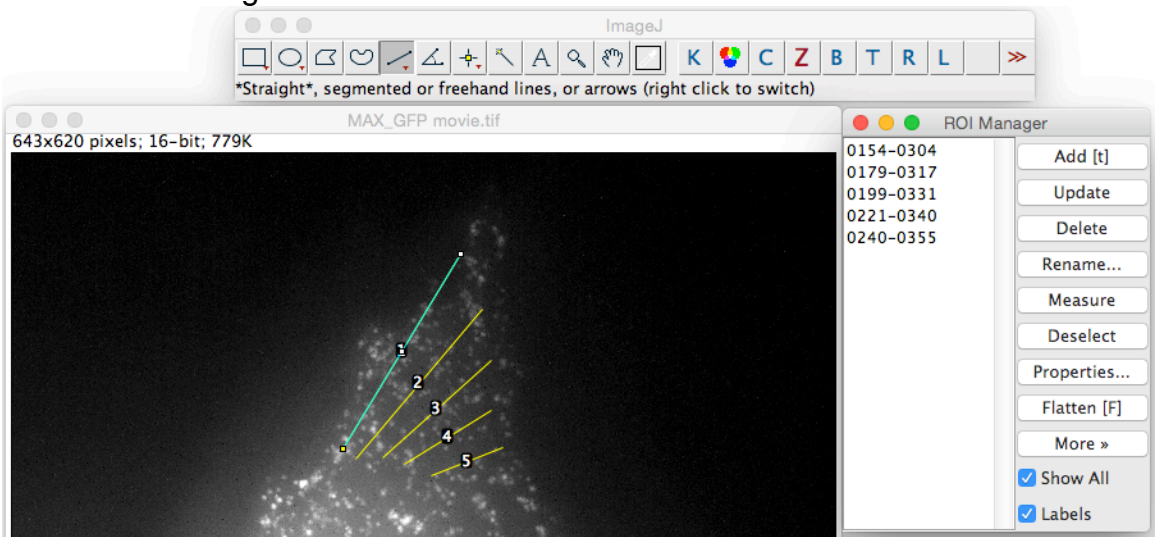
To make two-color kymographs from two channels of a movie:



[C] Make color kymographs

This tool will make two-color kymographs from line regions you create.
You need to have two movies, one for each channel. You can make these movies with the "Image Registration" application made by Sun Hong.

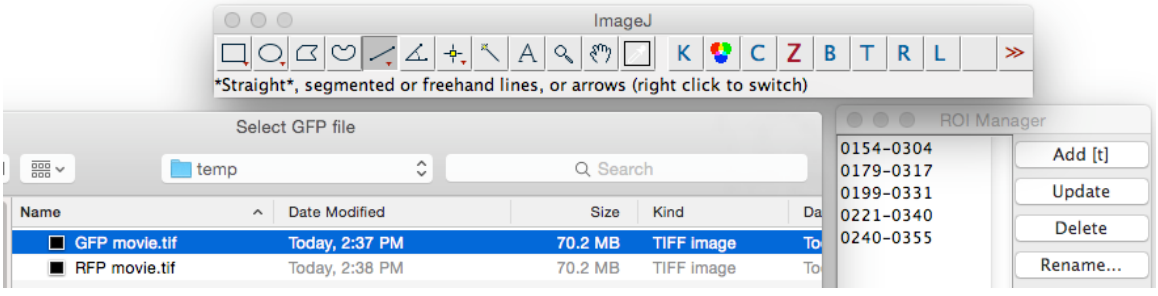
1. Create line regions



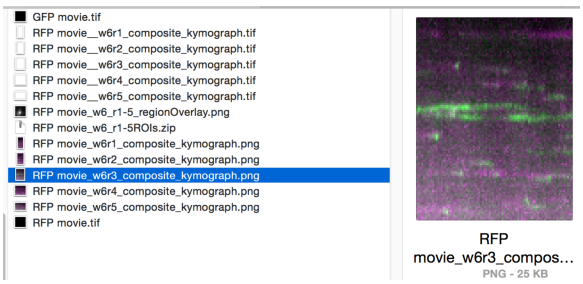
   a. Open your movie, e.g. GFP channel
   b. Optional: Create a maximum projection using the "Z" button on the toolbar
   c. Use the Line Tool to draw lines on the regions of the cells you want to make kymographs for. Save each region by pressing "t" on the keyboard or selecting "Add [t]" in the ROI manager.

