This repository contains a zip file ‘ptfm\_code’ which contains MATLAB ‘.m’ files for pTFM strain energy calculations. The main code ‘pTFM’ is designed for ‘.lif’ files but user can simply tune the code for their desired input format.

**Running the pTFM code:**

The code requires the input of the following:

**Initial pattern area** which can be quantified using the same code, which also provides area values as an output. Note: It is recommended to keep the same initial area for all the pTFM experiments, and the same initial area can be used for all the strain energy calculations.

**Pixel size** of the raw images

**Crop size** can be used to crop images if required. The input image can have single or multiple patterns in the single field of view, however for multiple patterns use crop function in the code to get the single pattern from multiple pattern image for analysis.

**Threshold value** for image binarization. User can change the value according to the brightness of the image to make sure pattern area is completely binarized

**Substrate Shear moduli** in Pascals

**Image series number** in the ‘.lif’ file is added in the ‘pos’ variable

If multichannel images are captured user can put specific **channel number** to be analyzed in the code.

For **time series** data mention the frame number set in variable N

Once you start running the code, it will ask for the ‘.lif’ file

**Running example files:**

The example file ‘pTFMExample.lif’ has images for two cells, where each image consists of 4 channels. The fourth channel contains the deformed pattern image which will be used to calculate the strain energy in pJ. Code inputs for example file are as follows: Initial Area (IA) = 2401.96, pixel size = 0.227, substrate shear moduli = 666.66 Pa