ABSTRACT

Title of Dissertation: A BIOPHYSICAL PERSPECTIVE ON COLLECTIVE CELL MIGRATION AND MATHEMATICAL MODELING IN PHYSICS FOR THE LIFE SCIENCES

Deborah Jeanne Hemingway, Doctor of Philosophy, 2018

Dissertation directed by: Professor Wolfgang Losert, Department of Physics
Professor Edward F. Redish, Department of Physics

This dissertation pulls from the fields of physics, biology, and education to address novel problems both in current biological research on collective cell migration and in a reformed introductory physics for life science (IPLS) course. In collective cell migration, cells communicate with each other via a number of means including via signaling pathways. In developing zebrafish, a select group of cells called the posterior lateral line primordium (pLLp) is known to communicate with each other via two types of signaling pathways, Wnt and Fgf. In this work, we examine another signaling pathway, BMP, to gain insight into its role in the migratory behavior of the pLLp. My results demonstrate that BMP signaling is vital to successful migration and show that BMP affects the

cohesiveness (cell-cell adhesions), directionality (direction of migration), and migratory speed of the cells in the pLLp. These results and insight were obtained through both modeling the biological system and utilizing concepts and analytical tools prevalent in physics.

As part of the continuing reforms for the IPLS courses at the University of Maryland, College Park (UMD) I proposed and developed a novel methodology for curriculum development that is based on my own experimental biophysics research on collective cell migration. As a researcher, I used the tools and principles of physics to gain insight in the biological system and in parallel, I propose that "cross-disciplinary authenticity" is achieved when the tools and principles of one discipline are utilized to gain insight into a secondary discipline. I outline the methodology for achieving such, include an example problem set that is based on my research, and discuss the results from the deployment of the problem set in the IPLS course.

A BIOPHYSICAL PERSPECTIVE ON COLLECTIVE CELL MIGRATION AND MATHEMATICAL MODELING IN PHYSICS FOR THE LIFE SCIENCES

by

Deborah Jeanne Hemingway

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Advisory Committee:
Professor Wolfgang Losert, Co-chair
Professor Edward F. Redish, Co-chair
Associate Professor Arpita Upadhyaya
Assistant Professor Kimberly Stroka
Professor Todd Cooke, Dean's Representative

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Chapter I: Introduction

This thesis presents research in the area of Biophysics, with the aim of gaining insights into biological systems through the application of the principles of physics. It builds on the highly interdisciplinary research field of biophysics and pulls from a third field, education. I have named this novel combination of research areas **biophysics education research.**

This dissertation is comprised of two main parts, with the first part focusing on experimental biophysics and the second part focusing on biophysics education. The experimental biophysics aim is to examine the role of cell-cell signaling in collective cell migration in *danio rerio* (zebrafish), more specifically Bone Morphogenetic Protein (BMP) signaling in the posterior Lateral Line primordium (pLLp). The biophysics education aim is to develop a problem set that is research-based and authentic for the Introductory Physics for the Life Science (IPLS) course at the University of Maryland, College Park (UMD). Most significantly these two parts are linked; the biomechanics of the pLLp is the inspiration for the membrane mechanics focused problem set.

1.1 Experimental biophysics brings together biology and physics.

As noted above, the first part of this dissertation examines the role of cell-cell signaling in collective cell migration in zebrafish, a well-established model system for

organismal development. This section introduces the concepts of collective cell migration, signaling, and particle image velocimetry, the main physics tool used.

1.1.1 Collective cell migration occurs throughout nature.

Collective cell migration occurs throughout nature both globally and locally in organisms. Global collective cell migration is coordinated motion that involves all cells in an organism. One example is Drosophila (fruit fly) development in which the distinct folding of the embryo into a band-like structure is critical for development [1, 2]. More prevalent is local collective cell migration which involves motion of a group of cells in an otherwise relatively stationary environment. Examples of local collective cell migration are wound healing and cancer metastasis, and many parts of normal morphogenesis [3-17].

In this thesis I study localized collective cell migration in a relatively well-defined, reliable developmental process, the migration of the posterior Lateral Line primordium (pLLp). In this system approx. 100-140 cells migrate along the length of the developing fish embryo, depositing small clusters of cells, to form a sensory organ that will allow the fish to sense fluid flow and pressure [18]. The pLLp is a particularly interesting model because it includes a transition from well-coordinated motion to separation of small clusters (which form neuromasts or sensory nodes) from the main body. Indeed, the transition in migration and in collective behavior results may apply to not only understanding development but also components of diseases such as cancer metastasis.

1.1.2 Collective cell migration in the pLLp is guided by signaling.

The cells in the pLLp, similar to other instances of local collective cell migration, respond to both environmental cues and cell-cell signaling [19–24]. The focus of prior work regarding signaling in the pLLp has been on Wnt and Fgf signaling, which account for the pattern in which neuromasts are generated and deposited by the migrating primordium [18, 25–27]. There has been little work done, however, on other signals.

Our colleagues observed a qualitative disruption in the migratory behavior of the pLLp when an additional signal, Bone Morphogenetic Proteins (BMP), is inhibited. The first part of this dissertation quantitatively analyzes this change. BMP signaling has been shown to affect collective cell migration in other contexts as well, most notably cancer metastasis [28–31].

For a thorough analysis of the role of BMP, several inhibitors are used–including two concentrations of a BMP inhibitor, an Fgf inhibitor, and a myosin inhibitor in addition to a control condition. The Fgf inhibitor was selected due to the known role of Fgf signaling in the pLLp and the myosin inhibitor was selected due to cell movement being regulated through actin contractility and polymerization [22, 32–34].

1.1.3 Using insights from fluid flow and soap films for analyzing collective cell migration.

We will compare and contrast the changes in collective migration after the different perturbations listed above by capturing movies of collective migration. The movies are analyzed with Particle Image Velocimetry (PIV), a software tool that was originally developed for use in fluid mechanics to track the velocity of particles in motion [35–37]. It was recently adapted for use in biological applications by Dr. Rachel Lee at the University of Maryland, College Park [11, 15, 38–40]. This dissertation work further analyzes cell motility as a group using a novel procedure that involves masking the region of interest in time-lapse videos of the migrating pLLp and overlaying the same on full-image PIV output to selectively obtain the results of PIV on strictly the migrating pLLp.

In addition to examination of this group of cells moving collectively, I also zoomed in to examine the junctions of multiple cells to gain additional insight. In soap films and similar non-living systems, junctions where multiple bubbles connect yield insight into the mechanical stability of the system [41]. To examine the junctions of cells, I developed a method of guided visual observation to quantify the number of quadrijunctions (where four cells intersect) present in the pLLp. Quadrijunctions are rare and mechanically unstable in soap film, thus finding relatively many such junctions indicates increased stability in the pLLp.

1.2 Biophysics Education Research

The second part of this dissertation builds on the first part as it pulls from biophysics research to develop research-based problem sets for an introductory physics course. The focus of the second part is biophysics education research, with the overarching aim of developing materials for an IPLS course that are not just authentic physics or authentic biology, but cross-disciplinary authentic (CDA), a new term I will introduce and define below.

1.2.1 Calls for reform result in new introductory physics for the life sciences course sequence.

Starting in the early 2000s, there were numerous calls from the professional medical and life science community calling for reform in the undergraduate life science curriculum for courses supporting biology, such as introductory physics for the life sciences (IPLS) [42, 43, 52–61, 44, 62, 63, 45–51].

A response to these calls was the National Experiment in Undergraduate Science Education (NEXUS) which produced reformed physics, math, and chemistry courses [64, 65, 74–83, 66, 84–93, 67, 94–98, 68–73]. The UMD Physics Education Research Group (PERG) was responsible for the physics component of the NEXUS project and created a reformed two-semester IPLS course sequence as a result.

The IPLS course has not just new content, but also reformed pedagogy to bridge disciplines. The course is student-centered and focuses on active learning with both

small- and large-group discussion. These discussions enable a strong interdisciplinary reasoning component for the class. This reformed course pays attention also to authenticity, considering what is relevant/true in a given discipline, and to interdisciplinarity, addressing broad problem topics [75, 99–104]. While this course makes great strides in establishing a more career-relevant experience for the IPLS students, more can be done. During the initial offerings of the course, both physics and biology faculty members and course developers agreed that the course content was biologically relevant, but the students did not always view it as such.

1.2.2 Cross-disciplinary authenticity attends to problem-set outcome.

The need to develop problem sets that the students viewed as being biologically relevant and useful led me to propose and define a new term, cross-disciplinary authenticity (CDA). This differs from the current standard which focuses on posing problems with authentic biology and physics. CDA gives explicit attention to the learning outcome of the problem, specifically that students recognize its biological-authenticity. In this part of the dissertation, I present a specific example of a CDA problem set that builds on the collective cell migration research in the first part of this dissertation. The goal of the problem set is to learn about developmental biology using concepts from physics, mainly the Laplace pressure equation.

Through an iterative design process, the design team and a network of our colleagues and peers made modifications until converging on a problem set that we considered to be CDA, using physics concepts to gain biological insight. However, after

interviews with members of the IPLS student body, we found that they did not view one of the problem set components as producing biologically-authentic results.

1.2.3 An Epistemological Bridge leads to interdisciplinary modeling.

One of the components of the problem set was a modeling component. The design goal was to have the students engage in interdisciplinary modeling of a biological system. The students in focus group interviews, however, rejected the simplified model of the biological system instead of engaging with it. They made statements indicating that biology was too complex to be modeled and recognized no value for modeling living systems, though they appreciated the utility of modeling non-living systems.

In overcoming this design failure, I drew from the work of Clement, 1993 [105]. The students in Clement's study had conceptual difficulties with physics when in applying what they learned in an anchor case to a target case. Clement therefore inserted a bridging case to assist with the transition of physics concepts from one scenario to another. Concretely, the students learned about a hand on a spring and then were asked to apply the concepts from this scenario to a book on a table. This method works because it is easier to comprehend a close analogy than a distant one; the bridge divides the analogy into two smaller steps that are easier to comprehend than one larger step. This is a conceptual gap (the concept that even a solid table exerts an upward force).

In the case of the IPLS students, however, there is a stark difference. The students did not have a conceptual barrier as Clement's students did. Instead, they had a problem with the appropriateness of engaging in modeling. They understood

conceptually the physics of each scenario, but they didn't see modeling as being an accessible tool for use in cases of living systems. They had an epistemological barrier, in that their difficulties were related to the nature of knowing instead of being difficulties with the concepts. In this scenario, I identified the need for an Epistemological Bridge.

1.2.4 Problem set deployment was successful.

After insertion of an Epistemological Bridge into the IPLS problem set, the students in focus groups successfully engaged in interdisciplinary modeling in all cases and the problem set was deployed in the large-scale IPLS classroom recitation. Although the class norm in the recitation is not to write down answers to the recitation worksheets as they are ungraded and typically not collected, still almost 40% of students engaging with the interdisciplinary modeling component, which is a baseline for success in the effectiveness of the interdisciplinary bridge.

1.3 Proposal to recognize the field of Biophysics Education Research.

The first aim of this dissertation, to examine the role of BMP signaling in the pLLp, resulted in bringing principles and concepts from physics in to examine the biological system. It was found that the BMP signaling is critical for directionality and speed of migration.

The second aim of this dissertation, to develop a CDA problem set for IPLS, brings together content and perspective from biology into physics education research.

The work brought forth a novel method for achieving CDA and an Epistemological Bridge to successfully create a research-based, CDA problem set.

In addition to the work put forth in this dissertation, I propose that the work, itself, lies in a new domain, biophysics education research, bringing together biology, education, and physics.

Chapter II: Background

In this chapter of my thesis, I present background work from research conducted in the fields of biology, physics, and education. I first present background from biology and physics regarding cellular biomechanics, zebrafish as an animal model, and micropipette aspiration. This information provides a base level of biological and physics background upon which I pull in components from education to construct an educational biophysics problem set.

2.1 Collective cell migration occurs when a group of cells moves together.

I first focus on a biological phenomenon, localized collective cell migration. Local collective cell migration occurs when a group of cells moves together as a unit within a relatively stationary environment [3, 4, 8–11, 13–16]. This occurs in instances such as morphogenesis (which is the development of organs and organisms) and wound healing [4, 12, 17, 106]. Even in cases where cells had been thought to migrate as individuals, such as in the developing neural crest and during cancer metastasis, recent work has highlighted that some collective character of migration is still present [107, 108]. In the immune response of collective cell migration, cells relay signals and collectively attack infectious sites, and in cancer metastasis, collective migration away from the primary tumor occurs even as local strands of cells break away from the original site [3, 4, 12, 109].

Each of the previous examples of collective cell migration share common traits of a group of cells moving as a single unit in an *in vivo* environment. (*In vivo* studies examine cell motility in the live environment of the organism as it is naturally occurring.) By examining collective cell migration in the robust, reproducible context of morphogenesis, we can further understand its complexities and expand our knowledge of this phenomenon, potentially identifying overlapping traits relevant multiple instances of collective cell migration.

2.2 Collective cell migration can be observed *in vivo* in zebrafish.

Observation of collective cell migration during development is possible in embryonic zebrafish (*Danio rerio*) when a certain group of cells migrates down the fish body [18]. The embryonic development of zebrafish provides an excellent model for observing collective cell migration *in vivo* and thus has become a prevalent animal model as their use in research is growing rapidly both in the US and abroad [110–114]. One aspect of the zebrafish development, in particular, the voyage of the posterior Lateral Line primordium (pLLp), proves to be an excellent *in vivo* model for examining the biomechanics that occur during collective cell migration as it is a relatively well-defined system [18, 25, 26, 115]. And although it is a well-known system, little work has been done examining the biomechanics of the system and only recently have researchers began to explore the 3D aspects of migration.

This work examines the physical parameters of cell migration and quantifies perturbations of cell movement by examining and perturbing the pLLp. These

perturbations are examined not only in a baseline control environment but moreover in an experimental setting where the main lines of communication (signaling systems) between migrating cells are affected by varied inhibitors in varying quantities.

2.2.1 The embryos of zebrafish are transparent and genetic modification is straightforward.

Embryonic transparency in zebrafish occurs because the pigment has yet to develop in the embryo and aids in the ease of the visualization [116]. In the absence of any genetic modifications, the embryos will be transparent. Genetic modification may allow a subset of the cells in select lines of zebrafish to produce a tag that fluoresces after being illuminated via a specific wavelength [117]. This illumination enables the visualization of cells as they move throughout the organism. The fully sequenced genome allows for genetic modifications, and the transparent embryo makes opportune visualization of the fluorescent cells convenient.

The zebrafish used in my research were housed at the National Institutes of Health's Eunice Kennedy Shriver National Institute of Child Health and Human Development (NIH/NICHD) and bred using standard protocols (Figure 1) [118].

Figure 1. Mature Zebrafish.
Photograph of mature (adult) female zebrafish housed at the National Institutes of Health. Mature zebrafish are allowed to spawn naturally, and embryos are harvested for imaging. Mature zebrafish are approximately 1.5 +/- .5 inches in length in captivity.

2.2.2 Zebrafish embryos are easily collected and a superb size for imaging.

To mate in the wild, at dawn female and male zebrafish will deposit their eggs and sperm into the water, and the fertilization of the eggs occurs external to the female zebrafish body [116]. In captivity, female and male zebrafish are typically housed in separate compartments. When the collection of fertilized embryos is needed, male and female zebrafish are housed together overnight, and the process of fertilization will naturally occur once the simulated day lighting is present. Fertilized embryos can then be

collected from the bottom of the housing container. This external fertilization process is very convenient for embryonic experimental purposes.

Another feature that contributes to the desirability of zebrafish as a model organism is their small size. Unlike other situations in which one can observe collective cell migration in vivo, such as in a mouse ear, zebrafish are unique in that the entire organism can be viewed under a microscope. Many zebrafish embryonic experiments, such as the ones discussed in this body of work, capitalize on the ability to view a group of cells moving together, traversing the length of the fish body which is uniquely observable in this animal model.

2.2.3 Transgenic cells in the pLLp can fluoresce green.

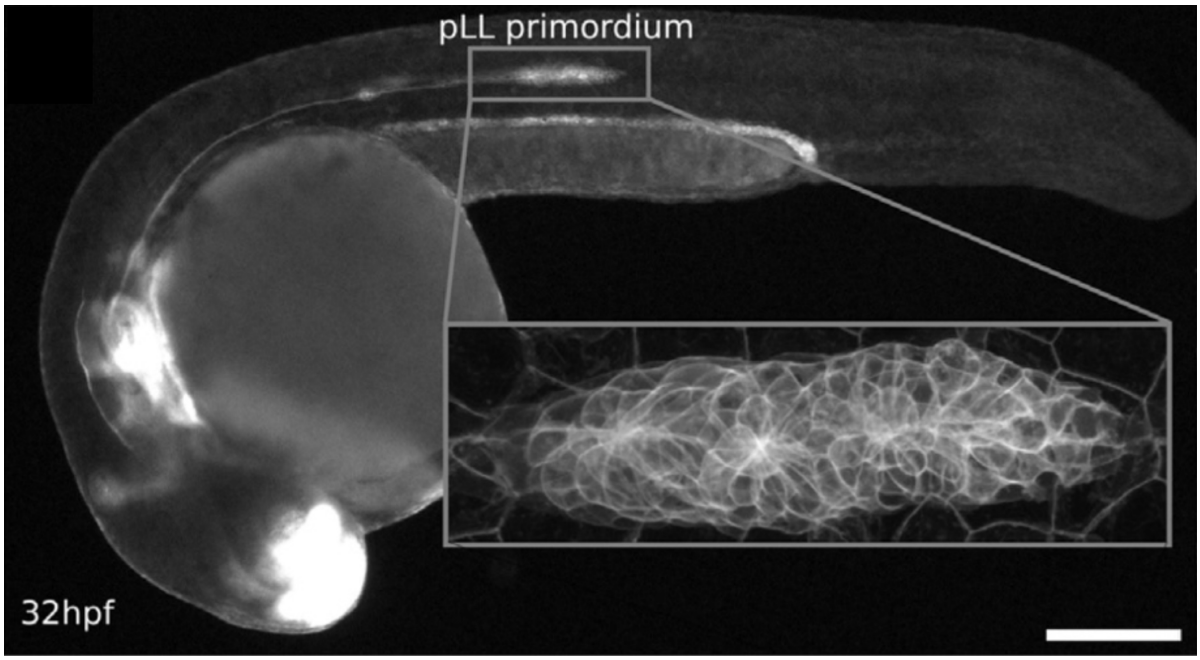
In the wild, zebrafish embryos are completely transparent during the first 22 hours post fertilization (hpf) as it is at this time that pigment begins to develop. Though possible, identifying specific cells, such as the cells in the pLLp, is difficult. It was, therefore, necessary to make modifications to the genetic makeup of the fish to enable imaging (through creating a transgenic zebrafish line). In this work, we utilized a popular transgenic line, the CldnB:lyn-GFP line (Cldn-B) [119].

The Cldn-B line was developed by first identifying a version of a green fluorescent protein (GFP) tag that is suitable and able to bind to the cell membrane. A GFP tag is well suited for this usage as it possesses the trait of fluorescing green when exposed to blue light [120]. In the transgenic construct, a GFP tag attaches to a specific cell membrane protein, Cldn-B where it is under the control of a Cldn-B promoter. All

the cells which express the Cldn-B protein appear green in color due to the GFP tag [121]. As a result, when exposed to blue light, the membranes of the cells fluoresce green. Since the cells in the pLLp express Cldn-B, the membranes of the cells in the pLLp are tagged with a GFP and therefore they can be easily observed via fluorescent microscopy.

2.2.4 The deposition of neuromasts is critical to zebrafish development and viability.

At approximately 24 hpf, the pLLp begins to migrate. This group of approximately 100-140 cells, travels along the horizontal myoseptum, which is a wall of connective tissue present during the embryonic stage [27, 122]. The group originates in a region near the developing head of the embryo, in the cephalic posterior lateral line placode. It then travels away from the head through the posterior region of the developing embryo to the end of the embryonic body, which is the tip of the developing tail.



Dalle Nogare 2017

Figure 2. Zebrafish embryo and pLLp.
Figure from Dalle Nogare 2017 [25]. Used with permission. Image shows pLLp in zebrafish embryo 32 hours post fertilization (hpf). The pLLp has deposited L1 neuromast. Inset shows a magnified view of an example pLLp. Scale bar is 200 μm.

As the pLLp collectively migrates down the posterior region of the developing embryo (Figure 2), it deposits groups of cells called neuromasts [18, 25]. The pLLp deposits approximately eight neuromasts over the course of the twenty-four-hour migration. These neuromasts develop as part of the lateral line which is a sensory organ whose function is to detect displacement in the surrounding aquatic environment in the developed zebrafish [25, 27, 123–125]. This process of neuromast deposition is critical to the viability of the fish.

2.3 Cellular biomechanics are crucial components to healthy cellular functioning.

Now that I have introduced the biological functioning of the pLLp, I would like to transition into a discussion on cellular biomechanics, a topic that brings together physics and biology. Here I discuss the Newtonian properties of pressure, motion, and force in the cellular environment and in micropipette aspiration. In Chapter 3, I investigate the cellular biomechanics of the pLLp and in the second part of my dissertation, I build on biomechanics (both micropipette aspiration and my research) to develop novel problem sets.

2.3.1 Cellular biomechanics yields insight into collective cell migration in cancer and in developmental processes.

Biomechanics can include concepts such as Young's modulus (elasticity), storage/loss modulus (viscoelasticity), general deformation of cell in response to a force, and other mechanical properties of the biological system. The examination of cellular biomechanics is useful for understanding healthy cells behave and for gaining insight into illnesses and diseases arising from cellular abnormalities. The examination of biomechanical parameters is commonly used to determine causes, methods of development, morphologic changes, and consequences of changes of disease [126, 127]. Recent work has paid increased attention to the physical forces involved in the collective

cell migration process, specifically regarding the forces cells exert on each other while migrating [128].

Pressure, for example, plays a role in cancer and in development. A standard model for a cell is a fluidic substance surrounded by an elastic cortical shell [129]. If the internal pressures of the cells are equivalent, the contact boundary will be planar. If they differ, the boundary will arc in the direction of lesser pressure, while also influenced by biological components. Some of these components include cell-cell adhesion and cortical actin networks that physically link the membrane and maintain the cortical tension in the cell [24]. The cortical actin network is a network of actin filaments that connect to each other and to proteins in the cell membrane. This influences cell surface mechanics and the control of cell shape [23, 130].

To anchor this phenomenon to biological relevancy, consider that pressure has implications for both the formation of carcinogenic tumors and in embryonic development. Prior work has shown that when external pressure is applied to malignant mammary cells, they revert to a normal growth phenotype [131]. Pressure differentials play an essential role in developmental biology, as in the formation of the sensory system's neuromasts during embryonic zebrafish development after deposition by the posterior lateral line primordium. Each pressure differential corresponds to differing forces from multiple components in the system.

2.3.2 Membrane forces balance in static scenarios.

Each cell in a group of cells has its own set of pressures and forces acting on it. As a simplified scenario, we consider a single cell from the group of cells to create a simplified model. For this single cell, we consider the balance of forces at a small area of its membrane as it is suspended in medium (Figure 3). There is an internal pressure (F_1) yielding a force on the select area coming from the internal components of the cell. There is an external pressure (F_2) from the surrounding medium on the cell. (In the second part of this thesis, the external pressure will be replaced with the suction from the micropipette and then by other cells.) There is surface tension to the left (F_3) and to the right (F_4). In this simplified model, these are due to the membrane tension in the cell. As this is a three-dimensional structure, there will also be forces in the z-dimension. For illustrative purposes, those are not shown here.

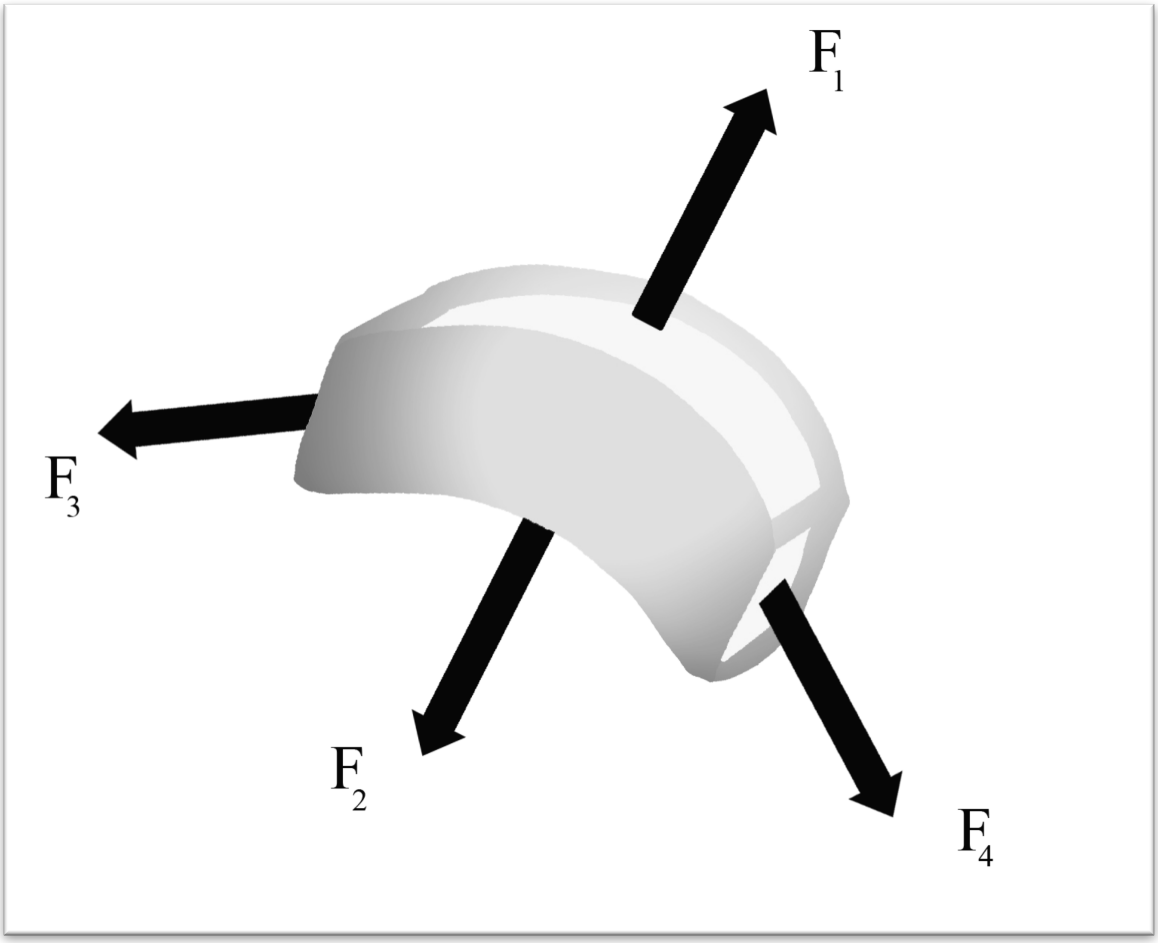


Figure 3. 2D Membrane Forces.

A schematic illustration of a select region of the membrane of a single cell and select forces acting upon the region. The membrane is shown in shades of gray. The membrane components and other subcellular components are not illustrated. The forces due to pressure and surface tension are shown via black arrows in their direction. The magnitude of force is not shown. The forces are as follows: F_1 is the force due to the internal pressure of the cell on the select membrane area. F_2 is the force due to the external pressure on the cell membrane. F_3 and F_4 are the forces due to tension.

The shape of the membrane sections gives us information about the balance of forces. This in turn tells us which parts are under greater pressure. In the example the pressure inside of the cell is greater than the pressure outside of it.

2.4 Micropipette aspiration allows for quantification of biomechanical properties.

Some techniques such as Brillouin microscopy, atomic force microscopy, optical traps, and micropipette aspiration can be used to characterize cellular mechanics [132–138]. One of these methods in particular, micropipette aspiration, is a classical technique in which enables quantification of cellular pressure, viscoelastic moduli, and deformability, stiffness, and geometric behavior [129, 139]. Combining micropipette aspiration with other techniques allows the study of cell volume, for example [140]. I am presenting background information on this technique in this chapter because it has a role in my educational work.

In the process of micropipette aspiration, the cellular membrane deforms in response to an application of a small amount of negative pressure via a micropipette on the surface of the single living cell. The cellular membrane deformation is measured by tracking the protruding deformation of the cell into the micropipette (Figure 4.) [139].

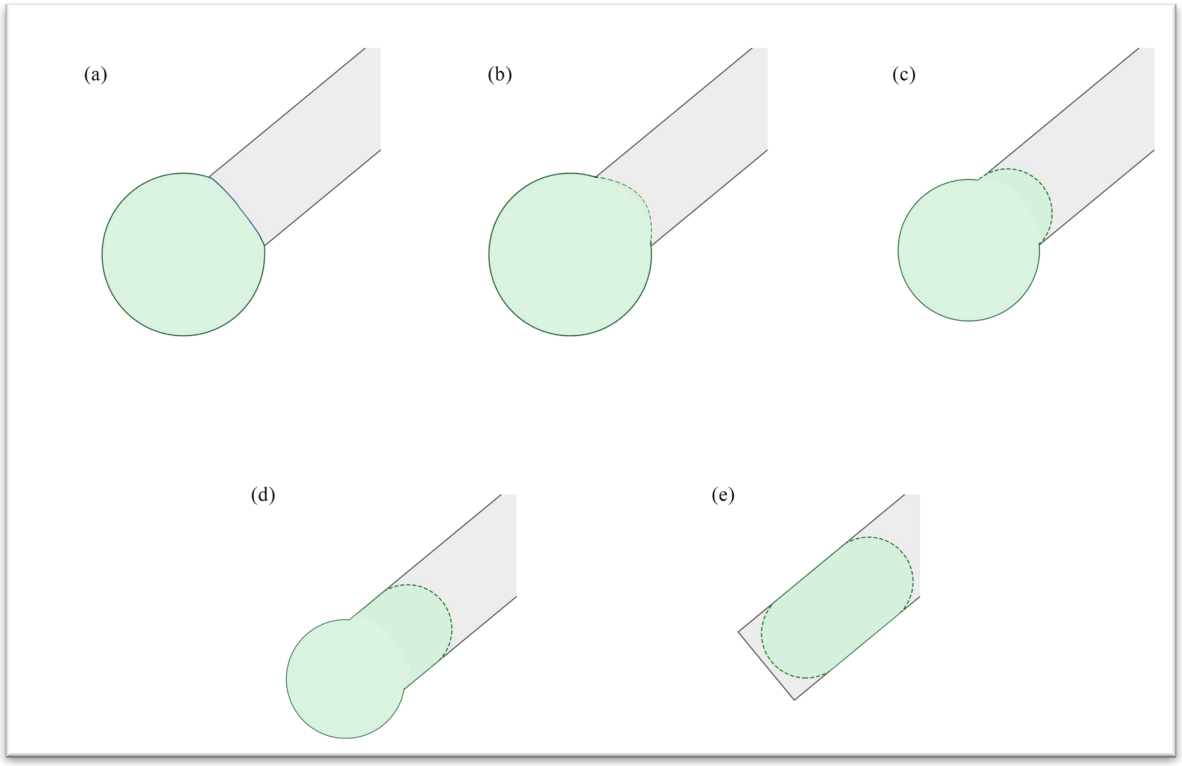


Figure 4. Stages of Micropipette Aspiration.

4. (a) – (e) An original schematic illustration of a single cell (solid green) being sucked into a pipette (gray) during the process of micropipette aspiration. The dotted lines represent the boundary of the cell once it is aspirated into the pipette. (a) the cell is touching the micropipette before aspiration begins. (b) The cell protrudes into the pipette as the aspiration process begins (c) The protrusion of the cell into the pipette at this point is equal to the diameter of the pipette and creates a hemispherical dome. (d) The cell is further aspirated into the pipette. (e) The entirety of the cell body has been aspirated into the pipette.

Applying our consideration of forces to the case of micropipette aspiration, we see that force F_2 is decreased. Thus in order for the forces to remain balanced, components of F_3 and F_4 must increase in the F_2 direction. If the surface tension of the cell remains constant, then F_3 and F_4 must rotate towards F_2. The rotating of F_3 and F_4 results in an increased curvature of the surface of the cell, which can also be expressed as a decrease in the radius. Coating the micropipette walls enables a minimization of

adhesion of the cell membrane to the walls thus minimizing any extraneous, interfering forces.

2.4.1 Laplace Pressure relates pressure to radius and surface tension.

In addition to understanding pressure differentials, we can also examine the relationship between pressure, surface tension, and radius. Considering the balance of forces acting on a given interface, one can obtain the Laplace pressure, determined from the Young-Laplace Equation [141, 142]. This describes the pressure difference (ΔP) between two substances as being equal to a uniform surface tension (γ) at the interface times the local mean curvature of the interface. R_1 and R_2 being the principal radii of curvature:

$$\Delta P = \gamma \left(\frac{1}{R_1} + \frac{1}{R_2} \right)$$

For the case of a perfect sphere, $R_1 = R_2 = R$, therefore:

$$\Delta P = 2\gamma \left(\frac{1}{R} \right)$$

In a review article, Laplace pressure is applied to the cell-micropipette system to which the research method of micropipette aspiration is being applied [129]. During the process, the external pressure is decreased via a micropipette suction system, whereby the inward force on the cell at a particular area in contact with the pipette is decreased. The result is a protrusion of the cell into the micropipette creating an area of increased curvature.

