# Documentation for myotube analysis code

## Initialising

Semi-automated Matlab code which tracks myocytes through the frames of brightfield images.

Save input videos as stk\_00*x*\_*y\_z.*tif

Where *x* is the video number (from 01 to 99), *y* is a description of the experiment (eg. *‘\_MD0-1 100perN2B27 10perHS 5min-01-Stitching-0*’) and *z* is the position number.

Example: *stk\_006\_MD0-1 100perN2B27 10perHS 5min-01-Stitching-02.tif*

This is the 6th video at position 2 for the 100% N2B27, 10% Serum experiment.

Run the Matlab code *CellTrackWorkFlowLiveImaging0221.m* in the same directory as your image files and the files *nearestneighbour.m* and *moore\_rayleigh.m*

## Videos per run

The number of videos to input is a balance. Tracking cells for longer gives more accurate behavioural data but myocytes are lost from tracking with each additional video. I use 5 videos of 30 frames per run (150 mins real time).

I do not use the first video of a run as this gives time for optical artifacts etc. to settle.

Inputs

**Image variables**

'Pixel width (microns):', [Important to get this right!!]

'Frame length (mins):'[Default is 5]

'Number of first video:'[Default is video 2]

'Number of last video:'[Default is video 6]

'Filename suffix:'[Main body of filename (see above)]

'Position:'[Default is 1]

**Image processing variables (Leave as defaults unless output is unreliable (see below).**

'Erosion factor:'[Default is 1]

'Opening factor:'[Default is 6]

'Dilation factor:'[Default is 2]

## Manual check

This code is not intended to define *all* myocytes in an image as this is very difficult without staining. The accuracy of tracking is improved by using a manual observation tool.

Left-click on unlabelled myocytes, right-click on any myotubes which have been mis-labelled. Press spacebar to finish.

**You do not need to label every myocyte!**

There are two strategies to use here:

1. Label only the unlabelled myocytes directly surrounding a labelled cell. This prevents the tracking code from ‘jumping’ between cells.
2. Label all of the myocytes. This provides more tracking data and gives a good estimate of myocyte density per image. It can be time consuming though!

## Display live tracking?

Get a frame-by-frame update of myocyte tracking. This will show if the code is functioning properly but uses a lot of processing time.

It is recommended to choose *yes* first time you input a new set of videos then *no* when you are happy with the result.

## Outputs

The code produces data on a variety of metrics. Below is a description of the ones which I have found useful.

### General

**MeanMBPersistence:** Mean persistence of motion of myocytes (dimensionless). Cell displacement / sum of distances moved at each timestep. Persistence of 1 is a cell which always moves in a straight line. Lower persistence indicates more randomness in the direction of motion.

**SDMBRotVel:** Standard deviation in velocity of angular motion (degrees). *This is what I use to summarise the angular variation in my model.*

**MeanMBRotVel:** Resultant angle of rotation (degrees). If motion is completely random, this should tend to zero. Anything above +/- 1 degree indicates a preferential direction of motion.

**MeanMBSpeed:** Average speed of motion of myocytes (um/min).

**SDMBSpeed:** Standard deviation in speed of motion of myocytes (um/min).

**Myoblasts/Myotubes:** Binary images of segmented myocytes/myotubes at the last stage. Useful to visualise to check reliability. I tend to delete these as they are quite large.

***RMTestMB:*** *Rayleigh-Moore test for preferential direction of motion. 1 indicates there is no significant preferential direction, 0 indicates a significant preferential direction with resultant angle* ***resultant\_phase.***

### Relevant only if all myocytes are labelled

**Myocytetot:** Total number of myocytes at frame 1.

**NearestNeighbourFinal:** Mean distance between myocyte nearest neighbours. *Only reliable for 1st frame.*

**NearestNeighbourSDFinal:** Standard deviation in distance between myocyte nearest neighbours. *Only reliable for 1st frame.*

## Trouble shooting

**Code does not run at all**

1. Check that all code files are in the same directory as images.
2. Check that the filename fits the type required.
3. Check that your version of Matlab is current and contains the necessary Toolkit add-ons.

**Too few myocytes/too many myotubes labelled**

As the code uses brightfield images, the contrast and focus of the image will affect the accuracy. Also, the sparser the cell density, the more accurate the discretisation code is.

1. Choose a region with fewer cells.
2. Change the default ‘image processing variables’ in the *inputs* comment box.