August 2020

Fluidization and Segregation in Confluent Models for Biological Tissues

Preeti Sahu
Syracuse University

Follow this and additional works at: https://surface.syr.edu/etd

Part of the Physical Sciences and Mathematics Commons

Recommended Citation
Sahu, Preeti, "Fluidization and Segregation in Confluent Models for Biological Tissues" (2020).
Dissertations - ALL. 1266.
https://surface.syr.edu/etd/1266

This Dissertation is brought to you for free and open access by the SURFACE at SURFACE. It has been accepted for inclusion in Dissertations - ALL by an authorized administrator of SURFACE. For more information, please contact surface@syr.edu.
ABSTRACT

Collective tissue dynamics, more specifically a tissue’s ability to fluidize and segregate, is imperative for proper embryonic development and normal physiological functioning. In this thesis, I use vertex models to understand how cell-scale properties govern large-scale collective behavior. I begin with the process of fluidization in ordered monolayers. By perturbing beyond the linear regime, I show that in confluent tissues the linear response does not correctly predict the non-linear behavior, which, in this case, is the ability to exchange neighbors and fluidize. We also construct a simple analytic ansatz that can predict the non-linear behaviour responsible for cellular motion in tissues. Shifting from fluidization to segregation, I next focus on two-dimensional (2D) binary mixtures. I show that a difference in cellular shape or size is insufficient to induce an emergent interfacial tension, and this leads to large-scale mixing. However, shape disparity can induce a small-scale demixing over a few cell diameters. We report a very similar de-mixing observed in an experimental co-culture of differently shaped Keratinocytes. This can be understood by examining the non-reciprocal energy barriers for neighbor exchanges at the interface, leading to micro-segregation. We next move on to three-dimensional (3D) binary mixtures that have an explicit interfacial tension between two distinct cell types. We find that they can undergo complete segregation, imparting unique geometric properties to cells at the interface. To understand the feedback between interfacial tension and cellular geometry, we develop simple toy models to probe the system’s response to
perturbations in cellular topology along the interface. Neighbor exchange processes in confluent tissues also involve perturbing the underlying topology with neighboring cells, and therefore are heavily regulated by the cell shape and inhomogeneity in surface tension. In all of the above cases, these local barriers govern the onset of unique collective behavior like- fluidization, microdemixing and novel geometric signatures.
Fluidization and Segregation in Confluent Models for Biological Tissues

By

Preeti Sahu

Integrated M Sc.
National Institute of Science Education and Research (NISER), 2015

Dissertation
Submitted in Partial Fulfillment of the Requirements for the degree of
Doctor of Philosophy in Physics

Syracuse University
August 2020
Dedicated to ma and baba...
ACKNOWLEDGEMENTS

My experience as a PhD candidate was very rich and fulfilling thanks to the valuable interactions and support I received from several individuals. I wish to express my sincere appreciation to my primary advisors- Prof. Lisa Manning and Prof. J. M. Schwarz for encouraging me to actively pursue what is best for me. Their immense passion and rigor for biology and theoretical physics inspired me throughout and shaped my future interests as well. I sincerely thank Prof. Cristina Marchetti for helping me to navigate through the early phases as a graduate researcher, more importantly how to take one step at a time. I wish to acknowledge Prof. Zhen Ma, Prof. Alison E. Patteson, Prof. Christian D. Santangelo and Prof. Jennifer L. Ross for being on my committee and for their critical reading of my thesis.

I wish to thank Daniel M. Sussman for being there as my post-doctoral mentor and teaching me the best practices for scientific presentations. I also thank my experimental collaborators - Aaron F. Mertz, Matthias Rübsam and Tony Tsai for extremely helpful conversations. I sincerely thank Matthias Merkel, Gonca Erdemci-Tandogan, Paula Sanematsu, Amanda Parker and Ojan K Damavandi for their mentoring on coding and writing. Many thanks to my colleagues - Michael Czajkowski, Adam Patch, Mahesh Chandrasekhar Gandikota, Sarthak Gupta, Ethan Michael Stanifer, Francesco Serafin, Elizabeth Lawson-Keister, Zachariah Steven Schrecengost and Julia Ann Giannini for making office time fun. Special thanks to my colleague Suraj Shankar for guiding me throughout my grad school.
I wish to acknowledge the support and love of my family, my better half- Sourabh S. Chauhan; my mother- Lata Sahu and my father- Sundhansu Sekhar Sahu. They kept me going on because, as my father always says- “When the going gets tough, the tough gets going.”
# Contents

1 Introduction ........................................ 1
   1.1 Collective behaviour and its importance in physiology ............... 2
   1.2 What drives collective fluidization and segregation? .......... 4
   1.3 How do cells move in a confluent tissue? ......................... 11
   1.4 Modelling tissues ........................................ 14
      1.4.1 Density-independent rigidity transition ...................... 16
      1.4.2 The unique energy landscape of confluent tissues .......... 18
   1.5 Mixtures of confluent tissues .................................. 21
   1.6 Outline ..................................................... 24

2 Linear and nonlinear mechanical responses can be quite different in 
confluent tissues ........................................ 27
   2.1 Characterizing fluidity in confluent models ......................... 28
      2.1.1 Computational model for a hexagonal monolayer ............. 31
      2.1.2 Neighbor exchange and non-linear rigidity .................. 32
   2.2 At what cell shape does non-linear rigidity vanish? ......... 34
      2.2.1 Many-cell system ......................................... 34
      2.2.2 Single cell prediction ..................................... 37
      2.2.3 Conclusion ................................................ 40
3 Small-scale demixing in a confluent mixture of two cell-types

3.1 Cell sorting in biology

3.2 Computational modelling of binary mixtures

3.3 Computational results

3.3.1 Fluidity and demixing in shape bidisperse mixtures

3.3.2 Fluidity and mixing in area bidisperse mixtures

3.3.3 Zero-temperature energy configurations

3.3.4 Zero-temperature T1 energy barriers

3.4 Experimental results

3.5 Appendix

4 Geometric signatures of interfacial tension between two cell types in 3D models

4.1 Introduction

4.2 Computational model

4.3 Results

4.3.1 Cell sorting due to interfacial tension

4.3.2 Effect of interfacial tension on cellular geometry

4.4 Toy models and their predictions

4.5 Conclusion

4.6 Appendix

5 Discussion and conclusion

Bibliography
List of Tables

A1  Shape Bi-disperse Dynamical Simulations .......................... 84
A2  Area Bi-disperse Dynamical Simulations ........................... 84
A3  FIRE minimization for $E_s/E_m$ ...................................... 85
A4  T1 energy barriers ...................................................... 85
List of Figures

1 Examples of collective organization in living systems at different lengthscales-ranging from (a) cancerous cells [6] (~25µm) to (b) fungi networks [7] (1 mm), (c,d) animals and fish schools (> 10cm) [8]. (a) is adapted from ‘Scaffold stiffness influences breast cancer cell invasion via egfr-linked mena upregulation and matrix remodeling.’: Anthony J. Berger, Carine M. Renner, Isaac Hale, Xinhai Yang, Suzanne M. Ponik, Paul S. Weisman, Kristyn S. Masters, and Pamela K. Kreeger., Matrix Biology, 85-86:80-93, 2020. (b) is adapted from ‘Mechanism of signal propagation in physarum polycephalum.’: Karen Alim, Natalie Andrew, Anne Pringle, and Michael P. Brenner, PNAS, 114(20):5136-5141, 2017.
For particulate systems, **rigidification** point can be defined as the density at which a slight increase in density, leads to an infinite pressure in the packing. This transition can shift to a higher density for systems with spatial order. For example, in 2D FCC crystals, the transition density is higher as compared to that of a disordered packing[24]. Adapted the figure panel from ‘Mean-field theory of hard sphere glasses and jamming.’: Giorgio Parisi and Francesco Zamponi, Rev. Mod. Phys., ISSN 15390756., 2010.
Possible candidates leading to the emergent interfacial tension between two cell types: (a) Competing mechanical forces- adhesion (Adh) and cortical tension (Ct), shown in a cell-doublet. $\mathbf{T}$ is the vector sum of both the mechanical forces. (b) Different sorting models explain sorting in terms of a difference in - cell-cell adhesivity (DAH), cortical tension (DCSH, DITH) or heterotypic vrs homotypic tension (HIT) [48]. $T_{AA}$ and $T_{BB}$ represent the homotypic tension and $T_{AB}$ represents the contact tension- also referred to as heterotypic interfacial tension or HIT. Adapted the figure panel from ‘Sorting at embryonic boundaries requires high heterotypic interfacial tension.’: Laura Canty, Eleyine Zarour, Leily Kashkooli, Paul Francois, and Francois Fagotto, Nat. Comm., 8:157, 2017. (c) Mechanical polarization can also explain a heightened interfacial tension. As adhesion molecules mature the contacts between like cells, the acto-myosin contractility may be regulated by an upstream signalling pathway. An up-regulation towards the outer interface and/or a down-regulation along the internal edges, can produce high-tension cables along unlike boundaries [49]. From ‘Knowing the boundaries: Extending the differential adhesion hypothesis in embryonic cell sorting’: Jeffrey D. Amack and M. Lisa Manning, SCIENCE, 212-215, 2012. Reprinted with permission from AAAS.
Segregation in particle-based models rely heavily on density fluctuation: (a) Confined mixtures with size bidisperse particles can segregate due to differential activity. [61] Used with permission of Royal Society of Chemistry, from ['Aggregation and segregation of confined active particles.'], Xingbo Yang, M. Lisa Manning, and M. Cristina Marchetti, 10:6477-6484, 2014]; permission conveyed through Copyright Clearance Center, Inc. (b) Non-reciprocal chemotactic behaviour leads to a nonequilibrium phase separation. [62] Reprinted figure with permission from ‘Active phase separation in mixtures of chemically interacting particles.’, Jaime Agudo-Canalejo and Ramin Golestanian, Physical Review Letters, 123:018101, 2019, Copyright (2019) by the American Physical Society.

Cells are slower at confluency: Single-cell trajectories on a polystyrene substrate for fixed time window of 90 minutes (a) sprawl farther for isolated cells (b) look somewhat caged even for cells at the leading edge of the monolayer [63]. Adapted the figure panel from ‘Locomotion of human skin keratinocytes on polystyrene, fibrin, and collagen substrata and its modification by cell-to-cell contacts.’:J. Drukaa, L. Bandura, K. Cieslik, and W. Korohoda, Cell Transplant., ISSN 09636897, 2001.

In order to make new neighbors, cells need to undergo an edge flip – in this figure, the blue edge flips to yellow. This change in topology, typically requires the system to go through an energy barrier $E_b$[67]. Used with permission of Royal Society of Chemistry, from ['Energy barriers and cell migration in densely packed tissues.'], D. Bi, J. H. Lopez, J. M. Schwarz, and L. M. Manning, 10:1885-1890, 2014]; permission conveyed through Copyright Clearance Center, Inc.
In segregated confluent monolayers, boundary cells find it difficult to move across the boundary. They face a discontinuous restoring force, the intensity of which increases directly with the interfacial tension. This effectively pins the boundary cells [71]. Adapted figure from ‘Soft yet Sharp Interfaces in a Vertex Model of Confluent Tissue.’: Daniel M. Sussman, J. M. Schwarz, M. Cristina Marchetti, and M. Lisa Manning, PRL, 120(5):58001, 2018.


In confluent systems, the fluid-solid transition point is marked by the onset of a caged trajectory. Cell types with this dynamical behaviour have been found to have smaller boundaries and more rounder shapes across several different space-filling models[81, 82, 84]. (a) Reprinted figure with permission from ‘Glass transitions in the cellular Potts model’, M. Chiang and D. Marenduzzo, EPL, 10.1209/0295-5075/116/28009, Copyright (2016) by the editor of Europhysics Letters. (b) Reprinted figure with permission from ‘Solid-Liquid Transition of Deformable and Overlapping Active Particles’, Benjamin Loewe, Michael Chiang, Davide Marenduzzo, and M. Cristina Marchetti, Physical Review Letters, 10.1103, 038003(6), Copyright (2020) by the American Physical Society. (c) Adapted from ‘Motility-driven glass and jamming transitions in biological tissues.’, Dapeng Bi, Xingbo Yang, M. Cristina Marchetti, and M. Lisa Manning., Phys. Rev. X, 10.1103, 2016.

(a) A typical energy landscape. (b) Energy minima for confluent models becomes shallow at lower temperatures, whereas for liquids with super-Arhenious behaviour, the effective stiffness about minima increases and eventually diverges as the temperature is cooled to the glass transition temperature $T_g$ [91]. Adapted from ‘Anomalous glassy dynamics in simple models of dense biological tissue.’, Daniel M. Sussman, M. Paoluzzi, M. Cristina Marchetti, and M. Lisa Manning, EPL, 121.3, 2018.
Due to the pinning effect at the heterotypic interface, one can observe geometric features unique to confluent tissues. For example, in 2D monolayers, the edge length becomes bimodal for increasing tension [71]. Reprinted figure with permission from ‘Soft yet Sharp Interfaces in a Vertex Model of Confluent Tissue.’: Daniel M. Sussman, J. M. Schwarz, M. Cristina Marchetti, and M. Lisa Manning, PRL, 120(5):58001, 2018, Copyright (2018) by the American Physical Society.

Energetics of an ordered T1 transition: (a) A T1 edge, highlighted in red, at its rest length, (b) the T1 edge shrinks to zero length (c) the T1 edge rotates by 90° and is then expanded. (d) A typical energy profile across the T1 transition plotted with respect to the T1 edge length $l$ during T1 junction remodelling, for $s_0$ 3.71, 3.72, 3.73 and 3.75 (light red to dark red). The T1 energy barrier for the lowest $s_0$ is the peak height (highlighted in blue vertical line).

Many-cell energy profile: (a-c) A snapshot from an ordered tesselation of 90 cells with $s_0 = 3.76$. A randomly chosen edge (highlighted in red), shrinks to zero length (left to right as directed by the arrows). (d) In this process, the total energy of the tissue, $E$, is plotted against the shrinking T1 edgelength $l$ for increasing values of $s_0$ (3.72 to 3.81 in steps of 0.01 and 3.810 to 3.825 in steps of 0.001) varying from red to green. The energy cut-off is shown by yellow dash-dot line. (e) The critical edgelength $l^*$ associated to the cut-off shown in (d) is plotted for each $s_0$ value in yellow circles. The dashed line indicates critical $s_0^*$ found for disordered tissues.
A geometric mechanism for formation of a uniform pentagon: The 6-sided polygon has five sides equal to each other and one that is allowed to be different subjected to the constraints that the polygon lies on a circle and its area remains unity. The angles correspond to two different types of sides ($\alpha$ and $\theta$) are highlighted in pink and green.

Single-cell energy profile: (a-c) For a single cell inscribed on a circle, the T1 edge (highlighted in red) shrinks to zero length (right to left as directed by the arrows). (d) In this process, the total energy $E$ is plotted against the shrinking T1 edgelength $l$, for increasing values of $s_0$ (3.72 to 3.81 in steps of 0.01) varying from red to green. (e) The critical edgelength $l^*$ associated to the drop shown in (d) is plotted for each $s_0$ value in blue dot-dashed line. The blue dashed line indicates critical $s_0^*$ found for disordered tissues.

Non-linear stabilization seen in ordered bulk systems can be produced in 4-cell system and single cell model: Critical edgelength $l^*$ plotted against $s_0$ is superimposed for both- many-cell (green circles) and 4-cell systems (magenta circles). In addition, the analytical prediction from the geometric mechanism explained in the text is shown in blue dashed line.
A1 **Four-cell energy profile:** (a-c) In an ordered initial configuration of 4 cells with, the T1 edge, shrinks to zero length (left to right as directed by the arrows). (d) In this process, the total energy of the 4-cell unit, $E$, is plotted against the shrinking T1 edgelength $l$ for increasing values of $s_0$ (3.72 to 3.81 in steps of 0.01 and 3.810 to 3.825 in steps of 0.001) varying from red to green. The cut-off for the energy is shown by the magenta dash-dot line. (e) The critical edgelength $l^*$ associated to the cut-off shown in (d) is plotted for each $s_0$ value in the magenta circles. The dashed line indicates critical $s_0^*$ found for disordered tissues.

A2 **Vibrational mode analysis:** (a) A sample zero mode for bulk ordered tessellation with $s_0 = 3.75$. (b) The number of zero modes sharply increases after $s_0 = 3.722$. (c) The number of zero-valued eigenvalues of an ordered tessellation at a fixed value of the shape index, $s_0 = 3.75$, is studied along a T1 reaction coordinate. At the cusp in the potential energy landscape, $l^*$, where the energy changes by several orders of magnitude, the number of zero modes begins to systematically decrease.

A3 **Computational results for the lengths of cell edges during a forced T1 transition in an ordered tessellation:** (a) In a bulk ordered system with $s_0 = 3.75$, we compute the lengths of edges on a cell undergoing a forced T1 transition, grouping the edges into “T1-adjacent” ($L_A$) and “non-T1-adjacent” ($L_B$) bins, which show different trends and become tightly constrained near the transition point, highlighted in (b) as the point at which the energy profile for $s_0 = 3.75$ has a cusp.
The transition length is minimum for a ratio of unity: For a single-cell ansatz, we allow the ratio $L_A/L_B$ to differ from unity. The transition points are plotted with respect to varying ratios for increasing $s_0$ values- 3.73 (red), 3.75 (green) and 3.81 (dark green).

Many-cell disordered energy profile: (a) In a disordered system of 90 cells, a randomly chosen edge undergoes a T1 transition for 50 different initializations. In this process, the relative energy of the tissue, $\Delta E(l)$, is plotted against the shrinking T1 edgelength $l$ for increasing values of $s_0$ (3.71 to 3.95 in steps of 0.04) varying from red (3.71) to green (3.83) to blue (3.95). The cut-off for the energy is shown by a horizontal pale blue line for reference. (b) Critical edgelength $l^*$ plotted against $s_0$ is superimposed for both many-cell (yellow circles) and 4-cell systems (magenta circles). The analytical prediction from the geometric mechanism explained in the text is shown in blue dashed line. The dark green box and whisker plot in blue shows the $l^*$ distribution in disordered systems.

Vertex model binary mixtures. (a) Schematic of vertex-based modeling of a tissue: A typical tessellation with two different types of cells highlighted. The energy depends only on a cell’s perimeter ($P_j$) and area ($A_j$). (b) A heat map of $\log_{10} D_{eff}$ as a function of $\Delta$ on the x-axis and $s_{av}$ on the y-axis. The phase points with: fluid-fluid ($s_{0,1}, s_{0,2} > 3.81$), solid-fluid ($s_{0,1} < 3.81$) and solid-solid ($s_{0,1}, s_{0,2} < 3.81$) components are denoted by circular, square and star-shaped markers, respectively. Black-filled markers, demarcated by a solid black line, denote mixtures with a $D_{eff}$ less than that of the chosen cutoff of 0.01. Region above this line denotes fluid-like behaviour on average.
Shape bidisperse fluid mixtures. (a) Snapshot of a $P_{av} = 3.85$, $\Delta = 0.4$, $N = 1600$ mixture. Scale bar denotes 10 length units. Yellow is used for solid-like cells ($s_0 = 3.65$) and blue for liquid-like ones ($s_0 = 4.05$).

(b)-(d) Various quantifications of demixing in shape bidisperse mixtures (curves colored from green to red in increasing order of shape disparity i.e $\Delta = 0.01, 0.1, 0.2, 0.3, 0.4$) are compared to a mixture with an extra heterotypic line tension of value 0.1, $s_0 = 3.97$ (black dashed curve).

(b) Demixing Parameter versus log(time). The final value ($DP_f$) as a function of $\Delta$ is shown in the inset. (c) Average cluster radius ($R$) versus time. (d) Pair correlation function of high-$s_0$ cells ($g_{22}$) versus radial distance for $t = 200 \tau_0^0$. The dashed grey line shows an exponential decay. The inset shows the decay lengthscale ($\xi$) in terms of the maximum possible lengthscale ($\xi_{HLT}$) with increasing disparity $\Delta$. Simulation details provided in Table S1.
8 

*Area bidisperse fluid mixtures.* (a) Snapshot of a $s_0 = 3.85$, $\alpha = 2.5$, $N = 1600$ mixture. Scale bar denotes 10 units. Yellow is used for larger cells ($A_0 = 1.43$) and blue for smaller ones ($A_0 = 0.57$). (b)-(d) Various quantifications of demixing in area bidisperse mixtures (curves colored from green to red in increasing order of size disparity i.e $\alpha = 1.0, 1.5, 2.0, 2.5$) are compared to a mixture with an extra heterotypic line tension of value $0.1$, $s_0 = 3.97$ and $A_0 = 1.0$ (black dashed curve). (b) Demixing Parameter versus $\log(time)$. The final value ($DP_f$) as a function of $\alpha$ is shown in the inset. (c) Average cluster radius ($R$) versus time. (d) Pair correlation function of small-$A_0$ cells ($g_{ss}$) versus radial distance in units of the smallest lengthscale for $t = 200 \tau_0^0$. The dashed grey line shows an exponential decay. The inset shows the decay lengthscale ($\xi$) in terms of the maximum possible lengthscale ($\xi_{HLT}$) with increasing disparity $\Delta$. Simulation details provided in Table S2.

9 

*Minimal energy configurations.* Systems with $N = 100, 400, 900$ cells (green to black) are energy minimized using the FIRE algorithm to get the total energy of the configurations- mixed($E_m$) and sorted($E_s$), for $a < s_{av} >= 3.85$, $K_a = 100$ with increasing disparity. The ratio $E_s/E_m$ versus $\Delta$. (b) The ratio $E_s/E_m$ is plotted versus $\alpha$ for $s_0 = 3.85$ and $K_a = 1$. Simulation details provided in Table S3.
Differential energy barriers in shape bidisperse fluid mixtures. (a) Energy $E(l)$ relative to $E_0$ is plotted against T1 edgelength $l$ for a typical shape bi-disperse T1 pair ($\Delta = 0.4, s_{av} = 3.85$). (b) Energy Barrier $E_b$ is plotted against signed disparity in shape $\Delta_s$. Positive and negative $\Delta_s$ values imply stiffer cluster in yellow and floppier cluster in blue respectively, in the 4-cell diagrams show above. Each solid curve represents the barrier for a heterotypic cell to get out of the cluster for $s_{av} = 3.79, 3.85, 3.88$ (from solid-like (orange) to liquid-like (green)) (c) Correlation plot for $s_{av} = 3.85$ between Differential Energy Barriers on the right y-axis $\delta(E_b)$ (in maroon triangles) and demixing relative to mixed scenario $DP_f$ on the left y-axis (in black discs). Shape difference $\Delta$ is plotted on x-axis. Simulation details provided in Table S4.
Micro-demixing observed in keratinocyte co-cultures. (a) Wild-type (Ctr) and E-cadherin knockout (E-cad\(^{-/-}\)) cell monolayer mixtures with nuclei labelled using immunofluorescence. (b) Histograms of cell shapes for Ctr and E-cad\(^{-/-}\) cells are compared across seven and six different isolates respectively, i.e. seven different mice. There is a clear difference in the shape index (\(\Delta = 0.31\)) of both cell-types, with shape indices of 4.08 ± 0.06 for Ctr cells and 4.38 ± 0.14 for Ecad\(^{-/-}\) cells. (c) Both cell-types, Ctr in yellow and E-cad\(^{-/-}\) in blue, start off initially mixed as shown the 0h (zero hours) snapshot. The co-culture evolves into a micro-segregated mixture, as shown in a 24h snapshot. (d) Voronoi tessellations (VT) of the same snapshots. (e) Solid maroon curve represents the time evolution of demixing parameter for the E-cad\(^{-/-}\) cell-type in the mixture as a function of time and averaged over five different isolates. This result is compared against a control of demixing curves for some initially well-mixed regions of monolayers of either all Ctr cells or all E-cad\(^{-/-}\) cells but with half the cells stained differently than the other half. Well-mixed regions of the control cells are shown in yellow, while the well-mixed regions of the E-cad\(^{-/-}\) cell-type is shown in blue.

(a) Effective diffusivity \((D_{eff})\) with respect to target shape parameter \(s_0\). Different curves are for monodisperse systems with \(K_a\) varying from 1 to 100. The solid horizontal line represents the cutoff at 0.01 used previously. The vertical dashed line denotes \(s_0^* = 3.813\). (b) Effective diffusivity in area bidisperse mixtures. Plot of the effective diffusivity \((D_{eff})\) with respect to increasing area dispersity \(\alpha\). Parameter are details provided in Table S2.
A2  Component-wise diffusivities and timescale to approach steady state. 
(a) Plot for effective diffusivity ($D_{\text{eff}}$) with respect to increasing shape dispersity $\Delta$. The solid lines are for the two different components with triangles and circles representing higher $s_0$ (type 2) and lower $s_0$ (type 1) respectively. The dashed curve represents the averaged $D_{\text{eff}}$.  
(b) The average time it takes for the system to achieve half of its steady state DP value, or $t_{1/2}$, is plotted against $\Delta$. The solid curves from 7(b) are used to compute $t_{1/2}$. The inset shows log-log plot of the same, with a linear fit in solid yellow line is $y = 3x + 3$. 

A3  Increasing temperature, diminishes observed demixing.  
(a) Plot for demixing parameter ($DP$) with respect to time (in units of $\tau_\alpha$). The solid curves represent an increasing temperature ($T$) from blue ($T = 0.005$) to maroon ($T = 0.04$). The curves are averaged over 280 ensembles. 

A4  Cortical tension in shape bidisperse mixtures of $\Delta = 0.4$. Left and right panels shows line tension maps for sorted and mixed scenarios for a $N = 900$ system respectively. Heterotypic edges are shown in dash-dot lines. Yellow and blue cells have $p_0 = 3.65$ and 4.05 respectively. They are followed by histograms for heterotypic(in black) and homotypic(colored) edges. Vertical lines show the mean values for each curve in their respective colors.
A5  *Cortical tension in area bidisperse mixtures of* $\alpha = 2.5$. Left and right panels shows line tension maps for sorted and mixed scenarios for a $N = 900$ system respectively. Heterotypic edges are shown in dash-dot lines. Yellow and blue cells have $A_0 = 0.57$ and 1.43 respectively. They are followed by histograms for heterotypic(in black) and homotypic(colored) edges. Vertical lines show the mean values for each curve in their respective colors.