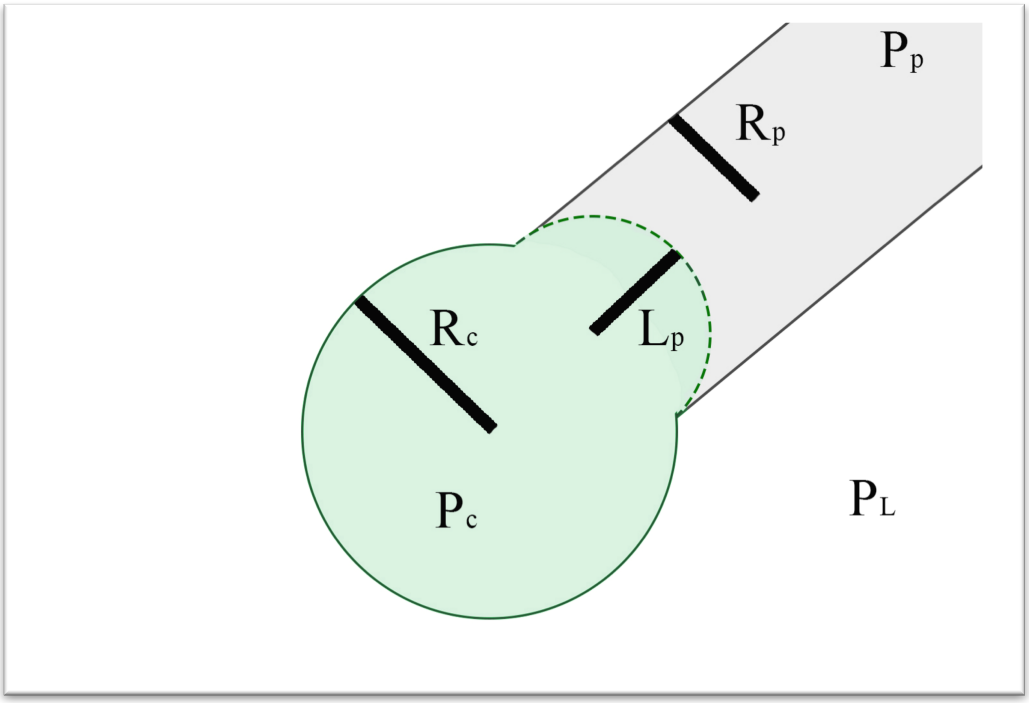


Figure 5. Laplace Pressure in Micropipette Aspiration.

A schematic illustration of a moment during the process of micropipette aspiration. A single cell (shaded solid green region) is being sucked into a pipette (shaded gray region). The dotted lines represent the boundary of the cell once aspirated into the pipette. The protrusion of the cell into the pipette at this point is equal to the diameter of the pipette and creates a hemispherical dome. The illustration includes the following labels: P_c - pressure inside of the cell; P_p - pressure inside of the pipette; P_L - fluidic pressure; R_c - radius of the cell; R_p - radius of the micropipette; and L_p - extension of the cell into the micropipette.

Referring to the figure and considering the pressure difference between the cell (P_c) and the surrounding fluid (P_L) in the general case, we obtain:

$$P_c - P_L = 2\gamma\left(\frac{1}{R_c}\right)$$

Considering the pressure difference between the cell (P_c) and the pipette (P_p) in the general case, we obtain:

$$P_c - P_p = 2\gamma\left(\frac{1}{L_p}\right)$$

Hochmuth *et al* [129] describe that as the cell is sucked into the micropipette from its initial state, the increased curvature will lead to the formation of a hemisphere. The hemispherical shape will exist when the ratio of the extension of the cell into the micropipette (L_p) and the radius of the micropipette (R_p) is equal to one.

$$\text{when } \frac{L_p}{R_p} = 1,$$

$$P_p - P_L = \Delta P = 2\gamma\left(\frac{1}{R_p} - \frac{1}{R_c}\right)$$

If the pressures in the liquid and pipette are known, the above can be used to determine the cortical tension by rearranging the above equation:

$$\gamma = \Delta P \left(\frac{R_p - R_c}{2}\right)$$

This equation allows us to compute cortical tension. Knowing the surface tension allows one to infer the pressure inside of the cell, which does not appear in this equation and may not be known. Examples of the size of changes in detectable parameters include +/-25 nm changes in membrane displacement and pressure changes as small as 0.1-0.2 pN/μm^2 [129]. Knowing these cellular parameters gives insight into biological components such as molecular motors, bleb growth and nucleus dynamics [143]. Additionally, the application of the Laplace pressure to the cell-micropipette parameters examined during micropipette aspiration provides an excellent opportunity for the designing of a research-based biophysics problem set, as shown in the second part of this dissertation.

2.5 Dual-cell interface examination reveals pressure difference.

After considering the cell-micropipette system, we now consider a slightly more complex biological system. In this section, I describe a two-cell system. The system includes two approximately spherical cells of the same type that are touching and suspended in medium. An examination of the interface of these two cells yields insight as to the internal pressures of the cells relative to each other and to the environment.

Recall from the prior discussion that if there is curvature in the boundary, then there must be a pressure difference on the alternate sides of the boundary. If the two cells in physical contact maintain identical pressures, there will be no curvature in the region of their touching membranes. It will be a straight line. This scenario (Figure 6) shows two similar cells modeled as a fluidic substance surrounded by an elastic cortical shell. Taking a cross-sectional slice of the two cells suspended in solution, we can consider the interface between the two cells and their environment. We determine that from the aforementioned equations regarding Laplace pressure in our simplified model that the two cells in Figure 6 (a) have identical internal pressures.

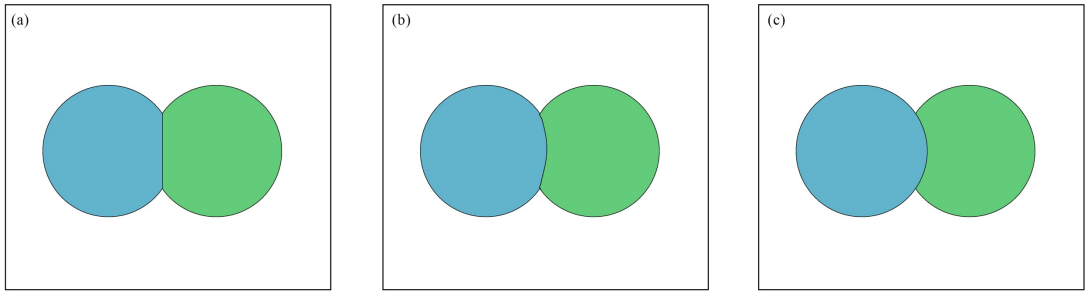


Figure 6. Modeling Two Cells.

6. (a) – (c). A schematic illustration of a given cross-sectional slice of two spherical biological cells of unspecified but similar type. (a). The cross-sectional slice of two cells of equal internal pressure with a planar interface. (b). The cross-sectional slice of two cells with slightly different internal pressures and an interface that protrudes in the direction of the cell of lesser pressure (the green cell). (c). The cross-sectional slice of two cells with much different internal pressures and an interface that protrudes in the direction of the cell of lesser pressure (green) so much so that the cell of greater pressure (blue) is able to maintain a spherical shape.

Alternatively, when the two touching cells do not maintain similar pressures, the cellular interface will arc towards the direction of lesser pressure, protruding into the cell of lesser internal pressure as is illustrated in Figure 6 (b).

Finally, we consider a third case in which the cell with the greater pressure, as illustrated in Figure 6 (c) maintains a spherical shape, illustrated by a circular cross-sectional slice. In this case, the internal pressure of the cell of lesser pressure is approximately similar to the pressure of the surrounding liquid, relative to the internal pressure of the cell of greater pressure.

Knowledge of the mechanics and formations of adjacent cells due to their internal pressure differences and the interplay of external forces among groups of cells provides a groundwork for studying cell migration and the involved signaling pathways.

2.5.1 Visual observation of circular shape yields insight into cell division.

In addition to computation methods, visual examination can yield information regarding cell behavior. Two such behaviors presented in this section are cell division and cell-cell adhesion.

For example, Figure 7 shows the boundaries of cells in the pLLp. Except for two of the cells, the cells in the image have irregular, non-circular shapes. The two circular cells are located in the back-end, upper-middle region of the pLLp and in the front-end, bottom region on the perimeter.

Hypotheses about the biological behavior of these cells can be formed through observation of their shapes. One hypothesis is that both of these cells are undergoing cell division. Since this is the first frame of a multi-frame time-lapse, the hypothesis can easily be confirmed. The time-lapse shows the front cell dividing in frame 13, and then both daughter cells return to an irregular shape. The back cell divides and the daughter cells return to an irregular shape in frame 68. In addition to observation of the biological behavior, we can also consider the principles of physics.

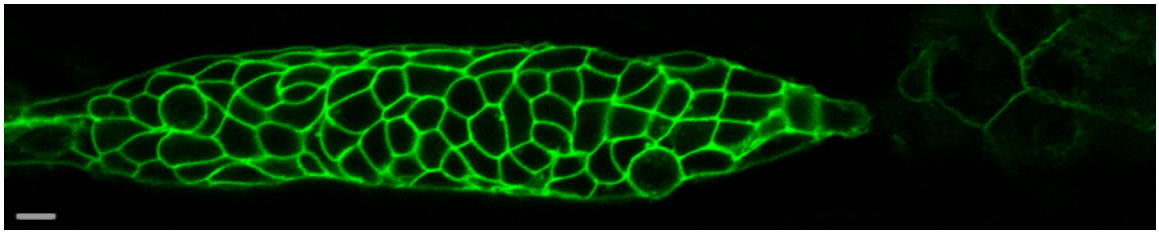


Figure 7. Confocal Image Slice Showing Migrating pLLp.

This figure shows a horizontal confocal slice of the pLLp in a control embryo. The green outlines in the image are cell membranes. There are approximately 90 cells in the image. The scale bar is 10 μm.

From physics we know that forces are involved in all physical scenarios. A biological scenario is a physical scenario; therefore, forces are involved. Pressure is the term that describes a force over a given area and plays a role in this scenario—in determining cell shape. In terms of the role that pressure plays in determining the shape of a dividing cell biologically, prior work has shown that the dividing cells are under high pressure [144]. This is consistent our observation of the boundary of the dividing cell.

2.5.2 Cell-cell junctions may also influence shape during cell division.

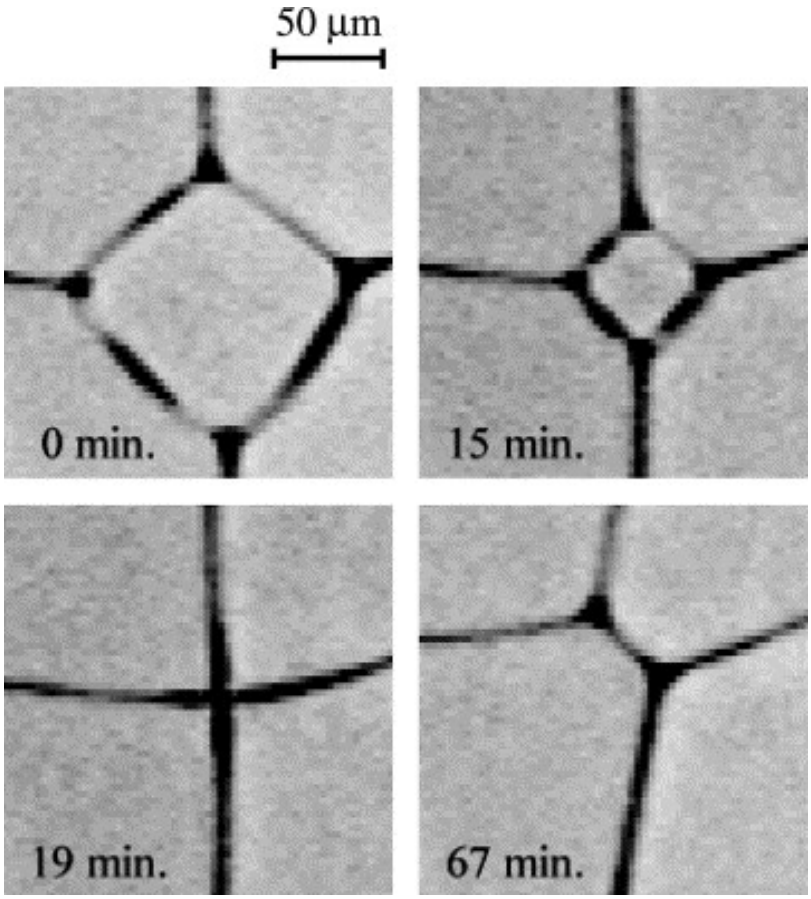
One hypothesis is that cell-cell junctions also contribute to the shape. As the cell is going to divide it could release cell-cell junctions with its neighbors, thus pulling away during cell division. Applying the principles of physics means—in the simplest model of a cell—that a cell would have to be at least partially attached to its neighbors because if the other cells were detached, the cell-cell junctions would be orthogonal, neglecting internal biological structures. Such shapes are not observed in the cells surrounding the dividing cells. Whether this is due to biological structures prohibiting the deformation or to partially released cell-cell junctions is an open question.

Cells under a known higher pressure without interference from being mechanically coupled from other cells, would display a circular shape. When cells are mechanically coupled, however, they also influence each other through specific cell-cell adhesion proteins. These include N-Cadherin, E-Cadherin, EpCAM, and Z0-1 and influence cell behavior in zebrafish development [18, 145, 154–156, 146–153].

2.5.3 Visual examination of cell boundary intersections may yield insight into changes in cellular functioning.

In addition to visual examination enabling the predictive nature of cell shape for future cellular behavior, the intersections of the cell membrane boundaries where multiple cells are in contact can yield insight into cellular functioning. Since there are a number of cells in the migrating pLLp, there are ample cell intersections throughout the collective group of cells.

Visual examination of these intersections allows the identification of perpendicular intersections where four cells are in contact, each single cell touching the other three in the juncture, termed a quadrijunction. An example of this type of intersection in a soap film, an inanimate non-biological system, is shown in the bottom left of Figure 8 [41].

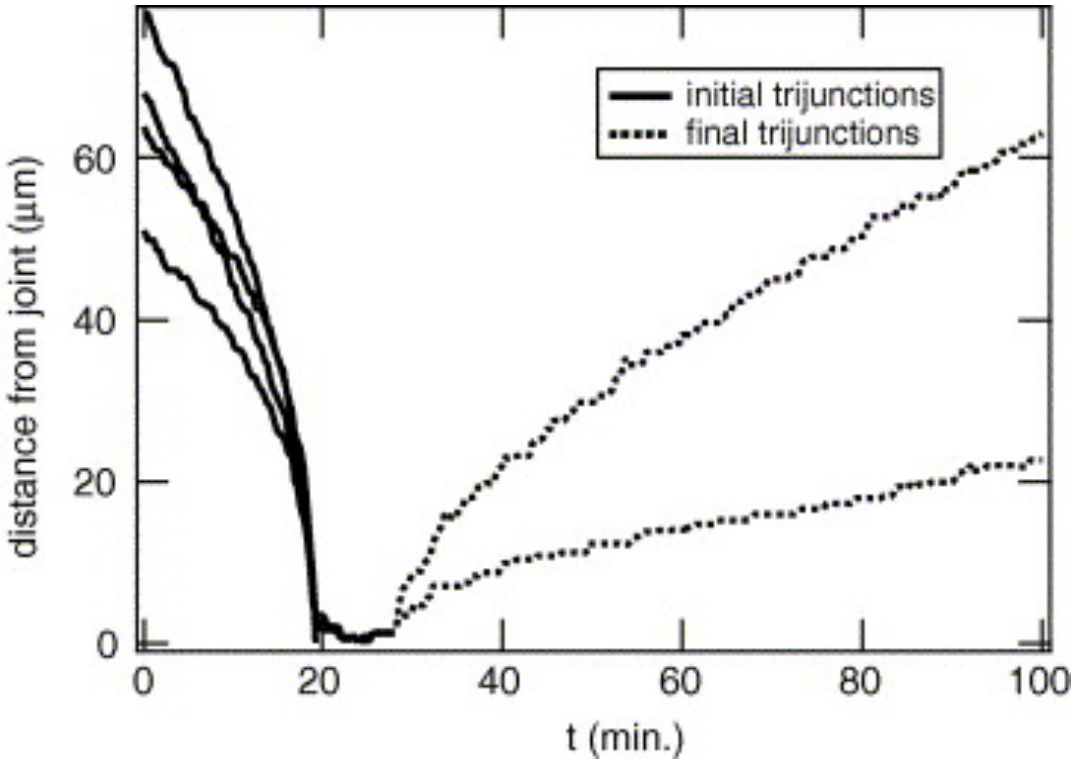


Lee and Losert 2005

Figure 8. Junctions in a Nonliving System.

From Lee 2005 [41]. Used with permission. Each of the four images illustrates a different type of juncture. The top left shows four trijunctions. The top right also shows four trijunctions. The bottom left is one quadrijunction. The bottom right shows two trijunctions.

In the soap film, it is very common to observe trijunctions throughout the environment. It is very rare, in contrast, to observe quadrijunctions. In the soap film scenario, these junctions can only occur when there is a perpendicular intersection of equal pressures. Figure 9 illustrates the time that is spent both in and out of the quaridjunction.



Lee 2005

Figure 9. Rarity of Quadrijunctions in Nonliving Systems.

From Lee 2005 [41]. Used with permission. This graph shows the distance that a trijunction is away from a quadrijunction location versus the time spent at each location. From this graph we observe that the majority of the time is spent out of the quadrijunction formation, illustrating the rarity of the event.

If these biological cells were modeled as merely nonliving membranes with no internal structures with no biological functioning, we would expect to observe no change in the number of quadrijunctures across the various conditions. What we observe, however, is that there is indeed a change in the quantity of these junctures. Therefore, when interpreting the observations, it is recognized that modeling these cells as nonliving membranes would not suffice. We consider biological parameters such as the cell cortex, actin filaments, and cell-cell adhesions in our biological interpretation of the results.

2.6 Cell signaling is critical for collective cell migration.

In addition to mechanical components between cells, there are also chemical components. As cells move collectively, whether it be in cancer metastasis, immune response, or morphogenesis, they communicate with each other through chemical signals which guide their behavior [5–8, 11–16]. Migrating cells respond to both environmental cues and cell-cell signaling [9, 13, 18, 20, 25, 157]. Cells also have specific cell-cell adhesions that physically link two cells together [158–160].

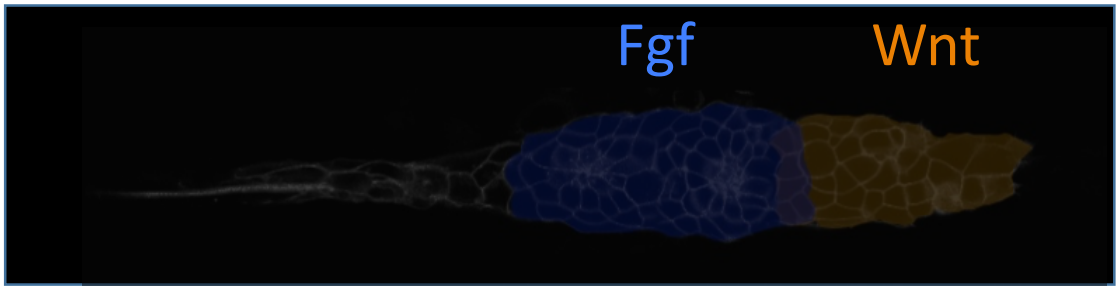
2.6.1 Cell signaling is prevalent in zebrafish lateral line development.

Paracrine signaling is a type of cell-cell signaling which operates over short distances and functions when a ligand (a particular signaling molecule) binds to a particular receptor (the location which accepts the ligand) on the cell surface [14, 20, 24]. For example, a Wnt ligand would bind to a Wnt receptor on the cell surface, triggering a cascade of events inside of the cell. (In this work, I do not focus on the internal cascade of events, only the communication between the cells.) This type of signaling is critical for the successful migration of the pLLp. While a number of signals are prevalent in the pLLp, this study focuses on the role of Wnt, Fibroblast Growth Factor (Fgf), and Bone Morphogenetic Protein (BMP) signals.

These three signals, Fgf, Wnt, and BMP, are all conserved in the animal kingdom [20]. Much of the prior work regarding the coordination of cell fate, morphology and migration in the pLLp focuses on the role of Wnt signaling and Fgf signaling [18, 25]. It

is well-established through this prior work that both Fgf and Wnt signaling guide the migratory behavior of the migrating pLLp [18, 25].

The pLLp can be characterized into two zones: the front (leading domain) and the back (trailing domain)[25]. The cells in the leading domain—where Wnt signaling is active—display different characteristics than the trailing domain. The cells in the leading domain are more mesenchymal in nature (flat and unformed) and the cells in the trailing domain are more epithelial in nature [25]. In the trailing domain, rosettes are being formed and Fgf signaling is active. In short, Wnt signaling operates in the leading domain and Fgf signaling operates in the trailing domain (Figure 10).



Dalle Nogare (unpublished)

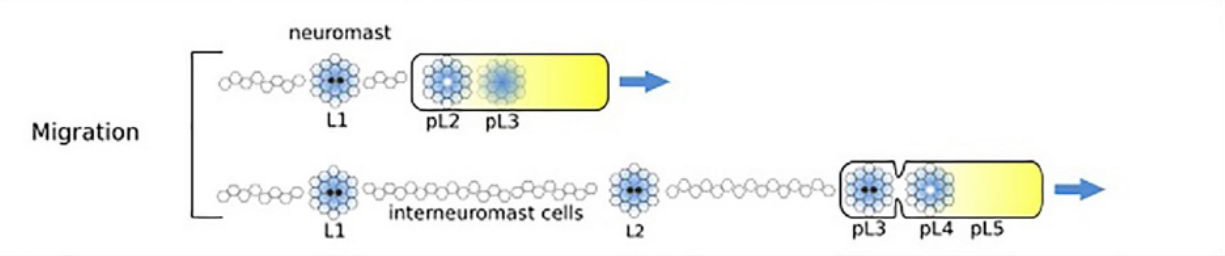
Figure 10. Fgf and Wnt Signaling Domains

From Dalle Nogare (unpublished). Used with permission. Composite image shows an example confocal slice of a pLLp during migration. Light regions indicate cell membranes. Color overlays indicate regions in which Fgf signaling and Wnt signaling are active. The active region for Fgf signaling is shown in blue in the trailing domain, and the active region for Wnt signaling is in orange in the leading domain.

Before the dual signals are present in the pLLp, only Wnt signaling is present. Beginning at approximately 18 hpf, Wnt signaling is expressed throughout the entire pLLp. The mechanism by which this occurs is an area active of research. By

approximately 24 hpf when the pLLp begins its migration, the known signaling pattern of Wnt in the leading domain and Fgf in the trailing domain is present [161, 162].

Wnt signaling is activated in a cell when the ligand Wnt10a binds to its receptor on the cell's surface [163, 164]. Wnt signaling, which is active over the entire pLLp, becomes restricted to the leading domain as Fgf signaling begins to be established in the trailing domain. In the leading domain, the cells that have Wnt signaling activity produce two Fgf ligands, Fgf3 and Fgf10a [25]. These same cells also express Fgf inhibitors, like Sef1 and Dusp6, which inhibit the activity of the Fgf receptor in the leading domain, ensuring that the leading domain is only responding to Wnt signals [165].



Dalle Nogare 2017

Figure 10. Wnt and Fgf Signaling in Neuromast Deposition.

Dalle Nogare 2017 [25]. Used with permission. Schematic showing generalized regions of operation of the signals in the pLLp. The active Wnt signaling region is shown in yellow and active Fgf signaling region is shown in blue. The arrow below the schematic shows the direction of migration, with Wnt signaling being in the leading cells and Fgf signaling being in the trailing cells. Deposited neuromasts are also shown in blue and interneuromast cells are outlined in gray Neuromast are described in this work.

Fgf ligands secreted by Wnt actives Fgf signaling in the trailing domain where Fgf receptors are expressed. These ligands bind to Fgfr1 and activate Fgf signaling. Once Fgf signaling is activated, it triggers the expression of a secreted Wnt inhibitor, thus making Wnt and Fgf signaling mutually inhibitory. Once Fgf signaling is established, it

plays a role in the formation and maintenance of rosettes. In contrast, the cells in the leading domain can be described as mesenchymal in nature and do not form rosettes (Figure 11).

2.6.2. Why investigate BMP?

BMP signaling has a less-established role in pLLp migration. As a signal, its role is to transmit information between cells, and it is present in multi-cellular organisms throughout the animal kingdom [166, 167]. BMP signaling was discovered for its role in bone and cartilage formation [167–172]. It is also known to play a critical role in other processes such as cell growth, cell apoptosis, and cell differentiation during embryogenesis; and fracture healing, joint integrity, and vascular remodeling as part of adult tissue maintenance [171–178]. Additionally, prior literature shows that BMP also plays a role in collective cell migration in embryonic chick development and mouse muscle formation [179–181].

Since BMP has been shown to have a role in collective cell migration in other scenarios, two questions naturally arise. The first is if BMP signaling has a role in the collective cell migration of the pLLp. The second is if it does, can we identify in what locations of the pLLp it is active.

Prior inhibitors were unsuccessful in targeting BMP and therefore, until recently, such questions could not be answered. Fortunately, a new chemical inhibitor of BMP was recently developed. This inhibitor, K02288, has a high specificity for BMP type I receptors and allowed us to examine the role of BMP in the pLLp [182].

2.7 Inhibitors can disrupt cell contraction and communication in cell signaling.

This work uses the inhibitors SU5402 (an Fgf inhibitor), K02288 (a BMP inhibitor), and Blebbistatin (a non-muscle myosin II inhibitor) to disrupt either intercellular communication or cellular contraction as described below. The controls were kept in the same amounts of DMSO. The embryo preparation and imaging used for this work were done by my colleagues in the Chitnis lab.

2.7.1 SU5402 inhibits Fgf signaling.

As discussed earlier, Fgf signaling is known to play a vital role in pLLp migration and is responsible for playing an active role in enabling communication between cells during cell motility in multi-cellular organisms. SU5402 is a chemical inhibitor which blocks the functioning of the Fgf receptor on the cell surface and thus prevents cells from responding to Fgf ligands [183]. This inhibitor, SU5402, is added to the medium in which zebrafish embryos are housed. In other studies, concentrations of this inhibitor range from 5 μM to 60 μM [184–186]. In our work, we used 5 μM SU5402, the lowest effective dose.

2.7.2 K02288 interrupts BMP Signaling.

K02288 is a recently-developed, highly-selective small molecule inhibitor of the BMP receptor kinase ALK-2 (ACVR1) [171, 172, 182, 187]. While much is known about the mechanisms of BMP signaling pathway, this work focuses on its role in pLLp migration. We used two concentrations of K02288: 10 μM and 15 μM. There has been limited work using K02288 in zebrafish, but what has been done used concentrations in the 10-20 μM range and noted that 40 μM resulted in unusable data [188, 189].

2.7.3 Blebbistatin disrupts a cell's contractility.

Cells are known to move via a multi-step process that includes protrusions, adhesions, and contractions [190, 191]. Non-muscle myosin II is a molecular motor inside of a cell that plays a role in cellular contraction, one of the key component of cell migration [192]. Blebbistatin is a chemical that inhibits the functioning of non-muscle myosin II. Since non-muscle myosin II is responsible for producing cellular contraction, the introduction of blebbistatin inhibits the contractile forces in the cell [193–195]. While it is known that the contractile forces in the cell are impacted, it is not known how the aspects of collective cell behavior such as pLLp migration are affected. This study seeks to identify how the behavior is altered in a quantifiable way. In this work we used 12.5 μM of blebbistatin. Other studies have shown effectiveness from 5 - 10 μM but used up to 50 μM [196, 197].

There have been some concerns regarding the phototoxicity and photoinactivation of Blebbistain after exposure to blue light [198, 199]. For our experiment, we used a significantly lower concentration and with significantly less time duration and intensity of light than that which has been shown to interfere.

2.8 Increasing knowledge is important both in research and in an education.

The first part of this thesis focuses on experimental biophysics, research done at the intersection of physics and biology. The generic aim of research is to increase knowledge and understanding of the world around us. In the case of collective cell migration in this work, I started with a set amount of knowledge of the given biological system. I then used physics tools and concepts to increase our understanding of the system. Finally, I will discuss how the results from using these tools will yield additional insight into this biological system, increasing our understanding of biology (Chapter 3).

In short, research starts with a base level of understanding that is expanded through the application of tools, principles, and concepts to reach a higher level of understanding. This operating practice for researchers in a laboratory also applies to students in a classroom in that we use tools to increase knowledge. In the second part of this thesis, I bring in a third field, education. Here I provide background for this second part, focusing on biophysics education research.

2.9 The field of Physics Education Research (PER) studies student learning in relationship to physics.

Typically housed within physics departments, researchers in the field of Physics Education Research (PER) study the aspects of student learning in the physics classroom. There is typically a wide variety of students who take University-level physics courses, such as physics, engineers, other science, health, and education majors among students from other disciplines across a given institution.

Some institutions such as the University of Maryland, College Park (UMD), offer courses specifically tailored to students from the given disciplines. One such example is a two-semester course sequence at UMD, the Introductory Physics Course for the Life Sciences (IPLS), designed specifically for life science and pre-health majors.

2.9.1 The medical and biological research communities issued calls for undergraduate education reform.

Over the past years, there have been a number of calls for undergraduate life science education reform from the professional medical and life science communities to reform the undergraduate biological science curriculum. These professionals felt like their undergraduate education insufficiently prepared them for their careers and was out of touch with the evolving world and current problems of the 21st century. A number of calls went out and were published in places such as the AAAS/NSF *Vision and Change* Report, the HHMI and the Association of American Medical Colleges' *Scientific*

Foundations for Future Physicians Report, and Bio2010: Transforming Undergraduate Education for Future Research Biologists among a number of national reports and publications [42, 43, 52–61, 44, 62, 63, 45–51]. There existed a mismatch between the undergraduate science curriculum and needs of professionals—both in content and in pedagogy.

In addition to these published documents, individuals also called on their colleagues to make a change. One such notable event occurred during a session at the 2010 AAAS meeting in which the recommendations made in the *Vision and Change* Report were being discussed. The co-chair of the session and first author of the *Vision and Change* Report, Dr. Carol Brewer, gave the following charge to the community:

> *"We all have work ahead of us to ensure that the transformations we make in undergraduate biology classrooms around the country reflect the biology we do in the twenty-first century. I am confident our community is up to the challenge. Because, after all, if not now, when? And if not us, then who?"* [46]

The pressing call is for the need to completely revolutionize the undergraduate education system for life sciences students as we know it. This is the background that describes the indisputable need to respond to the urgent call for reform.

2.9.2 The National Experiment in Undergraduate Science Education was the response.

The response to these increasingly active calls came from the Howard Hughes Medical Institute (HHMI) and the National Science Foundation (NSF). Following the

recommendations in these reports, they established the National Experiment in Undergraduate Science Education (NEXUS) Project in 2010. NEXUS was a successful multi-university multi-disciplinary project with the goal of achieving science and math course reform in biology education [65, 66, 78–87, 70, 88–97, 71, 98, 72–77].

The aim of the NEXUS project is to reform undergraduate science education for biological science students. This reform is accomplished through the redesign, development, and assessment of new interdisciplinary content and courses that more appropriately reflect the dynamic, interdisciplinary nature of modern-day scientific research and science-based medical practice.

The institutions involved in this project include researchers at Purdue University who focus on "integrating biology and chemistry"; the University of Maryland, Baltimore County who focus on "infusing math into biology"; the University of Maryland, College Park who focus on "teaching the physics of life"; and the University of Miami, who focuses on "using case studies to integrate scientific disciplines". Three distinct overseeing bodies coordinated the activities of each of the different institutions; they are the executive steering committee, the global assessment committee, and the advisory board.

2.9.3 Introductory Physics for the Life Sciences is UMD's component of the NEXUS project.

Having the "teaching the physics component" of the NEXUS project, (NEXUS-Physics), the Physics Education Research Group (PERG) at UMD introduced a reformed

Introductory Physics for the Life Sciences (IPLS) course in 2011. This interdisciplinary course developed a curriculum based on solid educational research that not only responds to the prior calls for reform but also increases the value which students place on interdisciplinarity and enhances their ability to transfer knowledge across disciplinary boundaries [79, 88].

This curriculum is a two-semester sequence with the two courses titled Physics 131 Fundamentals of Physics for Life Sciences I (PHYS131) and Physics 132 Fundamentals of Physics for Life Sciences II (PHYS132). The course content includes attention to atoms, molecules, and chemical energy, the thermodynamics of entropy and free energy, and has a statistical physics viewpoint [89, 90]. Reformed laboratories also reflect the process of science and the course content [91].

Additionally, the lectures are in a "flipped" formation with highly interactive components including ample group work in the main recitation and readings conducted prior to class. The course components specifically focus on student-centered learning, active learning, small group discussion, large group discussion, and interdisciplinary reasoning.

Additional work stemming from this project has been done on students' reasoning and the educator's positioning of the scientific domains [83, 200]. Results from other continued work demonstrated that upon viewing physics as being authentic and relevant to the life sciences, life science students shift in their identification with, affect towards, and ways of knowing physics [97]. While this course pursues the incorporation of authentic biological examples, more work is needed.

This dissertation discusses not only the role physics plays in determining cell biomechanics and results from experimental biophysics research (Chapter 3) but also the formation, content, and deployment (Chapters 4, 5, and 6 respectively) of a biologically-authentic, research-based problem set and the influence of the course educator (Chapter 7) in an IPLS setting.

Chapter III: The Effects of BMP Signal Inhibition in the Posterior Lateral Line Primordium Migration in Zebrafish

In this chapter I present ways in which the principles of physics can be applied to a select living biological system, the posterior Lateral Line primordium (pLLp) in zebrafish, to gain insight into the biological functioning of the system. This work was carried out in collaboration with Dr. Ajay Chitnis's biological research group at the National Institutes of Health (NIH) Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD).

3.1 Introduction

Collective cell migration, the migration of a group of cells as a single entity, is a hallmark of processes such as development, wound healing, and cancer metastasis [3-17]. The posterior lateral line primordium (pLLp) in *danio rerio* (zebrafish) presents a model to study collective cell migration (as introduced in Chapter 2). It is a group of approximately 140 cells which originate from a specific region in the head of the embryonic zebrafish (the epithelial placodes). The pLLp begins its migration around 22 hpf (hours post fertilization) and migrates along a well-defined track (the horizontal myoseptum), depositing neuromasts at regular intervals. After deposition, these neuromasts mature to form the lateral line sensory system, which plays a role in detecting water currents and prey, and enables collective swimming (schooling). The cell cohort in the pLLp has a coordinated migration pattern with cell-cell communication happening at

multiple levels involving the interplay of several signaling pathways. A few of these signaling pathways, namely Wnt, Fgf, Notch, and chemokine signaling, have been studied extensively.

