Immune cell imaging analysis routine HYK 2/19/2016

1. Copy imaging .TIF files from Metamorph into new directory. Files need to be acquired using Metamorph multi-dimensional plugin to be compatible with the MATLAB reader.
2. Run MATLAB. Add all the directories in the image processing suite to the current path, by pressing `Set Path’, followed by ‘add with subfolders…’
3. Edit the relevant scripts to process imaging files:

* Specify the directory location of the raw images, file prefix and output directory:

1 - preprocess, segment and track/main.m (line 3):  
  
indir = {'../rawdata'}; %input directory names

base = {'exp1'}; % prefix for Metamorph files

dirname = {'../processed'}; %output directory names

* Specify the segmentation and tracking parameters:

1 - preprocess, segment and track/main.m (line 9):

% image segmentation parameters

minsize = 45; % minimum size of a cell in pixels

maxsize = 600; % maximum size of a cell in pixels

maxecc = 8; % maximum eccentricity of a cell in pixels (1 = circle)

out = 'sch.mat'; % output file (segmentation only)

out2 = 'schtracked.mat'; % output file (segmentation + tracking)

% cell tracking parameters

Cxy = 40; % maximum travel distance for cell from frame-to-frame

Carea = 0.3; % maximal fold change in area

Cgr = 0.5; % maximal fold change in fluorescence level

maxskip = 1; % the total possible number of omitted frames for tracking

* Specify the number of pixels in the image, number of rows and columns in image tile, and the tiling order:   
  1 - preprocess, segment and track/mmpp.m (line 15):

X = 256; % the number of row pixels

Y = 256; % the number of column pixels

M = 5; %sqrt(S); % the number of row positions

N = 5; %sqrt(S); % the number of column positions

%% map stage positions

S = M\*N; % the total number of stage positions for this condition

mapping = reshape(1:S,M,N); % tiling order for the images

mapping = mapping(:,end:-1:1) + offset\*S;

* Specify the identity of the channels in the images in  
  1 - preprocess, segment and track/mmpp.m (line 35)

% DIC channel parameters, defined as previous with micromanager

C(1).cG = 255; % color - green

C(1).cR = 255; % color - red

C(1).cB = 255; % color - blue

C(1).doz = 0; % do a z-stack or not

C(1).zslices = 1; % number of z-slices

C(1).tlist = [];  
  
Note that the index of the array corresponds to the wavelength number acquired in Metamorph.

1. Run main.m to process images, perform cell segmentation and cell tracking.  
     
    Output files:  
     
   imgf\_XXXX.mat image files in MATLAB arrays, background subtracted

acq.mat image acquisition parameters

sch.mat MATLAB file containing image segmentation  
schtracked.matMATLAB file containing image segmentation and tracking

1. To access graphical user interface for manual cell tracking, run:

3 - image viewer with gates/pview.m

You will be prompted to enter the location of acq.mat, followed by the location of schtracked.mat. Afterwards, the images will appear. Use the scrolls bars above to scroll through the timelapse movie. To perform manual correction, press segment, then follow keyboard shortcuts below to edit cell segmentation and tracking.Keystrokes

Keypad with Numlock on

7 advance to previous frame with fluorescent image

9 advance to next frame with fluorescent image

4 advance to previous frame

6 advance to next frame

1 go to first frame of object

3 go to last frame of object

5 add object closest to mouse for current track

2 identify current object on screen

0 change current track / create new track

+ zoom in

- zoom out

\* remove all subsequent objects from track

z assign track to child

a approve of track

s disapprove track

u undo

p draw polygon to create new track

/ perform watershed split of two cells, choosing cell closer to cursor as object

\ draw dividing line to split two objects

[ track in the backwards direction

] track in the forward direction