**Processing Data (January 2014; Simina Ticau)**

Open Matlab 🡪 Make sure you're in the right directory

**Mapping:**

[fn fp]=uigetfile //choose header.mat from the first video

foldstruc.gfolder=fp

[fn fp]=uigetfile //choose header.mat from the second video

foldstruc.gfolder2=fp

imscroll(foldstruc)

look through the images in the first GUI (make sure it's on GLIMPSE); can change between the 2 videos and confirm the correct ones are open

click "MAPPING" to obtain the new GUI

* set both figures to GLIMPSE
* scroll to the correct frames (set avrg.=2)
* can change scale to make the spots brighter and easier to pick out: click "AutoScale" and change MaxIntensity; use scroll bar to modify
* make Pixel Number = 8
* zoom in to ¼ of the view
* click "NoFit"
* AddPoint (click one in top window and corresponding point in the bottom window)
  + choose 3 spots in this way --> should start fitting automatically (right click in top window if you agree, left click on bottom if you want to correct)

change the name of fitparms.dat in fig-files--> im-scroll--> mapping

date\_im1\_im2\_channel\_fitparms.dat (4\_16\_12\_323\_324\_488\_fitparms.dat)

**Drift Correction:**

[fn fp]=uigetfile //choose header.mat from the video

foldstruc.gfolder=fp

imscroll(foldstruc) //look at the images; leave GUI open

gui\_drift\_correction //to open up drift gui

* "Load File" //select file to open
* Adjust intensity using “+” and scrollbar
* "Select Area to Analyze" (use left button to select area)
* Select Range to Analyze (range of frames--> check in main GUI; in large box)
* Diam of Noise =1.5; Diam of Spots =5; Brightness of Spots =60; Min Frames in Tracks =50
* "Start Analysis" //wait for it to finish
* "View Track Lengths"
* select from these until you get complete coverage (can zoom in)
  + "Select from Length Plot" and click on track
* set both "ln X" and "ln Y Axis" to 4 (degrees of freedom) -->"Display Drift Correction"
* "Gaussian Refine Selected Tracks"
* "Save Project" - in fig-files--> Drift Correction GUI--> Projects (drift\_correction\_date\_file number.mat)

**load driftlist:**

[fn fp]=uigetfile \\header.mat for video

eval(['load ' [fp fn] ' -mat']) \\vid variable should show up

[fn fp]=uigetfile \\drift\_correction.mat file

eval(['load ' [fp fn] ' -mat']) \\Drift/driftlist variables should show up

help construct\_driftlist\_time \\help for function

drifts=driftlist\_time\_interp(Drift.drift\_correction\_cumfit\_glimpse,vid); \\makes drifts variable

foldstruc.DriftList=drifts.diffdriftlist;

driftlist=drifts.diffdriftlist;

save S:\Desktop\matlab\data\4\_16\_12\_325\_driftlist.dat driftlist (name of driftlist at the end .dat)

**//to load up at another time**

foldstruc.DriftList = driftlist;

**select DNA spots**

pay attention to spot brightness;

"Save AOI Information" Go; change default.dat in matlab -->data

**Setting low/high thresholds:**

* open up protein video
* select spots: Spot brightness 65, Diam.5, Noise 1.0
* input frame range
* select area (half the whole screen)
* select "Moving AOI" and "Gauss2d+Int"
* make sure high threshold is selected
* select Frame Range; click Frames
* Save AllSpots ->Save
* change default.dat in data (date\_number\_allspots.dat)
* to look at them: select Map Spots --> Map
* select low threshold; spot brightness = 6; make sure “low”; Frames
* save AllSpotsLow ->Save
* change default.dat in data (date\_number\_allspotslow.dat)

**Loading/Mapping AOIs from DNA to protein**

1. "Load Fitdata: marking spots"-> enter name of AOIs file (4\_16\_12\_323\_AOIs.dat) -> "GoButton"
2. "Load FitParms" -> enter name of mapping file -> "GoButton" -> "Map AOIs (out: x2y2)"
3. "Save AOI Information"-> "GoButton"-> change name of default.dat (date\_name\_mappedAOIs.dat)

**Integrate:**

* Map out AOIs
* select frame range
* "Moving AOI"
* "Int.linear interp."
* select pixel size (pixel number=5)
* "Fit AOIs"

when it's done --> change default.dat in data file

**Find intervals**

In Integration GUI

1. "load vid time base"-> DataOperation -> select header.mat of current file
2. "Set Detrend Frame Range"-> DataOpertaion (click beg/end of background; right click at the end)
3. "Detrend Trace" -> DataOperation
4. "Set mean/std frame range"->DataOperation->same as above
5. "Set Interval Frame Range" -> place cursor in "Interval Data" [1 1130]; hit return
6. Up 3.6
7. Down1.0
8. Find Intervals -> DataOperation

back in the 1st GUI:

"LoadAllSpots" -> type up name -> "Load"

"LoadAllSpotsLow" -> type up name -> "Load"

2nd GUI

Up: 1.5

radius: 1.5

(in the Binary Spot Trace)

"Spot Int" (starts up automatic spot finding of all spots"

"UI Save Interval Data Structure" to save everything

Removing background from AOIfits using fixed sigma:

* “Fit AOIs” over a short range, say 100 frames using “Gauss2d+Int”, “Moving AOI”, and a pixel number of 10
* load AOIfits file
* plot out sigma:
  + aoifits.dataDescription
  + figure(11);hist(aoifits.data(:,6),20);shg
* look at the plot/determine mean
  + logik=aoifits.data(:,6)<2; or logik=aoifits.data(:,6)<2 & aoifits.data(:,6)>0.5;
  + mean(aoifits.data(logik,6))
* load aoifits in each channel
  + aoifits1= 549
  + aoifits2= 649
* assign them to dat
  + dat1=draw\_aoifits\_aois\_v1(aoifits1,'y');
  + dat2=draw\_aoifits\_aois\_v1(aoifits2,'y');
* plot dat % 131 refers to AOI# in this case
  + figure(10); plot(dat1(:,2,131),dat1(:,3,131),'b');shg
  + figure(10); hold on; plot(dat2(:,2,131),dat2(:,3,131),'r');shg
* xcvkb