The process of pLLp migration is initiated when the Wnt signaling is expressed over the entirety of the primordium. (The triggering of this expression is a question of active research.) Wnt signaling expresses both Wnt ligands and Fgf ligands—fgf3 and fgf10. These ligands, however, cannot activate Fgf signaling in the leading domain, because the Wnt signaling pathway also leads to the expression of sef1 and dusp6, which inhibit the expression of the Fgf receptor. As development progresses over time, Fgf receptors begin to express in the trailing domain. Thus, Fgf3 and fgf10, which are secreted in the leading domain and diffuse to the trailing domain, activate Fgf signaling in the trailing domain. Fgf signaling leads to expression of dkk1b, which inhibits Wnt signaling in the trailing domain, making these two signaling systems mutually inhibitory.

Chemokines also play an important role in pLLp migration. In particular, the ligand cxcl12a (sdf1a) is expressed in a band of cells along the horizontal myoseptum, and its receptors cxcr4b and cxcr7b are expressed in the leading and trailing domains, respectively. Cxcr4b and cxcr7b bind to the ligand at varying affinities and create a gradient across the embryo across which the pLLp migrates.

The focus of this study is a signaling protein that has only recently been implicated in the control of pLLP migration: BMP (Bone Morphogenetic Protein). BMP is a protein based signaling process by which bone and cartilage are formed. In addition to performing these functions, bone morphogenetic proteins are a group of versatile morphogens that play roles ranging from influencing cell morphology to determining the

Dorsal-Ventral (DV) patterning of the embryo. Recent unpublished studies by my collaborators, the Chitnis lab, have now implicated BMP also in pLLp migration. The Chitnis group found that BMP ligands bmp2b, bmp4 and bmp5 are expressed in the leading domain of the primordium and that they are regulated by both Wnt and Fgf signaling (Chitnis Lab, unpublished).

One limitation we had while studying BMP signaling is in regards to studying the loss of function. Since BMP signals are involved in DV patterning, when the ligands are knocked, the embryos are dorsalized, and therefore we were not able to study its effects on the primordium. We resorted to using K02288, a drug which effectively and selectively inhibits BMP signaling. This drug inhibits the BMP Type I receptor.

In addition to K02288, we used additional drugs to manipulate other signaling systems in the pLLp. These drugs include SU5402 and Blebbistatin. SU5402 is an inhibitor of the Fgf receptor; when the receptor is inhibited, it cannot activate Fgf signaling. Epithelial cells assemble into neuromasts in the pLLp, apical constriction of epithelial cells to form neuromasts requires activated Non-muscle Myosin II (NM II). Blebbistatin inhibits NM II and results in disassembly of the neuromasts and stalling of migration.

The migration of the primordium is affected at different levels and intensities with various drug which target these signaling systems. When we treat the embryos with SU5402 and Blebbistatin, the neuromasts disassemble and the primordium stop migration altogether and there is no further deposition of neuromasts. When embryos are treated with K02288, migration of primordium slows down, but then it completes migration and deposits neuromasts at close intervals.

All the drugs affected signaling pathways and migration to varying degrees. To understand the differences between them, I carried out PIV analysis as explained in detail below. Using PIV to investigate this biological system enables a detailed, numerical examination of the distinctions among migratory behaviors. This analysis shows that disruption of BMP signaling reduces migratory coordination between the cells, particularly amongst the leading cells, both slowing migratory speed and altering directionality. Biologically, this points to the leading domain as the region responding to BMP signaling during migration.

3.2 Methods

At a high-level, the particular cell signaling of Fgf, BMP, and Wnt may influence the physical parameters of the cellular system. Our research methods combine normal biological practices for zebrafish with a modified software tool that is frequently used in fluid mechanics. This combination enables us to apply the principles of physics to the biological system to gain insight into the system regarding cellular dynamics with the aim of discovering how those parameters are linked to the cell's functioning.

3.2.1 Time-Lapse imaging of zebrafish via confocal microscopy.

Zebrafish were maintained under standard conditions in an aquatic facility of National Institutes of Health. Embryos were staged according to Kimmel *et al.* [118]. The fish line *Tg[cldnb:lynGFP]* [119] was used for all of the experiments.

For time-lapse acquisition, embryos were anesthetized at ~26hpf, after L1 deposition in 600 μM Tricaine (Sigma) and mounted in 0.8% low melting agarose (Nusieve GTG, Lonza) with the drug, in glass bottom chamber slides (Nunc). Egg water having the same amount of drug is added over the embryos to prevent drying of the agarose. Embryos for the conditions were treated with either 12.5 μM blebbistatin, 5 μM SU5402 (Tocris), 10 μM of K02288 (Tocris), or 15 μM of K02288 (Tocris). The controls were treated with DMSO.

Movies were acquired at frame intervals of 10 seconds using an inverted Leica SP5 confocal microscope with a 40X lens for the duration of the imaging time. Both the fluorescent and the differential interference contrast (DIC) channels were captured and used for analysis.

3.2.2 ImageJ and MATLAB® were used for image processing.

In preparation for the computational analysis, Image J [201] was used for initial processing of the time-lapse images. The images were imported into ImageJ using the *Bio-Formats Importer*. The channels were split upon import. (Figure 12 (a), Figure 12 (c)) The DIC channel was not altered.

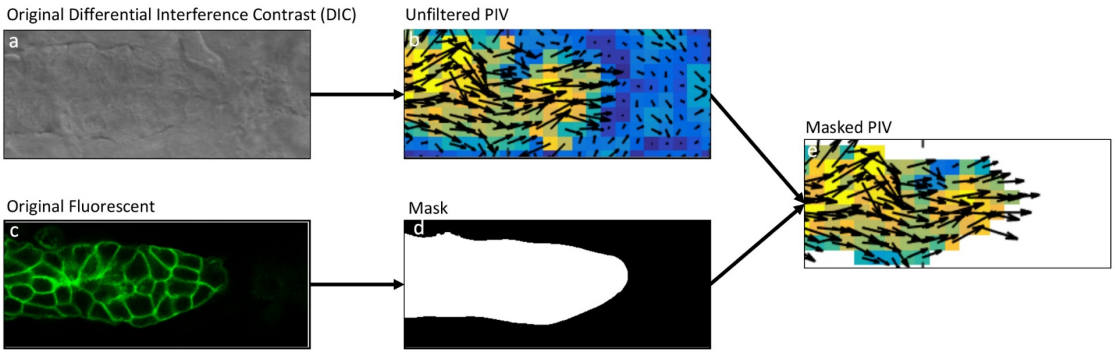


Figure 11. PIV Methodology Visualization

A dual text and image-based flow chart of software method. This flow chart uses text to describe the steps associated with each step of the process. (a) The Original Difference Interference Contrast (DIC) channel was one of two channels captured during imaging. (b) A custom software script written in MATLAB that implements Particle Image Velocimetry (PIV) was applied to the full original DIC channel. The colored boxes illustrate various speeds, and the velocity vectors are overlaid on the colored boxes. (c) The fluorescent channel images show GFP tag in the cell membranes. They were separated from the DIC channel during import into ImageJ. (d) A mask was created in ImageJ using the fluorescent channel to color the pLLp white and the background black, creating a mask. (e) The mask was overlaid onto the unfiltered PIV to select only the regions of collective cell migration in the frame. Only the velocity vectors associated with the region of interest remain and are included in the Matlab analysis.

The fluorescent channel was modified in such as way so that the pLLp was white and the background was black. This was accomplished by utilizing Huang [202] image thresholding then running a custom macro to remove outliers and fill holes. This resulted in a binary image with white regions where the pLLp is located on black background. The tiff image sequence was then saved and used as the mask for PIV. These black and white images served as a mask for the DIC channel images. (Figure 12 (d)) We created this mask so that only selected the region of interest in the frame, which is the migrating cells, could be analyzed. This process enabled the background to be excluded from the analysis (Figure 12 (e)).

For the analysis, a mask was created in ImageJ then utilized in MATLAB [35] to select the migrating cells of interest. In MATLAB, we used a technique traditionally

used in fluid mechanics called Particle Image Velocimetry (PIV) to conduct our analysis [36, 37]. This yielded vector flow fields for our region of interest, thus enabling analysis of directionality and speed during pLLp migration [11]. This process is visualized for an entire embryo (Figure 13).

In Matlab, PIV analysis was performed by means of using a custom script and the MatPIV MATLAB toolbox [37]. There are multiple parameters that can be manipulated to find the ideal set for analysis. Two of these parameters are the interrogation window size and the time between frames. To find the ideal window size, multiple box sizes were tested, including 8 x 8 pixel (1.78 μm x 1.78 μm), 16 x 16 pixel (3.57 μm x 3.57 μm), 32 x 32 pixel (7.13 μm x 7.13 μm), and 64 x 64 pixel (14.27 μm x 14.27 μm). An examination of two quality checks revealed that the 32 x 32 pixel window (7.13 μm x 7.13 μm) produced the highest quality. It is notable that of the box sizes, 7.13 μm is the closest to the diameter of a single cell.

In addition to tuning the pixel window size, we also adjusted the time frame of analysis. The image sequence was captured at 10 seconds between frames. The times of 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 seconds between frames were examined and revealed that 20 seconds between frames yielded the highest quality.

The images sequences were then analyzed with a 32 x 32 pixel window (7.13 μm x 7.13 μm) and 20 seconds between frames for the final analysis.

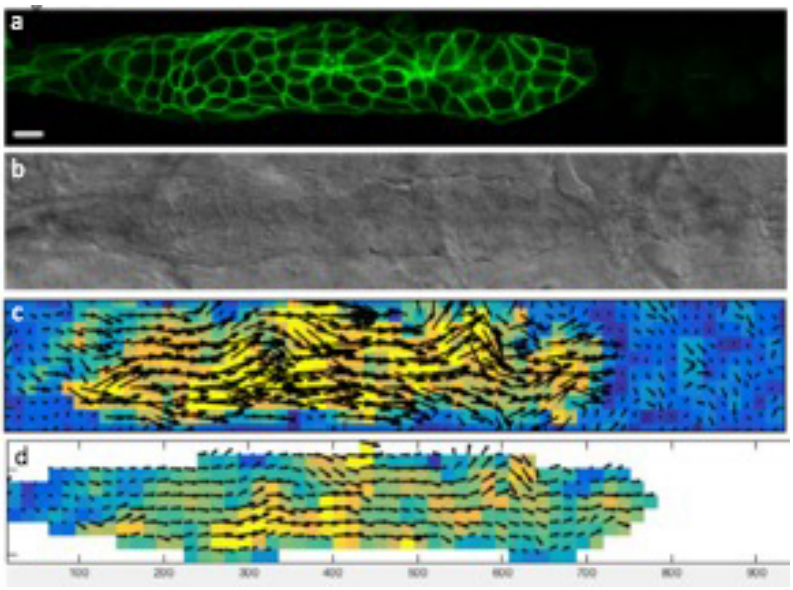


Figure 12. Components of PIV Visualization

Full frame visualization of analytical processes for a select single pLLp in a control embryo (a). Sample frame from the fluorescent channel depicting fluorescent cells in the pLLp. Scale bar is 10 μm. (b). Companion sample frame from the DIC channel (c). Assignment of velocity vectors to entire frame from PIV analysis (d). Selected region of interest generated from fluorescent channel mask.

In addition to comparing overall trends in migration, it is also useful to examine trends in migration based on location in the primordium, which allows the comparison of the leading region to the trailing region. To analyze trends based on location in the primordium, we established a moving reference frame. In this case, a moving reference frame means that instead of the origin (point 0,0) always being at the left, bottom corner, as it was with the prior graphs, it moves as the pLLp migrates. While the pLLp migrates along, the origin is always at the centermost vertical point of the leading edge. The origin moves to a new location in every frame, corresponding to the new location of the leading edge. The reference frame moves, tracking the pLLp as it migrates along, which enables

us to compare regions in the pLLp. Figure 14 identifies the location of the origin at three time points in an example image sequence.

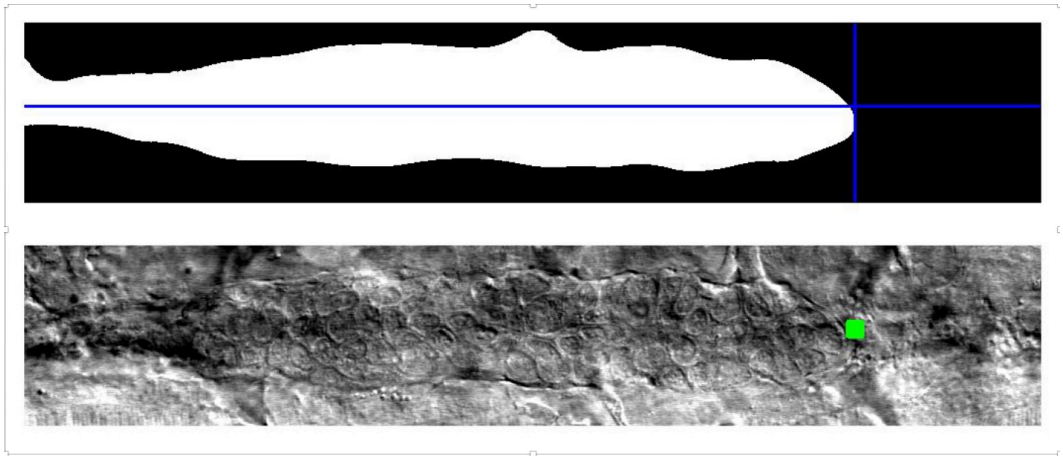


Figure 13. Moving Reference Frame Identification Marker.

An example mask and accompanying pLLp is shown at a distinct time point. The mask was created from the fluorescence channel and is binary black and white. The horizontal blue line in the mask is the centermost position of the pLLp. The vertical blue line is at the point of the leading edge. The intersection of these two blue lines is the origin. The DIC channel image is shown as a high-contrast image for visualization purposes. The green dot overlay identifies the location of the origin in the image.

The observations of cell junctions through visual examination can be made both in soap film, as is shown in the literature, and in the cells of the pLLp, as shown in this work. Figure 15 is a single confocal slice of a migrating pLLp from the control condition. There are a number of quadrijunctions in this frame, and a select juncture is highlighted and enlarged for illustrative purposes. Similar to shape of the soap film quadrijunction (Chapter 2), we observe the intersection of four cells comprising the intersection.

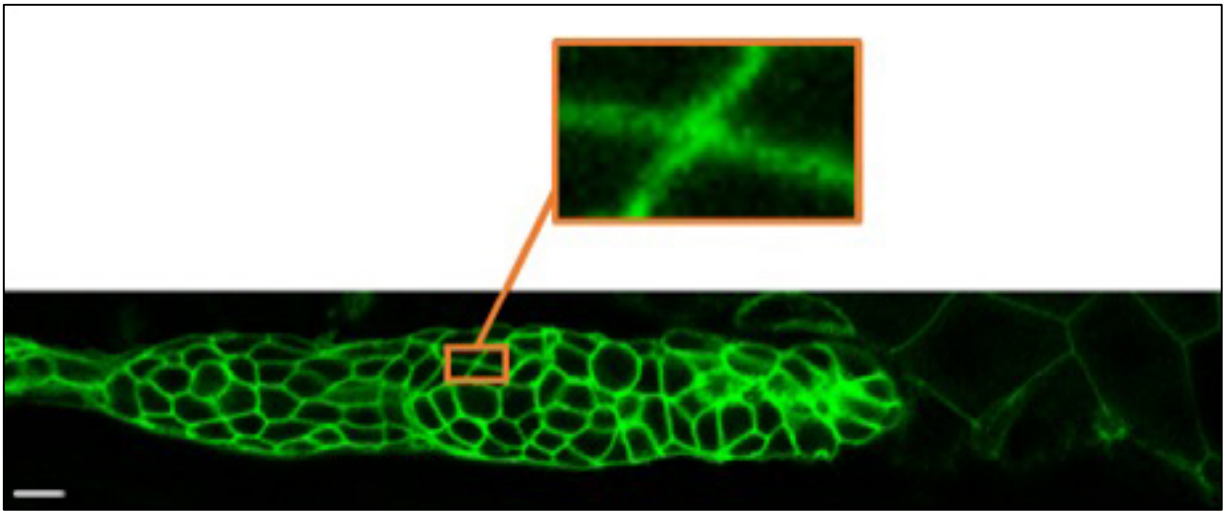


Figure 14. Quadrijunction in the pLLp.

A single confocal slice of a pLLp in migration. Zoomed in region is of a quadrijunction. Scale bar is 10 μm.

I selected movies (n=5) for each condition: control, 12.5 μM of blebbistatin, a non-muscle myosin II inhibitor (n=5); 10 μM of K02288, a BMP signal inhibitor (n=5); 15 μM of K02288 (n=5); and SU5402 (n=5), an Fgf signal inhibitor. In each movie, six frames were analyzed: three early frames at t = 0, 50, and 100 seconds and three latter frames at t = 500, 550, and 600 seconds. The quadrijunctions were manually identified through visual examination and selection of locations with intersections containing angles greater than $\pi/4$ radians.

3.3 Results

The aim of this investigation was to understand how inhibiting the BMP signaling pathway affects the migratory behavior of the pLLp. In addition to the insight gained through visual examination, the incorporation of the analytical tools of physics into this

traditionally biological scenario enables a more detailed, quantitative characterization of how the collective cell behavior changes across conditions. Analysis shows that inhibition of cell contractility, Fgf signaling pathway inhibition, and BMP signaling pathway inhibition all alter the migratory patterns of the pLLp. Additionally, each of the conditions showed distinct phenotypes regarding directionality and migratory speed.

3.3.1 BMP, Fgf, and Blebbistatin alter variance in directionality during pLLp migration.

Directionality (variance in direction) is first examined in the pLLp across the various conditions, as shown in Figure 16. The directionality component of each vector was obtained from a vector flow field created using PIV. To complete the analysis of directionality, the number of vectors in each direction of every frame was quantified for the entirety of the image sequence.

The five conditions are on the horizontal axis. The control condition (n=8) provides a standard of comparison for the remaining conditions. Embryos treated with: 12.5 μM of blebbistatin (n=5); 10 μM of K02288 (n=8); 15 μM of K02288 (n=8); and SU5402 (n=8).

The angular spread is on the vertical axis in bins between 0 and sqrt(2). Each trail has an average angular spread that ranges from approximately 0.4 to 1.2.

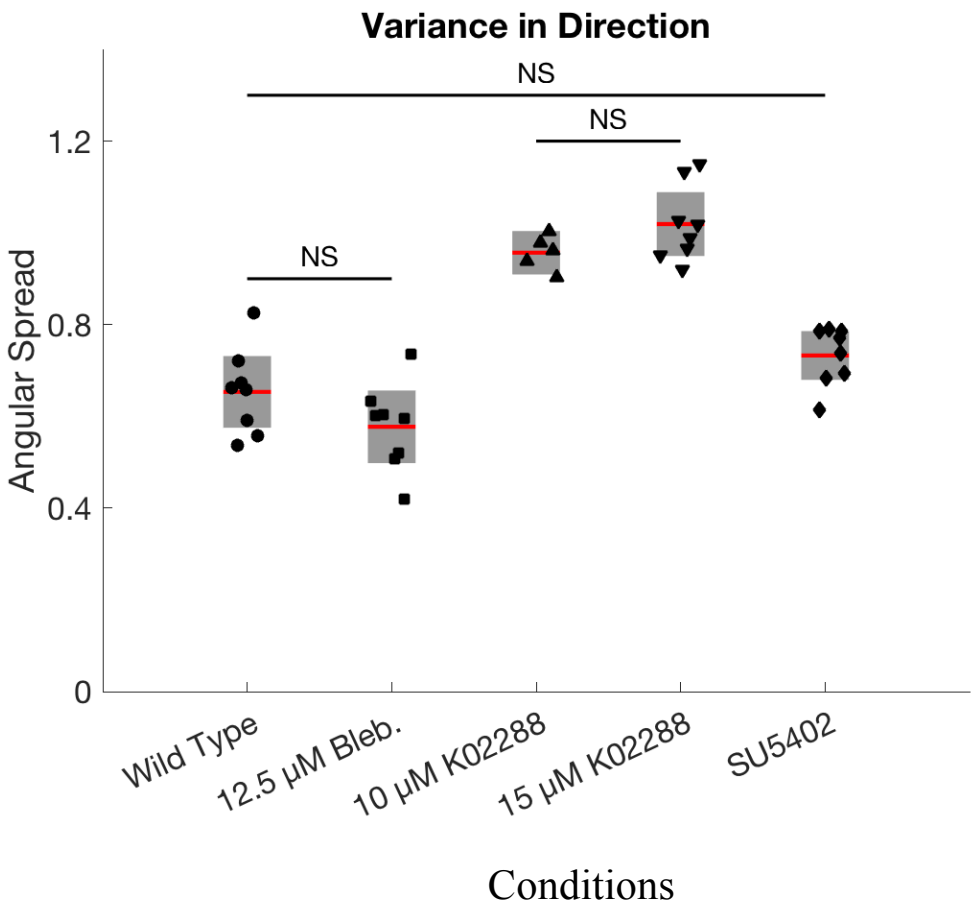


Figure 15. Variance in Direction.

The various conditions are on the horizontal axis. The angular spread increases along the vertical axis. Each black tick is the averaged angular spread for a given trial. The red bar is the averaged angular spread across all trials. The gray region is the 95% confidence interval for the averaged angular spread across all trials. NS marks conditions that are not statistically significantly different.

Table 1. ANOVA. Lists each of the inhibitors or control and the p-value for each comparison.

Angular Spread Significance

Condition 1	Condition2	p-value
Control	Bleb - 12.5uM	3.41E-01
Control	K02288 - 10uM	1.71E-06
Control	K02288 - 15uM	1.19E-08
Control	SU5402	2.99E-01
Bleb - 12.5uM	K02288 - 10uM	2.74E-08
Bleb - 12.5uM	K02288 - 15uM	9.94E-09
Bleb - 12.5uM	SU5402	4.26E-03
K02288 - 10uM	K02288 - 15uM	6.53E-01
K02288 - 10uM	SU5402	2.46E-04
K02288 - 15uM	SU5402	4.17E-07

This comparison shows that the angular spread is reduced in blebbistatin-inhibited cells when compared with the control group, though it is not a significant difference. When Fgf signaling is inhibited, as in done in the SU5402 inhibitor-treated conditions, there is no significant change in the directionality of the cells in pLLp, similar to that of the controls.

When BMP signaling is inhibited, however, there is a significant increase in the angular spread of the cells, though the difference between different amounts of inhibitors is not significant. This increase in angular spread indicates that the cells in the pLLp are migrating in a much less directed manner and is our first indication that BMP signaling is necessary for coordination between the cells in the pLLp.

An alternative method of analysis and visualization is to examine the conditions in accordance with the quantity of vectors in a given direction (Figure 17). This angle distribution graph shows the directionality of the different conditions, separated by

direction bins. The same overall trends shown in the previous graph (Figure 16) are present, but this visualization provides additional information about the nature of the differences regarding number of vectors in each direction.

The horizontal axis is the velocity direction. This was obtained from the vector flow field which can be visualized as an image of little arrows. Each arrow has a length, which corresponds to the speed of migration and is pointing in a given direction, which is the directionality.

We set zero along the x-axis, which is the direction of pLLp migration. Therefore, the arrows pointing along the x-axis are in the direction of migration and have a spread of 0. Moving in the direction of the positive y-axis in the image corresponds to moving to the left on the x-axis graph and moving in the negative y direction in the image corresponds to moving to the right on the x-axis.

The normalized count is on the vertical axis. The count is normalized to be the relative probability. The sum of the bar heights is less than or equal to 1 via $v_i=c_i/N$ where v_i is the bin value, c_i is the number of elements in the bin, and N is the number of elements in the input data. Biologically, this relationship suggests that the cells are aggregately coordinated as they move.

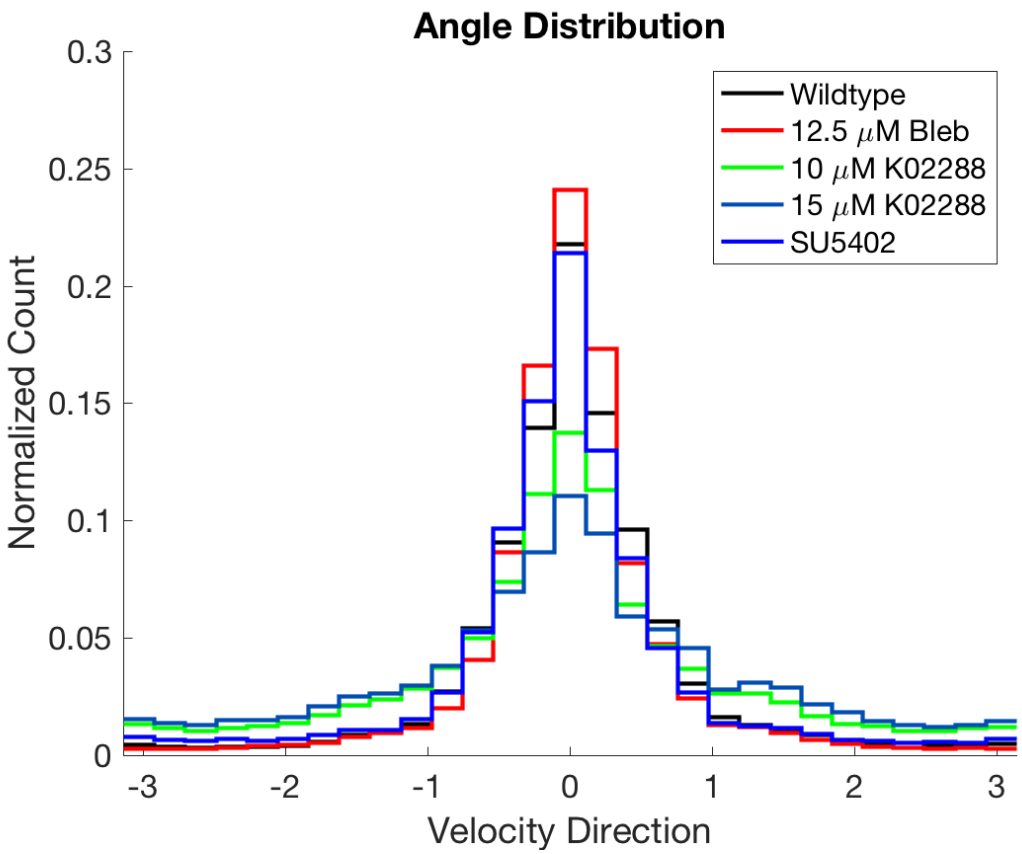


Figure 16. Count versus Directionality Comparison Graph.

Each line represents the cumulative distribution over the trials for each given condition. The velocity direction is in radians along the horizontal axis. There are 16 bins along the horizontal axis. The bin size is $\pi/8$. The vertical axis shows the normalized count.

The blebbistatin treated embryos (n=8) had the highest number of vectors whose directionality along the horizontal axis is $\pm \pi/16$ (from x=0), illustrating that the most common direction of migration is in the common direction of migration for each of the individual trials. A comparison of the control and of blebbistatin-treated cell conditions shows that blebbistatin-treated cells migrate with increased directionality as compared with the control, meaning that they migrate in a more direct manner than does the control, though we know from our ANOVA analysis that this change is not significant.

As opposed to the primordium cells that have been exposed to blebbistatin, which displayed an increase in directionality, primordium cells of embryos treated with 10 μM of K02288, a BMP inhibitor, (n=8) show a decrease in directionality. This is illustrated in the figure by a larger number of the vectors in directions other than $\pm \pi/16$ from $\Theta=0$. These results show that BMP plays a key role in the migration of the pLLp as the cells are moving in a less directed and less coordinated manner.

The critical role of BMP in pLLp migratory directionality is further confirmed by our analysis of the behavior of embryonic cells treated with 15 μM of K02288 (n=8). For these cells, the increased inhibition of the transduction of BMP signals corresponds with a further increase in the lack in directionality of the migrating cells. These results confirm that BMP plays a key role in the migration of the pLLp as the cells are moving in a less directed and less coordinated manner.

3.3.2 Directionality varies along the length of the primordium.

A moving reference frame enables examination of the angular spread for regions of the pLLp. This analysis yields insight into whether changes in angular spread are attributed to a global or local phenomenon, that is whether a given perturbation is affecting the entire pLLp or only a select region. An increased localized angular spread indicates reduced local coherence in the region of increased spread.

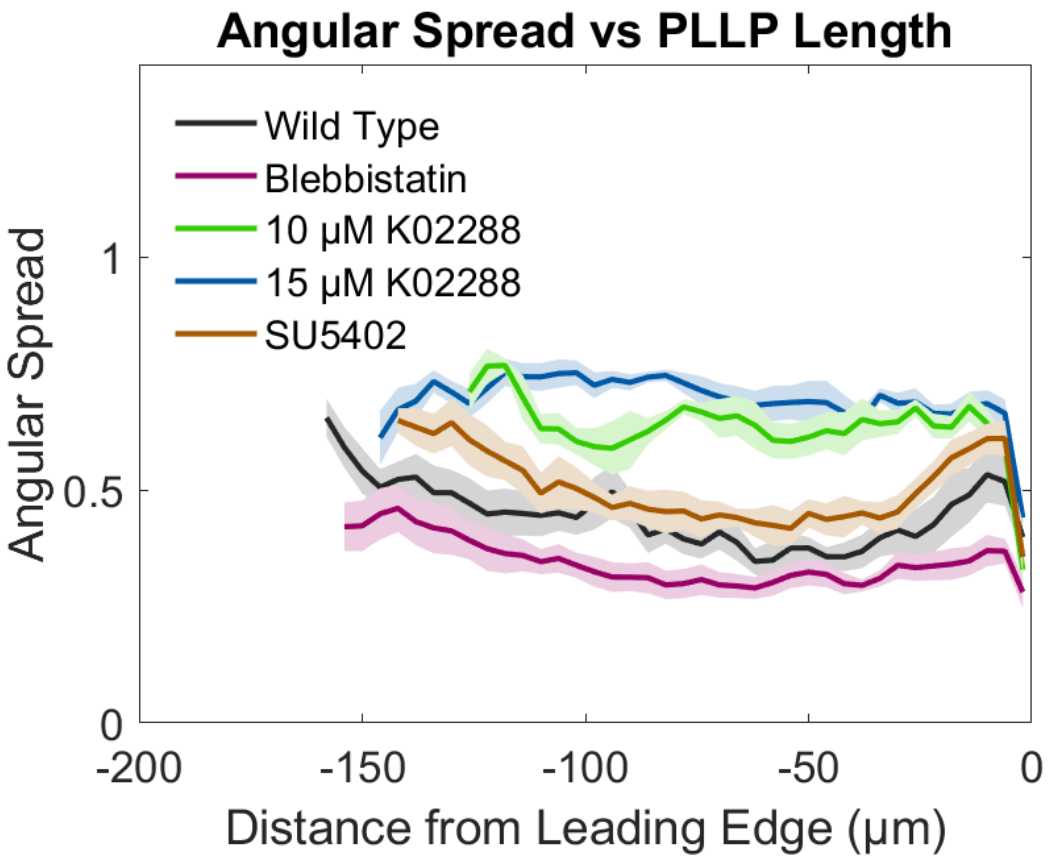


Figure 17. Angular Spread Versus pLLp Length.

Angular spread versus distance from leading edge comparison. Distance from the leading edge in microns is on the horizontal axis. The moving reference frame allows for the centermost point of the front most leading edge to be at the origin through the duration of the time-lapse. The angular spread is on the vertical axis. The darker line is the trend line, and the shaded region is the confidence interval.

Examining the control condition for the angular spread as a function of distance from the leading edge, there is a higher angular spread in the first 20 microns, then a dip, followed by a steady increase along the pLLp, away from the direction of migration. This pattern corresponds to visual observations, which are that the leading cells protrude outward and not necessarily in the direction of migration. The leading cells also switch places and rearrange themselves as the pLLp migrates. The angular spread increases

along the pLLp moving towards the trailing domain, where the rosettes are forming. The cells move into a circular formation in the formation of the rosette, which could contribute to an increase in the angular spread moving into the region where they are formed.

By comparison, the uptick in the leading domain is not observed in blebbistatin, as it is in the control. There could be a number of reasons for this difference. One reason could be that the frequency of protrusions drops with the contraction inhibition. Another hypothesis is that the magnitude of the protrusions drops. This is a point of ongoing investigation. What is known is that the cells in the leading domain move in a more directed manner.

For the blebbistatin case, there is an also increase in the angular spread moving into the trailing domain, where the rosettes are forming. There is the characteristic lift in the back, where the rosettes are forming. Blebbistatin disrupts the formation of rosettes, and this can be confirmed through visual examination of the movies, yet it could be that groups of cells are still being deposited, or at least rearranging, as an increase in angular spread is reflected in the trailing domain.

When comparing BMP signaling inhibited cells to the other conditions, the angular spread is increased, thus the directionality of the entire pLLp is reduced. There is not significantly higher spread in the first 20 microns than in the following center region; instead, there is an increased spread throughout the pLLp for both BMP signaling inhibited conditions.

For the 10 μM K02288 condition, there is a dip in the trailing domain followed by an increase in angular spread. This could be due to an organization that may occur as the

pLLp attempts to form rosettes, followed by the characteristic upward region that is attributed to neuromast deposition in the control condition.

For the 15 μM K02288 condition, such a trend is not present. For the angular spread as a function of distance from the leading edge, there is a fairly consistent spread across the length of the pLLp. This means that the cells are moving in an equally uncoordinated manner in the various regions of the pLLp. Additionally, this finding confirms that BMP signaling is critical for collective cell migration in the pLLp.