A6  *Differential energy barriers in area bidisperse mixtures.* (a) Energy $E(l)$ relative to $E_0$ versus T1 edgelength $l$ for a typical size bi-disperse T1 pair ($\alpha = 2.5, s_{av} = 3.85$). (b) Energy Barrier $E_b$ is plotted against area disparity $\alpha_s$ where large values on right and small values on left imply large-cell cluster in yellow and small-cell cluster in blue respectively. Each solid curve represents the barrier for a heterotypic cell to get out of the cluster for a fixed $s_0$ (varied from solid-like (orange) to liquid-like (green) - 3.82,3.85,3.88) (c) Correlation plot for $s_0 = 3.85$ between Differential Energy Barriers on the right y-axis $\delta(E_b)$ (in maroon triangles) and demixing relative to mixed scenario $DP_f$ on the left y-axis (in black discs). Size ratio $\alpha$ is plotted on x-axis. Simulation details provided in Table S4.
A7 Symmetric 4-cell T1 energy barriers for shape bidispersity. On the left is the color plot of energy barrier as a function of independently tunable shapes of T1 pair and non-T1 pair is plotted along x-axis and y-axis respectively. The dashed line represents monodisperse calculation ie for $\Delta = 0$. As expected it is red till it reaches the monodisperse transition point $s*_{0} = 3.813$, after which it becomes blue. Off-diagonal phase points depict bidisperse mixtures ie $\Delta \neq 0$. We see that it is necessary for the T1-pair to be fluid like, for vanishing barrier. On the right is a cross-section of the phase diagram on left. Energy barrier is plotted against area disparity for increasing values of $s_{0}$ 3.79 to 3.85.

A8 Symmetric 4-cell T1 energy barriers for area bidispersity. On the left is the color plot of energy barrier as a function of independently tunable sizes of T1 pair (blue polygons) and non-T1 pair(yellow polygons). On the left is when blue polygons are bigger than yellow. On the right is smaller blue cells sandwiched between yellow (BssB). $\alpha$ and $P_{0}$ are the area ratios and preferred shape index respectively. Dashed black line represents the monodisperse transition point $s*_{0} = 3.813$. This graph predicts the energy barriers to vanish at a shape index higher than $s*_{0}$ in highly bidisperse systems. On the right is a cross-section of the cumulative phase diagram on left. Energy barrier is plotted against area disparity for increasing values of $s_{0}$ 3.79 to 3.85.

A9 T1 transitions in shape and area bidisperse mixtures. (a)-(c) T1 topologies (shown as cartoons on axis extremities) and their barrier statistics for shape bidisperse mixtures. (d)-(f) T1 topologies (shown as cartoons on axis extremities) and their barrier statistics for size bidisperse mixtures. Parameters used are in Table A4.
Additional quantification of cell properties. (a) Cell displacements integrated over 24 hours in the Ctr-E-cad$^{-/-}$ mixtures and in the control Ctr-Ctr and E-cad$^{-/-}$-E-cad$^{-/-}$ monotypic monolayers (cells of the same type but different tag). Ten cells from each type of the 7 Ctr-E-cad$^{-/-}$ demixing videos and the 4 Ctr-Ctr and E-cad$^{-/-}$-E-cad$^{-/-}$ demixing videos are measured. We additionally show the root mean-square displacements for various time intervals for the same data sets. (b) The distribution of cell areas is shown for E-cad$^{-/-}$ (in red) and Ctr (in black). The inset shows the average across 6 and 7 isolates respectively, to show that area distributions are similar for both cell-types. (c) The ratio of number of E-cad$^{-/-}$ (KO) to Ctr cells is plotted against time for a typical experimental co-culture. The ratio approaches unity i.e. it is almost a 50:50 mixture, over the course of the experiment.
Additional quantification of experimental co-cultured monolayers. (a) The solid maroon curve represents the time evolution of the demixing parameter for the E-cad$^{-/-}$ cell-type in the Ctr-E-cad$^{-/-}$ mixture as a function of time, averaged over 5 different monolayers using five different isolates. The maroon curve should be compared against the almost flat demixing curves for monotypic mixtures composed of 50:50 differently tagged Ctr and E-cad$^{-/-}$ cells shown in yellow and blue dashed curves respectively, averaged over two monolayers using two different isolates each. (b) The initial (0h) Voronoi tessellation of the co-culture nuclei, is compared side by side to the final (24h) snapshot in the high calcium condition. Green cells and red cells depict Ctr (Ctl) and E-cad$^{-/-}$ (KO) cells respectively. (c) The pair correlation function is plotted for the initial and final snapshots in (b). Green, red and blue markers depict correlations between- homotypic Ctl, homotypic KO, and heterotypic Ctl-KO nuclei respectively. 

Sorting in fluid-like binary tissue with HIT: (a-b) Initial and final snapshots of a $s_0 = 5.5, \sigma = 1$ and $N = 512$ mixture, respectively. Both cell types denoted by red and blue polyhedra. (c) Quantification of segregation in terms of demixing parameter DP vrs simulation timesteps for different values of tension- 0(maroon), 0.01(pink),0.03,0.1,0.3,1(blue).
Cellular geometry changes around the high-tension interface: (a) Snapshot of the bilayer arrangement of sorted compartments for a high value of $\sigma = 20$. Only the blue cell type is emphasized here, colored by major axis length. Green is for elongated and blue for rounder cells. The white rods denote the long axis of the polyhedron. (b) Acquired cell shape index ($s$), plotted with respect to tension ($\sigma$), is higher for interfacial cells (green curve) as compared to the cells in bulk (blue curve). (c) Rose plot for the distribution of orientation angle of interfacial cells is shown for increasing tension ($\sigma$). The faint black curve represents the density for uniform distribution i.e $\sin(\theta)$. (d) The probability distribution of heterotypic facet area is shown for increasing tension ($\sigma$).

Interfacial cells are right-prisms: (a) a snapshot that highlights the cells at both sides of the interface. Cell types are tagged in blue and red and made translucent to make their centers visible (white solid spheres). The edges of red cells are depicted by solid red lines. (b) Height distribution ($z$) of the interface cells is plotted for increasing tension. The inset shows standard deviation $\Delta z$ with respect to tension $\sigma$. (c) Minimum distance along the XY plane between heterotypic cells is plotted for increasing tension. The inset shows the average of this distance with respect to tension. A distance of zero is highlighted by complete Registration $R = 1$. 
Global minima becomes registered for higher tension: (a) Shared surface area (shaded in dark for all snapshots) is computed as a function of the registration between the different cell types. The string of blue cells is allowed to move past the string of stationary pink cells. Snapshots for no, half and complete registration is shown for encircled points. (b) The change in the total energy of this system is plotted with respect to registry, for different values of tension ranging from 1 (pink) to 4 (blue) in increments of 0.25. (c) The ground-state registration is plotted for increasing tension. The solid curve represents the global minima. The dashed curves represent the local minima, parabolic in blue and cuspy in black. The inset zooms about the critical tension at which the transitions occurs between both types of minima.

Shape frustration might be less dominant for larger more fluid-like shapes: (a) Snapshot from the top-view of HCP toy model setup where both cell types are depicted in blue and red. Cell centers for the top and bottom layer are depicted by solid while spheres. (b) Shared surface area is computed as a function of the registration between both the layers. (b) The change in the total energy of this system is plotted with respect to registry, for the lower values of tension $\sigma = 0.001, 0.003, 0.01, 0.032, 0.1, 0.3$ (pink to blue) and a fluid-like shape of $s_0 = 5.5$.

Transition to complete registration shifts for fluid-like cell shape: A heat-map for the average steady-state registration is shown as function of cell shape $s_0$ at interfacial tension $\sigma$. Yellow denotes complete registration and blue denotes partial registry. The registry between heterotypic cell-pairs is averaged for 200 different initializations.
A1  (a) Plot for demixing parameter \(DP\) with respect to time in a bilayer arrangement. The inset shows the final demixing value with respect to cubic root of system size in solid black curve for a fixed tension of 1. The prediction from assuming that the interfacial cells share one-third of their facets with the other cell type, is plotted in dashed grey.

A2  (a) Plot for average observable area with respect to increasing tension.

A3  A sketch of the 9-cell set up where the different cell types are colored with pink and blue and the heterotypic boundary highlighted by red. The Voronoi centers are shown in grey filled circles. The black edges show initial cell-cell boundaries. The central cell’s boundary after perturbation is highlighted in blue.

A4  Properties of parabolic and cuspy minima  (a) Plot of stiffness of the parabolic minima with respect to increasing tension. (b) The restoring force for perturbations away from the cuspy minima is independent of the displacement on y axis for different values of tension (c) The value of restoring force at a fixed value of displacement is plotted against tension.

A5  Shape vrs surface energies at extreme tension values  Plot of change in total energy (in dashed orange), blue cell’s mechanical energy (in solid blue) and interfacial energy (in solid black) with respect to registry is shown for (a) \(\sigma = 0.001\) and (b) \(\sigma = 1\).

A6  Restoring forces seems to become non-zero for registered steady State: Plots of steady-state registry and restoring force magnitude for a fixed value of displacement is shown with respect to increasing tension for different values of \(s_0\) - 5.314 (maroon) to 5.8 (green).
Chapter 1

Introduction
1.1 Collective behaviour and its importance in physiology

Living organisms are capable of performing a variety of complex phenomena. Amongst growth, reproduction, adaptation, and so on, a very distinguishing feature is their ability to actively move and self-organize. Such emergent behaviour is observed in living organisms as large as animals to microorganisms as small as bacteria[1–5].

Figure 1: Examples of collective organization in living systems at different lengthscales- ranging from (a) cancerous cells [6] (~25μm) to (b) fungi networks [7] (1 mm), (c,d) animals and fish schools (> 10cm) [8]. (a) is adapted from ‘Scaffold stiffness influences breast cancer cell invasion via egfr-linked mena upregulation and matrix remodeling.’: Anthony J. Berger, Carine M. Renner, Isaac Hale, Xinhai Yang, Suzanne M. Ponik, Paul S. Weisman, Kristyn S. Masters, and Pamela K. Kreeger., Matrix Biology. ,85-86:80-93, 2020. (b) is adapted from ‘Mechanism of signal propagation in physarum polycephalum.’: Karen Alim, Natalie Andrew, Anne Pringle, and Michael P. Brenner, PNAS ,114(20):5136-5141, 2017.

Many biological systems- like cells in plant and animal tissues- may not seem to move as fast as birds or bacteria and yet, their functionality relies on the collective behaviour of cells that emerges gradually over time. Two of the most important collective phenomena for cells are, the abilities to flow- for example to change the
global shape of a tissue, and segregate- for example to separate two tissue types into compartments. Starting from their integral role during an organism’s formative stages[9–12], these processes also play a crucial part in the upkeep of normal functioning of organs[13], healing for wounded tissues[14, 15] and containment of the spread for diseased/infected tissues[16–18].

For example, epithelial tissues (Fig:2(b)) that form the outer lining of most of our organs, have the primary functions of protecting the cells inside and maintaining the shape and integrity of the organ as a whole. They do so by ensuring that cells have no gaps in between each other (called confluency) and by ensuring they form a collectively rigid outer covering[13, 18]. A very different behaviour emerges when the tissue becomes wounded. The tissue ceases to be rigid and begins to collectively fluidize[14, 15, 19]. This makes it easy for cells to flow and repair the gap that is created due to wounding and expedite the process of wound healing (Fig:2(a)).

Tissues, in general, comprise of many different kinds of cell types. They can spontaneously segregate into distinct domains, homogeneous in cell type, by a process called cell sorting. Rapid and sharp segregation is required in embryonic tissues[9–11] to ensure proper organ development. The importance of segregation is not just limited to developing tissues, but is thought to play a vital role in disease proliferation. For example, breast carcinoma cells are found to segregate from, and become enveloped by, healthy breast cells[18, 21] as shown in Fig:2(c). Such a spatial arrangement could potentially make a tumor less susceptible to metastasis.
Figure 2: Examples of collective behaviour in animal tissues. a) wound healing as shown by the closing of the circular gap [20]. Adapted the figure panel from ‘Physical forces during collective cell migration.’: Xavier Trepat, Michael R. Wasserman, Thomas E. Angelini, Emil Millet, David A. Weitz, James P. Butler, and Jeffrey J. Fredberg, Nat. Phys., ISSN 17452481, 2009. b) a healthy skin epithelial tissue [13]. Adapted the figure panel from ‘E-cadherin integrates mechanotransduction and EGFR signaling to control junctional tissue polarization and tight junction positioning.’: Matthias Rubsam, Aaron F. Mertz, Akiharu Kubo, Susanna Marg, Christian Jungst, Gladiola Goranci-Buzhala, Astrid C. Schauss, Valerie Horsley, Eric R. Dufresne, Markus Moser, Wolfgang Ziegler, Masayuki Amagai, Sara A. Wickstrom, and Carien M. Niessein, Nat. Commun., ISSN 20411723, 2017. c) a spontaneously segregated mixture of healthy (green) and carcinoma breast cancer cells (blue) [18]. Adapted the figure panel from ‘Testing the differential adhesion hypothesis across the epithelial-mesenchymal transition.’: Steve Pawlizak, Anatol W. Fritsch, Steffen Grosser, Dave Ahrens, Tobias Thalheim, Stefanie Riedel, Tobias R. Kiesling, Linda Oswald, Mareike Zink, M. Lisa Manning, and Josef A. Kas. New J. Phys. 17:0910011, 2015.

1.2 What drives collective fluidization and segregation?

A large body of theoretical and computational work has brought us a long way in understanding the physics governing collective phenomena in tissues. It is tempting to model biological cells as particles that interact with each other, similarly to particles interacting to form a fluid, for example. If the interaction between particles is short-ranged, i.e. no long-range attraction, then the interaction is purely repulsive and so
one would expect that a cellular monolayer would have the same bulk rheology as a dense packing of soft, repulsive discs. The onset of rigidity in these systems has been extensively studied as a function of the packing fraction, which is the control parameter for such systems. When discs pack more densely than a threshold value, they rigidify. The reverse process of fluidization—when tightly packed discs are able to rearrange and diffuse due to thermal noise, activity, external shear or a decrease in density—is also well explored. Although rigidification and fluidization occur in both crystalline systems, where particle positions follow a regular pattern, and in disordered systems, where the particle positions are patternless and jumbled, it occurs differently in the two systems. As crystalline systems pack more efficiently, the threshold density at which the system rigidifies is higher[22–24] as compared to disordered systems. In addition, the transition is first-order in crystals, as all particle interactions/bond strengths are essentially equivalent. In contrast, disordered systems undergo a random first-order transition, exhibiting features of both continuous and discontinuous transitions, and are still being studied today[25–27]. In practice, many materials exhibit an equation of state with two branches: an out-of-equilibrium branch that can be reached by rapid cooling or rapid density changes, and an ordered equilibrium branch that corresponds to the ground state as shown in Fig:3. In this thesis, I will explore if/how such ideas are applicable to biological tissues.

On the other hand, inanimate biomaterials like extracellular matrix (ECM), can also undergo significant change in bulk rheology under large strains [28]. But living matter like tissues can behave very differently from particulate systems. While some tissues do get rigidified due to over-crowding[29, 30] in a density-driven manner, the process of tissue fluidization can have counter-intuitive features due to confluency.

A lot of exciting experimental work on trying to understand tissue rheology from a biochemistry perspective— for example, epithelial to mesenchymal transition (EMT) in
animal tissues and auxin mediated directed growth in plant tissues⁸ – will not be directly addressed in this body of work. Instead, we focus here on how cell-scale mechanical properties influence collective behaviour, which should serve as a basis for future work that links biochemical signaling to cell mechanics.

**Cell sorting**, being such an ubiquitous and important process in biology, has been also studied extensively using several models. If one models cells as particles, what kinds of segregation mechanisms are relevant for tissues? In the context of tissues, segregation of different cell types is also known as cell sorting. Given the cell-as-particle perspective, then perhaps mixtures of two different kinds sort in a fashion similar to segregation of two immiscible liquids. For example, when oil sorts from water, an oil droplet has a surface tension associated with it that governs the way it grows by further coalescing with other droplets. The different versions of sorting models, which I will be describing next, basically try to explain what is contributing
to this surface tension in the context of tissue co-cultures.

The first among all is the **Differential Adhesion Hypothesis (DAH)** which was proposed by Steinberg in 1963 [33]. The idea of DAH is that tissues behave like immiscible liquids composed of motile cells that rearrange in order to minimize their interfacial tension. It additionally assumes that difference in surface tension of tissues are caused by differences in cell-cell adhesion. This assumption has been tested and well-understood in particle models. Interfacial surface tension, can drive bulk segregation via spinodal decomposition or nucleation processes[34–37]. In Lennard Jones mixtures[38, 39], a difference in cell-cell affinity is enough to create an emergent surface tension. Tension-driven coarsening is also observed in bio-polymers at the sub-cellular scales[40]. Entropic forces might be contributing to emergent surface tension as well [41]. For example, in entropy dominant mixtures, larger or more elongated units can cluster together due to depletion forces[42–44]. Therefore, particulate mixtures can segregate due to difference in – geometrical properties like size\(^1\) and shape, and cell-cell interaction like adhesivity. Difference in adhesivity, in particular, leads to emergent line tension in these systems. The same seemed to be true for tissues as well. Tissue surface tension (TST) was found to be directly proportional to cadherin (a type of cell adhesion molecule) expression[47], thereby supporting the DAH hypothesis.

Although DAH is one of the most accepted hypotheses in the field, it alone is not sufficient to explain sorting behaviour in germ-layer organization in Zebrafish [50], as measured cell adhesion does not correlate with tissue surface tension in three tissue types present in early development. Harris[51] and later Brodland[52], put forth a second candidate for a cell-scale mechanical property that should correlate with tissue surface tension, namely actomyosin contractility. According to Harris’, **differential surface contraction hypothesis (DSCH)**, cells with higher contractility should

\(^1\)Soft materials like foam segregate due to difference in size but are found to mix under large strains[45, 46].
Possible candidates leading to the emergent interfacial tension between two cell types: (a) Competing mechanical forces- adhesion ($\text{Adh}$) and cortical tension ($\text{Ct}$), shown in a cell-doublet. $\text{T}$ is the vector sum of both the mechanical forces. (b) Different sorting models explain sorting in terms of a difference in - cell-cell adhesivity (DAH), cortical tension (DCSH, DITH) or heterotypic vs homotypic tension (HIT) [48]. $T_{AA}$ and $T_{BB}$ represent the homotypic tension and $T_{AB}$ represents the contact tension- also referred to as heterotypic interfacial tension or HIT. Adapted the figure panel from ‘Sorting at embryonic boundaries requires high heterotypic interfacial tension.’ Laura Canty, Eleyine Zarour, Leily Kashkooli, Paul Francois, and Francois Fagotto, Nat. Comm., 8:157, 2017. (c) Mechanical polarization can also explain a heightened interfacial tension. As adhesion molecules mature the contacts between like cells, the acto-myosin contractility may be regulated by an upstream signalling pathway. An up-regulation towards the outer interface and/or a down-regulation along the internal edges, can produce high-tension cables along unlike boundaries [49]. From ‘Knowing the boundaries: Extending the differential adhesion hypothesis in embryonic cell sorting’: Jeffrey D. Amack and M. Lisa Manning, SCIENCE, 212-215, 2012. Reprinted with permission from AAAS.

have lower tissue surface tension. A very similar version of this theory by Brodland is the- differential interfacial tension hypothesis (DITH). Recent work on gastrulating Zebrafish embryos [53], has suggested that DITH is largely correct and encompasses DAH and DSCH, in the sense that both adhesion molecules and surface contractility contribute to the effective interfacial tension at an edge between cells of two different tissue types. Mertz et al., also found that DITH explains the surface
tension better than DAH alone\cite{54}. They compared a simple physical model of cohesive colonies as adherent contractile disks. The model captures the essential observations and suggests that the apparent surface tension in the large-colony limit is driven by acto-myosin contractility.

Both DAH and DSCH assume that all cells inside a cluster are mechanistically identical and that either adhesion or contractility at a cellular level contributes in some fashion to the macroscopic TST. In contrast, the DITH theory focuses on the properties of interfacial edges, allowing for the possibility that some edges are different from others. In support of this idea there are several pieces of evidence that suggest that cells respond differently when at the boundary versus when they are in bulk i.e they \textit{mechanically polarize} at the boundary\cite{49}. To understand this effect better, let us consider the smallest system - a doublet of cells. Since both the cells are at boundary, experiments have shown that, cadherin-mediated adhesion, reorganizes the actomyosin network to intensify contractility along the external edge of a doublet and down-regulate along the internal interface\cite{55}. Extending this idea to larger aggregates, one might expect the contractility to be much lower inside the aggregate than on the periphery. It has been recently shown that this effect at the periphery is similar to the effect of having an increased effective adhesion\cite{56}. According to this hypothesis called- \textbf{extended DAH}, the effective adhesion has orders of magnitude higher contribution from contractility than from adhesion. This suggests that TST is dominated by the contribution of mechanical polarization rather than adhesion and that cadherins act as a signaling molecule to regulate it. In this view, the TST may still scale with the expression of adhesion molecules, not because they have a direct mechanical effect but because their signaling modulates contractility.

The above variations of cell sorting are based on the idea that tissue behave like liquids, in that they tend to minimize their surface tension. Hence, an agglomerate
will always be a spherical droplet and in the presence of another agglomerate, it fuses to form another spherical droplet. But some biological tissues also have solid-like properties. For example, coalescence experiments in cancer cell lines show an arrested fusion that persists over 24 hours[57]. For these solid-like aggregates, an arrest is expected when the tissue surface energy precisely balances the resistance to plastic deformations in the solid. Also, the agglomerates formed by carcinoma cells either in co-cultures or homogeneous suspensions, are not spherical in shape[47]. They have protrusions and some even have holes. Therefore, one should look beyond these theories to explain tissue segregation.

The above variations also only address equilibrium systems, but equilibrium simulations are not sufficient to understand biological cells, which are inherently out-of-equilibrium. Therefore, self-propelled particle (SPP) models[58–60] are more suitable. Mixtures of SPP cells, with difference in size and self-propulsion[61] can segregate efficiently(Fig. 5(a)). A very similar mechanism of non-reciprocal interaction governs active phase-separation in mixtures with difference in chemical activities [62], as shown in Fig. 5(b).

In sum, most of the understanding about collective segregation in tissues is based on the fluid particle-like behaviour of cells. However, cells in confluent tissues have a more complex many-body interaction that relies heavily on the underlying topology given that many tissues are confluent, or no gaps between cells. To begin to understand this complexity, let’s study how cells move through confluent tissue.
1.3 How do cells move in a confluent tissue?

Both the seemingly disparate emergent phenomena—fluidization and sorting—can be understood in terms of cellular movement. For example— for fluidization to happen, the constituent cells should be able to uncage (Fig. 11) themselves from their initial neighborhood and travel freely for long distances. Similarly, if a binary mixture would spontaneously separate if fluid-like, but the rheology is solid-like, then particles at the boundary would find it very difficult to move across and enter into the other component. If instead the rheology is fluid-like and this transverse movement is easy, the components will end up mixing. Therefore, both these processes can be understood by focusing at how cells uncage in the bulk and at the boundaries respectively.

While a handful of biological cells - like RBCs, sperm cells, etc- do behave like particles, for a majority of cells in tissues, this assumption can be far from the reality. Cells in cohesive tissues, like the epithelia, are severely constrained by their neighbors.
and thus move much more slowly, if at all [63–66]², as shown in Fig. 6(b). Unlike molecular fluid particles, cells in such tissues cannot open up gaps between them, as this can rupture the protective lining which is essential to organs. Maintaining this confluency condition at all times is therefore an added restriction on cellular movement.

Figure 6 : Cells are slower at confluency: Single-cell trajectories on a polystyrene substrate for fixed time window of 90 minutes (a) sprawl farther for isolated cells (b) look somewhat caged even for cells at the leading edge of the monolayer [63]. Adapted the figure panel from ‘Locomotion of human skin keratinocytes on polystyrene, fibrin, and collagen substrata and its modification by cell-to-cell contacts.’: J. Drukaa, L. Bandura, K. Cieslik, and W. Korohoda, Cell Transplant., ISSN 09636897, 2001.

For tissue monolayers (2d) with no cell death or cell proliferation, this seemingly complicated collective cellular motion can be broken down into individual neighbor exchanges called T1 transitions[67]. T1 transition have been extensively studied in the context of foam community. In two-dimensions, a cell moving past its neighbors, undergoes an edge flip that puts it in contact with the cell ahead. One such edge flip, also known as a T1 transition, is shown in the Fig. 7. Edge flips like these help the cell to make new connections and break old ones. A series of such T1 transitions helps

²Quantifying cellular motion at confluency can be tricky as the motion of cell’s center might fail to capture the actual movement of the cell.
a cell to move to its final destination while maintaining confluency. Similar motions are also possible for 3D polyhedral cells except possibly in multiple different ways.\footnote{Some such T1 transitions are studied in 3D foams \cite{68}.}

In general, an edge flip involves a perimeter increase, and thus, it costs energy (as shown in Fig. 7) for a cell to undergo one of these neighbor exchange processes\cite{67, 69}. In analogy with activation energies required for Arrhenious processes, one can think of the T1 edgelength as a reaction coordinate\cite{67, 70}. In systems with negligible barriers, cells can move freely, making the tissue dynamics very similar to that of a fluid-like system.

Figure 7: In order to make new neighbors, cells need to undergo an edge flips an edge flip— in this figure, the blue edge flips to yellow. This change in topology, typically requires the system to go through an energy barrier $E_b$\cite{67}. Used with permission of Royal Society of Chemistry, from ['Energy barriers and cell migration in densely packed tissues.', D. Bi, J. H. Lopez, J. M. Schwarz, and L. M. Manning, 10:1885-1890, 2014]; permission conveyed through Copyright Clearance Center, Inc.

