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Data Analysis Protocol

**Before starting this protocol, make sure you have made the appropriate mapping files (488 to 532 and 532 to 640 if using)and have made the drift correction files for both the 532 and 640 channels if you are using them.**

1. Load the DNA before file into matlab
   1. [fn fp]=uigetfile 🡪 select correct file name and location from the images folder inside the matlab folder
   2. foldstruc.gfolder=fp
2. Load the DNA after file into matlab
   1. [fn fp]=uigetfile 🡪 select correct file name and location from the images folder inside the matlab folder
   2. foldstruc.gfolder2=fp
3. Load the Experiment file into matlab
   1. [fn fp]=uigetfile 🡪 select correct file name and location from the images folder inside the matlab folder
   2. foldstruc.gfolder3=fp
4. While the experiment file is the most recent file you’ve gotten, do the following command:
   1. eval(['load ' [fp fn] ' -mat'])
      1. This creates a vid variable which is needed to make the final driftlist
5. Use the drift correction file you made to make a real driftlist:
   1. [fn fp]=uigetfile 🡪 select correct file name and location from the fig-files🡪Gui\_drift\_correction🡪projects folder inside the matlab folder
   2. eval(['load ' [fp fn] ' -mat'])
      1. This creates a Drift variable
   3. Execute the following series of commands:
      1. drifts=driftlist\_time\_interp(Drift.drift\_correction\_cumfit\_glimpse,vid);
         1. This creates a drifts variable
      2. foldstruc.DriftList=drifts.diffdriftlist
         1. This puts the finalized driftlist into the foldstruc variable so it will be there when you open the imscroll gui
      3. driftlist=drifts.diffdriftlist
         1. Makes variable driftlist so you can save this specifically
   4. Save the finalized driftlist:
      1. save c:\matlab\data\(desired name of driftlist here.dat) driftlist
         1. Putting driftlist at the end will tell matlab to save that specific variable
         2. Example: save c:\matlab\data\03012016\_1336\_532driftlist.dat driftlist
6. Open Imscroll Gui
   1. imscroll(foldstruc)
      1. This will open the Gui so you can look the your files, pick spots, integrate over time, etc.
7. In upper left, change dropdown from “Folder” to “Glimpse”
   1. Changing the number above this from 1 to 2 and 3 etc changes the file you are viewing. 1 is gfolder, 2 is gfolder2, 3 is gfolder3, etc.
8. Make Map from DNA before to DNA after reaction mix addition
   1. Open DNA before file and scroll to appropriate location
   2. Orange Magnification box: select field 1 and click button
      1. This will zoom in on only the top field of view, where the DNA spots will be if they are blue. If red, select field 2
   3. Pick DNA spots using the orange Auto Spot Picking box
      1. Change the Spot Brightness to get boxes around the visible DNA spots
   4. Change to the DNA after file (should be 2 if you followed the above instructions) and go to the first frame.
   5. Make sure that the boxes overlap the spots that are there. They don’t have to be centered, just inside the boxes
      1. If they look very far off, you can move them with the “Move” button in the middle left. Clicking it brings up up/down/left/right arrows that allow you to manually move boxes so that they are inside the spots
   6. Upper Right Mapping box, pick Remove MT AOIs
      1. This will remove any boxes that are not present at this time.
   7. Fit these AOIs
      1. Change frame range (Upper right) to 1
      2. Hit the Fit AOIs button
      3. Once done, this will open a new window. Close it.
   8. In center of the gui, from dropdown, select Centering and click the GoButton next to it
      1. Scrolling over 1 in your video, the boxes will shift slightly so that the DNA spot should be in the center.
   9. Define this a field 2
      1. In Orange Mapping box, select “Define Field 2” and click the button next to it
   10. Return to the DNA before protein addition file (should be glimpse file 1)
       1. Manually move the DNA back to be around the DNA spots if you moved them above
   11. Fit these AOIs
       1. Put correct location into the frame range based on where the DNA image is within your file
          1. i.e. [58]
       2. Click Fit AOIs button (center)
       3. Once done, this will open a new window. Close it.
   12. Define this as Field 1
       1. In Orange Mapping box, select “Define Field 1,” and click the button next to it.
   13. In Orange Mapping box: select the Make map option from the dropdown and click the button next to it.
       1. This will make a map as done with the mapping files
   14. Remove points that are outside of 0.8 and -0.8
       1. See Mapping protocol for more details on this procedure
   15. Save map
       1. This creates a fig file in the matlab\fig-files\imscroll\mapping folder. Rename this appropriately
9. Picking DNAs for Analysis:
   1. Go to your DNA image in the pre-reaction DNA file
   2. Pick spots using the Auto Spot Picking orange box
      1. Change spot brightness to get all the spots you can see
      2. You can change the Frame Average (Bottom middle next to scroll bar) to 2 or 3 to make it easier to see your spots if they are dim
   3. Fit and center your AOIs
      1. Like before: put correct frame into frame range box, then click Fit AOIs
      2. Once done, this will open a new window. Close it.
      3. Centering🡪 GoButton
   4. Remove close AOI
      1. Orange Mapping box 🡪 Remove close AOI, number should be 3, click button (This removes DNAS that are really close together)
   5. Go through each quarter and remove bad AOIs
      1. Orange Magnification box: select field 1 Q1. It should then zoom to ¼ of the view
      2. Look at spots in the boxes. Are they too bright? Are there multiple spots within the boxes? Those should be removed.
         1. To remove: Click the Remo button (left middle) and click on the spot you want to remove
      3. Do this procedure for each quarter of the appropriate field of view
   6. Save your Spots!
      1. Center dropdown: Save AOI information 🡪 GoButton
      2. This creates a default.dat folder in the matlab\data folder. Change the name to something appropriate.
10. Looking at associations of protein over time:
    1. Position your DNA to where it will start.
       1. Load in your DNAbefore to DNA after mapping file
          1. Put file name.dat into the Input Filename box (center)
          2. Center dropdown: Load Fitparms 🡪 go button
             1. This will change the numbers in the mx21 bx21 my21 by21 box
          3. Center dropdown: Map AOIs (out: x2y2) 🡪 GoButton
             1. If you scroll over one, the AOIs will shift
       2. Load in your 488 to 532 mapping file
          1. Same method as above
          2. Map AOIs (out: x2y2) 🡪 goButton
             1. If you scroll over one, the AOIs will shift
    2. Change pixel number (upper right next to frame range) to 5
       1. Scrolling will make boxes shrink
    3. Change frame range to [1:end of video]
       1. Make sure now that the Frame average at the bottom next to the scroll bar is set at 1 or your analysis won’t work!!!!!
    4. Above Auto Spot Picking, change Fixed AOI to Moving AOI and Guass2d+int to Int. linear interp
    5. Hit the Fit AOIs button
       1. This should start a count up by 10 in matlab. Note that the start of this can take a while, so give it 5min or so to get going before you freak out.
    6. Once done, a new window will pop up. Leave it open this time, because you probably want to do more analysis in it.
    7. Once the new window opens, a large default.dat file will have appeared in the matlab\data folder. Change the name as this is your results file.
11. Figure out what you want to do with your data!
    1. Dwell Analysis? Time to First binding? See other instruction sheets for more info!