The inhibition of Fgf signaling follows a similar trend to that of the control but with disruption in the trailing domain. This could be because if Fgf is inhibited in the trailing region, as is known to be its active region, then the cells in this zone could take on characteristics that are more mesenchymal in nature.

3.3.3 Migratory speed varies across conditions.

An examination of the overall mean speed of the various conditions also yields useful insights (Figure 19). From our vector flow field created using PIV, the speed component of each vector was obtained. In the figure, the average speed (in μm/min) is on the vertical axis.

The five conditions are on the horizontal axis: the control condition (n=8), embryos treated with: 12.5 μM of blebbistatin (n=5); 10 μM of K02288 (n=8); 15 μM of K02288 (n=8); and 5 μM SU5402 (n=8).

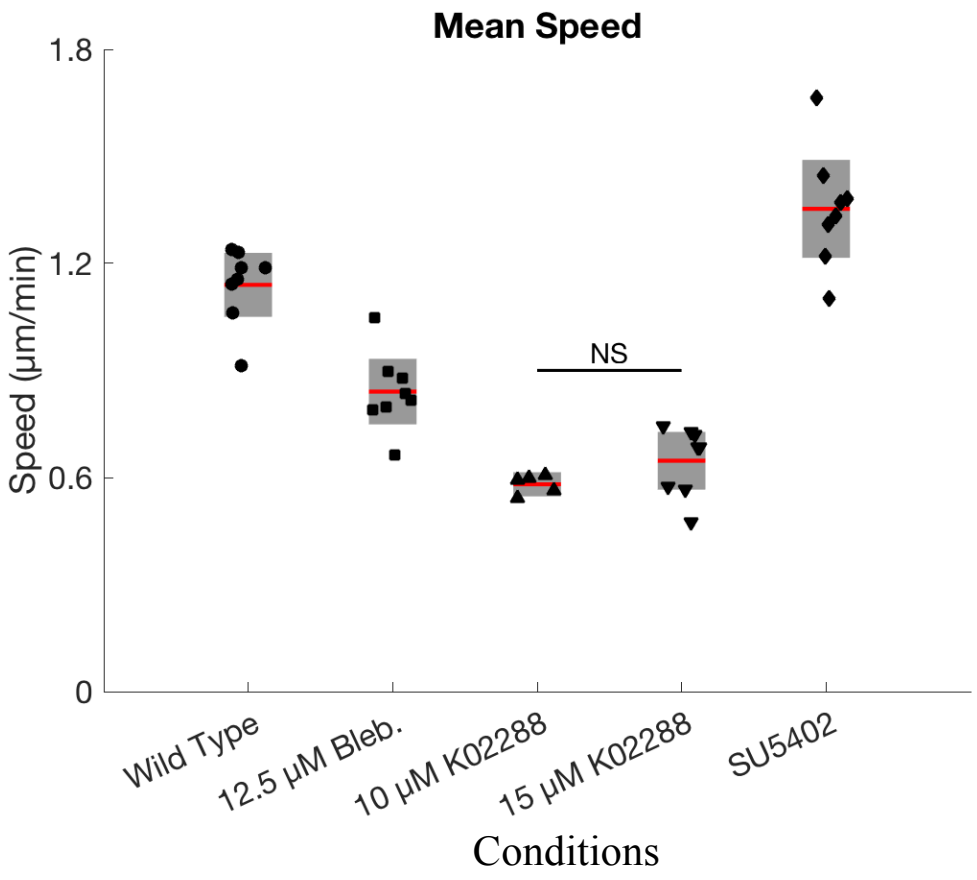


Figure 18. Mean Speed Across Conditions.

The various conditions are on the horizontal axis. The speed in microns per minute increases along the vertical axis. Each black tick is the averaged angular spread for a given trial. The red bar is the averaged angular spread. The gray region is the 95% confidence interval. NS marks conditions that are not statistically significantly different.

Table 2. Lists each of the inhibitors or control and the p-value for each comparison.

Speed Significance

Condition 1	Condition2	p-value
Control	Bleb - 12.5uM	9.97E-05
Control	K02288 - 10uM	1.89E-08
Control	K02288 - 15uM	1.79E-08
Control	SU5402	6.52E-03
Bleb - 12.5uM	K02288 - 10uM	3.27E-03
Bleb - 12.5uM	K02288 - 15uM	1.55E-02
Bleb - 12.5uM	SU5402	1.33E-08
K02288 - 10uM	K02288 - 15uM	8.49E-01
K02288 - 10uM	SU5402	9.93E-09
K02288 - 15uM	SU5402	9.92E-09

The controls migrate with a given speed, yet the Fgf inhibited embryos, inhibited with SU5402, have the fastest overall migratory speed. This could be because Fgf disrupts the formation of rosettes, allowing the cells to move along the direction of migration.

Blebbistatin-treated embryos moved with a decreased speed. Biologically, this condition could be because cell contractility is disrupted therefore the cells may have difficulties contracting, thus migrating. Additionally, one hypothesis is that if the migratory cells are mechanically couples, disruptions in the contractile motion of one cell may interfere with the protrusions of another. Though we do not see a disruption in the coordination between cells, as reflected in angular distribution, the decreased speed does indicate some difficulties with migration. Such effects of the disruption of contractility of one cell on the protrusions of the cells to which it is mechanically coupled could be the focus of future work.

The BMP inhibited embryos, 10 μM and 15 μM of K02288, are slower than the SU5402, the control, and the blebbistatin, with the embryos treated with 15 μM K02288 being the slowest condition. This supports the hypothesis that BMP is critical for collective cell migration in the pLLp.

3.3.4 Speed distribution differs across conditions.

The speed graph (Figure 20) shows the normalized count for the various speeds of the different conditions, with the control cells serving as the baseline for comparison. There are the same trends as in the previous graphs, but this visualization provides additional information. The speeds range from 0 μm/min to 8 μm/min.

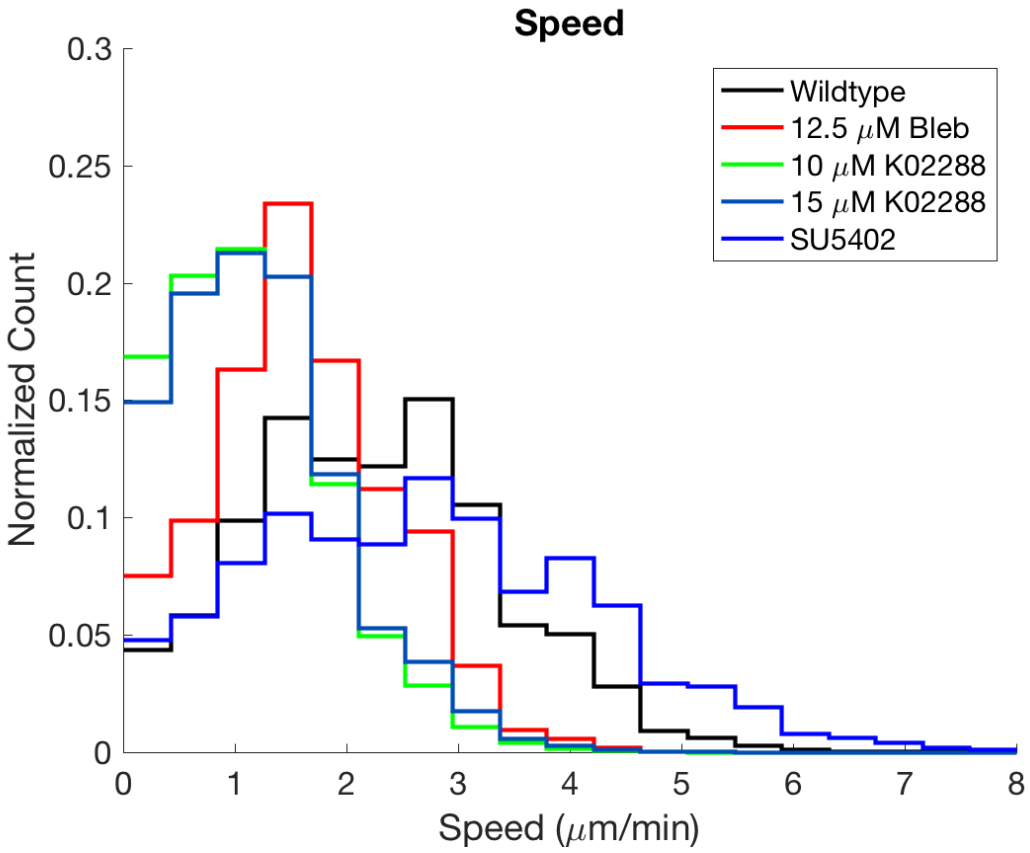


Figure 19. Speed Count for Conditions.

Comparison graph of four conditions. Each line represents the total count over the trials for each given condition. The speed of migration is in microns per minute (µm/min) along the horizontal axis. There are 16 bins along the horizontal axis. The bin size is .667 µm/min. The vertical axis shows the normalized count.

From Figure 20, the SU5402 condition has a number of cells in the 6-8 µm/min bin, much higher than the highest migratory speeds of virtually all of the other conditions. The controls have the next fastest migratory speeds followed by the blebbistatin, and BMP inhibited conditions.

For the control condition, the average speed is around 2 µm/min. The distribution is due to the individual cells in the migrating pLLp moving at a slower or quicker rate than the overall rate of migration. Biologically, this behavior makes sense as the cells in

the pLLp are rearranging themselves during migration to form rosettes and the leading cells are protruding and retracting throughout the migration.

The Blebbistatin-treated cells move slower as shown in the graph of normalized count vs. speed. Biologically, actin polymerization is a critical component of cell motion due to its role in cell contractility. From the graph, we observe that contractile inhibition leads to a reduction in speed.

For 10 μM and 15 μM of K02288, the speed component of each vector in the vector field was quantified within a range of 0 μm/min to 4 μm/min, with the average speed being between 1-2 μm/min. Biologically, the cells are performing various functions and thus may be moving at faster or slower speeds than the average pLLp speed of migration.

The migratory speed of the pLLp with Fgf inhibited ranges from 0 μm/min to 8 μm/min. This particular graph, again, does not distinguish between locations in the pLLp, but instead, it gives a total count of the number of vectors in the entire time-lapse that have a given speed. Fgf-inhibited cells have a similar number of speeds in the lower speed regions, but the speeds in the middle region have shifted to higher speeds when compared with the wildtype.

3.3.5 The migratory speed does not change over the duration of the imaging time.

The change in speed over time is shown in Figure 21. Though there is a change in the overall speed for each condition, there is no significant change in speed over time for any of the given conditions. The trends with Fgf signal inhibited (K02288) being the

fastest, followed by the control, contractility (blebbistatin), and BMP (SU5402) in the respective order, hold.

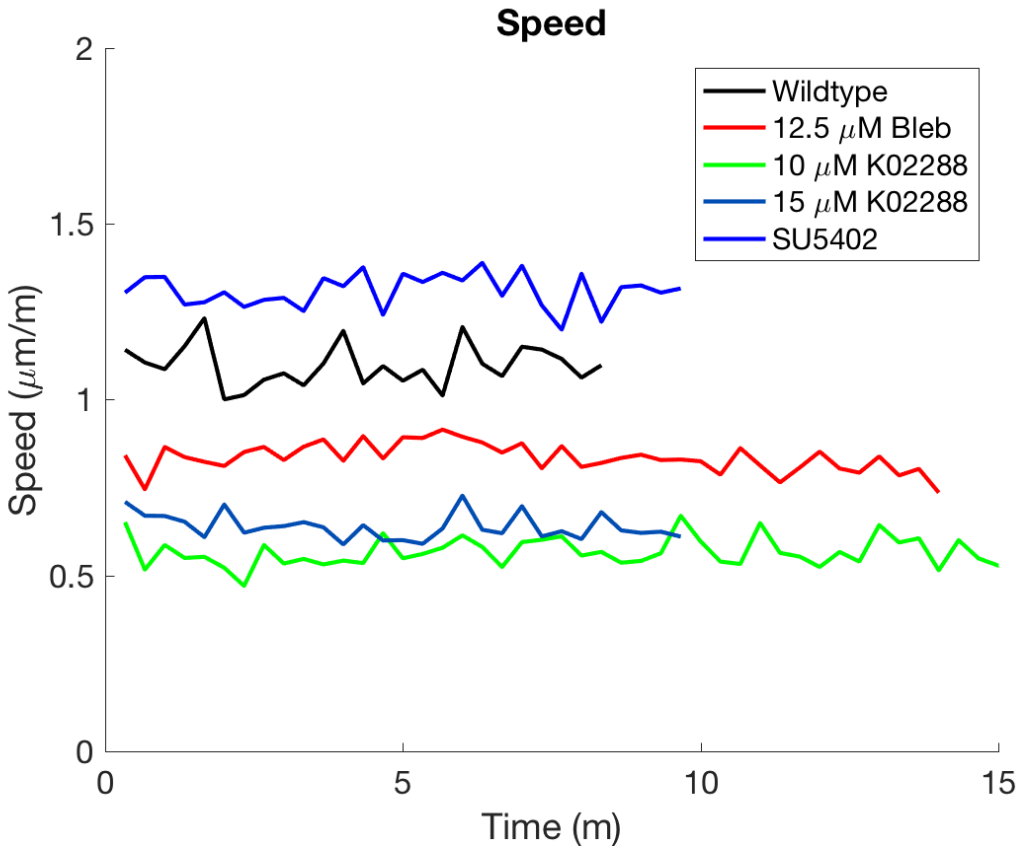


Figure 20. Change in Speed Over Time.

Each line represents the average over the trials for each given condition. The time in minutes (μm/min) is along the horizontal axis. The vertical axis shows the speed (μm/min) at the given time.

It should be noted, however, that the imaging time for the image sequences is less than 15 minutes. The total migratory time for the pLLp is 24 hours. This analysis only examines a brief component of the migration and cannot be used to make conclusions regarding the pLLp migration over the entire time of its migratory journey.

An examination of the speeds over time, though noisy, shows that speeds range from 0.4 μm/min to 1.4 μm/min. This means that overall, the pLLp maintains its overall speed migrating down the embryo for the duration of the time-lapse.

3.3.6 Speed versus pLLp length varies across conditions.

Through again utilizing a moving reference frame, as was shown in Figure 22, the migratory speeds for various regions of the pLLp across the various conditions can be compared. Such analysis yields additional insight into the migratory behavior, specifically addressing whether any changes in speed occur as a global or local phenomenon.

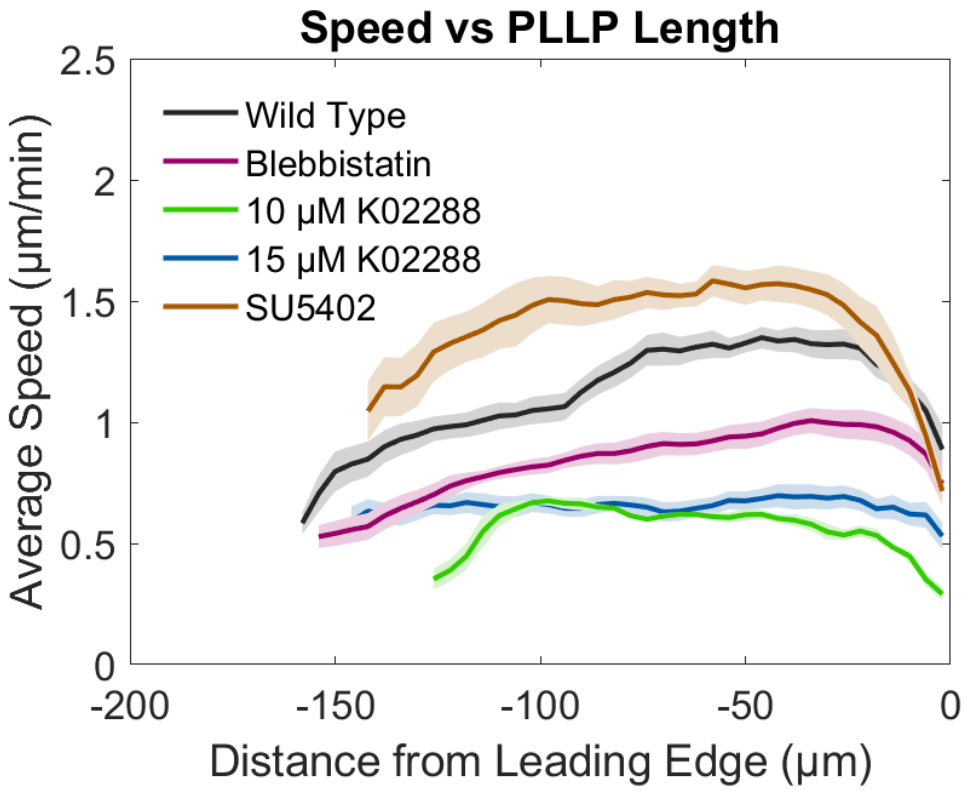


Figure 21. Speed versus pLLp Length.

Distance from the leading edge is in microns on the horizontal axis. The moving reference frame allows for the centermost point of the front most leading edge to be at the origin through the duration of the time-lapse. The average speed in μm/min is on the vertical axis. The darker line is the trend line, and the shaded region is the confidence interval.

Examination of the average speed versus the distance from the leading edge reveals differences across conditions. For the control condition, the speed decreases moving away from the leading edge towards the trailing domain. The trailing domain is the region where the rosettes are formed. Biologically, this region will eventually be deposited and thus entirely stop moving. This slowing of the soon-to-be-deposited cells is depicted in the portion of the graph farthest from the leading edge in the control.

The blebbistatin-treated embryos follow a similar trend, indicating that while a decrease in the contractile forces contributes to an overall decrease in speed, the underlying mechanisms and cell processes are still functioning.

The BMP-inhibited embryos follow a different pattern. Instead of the leading edge starting at a higher speed, followed by a decrease in speed moving towards the trailing edge, we observe that average speed follows a different pattern. For both the 10 and 15 μm K02288 there is a leading zone that is the same or slightly lower in average migratory speed as the trailing domain. This observation could indicate the BMP signal inhibition leads to a global slowing of the pLLp.

Additionally, in the 10 μm K02288 condition, there is a region where neuromast deposition may be occurring due to the drop off in the leading zone that is hypothetically connected to neuromast deposition. However, in the 15 μm K02288 condition, the trailing domain does not drop off as we see in the other conditions. Biologically, this could indicate difficulties in neuromast deposition in the 15 μm K02288 condition.

3.3.7 Quadrijunctions vary in number across conditions.

In addition to an examination of the migratory behaviors, we can also examine the cell intersections to gain additional insight into the roles of the signaling pathways. An examination of the quadrijunctions shows that the blebbistatin-treated embryos have a slightly increased–but not statistically significantly different–number of quardrijunctures from the control conditions (Figure 23). The embryos treated with the BMP and Fgf signal inhibitors are statistically significantly different. With the treatment of K02288,

we observe an increase in the number of quadrijunctions for embryos treated with 10 μM of K02288 and an even further increase in those treated with 15 μM of K02288. Embryos treated with SU5402 show a reduction in the number of quadrijunctions.

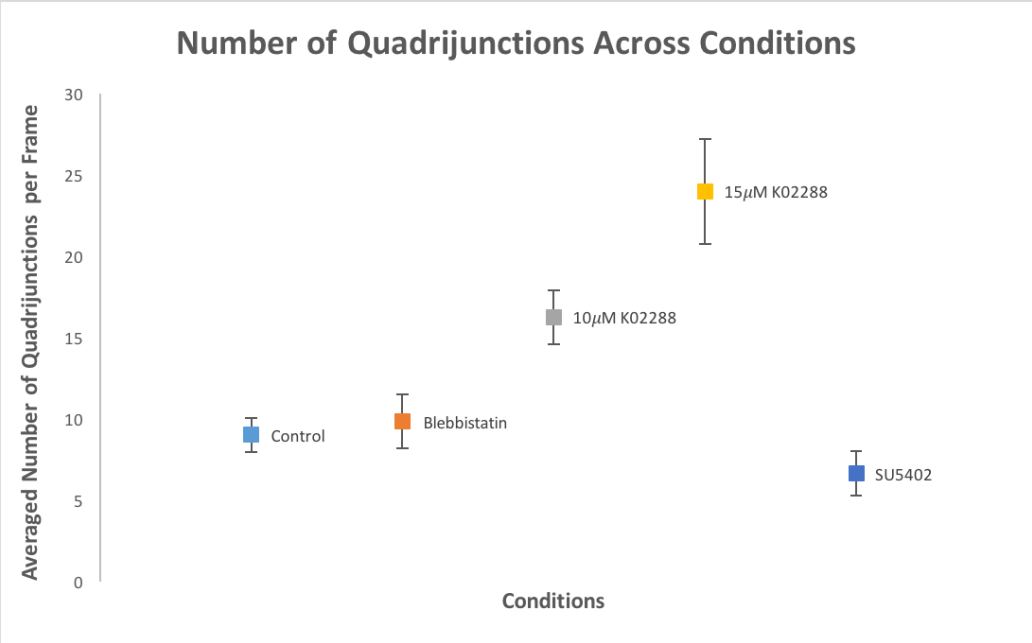


Figure 22. Prevalence of Quadrijunctions Across Conditions.

Crosshairs analysis graph showing the number of quadrijunctions across the various conditions. The error bars show one standard deviation for values in the condition data.

Considering the variation across the conditions, there is no statistical difference in the blebbistatin-treated condition when compared with the control. Although non-muscle myosin II is directly involved in actin and cell adhesion dynamics [203], its inhibition did not have a significant enough of an effect to be captured by this form of analysis. In regards to the two types of signal inhibition, we see differing effects in the quantity of quadrijunctions: the number of these junctions increases with BMP signal inhibition and decreases with Fgf signal inhibition.

From a biological perspective, the varying quantities of quadrijunctions confirms the need to consider other parameters beyond the cell membrane. One such parameter is the cell cortex and, more specifically, its actin scaffolding. We know that actin scaffolding reaches across a cell's membrane and into an adjoining cell. The cells in the quadrijunction would be more likely to align if they are aligned through actin scaffolding via actin cables that secure them to each other. The coupling of the cell cortexes through actin cables could provide an increased stability to the cell. The coupling of the cortex is an additional parameter and a differentiator of these living biological systems as opposed to inanimate objects.

Additionally, cell adhesions occur at points of cell-cell contact, and these cells are known to be mechanically linked through cell-cell adhesion proteins [22]. The increase in the number of quadrijunctions in the BMP-signal-inhibited cells could indicate an increase in these adhesions, namely N-Cadherin, E-Cadherin, EpCAM, and Z0-1, though this work is not able to discern which are being affected [18, 145, 154–156, 146–153]. It could also be due to an increase in the actin scaffolding held together by proteins traversing the adjoining membranes or coupled cortex, which would indicate an increase in the adherens junctions. Determination of the nature of the indicated increase in adhesions is the focus of future work.

While we observe a possible increase in the quadrijunctions in the BMP signal inhibited condition, we observe that cells with Fgf signaling inhibition show a reduced number of quadrijunctions as compared to all other conditions, including the control. This decrease could indicate a decrease in cell-cell adhesions (also it is notable that in this condition the neuromasts in the primordium disassemble and cells become more

relaxed). It has been shown that Fgf signaling contributes to the polarity of the cell (Fgf signaling plays a role in formation of neuromasts) and polarized cells intercalate amongst one another [204]. A reduction in polarity could lead to a lessening of cell intercalation and thus fewer cell-cell adhesions. Further examination of the biological causes of the suggested decrease in adhesions is the focus of future work.

3.4 Discussion

These results demonstrate that cells undergoing varying inhibitions of BMP signaling, Fgf signaling, and contractility will display varying phenotypical changes. For each condition, the change in speed of migration, the variance in directionality, and the presence of quadrijunctions yield insight into the pLLp system and demonstrate the vital role of BMP signaling in the pLLp.

3.4.1 Visual examination reveals varying degrees of cell-cell adhesions across conditions.

Recall (from Chapter 2) that quadrijunctions are where four objects meet in a perpendicular intersection. These are rare in inanimate fluidic systems (such as in soap bubbles). These types of junctions are, however, prevalent in varying degrees across the different experimental conditions as noted above. The presence of which indicates stability in the system. In comparison with our control, there is not a significant change with the blebbistatin-treated embryos, yet there are changes in the chemical signal

inhibited conditions. For BMP signal inhibition, we observe an increase in the junctions and for Fgf signal inhibition, there is a decrease. I propose that the presence of these junctions implies the presence of cell-cell adhesions. Therefore, Fgf signal impairment reduces cell-cell adhesions, and BMP signal impairment increases cell-cell adhesions. These results highlight the critical role of BMP signaling in the pLLp.

3.4.2 Directionality and speed are distinct parameters and separate targets.

Additionally, it has recently been shown that speed and direction are distinct parameters [38]. This work confirms that directionality and speed can be targeted independently in this biological system as well through the quantitative PIV analysis. It showed that while various drugs can impact directionality, speed and direction can be regulated separately, as we observed in our blebbistatin trials. Speed was reduced, but directionality was retained; a reduction in speed does not necessarily lead to a reduction in directionality.

3.4.3 BMP signaling may be responsible for cellular coordination in the pLLp.

Furthermore, results from the quantitative PIV analysis confirm that BMP plays a vital role in the collective cell migration of the pLLp. The inhibition of BMP signaling disrupts both speed and directionality. The inhibition of BMP signaling causes the cells in the pLLp to migrate in a less coordinated way, as is supported by the decrease in directionality that occurs.

Additionally, instead of distinct regions of increased and decreased directionality, the increased angular spread is present throughout the length of the pLLp. I, therefore, propose that BMP signaling is active in the leading domain. This is because inhibiting BMP yields an angular distribution that does not follow the district bi-phasic trend that we observe in the control condition. Instead, the trend throughout the length of the primordium is comparable to the trailing domain and the uptick in the control. I propose that inhibiting BMP effects the leading domain in that it causes an increase in the directionality.

Furthermore, BMP signal inhibition leads to a global slowing of the pLLp. Instead of a variation of speeds through the pLLp, with BMP inhibition the cells show much less variation in their speeds and a relatively consistent low speed in the 15 μm K02288 condition. Biologically, this could also support the hypothesis that BMP is active in the leading domain as this region does not maintain an increased speed. Additionally, if the leading domain slows and all of the cells are mechanically coupled, you would observe an overall slowing through the pLLp as we observe here.

In summary, BMP signaling-inhibition yields an increase in cell-cell adhesions, an increase in directional variance, and a reduction in the migratory speed. Fgf signaling-inhibited cells display a decrease in cell-cell adhesions, no change in directionality, and an increase in migratory speed. This relationship highlights the critical nature of both Fgf and BMP signaling pathways in the proper functioning of pLLp migration.

3.5 Further Analysis

There are numerous opportunities for continuing work on this project. Now that the quantitative analysis methods are fully developed, and we have discovered and confirmed the presence of BMP signaling in the pLLp, we have the opportunity to use these analysis tools to investigate other hypotheses about situations, including disrupting other signaling pathways (such as Wnt signaling) via chemical inhibition and perturbing other cellular mechanisms connected with migration.

Examples of such experiments may include inducing latrunculin, a chemical inhibitor which prevents actin polymerization, thus disrupting migration; inhibition of the p38 pathway, which is downstream of BMP and known to be associated with cell migration; and chemical inhibitors which both activate (BIO) and disrupt (IWR) Wnt signaling. It may be of interest to observe inhibiting both Fgf and BMP signaling simultaneously and comparing the result to the inhibition of Wnt signaling.

Further application of this work provides inspiration for the development of problem sets for life science students in physics courses, as is discussed in the second part of this thesis.

Interlude

This highly interdisciplinary dissertation spans the fields of physics, biology, and education. In the first section of this dissertation (Chapters 2 and 3), we focused largely on cellular biophysics, pulling from both physics and biology to conduct novel analysis. In the second section of this dissertation (Chapters 4-7) we move into leveraging this work through expanding its focus into an educational setting and pull from education to address our research aims.

In the first section, our focus on cellular biophysics led us to examine the migratory behaviors of and physical interactions amongst collective cells. Through leveraging tools and principles comment to the study of physics and applying them in a biological scenario, we gained insights into previously unknown biological functioning. This process of utilizing tools prevalent in one field of research (in this case, physics) to increase understanding in a second field (biology) is a process that I defined and termed and will be discussed further in the second section of this work addressing education research.

Additionally, in the first section, to engage in this research, we utilized multiple techniques from visual observation to analytical computations. Each technique enabled us to gain novel insight into our system of interest. Of notable interest is the analysis of the quadrijunction intersections of the cells in the pLLp and the biological implications of the perturbation of various parameters. I note that in this case modeling the systems as simply elastic membranes in contact was not robust enough to yield biological insight and proved an insufficient model. To address the effects of the various chemical

inhibitors, we discussed the plausible changes to the actin filaments and cell-cell adhesions. The creation and refining of a model will also be discussed in an educational setting section two.

As we move into our second section, we recognize the calls for reform for the undergraduate life science major curriculum that have been coming from the medical and biological research science communities. This work is situated in a reformed physics course that is part of the response to these calls. Pulling from the biophysics work presented in the first section, I developed a novel problem set for this course. This problem set is intended to be used in a group-work discussion session.

In the first section, we obtained results from asking questions about the biological system then utilizing physics to strengthen our depth of understanding of the system and answer some of these questions. This same methodology is further refined and detailed as a process for curriculum development. Additionally, in my experimental research, I discussed the limitations of modeling a non-living and living system and created a more robust model to address our biological questions. Engaging in such modeling was incredibly beneficial to my own research and is a focal point of the problem set I present in the second section of this dissertation.

My hope is that this unique combination of education, biology, and physics as presented in these two sections of my dissertation will inspire (1) physicists and engineers in academic to develop problem sets and activities that are most suited for the students in their physics-based courses and (2) the physics education research community to continue to recognize and use this work as a template for addressing the needs of the life science students in our physics courses.

Chapter IV: Achieving Cross-disciplinary Authenticity in Physics for the Life Sciences

Research has shown that when life-science students perceive physics problems as providing authentic insight into biological phenomena, they achieve more expert-like ways of knowing in physics [75, 97]. This work proposes a methodology for incorporating cross-disciplinary problems into interdisciplinary courses such as the introductory physics course for life science majors (IPLS) course at the University of Maryland, College Park. Building on current biophysics research, as was discussed in Chapter 3, and cell biomechanics, as was discussed in Chapter 2, we developed such a problem set. The development of this cross-disciplinary problem set is the focus of this chapter.

This chapter introduces and defines "*cross-disciplinary authenticity*", as advancing the understanding of a secondary discipline through the application of the concepts and methods from a primary discipline. We then discuss an innovative way to achieve it. Under the new definition, a physics problem set that is developed for use in an interdisciplinary course is not considered cross-disciplinary authentic unless it yields insight into the accompanying discipline.

This work focuses on a physics course that is designed for life science majors, therefore, authentic cross-disciplinary physics problems are activities that students perceive as yielding useful biological knowledge. I introduce problem set content in Chapter 5 and analyze the student response in Chapter 6.

4.1 Interdisciplinary courses are increasingly common.

While it is common to offer introductory physics courses for non-majors, in recent years there has been a rise in the number of specific introductory physics courses that are available for select groups of students, such as those students majoring in biology, architecture, or education [205]. An increasing number of institutions are offering introductory physics courses that are specifically for life science majors, often referred to as introductory physics for the life sciences (IPLS) courses, due to increased demand from the professional biological science research and medical communities [64–69]. And not only are does the demand arise just from those communities, but also it arises in part due to the substantial growth in numbers of students. At a number of universities and colleges, the number of biology and health-care majors taking physics surpasses the number of engineering students taking physics.

4.1.1 The IPLS course at UMD is part of the NEXUS project.

The number of IPLS courses offered is steadily increasing, yet at a number of universities, the physics courses for biological science and pre-health students greatly lack in biological-authenticity. At the University of Maryland College Park (UMD), our reformed IPLS course is the physics component of the National Experiment in Undergraduate Science Education (NEXUS), a project of the Howard Hughes Medical Institute and National Science Foundation created in response to calls from the medical

and biological science communities for a reformed undergraduate science curriculum [43, 44, 49, 55].

The development of course material in these IPLS courses often takes existing physics concepts and attempts to integrate a biological system with the physics. In this approach, a curriculum designer would first identify physics concepts then seek to identify biological scenarios in which to situate the physics concepts. This approach starts with the physics. More specifically, in this work, our approach starts with current biophysics research and seeks to show how identifying the critical physics can give authentic insight into the biology.

4.1.2 The IPLS course at UMD is particularly well situated for this work.

The NEXUS/Physics IPLS course is well situated to be an excellent location for the incorporation of cross-disciplinary authentic problem sets as it was developed with extensive interactions with biologists and chemists over a multi-year period. It was intentionally designed to provide support to life science majors for difficult physics concepts that they encounter in biology and chemistry, particularly those that cannot be studied in depth in those classes [78]. The NEXUS/Physics IPLS course is oriented toward competency development in an interdisciplinary context.

The course is was developed with a strong research effort on understanding both students' and faculty's interdisciplinary perspectives, including students' attitudes towards using physics in biology, watching them perform in authentic classroom situations, and exploring their views and responses through interviews [78, 82, 83, 92]. This makes the

course an excellent setting for further examining students' attitudes by extending our existing attention towards the use of novel cross-disciplinary authentic problem sets.

4.2 Incorporating authenticity in IPLS can be challenging.

This excellent situation in which to develop curriculum in introductory physics for the life science enables the exploration of what it means to develop problem sets that are both cross-disciplinary authentic and research-based. We begin our discussion by describing what it means to be authentic from the literature and then proposing a novel definition of cross-disciplinary authenticity before discussing why we should care about achieving it.

4.2.1 What is authenticity?

Merriam-Webster's dictionary provides this definition for the word "authentic":

1a: worthy of acceptance or belief as conforming to or based on fact; paints an authentic picture of our society

b: conforming to an original so as to reproduce essential features; an authentic reproduction of a colonial farmhouse

c: made or done the same way as an original authentic Mexican fare

2: not false or imitation: real, actual; an authentic cockney accent

3: true to one's own personality, spirit, or character is sincere and authentic with no pretensions

From this definition, authentic physics means that we are conforming to the spirit and character of physics -- being true to its principles and methods. Authentic biology would mean that we are conforming to the spirit and character of biology -- being true to its principles and methods. Taking this a step further, to achieve cross-disciplinary authenticity, one must be true to both disciplines.

