**The following code and data were used to generate the results in “Tissue Fluidity: A Double-Edged Sword for Multicellular Patterning” Rikki M. Garner, Sean E. McGeary, Allon M. Klein, Sean G. Megason bioRxiv 2025.03.01.640992; doi: https://doi.org/10.1101/2025.03.01.640992**

**Experimental datasets** (\Experimental\_Data\, 252 GB)

* Raw Data (\Raw\_Data\, 236 GB)
  + 2 Nikon nd2 image files
  + 2 excel files describing the sample being imaged
* Processed Data (\Processed\_Data\, 16 GB)
  + Maximum intensity projection images (\*\_MaxIP\_MATLAB.tiff, image files) for each sample imaged
  + Segmented images (\*\_MaxIP\_Segmentation.tiff, image files) for each sample imaged
  + Metadata (\*\_MaxIP\_Segmentation.mat, data files) for each sample imaged

**Simulation datasets** (\Simulation\_Data\, 75 GB)

* **Overview:** Raw and processed simulation files for simulations run for a fixed amount of time (\Fixed\_Time\ folder), simulations run to steady state (\Steady\_State\), and simulations run via a Monte Carlo method (\MC\). Each of these directory contains an end directory, named with the date the simulations were run, containing the simulation results from a parameter scan including:
  + Input file to initialize each simulation (\*\_in.mat)
  + Output file containing each simulation’s results (\*\_out.mat)
  + Bash shell scripts used to run and analyze the simulations on a server
  + MATLAB scripts called by the server to run and analyze the simulation
* **List of simulation datasets:**
* Fixed Time: Larger, lower resolution scan of kT, E\_homo, and viscosity
  + Location: \Fixed\_Time\Results20240221\
  + Used in: Fig. 5a
* Fixed Time: Smaller, higher resolution scan of kT, E\_homo, and viscosity
  + Location: \Fixed\_Time\Results20240408\
  + Used in:
    - Fig. 1c-i
    - Fig. 6a-e
* Fixed time, kT scan
  + Location: \Fixed\_Time\Results20240819\_1\
  + Used in:
    - Fig.1SuppFig2e
    - Make\_Fig2eim\_Fig2SuppFig1a\_SortvskT
    - Make\_Fig3a\_SortvskT
* Fixed time, E\_homo scan
  + Location: \Fixed\_Time\Results20240820\
  + Used in: Make\_Fig2fjn\_Fig2SuppFig1b\_SortvsEhomo
* Fixed time, E\_het scan
  + Location: \Fixed\_Time\Results20240819\_3\
  + Used in: Make\_Fig2gko\_Fig2SuppFig1c\_SortvsEhet
* Fixed time, viscosity scan
  + Location: \Fixed\_Time\Results20240820\_1\
  + Used in: Make\_Fig2hip\_Fig2SuppFig1d\_SortvsV
* Steady state, larger box size
  + Location: \Steady\_State\Results20240927\
  + Used in: Make\_Fig.1SuppFig2g
* Steady state, smaller box size
  + Location: \Steady\_State\Results20240221\
  + Used in:
    - Make\_Fig.1SuppFig2g
    - Make\_Fig3bcde\_Fig3SuppFig1\_KineticModelSteadyState
    - Make\_Fig5a\_KineticModel
* MC Global Swap
  + Location: \MC\Results20241017\
  + Used in: Make\_Fig1SuppFig2a-f
* MC Local Swap
  + Location: \MC\Results20241017\_1\
  + Used in:
    - Make\_Fig1SuppFig2abcde
    - Make\_Fig1SuppFig2f