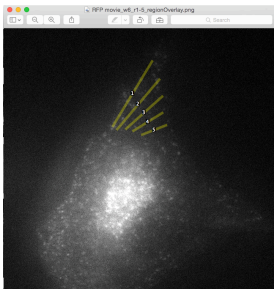
2. Make two-color kymographs

   a. Make sure you have saved any open images that you want. The tool will close all the open images without saving.
   b. Click "C" on the toolbar.
   c. The program will ask you to select the GFP movie, and then the RFP movie.



   d. The program will automatically make two-color kymographs and save them as .tif and .png files in the same folder as your movies. It will also save the regions as a .zip file and an image of the regions overlaid on the cell.





Options:

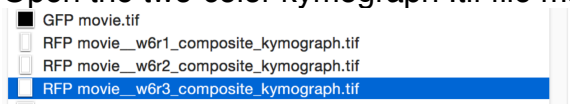
- If you already have line regions saved in a file, then leave the ROI manager empty and the program will ask for the .zip file of line regions.
- If you prefer red-green kymographs instead of red-magenta kymographs, hold SHIFT when you click “C” on the toolbar.
- In the file name of the kymographs, “w” stands for width of the region used to make the kymographs. You can change the width used in the text file “cmespots.txt.” A few lines under “Make color kymographs action tool,” change “kymographWidth = 6” to a different value. (This is currently line 171 of the code.)

[L] Measure line from kymograph

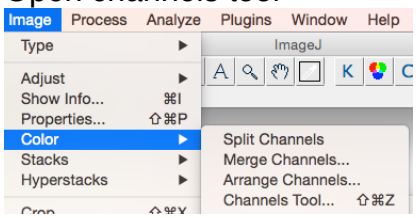
This tool measures lines you have drawn on kymographs and saves them with the same name as the kymograph.

<u>If you made a two-color kymograph:</u>

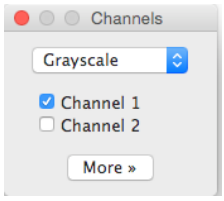
1. Open the two-color kymograph .tif file made in the previous step



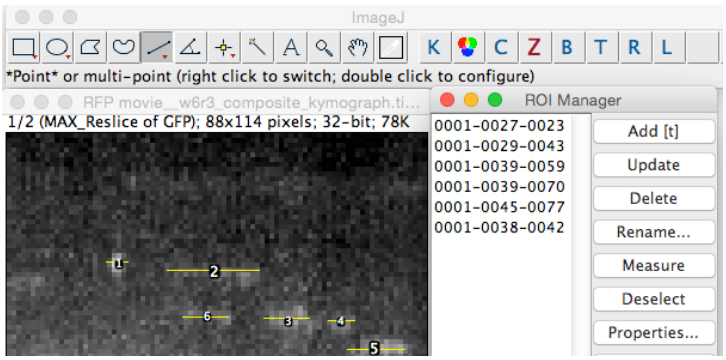
2. Open channels tool



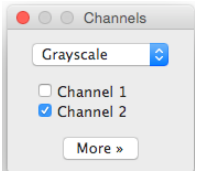
3. In the channels tool, switch to grayscale. Select Channel 1.



4. Use the line tool to draw lines across the fluorescence you see in the kymograph. Save each line by pressing “t” on the keyboard or clicking “Add [t]” in the ROI manager.



5. Once you have made selections for each line, then click [L] on the toolbar. The program will make measurements and save them as .xls and .txt files. The regions will be saved in a .zip file.
6. In the channels tool, switch to Channel 2 and repeat steps 4 and 5.



7. Open the measurement files. The “Length” measurement, multiplied by the time step of the movie, gives you the approximate lifetime of the event. You can plot these in Origin or Matlab. I have a Matlab program that will plot histograms of the lifetimes if you input the file name.

Options:

- If you want a measure of the frequency of events, open cmespots.txt and make “var measureInitiations = 1” instead of 0. This measures number of events (number of line ROIs) and divides by measurement area (kymograph height and thickness) and total time (kymograph width). The final units are in events/μm^2/s. For the measurement to have the right units, the “timeinterval” and “micronsperpixel” variables need to be correct for your movie (in cmespots.txt).