In contrast, for disciplined-based authenticity, prior work would, "use tools—such as concepts, equations, or physical tools—in ways and for purposes that reflect how the disciplines build, organize, and assess knowledge about the world" [75]. We are very distinct from this view of authenticity in that we are giving our life science students access to tools that are more commonly used in physics in order to build, organize, and assess knowledge about the biological world to use to engage in authentic biological exploration.

Our "cross-disciplinary" also differs from "interdisciplinary" as it is often used in the literature:

"...a process of answering a question, solving a problem, or addressing a topic that is too broad or complex to be dealt with adequately by a single discipline or profession ... [by] draw[ing] upon disciplinary perspectives and integrat[ing] their insights through construction of a more comprehensive perspective (Klein and Newell 1997, p. 393–394)." [103, 104]

In this definition of interdisciplinary, the mere incorporation of a broad problem would be included. This definition does not address the idea of authenticity or of outcomes instead focusing on the simple amalgamation. In contrast, cross-disciplinary authenticity maintains a specific aim and a specific purpose: to leverage tools and concepts available in a given discipline to increase the knowledge base of another discipline.

In our case, since we are studying a physics course for biologists, we want to be true to both disciplines. In developing our IPLS class, we aim for cross-disciplinary authenticity -- to have the students learn principles and tools of physics that they perceive as being of real and actual value to their knowledge of biology thus aiming to achieve cross-disciplinary authenticity through being true to both physics and biology. This means doing physics in a way that is authentically relevant to biology and providing our students with new tools that they perceive helps them gain insight into biological systems.

4.2.2 What is cross-disciplinary authenticity?

To achieve cross-disciplinary authenticity in a physics activity for biologists, one must compose a problem set where the basic level of biological knowledge must be increased through the application of the principles of physics. To achieve true cross-disciplinary authenticity, the knowledge of a secondary discipline (in our case biological

systems) becomes more advanced through the application of the primary discipline (in our case physics) then it was at the start.

4.2.3 Why do we care about achieving cross-disciplinary authenticity?

Cross-disciplinary authenticity is important because we know from prior literature that when students perceive a scenario as being authentic in a biological context, it increases student motivation, engagement, understanding, and retention [75, 81, 97, 100–102]. As educators, we aim to provide the best education possible for our students, and these factors contribute to student success. Additionally, part of the goals of reform as stated by the biological research and medical school communities is that students develop an appreciation and understanding for the value of other disciplines (physics, math, and chemistry) to biology and medicine.

Earlier work leads us to the conjecture that students' relationship towards physics shift if the students see using physics as relevant in an authentic biologic context [97]. During a case study, Violet moved from "I don't do symbols" to being able to think with the symbols and calling herself "more of a symbols person" and attributes that to her seeing the biological authenticity as illustrated by the following quotes:

> *"I like this course better now because I actually see the implementations in the biology rather than like no biology at all. Cause I like these questions like the ATP question and the heart blood rate flow. I was like ok that's cool it actually relates." (p 20)*

"Because me as a biologist, I like biology and chemistry like so much more than I did physics. But now that I can see the relationship between all three, it's kind of made me like physics more." (p 20)

Emotions are connected with doing physics, as the act of engaging with the concepts of physics may elicit an emotional response [206–209]. Physics alone may produce negative affect; increased frustration with physics, and a negative disciplinary identity [97, 210].

In prior work, it is shown that IPLS students more readily recognize the usefulness of the activity they perceive it as being useful in biology [81]. As we move towards more cross-disciplinary authentic contexts, our aim is that students perceive physics as more useful in biology that they would otherwise or prior to the course.

4.3 Adjusting curriculum development methodology is necessary for reform.

The curriculum for a given introductory physics for the life science course is commonly developed by textbook authors and individual professors. In many instances, existing problem sets are tweaked in an effort to incorporate elements of biology. A common approach is to start with physics and then search for a way to manipulate it into a problem that incorporates a biological situation. Here we are proposing a methodology for curriculum development that takes an alternative approach. In our method, a

curriculum developer starts with a biology concept when aiming to achieve cross-disciplinary authentic physics problem sets.

4.3.1 A common approach to curriculum development is to start with physics which may lead to biologically inauthentic problems.

A commonly used method for curriculum development among physics course designers is to establish physics learning goals first. If the curriculum is being developed for a physics course for life science majors, then the developer may select a given physics concept and then search for an analogous biological context and attempt to fit the biological context to the physics concept. They then build an activity.

While this method may work in that the final result may be biologically correct, often it is unsuccessful and results in problems that are not cross-disciplinary authentic. One such example of a problem that is not cross-disciplinary authentic is of a greased pig sliding down an inclined plane. This problem appeared in the Physics for Scientists and Engineers textbook, page 165, problem #61 [211]. The text reads:

> **61** ••• You and your friends push a 75.0-kg greased pig up an aluminum slide at the county fair, starting from the low end of the slide. The coefficient of kinetic friction between the pig and the slide is 0.070. (*a*) All of you pushing together (parallel to the incline) manage to accelerate the pig from rest at the constant rate of 5.0 m/s^2 over a distance of 1.5 m, at which point you release the pig. The pig continues up the slide, reaching a maximum *vertical height* above its release point of 45 cm. What is the angle of inclination of the slide? (*b*) At the maximum height the pig turns around and begins to slip down the slide, how fast is it moving when it arrives at the low end of the slide?

Tipler, et al. 2009

While this may be a novel problem with a touch of humor, it is not cross-disciplinary authentic because it yields no insight into a biological system.

Another problem of this nature is a problem labeled as being both biological and involving photosynthesis that appears in the Physics, Third Edition textbook, page 15, problem #37 [212]. The text reads:

> 37. • **BIO Photosynthesis** The light that plants absorb to perform photosynthesis has a wavelength that peaks near 675 nm. Express this distance in **(a)** millimeters and **(b)** inches.

Walker 2007

Some problems that start from physics may be considered biologically relevant but still not cross-disciplinary authentic because they do not yield insight into a biological system. An example of this is found in the College Physics, volume 1 textbook, page 479, problem #83 [213].

> 83. •**Biology** Blood takes about 1.50 s to pass through a 2.00-mm-long capillary. If the diameter of the capillary is 5.00 μm and the pressure drop is 2.60 kPa, calculate the viscosity of blood. Assume laminar flow. SSM

Freedman, et al. 2014

This problem set, while may be biologically relevant, still misses the final critical step of producing an increase in biological knowledge, and therefore is also not cross-disciplinary authentic.

It is notable that designers may start with the physics, but still end up with a cross-disciplinary authentic problem set that students perceive as yielding insight into biological systems. One such example of this is a problem set developed and deployed at the University of Maryland, College Park titled, “Insane in the Membrane”. This

problem set begins with identifying a base level of biological knowledge. It then uses physics to increase that level of biological knowledge. It was, however, developed by first identifying the physics concept that was desired to be taught. It provides an example of a problem set that was not developed in our proposed methodology yet still achieved cross-disciplinary authenticity. The problem set is in the University of Maryland's Physics Education Research Group's Open source textbook. Its introduction and question #6 are shown [214]. From this activity, students learn the physical mechanism behind a process that they have usually only been told happens.

How did life originate?

Many of the early models of life's origins proposed by biologists included as a crucial step the formation of **proto-cellular compartments** that could serve as distinct / discrete environments in which chemical reactions could take place. However, the exact structure and mechanism of their formation remained unknown.

In the 1960s laboratory experiments demonstrated that **phospholipids** could spontaneously assemble into bilayer membranes forming bacteria-sized containers (vesicles). Later experiments demonstrated that such vesicles could also form under simulated early-Earth conditions. Such experiments paved the way for a line of research investigating how these self-assembling membranes could have functioned in the evolution of living cells.

But how exactly does spontaneous membrane formation work? What are the mechanisms that drive this process? It turns out that an **understanding of the combined effects of energy and entropy** can help us make sense of this phenomenon.

6. Putting all this together, explain how phospholipids can spontaneously self-assemble into a lipid bilayer. Why this particular shape? (Why not a monolayer, or a trilayer?) Note that the individual phospholipid molecules are still free to move around within the bilayer, like a two-dimensional liquid; they're not bound together like a solid.

Dreyfus, et al. 2012

4.3.2 A novel approach to curriculum development is to start with the biology.

While we have acknowledged the possibility of creating cross-disciplinary authenticity through a traditional methodology (starting with the physics) we acknowledge the rarity of such problems and the difficulty of creating them. Therefore, we now put forth a methodological approach for the creation of such course content.

This work proposes and utilizes this approach and introduces a novel example problem set examining one of the roles that physics plays in gaining insight into a select biological system.

We aim to create problem sets that demonstrate the authentic use of physics inspired by modern biological research. Our dual goals are to have students see the value of mixing physics and biology perspectives (yielding insight into biological knowledge) and to obtain novel insight into the biological system, yielding insight into research questions. We seek to accomplish this by starting with a selected biological system and thoroughly examining the underlying physical concepts involved.

4.3.3 Starting with the biology can readily lead to achieving cross-disciplinary authenticity.

In our approach, one first starts with a given biological context. Once a biological system of interest has been selected, then the developer may ask questions about the scenario. From these questions, the developer begins by reflecting on the existing principles of physics and using physics as a lens to answer questions about the biological system. From this point, the developer then builds problem sets. To identify whether cross-disciplinary authenticity has been achieved, the developer identifies whether or not students perceive that insight into the biological system has been gained.

4.4 Problem sets can be both cross-disciplinary authentic and biologically research-based.

In addition to being cross-disciplinary authentic, one can build problem sets that are based on current biological research, biologically research-based. We use being biologically research-based in this context to mean that the biological system of choice is a system that is the focus of current biological science research and that the questions that are asked may be unknown to both the students and the researchers (as opposed to open research questions in the physics education research community).

One example of this incorporation, that ultimately inspired this work, was done by two of the course developers, Drs. Edward F. Redish and Wolfgang Losert. They created problems on inter-cellular signaling that were based on the research of Dr. Losert. My work, as discussed in this dissertation, carries out a study that not only builds lessons based on biophysics research but modifies and adapts it through research into student responses.

It is also notable that cross-disciplinary authenticity based on active research questions does not require a complete analysis of the research topic -- only a demonstration that the cross-disciplinary analysis produces a useful component in helping to build the full understanding of the research. In the work of Drs. Redish and Losert, the final result from a research perspective was not achieved, but they did produce a tool that yielded a useful insight and therefore achieves cross-disciplinary authenticity. A full analysis of the research would have been too in-depth for this course.

My work has this same property in that the students are not presented with the full research components as discussed in the first few chapters of this dissertation. They are merely presented with a biological scenario and then utilize physics to further their understanding of it.

Additionally, I discuss the development of these interdisciplinary-authentic, research-based problem sets with the hope of achieving three outcomes: raising awareness of their value within the physics education research (PER) community and beyond; inspiring and serving as a template for the establishment of new partnerships between members of the PER community and members of the biological science research community; and serving as a catalyst for the creation of future similar problem sets.

4.4.1 Utilizing Laplace pressure to gain insight into collective cell migration creates an excellent cross-disciplinary authentic, research-based example problem set.

To facilitate the development of our cross-disciplinary authentic, research-based problem set, we considered our colleagues' research at the National Institutes of Health (for the identification of a biological system for examination). Careful consideration of the issues in the biological system they used in their current research led to our choice of physical principles and the role they played in their system.

The University of Maryland not only maintains diverse research endeavors on our campuses but also benefits from collaborations with local federal and industrial laboratories. One such collaboration is with a developmental biology lab at the National Institutes of Health (NIH). This lab uses the popular animal model zebrafish, which is an

ideal model due to, in part, the transparency of its embryos. Additionally, genetic modification in select lines produces a fluorescent tag in certain cell membranes. This allows researchers to easily observe the cells of the fish as it develops as was discussed in Chapter 3.

In this research, specific attention is given to a select group of biological cells that move together during the development of the zebrafish called the posterior lateral line primordium (pLLp). The migration of the pLLp deposits subsets of this group of cells, called neuromasts, at various points in the developing fish. Neuromasts develop into a sensory organ (the lateral line). The lateral line is an organ that detects the pattern of water movement, vibration, and pressure changes along the fish body [18, 215].

There are many ongoing research groups examining this system, as it lets researchers observe the behavior of collective cell migration *in vivo*. This collectively migrating pLLp and neuromast deposition is a biological system is governed by the laws of physics. It is this system that we selected to use to as a foundation for developing our cross-disciplinary authentic, research-based problem set.

This particular biological system was chosen due to interest in the system and access to biological support; it was not selected for being especially amenable to simple physical models. One of the intriguing facets of this system is that cells' biomechanical interactions with each other and their environments are important to our understanding of cancer metastasis and atherosclerosis, in defining stem cell differentiation, during collective cell migration, and, in this case, in the deposition and development of the lateral line sensory cells in zebrafish.

In the development of our cross-disciplinary authentic, research-based problem set, now that we have a select biological system of interest we can move on to the next step. We now ask questions about the system and then find that viewing this biological phenomenon through a physics lens can yield valuable insights and increase our knowledge of the biological system.

4.4.2 Utilizing a physics lens may yield insight into the questions surrounding the biological system.

Some of the questions that one may ask about this particular system include but are not limited to:

> *How do the cells deposit into blobs? How do the cells "know" where to be deposited? How do they know where to migrate? Are there any physical cues in the environment? Is there a particular number in each blob? What do the biomechanical properties of the cells have to do with the system? How come the blobs of cells form? Why do the other cells continue to migrate? How do they know in which direction to travel? Do forces play a role? Could we learn something about this system by considering the known forces? How do the cells know which way to grow? What are the pressures involved? Are there different pressures in different locations? Why are the cells different shapes? Etc., etc., etc.*

This is only the start of a seemingly endless set of unanswered questions. Some of these questions may be unknown to our research collaborators, others unknown to the students in our IPLS course, and others unknown to both the researchers and the students. This last category—unknown to both researchers and students—may be the most intriguing, though all questions that are unknown to the students could be considered.

After identifying an animal model common in developmental biology, observing the behaviors of the biological system, and understanding the biophysical-based research questions, we contemplated the physical components that affect the mechanical properties and functioning of the cells. The mechanical properties and functioning of the biological cells are referred to as cellular biomechanical properties, and include the Newtonian properties of pressure, motion, and force in the cellular environment. Examination of the cellular biomechanical properties provides a deeper understanding of the biological system and reveals otherwise hidden biological insights, specifically by utilizing the Laplace Pressure equation discussed in Chapter 2.

Not only does consideration of these Newtonian properties provide a deeper understanding of the system, but these are properties that are already taught in the IPLS course. This presents an opportunity to develop a cross-disciplinary authentic, research-based problem set.

4.5 An iterative design process aligns all stakeholders' views.

To facilitate the problem set creation, I assembled and managed a design team that included two faculty members, two other graduate students, and myself. As a design

team, we first discussed the development of cross-disciplinary authentic, research-based problems internally. We then also connected with our peers, our colleagues, and the IPLS students, making changes after each discussion. The student response was a key part of this iterative design process. By some measures, the IPLS student response holds the most valuable input and is the most critical element as the problem set is being designed for IPLS student use.

The goal of the iterative design process is to align all stakeholders' views—of the design team, colleagues and peers, and students—towards a perceived outcome of authenticity, while acknowledging that each group commonly comes in with various levels of biological knowledge. This process involves incorporating feedback from not only the students, but from others in our research group, another biophysics research group on campus, and our collaborators. After feedback was received from each of these groups, the problem set is updated, and the iterative process continues until all parties have merged on a stable design (as defined by one or fewer requested revisions per problem viewing).

4.5.1 We obtained feedback from IPLS student focus groups, colleagues, and peers.

Our method for obtaining feedback from the students involves presenting the problem set to focus groups of three to four students who are currently enrolled in our IPLS course. These interviews were voluntary and were approximately 50 minutes in length. Students received a small monetary amount for their participation. The

interviews (n=10) were both video and audio recorded and then transcribed. The student response is detailed in Chapter 6.

For obtaining feedback from our colleagues, we distributed the problem set during a regularly scheduled Physics and Science Education (PHYS/SCIED) Research Group meeting and allowed our colleagues to work through the problem set in groups of three or four. The PHYS/SCIED Research Group is a group of faculty, researchers, lecturers, post-docs, and graduate students who are actively conducting research pertaining to physics education. These individuals are either physicists studying education or education specialists choosing to focus on physics. Their feedback was then incorporated into the next version of the problem set as part of the iterative design process.

I also distributed a version of the problem set at a regularly scheduled group meeting for the Losert Lab at the University of Maryland, College Park. This meeting is for Dr. Losert and the postdocs and graduate students who are doing research in his lab. These individuals have backgrounds in either biology, physics, or engineering, but do not have expertise in education. As with the PHYS/SCIED Research Group, the members of the Losert Lab were also allowed to work through the problem set in groups of three or four.

The design team then discussed the feedback received from both group deployments and made modifications to the problem set. The goal for myself and the other members of the design team, as the curriculum developers, was to get the views of members of these groups to align.

I consider one iteration as being made every time the problem set was changed whether that be due to design team discussions or feedback from the other stakeholders.

The problem set went through 21 of such iterations before arriving at the final version, which is viewed as cross-disciplinary authentic by all stakeholder groups. This final version was deployed in our IPLS course in the form of a recitation, a homework problem, a quiz problem, and an exam problem. The details of the final content are discussed in Chapter 5 and our analysis of student responses and how they were used to develop the activities are discussed in chapter 6.

4.6 This work can raise awareness, serve as a template, and serve as a catalyst.

We discussed the development of creating cross-disciplinary authentic, research-based problem sets with the aim of raising awareness of their value within the physics education research (PER) community and beyond; inspiring and serving as a catalyst for the establishment of new partnerships between members of the PER community and members of the biological science research community; and serving as a template for the creation of future similar problem sets.

This cross-disciplinary authentic, research-based problem set examines the collective cell migration in zebrafish through consideration of the relevant Newtonian properties of pressure, motion, and force. Student responses show that such a problem set is feasible but that work still needs to be done in order to enable biology students to engage effectively with physical modeling of research-oriented biological systems. We first gained an understanding of a biological system used in the research of our colleagues' biological laboratory at the National Institutes of Health. It was through first

developing an understanding of the biological system that we were able to make a determination regarding the physical parameters affecting the system.

This work provides a model for the establishment of new partnerships between members of the PER community and members of the biological science research community through discussions of the existing collaboration between laboratories at the University of Maryland and the National Institutes of Health. If an existing collaboration between a given physics group and a biological group does not exist, one can inquire with others in their network regarding a suitable biological group for a collaborative problem set development effort. Many biological scientists would enjoy having their research examined with a physics lens and discussed in an IPLS course.

For the PER community, we hope that additionally this work will serve as a catalyst for the continued creation of future problem sets that begin through the examination of modern biophysical research questions. This has the potential to benefit the entire life science community, which in turn produces our biological science researchers and physicians. Having well-educated researchers and physicians is a benefit to all.

Further work can include an examination of the mechanistic reasoning behind how the students experience authenticity [216, 217]. Attention regarding student perception on the cross-disciplinary authentic and understanding of Laplace pressure may yield greater insight into the development of future problem sets and the life science students' responses to them may be given. The problem set may be incorporated into the IPLS course at UMD and analysis conducted upon implementation. Additional study could also examine various other components of this and other interesting biological

systems and research methods. Such analysis may contribute to a more robust and relevant set of materials for ILPS students.

In summary, in this chapter, we introduced and defined the concept of cross-disciplinary authenticity, which is achieved through providing additional insight into a biological system through the application of physics. We also discussed a novel curriculum development methodology that involves starting with a biological system, instead of starting with a physics concept. We next examine the content outcome of utilizing this methodology (Chapter 5) as well as the student response to the problem set (Chapter 6).

Chapter V: Description of the Laplace Pressure Problem Set

To develop biologically-authentic, research-based course material for the Introductory Physics for the Life Science (IPLS) course at the University of Maryland (UMD), I draw from my own experimental biophysics research (detailed in Chapter 3). This work serves as an excellent biological scenario to examine through the lens of physics and biological base for the development of a problem set for our IPLS course.

This chapter demonstrates that development of a biologically-authentic, research-based set is possible and focuses on detailing the contents of the problem set. It should also be noted that while this material was designed for group work in an introductory physics course, optional modifications are suggested for deployment in upper-division courses. The student response to the portion that we deployed in the large-scale IPLS classroom at UMD will be discussed in Chapter 6.

Cross-disciplinary authenticity leverages tools and concepts available in a given discipline to increase the knowledge base of another discipline. Through an iterative design process, the design team developed such a problem set that is cross-disciplinary authentic in that students will come into the IPLS course—and more specifically begin this problem set—with a base level of biological understanding. The problem set enables—through the application of physics concepts—an increase in the students' understanding of biological systems, thus meeting the criteria for being cross-disciplinary authentic. The content of an example cross-disciplinary authentic problem set is the focus of this descriptive chapter. The problem set is intended to be completed as a group activity such as in a discussion session in which 3 to 5 students are working on the

problem set together. There is such an opportunity for this in the weekly discussion session of UMD's IPLS course.

Through 21 problem set iterations, revisions lead to a final version in which all stakeholders—the design team, our colleagues, peers, and the students—converged on a problem set that was perceived to be cross-disciplinary authentic. The 21st version of the problem set is detailed in this chapter along with discourse regarding the reasoning for the addition (or removal) of problem set components.

The problem set opens with providing motivation for the problem set for the students and a clear identification of their base level of knowledge. It then moves on to examine the concept of pressure differences in a non-living system (a pipe) before introducing interdisciplinary modeling. It then moves into the examination of a classical research method that relies on interdisciplinary modeling. The Laplace Pressure equation is then introduced accompanied by a derivation (or dimensional analysis activity depending on course level) and online simulation.

The problem set then moves back to the classical research method introduced earlier in the problem set, this time applying the Laplace Pressure equation to yield a quantitative result. The problem set then moves into an examination of a living system where the students will use the concept of pressure differentials, interdisciplinary modeling, and the Laplace Pressure equation to describe the system and produce a biologically-relevant quantitative result. The problem set concludes with encouraging the students to reflect on the learning accomplished and allows the educator to confirm an increase in biological knowledge. These components, as are found in version 21, the final version of the problem set, are detailed further in the remainder of this chapter.

5.1 Problem 1 provides motivation for the problem set.

5.1.1 Problem Text

The first problem text introduces the problem set and provides motivation for the students by framing the abstract problem by putting it into a realistic biological context from the first. It is designed as a question requiring a response to ensure that the students read and respond. The problem text for problem one is as follows:

> *In this problem, we will first investigate pressure in non-living scenarios such as in a pipe and in two bubbles before considering the role that pressure plays in morphogenesis. Considering the pressure of cells and of their surrounding environment is important as pressure plays a vital role in many biological phenomena such as morphogenesis, collective cell migration, and cancer metastasis. Describe qualitatively the role that pressure has in determining cell shape, citing biological examples.*

5.1.2 Problem Description

In the initial iteration of the problem set, this problem was not included. It was however discovered through the iterative design process (specifically through an interview with another faculty member at UMD) that the problem set lacked motivation

and the establishment of a base level of biological knowledge. The design team then developed the problem as a motivational paragraph to frame the problem set.

As the iterative design process continued, we observed in our student interviews that the students were not reading the motivational paragraph. After discussion, the design team changed the motivational paragraph to a problem to ensure student engagement. We then added a directive statement to the paragraph and shifted the numbering of the remaining problems. This was successful in future iterations for obtaining student engagement.

The directive in this problem is designed to encourage students to pull from their own prior basic biological knowledge. The entire problem set is designed in such a way as to examine this base level of knowledge through a physical lens and increase understanding of biological systems. The final problem of this problem set (Problem 11) is useful for assessing whether the students' level of biological understanding has increased and will be discussed later on in this chapter.

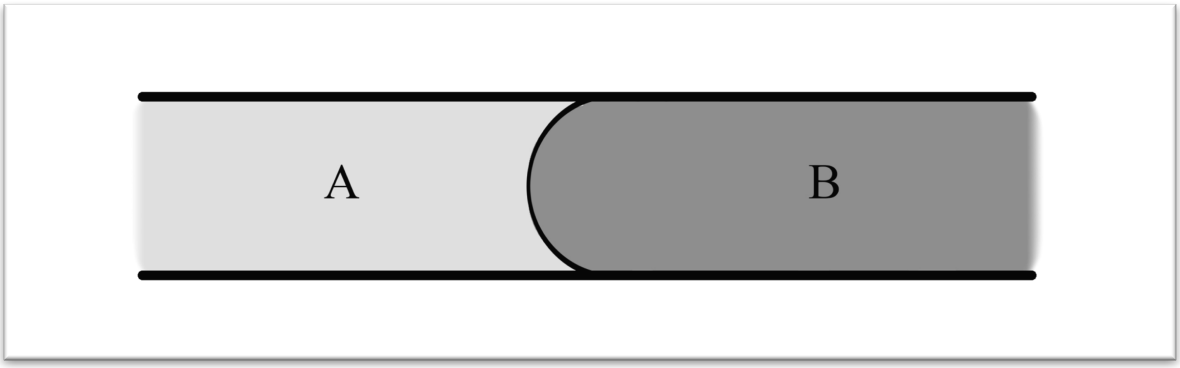
A broad range of responses to this problem are expected, as base levels of biological knowledge vary. (One semester of college biology is a pre-requisite for this physics course, and most students have completed two or more terms.) While there is no one ideal response, some responses may include aspects of cell morphology and cell differentiation or the idea that the characteristics that surround a cell will affect how the cell differentiates. As was discussed in Chapter 2, pressure has a strong impact on the development of the cell. One example is the constricting of a blood vessel which can cause a blood cell passing through it to elongate, thus altering the natural shape.

5.2 Problem 2 introduces the notion of determining pressure difference through visual inspection.

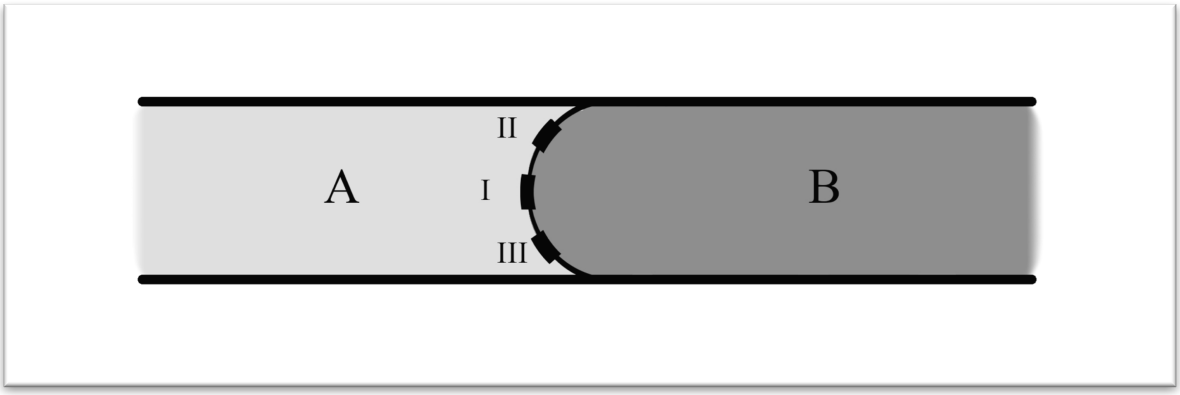
5.2.1 Problem Text

Following the motivational first problem, the second problem moves into an examination of pressure differentials through the consideration of fluids in a pipe. It was designed as a qualitative introduction to the concept of pressure differentials. The problem text for problem two is as follows:

> *2. (i) The illustration below shows a pipe containing two fluids. The view is of a cross-sectional slice of the pipe. The fluids in the pipe are separated by an elastic membrane that is fixed to the pipe. Consider the interface of the fluids as a hemispherical dome. Use words to describe how you could tell by looking if the two static, incompressible fluids in the pipe (pictured below) have the same or different internal pressures.*



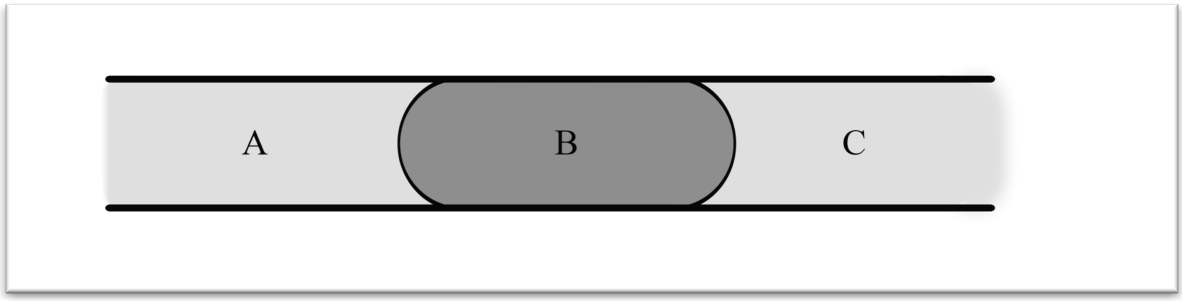
(ii) Draw free body diagrams for each of the small patches of surface area at the locations (I, II, and III) indicated below. State whether or not there is a net force at each location, and if there is, indicate the direction of the force.



(iii) In a different experiment with the same membrane materials, the curvature of the membrane is higher. What can you conclude about the pressures in the new scenario with regards to the initial scenario from part (i)?

(iv) In a different experiment with the same membrane materials, the curvature of the membrane is higher. What will change (if anything) in the free body diagrams for locations I, II, and III as drawn for part (ii)?

(v) Suppose there are now three fluids in the pipe, as pictured below. Fluid A and fluid B may or may not be the same. Rank the fluids in terms of greatest to least pressure.



5.2.2 Problem Description

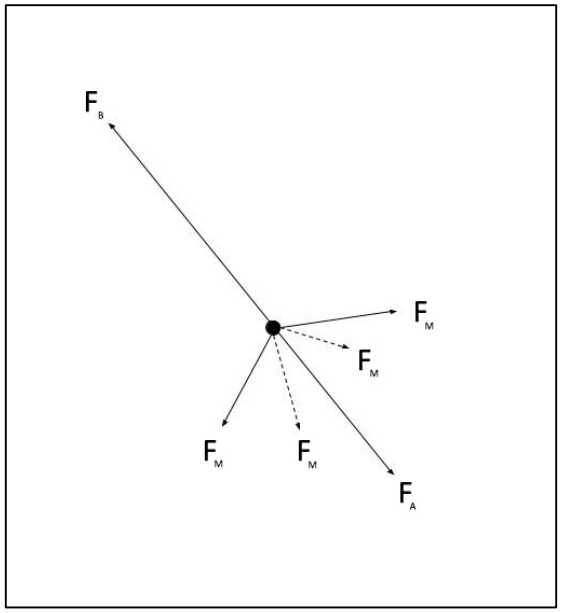
The problem begins by presenting a scenario depicting two fluids in a horizontal pipe. From visual examination, we know that fluid B has a greater pressure than fluid A as indicated by a curved membrane that is protruding from fluid A into fluid B. If fluid A and fluid B were exerting equal amounts of force on the membrane, then the membrane would be a flat surface. The fact that it is hemispherical tells us that one is pushing harder than the other. In this scenario, fluid B is exerting more force and thus has a greater pressure. While this is an intuitive result that almost every student will come up with, we want them to see how this result in fact follows from the basic principles of Newtonian mechanics.

The next component involves considering three separate small patches of surface area on the interface of the two fluids. The areas are located near the top horizontal boundary of the pipe (point I), the center (point II), and the lower boundary of the pipe (point III). The three distinct free body diagrams (FBD) for each of the selected points along the membrane should be created.

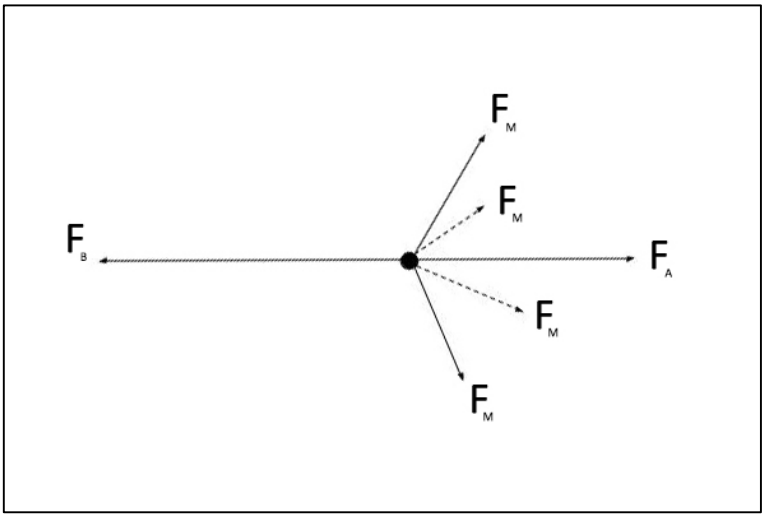
Each of the three created FBDs should include the forces from each fluid and surface tension and indicate that the net force is zero. Each FBD should resemble the others in terms of the magnitude of the vectors and relative direction, meaning the direction of the vectors in relation to each other within a given FBD. The vectors are distinct in direction; each diagram should be similar to the others in terms of magnitude. The original FBD could be rotated along the membrane to be placed in locations two and three.