Performing neighbour exchanges is a lot more difficult for cells trying to move across the boundary of two spontaneously segregated components. When a cell-type recognizes the other as biochemically distinct (we shall refer to them as heterotypic for the rest of this work), the two cells often heighten the tension along shared edges. In recent work studying such mixtures, the resistance faced by a cell trying to cross
the boundary increases linearly with the magnitude of interfacial tension between both components\cite{71}, as sketched in Fig. 8.

\begin{equation}
E = E_0 + c\gamma_0|\varepsilon_x|
\end{equation}

Figure 8: In segregated confluent monolayers, boundary cells find it difficult to move across the boundary. They face a discontinuous restoring force, the intensity of which increases directly with the interfacial tension. This effectively pins the boundary cells\cite{71}. Adapted figure from ‘Soft yet Sharp Interfaces in a Vertex Model of Confluent Tissue.’: Daniel M. Sussman, J. M. Schwarz, M. Cristina Marchetti, and M. Lisa Manning, PRL, 120(5):58001, 2018.

Neighbor exchange processes in the bulk and at shared boundaries can therefore be used as a simple probe to understand collective dynamics in confluent tissues. Now that we understand this, let us turn to how to quantitatively model confluent tissues.

1.4 Modelling tissues

Cells in many such tissues look polygonal, unlike disks, and fill the entire space with no gaps/opening in between. Cell-cell interactions are also very different from distance-based interaction potentials. As adjacent cells now mediate forces via shared common boundaries, the energy functional for each cell depends on the boundary length and area. Let us glance through some of the relevant computational and theoretical advancements made by the community in simulating confluent tissues. A confluent tiling can be generated using space-filling models like vertex and Voronoi tessellations (VT) and a cellular Potts model. A Cellular Potts Model (CPM)\cite{72} is a stochastic
model where cells in a monolayer are represented as regions in Fig. 9(a). The dynamic
evolution calculated with a Monte Carlo algorithm is based on a Hamiltonian that
includes interfacial tension, area conservation and active motility.

\[ H_{CPM} = \sum_\sigma \lambda_v (V_\sigma - V_\sigma^T)^2 + \sum_{(x,x')} J(\tau, \tau')(1 - \delta_{\sigma,\sigma'}) \]  \hspace{1cm} (1.1)

The first summation runs over all cells and penalizes the deviation of the cell’s
volume \((V_\sigma)\) from a prescribed target volume \((V_\sigma^T)\) with a coefficient \(\lambda_v\). The second
term sums the adhesion energies \((J)\) of all pairs at the cell boundaries where the
cells occupying adjacent lattice site pairs \((x,x')\) are different i.e. \(\sigma_x \neq \sigma_{x'}\). As \(J\) is
typically positive for heterotypic contacts i.e. \(\tau \neq \tau'\), cells tend to minimize their
surface area with other cells or the medium, making the adhesion term equivalent
to surface tension. Regarding the ease of implementation, a single cell in the CPM
clearly has a lot more than one degree of freedom, hence larger systems will be slower
to simulate.

The vertex model\[73, 74\] has a similar energy functional except with explicit
dependence on the perimeter up to second order. Each cell, as shown in Fig. 9(b)
tries to minimize this energy functional.

\[ H_{VM} = \sum_\alpha \frac{K}{2} (A_\alpha - A_\alpha^{(0)})^2 + \sum_{<i,j>} \Lambda_{ij} l_{ij} + \sum_\alpha \frac{\Gamma_\alpha}{2} L_\alpha^2 \] \hspace{1cm} (1.2)

The energy functional describes forces due to cell elasticity, acto-myosin bundles, and
adhesion molecules. The first term describes an area elasticity with elastic coefficients
\(K\), for which \(A_\alpha\) is the area of cell \(\alpha\) and \(A_\alpha^{(0)}\) is the preferred area, which is determined
by cell height and cell volume. The second term describes line tensions \(\Lambda_{ij}\) at junctions
between individual cells. Here, \(l_{ij}\) denotes the length of the junction linking vertices
i and j and the sum over $<i,j>$ is over all bonds. Line tensions can be reduced by increasing cell-cell adhesion or reducing acto-myosin contractility. The third term describes the contractility of the cell perimeter $L_\alpha$ by a coefficient $\Lambda_\alpha$, which could reflect, for example, the mechanics and contractility of the acto-myosin ring. The degrees of freedom of a single cell here are its vertices.

A **Self-propelled Voronoi model**[75], on the other hand, uses the same energy functional as Eq:1.2 but with different degrees of freedom. The degrees of freedom for a single cell here are the D (in D dimensions) associated with its own center and the cell shapes are given as the Voronoi tessellation of the centers. Therefore the shapes in the SPV models are not the minimum ones associated with Eq 1.2, but they are typically close[76]. It is also more efficient than all of the above models. One can easily incorporate activity by adding propulsion to cell centers. Homogeneously growing, confluent monolayers are very accurately described by Voronoi tessallations of their nuclei as shown superimposed in Fig .9(c). As both CPM and vertex models are difficult to code in 3D because they have so many degrees of freedom, a more computationally accessible way to study 3D tissues would be 3D voronoi models[77–79].

I will focus on the latter two models. What can we learn from such models for confluent tissues?

### 1.4.1 Density-independent rigidity transition

Recently, a unique kind of rigidity transition was discovered in both 2D[67] and 3D confluent models[77]. A dimensionless parameter called the shape index— the ratio of perimeter to the square root of area in 2D, and the ratio of surface area to the $2/3$ power volume in 3D— is a way of quantifying whether the cell is roundish or elongated. The shape index value is the lowest for rounder/spherical cells. In experiments, cell shapes are found to be larger for cells that are fluid-like[80] (for example- mesenchymal
cells) and smaller for solid-like celltypes (epithelial cells). A transition from a fluid-like to a rigid tissue is observed at a critical target shape index $s^* \sim 3.81$ in 2D and $\sim 5.41$ in 3D. Below this value, the tissue behaves like a rigid elastic solid. This rigidity vanishes when the shape target index exceeds this value and the tissue fluidizes.

This zero temperature result was further explored in 2D, using the SPV model with an added ingredient of activity. As found by Bi et al, a fluid to solid transition occurs at the same value of acquired shape of $3.81[75]$. For more solid-like cells, the acquired shape remains 3.81 and the trajectories are caged; whereas for liquid-like cells, shapes are larger and trajectories are diffusive, as shown in Fig: 11. Bi et al also demonstrated that the phase boundary depends on the activity. A very similar

shape-dependent fluid-solid transition is observed in other space-filling models as well\cite{75, 81, 82}, as depicted in Fig: 11.\footnote{With the help of analytical work, soft-solid\cite{83} like rheologies have also been explored at intermediate cell shapes.}

The zero temperature 2D transition point has also been observed in in-vitro experiments of lung epithelial monolayers\cite{85}. Furthermore, the cell shape correlates well with the bulk tissue fluidity as observed in both healthy tissues and the tissue from asthma patients. Similarly, these simple models have been surprisingly successful at describing the statistics and behaviour of many other biological tissues\cite{86–90} as well, suggesting that vertex-based models are a great candidate to study collective behaviour in confluent tissues.

\subsection{The unique energy landscape of confluent tissues}

Systems comprised of multiple particles have a complex multidimensional potential energy landscape with multiple minima as shown in Fig. 12(a). Typically the system
moves through minima by crossing energy barriers. In particulate systems, external stimuli like applied shear strain, can make it easier to cross barriers by *softening* the landscape, i.e. minima become effectively shallower. This means that as the curvature about a local minimum flattens the barriers also diminish. For a sufficiently large perturbation, the curvature vanishes with the energy barrier and the system moves into the nearest minimum.

But recent work on confluent monolayers have shown that its energy landscape has highly unusual properties like sub-Arrhenious scaling of dynamics with temperature[91]. In a counter-intuitive fashion, the landscape for these systems become stiffer with increasing temperature as shown in Fig:12(b).

The energetics at the interface between two heterotypic cell-types is also entirely
different from that of molecular fluid mixtures at equilibrium\[71\]. This leads to a much sharper segregation and pins the boundary cells by using discontinuous restoring forces. While higher fold vertices are generically unstable in the bulk\[69\], they’re stabilized along the high tension interface, as shown in Fig:13. Geometric signatures like these intensify directly with applied tension. Therefore strings of 4-fold vertices can be used as a visual hint about the presence of high tension cables in a monolayer.

In 3D tissues, a lot is yet to be unravelled about the landscape governing neighbor exchanges in bulk and at shared interfaces. Neighbor exchange barriers can be cumbersome to study in 3D tissues as a cell can create a new neighbor in a myriad number of ways. But, instead focusing on the barriers associated with a topological change at a heterotypic interface can greatly reduce the complexity, perhaps making it easier to understand.
Figure 13: Due to the pinning effect at the heterotypic interface, one can observe geometric features unique to confluent tissues. For example, in 2D monolayers, the edge length becomes bimodal for increasing tension [71]. Reprinted figure with permission from ‘Soft yet Sharp Interfaces in a Vertex Model of Confluent Tissue.’: Daniel M. Sussman, J. M. Schwarz, M. Cristina Marchetti, and M. Lisa Manning, PRL, 120(5):58001, 2018, Copyright (2018) by the American Physical Society.

1.5 Mixtures of confluent tissues

For the purpose of studying fluidization and segregation in monolayers, we use a 2D vertex model [70, 74, 86–89, 92–94], where the biomechanics of the \( j \)th cell of type \( \beta \) is given by the energy functional:

\[
E_{j,\beta} = K_a(A_{j,\beta} - A_{0,\beta})^2 + K_p(P_{j,\beta} - P_{0,\beta})^2, \tag{1.3}
\]

where \( A_{j,\beta} \) denotes the \( j \)th cell area of type \( \beta \) and the \( j \)th cell perimeter of type \( \beta \) is denoted by \( P_{j,\beta} \). Given the quadratic penalty from deviating for a cell’s preferred area and perimeter, \( K_a \) and \( K_p \) are area and perimeter stiffnesses, respectively, and both are independent of cell type. Physically, the area term represents the bulk elasticity of the cell, while the perimeter represents the contractility of the acto-myosin cortex with \( P_{0,\beta} \) denoting a competition between cortical tension and cell-cell adhesion. The total energy of the tissue is then defined as \( E = \sum_{j,\beta} E_{j,\beta} \). An important parameter in these models is the dimensionless shape index \( s_{0,\beta} = P_{0,\beta}/\sqrt{A_{0,\beta}} \) [70]. A regular hexagon has a dimensionless shape index of \( s_0 \approx 3.72 \), for example.
For studying fluidization, we use just one cell-type whereas for binary mixtures, we have two cell-types with $\beta = 1, 2$ and allow the cell types to have different parameters, $A_{0,\beta}$ and $s_{0,\beta}$. We will focus on cases of 50:50 mixtures where there is an equal number of each cell type, with either preferred shape disparity or preferred area disparity. Unless otherwise specified, the two components are uniformly distributed in the initial state. We set $K_p$ to unity for all systems.

We study the above energy functional from an energy minimization perspective as well as from a dynamical perspective in which the cells migrate within the monolayer. As for the latter, there is still much debate about how to model the motility of cells. We have chosen to model the motility of a cell by imposing a random active force on each vertex, i.e. each vertex undergoes over-damped Brownian motion at a fixed effective temperature $T$ with a conservative force contribution from the above energy functional and a second force contribution from a Brownian force. While there are other possible dynamical rules, we have found that, for example, the properties of an interface between two cells types with heterotypic line tension between them is rather robust to the specifics of the dynamical rule[71]. The equation of motion for each vertex is then iterated until the cells can adequately explore the entire system such that the system approaches a steady state, at least for most parameters we study. As the cells move, they may rearrange and come into contact with new cells. To implement such a T1 transition, an edge shared between two cells undergoes a $\pi/2$ rotation once the length of this edge is below some threshold length. The rotated edge then lengthens and allows for two different cells to now share an edge. A FIRE minimization [95] protocol is used for bulk energy minimization to compute the energy barriers associated with T1 transitions by constraining the length of a particular edge in the system such that a T1 transition occurs while allowing the remaining degrees of freedom in the system to relax. For studying fluidization, the edge can be anywhere
in the monodisperse system whereas for segregation, the edge must be at the interface shared between unlike cells.

We are also interested in comparing the behavior of these bidisperse systems to ones with an explicit heterotypic line tension (HLT), where cell types 1 and 2 recognize their joint interface as a heterotypic interface and, therefore, alter the line tension at that interface. Such interactions are common in cellular Potts models \([72, 96]\) and have also been studied in vertex and Voronoi models \([71, 97]\). In this case, we add an extra term to the cell energy to arrive at:

\[
E_{HLT} = \sum_{j,\beta} K_A (A_{j,\beta} - A_{0,\beta})^2 + K_p (P_{j,\beta} - P_{0,\beta})^2 + \gamma \sum_{\langle i,j \rangle} (1 - \delta_{\alpha\beta}) l_{ij},
\]

The latter sum is over all edges, \(l_{ij}\), between cells \(i\) and \(j\) with \(\delta_{\alpha\beta}\) representing a Kronecker delta such that there is additional line tension only between cells of two different types \(\alpha\) and \(\beta\). For simplicity, we assume that the additional tension, \(\gamma\), is the same for all heterotypic edges. We use open source cellGPU code for 2D simulations\([98]\).

We use a straightforward extension of this model to understand interfacial mechanics between two cell-types in a 3D tissue. A three-dimensional confluent tissue of \(N\) cells is created using Voronoi tessellation of the cell center as shown in the inset for Fig. 10. Individual cells now have preferred volumes \(V_0\) and surface area \(S_0\). In addition to the monodisperse form, there is surface tension between heterotypic cells. Therefore cells minimize their mechanical energy using the following energy functional:

\[
E_{HST} = \sum_{j} \left[ K_V (V_j - V_0)^2 + K_S (S_j - S_0)^2 \right] + \sigma \sum_{\langle i,j \rangle} (1 - \delta_{\alpha\beta}) A_{ij},
\]

where \(V_j\) denotes the the \(j\)th cell volume and \(S_j\) denotes its total surface area. \(K_V\)
and $K_S$ are volume and area stiffnesses, respectively. The preferred shape parameter can be defined as $s_0 = S_0/\langle V \rangle^{2/3}$. A regular BCC unit cell (truncated octahedron) has a dimensionless shape index of $s_0 \sim 5.31$. Particles are initialized in a segregated bilayer arrangement, with cells both the layers tagged as types $\beta = 1$ or $\beta = 2$ respectively. The later sum is over all the facets, $A_{ij}$, between heterotypic cells and $\sigma$ is the imposed surface tension. We use the code developed for 3D tissues[77].

1.6 Outline

In this thesis I shall focus broadly on two classes of problems. The first deals with fluidization in confluent tissues and the second investigates segregation in two-component confluent mixtures. Recent studies have systematically characterised the emergence of fluidity in disordered tissues[67, 75], but the same is not entirely understood for ordered tissues. Tissue surface tension (TST) and its pivotal role in compartmentalization is now well understood in the context of 2D monolayers[71]. However, it is not known if mixing cells with different cellular properties can induce an emergent TST. In 3D tissues, the effect of TST remains vastly unexplored. The onset of fluidity and interfacial stability, has been traditionally probed using linear shear response. But confluent tissues have topology-dependent interactions that lead to a landscape where linear response might fail to give the complete picture. As neighbor exchange processes in confluent tissues require a change in the underlying topology, we shall use neighbor exchanges– as a more fundamental non-linear probe – to understand fluidization in ordered tissues and segregation in 2D and 3D tissues.

With this as the overarching theme of my thesis, Chapter 2 begins with 2D ordered monolayers and explores the unusual relationship between their linear and non-linear responses. In disordered 2D vertex models, the shear modulus becomes zero precisely
when the cells can change neighbors and the tissue fluidizes, at a critical value of control parameter $s_0^* = 3.81$ [67]. However, the ordered ground states of 2D vertex models become linearly unstable at a lower value of control parameter $(3.72)$ [74, 86], suggesting that there may be a decoupling between linear and nonlinear response. We demonstrate that the linear response does not correctly predict the nonlinear behavior in these systems: when the control parameter is between 3.72 and 3.81, cells cannot freely change neighbors even though the shear modulus is zero. These results further highlight that the linear response of vertex models should not be expected to generically predict their rheology. We develop a simple geometric ansatz that correctly predicts the nonlinear response, which may serve as a framework for making nonlinear predictions in other vertex-like models.

Shifting from fluidization to segregation, in Chapter 3, I focus on 2D binary mixtures. We investigate whether a disparity in cell shape or size alone is sufficient to drive demixing in these mixtures. What is the biological implication of two different shape indices, for example? Consider two cell-types with the exact same area. The cell type that prefers to have a higher shape index can do so by increasing the cell-cell adhesivity, for example. Therefore, mixing two cell-types with a difference in cell-cell adhesivity, corresponds to studying mixing in cell-types with two different shapes. In reality, these adhesion molecules also affect the cell shape by signaling to either up-regulate or down-regulate contractility[99–101]. In the vertex model energy functional that we use, we have packaged both adhesion and contractility into the preferred shape index. Though more detailed models are possible, we find that our minimal model does indeed tell us something about how cells behave as indicated by the experiments. Surprisingly, we observe that both types of bidisperse systems robustly mix on large length scales. On the other hand, shape disparity generates slight demixing over a few cell diameters, a phenomenon we term micro-demixing.
This result can be understood by examining the non-reciprocal energy barriers for neighbor exchanges at the interface. Experiments with mixtures of wild-type and E-cadherin-deficient keratinocytes on a substrate are consistent with the predicted phenomenon of micro-demixing, which biology may exploit to create subtle patterning during developmental stages.

As difference in cellular properties like shape and size did not lead to bulk segregation in 2D, in Chapter 4, I move to study 3D mixtures that have explicit TST and study the mechanics of interface separating two tissue types. We find that cells rapidly sort to generate a tissue-scale interface between cell types, and cells adjacent to this interface exhibit signature geometric features including nematic-like ordering and bimodal facet areas along the surface. The magnitude of these features scales directly with the magnitude of imposed tension, suggesting that experiments might estimate the magnitude of tissue surface tension simply by studying cell shapes in a snapshot of a 3D tissue. To understand the origin of these features, we conclude by discussing the mechanism behind an emergent registration of both the boundary layers by using simplistic toy models. We find that slight perturbations to a perfectly registered state requires making new topological connections that trigger a discontinuous restoring force. Finally, in Chapter 5, I shall conclude with a summary of all the results and its implication on the ongoing research in broader tissue mechanics community.
Chapter 2

Linear and nonlinear mechanical responses can be quite different in confluent tissues

This chapter is based on work primarily presented in the article “Linear and nonlinear mechanical responses can be quite different in models for biological tissues” [102] co-authored by Preeti Sahu, Janice Kang, Gonca Erdemci-Tandogan and M. Lisa Manning, and published in the journal Soft Matter, in the year 2020. Both Janice Kang and I performed the simulations. I was responsible for preparing the first draft of the paper, and my co-authors provided valuable suggestions and corrections to add both to the conceptual understanding and the presentation of the work.
2.1 Characterizing fluidity in confluent models

The rheological properties of a biological tissue – how a tissue responds to stresses and strains – and the regulation of such properties are crucial for many biological processes. For example, mature skin tissue typically behaves like an elastic material, where cells maintain their neighbors and the tissue returns to its original shape after being stretched, just like a solid. However, in processes like wound healing, individual cells can change neighbors and migrate over long distances [14, 15] just as in a fluid. Tissues that transition between solid and fluid states have recently been shown to play an important role in development [103] and disease [85]. Thus, we would like to understand how the emergent material properties, such as the rheology, of the tissue are determined and regulated.

In traditional materials, the rheology of a material is usually characterized by a linear response variable, such as the shear modulus that describes how the mechanical stress in the material changes in response to a very small strain. More recently, it has been recognized that some biological materials behave very differently when they experience large strains instead of small strains. For example, extracellular matrix stiffens by several orders of magnitude when strained past a critical threshold [28]. In addition, it has recently been shown that models for heterogeneous confluent epithelial layers, with two cell types and an interfacial tension between them, has non-analytic cusps in the potential energy landscape so that the linear and nonlinear response are completely decoupled [91]. Both of these observations suggest that we should not necessarily expect the linear response of a biological tissue to predict its nonlinear response.

Biologists and biomedical engineers are often interested in processes that involve very large strains, such as convergent extension to elongate the body of a developing
embryo, or cells moving over tens or hundreds of cell diameters to close a wound. Therefore, it is important to understand whether the standard tools of linear response are valid in these systems, and if not, develop new approaches to predict the nonlinear response.

To explore this question further, we focus on homogeneous confluent epithelial monolayers composed of a single cell type. Vertex models represent these tissues as a 2D network of edges and vertices, and associate a mechanical energy with the shape of each individual cell in a tessellation. Such simple models have been surprisingly successful at describing the statistics and behavior of many biological tissues [86–88, 90, 104]. The mechanical energy associated with cell shape is based on experimental observations in cell doublets and triplets: cells with more cadherin-based adhesion tend to share longer joint interfaces, while those with higher cortical tension tend to have smaller shared interfaces [99, 100]. In all of this work, an important control parameter of the model is the dimensionless cell shape index $s_0$, which is the ratio between the cell’s cross-sectional perimeter and the square root of the cell’s cross-sectional area.

The next step is to understand how cell shapes influence the large-scale rheological properties. Within the framework of vertex models, Farhadifar et al. [86] and Staple et al. [74] performed a beautiful and comprehensive investigation of the linear response of ordered tessellations, which are the ground states of the vertex model. They demonstrated that the shear modulus of ordered ground states of the 2D vertex model disappear for all shapes with $s_0 > 3.722 = s_{\text{hex}}$, the perimeter to area ratio of a regular hexagon. In other words, the energy landscape is flat with respect to small perturbations for $s_0 > 3.722$. However, these works did not investigate the nonlinear response – how cells rearrange and change neighbors at larger strains.

In a confluent tissue with no cellular proliferation or death, the only way for a cell
to change neighbors and diffuse over large distances is to make a series of topological rearrangements, or T1 transitions. During this process, an edge between two cell shrinks to zero length and then a new edge grows between two new cells, as illustrated in Fig. 14(a-c). Many such exchanges lead to cell diffusion. Therefore, an important parameter that controls the nonlinear response of the tissue is the height of the mechanical energy barrier associated with a T1 transition.

Work by Bi et al. [70] on homogeneous disordered tessellations of cells demonstrated that the T1 energy barriers’ height depends sensitively on the target shape index ($s_0$) of cells. For the 2D vertex model, this energy barrier vanishes if for cells with shape parameter $s_0 > 3.81$. In addition, a careful numerical analysis showed that the shear modulus also vanishes at the same critical value of 3.81, which is different from the critical value of 3.722 identified in the ordered systems.

This presents an interesting open question, which is – what is the nature of the nonlinear mechanical response of ordered tissues? One possibility is that the energy barriers also vanish for $s_0 > 3.722$, similar to the scenario in jammed particulate matter, where there is an ordered and disordered branch to the equation of state and linear response is highly predictive of nonlinear response [22]. An alternate possibility is that the energy barriers for ordered tessellations vanish at some other value of $s_0$, indicating a decoupling between the linear and nonlinear response.

Understanding this point is important for several reasons. First, there are several examples where formation and maintenance of ordered 2D tessellations are important in biology, including the fruit fly wing [105], the sensory hairs in cochlea [106], and lens fibre cells in the vertebrate eye [107, 108]. In addition, scientists are investigating extensions to vertex models such as non-confluent systems [109, 110], and vertex models with additional signaling-based dynamics [111, 112]. Therefore, it is important to understand whether we should generically expect a strong correlation between
linear and nonlinear response in these extended models, or if the correlation observed
the simplest disordered homogeneous vertex model may be a special feature unique to
that model.

In this work we quantify the energy barriers to T1 transitions in an ordered tissue.
We find that although tissues with $s_0 > 3.722$ are linearly unstable, T1 transitions
cost finite energy up to $s_0^* = 3.81$, due to cusps in the potential energy landscape
along those trajectories in configuration space. This establishes that the linear and
nonlinear response of ordered tissues are decoupled – cells cannot change neighbors
even though the linear response indicates the tissue is floppy. To go beyond linear
response, we develop a simple, mean-field geometric construction that describes this
process and correctly quantitatively predicts features of nonlinear stabilization, and
discuss implications for extensions of vertex models.

### 2.1.1 Computational model for a hexagonal monolayer

To find the transition point based on T1 energy barriers, we simulate a 2D confluent
monolayer using a Vertex model [70, 74, 86–89, 92–94].

Vertex models describe the energy of a 2D tissue containing N cells as

$$E_j = \sum_{j}^{N} K_{A_j}(A_j - A_{0j})^2 + K_{P_j}(P_j - P_{0j})^2. \quad (2.1)$$

Here the first term represents cell volume incompressibility, and $A_j$ and $A_{0j}$ are the
actual and preferred areas of cell $j$. The second term models actomyosin contractility
and adhesion between the cells, where $P_j$ and $P_{0j}$ are the actual and preferred perimeter
of cell $j$. $K_{A_j}$ and $K_{P_j}$ are the area and perimeter moduli, respectively. We consider
the homogeneous case where all single-cell properties are equal ($K_{A_j} = K_A, K_{P_j} =
K_P, A_{0j} = A_0, P_{0j} = P_0$). The energy functional in Eq. 2.1 can be non-dimensionalized
in length \( \sqrt{A_0} \) resulting an effective target shape index \( s_0 = P_0/\sqrt{A_0} \) which has been shown to control rigidity or glass-like transitions in such systems \[70\].

