Last updated: 2024/11/12 – Max Hockenberry

The following instructions will guide you through processing the provided example 3D TFM dataset.

Before getting started, you will need to:

* Download the latest version of the Legant Lab 3D TFM code @
* Download MATLAB (most modern versions should work fine but haven’t been rigorously tested. We use MATLAB 2024b).
* Download the following MATLAB toolboxes:
  + Statistics and machine learning
  + Curve Fitting
  + Optimization toolbox
  + Parallel computing toolbox
  + Global optimization toolbox
  + Image processing toolbox
  + Partial Differential equations toolbox
  + Computer Vision toolbox

# Example dataset contents:

* 3D volumetric data set of a cytoplasmic GFP, SiR-DNA stained fibroblast migrating through a 5-micron wide, 40-micron long confinement. Individual channels are separated into separate folders while the bead folder also contains the reference frame where the cell is removed with SDS (Cell, DNA, and Beads folders).
* 2D masks of the cell shape and nuclei derived from cytoplasmic GFP and nucleus signal (Cell\_mask and DNA\_mask folders).
* Averaged swelling profile derived from imaging several confinements without cells present, measuring the SDS induced geometry specific swelling, and averaging the profiles (averagedSwellingProfile5x40.mat).
* 5x40 confinement FEA geometry in Abaqus format derived from hypermesh (5x40SolverDeck.inp).
* 5x40 confinement FEA nodal displacements solution in two parts (nodalDisplacements1.mat, nodalDisplacements2.mat).
* Example data set outputs including processed displacement field (ExampleDataSetDisplacements.mat), processed traction vectors (2024-11-11\_ComputedTractionsAlignedValues.mat), quality control plots (QA Plots folder), aligned STL of the substrate geometry with input images for 3D visualization (alignedSTLs folder), Amira traction and displacement vector and traction magnitude output folders (2024-11-11\_Disps, 2024-11-11\_Tractions, 2024-11-11\_TractionsContour).

# A note on FEA modeling:

We made use of hypermesh and optistruct to perform our FEA modeling but most FEA software should in principle be capable of reproducing the analysis necessary to compute the geometry specific Green’s Function. In general, you will need to compute a solution that contains the nodal displacements of each node (of the elements) in the data set for a unit point force in the X, Y, and Z directions. Thus for a model with 1000 nodes, you would expect a 3000 x 3000 matrix (1000 Nodes \* X \* Y \* Z displacements by each force in X Y Z). This matrix can then be used as the Green’s matrix assuming the input geometry and mechanical properties remain consistent with experimental data. For a more detailed analysis, consult original 3D TFM manuscript: Cite Wes’ paper.

# Computing Displacement Fields:

1. Launch MATLAB and set the path to include all the folders and subfolders in TFM.
2. Run main\_tracking\_script.m
3. A GUI will open to facilitate input of data.
4. Bandpass filtering
5. Track\_beads function inputs/adjustments based on data sets.
6. Swelling correction
7. Output images

A screenshot of a computer

Description automatically generated

A screenshot of a computer

Description automatically generated

A screenshot of a computer

Description automatically generated

A screenshot of a graph

Description automatically generated

A screen shot of a graph

Description automatically generated

A screen shot of a computer screen

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# Traction Computation:

1. Open processTFM\_General\_2024\_11\_11.m.
2. Edit the following variables.
3. Adjust XYZ
4. Output