To be technically correct, the FBDs should consider all three dimensions (dimensions *x*, *y*, and *z* being the traditional *x* and *y* along the flat surface of a paper and *z* protruding from the page) however students in our IPLS course typically neglected the *z*-dimension. Example FBD illustrations considering all three dimensions are below:

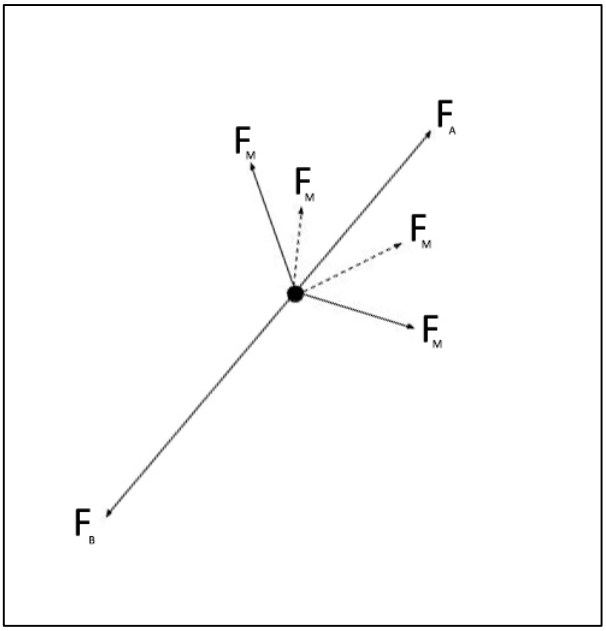
Location I:



Location II:



Location III:



Each of these illustrations shows six vectors. The vectors correspond to the force due to tension in the membrane (F_M) and the forces due to the pressures of the fluids ((F_A) and (F_B) for fluids A and B respectively). The critical point of the illustrations is that the tension vectors do not balance because of the curvature of the membrane. This is described further below.

There will be forces due to tension in the membrane in all three dimensions. The vectors due to the tension force in the membrane should be along the membrane, bent slightly inward towards the direction of fluid B. We chose to illustrate the vectors into the *z*-dimension via a dotted line. One may omit the vectors in the *z*-direction or modify the wording of the problem in such a way as to encourage the consideration of all three dimensions.

There are also forces due to the pressure of the fluids. Considering a tiny patch of the membrane, the pressure force of fluid B is greater than the pressure force of fluid A. This is shown by the magnitude of the vectors orthogonal to the membrane patch. The vector protruding into fluid A is greater than the vector protruding into fluid B. The difference in magnitude is proportional to the sum of the components of the forces due to membrane tension along the axis parallel to the forces due to pressure.

Additionally, since the scenario is static, all of the forces are in equilibrium; there is no net force. All of the components of each of the vectors along a given axis combine to cancel each other out. The main point is that we know that the tension vectors are not balanced so the pressure forces must balance them. This means that the net pressure force is out so the inner pressure must be greater than outer pressure (with "inner" here being on the side of the center of the curvature).

The problem continues by examining a different scenario (that does not have an accompanying illustration). The new scenario has a greater membrane curvature than the prior scenario, which means that it is a tighter circle and indicates that the pressure differential between the two fluids is a larger pressure differential. The difference in the pressures of the fluids in this scenario is greater than the difference in pressures of the fluids in the first scenario.

The FBDs will also adjust in that there will be a greater imbalance of the forces due to pressure. The difference in the magnitude of the pressures orthogonal to the patch of membrane will be greater, as the push of B on A is greater than the push of A on B by a greater amount. Additionally, the angle of the vectors due to the tensions forces to a line tangent to the membrane will be greater. They will deviate more from being perfectly parallel, in the direction more towards B to establish equilibrium. Also, the more curved the membrane is, the longer the force vectors will need to be, which confirms the tighter radius of the circle.

The following (final) component of the problem introduces a third fluid into the pipe. Instead of fluid B protruding into fluid A, we now examine fluid B is protruding outwards in the directions of both fluids A and C. From the previous parts of this problem, we know that fluid B is of greatest pressure. The curvature of the A/B interface exactly matches the curvature of the B/C interface. Since the curvature is the same curvature, the same pressure differential exists between fluid B and the fluids to either side. B is the greatest pressure, and A and C are at a less pressure but are equal to each other.

To confirm that the curvature of the A/B interface exactly matches the curvature of the B/C interface, one can measure them directly or either fold the printed document to overlay the curvatures or, if viewing electronically, can capture and overlay a screenshot. This component moves the students towards thinking about a more complex scenario and serves as preparation to move into problem 3.

5.3 Problem 3 facilitates interdisciplinary modeling.

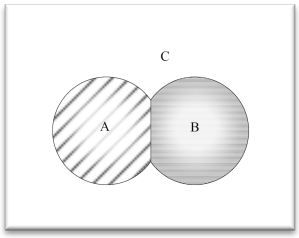
5.3.1 Problem Text

Problems one and two introduced the problem set and the physical concept of pressure differentials. Problem three is designed to investigate the pressure-differentials involved in more complicated non-living system before engaging in interdisciplinary modeling. This problem provides an opportunity for the students to engage in creating a toy model of a living system and discussing the limitations of such model. The problem text is as follows:

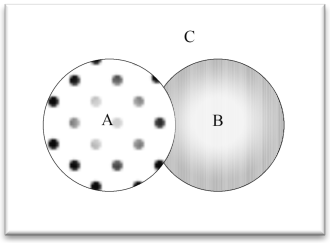
> *Suppose two bubbles are captured in the moment pictured. For each of the scenarios in the diagrams below, showing a cross sectional center slice of the bubbles, state whether the internal pressure of bubble A is greater than, less than, or equal to the internal pressure of bubble B, and how that relates to the pressure of the surrounding environmental pressure, C. Each bubble may or may not be made of the same material.*

(i) Using greater than or equal to signs, rank the pressures in the following scenarios. If the ranking cannot be determined, state what additional information would be needed.

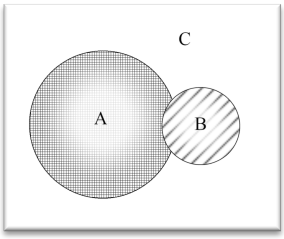
Scenario 1:



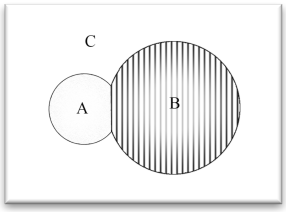
Scenario 2:



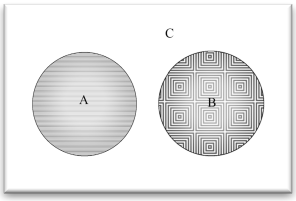
Scenario 3:



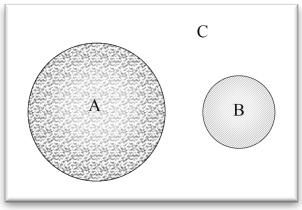
Scenario 4:



Scenario 5:



Scenario 6:



(ii) If you declared that any of the scenarios could not be fully ranked, what additional information would you need to know in order to fully determine the ranking, and what would that ranking be?

(iii) If instead of the cross-sectional slice of attached bubbles, the images above are now the view through an inverted microscope showing various different objects attached and suspended in solution. Each object is made of a phospholipid bilayer encasing a homogenous colloidal liquid. Would your responses for part (i) change? If so, how? If not, why not?

(iv) If instead of the above scenarios, the images above are now the view through an inverted microscope of spherical circulating tumor cells that are attached to each other and suspended in solution, would your responses for part (iii) change? If so, how? If not, why not?

5.3.2 Problem Description

This problem considers six scenarios that arise when two bubbles of the same or of different pressures and/or material and/or sizes are adhered together. The problem does not specify the material of which the bubbles are made. The bubbles share a common environment. The curvature at the interface is examined to identify which pressure is greater. In this question, each of the scenarios shows bubble A on the left, bubble B on the right, and the environment, labeled C. The pressures are then ranked in relation to each other.

This problem is designed in such a way that it is not necessary to use or even know the equation for Laplace Pressure, which relates the pressures to the surface tension and radius. One is, however, welcome to utilize it if so be desired.

In scenario one, there is no curvature at the interface of the bubbles. The intersection of these two bubbles forms a straight line (when considering the cross-sectional center slice). The pressure difference between the two balloons is zero. The environmental pressure is less than the pressures inside of the bubbles. The pressure of bubble A is equal to the pressure of bubble B and both are greater than the environmental pressure, C. This is true regardless of whether the membrane tensions are the same or not. Shorthand: A=B>C

In the second scenario, bubble A has a positive curvature, protruding into bubble B, which indicates that the pressure of bubble A is of a greater pressure than the pressure of bubble B. The pressure of bubble A and the pressure of bubble B are still greater than the pressure of the external environment, C because they are protruding outward. Shorthand: A>B>C

In the third scenario, bubble B has a protruding positive curvature into bubble A, which means of the pressure in bubble B is greater than the pressure of bubble A, though it is of a smaller size. The pressures of bubble A and of bubble B are both greater than the pressure of the external environment, C. Shorthand: B>A>C

In the fourth scenario, there is again no curvature at the interface. Though the bubbles are of different sizes (having different radii), the pressure difference between the two bubbles is zero. The surface tension must differ between the bubbles. The pressure

of bubble A is equal to the pressure of bubble B and both are greater than the environmental pressure, C. Shorthand: A=B>C

In the fifth and sixth scenarios, there is not enough information to state a relative pressure differential between bubbles A and B. Either the surface tension or the relative internal pressures is needed to determine the solution. In both scenarios, bubbles A and bubble B have greater pressures that the external environment. Shorthand: A>C and B>C

The third sub-component of the problem set, (3. (ii)), focuses on scenarios 5 and 6, in which the objects are not in contact.

Scenario 5: We know that the pressure of A is greater than the pressure in the external environment, C, and that the pressure of B is greater than the pressure in the external environment, C, because of the curvatures of each bubble. What we cannot make any statement about is the relative pressure differential between A and B because while they look like they have the same radius of curvature in Scenario 5, they could have different surface tensions and they could, in fact, be at a different pressure difference to the outside medium C.

Scenario 6: We know that again the pressure of A is greater than the pressure in the external environment, C, and that the pressure of B is greater than the pressure in the external environment, C, because of the curvatures of each bubble. What we cannot make any statement about is the relative pressure differential between A and B because while they look like they have different radius of curvature in Scenario 6, they could have different surface tensions and they could, in fact, be at a different pressure difference to the outside medium C. We do not whether or not they have the same surface tension, and since we do not, we cannot make a statement about the ranking.

The third and fourth components combine to present the opportunity to engage in interdisciplinary modeling. Elements include creating a toy model of two cells, and discussing the limitations of the model, and proposing how to make the toy model more robust. It may also be useful to note that in the literature, the model of a cell as being a liquid surrounded by an elastic cortical shell is a most popular model for and is surprisingly good at predicting cellular deformation in various scenarios [129].

The third component addresses a non-living system comprising two phospholipid bilayers encasing homogenous colloidal liquids and suspended in solution. A phospholipid bilayer can have a variation of tension; the surface tension may be unequal. The previous scenario contained homogenous liquids; the fact that the homogenous fluids are now homogenous colloidal liquids does not affect the scenario so long as the substrates are not creating internal structure.

The fourth component was specifically designed to elicit interdisciplinary modeling with the aims being to promote engagement with the model and to discuss the limitations of the model. There are two natural responses to this problem, both of which require engaging with the model.

The first response is to recognize that a cell can indeed be modeled as a phospholipid bilayer encasing a homogenous colloidal liquid, as a first-order approximation. This type of model is useful in the classical research method of micropipette aspiration as was discussed in Chapter 2. This response recognizes the scenarios in which this toy model is useful without further refinement.

The second response is to note the limitations of the model first and suggest additional parameters for inclusion. While in some instances, the approximation of a cell as a phospholipid bilayer surrounding a homogenous colloidal fluid is sufficient (as a toy model), there are a number of scenarios in which this approximation would not provide as robust of a model as needed.

For cases in which the toy model is not sufficient, biological contributions should be taken into account. Biologically speaking the cytosol contains a strong structure, the cytoskeleton, which changes and grows and attaches to the membrane. There is a cortical tension that encompasses the tension in the membrane along with the tension due to internal cellular and transmembrane structures. And an actual phospholipid bilayer membrane in a cell varies in tension along the membrane itself. There is a difference in cortical tension and a membrane or surface tension. All of these biological factors (and others) contribute and could be cited to create a more refined model.

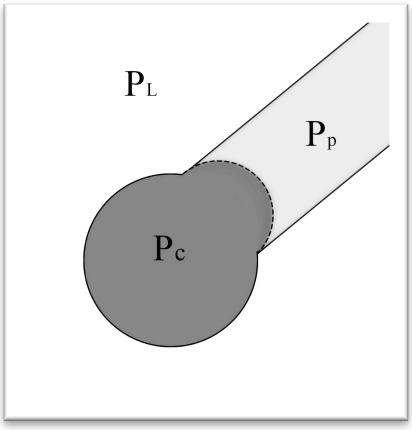
5.4 Problem 4 introduces a real-world research method.

After introducing the idea of interdisciplinary modeling in problem three, the fourth problem shows how such modeling is used in a classical, real-world research method. It was designed to provide an example for students on how these concepts are utilized in the real world. The problem text for problem four is as follows:

5.4.1 Problem Text

4. A classical research technique called micropipette aspiration involves aspirating a cell into a micropipette to determine its cellular mechanical properties. Let's investigate how this process works.

The illustration on the right depicts a single cell (dark gray) being sucked into a pipette (light gray) in suspension (white) during the process of micropipette aspiration. The protrusion of the cell into the pipette is equal to the radius of the pipette and creates a hemispherical dome.



The illustration includes the following labels: pressure inside of the cell P_c, pressure inside of the pipette P_p, and pressure in the surrounding liquid P_L.

Rank the pressures in terms of smallest to largest.

5.4.2 Problem Description

Using visual examination: P_L is less than P_c as is determined by observation of the boundary of P_L and P_c. P_p is also less than P_c as is determined by the visual examination of the boundary of P_p and P_c. In both of the instances we can make this

claim because P_c protrudes outward into P_L and P_p. We now examine the radius of curvature of P_c. The curvature between P_p and P_c is a smaller radius of curvature than the curvature between P_L and P_c.

We know from Problem 1 that, holding other parameters constant, as the circle of radius is smaller, the pressure difference is greater. The pressure difference between P_c and P_p must be greater than the pressure difference between P_c and P_L. P_p must therefore be less than P_L.

From the Young-Laplace Equation, $\Delta P = 2\gamma\left(\frac{1}{R}\right)$ in the case of a perfect sphere where (ΔP) is the pressure difference between the internal and external pressures; (γ) is the surface tension; and (R) is the principal radius of curvature (as noted in Chapter 2). From this we observe that since R is in the denominator, the change in pressures must be greater and therefore the smaller pressure must be even smaller. P_p is less than P_L. Shorthand: $P_c > P_L > P_p$

5.5 Problem 5 introduces the equation for Laplace Pressure.

The first four problems in the problem set have been entirely qualitative in nature. In problem five we introduce—for the first time in this problem set—the Laplace Pressure equation. This problem along with problems six through eight were designed to increase student comfort with this formula and with mapping meaning onto these symbols. The text of problem five is as follows:

5.5.1 Problem Text

5. In class you were given that for a sphere $\Delta P = 2\gamma/r$. Show how this is possible (true).

5.5.2 Problem Description

This problem was developed for multi-level use; one may differentiate wording according to level of course and desired response. In upper-division courses, a derivation for the Laplace Pressure equation may be appropriate. In lower-division courses, the problem can be modified to confirm by dimensional analysis. Dimensional analysis shows how it is possible. A derivation shows how it is true. The wording may be selected according to one's needs.

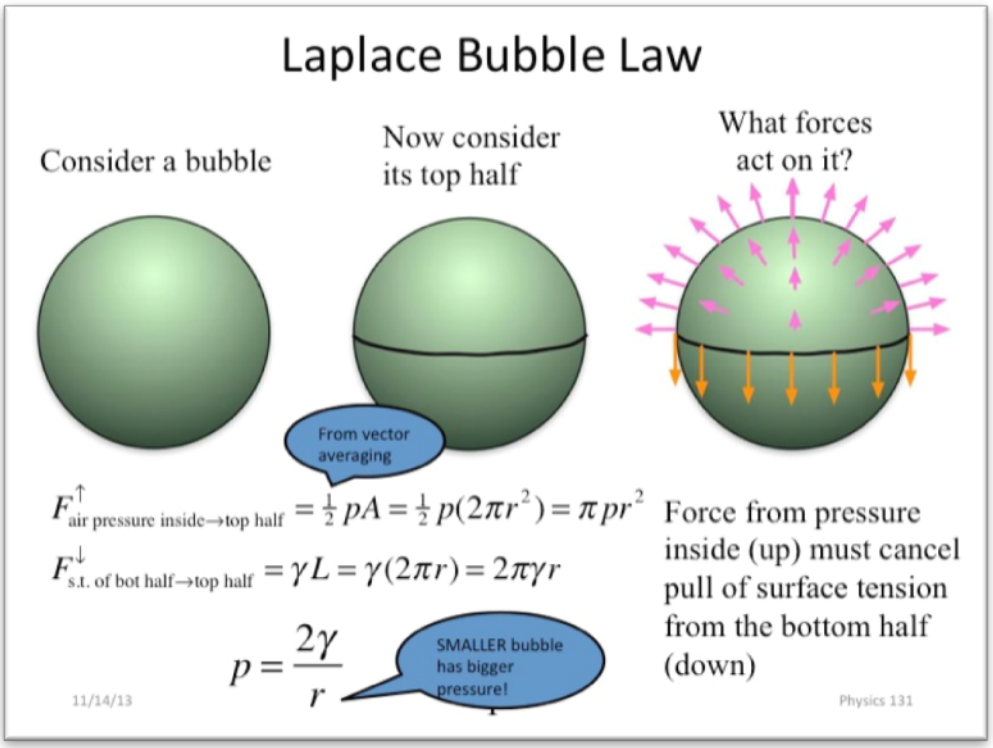
To conduct the dimensional analysis:

Pressure has units of force per length squared. Gamma has units of force per length, and radius has units of length. Force per length squared is equal to force per length squared.

To conduct the derivation:

Considered a perfect hemisphere in three dimensions, oriented so that the flat portion lies along the horizontal X-axis, extending in the Z direction, as to have no Y component. The radius of the hemisphere is defined to be R. There is a given internal pressure (P) inside of the hemisphere and a surface tension (γ).

To derive the Laplace pressure, we will first consider the sum of the forces in the vertical direction. Given that pressure, a scalar quantity, is defined as being the amount of perpendicular force (F) applied over a given surface area (A) ($P = \frac{F}{A}$), rearranging to force is pressure times area ($F = P * A$), allows us to solve for force due to pressure. Below is a slide from the first-semester course on Laplace Pressure, illustrating a bubble and the forces acting on it.



First, we will consider the sum of the forces in the vertical direction. Take the centermost point of the flat plane of the hemisphere lying along the X-and-Z-axes. Extending a vector from this point to any point on the domed surface of the hemisphere

will give a distance (R). First, we will identify a center distance vector that begins at the centermost point of the flat plane and extends vertically upwards, parallel to the Y-axis.

At the point which this vector intersects the surface of the hemisphere, we will consider the surrounding small region of surface area. Area must be considered because pressure is determined by considering the force over a given area, not over a given point. At this small selection of surface area, there will be a force, directed outwards due to the internal pressure inside of the domed hemisphere. This force will be normal to the surface area and point directly upwards, along the Y-axis.

Now let us consider another small region of the surface of the hemisphere some distance away from the surface region surrounding our center position. The vector indicating the distance from the centermost point of the flat plane of the hemisphere (located along the X-and-Z axes) to the surface of the hemisphere is still the distance R. We will now define theta to be the angle between our original R vector and the R vector associated with the new position.

The location where this vector reaches the surface of the hemisphere will also have a force due to pressure at this small region that is normal to the surface. This vector indicating the outward force due to internal pressure will have both horizontal and vertical components. The new vector of interest will be the vertical component of the force due to pressure vector.

To isolate the vertical component of this force due to pressure, we first considered theta, which is the angle between our new and original R vectors and, by the rules of trigonometry, is also the angle between our external pressure vectors and our vertical component of force due to internal pressure. The vertical component of force due to

pressure is the cosine of theta times the magnitude of the original force due to pressure vector. This stems from the rules of trigonometry which define the cosine of the angle as being the adjacent side over the hypotenuse of a given right triangle.

Now that we have the vertical component of the force due to pressure, let us consider the second variable in the ($F = P * A$) equation, the area. First consider the same vector as was considered while determining the force due to pressure that begins at the central point on the flat horizontal plane of the hemisphere and is directed to a given point at the surface. Now instead of anchoring this vector to a fixed location on the surface of the hemisphere, allowed it to move freely about the Y-axis while keeping it anchored at the central point.

If we allowed this vector to travel along the Z-axis, maintaining a constant position on the X and Y axes, it would in essence draw a circle on the surface of the dome, if one were observing the hemisphere from above. This circle would be located on the same Y-plane, parallel to the bottom plane of the hemisphere. The area of this newly identified circle would be two times π times r, where r, the radius of the circle, is given as R times the sine of theta ($R\sin(\theta)$) by the aforementioned rules of trigonometry.

Now let us consider the third dimension to determine the surface area. As you move the newly created circle along the surface of the hemisphere in the Y dimension, the radius of the circle, r, will be changing, as confirmed by the fact that the sine of theta is changing. To incorporate the surface area of the hemisphere covered by this change, we include r times the derivative of theta. Combining this with our previously determined value of $2\pi R$, we now consider the change in area to be $2\pi R^2\sin(\theta)d\theta$

Referring back to our equation ($F = P * A$), we now know that the derivative of force is equal to the pressure times the cosine of theta times the constants 2π times the radius of the hemisphere squared times the derivative of theta. ($dF = P\cos(\theta) * \sin(\theta)\, 2\pi R^2 d\theta$).

Integrating, we arrive at ($F = P\pi R^2 \int_0^{\pi/2} 2\cos(\theta) * \sin(\theta)\, d\theta$). Given that $\int_0^{\pi/2} 2\cos(\theta) * \sin(\theta)\, d\theta$ is equivalent to $\int_0^{\pi/2} \sin(2\theta)\, d\theta$, we solve this integral to be 1. To simplify our force in the Y-direction due to pressure to be equal to the constant π times the squared of the radius times the pressure. ($F = \pi R^2 P$).

Combining this with force due to tension, where force is equal to surface tension (γ) times the given length (L), we have ($F = \gamma L = \gamma 2\pi R$). We set these two force equations equal and arrive at ($F = \gamma 2\pi R = \pi R^2 P$). Solving for P, we have two times the surface tension divided by the radius ($P = 2\gamma/R$).

5.6 Problem 6 furthers student familiarity with Laplace Pressure.

Problem six, coupled with problems five, seven, and eight, were designed to increase student comfort with the equation for Laplace Pressure. This problem was designed to encourage mapping meaning onto the symbols and exploring the relationships between the variables in the equation. The text of problem six is the following:

5.6.1 Problem Text

6. (i) Now that you are familiar with the Laplace Pressure equation, consider the following scenario: Two attached bubbles are in an environment of constant atmospheric pressure. The internal pressure of each of the attached bubbles remains constant, as does the surface tension. What happens to the curvature of the bubbles as atmospheric pressure increases? What happened to the radius?

(ii) Now holding atmospheric pressure, and the internal pressure of the bubbles constant, what happens to the curvature when surface tension increases?

(iii) Now holding atmospheric pressure and surface tension constant, what happens as the internal pressure of the bubbles decrease?

(iv) Now let's examine the interface of the two bubbles. If the pressure of the first bubble is greater than the pressure in the second bubble, what does the interface of the two bubbles look like?

5.6.2 Problem Description

The problem commences by considering a scenario that does not typically occur in everyday interactions—the fact that the internal pressures of each of the bubbles remain constant. Neglecting this fact leads to an answer that the curvature and radius

both decrease. The correct response is that the curvatures and radius will both increase. As the external pressure increases, the pressure differential decreases. Gamma remains unchanged.

In the second component, both the atmospheric pressure and the internal pressure of the bubbles are constant therefore the pressure differential is constant. If surface tension increases, the curvatures will increase proportionately.

In the third component, the atmospheric pressure and surface tension are constant; in the equation gamma and external pressure are constant. As internal pressure decreases, the pressure differential will decrease therefore the curvatures will increase.

The fourth component may also be modified according to the level of the students. The information in the quotes may or may not be inserted: "Two bubbles are located at a distance of separation less than the sum of their two radii." This insertion may be included for upper-level courses and for Teaching Assistant instruction. This is omitted for introductory level courses as it adds an element of unnecessary complexity at that level.

If the pressure of the first bubble is greater than the pressure in the second bubble, at the interface, the curvature of the bubbles will be in the direction of the center of the second bubble.

Problem 7 uses a simulation to visualize the effects of Laplace Pressure.

The seventh problem continues the focus on the equation for Laplace Pressure but now moves to an online simulation platform. This problem was designed to increase

student comfort with the formula while allowing them to visually examine the effects of adjusting various parameters. The text of problem seven is as follows:

5.7.1 Problem Text

> *7. Consider the simulation found via the link on your course website or by visiting: http://ggbtu.be/m2118515. Use the simulation to create each of the scenarios in question 6 and test your answers. For each of the above parts (i-iv) state whether your answers agree or disagree.*

5.7.2 Problem Description

This question asks students to engage with a simulation I created using the online developmental platform GeoGebra.

> The simulation has the following introduction:
>
> *Below you will find a simulation of two bubbles. The bubble on the left with the green boundary is bubble one, and the bubble on the right with the blue boundary is bubble two. What is shown on the screen is a two-dimensional cross-sectional area of the three-dimensional bubbles.*
>
> *You can change the parameters of the two bubbles and their environment via the sliders in the left-hand tool bar by sliding the black dot along the*

gray line. The scenario is set up now so that bubble one should have a greater pressure that bubble two. Feel free to play with the parameters as much as you like, but if your lines disappear, your bubble "popped"!

Parameters:

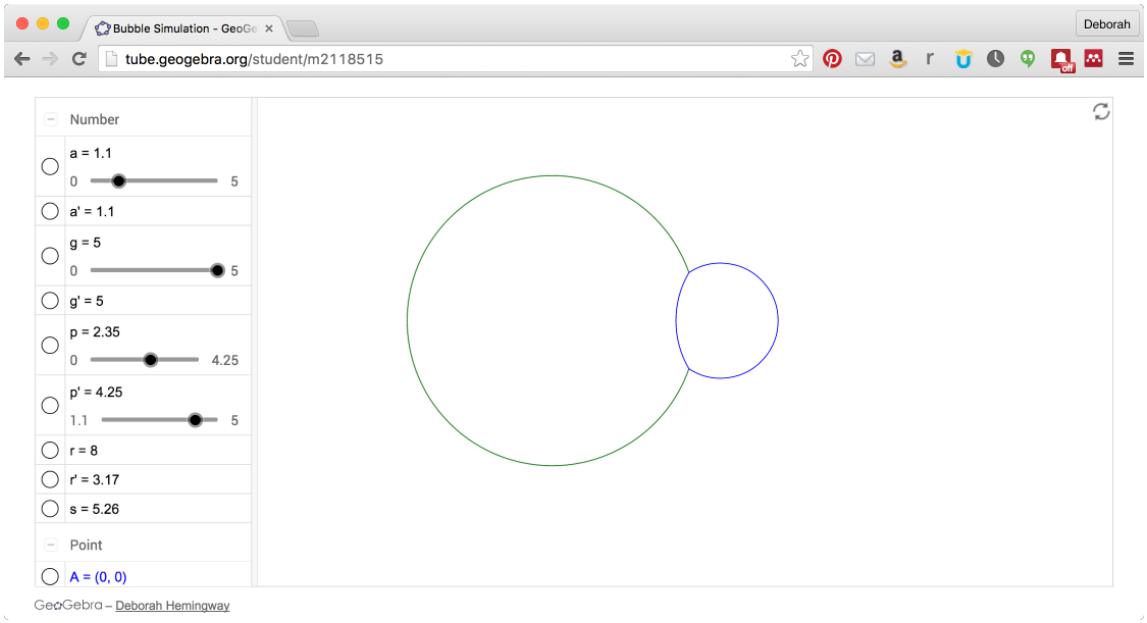
"a" is the atmospheric pressure

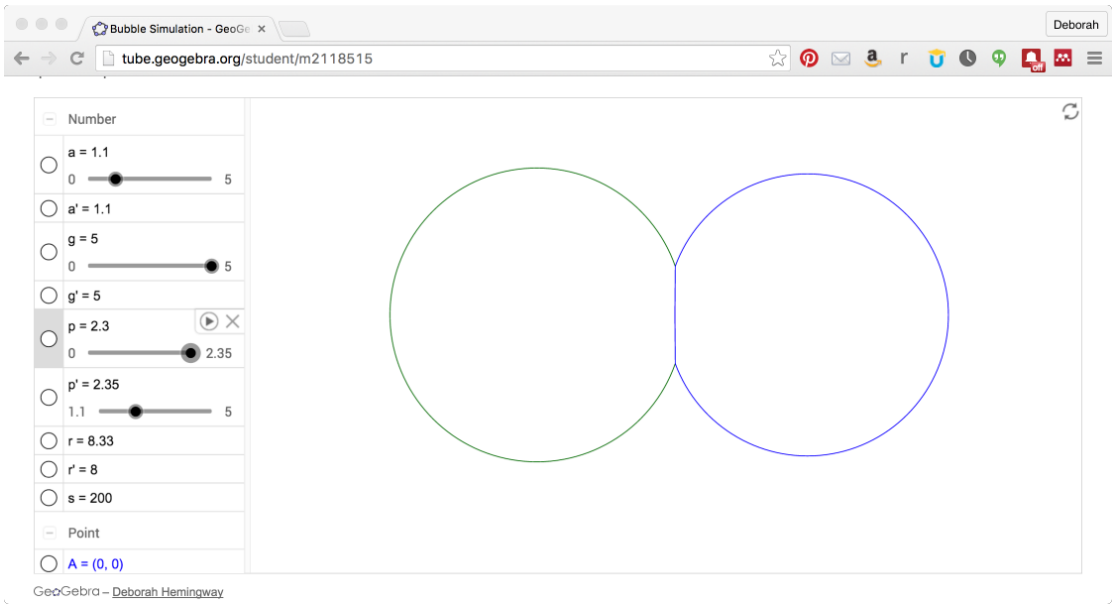
"g" is the surface tension

"p" is the pressure in bubble one

"p'" is the pressure in the bubble two

Two possible scenarios for the parameters set by the students:





The completion of this problem provokes intuition refinement or answer confirmation, depending on prior responses.

5.8 Problem 8 addresses any prior mistakes in applying the equation for Laplace Pressure.

The eighth problem is the final problem that deals explicitly with the Laplace Pressure equation. It was designed to provide an opportunity to the students to reconcile any mistakes of misconceptions in their prior problem responses. The problem text is as follows:

5.8.1 Problem Text

8. If any parts of your answers to questions 6 and 7 did not agree, what happened? If they did agree, why do you think that is?

5.8.2 Problem Description

This problem is provided an explicit opportunity to discuss and rectify any disagreements. Answers will vary based on prior response.

If the answers do not agree, one may have engaged in the wrong assumption about whether the pressure differential was increasing or decreasing or about whether surface tension was holding constant or non-constant or failing to recognize the inverse relationship between the radius and pressure differential.

Additionally, it may be beneficial to recognize that in our own real-world experiences, what we commonly experience is constant atmospheric pressure. We do not frequently encounter scenarios containing a varying pressure differential or scenarios when multiple variables are held constant.

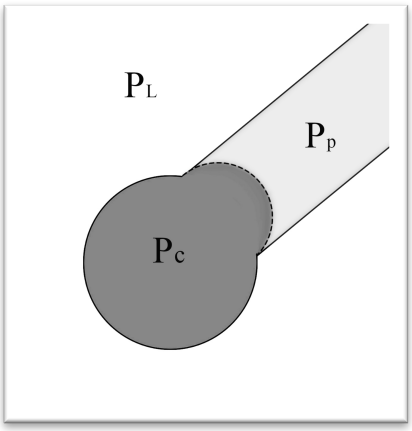
5.9 Problem 9 provides a quantifiable example of the application of the Laplace Pressure equation.

The ninth problem returns to the classical research method introduced earlier in the problem set, this time applying the equation for Laplace Pressure to yield a

quantitative result. This problem was designed to build off of the qualitative responses of the students in problem four. The problem text for problem nine is as follows:

5.9.1 Problem Text

9. Consider the micropipette system introduced in question 4, pictured at right, and the Laplace pressure formula from question 5.



(i) What is the surface tension of the cell in terms of the radii and the given pressures?

(ii) If the pressure in the pipette is 1 kPa less than the pressure in the surrounding liquid, estimate the surface tension of the cell.

5.9.2 Problem Description

This problem first involves manipulating the Laplace Pressure equation, to yield:

$$\gamma = (P_p - P_L)\left(\frac{R_p - R_c}{2}\right)$$

The second component involves combining estimation with arithmetic. The pressure difference is given as 1 kPa. A common estimation of cellular radius size is 5 μm. One could estimate the radius of the pipette to be 3 μm. This would yield a surface tension of 10^{-3} N/m.

5.10 Problem 10 applies lessons learned to an *in vivo* scenario.

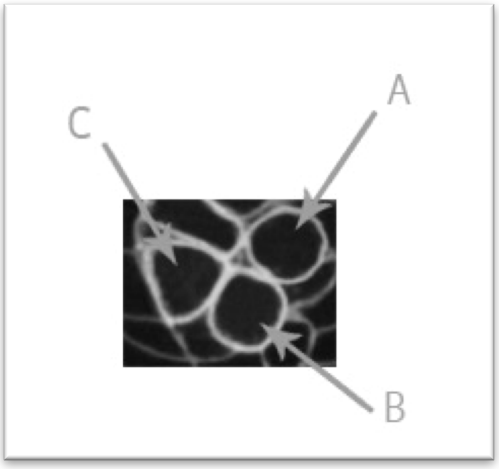
The tenth problem of the problem moves into an examination of a living system. The students will use all of the tools they learned throughout the problem set including the concept of pressure differentials, interdisciplinary modeling, and the Laplace Pressure equation to describe the system. They will also discuss the limitations of applying a toy model to the system and where the model breaks down. The problem text is as follows:

5.10.1 Problem Text

> *10. Now that we have investigated these different scenarios, let's see how these principles apply to an in vivo biological scenario. During zebrafish development, a group of migrating cells deposits neuromasts, which are clusters of cells that develop into sensory organs.*
>
> *(i) Some cells have irregular geometry. This could occur for a number of reasons. What are some biological or physical reasons that could contribute to a cell's irregular geometry?*

(ii) When the cells do have irregular geometry, which parts of the Laplace Pressure formula are affected and in what way?