### 2.1.2 Neighbor exchange and non-linear rigidity

Cell neighbor exchanges happen through T1 transitions. A typical T1 process is shown in Fig. 14(a-c). As the T1 edge \( l \) shrinks from its rest length, \( l_0 \), (Fig. 14(a)), it eventually achieves a transition state at \( l = 0 \) with a 4-fold vertex where all 4 cells are neighbors (Fig. 14 (b)). This is followed by a \( 90^\circ \) reorientation of the T1 edge and expansion along the new direction (Fig. 14 (c)). We find that the mechanical energy of the tissue is maximized at the transition state with the 4-fold coordinated vertex. As in previous work, we describe the difference between the initial energy and maximum energy as an energy barrier that must be overcome for cells to change neighbors. In analogy with activation energies required for diffusion in Arrhenius processes, we can then think of the T1 edge-length \( (l) \) as a reaction coordinate \[67, 70\].

We focus on the first part of the T1 process for the rest of this paper, as this is sufficient to compute the energy barrier (shown in blue vertical line in Fig. 14). We choose the sign convention as positive for this part of the transition, which is different from the convention used for \( l \) in work that studies both sides of the transition \[70\].

The difference between the peak energy \( E_f \) and the initial energy \( E_i \) gives the T1 energy barrier (Fig. 14 vertical line in blue),

\[
\Delta E(l) = E_f - E_i = E(l) - E(0).
\]

(2.2)

For the bulk simulations, we use the open source cellGPU code \[98\]. A FIRE minimization protocol \[95\] is used for bulk energy minimization. The initial FIRE step, \( dt \), is set to 0.01. The T1 protocol is such that a T1 transition forms whenever
Figure 14: **Energetics of an ordered T1 transition:** (a) A T1 edge, highlighted in red, at its rest length, (b) the T1 edge shrinks to zero length (c) the T1 edge rotates by 90° and is then expanded. (d) A typical energy profile across the T1 transition plotted with respect to the T1 edge length $l$ during T1 junction remodelling, for $s_0$ 3.71, 3.72, 3.73 and 3.75 (light red to dark red). The T1 energy barrier for the lowest $s_0$ is the peak height (highlighted in blue vertical line).
the distance between two vertices is less than a critical value, $l_c$. We chose $l_c = 0.006$ for the ordered tissue simulations. As discussed in this chapter’s Appendix, we apply the same procedure to compute the transition point in disordered systems. Unlike ordered systems, which have a unique hexagonal initialization, in a disordered systems we average the energy barrier profile over different initializations. See the Appendix-1 for more details.

Recent work [113] has shown that the transition point in vertex models is unaffected by the choice of $K_A$. Here, we choose $K_A = 100$, which enforces that cells remain close to their preferred area $A_0 = 1$.

2.2 At what cell shape does non-linear rigidity vanish?

2.2.1 Many-cell system

To test the transition point of ordered tissues subject to a specific non-linear perturbation, we construct a rectangular periodic box that can accommodate an integer number of hexagons, with a length-to-width ratio of $\frac{3m}{2\sqrt{3}n}$,

where $m$ is the number of hexagons along the vertical axis and $n$ is the number of hexagons along the horizontal axis. We investigate small systems with $N = 90$ such that $n = 9$ and $m = 10$, simulated using cellGPU code.

A random edge of the ordered confluent tissue is chosen to undergo a T1 transition, and the energy profile is analyzed across different $s_0$ values. A typical T1 edge, with its neighbourhood, is shown in Fig. 15 (a) along with energy profiles for different $s_0$ values (Fig. 15(d)). For values of $s_0 < 3.722$, any perturbations of edge lengths costs finite energy, as illustrated by the red curve in Fig. 15(d). For values of $s_0 > 3.722$, we find that small perturbations of $l$ require zero energy as previously predicted [74, 86] using linear response. This is indicated by values of $E(l)$ near zero on the left-hand
Figure 15: **Many-cell energy profile:** (a-c) A snapshot from an ordered tessellation of 90 cells with $s_0 = 3.76$. A randomly chosen edge (highlighted in red), shrinks to zero length (left to right as directed by the arrows). (d) In this process, the total energy of the tissue, $E$, is plotted against the shrinking T1 edgelength $l$ for increasing values of $s_0$ (3.72 to 3.81 in steps of 0.01 and 3.810 to 3.825 in steps of 0.001) varying from red to green. The energy cut-off is shown by yellow dash-dot line. (e) The critical edgelength $l^*$ associated to the cut-off shown in (d) is plotted for each $s_0$ value in yellow circles. The dashed line indicates critical $s_0^*$ found for disordered tissues.
side of Fig. 15(d). A similar transition can be seen by studying the normal modes of the system. We find that the number of nontrivial normal modes with zero frequency (i.e. “zero modes”, Fig. A2) is zero for $s_0 < 3.722$ and immediately rises to $3N$, where $N$ is the number of cells in the tessellation, for $s_0$ slightly above 3.722.

But as the T1 process proceeds further, the energy becomes finite at a critical lengthscale $l^*$. In practice, we identify $l^*$ as the point at which the energy first rises above a cutoff value of $10^{-7}$ shown by the dashed yellow line in Fig. 15(d). We find that $l^*$ diminishes with increasing $s_0$, and drops to zero at $s_0^* = 3.81$, which is the same value identified in disordered systems, as shown in Fig. 15(e). We note that the lowest value of $l^*$ accessible in our simulations is limited by the T1 threshold length, $l_c = 0.006$.

We focus on T1 processes for the transition path through configuration space because they are simple to parameterize and correctly capture the rigidity transition in disordered systems [70]. However, there are other possible transition paths, including one of the $3N$ nontrivial zero modes identified by a normal mode analysis. However, a visual inspection of these modes shows no obvious spatial structure (Fig. A2(a)) and because there are so many modes with the same degenerate eigenvalue (where any linear combination of them would also be a zero mode), an exhaustive search of these possibilities is beyond the scope of this work. Nevertheless, we find that as we execute a T1 trajectory, the number of zero modes starts to decrease precisely at the cusp in the energy landscape Fig. A2(c), suggesting that some zero modes start to cost finite energy at that point in configuration space.

Our data is consistent with the hypothesis that the energy landscape is locally flat in many directions for $s_0 > 3.772$, but that any finite displacement in configuration space will cost finite energy if the displacement is large enough. More work to study many paths in configuration space would be required to confirm this hypothesis.
2.2.2 Single cell prediction

In both many-cell and 4-cell systems (Fig. A1), the ordered polygons that undergo a T1 transition start out as perfect hexagons but become pentagons as the edgelength \( l \) shrinks to zero (Fig. 15(c) and Fig. A1(c)). For disordered systems, the formation of a pentagon was proposed as a mean-field lower bound on the T1 transition point previously by Bi et al [70].

The geometric ansatz

Here we construct a simple geometric ansatz to predict the T1 edgelength \( l^* \) at which the energy barrier becomes non-zero. We restrict ourselves to study a polygon whose vertices lie on a circle of radius \( R \) (Fig. 16(a)). This constraint is a simple way to enforce that the polygon remains roughly isotropic, consistent with our observations from simulations. To model the ordered case, we enforce that the polygon has six sides, one of which is constrained to shrink and subtends an angle \( \theta \) at the center. We assume the remaining sides adjust themselves to be of equal length, which minimizes the remaining perimeter subject to having one constrained edge, as illustrated in Fig. 16.

In Appendix-1 we show that the minimum energy geometry in numerical simulations is slightly more complex than our simple ansatz, because the non-T1 sides of the polygon have two different edgelengths instead of one. On the other hand, if we compare the equal-edge assumption to a generalized ansatz (Fig. A3), the simplest one-edgelength assumption generates a lower bound on the transition length \( l^* \) (See Fig. A4) that is highly predictive, as shown below.
Figure 16: A geometric mechanism for formation of a uniform pentagon: The 6-sided polygon has five sides equal to each other and one that is allowed to be different subjected to the constraints that the polygon lies on a circle and its area remains unity. The angles correspond to two different types of sides ($\alpha$ and $\theta$) are highlighted in pink and green.

**Calculation for the T1 barrier**

We can then study the perimeter change of this polygon as it transforms from a uniform hexagon to a uniform pentagon. We constrain the area of the polygon to unity to account for incompressibility of cells.

The area of the polygon can be written in terms of the area of six triangles that make up the polygon. Five of them are congruent to each other, since they subtend the same angle $\alpha$ at the center and the sides are of length $R$ (triangle $\triangle \alpha$, labelled in violet in Fig. 16). The leftover triangle subtends angle $\theta$ at the center and will be referred to as $\triangle \theta$.

The area constraint ensures $5A_r(\Delta \alpha) + A_r(\Delta \theta) = 1$. Substituting the area in terms of angles and radius $R$, the radius of the circle is determined as a function of $\theta$:

$$R(\theta) = \sqrt{\frac{2}{\sin(\theta) + 5\sin(\alpha)}}$$

where $\theta + 5\alpha = 2\pi$.

Adding all the edgelengths, the total perimeter $P$, of the polygon is $P(\theta) =$
Figure 17: **Single-cell energy profile:** (a-c) For a single cell inscribed on a circle, the T1 edge (highlighted in red) shrinks to zero length (right to left as directed by the arrows). (d) In this process, the total energy $E$ is plotted against the shrinking T1 edgelength $l$, for increasing values of $s_0$ (3.72 to 3.81 in steps of 0.01) varying from red to green. (e) The critical edgelength $l^*$ associated to the drop shown in (d) is plotted for each $s_0$ value in blue dot-dashed line. The blue dashed line indicates critical $s_0^*$ found for disordered tissues.
\[ 2R(\theta)\{\sin(\theta/2) + 5\sin(\alpha/2)\} = \sqrt{\frac{2}{\sin(\theta) + 5\sin(\alpha)}} \{\sin(\theta/2) + 5\sin(\alpha/2)\}. \]

For this T1 process, the edge facing \( \theta \) mimics the T1 edge that shrinks to zero as shown in Fig. 17(a). This T1 edge-length can be easily determined from \( \theta \) as \( l(\theta) = 2R(\theta)\sin(\theta/2) \).

For a cell of unit area the total vertex energy depends only on the deviation of the perimeter from its target value. The target perimeter equals the actual perimeter when the angle \( \theta^* \) associated with a T1 edgelength \( l^* \) satisfies the following analytic equation:

\[
P(\theta^*) = P_0 = \sqrt{\frac{2}{\sin(\theta^*) + 5\sin(\alpha^*)}} \{\sin(\theta^*/2) + 5\sin(\alpha^*/2)\}. \tag{2.3}
\]

The critical shape nearly reproduced

For each value of \( P_0 \), this equation then identifies the \( l^* \) at which the energy barrier goes to zero, as shown in Fig. 17(d). These results are quantitatively consistent with the results for \( l^* \) for the 4-cell and bulk simulations, demonstrating that a very simple geometric ansatz predicts the onset of nonlinear stabilization in the ordered vertex model. All the models exhibit very similar behavior, as shown in Fig. 18, with \( l^* \) dropping to zero when \( l^* \sim 3.81 \).

2.2.3 Conclusion

We have demonstrated that the ordered ground states of the frequently-used 2D vertex model for biological tissues are stable with respect to localized cell rearrangements when the target shape parameter \( s_0 \) is between 3.72 and 3.81. This is surprising, as previous analytic calculations for the linear response highlights that the ordered states become linearly unstable for all \( s_0 \) values greater than 3.72 \([74, 86]\).

We demonstrate this nonlinear stabilization in a full simulation of the vertex model,
Figure 18: **Non-linear stabilization seen in ordered bulk systems can be produced in 4-cell system and single cell model**: Critical edge length $l^*$ plotted against $s_0$ is superimposed for both many-cell (green circles) and 4-cell systems (magenta circles). In addition, the analytical prediction from the geometric mechanism explained in the text is shown in blue dashed line.

and also in two toy models, one of which is analytically tractable. In all three models, we find that for values of $s_0$ between 3.72 and 3.81, small perturbations to the structure cost zero energy, in line with previous calculations of linear response. However, there is a finite scale of perturbation at which the energy suddenly becomes non-zero. In ordered systems, we characterize this behavior in terms of the edge-length $l^*$ at which the energy first becomes non-zero, and find that $l^*$ decreases monotonically from the ordered edge length $l_0$ at $s_0 = 3.72$ to zero at $s_0 \sim 3.81$. In the simplest analytically tractable and purely geometric model, we see that $l^*$ vanishes precisely at $s_0 \sim 3.81$ because that is the point at which an isotropic pentagon costs zero energy.

As discussed in the Appendix, a very similar analysis can be performed on disordered configurations of the 2D vertex model. While the data is noisier due to the disorder in edge length, it is clear that in disordered tissues the smallest values of $l^*$ remains on the order of the average edge length in the tissue for all $s_0 < 3.81$, and drops
precipitously to zero for \( s_0 > 3.81 \). This Heavyside-function-like behavior is consistent with the hypothesis that disordered tissues also destabilize when it is possible for an isotropic pentagon to form at zero cost, as postulated previously \([70]\). An interesting direction for future work would be to carefully characterize how the statistics of short edge-lengths and \( l^* \)'s vary as a function of system size and model parameters in disordered systems, extending previous work demonstrating the importance of edge length statistics to rigidity in Vertex models \([114]\).

Overall, this result is interesting because it suggests that unlike particulate glassy materials, where there are two branches to the equation of state associated with ordered and disordered states \([22]\), vertex models are ultimately destabilized at the same point (or at least very nearly the same point) on the state diagram, at \( s_0 \sim 3.81 \), regardless of the degree of disorder.

This deep connection between ordered and disordered states is only possible because the potential energy landscape of vertex models is non-analytic, or “cuspy”. Unlike most particulate matter, in vertex models there is a decoupling between the linear response and the non-linear response. In this specific case, the energy landscape for the ordered tissue is perfectly flat in a ball of radius \( l_0 - l^* \) from the ordered ground state, and then rises sharply from zero starting at \( l^* \). This cuspy landscape has already been identified and implicated in other processes in 2D vertex models, including unexpectedly sharp interfaces between two tissue types \([91]\). In that work, it was demonstrated that the cuspy landscape is independent of the exact form of the model (i.e. Vertex vs. Voronoi). It was also argued that we should expect non-analytic behavior in any model with topological interactions between cells, where neighbors are defined as those that share an edge, instead of metric interactions, where neighbors are defined by how far apart they are. Additional work by some of us confirms that many types of models with topological connections, including underconstrained fiber networks,
exhibit universal behavior governed by an underlying geometric incompatibility [115]. Therefore, it is interesting to conjecture that any model with topological interactions, such as those for bird flocks and certain biomimetic- and meta- materials, might have similar features with deep connections between ordered and disordered states.

Another hint at this deep connection comes from beautiful work by Moshe et al [83], who develop an analytic model based on intrinsic metrics for periodic vertex lattices. In that work, they focus on an elastic model with no rearrangements where deformations from target metrics are quadratically penalized, and they predict from first principles that for $s_0 > 3.72$, the energy landscape in the space of metrics is also perfectly flat. It would be interesting to see if extensions of that framework might be able to account for nonlinearities, and perhaps find some non-analyticity in the space of metrics, in order to explain non-linear stabilization in real space. If possible, our work suggests that may be a productive path towards a first-principles prediction of rigidity in a disordered system, which would be very exciting.

A related manuscript that also highlights the importance of flat energy landscapes in ordered and disordered cellular systems is the work by Noll et al [116] on isogonal modes in force-balanced tension networks. In that work, a different version of the vertex model, without a $P^2$ term in the energy functional to act as a restoring force, is coupled with myosin dynamics. The form of feedback chosen to model the myosin dynamics, which has recently been confirmed in experiments on fruit flies [111], introduces a different type of restoring force that permits mechanically stable cellular networks. Although their myosin-feedback model and our standard ordered vertex model both possess zero-energy linear modes, their zero modes must be angle-preserving while perturbations associated with our T1 transitions explicitly change angles. Given this, it would be interesting to study how the functional form of restoring forces in the energy functional for vertex models impacts the linear and nonlinear stability of
cellular networks.

Finally, this work focuses on vertex models in the absence of fluctuations, i.e. at zero temperature. An interesting future direction would be to study how the effective linear response and nonlinear stability changes as a function of temperature or self-propulsion. For example, in ordered systems with $3.72 < s_0 < 3.81$ one might expect that at low temperatures, fluctuations typically remain small and only probe the linear regime with no shear modulus. At higher temperatures fluctuations would regularly probe the nonlinear response, so the effective linear response has a finite shear modulus. Moreover, active or driven fluctuations with a persistence time would sample these non-linear regions in different ways, perhaps leading to very rich behavior.

Given the existence and importance of ordered cellular networks in epithelial layers in developmental systems ranging from fruit flies to vertebrates, our results might impact how we think about their form and function. Specifically, we suggest that the mechanical properties of such tissues are quite exotic, with interesting nonlinearities and possible fluctuation-induced solidification. We speculate that perhaps some biological tissues tune themselves to take advantage of these interesting properties and functions.

2.3 Appendix

Four-cell system

As a simpler system to understand the observed behaviour of transition point $l^*$ in ordered systems, we study a mean-field model for a T1 process composed of a 4-cell hexagonal system. We study the non-linear response to a T1 transition, by minimizing Eq. 2.1 in the main text for every value of T1-edgelength $l$ between the edge’s rest length and zero across different values of $s_0$ as shown in Fig. A1. To perform
such minimizations, we must constrain the geometry using symmetry considerations. Specifically, a 4-cell system comprised of hexagons is expected to have a total of 16 vertices and hence 32 degrees of freedom (DOFs). If we assume symmetry about the x- and y-axes, this reduces the system to eight orthogonal DOFs. For each energy minimization step the T1 length is fixed, resulting in seven DOFs. Since there are 8 constraints (2 on each cell) imposed by the energy functional, we can solve the resulting system of equations uniquely.

A typical T1 edge is shown in Fig. A1(a-c) along with energy profiles for different $s_0$ values shown in Fig. A1(d). Similar to the many-cell system, infinitesimal perturbations
cost energy for $s_0 < 3.722$. For $s_0 > 3.722$, perturbing the system a small amount
costs zero energy, but as the T1 proceeds further into non-linear regime, the energy
becomes non-zero after a threshold value of $l^*$. This $l^*$ goes to zero as $s_0$ approaches
$\sim 3.813$ as shown in Fig. A1(e). We observe that the energy profile is qualitatively
similar to that of a many-cell system (Fig. 15) which confirms that a simple 4-cell unit
is a suitable mean-field model for T1 processes in ordered tissues.

**Vibrational mode structure of bulk ordered systems**

In vertex and other network models, an index theorem \[117, 118\] relates the number
of constraints, degrees of freedom, zero modes, and the number of states of self-stress.
Normally, in jammed systems, the states of self-stress only arise when the system is
overconstrained. However, recent work on disordered vertex models (and also under-
constrained fiber network models) has shown an inherent geometric incompatibility
that generates states of self-stress at a critical point in the shape parameter. These
states of self-stress are not associated with additional constraints and they rigidify the
system (i.e. remove all the non-trivial zero modes) \[115, 119\].

To study this in our ordered system, we compute vibrational modes using standard
techniques \[115, 119\], by evaluating the dynamical matrix of second derivatives of
the vertex model energy with respect to vertex positions, and diagonalizing it to
identify eigenvalues and eigenvectors. The total number of zero modes in the system
is computed by counting all the modes with eigenvalues below a very small threshold,
which we chose to be $10^{-8}$.

In the ordered case, we find something similar to disordered systems. Apparently,
the geometric incompatibility introduces self-stresses in response to linear perturbations
starting at $s_0 = 3.722$. Even though naive constraint counting suggests the system
is floppy for any value of $s_0$, we find that the system is rigid for $s_0 < 3.722$, where
all the non-trivial eigenmodes have positive eigenvalues, consistent with phonons in a finite system. For $s_0 > 3.722$ the system is floppy with an extensive number of zero modes (Fig. A2(b)). Although the number of zero modes decreases between 3.722 and 3.81, there is no obvious signature in the linear spectra, suggesting that self-stresses only occur in response to nonlinear perturbations between those values of the control parameter.

We also plot an example of a zero mode for a system that is linearly unstable with $s_0 = 3.75$ (Fig. A2(a)). Since there are an extensive number of such degenerate modes, we do not expect that an individual mode such as this one demonstrates any useful features of the energy landscape.

![Vibrational mode analysis](image)

**Figure A2 : Vibrational mode analysis:** (a) A sample zero mode for bulk ordered tessellation with $s_0 = 3.75$. (b) The number of zero modes sharply increases after $s_0 = 3.722$. (c) The number of zero-valued eigenvalues of an ordered tessellation at a fixed value of the shape index, $s_0 = 3.75$, is studied along a T1 reaction coordinate. At the cusp in the potential energy landscape, $l^*$, where the energy changes by several orders of magnitude, the number of zero modes begins to systematically decrease.

We also study the eigenspectrum for a single system during the course of a T1
perturbation (i.e. as we manually shrink the T1 edge). We find that the number of zero modes decreases as the T1 edgelength shrinks below the transition value (Fig. A2(c)). This suggests that there might be a local rigidification affecting the cells neighboring the T1 edge.

Comparison of the analytic nonlinear ansatz to numerical data

In our analytic ansatz, we assume that cell shapes are isotropic with equal length edges except for the T1 edge that shrinks to zero. Here, we show numerical data from bulk simulations for the shapes of cells undergoing a T1. We focus on the observed edge average and standard deviation (error bars) of an edge length, which we have grouped into “T1-adjacent” edges ($L_A$) and “non-T1-adjacent” edges ($L_B$). For a fixed value of shape $s_0 = 3.75$, we find that along a T1 process, the distributions converge to different mean values near the transition point $l^*$, as shown in Fig. A3. Specifically, for this case, the ratio $\xi = L_A/L_B$, is 1.21. This is different from our initial assumption of equal edgelengths. Hence we check the robustness of the single-cell results with respect to $\xi$.

We next generalize the single-cell calculation to accommodate possible differences between $L_A$ and $L_B$ i.e $\xi \neq 1$. To implement this, we start with a polygon very similar to the one displayed in Fig. 16, with the equal-edge criterion lifted. We instead have the three “non-T1-adjacent” edges of the same length ($L_A$) and the two “T1-adjacent” edges of length $L_B$, such that $L_A/L_B = \xi$. We then study the perimeter change of this polygon as it transforms from a hexagon to a pentagon, for a fixed $\xi$, in an area-preserving manner. An intermediate polygon for each of the extreme ratios is displayed in Fig. A4.

We find that, for $s_0 > 3.722$, the preferred perimeter is attained for several combinations of $\xi, l^*$. But $l^*$ corresponding to these newly found roots, is always
Figure A3: Computational results for the lengths of cell edges during a forced T1 transition in an ordered tessellation: (a) In a bulk ordered system with $s_0 = 3.75$, we compute the lengths of edges on a cell undergoing a forced T1 transition, grouping the edges into “T1-adjacent” ($L_A$) and “non-T1-adjacent” ($L_B$) bins, which show different trends and become tightly constrained near the transition point, highlighted in (b) as the point at which the energy profile for $s_0 = 3.75$ has a cusp.
higher than the one for $\xi = 1$. Hence, the simple ansatz we initially chose provides a robust lower bound for transition lengths (Fig. A4). In addition, the variation in $l^*$ is quite small across a range of ratios $\xi$, indicating the simple geometric ansatz with only one length scale is quite a good predictor of the nonlinearity. Therefore, we focus on this simplest case in the main text.

**Figure A4:** The transition length is minimum for a ratio of unity: For a single-cell ansatz, we allow the ratio $L_A/L_B$ to differ from unity. The transition points are plotted with respect to varying ratios for increasing $s_0$ values- 3.73 (red), 3.75 (green) and 3.81 (dark green).

**Comparison to disordered packings**

To compare our results on ordered systems to those in disordered systems, we investigate the onset of non-linearities in maximally disordered systems. We then study the properties of systems with shrinking T1 edges from 50 different initializations. As in most previous work in this field, we assume homogeneous line tensions. The disorder is introduced to the initial conditions in a standard way, used in both jammed
particle packings [25] and also in previous work on vertex models[67]. Specifically, we randomly uniformly distribute $N$ points on a 2D plane. Next, we generate the unique Voronoi tessellation of those $N$ points, which generates a random cellular network with 3-fold coordinated vertices. We then use standard minimization algorithms to find the local minimum for the vertex model energy functional that is closest to the initial condition in the potential energy landscape. During the initial equilibration process we use a higher $l_c = 0.15$ which allows the system to explore more states on the trajectory towards a local energy minimum. Once the system has arrived at a mechanically stable state, we start the same process of shrinking a random edge to a length as small as $l_c = 0.006$. Since this initial energy now is not necessarily zero for $s_0 > 3.722$, we look at the relative energy $\Delta E(l)$ from initial state at every edgelength. We bin every T1 edgelength into 40 bins. To look at the average trend of these profiles as a function of increasing shape, we average $\Delta E(l)$ for every bin. We have used the same color scheme as in previous plots, and so one can see that for the disordered case, the energy remains high at all values of $l$ throughout the entire range explored previously ($s_0$ in 3.71-3.83). For $s_0 > 3.83$, the average energy drops precipitously at an $l$ value smaller than the average. It is important to note that we have focused on average values in Fig. A5(a), but there are large fluctuations in edgelength due to the disorder, and the system will be unstable if any edge in the system can move at zero cost. Therefore, to find the $l^*$ for a given configuration, we should focus on the lowest $l^*$, not the average, as shown in Fig. A5(b).

For this energy profile, we use the same energy cut-off and identify the critical edgelength $l^*$ for an edge in every ensemble. We find that in general this ensemble exhibits a wide distribution of $l^*$s because disordered systems have a variety of edgelengths. Therefore, we represent this data using a box and whisker plot as shown in Fig. A5(b).
Figure A5: Many-cell disordered energy profile: (a) In a disordered system of 90 cells, a randomly chosen edge undergoes a T1 transition for 50 different initializations. In this process, the relative energy of the tissue, $\Delta E(l)$, is plotted against the shrinking T1 edgelength $l$ for increasing values of $s_0$ (3.71 to 3.95 in steps of 0.04) varying from red (3.71) to green (3.83) to blue (3.95). The cut-off for the energy is shown by a horizontal pale blue line for reference. (b) Critical edgelength $l^*$ plotted against $s_0$ is superimposed for both many-cell (yellow circles) and 4-cell systems (magenta circles). The analytical prediction from the geometric mechanism explained in the text is shown in blue dashed line. The dark green box and whisker plot in blue shows the $l^*$ distribution in disordered systems.
As previous work suggests that linear curvature does not vanish until approximately 3.81 for disordered systems, for \( s_0 < 3.81 \) one should expect the energy to grow as soon as the edge starts shrinking, so that \( l^* = l_0 \). As for \( s_0 > 3.81 \) the system is fluid so it should be possible for some edges to shrink to zero length at no energy cost, so that \( l^* = 0 \).