**Code to analyze data** (\Code\)

* Pre-processing of experimental data (\Code\SortingAnalysis\Experimental\_Preprocessing\)
  + Scripts
    - Max intensity projection: performMIP
    - Segmentation: performPixelClassification
  + Used in: Fig1h-i, Fig1SuppFig1, Fig. 6, Fig6SuppFig2
* Sorting analysis (\Code\SortingAnalysis\)
  + Skeleton-based domain size calculation (\Code\SortingAnalysis\Skeletonization Based Domain Size Calculations)
    - Scripts
      * Main domain size calculation:
        + calculateDomainSize\_SkelDist\_SizeDeptFill
      * Wrapper for Simulations:
        + performSkeletonWidthDomainSizeCalc\_Sims\_FeedFile\_O2
      * Wrappers for Experiments:
        + performSkeletonWidthDomainSizeCalc\_Expmts\_Wrapper
        + performSkeletonWidthDomainSizeCalc\_Expmts\_FeedFile\_O2
    - Used in: Fig1h-i, Fig1SuppFig1, Fig. 6, Fig6SuppFig2
  + Structure factor-based domain size calculation(\Code\SortingAnalysis\Structure Factor Based Domain Size Calculations\)
    - Scripts
      * calculateDomainSizeFromStructureFactor
      * calculateRadialAverage
    - Used in: Fig1SuppFig2
  + Fitting steady state sorting curves
    - Scripts
      * createFitAsymHill
      * evalInverseAsymHill
    - Used in: Fig3, Fig3SuppFig1
* Neighbor exchange analysis of simulations (Fig. 1f)
  + Scripts:
    - calculateNeighborExchangeRate
    - movingLinearRegression
  + Used in: Fig. 1f

**Code to run simulations** (\Code\Simulation\_Code\)

* Simulation code is distributed into separate folders for running simulations for a fixed amount of time (as would be measured in the laboratory) (\Code\Simulation\_Code\Fixed\_Time \), to steady state (as would be measured in the laboratory) (\Code\Simulation\_Code\Steady\_State \), or using the Monte Carlo method (\Code\Simulation\_Code\MC \). Each folder contains the following files.
  + To initialize simulation files with the appropriate parameters
    - SetUpAndRun\*.m
  + To run the simulations, call simSorting\_FeedFile\*.m with the file name of the simulation file to be run as the first argument
* Make sure to run chooseModelParameters.m to determine the default simulation parameters before you initialize the simulations.

**Code to generate figures** (\Code\Figure\_Panels\_Code\)

* Fig 1
  + c-f: Make\_Fig1cdef\_KineticModel.m
  + g-i: Make\_Fig1ghi\_AND\_Fig6\_Fig6SuppFig1\_Fig6SuppFig2\_FitSimsToExpmts.m
* Fig. 2
  + Make\_Fig2eim\_Fig2SuppFig1a\_SortvskT
  + Make\_Fig2fjn\_Fig2SuppFig1b\_SortvsEhomo
  + Make\_Fig2gko\_Fig2SuppFig1c\_SortvsEhet
  + Make\_Fig2hip\_Fig2SuppFig1d\_SortvsV
* Fig. 3:
  + Make\_Fig3a\_SortvskT
  + Make\_Fig3bcde\_Fig3SuppFig1\_KineticModelSteadyState
* Fig. 4: Make\_Fig4ef\_EquillibriumStatMech
* Fig. 5: Make\_Fig5a\_KineticModel
* Fig. 6: Make\_Fig1ghi\_AND\_Fig6\_Fig6SuppFig1\_Fig6SuppFig2\_FitSimsToExpmts.m
* Fig1SuppFig. 1: Make\_Fig1SuppFig1\_calculateDomainSize\_SkelDist\_SizeDeptFill
* Fig1SuppFig. 2:
  + abcde: Make\_Fig1SuppFig2abcde
  + f: Make\_Fig1SuppFig2f
  + g: Make\_Fig1SuppFig2g
* Fig2SuppFig. 1
  + Make\_Fig2eim\_Fig2SuppFig1a\_SortvskT
  + Make\_Fig2fjn\_Fig2SuppFig1b\_SortvsEhomo
  + Make\_Fig2gko\_Fig2SuppFig1c\_SortvsEhet
  + Make\_Fig2hip\_Fig2SuppFig1d\_SortvsV
* Fig3SuppFig1: Make\_Fig3bcde\_Fig3SuppFig1\_KineticModelSteadyState
* Fig6SuppFig1: Make\_Fig1ghi\_AND\_Fig6\_Fig6SuppFig1\_Fig6SuppFig2\_FitSimsToExpmts.m
* Fig6SuppFig2: Make\_Fig1ghi\_AND\_Fig6\_Fig6SuppFig1\_Fig6SuppFig2\_FitSimsToExpmts.m