(iii) What can you say about the pressure difference of cells A, B, and C?



(iv) If the surface tension of these cells ≈ 0.2 mN/m, estimate the pressure difference between cell A and its surroundings.

5.10.2 Problem Description

This problem is the apex of the problem set as it incorporates biological authenticity and modeling. As has been discussed, it has been shown from prior literature that students, upon perceiving a problem as biologically authentic, more easily identify with physics, have a more positive affect towards, and become more expert-like in their ways of knowing [97]. By incorporating biological authenticity, this question seeks to help students embrace the physical concepts presented and remove the barriers that they face when approaching physics problems.

The second important aspect of this problem is its modeling component. One of the tools commonly used by research scientists in order to understand the world around them is to create models. It is through making models that insight is gained into the critical characteristics of a given system. Through making the model, the simplest components of the systems are identified along with reasoning as to those that must be included or may not be included. This problem allows students to participate in this as a complex biological phenomenon is being simplified and a basic model is being applied to it. This step of simplification is the first step in developing any model that describes a system, in this case, a biological phenomenon. [218, 219]

As is done in scientific research, the students will be asked to describe where and why this model breaks down, in the event that it does break down. They discuss what is needed to be done differently to make a better model so that it could be a case of modeling the biological system. Creating a full model that accurately and completely describes the exact pressure difference between the cells of the neuromast is too complicated for the nature of this course but could be considered for a future more advanced biophysics course. Nevertheless, creating a basic model and this line of thinking is beneficial for the students, and therefore is an important component of this problem.

This problem opens by considering a more intricate scenario that the bubble-based scenarios of Problem 3. The equation for Laplace Pressure should be considered along with the examination of a cluster of cells called a neuromast, which is deposited by a group of migrating cells during zebrafish development (which is the biological scenario discussed in Chapter 3).

At UMD, the students, before seeing this problem, will have had a course lecture in which Laplace Pressure is discussed. In the course of this discussion, the students are asked to consider a spherical bubble, then consider the top half of the bubble, a hemisphere, and identify what forces act on it. The students are given that the force from pressure inside the bubble defined as up, must cancel the pull of the surface tension from the bottom half, defined as down, along with the following formulas:

$$F^{\uparrow}_{\text{air pressure inside}\rightarrow\text{top half}} = \tfrac{1}{2} pA = \tfrac{1}{2} p(2\pi r^2) = \pi p r^2$$

$$F^{\downarrow}_{\text{s.t. of bot half}\rightarrow\text{top half}} = \gamma L = \gamma(2\pi r) = 2\pi\gamma r$$

$$p = \frac{2\gamma}{r}$$

Courtesy Dr. Edward F. Redish – PHYS 131 Course Slides

The takeaway point from the side is a note regarding the radius (r) in the denominator with the text reading "SMALLER bubble has bigger pressure!" This is our physics lens that we will utilize to examine this physical system.

Examining each of the components further, we see that the first sub-question is the culmination of the above parts to this problem as is incorporates biological relevance as the students now examine their model. The ideal response would recognize that the irregular geometry of the cells causes the geometric components thus derived pressure components of their model to break down. The pressure formula was derived based on the geometry of a perfect hemisphere whereas the cells in this biological environment are not. However, if assuming the derivation is an approximation, the students may indicate

that the radius, in the denominator, if actually smaller than predicted by the hemispherical approximation, will cause an increase in the actual change of pressure while if actually larger than predicted by the hemispherical approximation, will cause a decrease in the actual calculated pressure difference.

The students can also mention other biological factors that are not taken into consideration in this model such as cell-to-cell junctions, changes in surface tension, and internal cellular pressures arising from subcellular structures, along with other factors that will contribute to adjustments to and additional parameters in the model.

The second part addresses what components break down. The radius is the most common response. Students may also comment on the lack of uniform surface tension or possible pressure differentials of multiple touching cells.

The third part ranks the cells in terms of pressure. This requires usage of the toy model for cellular pressure. In this model, cells C and B are in contact with a flat interface. If the students consider the interface between A and B to be flat, they may rank all cells as equal. They are all greater than the environment. If the students consider cells A and B to not have a significant enough interface, then C is equal to B and the relative pressure to A cannot be determined.

The fourth sub-question is another estimation problem. After identifying and relating relevant aspects, this simplifies to a direct "plug-and-chug" question as the students are given the formula from the previously mentioned course slide and merely need to estimate the radius of a cell. They also need to approximate each cell as a hemisphere, which I hypothesize will cause some of the authentic biological relevance to

be lost to the students, unless the role of modeling is properly framed. Regardless, plugging the values gives a pressure difference along the order of 40 N/m^2.

5.11 Problem 11 concludes the problem set and allows for reflection on learning.

The eleventh problem concludes the problem set with returning to a qualitative problem. This problem was designed for the dual purpose of promoting reflection on the learning accomplished for the student and allowing the educator to confirm an increase in biological knowledge, thus having achieved cross-disciplinary authenticity. The problem text is as follows:

5.11.1 Problem Text

> *11. Describe qualitatively the role that pressure has in determining cell shape, citing biological examples.*

5.11.2 Problem Description

This question allows the students to reflect on what they learned throughout the problem set and refine their answer to Problem 1. In Problem 1 the students recognize the base level of knowledge that they have about a biological system. Throughout the course of the problem set, they utilize a physics lens and gain a physics tool that they can

then use to gain more insight into biological systems. This problem set is successful in being biologically-authentic if the students, coming in with a base level of biological knowledge, have now used tools and principles of physics to heighten their level of understanding of biological systems.

5.12 This problem set will be accessible to educators worldwide.

After undergoing an iterative design process (described in Chapter 4), this chapter has demonstrated that the development of a biologically-authentic, research-based set is possible and has detailed the contents of the problem set. The student response to this problem set will be discussed (in Chapter 6), components of which include interview response data that formulated the development of the problem set and large-scale deployment after biological-authenticity was achieved.

For educators wishing to deploy this material, this problem set may be accessed through the existing UMD PERG Wiki and will also be available through the IPLS Physics Portal (once the portal is fully developed). A copy is included in Appendix A. Our hope is that it will be utilized by educators worldwide—not only as a complete problem set for usage in the classroom but also as a template for the development of additional like problem sets.

Chapter VI: Collection and Evaluation of Student, Peer, and Colleague Response in Iterative Design Process

This dissertation has discussed not only the critical role of physics in cell biomechanics (Chapter 2) and in experimental biophysics research (Chapter 3), but we have also discussed the formation of biologically-authentic, research-based problems (Chapters 4 and 5).

This chapter examines the student response during the problem set development process and during large-scale deployment. During the development process, incorporating student response led to both changes in the problem set and the identification of a new methodology—the epistemological bridge. We will also discuss student response to the deployment of this problem set in the large-scale classroom, which was at least moderately successful.

6.1 We are motivated by the stance that authenticity leads to understanding.

When life-science students perceive given physics problems as providing authentic insight into various biological phenomena, they achieve more expert-like ways of knowing in physics [75, 97]. At an increasing number of universities, such as at the University of Maryland (UMD), students pursuing biological science and pre-health degrees will enroll in an IPLS course [66]. Our reformed two-semester calculus-based IPLS curriculum is the physics component of the National Experiment in Undergraduate Science (Education (NEXUS/Physics) project, a response to calls from the professional

medical and biological science communities for a reformed undergraduate science curriculum [66, 77, 83, 88, 90, 93].

6.2 The initial problem set was perceived as biologically-authentic by the design team prior to the first iteration.

Our aim was to create a problem set for students that demonstrates the authentic use of physics in gaining insight into biological systems. The dual goals of this are (1) having students see the value of mixing physics and biology perspectives and (2) obtaining novel insight into the biological system, meaning that the students are using physics to improve their present biological knowledge.

We utilized an iterative design methodology to develop the problem set. We started with a research-based problem set and went through an iterative design processes until all stakeholders had converged on a problem-set that was both research-based and biologically-authentic (as discussed in Chapter 4). Our first iteration was a short four question problem set. The design team perceived this problem set as being biologically authentic. The 22nd (final) iteration is an 11 question problem set that is perceived as being biologically authentic by all stakeholders.

6.2.1 Discussion with peers during first iteration did not converge on biological-authenticity.

After the first version of the problem set was developed, we discussed the problem set with our peers at UMD. They did not perceive the problem set to be biologically authentic, so we made adjustments accordingly. This completed the first iteration.

We then went through eleven cycles of the iterative design process. Throughout these cycles, the design team presented the problem set to faculty, research assistants, and other experts. Each time we collected feedback on the problem set and incorporated the feedback before presenting to a different group of individuals.

6.2.2 Biological-authenticity was not achieved amongst the design team, our peers, and our colleagues until the 11th iteration.

We went through 11 iterations before converging on a problem set that was considered biologically-authentic by the design team, our peers, and our colleagues. We converged on an agreed-upon biologically-authentic, research-based problem set before beginning student interviews. I organized and managed the design team which included two faculty members, two graduate students, and myself. Our peers and colleagues were faculty members, researchers, lecturers, postdocs, and graduate students from my network with backgrounds ranging from education to physics to biology who were currently involved with research either at the University of Maryland, College Park or the

National Institutes of Health. The nature of the changes ranged from grammar and clarity to conceptual and epistemic.

The next iteration of the problem set incorporated student input, as the final—and most important—group of stakeholders. The student response was taken into consideration for four of the iterations with results from multiple students in each iteration. All stakeholders' views aligned for the final problem set that was reached after the 21st iteration.

6.3 Our iterative design development process enabled the successful production of a problem set that produces desired epistemological shifts in students.

Including student response in the iterative design process allowed us to realize the parts of the problem set that did not lead the students to our desired learning outcomes. The most dramatic of these realizations occurred during version 11 of the problem set. When this occurred, we built on theory developed for conceptual improvement, which we adapted and applied to our problem set, which ultimately lead to the largely successful realization of our desired learning outcomes.

6.3.1 Version 11 of the problem set did not provoke student engagement in interdisciplinary modeling.

The first round of student interviews occurred at version 11. (Additional interviews occurred at versions 17, 18, 19, 20, and 21.) These interviews were in the form of 3-4 person focus groups. All interviews lasted 50 minutes and were both audio and video recorded. Four sets of focus group interviews were conducted for problem set version 11.

Version 11 of the problem set contained a set of questions that were designed to elicit student engagement in interdisciplinary modeling. This specific subset of questions was perceived by our design team, peers, and colleagues as appropriate and accurate. Students were asked about the role pressure plays in biological systems and other scenarios, both biological and non-biological. However, student focus groups showed that all four (out of four) student groups did not engage in interdisciplinary modeling. Each of the groups gave similar responses, indicating that the biology was too complex to be modeled and that the toy model that they used to model a physical, non-living system was useless for gaining insight into the biological system. The students did not perceive the task as being biologically authentic or even relevant therefore this version of the problem set does not achieve cross-disciplinary authenticity.

The full intention of the design team was that the problem set would facilitate the students' consideration of how physics constrains the biology and where the biology limits the relevance of the simple model and causes it to break down. In each of the interviews, we see the students reject the model, making statements reasoning why the

principles of physics do not apply to living cells, but stopping there. The fact that the students did not engage in creating a simplified biological model is a failure in problem design and spurred the design team to make further problem set revisions.

6.3.2 A goal of the problem set is to move from the modeling of a physical system to interdisciplinary modeling.

In the first section of the problem set, before addressing the zebrafish cellular system, the students are presented with multiple scenarios of two objects either in contact or distanced (Fig. 25). The students are first asked to consider the shapes as bubbles [modeling of nonliving systems]. In early iterations of the problem set, the students were next asked to consider the shapes as cells, modeling of a living system.

After the introductory section, the students consider six scenarios of two non-living, fluid-filled bubbles captured in a moment (Figure 25). The bubbles are simply membranes filled with unspecified liquids, both in and out of contact. The problem set next models the same situation as a comparison of the internal pressure of two cells that are either touching or separated, suspended in fluid.

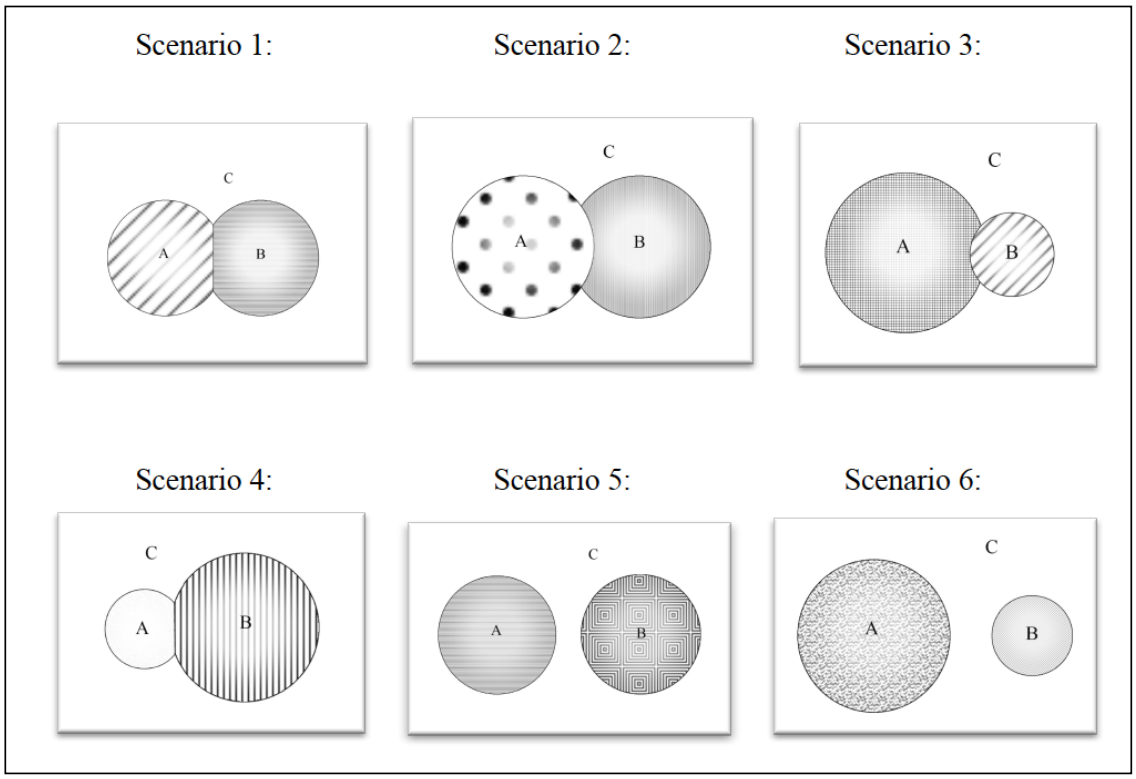


Figure 23. Problem Set Sample.

From Problem 3 on the Laplace Pressure Problem Set. Interfacing or close proximity bubble scenarios.

Through the consideration of this model, the simplest components of the systems are identified. The aim of this problem is to allow students to participate in the process of simplifying a complex biological phenomenon, as simplification is the critical step in developing any model that describes a physical system.

6.3.3 The initial problem set did not elicit engagement in interdisciplinary modeling.

As is often done in scientific research, in the problem set the students are asked to describe where and why this model breaks down. They should discuss what further

parameters could be considered to refine the model. Though creating a model that accurately describes the exact pressure difference between the cells is too complicated for this IPLS course, it could be considered for a future more advanced biophysics course. Nevertheless, creating a basic biological model can be beneficial for researchers as for students and therefore is an important component of this problem.

There is value in using this model and in considering when, where, and under what circumstances a given model is appropriate. Yet in every interview (four of four interviews), we saw the students reject engaging with living-systems modeling. The most common reason given for rejecting the model was that the biological system of a living cell was "too complex" and that no insight could be gained from the consideration of a toy model. The students should consider the following: how the physics constrains the biology, where the biology limits the model, what causes the model to break down, and when and where applying a toy model would be useful. However, the students conclude that this model is too simple; it does not apply due to the complexity of the biological cell and no insight can be gained. The bottom line is that we want them to discuss where the model breaks down instead of avoiding the modeling all together.

6.3.4 After the initial student interviews in iteration 11, the other stakeholders contributed to iterations 12-17.

This valuable feedback obtained from the student focus groups (n=4) for iteration 11 was then taken into consideration by the design team. I then returned to discussions within our design team as well as with our peers and colleagues and made updates to the

problem set during iterations 12 – 17. In this time, I identified a new methodology—the epistemological bridge.

It was during discussion of student interviews and review of the problem set that one of the members of the design team, Kim Moore, suggested the insertion of an intermediary step in an attempt to address the previously mentioned design flaw. Together Kim and I created such a step that would serve as a first order physical approximation of a cell. Further interviews with the students revealed that this intermediary step acted as an epistemological bridge and was successful in enabling the students to engage in interdisciplinary modeling.

6.4 We modified and utilized an adapted bridging methodology to elicit change in student response.

Before the insertion of the epistemological bridge, our problem was that students were failing to engage in interdisciplinary modeling, which is an epistemic difficulty. By epistemic difficulty, I mean that the difficulty is epistemological in nature. Epistemology is, according to the Merriam-Webster dictionary, the study or a theory of the nature and grounds of knowledge especially with reference to its limits and validity. Epistemology deals with knowledge: how do we know and what does it mean to know something? [220, 221]

This type of epistemic difficulty is similar at some level to the conceptual difficulties students have with physics. These conceptual difficulties have been studied extensively. For example, in a 1993 publication by J. Clement, researchers addressed

student difficulties in dealing with preconceptions in physics [105]. These researchers propose a strategy that involved inserting a bridging case to link an anchor to a target example.

The target example was a book on a table with the goal of having students recognize that static objects could exert forces. Before intervention, 76% of their population indicated that a table does not exert a force on a book resting on it. This is the target scenario. In the same population, 96% of the students indicated that a spring will exert a force on an individual's hand when pushing down on the spring. This scenario served as the anchor.

The intermediary step is the insertion of a conceptual bridging case. The bridging case is a scenario in which a book is placed on a thin flexible board. This bridging analogy is "*the strategy of finding an intermediate third case that shares features with both the original case and the analogous case*" (Clement 1993).

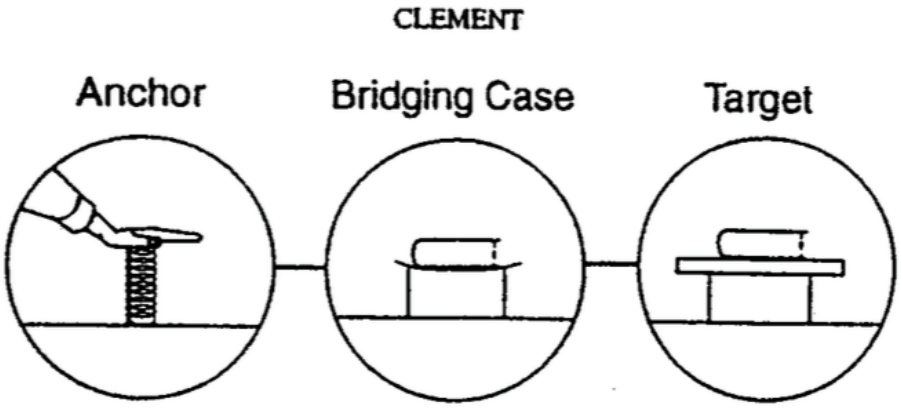


Figure 1. A bridging analogy.

J. Clement, J. Res. Sci. Teaching 30 (10), 1241 (1993)

Figure 24. Clement Conceptual Bridging Strategy

This technique proved successful for Clement's students to move from the anchor to the target and has been widely utilized since publication [222–226].

6.4.1 We are introducing a new theoretical construct, the epistemological bridge.

Clement introduced a conceptual bridging case to facilitate students moving from the anchor to the target. It is common to think of bridges as conceptual bridges, but this does not have to be the case. In the case of the Laplace Pressure Problem Set, the students did not have a conceptual need for a bridge. The barrier for moving from the anchor to the target was not a conceptual one but an epistemological one.

Here we present a new theoretical construct, the epistemological bridge. The epistemological bridge is designed to provide framework for the students to engage in successful interdisciplinary modeling. The insertion of the epistemological bridge components enabled the students to engage in biological modeling, the aim of this component of the problem set.

6.4.2 The epistemological bridge is the intermediary component.

It was not until we introduced an intermediary component between the non-living and living systems component that we saw the students engage in modeling for the living system. The intermediary component asked the students:

"*If instead of the cross-sectional slice of attached bubbles, the images above are now the view through an inverted microscope showing various different objects attached and suspended in solution. Each object is made of a phospholipid bilayer encasing a homogenous colloidal liquid. Would your responses for part (i) [the non-living system] change? If so, how? If not, why not?"*

6.5 Students engage in interdisciplinary modeling in interviews for version 17.

As mentioned, in the initial versions of the problem set, the students do not attempt to engage in interdisciplinary modeling (addressing why the model breaks down), illustrating a lack of the interdisciplinary shift of perspective that would allow them to consider the implications of the physics for the biology. Modeling is a useful tool in many types of research (and in life in general). There is value in considering when, where, and under what circumstances this (or any) model is appropriate. In the discipline of physics, for example, we use the approximations of "massless strings" (neglecting mass) and "frictionless pulleys" (neglecting friction) as minimal models in certain scenarios. In this problem set, the goal of the design team was to hear the students discuss specific parameters that were not being included in the model such as "intercellular component-less cells" (neglecting the actin cytoskeleton) for example.

6.5.1 Insertion of intermediary component elicits interdisciplinary modeling.

After the insertion of this intermediary component, the epistemological bridge, the students readily engaged in interdisciplinary modeling in all six focus group interviews. The move from the anchor scenario to the bridging case to the target scenario is successful for each group.

Success is defined as engagement with the interdisciplinary model and to recognize the interplay of physics and biology which is an epistemological issue as the physics constrains the cell. The cell, which is a biological system, is also a physical system. Part of the instructional design goal is getting the students to deeply think about what they are doing and not just accept results.

6.5.2 In interviews, students discuss where the model breaks down.

The ideal response is that students will reflect on and engage with the model and explicitly state where the model would break down (Response #1). The second successful response is that students will recognize that biological systems and that they follow the laws of physics and can be approximated as a toy model (Response #2).

Below is an excerpt from a focus group interview of the students discussing the problem set and providing an answer in line with Response #1:

"So the tumor cells are cells, and for [problem] two even though they didn't say it was a cell—they said phospholipid bilayer—but the first thing that came to mind

was cell. Because cells have a phospholipid bilayer… It should stay the same. Though a cell has more internal structures in the cytoplasm. So I guess it not completely similar, and cells have things pushing against the membrane… but overall should stay the same."

The students discuss some of the biological structures that were not taken into consideration with the bridging scenario but then recognize that the cell can be approximated as the model.

6.5.3 The epistemological bridge enables students to move into interdisciplinary modeling.

Clements's flexible board case is a conceptual bridge, as the aim is to get students to see a desktop as a bit flexible. The bridging example in this problem, the phospholipid bilayer encasing a homogenous colloidal liquid, is not providing the students a new way to look at the biophysics of the cell. What it is doing is providing them epistemological reasons to move to the target scenario.

It is providing the students permission to apply the laws of physics to the biological system. It stems from epistemological reasons instead of conceptual reasons as it cues the students to realize that it is acceptable to use a simplified model. It is working differently than phenomenological primitives activation in that we are not activating new conceptual resources and instead new epistemological resources are being activated [227–232].

To further clarify, in the flexible board case, when the students moved from spring to solid board, the problem was not that they thought that the solid board on the desk was too complicated to be modeled by the spring. The students simply did not recognize the desk as exerting force. The bridging case allowed the students to realize that the desk is indeed exerting force on the book.

In our case, moving from the bubbles (the anchor) to the cells (the target) directly did not work. In contrast to the flexible board case, however, the lack of movement was not due to a conceptual reason. It was that the students thought that the biological scenario was too complex and the principles of physics would not apply, which is an epistemological reason. The insertion of the intermediate step allowed for the epistemological bridge to be crossed.

6.6 The problem set was fully deployed after 21 iterations.

After 21 iterations of the problem set, the design team, our colleagues, peers, and students had finally converged on a problem set that all parties deemed as biologically authentic. This means that all stakeholders agreed that biological insight would be gained by going through the problem set. At this point it was considered ready for deployment in the IPLS classroom.

This problem set in its entirety is an 11 question collection that takes many typical IPLS students multiple hours to complete. For our IPLS course, we have a 50-minute recitation session. Due to the time constraints of the recitation, we opted to select a portion of the problem set for deployment; the students worked approximately four to five problems in recitation.

6.6.1 Deployment uses may vary according to needs and course level.

This problem set may be utilized in other manners of deployment for IPLS students such a series of homework problems, over multiple recitations or as a group exam. Deploying it in a series of homework problems could be beneficial if the students are learning about pressure over a multi-week period, so that they build a theme over the weeks in the homework set. Depending on the number of problems selected, they could be coupled along with other homework problems as needed. Another option is to deploy it as multiple (two to three) recitations.

Another deployment option is to deploy a portion of the problem set as a group exam. The beginning parts of the problem set could be introduced in a recitation, and the remaining parts administered in a group exam format. For an upper level course, this could be deployed as a single problem set.

6.7 UMD large-scale class deployment occurred in the form of a recitation.

Recitation sections at UMD are 50-minute sessions occurring immediately before a two-hour laboratory period. Students work in groups of four and work together to complete a worksheet. The worksheets are not (typically) collected and are not graded.

Due to length and time constrictions, for deployment in our IPLS course at UMD, we opted to select a portion of the problems for deployment. The design team selected:

- Problem one; motivation question
- Problem two; parts (i) – (v) only; nonliving physical modeling of fluid(s) in a tube
- Problem three; interdisciplinary modeling
- Problem ten; *in vivo* application
- Problem eleven; reflections on learning

We were aware that it was more than the students could accomplish in the 50-minute time frame, and we had no groups complete the entire recitation. Most groups completed the first three problems.

The class norm in the recitation is for the papers not to be collected. The students generally work in groups of four and sometimes take notes. For this recitation, we asked the students to physically write down the answers and turn in their papers for analysis. As mentioned, this was very different for the class, but we still had over 200 students comply (n=214).

The results were then analyzed by the lead team designer, and inter-rater reliability was conducted for 50% of the student responses. We were particularly interested in the inter-disciplinary modeling component (Problem 3) as this is the target of our epistemological bridge.

6.7.1 There are five categories of student response types.

For this discussion, recall the fourth component of question 3 of the problem set:

> *(iv) If instead of the above scenarios, the images above are now the view through an inverted microscope of spherical circulating tumor cells that are attached to each other and suspended in solution, would your responses for part (iii) change? If so, how? If not, why not?*

This question is asking the students to move from the bridging case to the target case. The student responses to this question can be organized into the following five categories:

1. Yes, because [specific parameters to refine/strengthen the model]
    - actin polymerization or myosin contractility or cytoskeleton or other subcellular structures that regulate cell shape, *etc.*
    - transmembrane glycoproteins, adhesion molecules, substrates that adhere cells together, *etc.*
    - Diffusion, channels, passing of materials through membrane, *etc.*
2. No, because [laws of physics/simplified model]
    - cells are physical systems, follow laws of physics, *etc.*
    - simplified model, good approximation, *etc.*
3. Yes, because [generic reasons]
    - not engaging with the model *i.e.* biology is a "black box" and is "too complex" to be able to gain any insight from a simple model, *etc.*
4. No, because [generic reasons]
    - not engaging with the model *i.e.* biology is a "black box" and is "too complex" to be able to gain any insight from a simple model, *etc.*
5. Equivocation
    - reasoning which did not support claim, *etc.*

6.7.2 The percentage of student responses varied across response types.

For the students we found the following breakdown of responses:

Table 3. Student Response to Target Scenario.

Response Category	Percentage of Students Giving Response
Yes, because [specific parameters to refine/strengthen the model]	19
No, because [laws of physics/simplified model]	19
Yes, because [generic reasons]	10
No, because [generic reasons]	36
Equivocation	16

Student response to the target scenario question. The question reads: "(iv) If instead of the above scenarios, the images above are now the view through an inverted microscope of spherical circulating tumor cells that are attached to each other and suspended in solution, would your responses for part (iii) change? If so, how? If not, why not?"

From this set, 38% of students gave written, favorable responses (Responses 1 and 2). And while a significant percentage of students responded with non-answers or did not show signs of engaging in modeling, that does not necessarily mean that they did not engage. It is notable that it is not the class norm to write on let alone provide robust answers as the recitation documents, as the recitations are not typically collected or graded. The student behavior in recitation focuses on their interactions and discussion with their peers instead of writing on paper. It is highly likely that these positive responses are a lower bound to the number of students having discussions of the type we were seeking.

6.7.3 The epistemological bridge is at least partially successful even in large N settings.

In the student interviews after the insertion of the epistemological bridge (n=6), we saw the epistemological bridge work successfully in all cases. We know that the epistemological bridge works when students are engaged (such as when they are being interviewed). This large N study shows that it can also works voluntarily in the recitation setting. The evidence shows that it is working for a least a significant fraction of the students (~40%), which is superior to none (0%) in the interviews before the insertion of the epistemological bridge.

6.8 The creation of additional research-based, biologically-authentic problem sets is needed.

In addition to providing an example of a research-based, biologically-authentic problem sets for IPLS students, this work hopes to raise awareness in the PER community of the need to develop such problem sets and to serve as a catalyst for the creation of future like problem sets that enable and encourage interdisciplinary modeling. This work has already served as a catalyst for the creation of additional homework and exam questions in the IPLS course.

Furthermore, as we have now examined the formation (Chapter 4) and deployment of this biologically-authentic, research-based problem set (Chapter 5), we now focus on another aspect of classroom environment—and that is the influence of the instructor in an IPLS setting (Chapter 7).

Chapter VII: How an Educator Characterizes Scientific Domains and Disciplinary Relationships

In this dissertation we have discussed the importance of and method for producing biologically-authentic, research-based course material (Chapter 4). We presented an example problem set (Chapter 5) based on current biophysics research (Chapter 3). We also discussed incorporating student feedback into the design process (Chapter 6) to achieve biological-authenticity for all stakeholders. This chapter focuses not on the development and deployment of the biologically-authentic course material but on the more nebulous component of the positioning of the disciplines of physics and biology in the classroom.

As educators in an academic setting, we can unwittingly position historically distinct disciplines in such a way as to communicate overarching messages about these disciplines in the classroom while lecturing. They may, for example, be positioned as distinct and different from each other or as more similar and less-siloed as was observed in this work. And also, as observed in this work, such positioning and the shift in such positioning, may not be obvious to educators and furthermore may influence students in unintended ways. Therefore a thorough examination of the positioning of the disciplines in the classroom is an important first step in understanding what messages are being communicated to the students, and further work could consider if their uptake influences student behavior or learning outcomes.

7.1 Instructors may unwittingly influence the Introductory Physics for the Life Science classroom environment.

As instructors, lecturers, teachers, and educators, we are responsible for the spirit and the environment of our classrooms. Anecdotal classroom evidence from both educators and students at the University of Maryland, College Park (UMD) indicates that many life science students still experience difficulties even in our reformed Introductory Physics for the Life Sciences (IPLS) course. Here we analyze how one educator (the course instructor) positioned scientific disciplines in relation to one another over a two-semester time span, specifically focusing on the nature of physics, the nature of biology, and the nature of math. In the first iteration of the course, the nature of physics and the nature of biology were positioned as being different and distinct. In the second iteration, they were positioned and being similar and less-siloed. The nature of math, in contrast, was consistently positioned across iterations.

This work examines statements made by the educator during lecture regarding the nature of physics and nature of biology, which changed between iterations, while emphasizing the consistency in the positioning of the nature of math. During this two-semester course, the design research team gave formative feedback to the educator regarding how students were interpreting comments about the disciplines and their relationships. Our analysis of the educator's discourse in the classroom demonstrates shifts in the messages the educator sends about the domains of physics and biology. In year one, physics and biology were largely described as distinct and different, but in year two they were more frequently described as similar, complementary, and overlapping.

7.2 Statements regarding the nature of physics and the nature of biology were prevalent in the IPLS course.

The reformed IPLS course at UMD is the physics component of the National Experiment in Undergraduate Science Education (NEXUS-Physics) project, a response to calls from the medical and biological science communities for a reformed undergraduate curriculum [66, 88]. Other research initiatives regarding the NEXUS-Physics project have contributed accounts of what students are getting out of these experiences but has left underspecified what instructors in these environments are communicating regarding the nature of given disciplines and what such communications may do to enable or constrain students' learning opportunities [89, 90]. How faculty position the disciplines relative to one another may influence the way students seek or build connections between the disciplines, therefore a thorough examination of this positioning is the first step in examining this potential influence.

7.2.1 We analyzed the positioning of the disciplines by the classroom educator.

When lecturing in front of a classroom, educators may find themselves making statements without realizing their implications. The commentary can thus shift drastically without detection from the educator that such a shift is occurring. We, as educators, communicate many of our expectations for students, only some of which are deliberate and intentional [233]. Dramatic, unconscious internal shifts may occur in

educators that do not require a defining moment of clarity. This work analyzes the nature of such a shift with hopes of raising awareness of this phenomenon within the Physics Education Research (PER) community.

Furthermore, an analysis of the changing number of times each given discipline was mentioned yields interesting results for reflection and provides a novel analysis approach to the community.

7.2.2 Observation occurred during the small N classroom of NEXUS/Physics.

In this work, we examine two sequential implementations of the NEXUS/Physics course (first and second semester) to discern how the educator shifted in the presented relationship between the disciplines of physics and biology as well as the unchanging statements on the nature of math. This two-semester course observation was the first implementation of the NEXUS/Physics project and occurred during the 2011-2012 academic year.

