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Instructions for getting imscroll to select spots so you can look at intervals of binding.

1. Open appropriate video file and driftlist
2. Put frame range into the brackets near the top of imscroll gui window
   1. i.e. change [1:10] default to [1:1206]
3. Make sure above frame picking area, thing is set to Gauss 2d + int and moving AOI
4. Go to a part in the video where you can see a good number of spots
5. Pick the spots as you would for DNA, etc., making sure that you are only picking the spots and not blank area.
6. In box where you pick spots, change bottom dropdown menu to “Frame Range” and make sure the button next to the dropdown says High
7. The button that usually says pick will now say “Frames.” Click this
   1. In the matlab window, you will get a print out of 500 and 1000 so you know it’s working.
8. In same dropdown as before, select “save all spots” and Click save button that appears
   1. Change the default file in the data folder to allspotshigh
9. Set dropdown back to pick frm, and set a threshold such that the entire surface of the microscope image is covered
10. Pick Frame range from dropdown, change the button next to it to low by clicking on it and push the Frames button
    1. See 500 and 1000 again to know it’s working. This one takes longer and will be a larger file if you did it right.
11. In same dropdown, select save allspotslow and click the save button that appears
    1. Save the default file in the data folder as allspotslow
12. Open up the trace viewing window and load data from experiment into both top and bottom viewing panes
13. Look through data and find an ideal spot
14. In dropdown under DataOperation button, pic “load vid time base” then push DataOperation button.
    1. It will open a finder window. Pick the video for which you integrated your spots
15. In same dropdown, pick “Set Detrend Frame Range” 🡪 DataOperation
    1. This will bring up the cross spot picking cursor. Click on the parts in your ideal spot that correspond to when nothing is associated (ie the background)
16. Dropdown should now say “Detrend Trace”. Click DataOperation
17. Dropdown should now say “Set mean/std frame range”. Click Data Operation and select the background as in step 15.
18. From Same dropdown, select “Set interval frame range”. Click on the “Interval Data” and then press enter.
19. Set Up to 3.6 and down to 1.0 (below Interval data and dropdown)
20. From same dropdown, select “Find Intervals” and click DataOperation
    1. This should select the intervals that have assocations in your ideal spot.

**IF YOU DID NOT JUST DO THE LOW AND HIGH TRESHHOLDS (otherwise skip to 23)**

1. In Imscroll window: Load your high threshold by selecting “LoadAllSpots” (in the picking spots box area dropdown), put name in the lower left input file name area and click Load in the pick spots box. Make sure the tiny button next to the dropdown says High.
2. In Imscroll window: Load your low threshold by selecting “LoadAllSpotsLow” (in the picking spots box area dropdown), put name in the lower left input file name area and click Load in the pick spots box. Make sure the tiny button next to the dropdown says Low.

**Back in the Viewing Spots Window:**

1. Set Up to what you used to get good results and in top dropdown next to plot, select Binary Spot Trace, and set Radius to 1.5 (appears when you change the upper window dropdown to Binary Spot Trace) (or 1.8 if you data is slightly blurry) in the box that appears above it.
   1. **NOTE:** Good settings for event threshold will vary widely by experiment. After you Spot Int, you should look through the data and see how well the spot picker did. If it did poorly, adjust the event threshold stuff to make it look better. In the end, you’re probably going to have to look through the spots to make sure, but ideally, you don’t want to have to edit more than ½.
2. Click the Spot Int. Button at the bottom of the window
   1. This should start a countup in the regular matlab window where it will go through all of your spots and find where associations happen and try to call them based on your thresholds. It works ok.
3. Save you data by picking the “UI Save Interval Data Structure” in the dropdown under the DataOperation button and naming the file appropriately .

Viewing Intervals

1. In the top viewing window, use the left dropdown and select “Plot Cumulative InputTrace” and push the Cumul. Operation Button above it.
   1. This will bring up a trace of the interval called.
2. If the Interval looks bad, you can look at the spots and manually fix it.
   1. In the left dropdown, select “Edit Intervals”
   2. A + 1 – bar will appear below the top viewing window.
   3. Select the + to add or combine intervals and click on the interval you would like to define (ie click twice, one on each side)
   4. Select the – to remove an interval and click on both sides to remove it.
   5. Once you have changed it, save it using “UI Save Interval Data Structure” as done above, but add a number up to what you have changed
   6. After editing, you will need to reselect “Plot Cumulative InputTrace” and push the Cumul. Operation Button above it.
3. You can also remove bad spots from your data using the dropdown and the Delete InputTrace 🡪 Cumul. Operation combo.
   1. After deleting any, your numbers for the top and bottom will be off, so, you will need to make sure you to reselect “Plot Cumulative InputTrace” and push the Cumul. Operation Button above it periodically, otherwise you will be confused.
4. Do this for all your spots.

Making figures:

1. Open your Cumulative Input file you’ve been editing using the [fn fp]=uigetfile command and picking the corresponding intervals file

2. eval(['load ' [fp fn] ' -mat']) command to read the file

3. cia=Intervals.CumulativeIntervalArray to pull the interval array data from the matlab file and name is cia.

This should give you a matrix with 7 columns and the number of rows corresponding to the number of frames in your movie (ie a 1254x7 matrix)

4. Use plots program written by Simina to make duration histograms

Command example: plots3(cia(:,:), [0:10:100]). The (:,:) indicates to use the entire subset of spots. You can do a subset of them by specifying a number range (ie 2974-3716). **HOWEVER:** These numbers correspond to **EVENTS**, NOT spot numbers. The 7th column in the matrix gives you what spot the event comes from, so you can look at where the spots you want to include begin and end by putting in cia(number:number, 7) of a 10 spot range (ie cia(100:110, 7) which will give you what’s in the 7th column for these 10 numbers to see what DNA spot in your data this corresponds to. You can then use this range specifically for the graphs (if you decide to not curate all the spot picking done by the computer for example).