As shown in Fig A5, our data is in line with these expectations. For \( s_0 < 3.81 \), \( l^* \) is large and approximately equal to \( l_0 \), while for \( s_0 > 3.81 \), there are some edges for which \( l^* \) approaches zero, resulting in a near discontinuity in the plot.
Chapter 3

Small-scale demixing in a confluent mixture of two cell-types

*This chapter is based on work primarily presented in the article “Small-scale demixing in confluent biological tissues” [113] co-authored by Preeti Sahu, Daniel M. Sussman, Matthias Rübsam, Aaron F. Mertz, Valerie Horsley, Eric R. Dufresne, Carien M. Niessen, M. Cristina Marchetti, M. Lisa Manning and J. M. Schwarz, and published in the journal Soft Matter, in the year 2020. I carried out the simulations while Matthias Rübsam performed most of the experiments. We did the image analysis together. I was responsible for preparing the first draft of the paper, coordinating the collaboration meetings and my co-authors provided valuable suggestions and corrections to add both to the conceptual understanding, the writing of the experimental sections and the presentation of the work.*
3.1 Cell sorting in biology

Liquid-liquid phase separation, i.e., demixing, drives patterning. In materials science, demixing between two liquids is typically driven by the energetics of interfacial tension overcoming entropy-driven mixing [34]. By cooling a material, one can tune between a mixed state at high temperature and a demixed state at low temperature. Depending on the material and quench rate, this transition can occur continuously via spinodal decomposition or discontinuously via nucleation [35, 120]. In order to distinguish between mechanisms it is often useful to analyze the lengthscales of emergent patterns: nucleation and spinodal decomposition give rise to characteristic lengthscales that then coarsen, while in the absence of interfacial tension fluids will mix down to the scale of single molecules. These and related demixing phenomena have been studied numerically using multicomponent Lennard-Jones mixtures in which particles have a fixed shape and an interaction potential that depends on the distance between. The potential also energetically distinguishes between particles of different types to model interfacial tension [39].

In biology, demixing at the subcellular scale can lead to compartmentalization within cells [40], while in a developing organism, demixing can lead to compartmentalization among cells of different type, otherwise known as cell sorting. In fact, interfacial tension-driven demixing has long been invoked to explain cellular patterning. The first among such ideas is the Differential Adhesion Hypothesis (DAH), proposed by Steinberg in 1963 [121], to explain patterns in the spatial sorting of progenitor cells, such as ectoderm and mesoderm, during embryonic development. The DAH postulates that tissues behave like immiscible liquids composed of motile cells that rearrange in order to minimize their interfacial tension caused by differences in cell-cell adhesion. Building on the DAH, Harris [122], and later Brodland [123], have highlighted the
importance of other contributors to interfacial tension, including regulation of the acto-myosin cortex. There is an emerging consensus [55, 124–127] that adhesive molecules help to regulate cortical acto-myosin, which can strongly impact cell sorting. However, it remains controversial whether differential adhesion or differential cortical tension alone is sufficient to generate the level of cell sorting and compartmentalization observed in embryos and cell culture systems [18, 128–133]. Several experiments have suggested that additional processes such as specialized cell-cell signaling [130] or cellular jamming [18] enhance or disrupt sorting in living tissues.

One major difference between immiscible liquids composed of cells and immiscible liquids composed of soft spheres is that in the latter case, the particles have a distance-dependent interaction, while in epithelial layers and even some 3D tissues, the cells are confluent— they can change their shape to completely fill space—and so their interaction is shape-based. To reflect this property, confluent tissues have been studied theoretically and computationally using vertex or Voronoi models [73, 97, 134, 135], where cells are constructed from tessellations of space with no gaps between cells. As active fluctuations drive cellular rearrangements, cells must deform so that no gaps open up between them. This suggests cells are subject to strong geometrical and topological constraints. For example, in flat 2D tilings with three-fold coordinated vertices, the average number of neighbors must be precisely six. This constraint leads one to predict that a rigidity transition should occur when neighbor exchange between six-sided cells cost zero energy, i.e. when cells can form regular pentagons at zero cost [75, 136]. This prediction has since been realized in experiments [137] and is distinct from rigidity transitions in particulate systems [138, 139].

Does such an interaction potential with non-trivial geometrical and topological constraints affect the fundamental definition of surface tension? Work on bidisperse foams modeled as ordered vertex models demonstrate that, in equilibrium, demixed
cells of two different areas have a lower energy than a mixed system and so demixed states are energetically preferred \[46\]. However, disperse-in-area foams under large shear strain will mix \[45\]. If we think of the shear strain as a temperature-like variable, then these findings are similar to particulate systems.

On the other hand, recent work by some of us demonstrates that so-called heterotypic contacts in vertex models can drastically affect the notion of interfacial tension \[140\]. Heterotypic contacts, where cells recognize neighbors of a different cell type, can be modeled in two-dimensional vertex models with a higher or lower line tension along interfaces between cells of different types, or heterotypic line tension. Such a rule results in very sharp, yet deformable, interfaces \[140\] where surface tension measured by macroscopic deformation of an overall droplet shape gives a value in line with equilibrium expectations, yet, surface tension measured from interfacial fluctuations is at least an order of magnitude larger. This discrepancy is due to discontinuous pinning forces generated during topological rearrangements between cells of different types. That is, it is a consequence of the shape-based nature of the interactions.

Here, we explore the possibility of interfacial-tension-driven demixing in the absence of explicit heterotypic tension in both modeling and in experiments. From the modeling side, we consider a two-dimensional vertex model with two different cell types. Particulate mixtures can demix when a miscibility parameter, the ratio of the strength of the distance-dependent interaction between dissimilar particles as compared to similar particles, becomes less than one. Since in vertex models the interaction is shape-based, it is natural to ask if binary vertex model fluids consisting of mixtures of cells with different preferred cell shapes and/or sizes, accounting for differential adhesion, cortical tension or volume, demix even in the absence of specialized heterotypic interactions. In other words, is there an emergent effective
interfacial tension between two cell types that is sufficient enough to sort cells? Should the answer be yes, then one can imagine that the sorting of progenitor cells occurs very early on in the development process before robust heterotypic interfacial tensions are established. Should the answer be no, then cells must establish heterotypic interactions before sorting can occur, suggesting a more important mechanical role for cell recognition receptors than previously thought. The topological nature of the discontinuous pinning forces stabilizing interfaces in vertex model fluid mixtures tells us that once such recognition is in place, a finite active force is required to overcome the discontinuity [140]. Interestingly, a recent study with both in vitro experiments and cellular Potts model simulations suggests that a large heterotypic line tension is required for cell sorting [96], although the mechanism was left unresolved.

In searching for whether or not large-scale interfacial tensions and, therefore, cell demixing are emergent/collective properties of such binary vertex model fluid mixtures, we do not observe large-scale demixing. However, we do observe small-scale demixing in mixtures with differential adhesion, which is not thermodynamic in origin and which we term micro-demixing. We find that this behavior arises from dynamical trapping due to energy barriers to neighbor exchanges (T1 transitions) that depend on configuration of the type of cell. We then ask if the predicted phenomenon of micro-demixing can be realized in cellular systems.

To begin to answer this question, we experimentally study monolayers of mixtures of wild-type primary keratinocytes, denoted as Ctr cells, and primary keratinocytes in which the E-cadherin has been knocked down, denoted as E-cad−/− cells. Cadherins, such as E-cadherin and P-cadherin, are crucial components of adherens junctions (AJs) that couple intercellular adhesion to the cytoskeleton via α- and β-catenin [141], the former of which can interact directly with actin and other actin binding proteins. It been even more recently established that E-cadherin plays a central role in the
mechanical circuitry coordinating adhesion, contractile forces and biochemical signaling
to drive polarized organization of tension observed in stratified epidermal layers [101].
Given the central role of E-cadherin, E-cad−/− keratinocytes affect the mechanical
circuitry via, for example, decreased adhesion site lengths [101]. Since the wild-type
keratinocytes contain both P- and E-cadherin, two-dimensional mixtures of the wild-
type keratinocytes with E-cad−/− keratinocytes are ideal for testing whether or not
differential adhesion leads to large-scale demixing or not, even in the absence of
heterotypic tensions. In addition to the lack of large-scale demixing in the experiments,
we also find evidence for small-scale demixing as predicted in our two-dimensional
vertex model, further bolstering the use of this class of models as a predictor of tissue
rheology.

3.2 Computational modelling of binary mixtures

Cells are biomechanical (and biochemical) constructs that are not in equilibrium, i.e.
they are driven by active forces. Given our question of mixing, we study a confluent
monolayer of cells of different types. The biomechanics of the jth cell of type β is
given by the energy functional:

\[ E_{j,\beta} = K_a(A_{j,\beta} - A_{0,\beta})^2 + K_p(P_{j,\beta} - P_{0,\beta})^2, \]  (3.1)

where \( A_{j,\beta} \) denotes the jth cell area of type β and the jth cell perimeter of type β is
denoted by \( P_{j,\beta} \). Given the quadratic penalty from deviating for a cell’s preferred area
and perimeter, \( K_a \) and \( K_p \) are area and perimeter stiffnesses, respectively, and both
are independent of cell type. Physically, the area term represents the bulk elasticity
of the cell, while the perimeter represents the contractility of the acto-myosin cortex
with \( P_{0,\beta} \) denoting a competition between cortical tension and cell-cell adhesion. The
total energy of the tissue is then defined as \( E = \sum_{j,\beta} E_{j,\beta} \). An important parameter in these models is the dimensionless shape index \( s_{0,\beta} = P_{0,\beta}/\sqrt{A_{0,\beta}} \). A regular hexagon has a dimensionless shape index of \( s_0 \approx 3.72 \), for example.

To study binary mixtures, we fix \( \beta = 1, 2 \) and allow the cell types to have different parameters, \( A_{0,\beta} \) and \( s_{0,\beta} \) (see Fig. 6a). What is the biological implication of two different shape indices, for example? Consider two cell-types with the exact same area. The cell type that prefers to have a higher shape index, can do so by increasing the density of adhesion molecules, for example. Therefore, mixing these two differently adhering cell-types corresponds to studying mixing in cell-types with two different shapes. In reality, these adhesion receptors also affect the cell shape by signaling to either up-regulate or down-regulate contractility. In the vertex model energy functional that we use, we have packaged both adhesion and contractility into the preferred shape index. Though more detailed models are possible, we find that our minimal model does indeed tell us something about how cells behave as indicated by the experiments presented below. We will focus on cases of 50:50 mixtures where there is an equal number of each cell type, with either preferred shape disparity or preferred area disparity. Unless otherwise specified, the two components are uniformly distributed in the initial state. We set \( K_p \) to unity for all systems.

We study the above energy functional from an energy minimization perspective as well as from a dynamical perspective in which the cells migrate within the monolayer. As for the latter, there is still much debate about how to model the motility of cells. We have chosen to model the motility of a cell by imposing a random active force on each vertex, i.e. each vertex undergoes over-damped Brownian motion at a fixed effective temperature \( T \) with a conservative force contribution from the above energy functional and a second force contribution from a Brownian force. While there are other possible dynamical rules, we have found that, for example, the properties of an
interface between two cells types with heterotypic line tension between them is rather robust to the specifics of the dynamical rule \[140\]. The equation of motion for each vertex is then iterated until the cells can adequately explore the entire system such that the system approaches a steady state, at least for most parameters we study. As the cells move, they may rearrange and come into contact with new cells. Such rearrangements are known as T1 transitions. To implement a T1 transition, an edge shared between two cells undergoes a $\pi/2$ rotation once the length of this edge is below some threshold length. The rotated edge then lengthens and allows for two different cells to now share an edge. As for the energy minimization approach, in addition to comparing the minimum energy configurations of both demixed and mixed states, we will also compute the energy barriers associated with T1 transitions by constraining the length of a particular edge in the system such that a T1 transition occurs while allowing the remaining degrees of freedom in the system to relax. See the Methods section for more details.

We are also interested in comparing the behavior of these bidisperse systems to ones with an explicit heterotypic line tension (HLT), where cell types 1 and 2 recognize their joint interface as a heterotypic interface and, therefore, alter the line tension at that interface. Such interactions are common in cellular Potts models \[72, 96\] and have also been studied in vertex and Voronoi models \[97, 140\]. In this case, we add an extra term to the cell energy to arrive at:

$$E_{HLT} = \sum_{j,\beta} K_a (A_{j,\beta} - A_{0,\beta})^2 + K_p (P_{j,\beta} - P_{0,\beta})^2 + \gamma \sum_{\langle i,j \rangle} (1 - \delta_{\alpha\beta}) l_{ij}. \quad (3.2)$$

The latter sum is over all edges, $l_{ij}$, between cells $i$ and $j$ with $\delta_{\alpha\beta}$ representing a Kronecker delta such that there is additional line tension only between cells of two different types $\alpha$ and $\beta$. For simplicity, we assume that the additional tension, $\gamma$, is
the same for all heterotypic edges.

3.3 Computational results

3.3.1 Fluidity and demixing in shape bidisperse mixtures

To single out the effect of shape dispersity, we first vary the preferred shapes under the constraint that the preferred/target area is the same across cell types, $A_{0,1} = A_{0,2} = 1$.

Previous work on the vertex model has identified a regime in parameter space dominated by a coarsening instability [73, 135], where some cells shrink in size and others grow. We expect that heterogeneous $s_0$ values might amplify this instability, as heterogeneity amplifies differences between the cells. To prevent area dispersity from affecting the results in these mixtures, we choose $K_a = 100$, which is sufficient to reduce fluctuations in area $A$ from target area $A_0$ to a standard deviation of less than 1%, preventing the onset of the coarsening instability. Moreover, Fig. A1 shows that increased area stiffness does not significantly impact the fluidity of homogeneous tissues, as measured by the effective diffusivity (Eqs. 3.6-3.8), denoted as $D_{eff}$, which is the ratio of the diffusion constant in the presence of interactions to that in the absence of interactions. The onset of a finite effective diffusivity as a function of the shape index remains near $s_0 \approx 3.81$ with increasing $K_a$.

Next, we investigate how shape disparity affects the fluidity of the tissue. We find that the most effective way to represent the phase space of the two-component system with two shape indices $s_{0,1}, s_{0,2}$ is by the average value of the shape index, $s_{av} = (s_{0,1} + s_{0,2})/2$, and the shape disparity, which is the difference between the two values, $\Delta = s_{0,2} - s_{0,1}$ with $s_{0,2} > s_{0,1}$. Figure 6b is a heat map of the effective diffusivity of binary mixtures as a function of $s_{av}$ and $\Delta$. We see that there is a boundary between
fluid-like and solid-like, demarcated by the thick solid line, as determined by the $D_{eff}$ threshold. Interestingly, for $\Delta = 0.3$, this boundary does not match up with the fluid-solid boundary for the monodisperse case for the Brownian limit of a self-propelled Voronoi model at a similar temperature, which is near $s_0 = 3.81$ [75]. Moreover, the solid-fluid mixtures depicted by squares, have a fluid-like diffusivity above the boundary line. This indicates that the fluid-like species in the mixture are sufficient to fluidize the entire tissue, which is additionally confirmed by analyzing the diffusivities of each component (Fig. A2a).

![Figure 6: Vertex model binary mixtures. (a) Schematic of vertex-based modeling of a tissue: A typical tessellation with two different types of cells highlighted. The energy depends only on a cell’s perimeter ($P_j$) and area ($A_j$). (b) A heat map of $\log_{10} D_{eff}$ as a function of $\Delta$ on the x-axis and $s_{av}$ on the y-axis. The phase points with: fluid-fluid ($s_{0,1}, s_{0,2} > 3.81$), solid-fluid ($s_{0,1} < 3.81$) and solid-solid ($s_{0,1}, s_{0,2} < 3.81$) components are denoted by circular, square and star-shaped markers, respectively. Black-filled markers, demarcated by a solid black line, denote mixtures with a $D_{eff}$ less than that of the chosen cutoff of 0.01. Region above this line denotes fluid-like behaviour on average.](image)

After understanding how $\Delta$ affects diffusivity in a mixture, let us now understand its role in bulk demixing for a fixed $s_{av} = 3.85$. A snapshot of a typical long-time configuration for such a mixture is shown in Fig. 7a. By eye, it appears that demixing does occur at very small scales, due to some clustering of the cells with larger $s_0$. The system maintains this small-scale structure at long times. No large-scale demixing
is observed. Hence, we shall refer to this process as *micro-demixing*. To quantify micro-demixing and highlight its long-time steady state, we study three observables.

The first is the demixing parameter $DP$, which measures the average environment of each species, quantifying whether it is more likely to be surrounded by similar (homotypic) or dissimilar (heterotypic) cells. Defining $N_s$ as the number of similar neighboring cells and $N_t$ as the total number of neighboring cells,

$$DP = \langle DP_j \rangle = \left\langle 2\left(\frac{N_s}{N_t} - \frac{1}{2}\right)\right\rangle,$$

where the brackets denote averaging over all cells in the tessellation. In a completely mixed state, $DP = 0$, whereas in a completely sorted mixture, $DP = 1$, in the limit of infinite system size.

The demixing parameter as a function of time is shown in Fig. 7b. The value of $DP$ is initially zero since the two cell types are initially seeded at random, and saturates to a small non-zero value at long times. The final steady state value, $DP_f$, increases with increasing shape disparity $\Delta$, as shown in the inset to Fig. 7b, and the length of time required to reach the steady state also increases with increasing $\Delta$ (Fig. A2(b)). For comparison, the dashed black line in Fig. 7b illustrates the demixing parameter as a function of time for a model with heterotypic line tension. In the HLT case, $DP$ rises very quickly to a value close to unity as one species rapidly forms a circular droplet, in a manner similar to that expected for conventional liquid-liquid binary mixtures.

We then measure the average cluster radius $R$ by quantifying the average radius of gyration of the dispersed component. In the case of shape bidisperse mixtures, the more fluid-like (larger $s_0$) component tends to be dispersed. The average cluster radius (Fig. 7c) shows a small growth in time, which appears to saturate at long times, although the data is noisier given the cluster statistics sampling rate. The steady
state radius tends to increase with increasing $\Delta$. In all cases studied, clusters have an average radius of less than $2.5 \pm 0.2$. For comparison, the dashed line shows a system with HLT, which we expect to saturate as a nearly circular droplet of one species embedded inside the other species. For the system size we study, this would correspond to a cluster of radius 8, which is close to the observed steady state value of $7.2 \pm 0.2$.

To further quantify the structure of this micro-demixed state, we study the pair correlation function, $g(r)$, which describes the normalized probability of finding a cell center a given distance from another cell center. In homogeneous fluids and amorphous solids, this function exhibits short range order with peaks occurring at distances that are integer multiples of the typical spacing between two cells. The envelope of these peaks falls off with distance and eventually approaches unity, highlighting that these materials are disordered over larger lengthscales. In bidisperse mixtures, we compute the correlation between each species $\beta$ separately, defined by the relative position vectors $(r^{(\beta)})$ between two cells of type $\beta$:

$$g_{\beta\beta}(r) = \frac{1}{2\pi r N_{\beta} \rho_0} \sum_i \sum_j \delta(r - r^{(\beta)}_{ij}).$$

(3.4)

For a completely sorted mixture, $g_{\beta\beta}(r)$ should exhibit an envelope that falls off exponentially, with a length scale $\xi$ that corresponds to the average cluster radius. In the HLT mixtures, where a single droplet forms, we see such a structure, as shown by the dashed black line in Fig. 7d. We extract a length scale of $\xi_{HLT} = 4.5 \pm 1.2$, which is very similar to the steady state average cluster radius shown in Fig. 7c. For comparison, we measure $\xi$ for all shape bidisperse mixtures and compare it to $\xi_{HLT}$ by computing $\xi/\xi_{HLT}$ (see inset to Fig. 7d). We find this ratio to be quite small, consistent with previous results.
Figure 7: Shape bidisperse fluid mixtures. (a) Snapshot of a $P_{av} = 3.85$, $\Delta = 0.4$, $N = 1600$ mixture. Scale bar denotes 10 length units. Yellow is used for solid-like cells ($s_0 = 3.65$) and blue for liquid-like ones ($s_0 = 4.05$). (b)-(d) Various quantifications of demixing in shape bidisperse mixtures (curves colored from green to red in increasing order of shape disparity i.e. $\Delta = 0.01, 0.1, 0.2, 0.3, 0.4$) are compared to a mixture with an extra heterotypic line tension of value 0.1, $s_0 = 3.97$ (black dashed curve). (b) Demixing Parameter versus log(time). The final value ($DP_f$) as a function of $\Delta$ is shown in the inset. (c) Average cluster radius (R) versus time. (d) Pair correlation function of high-$s_0$ cells ($g_{22}$) versus radial distance for $t = 200 \tau_0^s$. The dashed grey line shows an exponential decay. The inset shows the decay lengthscale ($\xi$) in terms of the maximum possible lengthscale ($\xi_{HLT}$) with increasing disparity $\Delta$. Simulation details provided in Table S1.
3.3.2 Fluidity and mixing in area bidisperse mixtures

After studying the impact of shape disparity in cell sorting, we next study the effect of dispersity in area. The mixture is now comprised of equal numbers of cells with $A_{0,1} < A_{0,2}$, where we take $\sqrt{A_{0,1}}$ as the unit of length. Both types have the same $s_0$, or $s_{0,1} = s_{0,2}$. We have taken care to ensure that our area bidisperse mixture are also in a fluid region of the phase diagram (Fig. A1b) by checking that $D_{\text{eff}} > 0.01$. For the results shown here, the shape index is fixed at $s_0 = 3.85$ to mimic fluid-like cells. We define the ratio of the preferred areas as $\alpha = A_{0,2}/A_{0,1}$.

Visual inspection of a snapshot from a simulation of an area bidisperse mixture with high $\alpha = 2.5$ at long times demonstrates that observing cluster formation by eye is difficult, particularly given the disparity in area fraction between the two cell types (see Fig. 8). The DP has been measured and is smaller than those found in shape bidisperse mixtures (Fig. 7b). Since the large-$A_0$ cells occupy more than half of the total area, we perform our cluster analysis on cells with $A_{0,1}$. As shown in Fig. 8c, the final clusters have an average cluster radius that is typically less than two cell diameters and becomes smaller as $\alpha$ increases. Similarly, Fig. 8d illustrates that $g_{ss}(r)$ also shows no sign of bulk demixing, with a structural length scale that is always less than $0.2 * \xi_{\text{HLT}}$, and decreases with decreasing $\alpha$, as seen in the inset to Fig. 8d.

3.3.3 Zero-temperature energy configurations

Our finite-temperature simulations suggest that large-scale sorting is not preferred in these mixtures. To understand this, we study an ensemble of energy minimized states. If the mixed state has a lower energy at zero temperature, then we expect that energetics cannot drive demixing at finite temperature. Therefore, we compare the energy of two initial states of $N = 400$ cells: a sorted system where all of the cells with cell centers in the left half of the box are labeled type 1, and the remainder
Figure 8: *Area bidisperse fluid mixtures.* (a) Snapshot of a $s_0 = 3.85$, $\alpha = 2.5$, $N = 1600$ mixture. Scale bar denotes 10 units. Yellow is used for larger cells ($A_0 = 1.43$) and blue for smaller ones ($A_0 = 0.57$). (b)-(d) Various quantifications of demixing in area bidisperse mixtures (curves colored from green to red in increasing order of size disparity i.e. $\alpha = 1.0, 1.5, 2.0, 2.5$) are compared to a mixture with an extra heterotypic line tension of value 0.1, $s_0 = 3.97$ and $A_0 = 1.0$ (black dashed curve). (b) Demixing Parameter versus log(time). The final value ($DP_f$) as a function of $\alpha$ is shown in the inset. (c) Average cluster radius ($R$) versus time. (d) Pair correlation function of small-$A_0$ cells ($g_{ss}$) versus radial distance in units of the smallest lengthscale for $t = 200\tau_0^0$. The dashed grey line shows an exponential decay. The inset shows the decay lengthscale ($\xi$) in terms of the maximum possible lengthscale ($\xi_{HLT}$) with increasing disparity $\Delta$. Simulation details provided in Table S2.

are labeled type 2, and a mixed system where cell types are randomly assigned. We use FIRE minimization [142] to identify the nearest local energy minimum for 250
realizations in each of the two scenarios.

Figure 9a shows the ratio between the energy of states with sorted initial conditions ($E_s$) and mixed initial conditions ($E_m$) in the case where type 1 and 2 cells have different shape parameters. At larger system sizes, there is a clear trend that the sorted states typically possess higher energies than the mixed states, so that the ratio rises above unity as the shape disparity $\Delta$ increases. This indicates that there are no energetic forces driving the demixing in larger systems. We have also quantified the effective interfacial line tension (Fig. A4, Fig. A5) using a method developed previously by some of us [143]. We find that there is no emergent line tension in any of these mixtures, which is consistent with our energy calculations. In Fig. 9b, which shows the ratio of energies between sorted and mixed states for cells with area dispersity, the trend is even clearer. Again, sorted states have significantly higher energy compared to mixed states as the area dispersity $\alpha$ increases.

3.3.4 Zero-temperature T1 energy barriers.

Although the zero temperature energy calculations above help us understand the lack of macroscopic demixing in mixtures with no heterotypic interfacial tension, they do not explain the small-scale demixing seen, for example, in Fig. 7b. Since both cell types are subject to the same geometrical and topological constraints and rearrange via T1 transitions, we now turn to an energetic analysis of T1 transitions for the bidisperse system.

