Single Molecule Data Analysis

MDW 5/28/2014

**Mapping:**

Open your mapping file:

[fn fp]=uigetfile %allows you to pick your image file to analyze

Pick the file of mapping DNA images (matlab🡪images🡪date folder 🡪 correct file number ie sticau01876)

foldstruc.gfolder=fp

imscroll(foldstruc) %opens the GUI for image analysis

**Once new GUI window pops up:**

1. Change “Folder” in left top to “GLIMPSE”
2. Move over in movie until you reach the images.
   1. Image: top: green, bottom: Red, then top again is blue (no bottom present)
3. Push Fullscreen button at top box
4. Change from autoscale to manual
5. Pull top bar all the way right, then adjust bottom bar to right to get good contrast while in green

**Mapping Green (542 laser) and Blue(488 laser)**

1. Go to First Green image that is fully bright (ie usually 2 clicks in from where it first appears)
   1. Want to be bright, but before any spots have bleached.
2. Orange “Auto Spot Picking” box 🡪 Pick Frm
   1. Blue boxes will select spots
3. Lower “Spot Brightness” in Orange spot picking box and push Pick Frm until all the spots you can see are picked and yet the program isn’t picking imaginary spots (can be as low as 15 sometimes)
4. Scroll to Blue image taken at same location
5. “Mapping” 🡪Remove MT AOIs from dropdown
   1. This allows us to select DNA with both a blue and green fluorophore.
6. Put frame number inside [] in the frame range box (ie [79])
7. Click ProxMap
   1. **NOTE: button turns bluish and will say clicked for rest of time, so you only need to do this if it’s the first round or if you have to close matlab**
8. Push “Fit AOIs” button in upper right
   1. This will bring up a new window with blank graphs. It will take a few seconds. Close it once it’s opened.
9. Select “Centering” from the dropdown menu next to the “GoButton”. Click the GoButton
   1. The spots should now be in the center of the blue boxes
10. Define this as Field 1 by selecting the “Define Field 1” option from the “Mapping” orange box and pushing the button
11. Move back to the green image from before
12. Repeat sets 6-9 (skip 7 because it stays on for the remainder of the time)
13. Define this as Field 2 by selecting the “Define Field 2” option from the “Mapping” orange box
14. Make Map by selecting the “Make Map” option from the dropdown menu in “Mapping” and pushing the button.
15. Select Remove X2 AOI from the dropdown in “Mapping” and push button
    1. Remove spots in the X2 graph that are outside the 1 to -1 range by clicking near spots you want to remove. Right click when you have removed everything you wanted to.
16. Select Remove Y2 AOI from the dropdown in “Mapping”
    1. Remove spots as down in 15
17. Repeat 15 and 16 again each time, this time aiming for y values between 0.6 and -0.6.
    1. **NOTE: it makes a new map and that will change the position in the graphs**
18. Rename fitparms.dat file with name that tells how many regions you’ve mapped so far and what colors
    1. **Note: This is only so that if you mess up, you don’t have to completely start over.**
19. Move to next green image taken
20. Repeat steps **1-9**
21. After selecting the new spots for the next location in the blue field, save these spots alone by selecting the “Save AOI information” from the dropdown next to the “GoButton”
22. Mapping box🡪”Add to Field 1”
    1. Adds spots in blue field to previously ID’s spots in Blue
23. Mapping box 🡪 Restore preAddition
    1. Returns you to only the blue boxes you had when you picked them for this frame rather than all the blue boxes in your map
24. Move back to the Green that corresponds to this location
25. Repeat Steps 6-9
    1. **NOTE: Do not remove any spots at this point. Otherwise, your mapping won’t work**
26. Mapping Box🡪 “Add to Field 2”
    1. This adds the new green spots to the mapping file
27. Mapping Box🡪 “Make Map”
    1. Repeat Steps 15-17
28. Repeat Steps 19-28 for the next two locations used for mapping

**Mapping Green To Red Field:**

1. Find a previous Red mapping file (can be ANY one) and put file name.dat into Input Filename box🡪 Gobutton
   1. Numbers in box below should change if it works
2. Go to Green/red image and zoom in so you are only looking at the green.
   1. Select Spots as before
3. To make to Red: Map AOIs (out: x2y2) 🡪GoButton
   1. NOTE: You will need select field 2 in the Magnification box to now view the Red. Once you do, you should see that your blue boxes now appear in this channel.
4. Remove MT AOIs as done above.
5. Fit AOIs as above (Make sure proxmap is still on)
6. Define this as field 2
7. Inv Map AOIs (out x1y1) 🡪 GoButton to move the boxes back to the top field of view
8. Fit AOIs as above
9. Define as Field 1
10. Mapping box: Make Map and push button.
11. Repeat same procedure as above, using Map Out/Inv Map instead of scrolling over to move spots between the top and bottom fields
    1. NOTE: You will need to use Restore preAddition **before** you Inv Map AOIs.

**Drift Correction:**

1. [fn fp]=uigetfile %allows you to pick your image file to analyze

Pick the file your **protein** Data (matlab🡪images🡪date folder 🡪 correct file number ie sticau01883)

foldstruc.gfolder=fp

imscroll(foldstruc)

1. Go about ½ way through the movie and Pick Spots and adjust the picking parameters to get good selection
   1. Write down the parameter #’s for Noise Diam., Spot Diam., and Spot Brightness
2. gui\_drift\_correction
   1. Brings up Gui for doing drift correction
3. Click Load file
   1. Images🡪date🡪pick folder with your **protein** data you were looking at before
4. In gui\_image\_display, drag top slider bar left
5. Click Select Area to Analyze
   1. Click and drag over the region of image you want to look at (the one with spots)
6. Put in Spot Detection Paramters
   1. These are the numbers from Step 2
7. Set Min frames in tracks to 30.
8. Click Start Analysis
   1. This should be fairly quick
9. Click View Track Lengths
10. Zoom in on a red line
11. Click Select from length plot
    1. Pick a red line
12. Redo to pick 8-10 different spots or as many as necessary to have 100% coverage of the video.
13. Polynomial Options
    1. Set X and Y to 4
14. Display Drift Correction
    1. Want to have the fit look pretty good.
    2. If not, try picking more spots/eliminating spots near the edge
    3. To edit: using Track Editing options and scroll through (spots selected are in green). Click deselect spots to remove undesired spots.
15. Click Gaussian refine selected tracks
    1. This will take awhile
16. Click Save Project button
    1. Fig-files🡪Drift Correction Gui🡪Projects (drift\_correction\_date\_file number.mat)