**Step by Step puncta analysis:**

**Scripts stored in MATLAB scripts 🡪 Tracking with Mingyucode 🡪 Live-cell puncta analysis 🡪 Processing or Analysis**

**Part 1:**

Calculate illumination bias that needs to be subtracted from each image prior to tracking. Requires the camera specific CMOS file.

1. Run BiascalcNuc.
2. Run BiascalcSignal for YFP and mCherry channels right after the first script. If workspace is cleared after running the biascalcnuc script, load the CFP.mat bias file before running Biascalcsignal or else you will get the error “undefined function or variable “bias””.

**Part 2:**

Track single cells and measure desired signals.

1. Timelapsepuncta\_BackgroundIllumcorr/Parallel well. Puncta size 4 and strel radius of 8 works the best for MCF10A. Make sure MATLAB knows the location of the correct punctainfo\_new otherwise you get an error “expected two curly brackets…..”. Data is saved as tracedata\_withPuncta\_(row)\_(col)\_(site).

**Part 3:**

Various scripts for plotting data obtained from Timelapse.

1. Viewtraces\_Panel\_CDK2IF\_allreddots\_puncta. Spikes in puncta (foci detected only for a single frame) have been removed by Harsha. It removes puncta >10 and replaces with previous frame value. Can change this number in line 112 of this script. Data is saved as Row\_col\_gatedTracedata\_reddots.
2. Align\_mitosis\_traces\_segregated\_withPuncta. Output is “keyinfo”.
3. Manuallyclassifygoodcell\_includesNucArea \_andpuncta. This scripts requires “keyinfo” data as input. Make sure you check which number to use to classify a trace as emerge or low. Also, skip traces (press enter) that do not last long enough to be classified as inc or low with confidence. The current parameter that I have is the trace needs to exist for at least 5 hours after anaphase to be considered. For a trace to be classified as “emerging”, the cell needs to be in CDK2low state for at least 5 hours but not more than 10 hours. Any cell that remains quiescent for more than 10hours should be classified as “low”.
4. Align\_mitosis\_traces\_manuallyselected\_allpunctaplots or Align\_mitosis\_traces\_manuallyselected\_allpunctaplots\_rand300 (randomly selected 300 traces).

**Finding the dependencies for a MATLAB code:**

The list of functions required or called by any scripts can be documented by using the following command in MATLAB:

[fList,pList] = matlab.codetools.requiredFilesAndProducts('Timelapse\_puncta\_backgroundcorr.m')

\*Make sure the current folder is where the script is located and also the path to the master folder with all the functions is added to MATLAB before running the above command.

Instructions from MATHworks site: https://www.mathworks.com/help/matlab/matlab\_prog/identify-dependencies.html

List of functions called by Timelapse\_punctabackgroundcorr:

'X:\MATLAB SCRIPTS\TrackingwithMingyucode\Functions\ThreshImage.m'

'X:\MATLAB SCRIPTS\TrackingwithMingyucode\Functions\adaptivetrack\_9\_puncta.m'

'X:\MATLAB SCRIPTS\TrackingwithMingyucode\Functions\apriori\_markermask.m'

'X:\MATLAB SCRIPTS\TrackingwithMingyucode\Functions\bgsubmasked\_global\_2.m'

'X:\MATLAB SCRIPTS\TrackingwithMingyucode\Functions\blobdetector\_4.m'

'X:\MATLAB SCRIPTS\TrackingwithMingyucode\Functions\blockpercentile\_blockimage.m'

'X:\MATLAB SCRIPTS\TrackingwithMingyucode\Functions\bridge.m'

'X:\MATLAB SCRIPTS\TrackingwithMingyucode\Functions\excludelargeandwarped\_3.m'

'X:\MATLAB SCRIPTS\TrackingwithMingyucode\Functions\getcurvature.m'

'X:\MATLAB SCRIPTS\TrackingwithMingyucode\Functions\getcytoring\_3.m'

'X:\MATLAB SCRIPTS\TrackingwithMingyucode\Functions\getdapimask.m'

'X:\MATLAB SCRIPTS\TrackingwithMingyucode\Functions\getdeflections.m'

'X:\MATLAB SCRIPTS\TrackingwithMingyucode\Functions\interpolateframes.m'

'X:\MATLAB SCRIPTS\TrackingwithMingyucode\Functions\markershed.m'

'X:\MATLAB SCRIPTS\TrackingwithMingyucode\Functions\markershed\_apriori.m'

'X:\MATLAB SCRIPTS\TrackingwithMingyucode\Functions\postprocessing\_nolinking.m'

'X:\MATLAB SCRIPTS\TrackingwithMingyucode\Functions\punctadetect\_new.m'

'X:\MATLAB SCRIPTS\TrackingwithMingyucode\Functions\punctainfo\_new.m'

'X:\MATLAB SCRIPTS\TrackingwithMingyucode\Functions\registerimages.m'

'X:\MATLAB SCRIPTS\TrackingwithMingyucode\Functions\secondthresh.m'

'X:\MATLAB SCRIPTS\TrackingwithMingyucode\Functions\segmentdeflections\_bwboundaries.m'

'X:\MATLAB SCRIPTS\TrackingwithMingyucode\Functions\splitdeflections\_4\_bwboundaries.m'

'X:\MATLAB SCRIPTS\TrackingwithMingyucode\Functions\threshmask.m'

'X:\MATLAB SCRIPTS\TrackingwithMingyucode\Functions\timelapsesetup\_4.m'

**List of functions called by the various plotting scripts:**

'X:\MATLAB SCRIPTS\TrackingwithMingyucode\Functions\gate\_Cdk2\_8\_mother.m'

'X:\MATLAB SCRIPTS\TrackingwithMingyucode\Functions\gate\_Geminin\_9\_mother.m'

'X:\MATLAB SCRIPTS\TrackingwithMingyucode\Functions\gathertracedata\_4\_allreddots\_puncta.m'

'X:\MATLAB SCRIPTS\TrackingwithMingyucode\Functions\getmotherstatsonly.m'

'X:\MATLAB SCRIPTS\TrackingwithMingyucode\Functions\getstats.m'

'X:\MATLAB SCRIPTS\TrackingwithMingyucode\Functions\linkancestry\_allreddots.m'

'X:\MATLAB SCRIPTS\TrackingwithMingyucode\Functions\parseArgs.m'

'X:\MATLAB SCRIPTS\TrackingwithMingyucode\Functions\smoothignorenans.m'

'X:\MATLAB SCRIPTS\TrackingwithMingyucode\Functions\subaxis.m'