Specifically, we study the statistics of energy barriers in bidisperse systems, where there are nine types of T1 transitions possible. While we present data in the Appendix for symmetric cases where two of the cells are of type 1 and two of are type 2, we focus here on asymmetric systems where 3 of the cells are of one type and one is of another type. As illustrated by the 4-cell cluster diagrams in Fig. 10, such 3:1 arrangements
Figure 9: Minimal energy configurations. Systems with $N = 100, 400, 900$ cells (green to black) are energy minimized using the FIRE algorithm to get the total energy of the configurations- mixed($E_m$) and sorted($E_s$), for a $s_{av} = 3.85$, $K_a = 100$ with increasing disparity. The ratio $E_s/E_m$ versus $\Delta$. (b) The ratio $E_s/E_m$ is plotted versus $\alpha$ for $s_0 = 3.85$ and $K_a = 1$. Simulation details provided in Table S3.

naturally represent the cost of one cell type invading an interface composed of cells of a distinct type, which determines the dynamic stability of such an interface.

Similarly to previous work [75, 136], we compute the T1 energy barrier height by measuring the global tissue energy as we force a single edge in our bidisperse simulation to shrink to zero length while minimizing the energy and allowing the other degrees of freedom to relax, as shown in Fig. 10a. The energy barrier $E_b$ we report in Fig. 10b is the difference between the final energy $E(l = 0)$ at the 4-fold vertex and the initial energy $E_0$, or $E_b = E(l = 0) - E_0$, averaged over 250 edges with the same topology in small simulated tissues with $N = 80$ cells.

Figure 10a illustrates a particular type of (3:1) T1 energy profile where a single cell with shape parameter $s_0^{\text{1}}$ invades a 3-cell cluster formed by cells with $s_0^{\text{cluster}}$. We define a signed shape disparity $\Delta_{\text{sign}} = s_0^{\text{1}} - s_0^{\text{cluster}}$ to distinguish it from a T1 with
cell types swapped. Negative $\Delta_{\text{sign}}$ indicates that a more stiff cell is invading a cluster of floppy cells. We have checked that energy barriers are statistically identical for cells entering or exiting a cluster. Because cells are as likely to leave a cluster as to enter it, this suggests that clusters of a given cell type will not grow or shrink over long-time or length-scales.

Figure 10b highlights that the energy barriers associated with these (3:1) transitions systematically increase as the magnitude of the shape dispersity $\Delta_{\text{sign}}$ increases. In other words, it becomes energetically more difficult for a single cell to invade or leave a cluster of a different cell type as the shape dispersity between the two types increases. Perhaps more importantly, it also shows that these energy barriers are not symmetric around zero; there is a systematic difference between a stiffer cell invading a floppier cluster and vice-versa, especially for lower values of $s_{av}$ as the system approaches the jamming transition. Stiffer clusters tend to be more difficult to break up than floppier clusters. To characterize this effect, we define the energy barrier disparity between invading stiffer and floppier clusters as $\delta E_b(\Delta) = E_b(\Delta) - E_b(-\Delta)$.

To test whether this mechanism might be relevant for the micro-demixing we observed in our finite-temperature simulations, we directly compare the demixing parameter associated with the final, steady state in each simulation, $DP_f$ to the energy barrier disparity $\delta E_b$ as a function of shape dispersity $\Delta$, as shown in Fig. 10c. This plot shows a quite strong correlation between the two quantities, suggesting that this mechanism is a very likely driver of micro-demixing. To further test this idea, we have increased the temperature for the $\Delta = 0$ mixtures and found $DP$ to vanish at temperatures higher than the differential energy barrier, as shown in Fig. A3.

A similar analysis can be performed for area bidisperse mixtures as shown in Fig. A6. An important difference from the shape bidisperse case is that while there is a clear connection between cell shape and tissue rheology (stiffer cells have a smaller
Figure 10: *Differential energy barriers in shape bidisperse fluid mixtures.* (a) Energy $E(l)$ relative to $E_0$ is plotted against T1 edgelength $l$ for a typical shape bi-disperse T1 pair ($\Delta = 0.4, s_{av} = 3.85$). (b) Energy Barrier $E_b$ is plotted against signed disparity in shape $\Delta_s$. Positive and negative $\Delta_s$ values imply stiffer cluster in yellow and floppier cluster in blue respectively, in the 4-cell diagrams show above. Each solid curve represents the barrier for a heterotypic cell to get out of the cluster for $s_{av} = 3.79, 3.85, 3.88$ (from solid-like (orange) to liquid-like (green)). (c) Correlation plot for $s_{av} = 3.85$ between Differential Energy Barriers on the right y-axis $\delta(E_b)$ (in maroon triangles) and demixing relative to mixed scenario $DP_f$ on the left y-axis (in black discs). Shape difference $\Delta$ is plotted on x-axis. Simulation details provided in Table S4.

$s_0$, there is no such connection between area and rheology. Moreover, there is very little evidence for micro-demixing, and so we expect the signal to be much weaker. Nevertheless, we can define a quantity $\alpha_s = A_{0}^{\text{cluster}}/A_{1}^{\text{target}}$ that is less than unity if a larger cell is invading a cluster of smaller cells and greater than unity otherwise. Fig. A6b suggests that large-cell clusters are more difficult to invade than small-cell clusters, although the differential energy barrier is quite a bit smaller than for the case of shape bidispersity. In particular, the case where $s_0 = 3.85$ is highlighted in
Fig. A6c showing a correlation between demixing and $\delta E_b$, although the amplitude of both effects is quite small.

### 3.4 Experimental results

To test our modeling against experiments, we first study monolayers of primary keratinocytes (Ctr) in the presence of high calcium, whose presence initiates intercellular junction formation (see Fig. 6a). Under such conditions, the monolayer is confluent in the sense that there are essentially no gaps between cells. We also test whether the confluent monolayer is fluid-like by measuring the displacement of cells over the course of 24 hours. While some number of neighbor exchanges indeed take place, and while the integrated displacement of the cells is several times a typical cell length, we find that the mean square displacement of these cells is typically of the order of a single cell size (Fig. S10a). This places some limitations on the scale of demixing that is expected, but we nevertheless see a level of micro-demixing that is comparable to what we observe in our simulations (Fig. A6e).

Since the shape index is an important parameter in our theory, we measure this quantity for Ctr cells in the monolayer and obtain an average shape index of $4.08 \pm 0.06$. The full histogram is plotted in Fig. 6b. Confluent monolayers of primary keratinocytes but with E-cadherin knocked-out, or E-cad$^{-/-}$ cells, again in the presence of high calcium, are then studied to check for confluency (Fig. 6a) and fluidity (Fig. S10a). We then measure an average shape index of $4.38 \pm 0.14$ for the E-cad$^{-/-}$ cells (Fig. 6b). A T-test reveals that the difference in the two shape index histograms is statistically significant with a P-value of 0.0008. The difference in the shape index corresponds to $\Delta = 0.31$. Since we explore both differential adhesion and differential size, we also measure the areas of each cell type in the monolayer and found no statistically
significant area difference. See Fig. S10(b). In other words, the monolayer mixture tests differential adhesion, as opposed to both differential adhesion and bidisperse areas.

Next, we study the monolayer of approximately a 50:50 Ctr/E-cad$^{-/-}$ mixture in the presence of high calcium over the course of 24 hours. Again, a major complication in our comparison is that while both types of cells in the mixture exert active forces on their environment the typical displacements over this time frame are small. Nevertheless, after constructing a Voronoi tessellation for snapshots of the monolayer taken every hour, we measure the demixing parameter (DP) for each cell type, accounting for the fact that the mixture is not precisely a 50:50 mixture (see Fig. S10c). In doing so, we measure number of neighbors for each cell type and subtract off the corresponding number fraction. Figure 6e shows the DP parameter as a function of time for the E-cad$^{-/-}$ cells in the mixture. This parameter increases from zero (within one standard deviation) and appears to saturate after approximately 19 hours to around 0.15, albeit with some fluctuations. Such values of the DP parameter are consistent with our computational observations of micro-demixing in the differential adhesion case.

We argue that these results suggest the small-scale demixing is a consequence of large differences in differential adhesion. To rule out this being a consequence of the natural variability in adhesion even within one cell type, we also measure the demixing parameter in monolayers of just Ctr cells and of just E-cad$^{-/-}$ cells by staining half of the cells with one type of stain and the remaining half with a second type of stain (checking for an artificial “demixing” due to variability in these two monotypic monolayers). We find that the demixing parameter does not increase over time on average for either the Crt cells or the E-cad$^{-/-}$ cells when looking at initially well-mixed regions (Fig. 6e) or the entire monolayer (Fig. S11a). In addition to the DP, we also compute the pair-correlation function for the experimental system.
Figure 11: Micro-demixing observed in keratinocyte co-cultures. (a) Wild-type (Ctr) and E-cadherin knockout (E-cad\(^{-/-}\)) cell monolayer mixtures with nuclei labelled using immunofluorescence. (b) Histograms of cell shapes for Ctr and E-cad\(^{-/-}\) cells are compared across seven and six different isolates respectively, i.e. seven different mice. There is a clear difference in the shape index (\(\Delta = 0.31\)) of both cell-types, with shape indices of 4.08 ± 0.06 for Ctr cells and 4.38 ± 0.14 for Ecad\(^{-/-}\) cells. (c) Both cell-types, Ctr in yellow and E-cad\(^{-/-}\) in blue, start off initially mixed as shown the 0h (zero hours) snapshot. The co-culture evolves into a micro-segregated mixture, as shown in a 24h snapshot. (d) Voronoi tessellations (VT) of the same snapshots. (e) Solid maroon curve represents the time evolution of demixing parameter for the E-cad\(^{-/-}\) cell-type in the mixture as a function of time and averaged over five different isolates. This result is compared against a control of demixing curves for some initially well-mixed regions of monolayers of either all Ctr cells or all E-cad\(^{-/-}\) cells but with half the cells stained differently than the other half. Well-mixed regions of the control cells are shown in yellow, while the well-mixed regions of the E-cad\(^{-/-}\) cell-type is shown in blue.
and find that while it contains less structure than the simulations, it also exhibits a correlation length over several cell diameters (see Figs. S11b and c).

While the qualitative and semi-quantitative comparison between the degrees of micro-demixing observed in experiments and vertex model simulations is promising, we must also acknowledge that there are several differences between the two settings. Taking into account such differences and determining how the micro-demixing is potentially affected is a future avenue for investigation. For instance, our computations so far focus on 50:50 mixtures and do not take into account the potentially persistent motion of cells. Another difference is the apparent timescale over which the micro-demixing occurs from various initial conditions: in the experiments the demixing seems to occur during a time in which the cells move not much more than a typical cell size; in contrast our simulations require many $\tau$ to reach comparable levels of demixing from a random initial configuration. Additionally, while we expect the differential energy barriers to remain at least for some range of persistence, the value of the DP parameter will depend on that just as the steady state value of the DP parameter depends on temperature in our over-damped Brownian simulations. Further differences may include the effect of differential motility, differential mechanical stiffnesses of the cells, and differential cell division and death (which can itself affect the diffusivity of cells [144]). Therefore, to more rigorously test the computations against the experiments, future experimental work with detailed cell tracking within the monolayer and the prevention of cell birth with the introduction of mitomycin, as well as additional computational work, needs be implemented.
Methods and Materials

Simulation details: We simulate a vertex model where the degrees of freedom evolve according to over-damped Brownian dynamics \[145\]. Specifically, each vertex \( i \) located at coordinate \( \mathbf{r} \) experiences a Brownian force \( \mathbf{F}^B \) with \( \mathbf{F}^B_i = \xi_i \), where \( \xi_i \) is white noise with zero mean and \( \langle \xi_{\gamma i}(t)\xi_{\lambda k}(t') \rangle = 2T\delta_{\gamma \lambda}\delta_{ik}\delta(t - t') \) with \( \gamma \) and \( \lambda \) denoting spatial components and in units of \( k_B \) equal to unity. In epithelial layers, we expect that fluctuations are driven by active cytoskeletal components, and hence the \( T \) is an effective temperature that represents the magnitude of this activity (Ref. \[146\]). The equation of motion for a single vertex, therefore, takes the form

\[
\dot{\mathbf{r}}_i = \mu \mathbf{F}_i + \mu \mathbf{F}^B_i,
\]

with \( \mathbf{F}_i = -\nabla_i E \), where \( E \) is the total energy as defined in the Model section. The force \( \mathbf{F}_i \) is a non-local effective mechanical force experienced by the \( i \)th vertex of the \( j \)th cell and hence represents the cell-cell interactions. In the absence of mechanical interactions, an isolated cell performs a random walk with a long time effective diffusion rate of \( T/\mu \). Unless otherwise specified, \( \mu = 1 \). Finally, the Euler-Murayama integration method is used to update a discretized version of the equations (one for each vertex). One simulation unit time is referred to as \( \tau \). For a system with no dispersity, a cell with a shape parameter \( s_0 \) of 3.85, typically requires 1000\( \tau \) to move its own length, i.e. \( \tau^0_s = 1000\tau \). We typically simulate up to times several hundred times greater than \( \tau^0_s \). We also note that other models with directed cell motility are possible, including an Active Vertex Model \[147\] and the SPV model where cells are self-propelled due to an active force with persistence \[75, 97\].

In vertex models, one needs to take care of cellular rearrangements explicitly \[73, 75\]. In the absence of cell division or death, such rearrangements correspond to T1
transitions in which one edge shrinks to zero length and two new cells are connected via a new growing edge. In simulations, if an edge length falls below some threshold length \( l_c \), then we rotate the edge by \( \pi/2 \) and reconnect the topology of the surrounding cells to generate a local neighbor-exchange. Unless otherwise specified, \( l_c \) is set to 0.04. The noise is controlled by temperature (T) which is set to 0.01.

Past work has demonstrated that the mechanical properties of vertex models depend sensitively on the shape parameter \( s_0 \) and temperature \( T \). Specifically, these models exhibit rigidity [136] or glass [148] transitions where the system transitions from more solid-like to more fluid-like. At \( T = 0 \), the 2D vertex model exhibits a rigidity transition as a function of cell shape parameterized by \( s_0 \). Above a critical value of target shape index \( s_0^* \sim 3.81 \), cells are able to move past each other with very small energy cost and below which they cannot. To understand this transition, one analyzes the energetics of how cells move past each other via T1 transitions. A minimal four cell calculation with fixed unit area hexagonal cells revealed that if the two cells that would no longer share an edge after the edge swap formed regular pentagons, then the energy barrier for the formation of a four-vertex vanishes, suggesting that pentagon shape formation is a geometrically compatible transition pathway for three-fold coordinated lattices. Interestingly, the shape parameter for a regular pentagon is \( s_0 = 2\sqrt{5}(5 - 2\sqrt{5})^{1/4} \approx 3.812 \approx s_0^* \). In the presence of activity or temperature, vestiges of this zero-temperature rigidity transition have been found in a glassy transition between fluid-like and more solid-like behavior in an active Self-Propelled Voronoi (SPV) model [75] and a Brownian Voronoi model [148].

Given the complex phase behavior of such vertex models, we want to ensure that the mixtures are fluid-like. To do so, we first measure the Mean-Squared Displacement (MSD). To account for global tissue motion possible in these types of models [149], we define the displacement of each cell in a time window \( t \), \( x(t) \), as the distance the cell
traveled in time $t$ minus the total displacement of the entire system of cells over that same time interval. Then the MSD is defined as

$$MSD(\Delta t) \equiv \langle (x(t + \Delta t) - x(t))^2 \rangle,$$  \hspace{1cm} (3.6)

where $\langle \cdot \rangle$ denotes an average over all cells in the tissue and all times $t$. The self-diffusivity $D_s$, is defined by assuming the long-time behavior of the system is diffusive,

$$D_s = \lim_{t \to \infty} \frac{MSD(t)}{4t}.$$  \hspace{1cm} (3.7)

To understand whether cells are being constrained by their neighbors, we compare $D_s$ to the bare, or non-interacting, diffusion constant $D_0$. For a non-interacting Brownian particle at temperature $T$ with mobility $\mu$, the Fluctuation-Dissipation theorem states that $D_0 = \mu k_B T$, where $k_B$ is Boltzmann constant. We set $\mu k_B$ to unity. The effective diffusivity is given by

$$D_{eff} = \frac{D_s}{D_0}.$$  \hspace{1cm} (3.8)

Systems with small $D_{eff}$ are more solid-like, while systems with large $D_{eff}$ are more fluid-like. In practice, we use a threshold of 0.01 to distinguish between these different behaviors, in line with previous work [75].

Self-diffusivity time $\tau_s$ is defined as the time taken by a cell to move its own length. For a 2D system, one can use Eq:3.7 to compute $\tau_s = 1/4D_s$. Dispersity in the system can affect this average motion. Hence we convey time in units of $\tau^0_s$ which we define as the self-diffusivity of cell, with $s_0$ of 3.85, in absence of any dispersity. To study micro-demixing, we run simulations that are 200 $\tau^0_s$ long i.e. long enough for cells to explore the entire system multiple times.
Using Brownian vertex model simulations, we show that two-dimensional mixtures, bidisperse in preferred shape and in preferred area, have robust fluid-phase mixing at large scales in the absence of an explicit heterotypic line tension distinguishing between the two cell types. Energy minimization at zero temperature further supports this finding: mixed systems have lower energy than sorted ones, so that bidispersity is not sufficient to energetically stabilize an interface between the two fluids. For shape bidisperse mixtures, we find that, in spite of having solid-like cells making up half the mixture, the mixtures are still able to fluidize in some parameter regimes of the vertex model. Furthermore, although this large scale mixing occurs, we find persistent and equally robust micro-demixing in shape bidisperse mixtures, where clustering of the same cell type over sub-system-spanning length scales is observed.

To understand micro-demixing in shape bidisperse mixtures, we establish a correlation between micro-demixing and zero-temperature differential energy barriers for neighbor exchanges (T1 transitions) between four cells at the heterotypic boundaries. Specifically, we find that the energy barriers for a fluid cell type to “invade” a cluster of stiff cells is typically higher than for a stiff cell to “invade” a cluster of fluid cells. This difference in energy barriers creates a bias towards the small-scale clustering of stiffer cells. For area bidisperse systems, the differential energy barriers for neighbor exchanges are smaller than for the shape bidisperse case, and we find a negligible amount of micro-demixing. Our differential energy barrier calculations at zero temperature also yields a prediction for the temperature above which the micro-demixing does not occur—a prediction that has indeed been verified in our simulations.

The computational observation of robust mixing on large scales for both types of mixtures may be surprising, given that the shape-based interaction distinguishes between the two cell types just as changing the strength of the distance-dependent interaction between two particles of different types in thermal Lennard-Jones mixtures.
In the particulate case, there is either large-scale demixing or no demixing (depending on the miscibility), while in the cellular case, there can be micro-demixing. This suggests that vertex models may be more relevant for characterizing cell sorting in dense cellular mixtures than other coarse-grained modeling approaches.

What about comparisons with athermal particle systems? Athermal two-dimensional bidisperse particulate mixtures of different size discs with purely repulsive forces, such as models for granular particles with no (or little) friction, are not expected to sort at small size disparities [42]. Only as the size dispersity increases does sorting occur due to entropic depletion forces [43]. Entropic depletion forces do not apply to a confluent tessellation in which the packing fraction is fixed at unity, though may to some extent apply to Voronoi models. Depletion forces also drive demixing in vertical vibrated shape bidisperse mixtures of rods and spheres [44]. Interestingly, size bidisperse mixtures of active particles can sort in the absence of any attractive forces [61]. The sorting here is due to an asymmetry in the energy barrier between one smaller particle passing through two larger particles as compared to one larger particle passing through two smaller ones. Given the above analogy, a vertex model fluid mixture perhaps has more in common with an active, disordered binary packing than with a thermal fluid mixture with differential adhesion.

How different is the vertex model examined here applied to cells in comparison to the vertex models applied to foams? The robust mixing observed in area bidisperse systems at zero temperature is indeed counter-intuitive when compared with area bidisperse foams in ordered hexagonal states [46]. In this case, the system demixes at zero temperature given an additional perturbative energetic cost to an interface between cells of slightly different areas. Only for large applied shear strains do area bidisperse foams mix [45]. Understanding differences between foam and vertex models is therefore an interesting area for future study. Foam models lack the $P^2$ contribution
to the energy functional (Eq.1.3) and this restricts the fluid-like phase space accessible to such models, perhaps contributing to differences between them.

To determine whether or not our micro-demixing prediction is directly relevant for biology, we conduct experiments with cellular monolayers consisting of both wild-type keratinocytes and E-cadherin-knock-out keratinocytes. Such mixtures allow us to study differential adhesion and its effect on cell sorting in the absence of heterotypic tensions. We find evidence for micro-demixing with a saturated demixing parameter that agrees with our prediction to within one standard deviation of the experimental value. Moreover, we do not observe large-scale demixing over the time scale of the experiment. Over longer time scales, the monolayers gradually become multi-layered, an effect we have yet to incorporate into our computational modeling of confluent cellular mixtures.

In addition to cell persistence, differential motility, and cell birth/death, we also have yet to explore the effects of small lapses in confluency, which could arise given the lack of E-cadherin in the modified cell type. Such exploration will allow for even more rigorous quantitative comparison between the modeling and the experiments.

Our computational and experimental results bring an understanding to earlier work demonstrating that sorting at embryonic boundaries requires high heterotypic interfacial tension [96]. Given our T1 energy barrier analysis encoding both the topological and geometrical constraints of confluent packings, we now understand why these mixtures robustly mix. This robustness suggests that despite some difference in shape and size, progenitor cells can readily mix throughout the embryo. To demix (or sort), progenitor cells have developed biochemical means of recognizing whether neighboring cells are of the same type or a different types. And while a small amount of heterotypic line tension can generate stable interfaces [140] in the absence of fluctuations, correlated fluctuations may be able to overcome such barriers. Our analysis gives a new way to understand bulk behavior based on cellular rearrangements.
in such confluent mixtures. In other words, based on the analysis of T1 energy barriers between different cell types, experimentalists can predict whether or not different cell types will mix or not mix in the bulk.

Finally, the micro-demixing effect observed both in our computations and in our experiments could be utilized in biology to create more subtle patterning. For instance, when randomly tagging a tessellation half with one cell type (and half with another cell type), one of the cell types percolates through the system [150]. However, if there is now some spatial correlation in the tagging introduced even at the small scale, such that the tagging of one cell type is positively correlated with tagging a neighboring cell of the same type, then the percolation transition point can be altered, transitioning from a tenuous spanning structure to one that is more robust and more able to respond to changes in the environment.

3.5 Appendix

Simulation Parameters

Here we provide tables for the parameters used for each aspect of the simulations. For our dynamical simulations, the systems are equilibrated for time, $t_{eq}$, and subsequently run for a longer time. For our FIRE simulations, simulations typically run until the maximum force experienced by a vertex reduces below a threshold value of $10^{-13}$.

Effect of area stiffness on fluidity

High shape-disparity can amplify coarsening in mixtures, resulting in further enhanced disparity in cell areas. To prevent this coarsening from occurring, we increase the area stiffness $K_a$ to 100. To make sure this does not affect the fluid phase seen in monodisperse mixtures, we study the effective diffusivity as a function of the target
shape parameter for several $K_a$ values. We find that $K_a$ barely affects the diffusivities and that the large changes in curvature of $D_{eff}$ versus $s_0$ remain close to 3.81 such that larger values of $K_a$ do not significantly affect the fluidity of the cells. See Fig. A1.

**Diffusivity of area bidisperse mixtures**

Monodisperse systems with $s_0 > 3.81$ have a fluid-like diffusivity. Here we check diffusivity for mixtures having the same $s_0 = 3.85$ for all cell types but bidisperse in size. We see that the average fluid-like diffusivity remains unchanged. See Fig. A1b.
Table A3: FIRE minimization for $E_s/E_m$

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Ensembles</td>
<td>250</td>
</tr>
<tr>
<td>2. $s_{av}, s_0$</td>
<td>3.85</td>
</tr>
<tr>
<td>3. $\alpha$</td>
<td>1.0-2.5</td>
</tr>
<tr>
<td>4. $\Delta$</td>
<td>0-0.12</td>
</tr>
<tr>
<td>5. $dt$</td>
<td>0.01</td>
</tr>
<tr>
<td>6. $K_a$</td>
<td>$1(\alpha) &amp; 100 (\Delta)$</td>
</tr>
<tr>
<td>7. $T$</td>
<td>0.01</td>
</tr>
<tr>
<td>8. $N$</td>
<td>100,400,900</td>
</tr>
<tr>
<td>9. Maximum FIRE steps</td>
<td>$10^5$</td>
</tr>
<tr>
<td>10. $l_c$</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Table A4: T1 energy barriers

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Ensembles</td>
<td>250</td>
</tr>
<tr>
<td>2. $s_{av}$</td>
<td>3.79-3.88</td>
</tr>
<tr>
<td>3. $\alpha$</td>
<td>1.0-2.5</td>
</tr>
<tr>
<td>4. $s_0$</td>
<td>3.82-3.88</td>
</tr>
<tr>
<td>5. $dt$</td>
<td>0.01</td>
</tr>
<tr>
<td>6. $K_a$</td>
<td>$1(\alpha) &amp; 100 (\Delta)$</td>
</tr>
<tr>
<td>7. $T$</td>
<td>0.01</td>
</tr>
<tr>
<td>8. $N$</td>
<td>80</td>
</tr>
<tr>
<td>9. Maximum FIRE steps</td>
<td>$10^5$</td>
</tr>
<tr>
<td>10. $l_c$</td>
<td>0.04</td>
</tr>
<tr>
<td>11. $\Delta$</td>
<td>0-0.12</td>
</tr>
</tbody>
</table>

Component-wise diffusivity and timescales in shape bidisperse mixtures

We study the diffusivities of individual components for mixtures with fixed $s_{av} = 3.85$. Although increased dispersity signals a solid-fluid mixture, we see that the average behavior remains fluid-like up to high dispersities. Hence, we measure the diffusivity of each component to determine if the solid-like cells diffuse (Fig. A2a). We find that a fluid-like component is indeed able to help the solid-like cells diffuse.

