**Guideline: Analyzing Tissue Mechanics using Particle Image Velocimetry from Live Cell Imaging**

1. **Software:** MATLAB R2019b & PIVLab v2.54 Plugin. (<https://www.mathworks.com/matlabcentral/fileexchange/27659-pivlab-particle-image-velocimetry-piv-tool-with-gui>)
2. **Before running:** Set path to all codes provided.
3. **PIV Settings:**
   * Run images on PIVLab to obtain PIV data.
   * Export all results to Matlab workspace. This will export PIV data for time series (x, y, u\_original,v\_original, u\_filtered, v\_filtered) in the form of cell structure array, each cell represents a timepoint.

A screenshot of a computer

Description automatically generated

* + Sample data provided as the image sequence of one movie and its output of PIVLab (pivdata.mat) and the segmented image of first frame.

1. **Bayesian estimation of tissue mechanics property: Elasticity (E), nu, eta from PIV:**
   * Import segmented cell data as segmentation mask into Matlab workspace. Name the variable segmentation\_mask.
   * Find strain tensors from PIV data.

strain\_tensors = computeStrainTensor(x,y,u\_original,v\_original); %use u\_filtered and v\_filtered if the PIV data were filtered for outliers

* + Run the Bayesian modeling on the PIV data to obtain E, nu and eta. Use mean E, nu and eta.

mechanical\_results = CellularMechanicsAnalysis2.analyzeCellularMechanics(strain\_tensors{1}, segmentation\_mask);

* + Output Window: Visualize the cell morphology detection for the user to double check the segmentation.

A collage of different colors

Description automatically generated

* + Output in Command Window: Acceptance rate has to be > 30%

A white text with black text

Description automatically generated

1. **Measuring stress & tension:** 
   * Run Tissue Analyzer.

analyzer = TissueStressAnalysis2();

* + Run the method in Tissue Analyzer to obtain stress and tension.

[stress\_tensor, tension\_map] = analyzer.calculateStressAndTension(x, y, u\_original, v\_original, strain\_tensors, E, nu, eta, dt); *%dt in second(s), in the provided sample data, dt = 1800*.

* + Export mean value for each time frame as .csv.

TissueStressAnalysis2.exportMeanValuesPerTimepointMasked(stress\_tensor, tension\_map, 'timepoint\_means.csv');