User's guide for TFM_v1

This software is licenced under the GNU General Public Licence 3.0 or later.
Please read the README.md and LICENCE files for more information.

Installation
1. Download from this repository the software and unzip it
2. Download the localization routine from: http://ciml-e12.univ-mrs.fr/App.Net/mtt/
   NOTE: this matlab program is provided under a licence of the university of Marseille
3. Unzip the file MTT.zip in the directory TFMv1 where the Matlab routines are located.

Preparation of the files:
1. Open the movie in ImageJ as stack of images
2. Run the macro "Crop_and_save.ijm"
3. Select the regions of interest (ROI) with the "Rectangle" instrument and add them to the ROI list using the key 't'. NOTE: when cropping the cell be sure to include a region of at least 5-10 pixel of not moving beads. Exclude from the analysis cells that are too close to the border or to other cells.
4. When finish click on 'OK'
5. The macro proposes a mask of the cell: you are satisfied with it click on "OK". If you are not satisfied click on "Not ok", select manually a close region with any instrument for selection (e.g. "Freehand" or "Oval") and click on "Continue"

Analysis
6. Crop and prepare beads and mask files using the crop_save_mask.ijm in Fiji/Imagej
7. In Matlab navigate to the position where the macro TFM_v1.m is placed
8. Open and run the macro TFM_v1.m. This file
   a. changes the path to make accessible the localisation and TFM routines
   b. loads the parameters for the computation
   c. run the analysis
   d. if required produce movies of the different results
9. Fill the dialogue with the parameters required:
   a. Pixel size (m): size of the pixel of the images in meters
   b. Time interval (s): time resolution in seconds (for movies)
   c. Young modulus (Pa): rigidity of the gel
   d. Poisson ratio: for isotropic elastic materials is 0.5
   e. Regularization: Tikhonov regularization parameter, limit effect of short range bead movements (generally kept around 5e-19)
   f. Window size (pixel): resolution of the displacement and force map, we advice to use a value of 4px, not too small to smooth noise, not too large to make it to coarse-grained
   g. Reference frame (0 to select file): the image of the beads in relaxed state (for dynamics TFM better use the first image taken before the cell contact the surface). Setting this parameter the user is asked to provide a .tif file as source of this data (the image must be of the same size in pixel of the rest of the movie)
10. A dialog asks if movies of the analysis are needed

11. A dialog window asks to choose the *Name*_movie.tif file containing the images of the beads.

Output

The file provide three files as output:

- *Name_results.mat*: the table containing the following variables

Frame	Sequential frame number
U	Contractile energy in the cell (J)
Mu	Trace of contractile moments (N*m)
Ftot	Sum of the magnitude of the forces in the cell (N)
Pmoy	Average magnitude of the stress (Pa)
Tmax	Maximal magnitude of the stress (Pa)
Fvect	Magnitude of the sum of the force vectors in the cell (N)
surface	Area of the cell (m^2)
Theta	Angle formed by the main moment eigenvectors and frame
Mu1	Moment matrix first eigenvalue (N*m)
Mu2	Moment matrix second eigenvalue (N*m)
nbeads	Number of beads in the cell area

Name_results.txt: same as above in CVS format

Name_displ.mat: the structure *displ* containing at each time point the following variables

deplacementX	Interpolated displacement in X matrix
deplacementY	Interpolated displacement in Y matrix
BWmask	Mask of the cell in the new grid size
gridX	Grid on which forces and displacement are computed (X position)
gridY	Grid on which forces and displacement are computed (Y position)
positionX	Position of the beads (X component)
positionY	Position of the beads (Y component)
deplaceX	Bead displacement (X component)
deplaceY	Bead displacement (Y component)

interval	Number of pixel taken to make the grid
pix	Size of the pixel in meter
regx	Registration parameter (X component)
regy	Registration parameter (Y component)
numbeads	Number of beads in the cell area

Name_force.mat: the structure containing at each time point the following variables

TractionX	X component of the traction force along the grid matrix
TractionY	Y component of the traction force along the grid matrix
gridX	Grid on which forces and displacement are computed (X position)
gridY	Grid on which forces and displacement are computed (Y position)
alphadef	Tychonov regularization parameter

Name_param.mat: the structure containing at each time point the parameters necessary for the analysis and the movie reconstruction.

Name_energy.fig: graph of the contractile energy of the cell vs time (seconds).
Name.fig: graph showing the beads detection in the reference frame (useful to check whether the beads localization works correctly with the used setting).

If the option for movies has been selected the software will generate the following .avi movie (color coded) with the quiver per time frame:
- *Name_bead.avi:* movie of the beads and (overlaid) tracked position and quiver plot.
- *Name_displ.avi:* movie of the interpolated bead displacement (color coded) and (overlaid) quiver plot.
- *Name_force.avi:* movie of the interpolated stress field (color coded) and (overlaid) quiver plot.
- *Name_energy.avi:* movie of the contractile energy measure as local scalar product of the force and displacement field.