For the demixing observed in shape bidisperse mixtures, as mentioned in the main text, we observe that for most of the $\Delta$, the DP saturates to a final value. We check if the timescale associated with this saturation increases with dispersity since Fig. A2a
Figure A1: (a) Effective diffusivity ($D_{\text{eff}}$) with respect to target shape parameter $s_0$. Different curves are for monodisperse systems with $K_a$ varying from 1 to 100. The solid horizontal line represents the cutoff at 0.01 used previously. The vertical dashed line denotes $s_0^* = 3.813$. (b) Effective diffusivity in area bidisperse mixtures. Plot of the effective diffusivity ($D_{\text{eff}}$) with respect to increasing area dispersity $\alpha$. Parameter are details provided in Table S2.

demonstrates that the solid components (of high dispersity mixtures) do not diffuse as much. We define $t_{1/2}$ as the average time taken by the system to get to half of its final DP. We observe that this half-time increases exponentially with $\Delta$, as shown in the inset of Fig. A2b.

**Increasing temperature decreases micro-demixing**

Since we hypothesize that micro-demixing is due to kinetic traps between energy barriers to neighbor exchanges, raising the temperature so that cells should be able to surmount such energy barriers should lead to complete mixing. We, therefore, study the micro-demixing as a function of an increased temperature in a mixture with fixed dispersity $\Delta = 0.2$. We observe that increasing temperature indeed leads to complete mixing, i.e. the demixing parameter goes to zero. For an increased temperature we use the relation $\tau_\alpha \propto T^{-3/2}$, reported in [148], to re-scale the x-axis. The lower temperatures systems have yet to reach a steady state demixing value. One can use a
Figure A2: Component-wise diffusivities and timescale to approach steady state. (a) Plot for effective diffusivity ($D_{eff}$) with respect to increasing shape dispersity $\Delta$. The solid lines are for the two different components with triangles and circles representing higher $s_0$ (type 2) and lower $s_0$ (type 1) respectively. The dashed curve represents the averaged $D_{eff}$. (b) The average time it takes for the system to achieve half of its steady state DP value, or $t_{1/2}$, is plotted against $\Delta$. The solid curves from 7(b) are used to compute $t_{1/2}$. The inset shows log-log plot of the same, with a linear fit in solid yellow line is $y = 3x + 3$.

Cortical tension for sorted vs. mixed configurations

An emergent line tension between two different kinds of cells must show a high line tension along heterotypic edges and lower line tension along the homotypic edges. Hence, for both the sorted and mixed scenarios (Fig. 9), we study a line tension map where the thickness of the edge is linearly proportional to its line tension. A positive value is colored in red and a negative value is colored in blue. The cortical tension for each edge can be computed using the method suggested in Ref. [143].
Figure A3: *Increasing temperature, diminishes observed demixing.* (a) Plot for demixing parameter ($DP$) with respect to time (in units of $\tau_\alpha$). The solid curves represent an increasing temperature ($T$) from blue ($T = 0.005$) to maroon ($T = 0.04$). The curves are averaged over 280 ensembles.

The cortical tension analysis conveys the fact that there is no emergent line tension due to bidispersity in the mixtures we study. The mean heterotypic line tension (black vertical line) is less than or equal to the mean homotypic line tension (colored vertical line) for all the scenarios. See Figs. A4 and A5.

**Differential T1 energy barriers in area bidisperse mixtures**

We present data supporting the notion that the differential energy barriers are smaller for the area bidisperse mixtures as compared to the shape bidisperse ones. We focus on larger cells trying to invade a cluster of smaller cells and vice versa to determine the stability (or lack thereof) an interface. See Fig. A6. We also present data for other types of topologies for both shape and area bidisperse mixtures for completeness (see Fig. A9).

Finally, to study differential T1 energy barriers in a simplified setting, we consider four cells connected to each other symmetrically. The energy is minimized with respect to a diminishing T1 edge length $l$ using MATLAB. The area stiffness is kept very high and the initial condition is recursively fed from a longer $l$ energy minimized
Figure A4: Cortical tension in shape bidisperse mixtures of $\Delta = 0.4$. Left and right panels shows line tension maps for sorted and mixed scenarios for a $N = 900$ system respectively. Heterotypic edges are shown in dash-dot lines. Yellow and blue cells have $p_0 = 3.65$ and $4.05$ respectively. They are followed by histograms for heterotypic (in black) and homotypic (colored) edges. Vertical lines show the mean values for each curve in their respective colors.
Figure A5: Cortical tension in area bidisperse mixtures of $\alpha = 2.5$. Left and right panels show line tension maps for sorted and mixed scenarios for a $N = 900$ system respectively. Heterotypic edges are shown in dash-dot lines. Yellow and blue cells have $A_0 = 0.57$ and 1.43 respectively. They are followed by histograms for heterotypic (in black) and homotypic (colored) edges. Vertical lines show the mean values for each curve in their respective colors.
Figure A6: Differential energy barriers in area bidisperse mixtures. (a) Energy $E(l)$ relative to $E_0$ versus T1 edgelength $l$ for a typical size bi-disperse T1 pair ($\alpha = 2.5, s_{av} = 3.85$). (b) Energy Barrier $E_b$ is plotted against area disparity $\alpha$, where large values on right and small values on left imply large-cell cluster in yellow and small-cell cluster in blue respectively. Each solid curve represents the barrier for a heterotypic cell to get out of the cluster for a fixed $s_0$ (varied from solid-like (orange) to liquid-like (green) - 3.82, 3.85, 3.88) (c) Correlation plot for $s_0 = 3.85$ between Differential Energy Barriers on the right y-axis $\delta(E_b)$ (in maroon triangles) and demixing relative to mixed scenario $DP_f$ on the left y-axis (in black discs). Size ratio $\alpha$ is plotted on x-axis. Simulation details provided in Table S4.

We can accommodate different sizes and shapes as long as cells of different types are positioned symmetrically about both $x$ and $y$ axis and make the cells sharing the T1 edge (T1 pair) have different properties from the non-T1 pair. The formula used to compute energy barrier is $E(l = l_H) - E(l = 0)$, where $l_H$ is the edge length of a uniform hexagon with unit area.

To study the effect of shape bidispersity (Fig. A7), the energy barrier (red when non-zero and blue when vanishingly small) is plotted with respect to the shape of T1 pair ($x$-axis) and the shape of the non-T1 pair ($y$-axis), which can be independently
Figure A7: *Symmetric 4-cell T1 energy barriers for shape bidispersity.* On the left is the color plot of energy barrier as a function of independently tunable shapes of T1 pair and non-T1 pair is plotted along x-axis and y-axis respectively. The dashed line represents monodisperse calculation i.e. for $\Delta = 0$. As expected it is red till it reaches the monodisperse transition point $s_0^* = 3.813$, after which it becomes blue. Off-diagonal phase points depict bidisperse mixtures i.e $\Delta \neq 0$. We see that it is necessary for the T1-pair to be fluid like, for vanishing barrier. On the right is a cross-section of the phase diagram on left. Energy barrier is plotted against area disparity for increasing values of $s_0$ 3.79 to 3.85.

Figure A8: *Symmetric 4-cell T1 energy barriers for area bidispersity.* On the left is the color plot of energy barrier as a function of independently tunable sizes of T1 pair (blue polygons) and non-T1 pair(yellow polygons). On the left is when blue polygons are bigger than yellow. On the right is smaller blue cells sandwiched between yellow (BssB). $\alpha$ and $P_0$ are the area ratios and preferred shape index respectively. Dashed black line represents the monodisperse transition point $s_0^* = 3.813$. This graph predicts the energy barriers to vanish at a shape index higher than $s_0^*$ in highly bidisperse systems. On the right is a cross-section of the cumulative phase diagram on left. Energy barrier is plotted against area disparity for increasing values of $s_0$ 3.79 to 3.85.
varied. A similar analysis is done for mixtures with bidisperse areas (Fig. A8). We observe differential energy barriers in both cases with, again, the size of the barrier generically larger in the shape bidisperse case as compared to the area bidisperse case, even in this simplified calculation. Re-phrasing this in terms of invading a cluster of the opposite type, one can think of these as invading doublets of opposite kind.

**Additional experimental features**

Figure S10 provides additional information regarding the motility of the cells (Fig. S10a), the distribution of cell areas (Fig. S10b), and the ratio of the numbers of the two cell types during the course of the experiment (Fig. S10c). There is little difference in the amount of the displacement the two different cell types undergo within 24 hours either in the monotypic monolayers or in the combined monolayers. There is also minimal difference in the distribution of cell areas for the two different cell types as measured in the respective monotypic monolayer (Fig. S10b). Finally, Fig. S10c demonstrates that the Ctr-E-cad−/− mixtures remain approximately 50:50 mixtures over the duration of the experiments.

We verify that natural variability in adhesion from cell-to-cell does not drive micro-demixing by calculating the demixing parameter (DP) for monotypic monolayers with half the cells tagged with one type of stain and the other half tagged with a second stain (Fig. S11a). We find that the demixing parameter does not increase (or decrease) on average with time, strongly suggesting that differential adhesion is indeed what is driving the micro-demixing.

In addition to computing the demixing parameter for the co-culture, we also study the pair correlation functions between all the three possible cell-type pairs in the co-culture in the high calcium condition (Figs. S11b and c). As the demixing parameter value is rather consistent with the prediction, the pair correlation function also
Figure A9: T1 transitions in shape and area bidisperse mixtures. (a)-(c) T1 topologies (shown as cartoons on axis extremities) and their barrier statistics for shape bidisperse mixtures. (d)-(f) T1 topologies (shown as cartoons on axis extremities) and their barrier statistics for size bidisperse mixtures. Parameters used are in Table A4.
indicates a small-scale correlation across a couple of cell diameters. The experimental pair-correlation curve is more structureless than the predicted curve, which potentially can be understood given the variability in cell areas found for both the cell-types shown in Fig. S11c.

Figure A10: Additional quantification of cell properties. (a) Cell displacements integrated over 24 hours in the Ctr-E-cad−/− mixtures and in the control Ctr-Ctr and E-cad−/−-E-cad−/− monotypic monolayers (cells of the same type but different tag). Ten cells from each type of the 7 Ctr-E-cad−/− demixing videos and the 4 Ctr-Ctr and Ecad−/−-Ecad−/− demixing videos are measured. We additionally show the root mean-square displacements for various time intervals for the same data sets. (b) The distribution of cell areas is shown for E-cad−/− (in red) and Ctr (in black). The inset shows the average across 6 and 7 isolates respectively, to show that area distributions are similar for both cell-types. (c) The ratio of number of E-cad−/− (KO) to Ctr cells is plotted against time for a typical experimental co-culture. The ratio approaches unity i.e. it is almost a 50:50 mixture, over the course of the experiment.
Figure A11: Additional quantification of experimental co-cultured monolayers. (a) The solid maroon curve represents the time evolution of the demixing parameter for the E-cad⁻/⁻ cell-type in the Ctr-E-cad⁻/⁻ mixture as a function of time, averaged over 5 different monolayers using five different isolates. The maroon curve should be compared against the almost flat demixing curves for monotypic mixtures composed of 50:50 differently tagged Ctr and E-cad⁻/⁻ cells shown in yellow and blue dashed curves respectively, averaged over two monolayers using two different isolates each. (b) The initial (0h) Voronoi tessellation of the co-culture nuclei, is compared side by side to the final (24h) snapshot in the high calcium condition. Green cells and red cells depict Ctr (Ctl) and E-cad⁻/⁻ (KO) cells respectively. (c) The pair correlation function is plotted for the initial and final snapshots in (b). Green, red and blue markers depict correlations between homotypic Ctl, homotypic KO, and heterotypic Ctl-KO nuclei respectively.
Chapter 4

Geometric signatures of interfacial tension between two cell types in 3D models
4.1 Introduction

One of the most important collective phenomena for biological cells is their ability to segregate with different cell types forming distinct compartments based on type in a process called cell sorting. Starting from its integral role during an organism’s formative stages [9–12, 151], these processes also play a crucial part in the upkeep of normal functioning of organs [13, 152] and containment of the spread for diseased/infected tissues [16–18, 129]. From the rich literature on cellular mixtures, cell sorting mechanisms can be classified into two broad categories—(a) biochemical/morphogen gradients [111, 153] and (b) difference in mechanical properties at the level of single-cell. These properties can be cell-cell adhesivity [33, 154], acto-myosin contractility [51–54], a mechanochemical coupling between both [49, 56, 155] or an explicit interfacial tension between unlike cells [48, 72, 97, 140].

Much of the cell sorting phenomenon has focused on particle mixture simulations as a basis for understanding, where the mechanism relies heavily on active fluctuations [156–160]. However, an essential feature of cell sorting that is observed in experimental co-cultures is that the interface is much sharper than what is expected from a particulate mixture [93, 161–165]. While such a straight and sharp interface is difficult to obtain merely by diffusive morphogens, heterotypic interfacial tensions (HIT) in confluent (no gaps between cells) tissues can generate a sharp interface easily [113, 140, 166]. These models are rooted in vertex models for confluent tissues. While there has been some understanding of how HIT drives cell sorting in two-dimensions using vertex models, there has been very limited work in three-dimensions focused on macroscopic features of coalescence in the zero-fluctuation limit [167]. This chapter intends to fill in the gap in making predictions for 3D cell sorting in confluent tissues.
Should HIT drive cell sorting in 3D, we first ask: how does one experimentally measure HIT? The current paradigm of experimental techniques to estimate the presence of HIT, such as laser ablation, are difficult to perform within a 3D tissue. But indirect measures, like single-cell assays, suggest that HIT can create robust cell sorting in 3D tissues [13, 53, 168]. In laser ablation experiments, cuts are made to ablate a cell-cell junction with the help of a pulsed laser. The resultant relaxation dynamics can help determine line tensions [169]. One the other hand, single-cell assays such as the dual pipette aspiration assay, are helpful in comparing contact preferences with like and unlike cells, by using contact angles and separation dynamics in cell doublets as a readout. However, using isolated cells for measuring effective tension might provide an incomplete picture as a confluent neighborhood can significantly change its mechanics [48, 140]. Recent work has shown that geometrical properties of interfacial cells in a confluent neighborhood can be directly affected by increased interfacial contractility and tension [140, 170, 171]. Hence, with the recent advancement in tissue segmentation techniques, cellular geometry can perhaps be used as a simpler and more direct readout of HIT in 3D mixtures, since contractility and tension can affect cell shape.

To explore the efficiency of HIT as a sorting mechanism in a fully 3D model for confluent tissue, we consider a 3D Voronoi model [77] with two distinct cell types that can modulate the higher interfacial tension along the heterotypic surface. The cell types are otherwise mechanically identical. For a fluid-like particulate mixture, the system is expected to naturally evolve to a configuration that reduces the shared surface area, and in the presence of large enough fluctuations, can also achieve complete spatial segregation with a blurred interface [172–174]. On the other hand, in confluent mixtures, in addition to optimizing the shared interfacial area, cells need to balance their individual mechanical stability as well. With the help of vertex
models [70, 77, 86, 88, 89, 92–94, 134, 135], it was recently found that cell shapes may determine the bulk rheology of confluent tissues. The cell shape can be quantified as the dimensionless ratio of perimeter $P$ to area $A$ i.e. $P/\sqrt{A}$ in 2D [70, 84], and of surface area $S$ and volume $V$ i.e. $S/V^{2/3}$ in 3D [77].

The model predicts that rounder cells with smaller shape index have a solid-like rheology whereas elongated shapes are more fluid-like. While we discover that fluid-like tissues do undergo robust and sharp compartmentalization, we also find that the tug of war between interfacial and bulk mechanical forces gives rise to unique geometrical signatures at the shared interface for both solid-like and fluid-like tissues. Some of these signatures can be directly understood by extending findings in 2D mixtures with HIT [140], owing to the highly topological nature of cell-cell interactions in both 2D and 3D confluent systems. In this work we take a step further and understand the integral role of cell shapes in the emergence of these signatures. We also study the effect of HIT on the geometry of surrounding cells. To understand the physical mechanisms that govern this unique relationship between HIT and cellular geometry, we develop simple toy models. We conclude by verifying the zero-fluctuation predictions from these models in bulk tissues.

### 4.2 Computational model

To understand the interfacial mechanics between two cell types, we use the recently developed 3D Voronoi model [77]. A simulation space with periodic boundaries is created using Voronoi tessellation of the cell centers of $N$ cells. Individual cells now have preferred volumes $V_0$ and surface area $S_0$. The combination of volume incompressibility and surface area regulation due to adhesion and contractility generate a preferred cell shape index $s_0 = S_0/V_0^{2/3}$. For example, a regular BCC unit cell (truncated
octahedron), has a dimensionless shape index of $s_0 \sim 5.31$ [175]. Here we set $V_0$ to 1. Half of the cells are tagged differently, creating a mixture of two cell types- $\beta = 1$ or $\beta = 2$, with a heterotypic interface between cells of different type. In addition to the original monodisperse energy functional, we impose an additional surface tension along the heterotypic interface. Therefore cells minimize their mechanical energy using the following dimensionless form of the energy functional:

$$e = \sum_i \left[ k_v (v_i - 1)^2 + (s_i - s_0)^2 \right] + \sum_{\langle i,j \rangle} (1 - \delta_{\alpha\beta}) \sigma a_{ij}, \quad (4.1)$$

where $v_i$ denotes the $i$th cell volume and $s_i$ denotes its surface area, non-dimensionalized by $V_0$. The unit of length is defined such that the average cell volume $\langle V_i \rangle$ is 1. $k_s = K_V/K_S$ sets the ratio between volume and area stiffnesses, and is also set to unity. The second term imposes an additional surface tension between heterotypic cells. The sum is over all facets with area, $a_{ij}$, shared between cells $i$ and $j$ of types $\alpha$ and $\beta$ respectively. The surface tension $\sigma$, for simplicity, is assumed to be the same for all facets and is non-dimensionalized by $K_S V_0^2$.

Biological cells can establish heterotypic tension by coregulating the acto-myosin network and adhesion molecules [49]. A biologically relevant estimate of tension, for example in the case of ectoderm-mesoderm co-cultures in Xenopus [48], would be around $\sigma \sim 2$. For systems with fluctuations, we analyse the dynamics of over-damped self-propelled particles with a high angular noise, which effectively leads to Brownian dynamics at the timescales relevant to us. While there are other possible dynamical rules, recent work on 2D mixtures has shown that the properties of an interface between two cells types with HIT between them is rather robust to the specifics of the dynamical rule [140]. For systems that require zero-fluctuation behaviour, we use conjugate gradient minimizer. See the Appendix. 4.6 for more details.
4.3 Results

4.3.1 Cell sorting due to interfacial tension

To test if HIT leads to a significant segregation in 3D tissues, in a manner similar to that of 2D mixtures [113], we focus on a fluid-like parameter regime [77] where cells undergo diffusive motion ($s_0 > 5.41$). For a fixed shape index $s_0 = 5.5$, we start from an initially mixed configuration Fig 12(a) for $N = 512$ system size. We let the system evolve long enough so each cell on an average gets to explore a distance equivalent to the simulation box length. Let us now understand the role of HIT on the bulk demixing. For a fixed $\sigma = 1$, a final configuration for such a mixture is shown in Fig 12(b). It is clearly segregated as compared to the initial snapshot. Some fraction of ensembles are able to create a planar interface as well.

To quantify this demixing, we study the demixing parameter $DP$ [113]. It measures the average neighborhood composition of every cell. In a completely mixed state, $DP = 0$, whereas in a completely sorted mixture, $DP = 1$, in the limit of infinite system size. However, as large system sizes can be time-consuming, we compute the maximum attainable value of demixing ($DP_{\text{max}}$) by looking at minimal surface configurations Fig. A1. Hence we plot the system’s demixing parameter relative to this maximum value in Fig 12.

The value of demixing is zero at the beginning as both the cell types are seeded at random positions. But it soon attains a high value, very close to $DP_{\text{max}}$. In the presence of HIT, the value of demixing increases quickly indicating that it can efficiently create robust segregation- very similar to a liquid-liquid particulate mixture and 2D confluent mixtures.

With non-zero fluctuations, we find that HIT efficiently leads to a large amount of segregation in mixed 3D tissues. We also observe that with higher values of tension, the
Figure 12: Sorting in fluid-like binary tissue with HIT: (a-b) Initial and final snapshots of a \( s_0 = 5.5, \sigma = 1 \) and \( N = 512 \) mixture, respectively. Both cell types denoted by red and blue polyhedra. (c) Quantification of segregation in terms of demixing parameter DP vrs simulation timesteps for different values of tension- 0 (maroon), 0.01 (pink), 0.03, 0.1, 0.3, 1 (blue).

The initial phase of the sorting process becomes faster as shown by the delayed plateauing for smaller values in Fig. 12. This confirms that heterotypic surface tension is very effective at compartmentalization in 3D, as expected. While biological cells are capable of upregulating tension cables along heterotypic interfaces via biochemical pathways, it is very difficult to directly measure this tension within a 3D tissue. Can features of individual cells at the interface help us quantify such tensions?
4.3.2 Effect of interfacial tension on cellular geometry

A visual inspection of the segregated mixture shown in Fig 12(b) indicates that the interfacial cells may be more elongated and nematically ordered as compared to the cells in the interior. This hints at an integral relationship between the applied tension and the surrounding cellular geometry. To delve deeper into the exact ways in which the surrounding cells deform, we set up a maximally segregated mixture. Here both compartments are placed side by side, similar to the previous work by Sussman et al [140]. We then study the cellular geometry as a function of the applied interfacial tension. Shape elongation along with the prominent stacking of cells (alignment of the polyhedral long-axes in Fig. 13(a)) can be observed here as well. To quantify the effect, we study three observables.

The first is acquired cell shape index \( s \), which measures the shape of cells in steady-state. This helps us quantify whether or not the otherwise homogeneous cells remain homogeneous after HIT is established. We measure the shape of both-interfacial cells \( s_{\text{boundary}} \) and the interior cells \( s_{\text{bulk}} \). This further helps isolate the shape changes in the immediate neighborhood of the interface. Individual cells have a final volume \( V_i \) and surface area \( S_i \). Hence, \( s_{\text{boundary}} \) is defined as:

\[
s_{\text{boundary}} = \left\langle \frac{S}{V^{2/3}} \right\rangle_{\text{boundary}},
\]

and \( s_{\text{bulk}} \) is similarly averaged over the interior cells. In the absence of HIT \( (\sigma = 0) \), both shapes have the same value of \( s_0 = 5.5 \).

Both the shapes are shown as a function of increasing tension in Fig. 13(b). With small values of tension, the shapes are very similar at the beginning but they gradually saturate at a higher value of disparity. This means that with a higher interfacial tension, the neighboring cells become more elongated, whereas the interior cells become
somewhat compact/round.

To study the alignment between cells, we next measure the orientation of interfacial polyhedra. We define orientation vector of a cell as the major axis of its moment of inertia tensor. We then plot the angular distribution of the angle made by each vector with respect to the normal to the interface ($\theta$), which in this bilayer arrangement is simply the z axis. For a homogeneous system with no HIT, the angles are very close to the random distribution density function in 3D, which is proportional to $\sin(\theta)$. But with a slight increase in tension, the cells polarize and orient themselves perpendicular to the interface, as depicted in Fig. 13(c).

Lastly, we study polygonal faces that make up the heterotypic interface by plotting the facet area distribution with respect to increasing tension. This is in analogy to the measurement of edge lengths in the 2D work [140]. With no HIT, the distribution is somewhat uniform, whereas, with increasing tension it becomes bimodal, as shown in Fig. 13(d). This means that the facets are either large or vanishingly small at high tensions. With the help of smaller facets, the interfacial vertices come very close to having a vertex coordination higher than the normal tetrahedral coordination, similar to the 4-fold vertices observed along 2D tension cables. An average larger facet also increases steadily in area as shown in Fig. A2. As a similar bimodality is observed in 2D as well [140], the origin of these signatures might be based on topological interactions between the cells.

With the help of planar interface, we discover that HIT indeed affects the geometry of the surrounding cells, inducing shape changes and nematic-like ordering in an otherwise homogeneous collection of cells. To understand the origin of this feedback, we focus on the specific geometry of an interfacial cell and ask: what does it take for the system to attain this precise geometry?

A typical interfacial neighborhood is shown in Fig. 14(a). A right prism can be
Figure 13: Cellular geometry changes around the high-tension interface: (a) Snapshot of the bilayer arrangement of sorted compartments for a high value of $\sigma = 20$. Only the blue cell type is emphasized here, colored by major axis length. Green is for elongated and blue for rounder cells. The white rods denote the long axis of the polyhedron. (b) Acquired cell shape index ($s$), plotted with respect to tension ($\sigma$), is higher for interfacial cells (green curve) as compared to the cells in bulk (blue curve). (c) Rose plot for the distribution of orientation angle of interfacial cells is shown for increasing tension ($\sigma$). The faint black curve represents the density for uniform distribution i.e $\sin(\theta)$. (d) The probability distribution of heterotypic facet area is shown for increasing tension ($\sigma$).

defined as a polyhedron with flat top and bottom facets, and perpendicularly aligned lateral facets. The cells here seem to closely resemble the geometry of a one-sided prism, with the flat side at the interface. The unique shape can be attained in a Voronoi tessellation only by fulfilling two conditions- (a) cell heights are arranged in a plane and (b) interfacial pairs align their centers so the distance between centers in the XY plane is minimized. We quantify both of these criteria and find them both fulfilled for higher values of tension, as shown in Fig. 14(b-c).
Figure 14: Interfacial cells are right-prisms: (a) a snapshot that highlights the cells at both sides of the interface. Cell types are tagged in blue and red and made translucent to make their centers visible (white solid spheres). The edges of red cells are depicted by solid red lines. (b) Height distribution \( z \) of the interface cells is plotted for increasing tension. The inset shows standard deviation \( \Delta z \) with respect to tension \( \sigma \). (c) Minimum distance along the XY plane between heterotypic cells is plotted for increasing tension. The inset shows the average of this distance with respect to tension. A distance of zero is highlighted by complete Registration \( R = 1 \).

We find that with an applied tension, the cell shapes become more prism-like. This makes the cell centers resist any deviation in height and prefer to register themselves along the interface. From the 2D work we understand that perturbations away or towards the interface are very costly [140], but the same is not known for cell-cell registration. Therefore we extend this calculation with respect to a perturbation in the cell-cell registration. We find that the final energy is exactly same to the linear order. Hence, we focus on the registration for the remainder of this chapter.
4.4 Toy models and their predictions

With the help of fluctuations, we discovered that cells adjacent to a high tension interface prefer a prismatic shape, and they attain it by registering their cell centers. To explore the system’s energetic preference to registration, we set up ordered toy models and study the response at zero-fluctuation.

Our first toy model is initialized in a BCC lattice, as a ground state for the shape $s_0 \sim 5.31$. This is similar to our recent work on ordered hexagonal monolayers [102], but with a different form of local perturbation. In this work, we study the response of the system to a change in registry, which is defined as:

$$R = 1 - \frac{d}{l_0},$$

(4.3)

where $l_0$ is the lattice spacing and $d$ is the distance between centers along the interfacial plane. We enable a string of polyhedra, to slide past the string below (shown in Fig. 15(a) insets), with an extra surface tension along the shared interfacial strip between both the sets of polyhedra. We compute the shared surface area and total energy of the system. We also find the global minima for different values of tension.

We find that the shared surface area is minimized for increased registration values (shown in Fig. 15(a)). While this suggests that maybe complete registration is the energetically preferred state, surprisingly, that is true only after a threshold value of tension. This can be seen by plotting the change in total energy with respect to increasing registration. One can observe the two kinds of minima in the system—parabolic and ‘cuspy’[140]. While the former is the common one with a spring-like potential in locally around the minima, the later has a discontinuity in its slope due to topological pinning expected from a 2D calculation shown in Sec. A4.6. Physically
this means that there is a discontinuous restoring force for the slightest perturbation in registry, as shown in Fig. A4(b), leading to a topological pinning. The parabolic minima, on the other hand, have a continuous and linear restoring force, the slope proportional to the stiffness of the parabola.

We plot the registration of energy minima as the tension increased in Fig. 15(c). We have two different branches corresponding to the two types of minima discussed before. For very low tension, shape preference dominates (as shown in Fig. A5(a)) and the system stays in BCC configuration. For moderate values of tension the system stays in the parabolic branch, but continuously transitions to non-zero registry. Just below the critical value of $\sigma_c \sim 3$, both the shape and interfacial energies become comparable (Fig. A5(b)) and at $\sigma_c$ the system discontinuously transitions to the tension-dominated-cuspy branch. The registrations values also jumps to $R = 1$. This branch originates at $\sigma \sim 2$.

Both kinds of minima become more and more stable for higher tension values. This is shown using stiffness for parabolic minima in Fig. A4(a) and restoring force for the cuspy minima in Fig. A4(c).

In summary, we find that the physical mechanism that drives registration at high tension values is very similar to that of 2D systems. However, the story changes at lower values of tension where shape frustration begins to play a dominant role. This leads to a minima that is partially-registered and has a spring-like response to small perturbations, i.e. states are no longer topologically pinned. As cell shape preference can play an integral role in determining the ground-state registration and its mechanical properties, we explore this effect for different preferred shape indices. Can cells with higher shape index, which are more fluid-like, have lesser effect on the ground-state registration?

To understand this, we develop a model that is also ordered but not constrained
Figure 15: Global minima becomes registered for higher tension: (a) Shared surface area (shaded in dark for all snapshots) is computed as a function of the registration between the different cell types. The string of blue cells is allowed to move past the string of stationary pink cells. Snapshots for no, half and complete registration is shown for encircled points. (b) The change in the total energy of this system is plotted with respect to registry, for different values of tension ranging from 1 (pink) to 4 (blue) in increments of 0.25. (c) The ground-state registration is plotted for increasing tension. The solid curve represents the global minima. The dashed curves represent the local minima, parabolic in blue and cuspy in black. The inset zooms about the critical tension at which the transitions occurs between both types of minima.

to a string. Instead it is free to move along the 2D interface to change its registration. With this flexibility, we can explore the energetics of more elongated cell shapes. The interfacial layers are placed in HCP format as shown in Fig. 16 while, there are buffer cells placed above and below the interface in a disordered fashion and are allowed to relax in course of the perturbation.

We compute the shared surface area and change in total energy for fluid-like
shapes. We find that similar to the BCC toy model, the shared surface decreases with registration. From preliminary results shown in Fig. 16 for $s_0 = 5.5$ and the increasing values of tension, we observe that the ground-state registration could be much closer to complete registration as compared to the BCC shape index. This suggests that in disordered fluid-like systems interfacial tension might dominate shape preferences in determining interface geometry and response.

Figure 16 : Shape frustration might be less dominant for larger more fluid-like shapes: (a) Snapshot from the top-view of HCP toy model setup where both cell types are depicted in blue and red. Cell centers for the top and bottom layer are depicted by solid while spheres. (b) Shared surface area is computed as a function of the registration between both the layers. (b) The change in the total energy of this system is plotted with respect to registry, for the lower values of tension $\sigma = 0.001, 0.003, 0.01, 0.032, 0.1, 0.3$ (pink to blue) and a fluid-like shape of $s_0 = 5.5$.

Finally, we verify the toy model predictions by looking at perturbations about the steady state registration for different parts of the shape-tension phase space. We
expect the boundary between registered and off-registered to coincide with \( \sigma_c(s_0) \) - as it demarcates the cusp-like registered minima from the parabolic minima. From preliminary data of the magnitude of restoring forces at a fixed perturbation of \( \epsilon = 5 \times 10^{-4} \), the transitions seem to go hand in hand, as shown in Fig. A6.

Figure 17: Transition to complete registration shifts for fluid-like cell shape: A heat-map for the average steady-state registration is shown as function of cell shape \( s_0 \) at interfacial tension \( \sigma \). Yellow denotes complete registration and blue denotes partial registry. The registry between heterotypic cell-pairs is averaged for 200 different initializations.

4.5 Conclusion

Using a Voronoi model with non-zero fluctuations, we show that three-dimensional binary mixtures, with heterotypic interfacial tension (HIT), sort robustly. In addition to this global collective dynamics, HIT also modifies the otherwise homogeneous mixture to have a prism-like geometry for cells surrounding the interface. With the help of zero-fluctuation energy minimization techniques, we find that the onset of these
signatures may depend on both the tissue rheology and the magnitude of interfacial tension.

To understand the onset of these geometric signatures, we use cell-cell registration at the interface as a probe to check the stability of these prism-like structures. We construct two simple toy models and study the energetics with respect to registry. We find that for an interfacial tension $\sigma > \sigma_c$, the ground state is completely registered - that gives rise to the prismatic geometry. These states are topologically pinned, due to cusplike pinning forces, previously observed in 2D mixtures with HIT. But for tension $\sigma < \sigma_c$, interfacial energy is dominated by shape frustration and hence the ground state can be much less registered. Surprisingly, the linear response of these minima is spring-like and not cuspy. Preliminary data suggests that $\sigma_c$ decreases for a fluid-like tissue. This would mean that decreasing the tissue fluidity toward a solid-like state may reduce topological pinning at the tissue interfaces.

Topological pinning might be affecting the cell sorting dynamics as well. Presumably, less pinning can lead to seamless coarsening of nearby droplets, while more pinning can hinder the sorting process. Nevertheless, we have shown that a small change in cell-cell interaction along an interface can lead to complete sorting in 3D tissues. This supports the claim that HIT can be an efficient candidate during early embryonic stages to create sharp compartmentalization [48, 166].

We have also shown that a change in the magnitude of the interfacial tension can have a pronounced effect on the neighboring cellular geometry - by making interfacial cells longer and a more oriented prism-like polyhedra. The facet areas also become larger. With the advancement of 3D segmentation techniques [176–180], one can use these signatures as a toolkit to probe interfacial tensions in a 3D tissue. Therefore, cells themselves can tell us about relative magnitudes of tissue surface tension through shape changes!
For a planar geometry like that of compound epithelium, one can also study the interaction between two nearby interfaces, and its effect on the signatures. The prism-like geometry of cells can be visually detected by the double-edged polygons seen using En Face imaging technique[13, 181]. As a lot of the current segmentation algorithms are tailored for 2D cross-sections of 3D tissues, these geometric signatures can be further characterized along the cross-sections [182]. In general 3D tissue have complex interfacial geometries. Hence, one of the future avenues of this work would be to study the dependence on the curvature of the interface.

Finally, cells usually move more slowly when confluent, i.e. they are surrounded by neighbors with no gaps in between. The previous work on 3D sorting is mostly fluctuation-based and hence relies on cellular movement. Hence, the mechanisms we have uncovered at the zero-fluctuation limit might be more relevant for biological systems. For example, a natural extension of our general framework is to have two different cell shapes mixed together. Unique extrusion behaviour can emerge due to differential pinning of cells [113].

So far tissue interfacial tension and cell shapes have been used in vertex models to quantify bulk behaviour like segregation and fluidization. Our work suggests that tension and cellular shape can coordinate and give rise to novel spatial patterns in a 3D tissue. For example, maybe elongated cells near the interface may become better suited for oriented cell divisions. We speculate that perhaps biology can make use of this subtle feedback for their own advantage.

Acknowledgement

We thank Paula Sanematsu and Matthias Merkel for helpful discussions.
4.6 Appendix

Maximum demixing value with respect to system size

For a small system size, the demixing cannot attain the maximum possible value of unity as the number of heterotypic facets is not negligible as compared to the number of homotypic facets. Therefore we look at demixing in a segregated arrangement as a function of system size.

The simple assumption that a given interfacial cell shares only one-third of its facets with heterotypic neighbors, reproduces the final demixing values fairly well. This is shown in the inset of Fig. A1

Figure A1: (a) Plot for demixing parameter ($DP$) with respect to time in a bilayer arrangement. The inset shows the final demixing value with respect to cubic root of system size in solid black curve for a fixed tension of 1. The prediction from assuming that the interfacial cells share one-third of their facets with the other cell type, is plotted in dashed grey.

Even though the bilayer geometry is the least surface area configuration for a cubic simulation space like ours, for a mixed initial condition it is actually hard to achieve. In general, compartments with different topologies can be observed- for example with
one or two holes. Many of these dynamically transform into the most stable- planar compartment that has no holes.

**Increase in the average observable facet area**

We observe that with HIT, heterotypic facet areas become bimodal, i.e. there are a majority of vanishingly small faces and the rest are larger faces. From an experimental point of view, vanishingly small facets are difficult to detect and hence, we plot the average of larger facets in Fig. A2. We see that it increases directly with tension.

![Figure A2: (a) Plot for average observable area with respect to increasing tension.](image)

**Interfacial energy with respect to registration**

For a perfect prism-like geometry along 3D interfaces, we need cells to be at the same distance from the interface as well as the cell pairs across the interface must have their centers aligned i.e. registered. While the former condition is already understood using simple square lattice geometry calculation in a recent work [140], the latter remains to be studied. Hence we use the exact same setup 9 neighboring cells, but with a
different local perturbation. As shown in Fig. A3, we consider a small perturbation of the central cell along the interface by an amount $\epsilon$, redraw the Voronoi tessellation and calculate final energy.

Figure A3 : A sketch of the 9-cell set up where the different cell types are colored with pink and blue and the heterotypic boundary highlighted by red. The Voronoi centers are shown in grey filled circles. The black edges show initial cell-cell boundaries. The central cell’s boundary after perturbation is highlighted in blue.

The initial energy of the 9-cell system is-

$$E_i = 9k_A(a - a_0)^2 + 9(p - p_0)^2 + 3\gamma_0 l_{ij},$$  \hspace{1cm} (4.4)

where $\gamma_0$ is the interfacial tension, $l_{ij}$ is the length of edges shared between unlike cells. By making the assumptions of $s_0 = 1$ and $p_0 = 4$ to start from the ground state, we reduce the expression to-

$$E_i = 3\gamma_0,$$  \hspace{1cm} (4.5)

After displacing the central cell to the right, the new total energy to leading order
becomes-

\[ E_f = 3\gamma_0 + \gamma_0(\sqrt{2} - 1)\epsilon_x + \left(\frac{3k_A}{2} + (22 - 14\sqrt{2} + \gamma_0\left(\frac{20\sqrt{2} - 8}{32\sqrt{2}}\right))\right)\epsilon_x^2, \quad (4.6) \]

We see that to the linear term, the energy has exactly the same cuspiness that was observed for perturbation in the distance from the interface. The coefficient of \( \gamma_0\epsilon^2 \) is double that of the original perturbation, indicating the non-linear stiffness is higher for sideways perturbation.

**BCC toy model**

We further explore the properties of both kinds of minima in Fig A4. We also look at the contribution from shape frustration and surface energy to the total energy change of the system in Fig. A5.

**Details about self-propulsion dynamics**

For dynamical simulations, we use the static model developed for 3D confluent tissues [77], but with added cellular activity. The model essentially allows active fluctuation during time evolution of cells, that are polyhedra that we get from a space-filling Voronoi tesallation of the periodic simulation box. The energy functional given by Eq. 4.1, provides the mechanical force \( \mathbf{f}_i = \partial e/\partial \mathbf{r}_i \) on cells due to changes in cell shape and/or shared interface area. For introducing activity in this dynamics, one needs to choose the frame of reference for example a static extra-cellular fluid. In a self-propelled system, the \( i^{th} \) cell has a polarization vector \( \mathbf{n}_i = \) and exert an active force of \( v_0/\mu \) on the static media, where \( \mu \) is the mobility of the cell and \( v_0 \) is the
Figure A4: Properties of parabolic and cuspy minima

(a) Plot of stiffness of the parabolic minima with respect to increasing tension. (b) The restoring force for perturbations away from the cuspy minima is independent of the displacement on y axis for different values of tension (c) The value of restoring force at a fixed value of displacement is plotted against tension.

Self-propulsion speed. Setting $\mu = 1$, the dynamical equation looks like-

$$\frac{d \mathbf{r}_i}{dt} = v_0 \hat{n}_i + \mathbf{f}_i. \quad (4.7)$$

The polarization vector evolves with a white Gaussian noise on a unit sphere, with the diffusion coefficient given by-

$$\frac{d \hat{n}_i}{dt} = \sqrt{2D_r} \left( \mathbf{E} - \hat{n}_i \hat{n}_i \right) \dot{\xi}_i. \quad (4.8)$$

where $\mathbf{E}$ is the 3x3 identity tensor, the dyadic product of the polarization vector with itself is given by $\hat{n}_i \hat{n}_i$ and $\xi_i$ is the white Gaussian noise with $\langle \xi_i \rangle = 0$ and $\langle \xi_i(t) \xi_j(t') \rangle = \delta_{ij} \delta(t - t') \mathbf{E}$. 
Figure A5: *Shape vrs surface energies at extreme tension values*  Plot of change in total energy (in dashed orange), blue cell’s mechanical energy (in solid blue) and interfacial energy (in solid black) with respect to registry is shown for (a) $\sigma = 0.001$ and (b) $\sigma = 1$.

We use a step size $\Delta t = 0.01$ and a total time that is long enough to diffuse the length of the side length of the simulation box, keeping in mind the self-diffusivity timescale in homogeneous systems.

**Preliminary data for verifying restoring forces in the bulk**
Figure A6: *Restoring forces seems to become non-zero for registered steady State:* Plots of steady-state registry and restoring force magnitude for a fixed value of displacement is shown with respect to increasing tension for different values of shape $s_0$ - 5.314 (maroon) to 5.8 (green).
Chapter 5

Discussion and conclusion
In this thesis our overarching goal is to understand and predict collective fluidization and segregation in confluent tissues. Space-filling models like the vertex models have been successful in predicting cell shapes and some features of collective fluidization in cellular monolayers. However, some fundamental questions about their mechanical response and segregation behavior is yet to be understood completely.

Most of our intuition about material rheology is built on particle-based systems. For example, monodisperse molecular/particulate systems have a high-dimensional potential energy landscape where both the curvature about the minima as well as the barriers between adjacent minima help predict the rheology of the material. The curvature is a readout for the mechanical stability of the minima and can be accessed via vibrational modes of the material. This is intimately coupled to the barriers, which are a proxy for mechanical instability. For example, as the barrier between neighboring minima diminishes, so does the stiffness of the original minima- as one would expect for an analytic energy function. This leads to a rearrangement and a subsequent fluidization.

Through our work on ordered vertex model, we show that the same is not true for confluent tissues. This is because the energy landscape has a cusp-like roughness that makes it non-analytic. This decouples the linear and non-linear behaviour entirely, which makes it harder to use linear response as a tool for understanding tissue rheology. How can we predict the tissue rheology then? We propose a simple analytic tool that accurately predicts the cuspiness in confluent landscapes. This helps understand neighbor exchange process in monolayers, and predict the onset of a fluid-like rheology. As the work was based on zero-fluctuation behaviour of tissues, an interesting future avenue would be to study the effect of temperature and activity on the non-linear stability.
Similarly, mixing a liquid-liquid mixture composed of two kinds of molecules with
difference in cellular properties like cell-cell adhesivity, cell shape or size, leads to
an emergent interfacial tension between both kinds of molecules. This helps them
segregate into spatially distinct domains by a process called cell sorting. Cellular
shapes in confluent tissues depend directly on cell-cell adhesion. For example, a higher
adhesivity can stably create longer shared boundaries with neighboring cells, leading
to a more elongated cell shape. Therefore in our work on 2D confluent mixtures, we
explore the role of shape and size bidispersity, in the collective segregation.

We find that in the absence of an explicit interfacial tension, such differences lead
to large-scale mixing. However, we discover a robust small-scale demixing, spanning
few cell diameters, that arises due to shape differences. We also realize this in an
experimental co-culture of Keratinocytes. This phenomena is very different from
sorting in particulate mixtures, as there is no energetic preference to form segregated
domains and therefore no fluid-like coarsening of the micro-domains. Instead, it is due
to a dynamical trapping that arises from non-reciprocal neighbor exchange barriers
at the interface. This suggests that in a developing embryo, the progenitor cells can
remain mixed in spite of cell shape and size differences. The subtle patterning might
help to create a tenuous spanning micro-domain that readily compartmentalizes as
soon as a biochemical means to recognize the other cell type develops.

Tissues use biochemical pathways to detect the other cell type and up-regulate the
tension along shared interfaces, thereby establishing heterotypic interfacial tension
(HIT) between the distinct cell types. While there has been some understanding of
how HIT drives cell sorting in two-dimensions using vertex models, there has been very
limited work in three-dimensional vertex models. Our work on 3D confluent mixtures,
intends to fill in the gap in making predictions for 3D cell sorting in confluent tissues.
We show that binary mixtures sort robustly in presence of HIT. In addition, HIT in the
otherwise homogeneous mixture gives rise to cells at the interface with a distinct prism-like geometry. To better understand this unique relationship between heterotypic tension and cellular geometry, we develop toy systems study their response in absence of fluctuation. As cells usually move more slowly at confluency, the mechanisms we have uncovered in the zero-fluctuation limit might be more relevant as compared to previous work in the field of particulate mixtures. For example, a natural extension of our very general framework is to have two different cell shapes mixed together. Unique extrusion behaviour can emerge due to differential interfacial pinning of cells.
Bibliography


[14] Xavier Serra-Picamal, Vito Conte, Romaric Vincent, Ester Anon, Dhananjay T. Tambe, Elsa Bazellieres, James P. Butler, Jeffrey J. Fredberg, and Xavier Trepat. Mechanical waves during tissue expansion. *Nat. Phys.*, 2012. ISSN 17452481. doi: 10.1038/nphys2355.


[27] Srikanth Sastry. Critically jammed, 2016. ISSN 10916490.


[54] Aaron F. Mertz, Shiladitya Banerjee, Yonglu Che, Guy K. German, Ye Xu, Callen Hyland, M. Cristina Marchetti, Valerie Horsley, and Eric R. Dufresne.


[58] M. Cristina Marchetti, Yaouen Fily, Silke Henkes, Adam Patch, and David Yllanes. Minimal model of active colloids highlights the role of mechanical interactions in controlling the emergent behavior of active matter, 2016. ISSN 18790399.


[61] Xingbo Yang, M. Lisa Manning, and M. Cristina Marchetti. Aggregation and


[88] Kevin K. Chiou, Lars Hufnagel, and Boris I. Shraiman. Mechanical stress


[164] Bruno Monier, Anne Pélissier-Monier, and Bénédicte Sanson. Establishment and maintenance of compartmental boundaries: Role of contractile actomyosin barriers, 2011. ISSN 1420682X.


Curriculum Vitae

AUTHOR: Preeti Sahu

NATIONALITY: Indian

DATE OF BIRTH: 09 June, 1992

DEGREES AWARDED:

Integrated MSc. in Physics,
National Institute of Science Education and Research,
Jatni, India (2010 - 2015)

PROFESSIONAL EMPLOYMENT:

Graduate Teaching Assistant, Department of Physics, Syracuse University (2015 - 2016)
Graduate Research Assistant, Department of Physics, Syracuse University (2016 - 2020)

PUBLICATIONS:


3. Preeti Sahu, Daniel M. Sussman, Matthias Rubsam, Aaron F. Mertz, Valerie Horsley, Eric R. Dufresne, Carien M. Niessen, M. Cristina Marchetti, M. Lisa


*equal contribution

AWARDS:

- Soft and Living Matter(SLM) travel grant of value 1000 USD. (2018)

- Khorana fellowship for a 10-weeks summer internship in Indiana University, IN, USA. (2014)

- Selected for the Visiting Students Program at Harish-Chandra Research Institute, Allahabad, India and the S N Bhatt Memorial Excellence Fellowship Program at International Centre for Theoretical Sciences, Bangalore, India. (2014)

- Runners-up team in a national-level science quiz competition in India- MI-MAMSA (2013).

- Generous fellowship by Govt. of India under Kishore Vaigyanik Protsahan Yojana for 4 years. (2011)

- Fellowship by Govt. of India under Innovation in Science Pursuit for Inspired Research for 1 year. (2010)

PRESENTATIONS:

Invited Talks

- “Role of neighbor exchange in fluidization and de-mixing of confluent tissues”, APS March Meeting, Boston, USA, Mar 2019 [Link]
Contributed Talks & Posters

Talks

- “Signatures of tissue surface tension in 3D models with two tissue types:” Cytoskeleton in Tissue Morphogenesis (Zoom) Microsymposium, April 2020
- “Signatures of tissue surface tension in 3D models with two tissue types”, APS March Meeting (via DSOFT Virtual Meeting) Mar 2020
- “Small-scale demixing in confluent cellular monolayers”, PCTS, Princeton University, Jan 2020
- “Geometric signature of surface tension in confluent tissues”, NISER, Jatni, India, Dec 2019
- “Role of morphological differences in cell sorting”, MNIT, Jaipur, India, Dec 2018
- “Mechanical difference insufficient to create sorting in confluent mixtures”, APS March Meeting, Los Angeles, USA, Mar 2018

Posters

- “Geometric signatures of tissue surface tension in 3D models with two tissue types”, PCTS, Princeton University, Jan 2020
- “Geometric signature of surface tension in 3D tissues”, thematic meeting, Aussois, France, Oct 2019
- “The search for physical mechanism of cell sorting in bidisperse confluent tissue”, PCTS, Princeton University, Feb 2018
• “Understanding how cells group together in confluent biological tissues”, Summer School in UMass, Amherst, USA, Feb 2017

• “Understanding how cells group together in confluent biological tissues”, Stevenson Biomaterials Poster Session, Syracuse Biomaterials Institute, Syracuse University, NY, USA, Feb 2017

SCHOOLS & CONFERENCES:

• APS March Meeting (via DSOFT Virtual Meeting)- March 2020 (Contributed Talk)

• Mechanics in Morphogenesis workshop, at PCTS, Princeton University, Jan 2020 (Poster Presentation and Contributed Talk)

• Conference on Recent Topics in Statistical Mechanics, National Institute of Science Education and Research, Jatni, India, Dec 2019 (Contributed Talk)

• APS March Meeting in Boston, USA- March 2019 (Invited Talk)

• Biology and Physics Confront Cell-Cell Adhesion thematic meeting, Aussois, France, Oct 2019 (Poster Presentation)

• 3rd International Conference on Soft Materials, Malaviya National Institute of Technology, Jaipur, India Dec 2018 (Contributed Talk)

• APS March Meeting in LA, USA- March 2018 (Contributed Talk)

• Summer School- Soft Solids and Complex fluids, UMass, Amherst, Feb 2018 (Poster Presentation: Confined Nematic Defects as Active Particles)

• Monsoon School- Physics of Life at NCBS-ICTS, Bangalore, India, JUN 2013
TEACHING EXPERIENCE \textit{Graduate Mentor}

- Primary mentor for Prof. Lisa Manning: REU student- Janice Kang (summer 2018)

- Co-mentor for Prof. Lisa Manning: REU student- Allie Brown (summer 2016)

\textit{Graduate Teaching Assistant (GTA)}

- PHY 221: General Physics Laboratory I (Spring & Fall 2016)

- AST 101: Our Corner of the Universe (Fall 2015)

PROFESSIONAL SERVICES:

- Helped to referee for a manuscript in Biophysical Journal.

- Actively volunteered for CUWiP (2016) and for Institute for Complex Adaptive Matter workshop on Smart and Active Matter at Syracuse University (June 2016),

- Tabling for GSNP,GSOFT and DBIO in APS MARCH meetings.

NON-ACADEMIC ACTIVITIES:
• Hindustani Classical Vocalist and Graduate president of Society for the Promotion of Indian Classical Music And Culture Amongst Youth (SPICMACAY) Syracuse chapter

• Student Representative for Under Graduate Committee and Physics Student Seminar Convener at college