During this first iteration, there were approximately 25 students in the course. The course met three times a week for weekly lecture and also had a weekly recitation and lab component. (The recitation and lab were not considered for this analysis.) The course contained many of active learning components including small group discussion and clicker questions.

In addition to receiving feedback from the students throughout the first semester, the educator also was interacting with an interdisciplinary design team who was analyzing and discussing what they were observing. The design team also had a meeting

with the education between the first and second semesters of the course in which they explicitly discussed the positioning of the domains.

7.2.3 Methods include reviewing recorded lecture video and analyzing field notes.

Video data was collected by my colleagues during the live lecture from the first and second iterations of the course, in Fall 2011 and Spring 2012 respectively. Analytical review of recorded course lecture was used for this work along with field notes from those present in the classroom.

The initial implementations of the course were recorded by three separate video cameras - two on student groups and one focused on the educator. This work analyzes the instructor view recordings of the first 15 out of 45 lectures of the first semester of the course across two different implementations. We coded these 15 lectures with eleven separate codes ranging from discussion on the nature of biology, to discussions on the use of multiple representations. On average, coded discussions are approximately 30-60 seconds in length but range from six seconds to over eight minutes.

7.2.4 The design team developed a coding scheme for tagging discussions.

This work focuses on the discussions regarding nature of physics (NOP), nature of biology (NOB), and nature of math (NOM). Each of these along with four others (Nature of Science, Nature of Medicine, Nature of Chemistry, Nature of the Course) were tagged throughout the course lectures. Though all codes were considered for analysis,

this work focuses on the NOP, NOB, and NOM. The annotations for these three are below:

- *Nature of Physics (NOP): Applied to comments/conversations referencing what Physics is, the way physicists think/work, and how Physics has developed/changed over time.*
- *Nature of Biology (NOB): Applied to comments/conversations referencing what Biology is, the way biologists think/work, and how Biology has developed/changed over time.*
- *Nature of Math (NOM): Applied to comments/conversations referencing how math is used in the course, what aspects of mathematical sophistication are emphasized in the course, how mathematicians think/work, what mathematics is, and how numbers are perceived.*

For reliability checks, an independent reviewer viewed and coded data from six arbitrarily selected distinct lectures to serve as a comparison. The results from this reviewer were approximately identical with all eleven codes matching, and start and end times matching within 30 seconds. The reliability checks were then discussed with the independent reviewer and minor discrepancies were resolved.

Our codes and commentary were also compared with commentary created by members of the research team made while present during the initial presentation of the lectures. The interpretations and commentary were also discussed on a continual basis with researchers from the UMD PER group for confirmation purposes. The analysis for

the changing numbers of times the disciplines were discussed was created using MATLAB® [35].

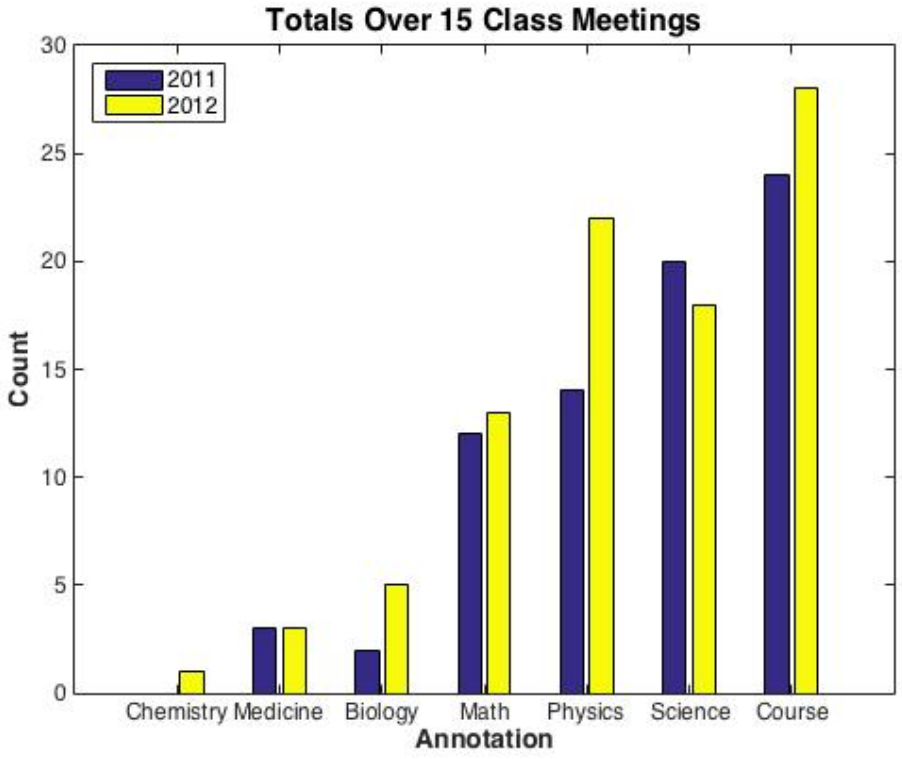


Figure 25. Total count for Each Annotation

The total times that each code appeared in the presentation of the course Fall 2011 or Spring 2012.

7.3 Results from the first course iteration show a distancing in the Nature of Physics and Nature of Biology.

In the first iteration of the course, our results demonstrate that the educator positions the disciplines of physics and biology as being separate and unique. The disciplinary differences are regarding: (1) variation in the mathematical preparedness of distinct groups of students, (2) derivation of knowledge in the disciplines, and (3)

differences in the ways the disciplines think. In the first course iteration, there are five instances in which NOB and NOP appear together. An instance is a time in which the lecture was tagged with both NOB and NOP either in overlap or in succession. In four of these five discussions, the educator highlights clear differences between the disciplines. One instance contains a mixed message by first discussing a similarity between biology and physics then transitioning to discussing differences. We now describe the substance of the differences that the educator describes.

7.3.1 Differences in mathematical preparedness were discussed.

Throughout the first course iteration (Fall 2011), the educator establishes as salient the students' mathematical preparation through statements indicating that this course was originally targeted to a specific type of student-- the student who is defined as being "less well prepared in mathematics". The following discourse occurred during lecture.

> *Educator: So here is the reason why we are doing what we are doing. Over the past decade there has been a lot of calls to modernize the undergraduate education for biology and pre-medical students... Calls for a multi-disciplinary, competency-based science education both for biology and pre-med students that is going to be less constrained and restricted than the previous environment.... But the point of this course…was a course that was created originally for a whole crew of different kinds of students but mostly students who were less well*

prepared in mathematics. And what they did is they took the engineering course, and they cut it back, and they shortened it up.

In this part of lecture, the educator is talking about the development of the NEXUS/Physics course. Many of the students enrolled in this course are aspiring health professionals majoring in biological sciences. In this segment, these students are grouped together as not well prepared in mathematics and are in contrast to the group of those that would be taking the engineering course, thus drawing a distinction between students from various academic domains. While the educator is talking about the course reforms, he is still emphasizing that this is for students who are less well prepared in mathematics.

7.3.2 Differences in derivation of knowledge were discussed.

In the first iteration of the course, biology is positioned as a discipline where one should be able to summon knowledge that has been learned from authority where physics is positioned as a discipline in which one should not memorize facts to disgorge but should be able to generate knowledge. As one example, the students are expected to be familiar with the anatomy of mammalian and fish respiratory systems and to recall on demand during a course discussion.

Eugene: My answer was sort of like hers, but I said that instead of being in the outside, it started developing those ridges on the inside so there is a larger volume, you could pack all of those ridges in the same surface area.

Educator: And what might you call that structure that you are building?

Eugene: In fish, they're lungs. In mammals, we call those the bronchi of our lungs.

Educator: Fish don't have lungs.

Eugene: Oh I mean gills.

...

Educator: Right. Yeah. Great. So you're supposed to have had biology before you take this course so I'm gonna feel free to toss in questions like that. And since I'm not a biologist, ...you'll have to keep me honest if I go astray on the biology. Okay? Because you probably know a lot more biology than I do.

When a student makes an error in his recall of the anatomy, the error is quickly challenged by the educator, then rectified by the student. The educator then states that the students should be familiar with biology because they are "supposed to have had biology" implying that the educator will expect recall of biological facts. The course educator then further distances himself by claiming that he is "not a biologist", thereby excusing himself from the requirement to recall biological facts, as those labeled "biologists" are asked to do. This separation of expectations further distances the disciplines from each other.

As the discussion in this day of class continues, the way that physicists approach problems is positioned as being different from the way that biologists approach problems.

Educator: And he [the speaker at a physics colloquium] basically explained that with a… very simple model, throwing away most of the biology, just focusing. So this is the way physicists do biology, and this is the way physicists do physics.

This example demonstrates an "us-versus-them" mentality by describing physicists as disregarding the complexity of the biological system being examined.

7.3.3 Differences in thinking were discussed.

During a lecture mid-way through the semester, the educator led an explicit discussion highlighting the differences between biology and physics, in which the educator's comments associate "thinking and reasoning" with physics and "memorizing" with biology. The educator opens this discussion by describing a conference on teaching physics for biology students in which he was encouraged to teach "them" how to think like "us". The telling of this story communicates a barrier between the disciplines. The educator comments that they (the "experts") have focused on this, the difference, for thirty years.

The educator then states explicitly that there is a gap, meaning that the different disciplines are separate and unique. The educator sets up two separate columns on the board, further separating the disciplines as distinct. The remaining activity involves placing generalizations about the nature of each discipline in the respective column. There is only one comment, "breaks into pieces" that is later placed in the middle as

representing both “thinking like a physicist” and “thinking like a biologist”. All other comments are placed in one column or the other.

Educator: I'm interested in what's your perception at this point. So what do you think is different about what I do from what you've seen in your biology classes? What do we do differently in physics? (pause) And there's no right answer to this, right? I am asking about your perception.

Jasper: I don't know. I'm kind of having trouble seeing a difference. I guess I just kind of think of it as like they're sciences.

Educator: OK. You think it's similar.

Sameer: I mean, I'm not exactly sure how to explain it but I’m seeing that physicists approach problems differently than biologists do.

Educator: And can you try to be specific in some way? Meaning?

Sameer: Meaning... I don't really know how... I don't know how a physicist does... but it seems that the way you go about doing a problem does feel different.

Educator: Does feel different. OK.

When the educator requests student input, he does so by asking only for the differences. By not asking for similarities, the educator highlights the distinctions and positions the disciplines further apart. Even though the first student to speak states that he is “having trouble seeing a difference,” the educator listens to the student but does not provide additional comment nor does he ask the student to elaborate. This acknowledges

the student but does not give credit to his stance. In contrast, the next student who offers a difference is asked to elaborate.

The educator continues to listen to students' general comments about the differences until a student describes biology as being more qualitative and positions chemistry closer to physics as working with "a lot of mathematical modeling and quantitative data". The discussion continues with students making statements highlighting differences in physics and biology. The students make comments and the educator writes terms in either column (see Table 4), with the exception of "break into pieces" which is the first and only comment to be placed into the middle of the two columns.

The discussion ends with the addition of the final comment under the "Thinking like a physicist" column by the educator, stating "Think and reason with equations". No accompanying comment is added to the "Thinking like a biologist" column. This is an example of the positioning of the disciplines as distinct and different.

Table 4. Contents from Blackboard Columns.

Contents from Columns on Blackboard that were written by the professor during an in-class discussion

Thinking like a physicist	Thinking like a biologist
Simple models	More complicated models
Just do it	Think about big picture
More quantitative modeling & data	More qualitative
Connect models to basic principles	Connect models to complex data
Idealized models	(No companion counter listed)
Clean principles	Hard to separate principles
Memorize eqs (equations) & apply	Memorize concepts and (illegible)
Think and reason with equations	(No companion counter listed)

7.4 Results from the second course iteration show a shift in the positioning of the Nature of Physics and Nature of Biology.

A thorough examination of the second iteration of the course reveals a shift in how the nature of physics and nature of biology are described. During the second iteration, the NOP and NOB were described as being more similar than different, and less silo-ed. Similarities within NOB and NOP are noted through discussions regarding their changing nature, use of mathematics, and focus on understanding physical systems. An extended class discussion on the differences between the natures of the disciplines is not present in this iteration. In the second year, there are seven instances in which NOB and

NOP appear together. Six of these seven highlight clear similarities between the natures of the two disciplines. One contains a mixed message as it begins by highlighting a generalized difference but then contains a counter example of a similarity. This section highlights the types of similarities the educator discusses.

7.4.1 Similarities in temporal change were highlighted.

On the first lecture of the semester, the educator interjects into his lecture statements regarding how physics is changing, an example of how biology has changed as well.

> *Educator: Biology and medicine- everything has changed. When I was in high school, they didn't tell me the right number of chromosomes for human beings. You'd think that they could count that. It turns out it is not as easy as it looks, but all of this changes. So there are changes that are happening fast. And you are going to see them.*

The disciplines are aligned with each other in that both are changing disciplines in which novel discoveries are being made, but while they are more aligned, we do still see elements of the "biology as facts" positioning that was present in the first iteration. During a subsequent lecture, the educator again highlights the similarities amongst these disciplines again.

Educator: Equations like this where the quantity is related to the rate of change in the quantity just occurs in science all over the place. And you'll see it in biology. You'll see it in medicine. You'll see it in physics.

In this instance, the educator points out that the examination of an object's rate of change occurs across the aforementioned disciplines. The educator lists biology, medicine, and physics as all being similar in this regard.

7.4.2 Similarities in being a part of science were discussed.

During a discussion on NOB, the educator makes statements on the use of mathematics in the sciences and implicitly includes biologists in the category of a science alongside of physics.

Educator: Math is extremely important in science. And this is why they make you [biological science students] take math classes… there's lots of things that is really important about the science that is added on top of the math that makes it much more challenging than the way the math is done in the math classes.

The educator communicates that they take math classes so that it can be used in the sciences. The educator in this message sets up why this class will be relying on the mathematics that the students have already learned.

7.4.3 Similarities in examining physical systems were discussed.

Embedded in a discussion on the importance of using math in physics, the educator describes the aligning of both physics and biology in the context of working with a physical system as a critical point.

> *Educator: A critical point about the whole process of using math--and it's not it's not just math 'cause once we talk about this you will be able to see that this is very much the same as a lot of what you do in qualitative biology--um is that we are going to begin with some physical system. And it could be a biological system. All systems are physical systems.*

The educator explicitly brings in biology by commenting on the similarity about both examining physical systems. This shows an aligning of physics and biology in that biologists work with physical systems just as physicists do and is an example of the disciplines being positioned as similar and less-siloed.

7.5 What Contributed to the Educator's Shift in Messaging?

While we cannot say with certainty what contributed to the educator shifting the messaging about NOP and NOB in the classroom, we can point to a couple of potential influences. During the first iteration of the course, a research team was actively recording students in class and interviewing them outside of class. In between the first

and second semester of the first iteration of the course, the research team had a meeting with the educator in which they showed him examples of how his messaging about the disciplines was being taken up by students *(e.g.* interview transcripts.).

Upon reflection of this data, the educator noted that some of the ways the students were positioning the disciplines were not how he intended. Additionally, in the second iteration of the course, a biophysics colleague joined the instructional team. This colleague viewed biology and physics as much more congruent than the original educator, and they had many discussions on how physics and biology were similar. It is likely that both of these types of discussions–with both the research team and the biophysics colleague–contributed to the shift in the educator's messaging from one iteration to the next.

7.6 Nature of Math did not shift.

While we see a shift in the positioning of the nature of physics and nature of biology from the first iteration of the course to the second, there is no such shift observed in the positioning of the nature of math.

> *Educator: We are going to assume you've had a calculus course. I'm not actually going to use a lot of calculus because my experience is that the students who have had calculus courses don't understand it in the way they need to for the physics. And therefore the calculus we do, we're going to build up from scratch, and it's going to look a little bit different from the way that it looked in your calculus*

class. And I'm going say some things, which I would prefer you not pass on to your mathematician professors.

Class:[Snickers]

The instructor shares his experience with the students, indicating that he believes the students' background in calculus does not equip them with the depth of understanding that they need to apply these mathematical principles to physics.

During the second iteration, the educator again indicates that the students are not prepared to use math in physics:

Educator: A big deal in what we're doing in physics, and one of the reasons that we have the physics class for you folks is that math is extremely important in science. And this is why they make you take math classes. But in a lot of their classes, you don't get to the way that the math is used in the science until quite late, like junior, sometimes even senior year. Sometimes you don't even get to see it until graduate school. [Then] it is really too late. You need to build your sense of how it works because it is not at all like it works in the math class. There are lot of things that are really important about the science that are added on top of the math that makes it much more challenging than the way the math is done in the math classes.

Maintaining consistency with the first iteration of the course, the educator believes the students background in math does not equip them with the depth of

understanding that they need to apply these mathematical principles to physics as the science is described as being added on top of the math, making it more challenging.

7.7 As educators our positioning can shift even if we are unaware.

Our analysis shows that the first iteration of the course included comments that positioned the nature of physics as distinct and different from the nature of biology (4 out of 5 instances). The second iteration revealed communications describing the nature of physics and the nature of biology as similar and less-siloed (6 out of 7 instances). During the first iteration of the course, the educator led a lengthy eight-minute discussion on the differences between "thinking like a physicist" and "thinking like a biologist". This discussion is notably absent from the second iteration of the course.

Our analysis also showed that the first iteration of the course included nine comments regarding the nature of math, and that the second iteration of the course included 10 comments. In nine out of nine instances in the first iteration, and in 10 out of ten instances in the second iteration, the educator described that as being different than the sciences, including physics, and/or described the students as being ill prepared for handling the math in the way that it was needed in the course.

In follow-up reflections by the educator, he denied any change in his messaging about disciplinary similarities and difference from the first and second iterations of the course. It was only after reviewing this analysis that the educator acknowledged a shift in the communicated nature of the given disciplines.

For the PER community, these results demonstrate that without being aware, educators can shift in communicated messages. We, as educators, may believe that we undergo perhaps only small internal shifts that are undetectable externally. Even in the presence of a recognized internal shift, we may not acknowledge any shift in the external messages we communicate. The messages sent to students are sometimes hidden, not only from the students, but also from ourselves. Additionally, changing the way students engage in mathematical reasoning in an IPLS course may require a shift in the positioning of the nature of math and also in the positioning the students as being capable and competent in mathematics.

This work provides a critical first step in exploring the positioning of the disciplines in the classroom. Upon gaining an understanding so to what messages are being communicated to the students, and further can now consider if the uptake of these messages influences student behavior or learning outcomes. Further study regarding a change in the discussions on the nature of the given disciplines may also effect of these communicated statements on students. Such analysis may contribute to raising instructors' critical awareness of the disciplinary messages they communicate in their courses. Another component of the classroom dynamics is the positioning of the disciplines from the students, as they are sources of knowledge, not just receivers.

Chapter VIII: Conclusion

This dissertation pulls from and links the disciplines of physics, biology, and education. Chapter 3 brings together biology and physics to explore collective cell migration. Chapters 4-7 build upon Chapter 3 and pull from a third research area, education, to develop a methodology for developing cross disciplinary authentic curricula.

In my experimental biophysics research (Chapter 3), I discussed my observations regarding the role of BMP signaling in the posterior Lateral Line primordium (pLLp) of zebrafish. As this group of cells migrate *in vivo* during embryonic development, they communicate with each other via signaling systems. Two of these signaling pathways (Wnt and Fgf) have been widely studied and are known to play a role in the migration of the pLLp. In this work, I examined the role of an additional signaling pathway (BMP) which was recently observed to play a role in collective migration by the Chitnis lab. I used physics-based research tools and methods to examine the role of BMP in the pLLp.

From my results, I propose that BMP is crucial for collective cell migration in this system and that it is active in the leading domain. I found that inhibiting BMP signaling in the pLLp altered multiple components of migration including the directionality, the migratory speed, and the prevalence of quadrijunctions.

Specifically, I observed that in the control, the leading and trailing domains were distinct with the leading domain more directed and the trailing domain less directed in migration. This trend is not present in the BMP-inhibited condition as there is a lack of distinct regions. Additionally, upon BMP inhibition, I observed an overall increase in

angular spread, meaning that the cells migrate in a less directed manner. As this overall increase is comparable to the trailing domain and the uptick in the control, I propose that inhibiting BMP effects the leading domain.

My observations in regards to the migratory speed of the pLLp also support the critical nature of the role of BMP and its position in the leading domain. I observed an overall decrease in the mean speed of the BMP-inhibited embryos as compared with the control. The overall slowing could be caused by the fact that the cells are mechanically coupled. A slowing in the leading domain could slow the entire pLLp. Additionally, in our control, there are distinct regions of higher and lower speeds in the pLLp. This trend is not observed in the BMP-inhibited condition as there is again a lack of distinct regions. The leading domain does not exhibit an increased speed when compared with the trailing domain. This slowing also supports the role of BMP signaling in the leading domain.

In addition to the trends in directionality and speed of the pLLp, I also examined cell shape and intersections which further reinforced the critical role of BMP signaling in this system. I observed this in the prevalence of quadrijunctions in the pLLp. (A quadrijunction is the intersection of four cells that join at a perpendicular intersection.) In the BMP-inhibited condition, I observed a significant increase in the number of quadrijunctions throughout the pLLp as compared to the control. Biologically this could indicate an increased coupling of the cells. I propose that there may be an increase in cell-cell adhesions either due to an increase in actin scaffolding and/or adhesion proteins.

In regards to my aim of using physics-based research tools and methods to examine and make analytical statements regarding the role of BMP in the pLLp, I was able to confirm and identify that BMP is vital to collective cell migration in the pLLp and

make quantitative statements regarding the change in directionality, speed, and junctions. Furthermore, from my results, I propose that BMP is activated in the leading domain of the pLLp.

Transitioning to my work in the field of biophysics education research, I note that in my experimental biophysics work, I started with a base level of understanding of the biological system. I then used tools and concepts from physics to increase our level of biological knowledge of the system. This idea of explicitly starting with biology and then using physics to gain insight into the biology was successful in the research lab. I then built upon this methodology to develop new curriculum in a physics classroom for biology students.

The second section of my dissertation focused on achieving a concept that I defined and termed "cross-disciplinary authenticity". Situated in a reformed undergraduate physics course for life science majors (IPLS), my aim was to develop a group-work based problem set that all stakeholders—including students—perceive as achieving cross-disciplinary authenticity through using physics to yield insight into biological systems. I prosed cross-disciplinary authenticity as a general term for starting with a base level of knowledge in a given discipline and then using the tools and concepts of a secondary discipline to increase one's level of knowledge in the original discipline.

I also presented a novel example of the development of such a problem set that achieves cross-disciplinary authenticity through the development of a research-based problem set. To build this problem set, I built off of my own experimental research on collective cell migration in zebrafish. I started with the biological then identified

questions about the system and used a physics lens to build the problem set with the goal of achieving cross-disciplinary authenticity.

I then used an iterative design process to further refine the problem set. The iterative design process took into account feedback from all stakeholders: the design team, our colleagues, our peers, and the students. The aim was to develop a problem set that all stakeholders view as yielding insight into a biological system, and thus achieving cross-disciplinary authenticity.

In the iterative design process of the problem set development, feedback was obtained through student focus group interviews. One component of the problem set was designed to facilitate modeling of two cells. In the early iterations, this component moved straight from modeling a non-living system (two bubbles in contact) to a living system (two cells in contact). This, however, was not a successful presentation as the students did not engage in modeling of the living system and did not see modeling as an authentic or relevant skill (that did not yield insight into the biology).

This design failure led me to identify and term an "epistemological bridge". The epistemological bridge is similar in some regards to a conceptual bridge, which is commonly discussed in the literature, and assists students in overcoming conceptual difficulties [105]. The most obvious similarity is that the epistemological bridge also includes an intermediary component that enables the students to reach the target scenario. The insertion of the intermediary component in this case enabled the students to overcome the epistemological difficulties that existed in the earlier versions and successfully engage in interdisciplinary modeling.

Deployment of the problem set in a large-scale classroom showed that a large number of the students did indeed engage in interdisciplinary modeling. Thus the aim of developing a problem set that is achieves achieving cross-disciplinary authenticity through using physics to yield insight into biological systems was successful.

And finally, in addition to written content available in the reformed IPLS, I also examined the ways in which one instructor positioned the disciplines of biology, physics, and math during lecture throughout the first and second semesters that the course was taught. Although the instructor denies that any shift occurred in his positioning of the disciplines, the results show that a shift occurred from positioning the disciplines of biology and physics as being distinct and different in the first semester to being similar and less-siloed during the second semester. This demonstrates that without being aware, we as educators can shift in the external messages we communicate to the students.

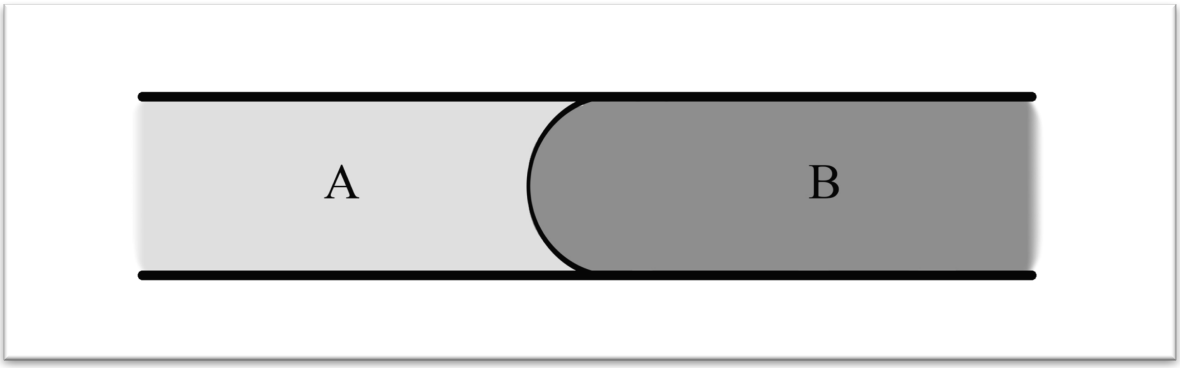
In conclusion, throughout this work we have seen that bringing together the fields of biology, physics, and education can be successful in producing new and innovative results. My hope is that this work will be built upon and inspire other researchers to achieve cross-disciplinary authenticity both in their research and in their classroom.

Appendix A

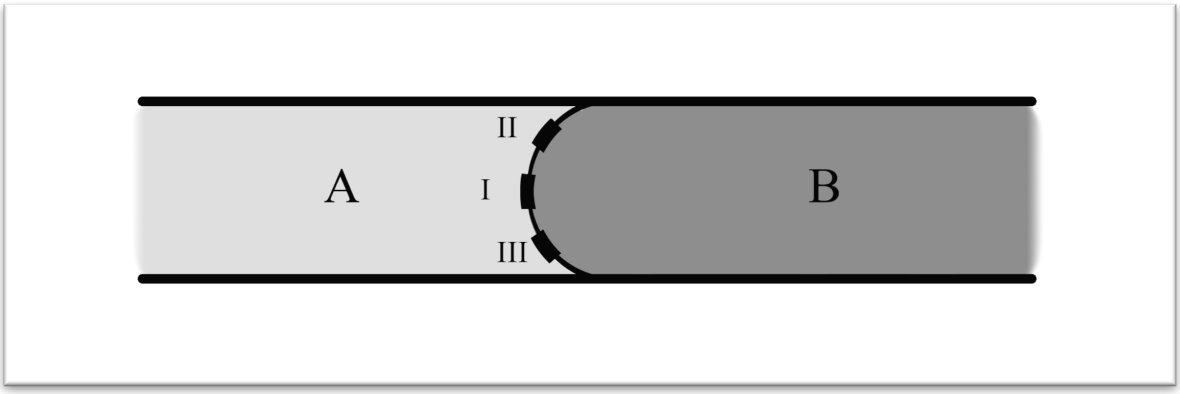
Laplace Pressure in Cells

1. In this problem, we will first investigate pressure in non-living scenarios such as in a pipe and in two bubbles before considering the role that pressure plays in morphogenesis. Considering the pressure of cells and of their surrounding environment is important as pressure plays a vital role in many biological phenomena such as morphogenesis, collective cell migration, and cancer metastasis. Describe qualitatively the role that pressure has in determining cell shape, citing biological examples.

2. (i) The illustration below shows cross-sectional slice of a pipe containing two fluids. The view is of a cross-sectional slice of the pipe. The fluids in the pipe are separated by an elastic membrane that is fixed to the pipe. Consider the interface of the fluids as a hemispherical dome. Use words to describe how you could tell by looking if the two static, incompressible fluids in the pipe (pictured below) have the same or different internal pressures.



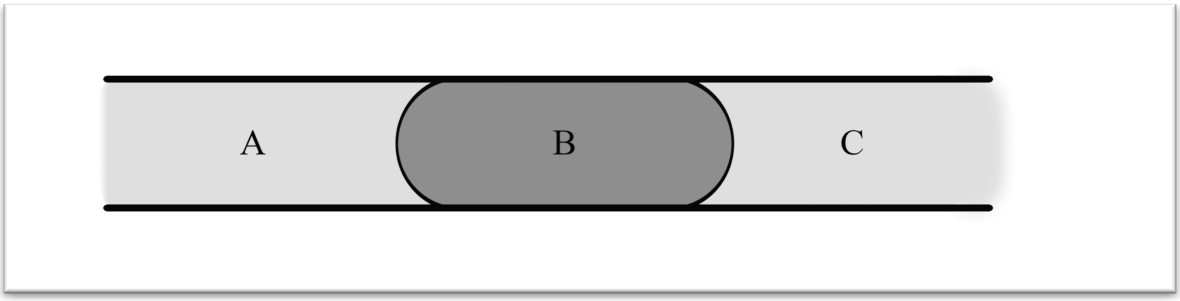
(ii) Draw free body diagrams for each of the small patches of surface area at the locations (I, II, and III) indicated below. State whether or not there is a net force at each location, and if there is, indicate the direction of the force.



(iii) In a different experiment with the same membrane materials, the curvature of the membrane is higher. What can you conclude about the pressures in the new scenario with regards to the initial scenario from part (i)?

(iv) In a different experiment with the same membrane materials, the curvature of the membrane is higher. What will change (if anything) in the free body diagrams for locations I, II, and III as drawn for part (ii)?

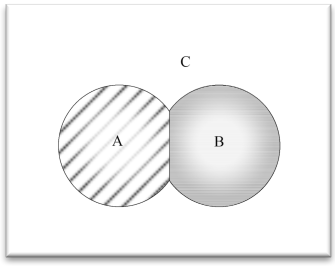
(v) Suppose there are now three fluids in the pipe, as pictured below. Fluid A and fluid B may or may not be the same. Rank the fluids in terms of greatest to least pressure.



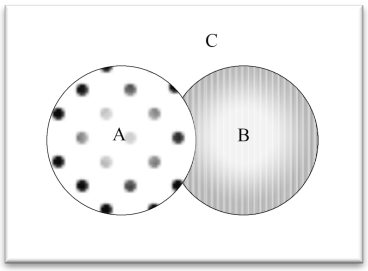
3. Suppose two bubbles are captured in the moment pictured. For each of the scenarios in the diagrams below, showing a cross sectional center slice of the bubbles, state whether the internal pressure of bubble A is greater than, less than, or equal to the internal pressure of bubble B, and how that relates to the pressure of the surrounding environmental pressure, C. Each bubble may or may not be made of the same material.

(i) Using greater than or equal to signs, rank the pressures in the following scenarios. If the ranking cannot be determined, state what additional information would be needed.

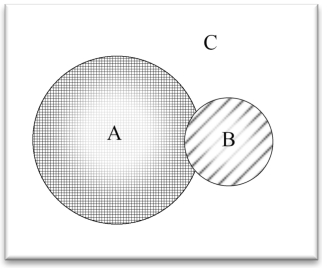
Scenario 1:



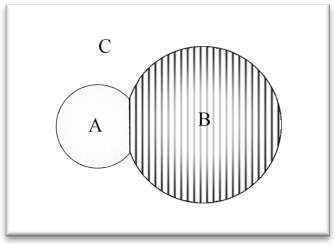
Scenario 2:



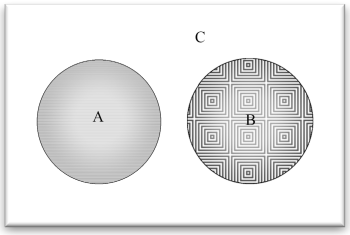
Scenario 3:



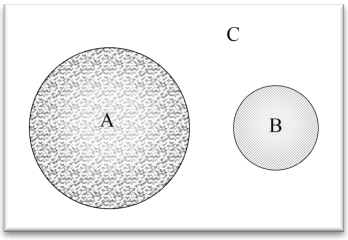
Scenario 4:



Scenario 5:



Scenario 6:



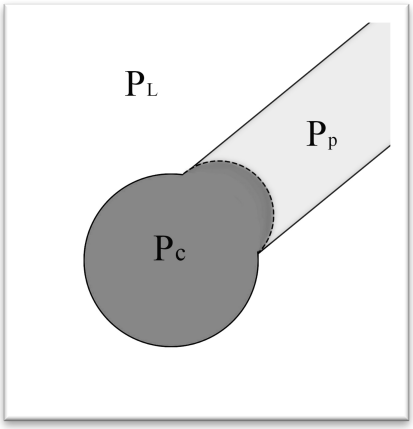
(ii) If you declared that any of the scenarios could not be fully ranked, what additional information would you need to know in order to fully determine the ranking, and what would that ranking be?

(iii) If instead of the cross-sectional slice of attached bubbles, the images above are now the view through an inverted microscope showing various different objects attached and suspended in solution. Each object is made of a phospholipid bilayer encasing a homogenous colloidal liquid. Would your responses for part (i) change? If so, how? If not, why not?

(iv) If instead of the above scenarios, the images above are now the view through an inverted microscope of spherical circulating tumor cells that are attached to each other and suspended in solution, would your responses for part (iii) change? If so, how? If not, why not?

4. A classical research technique called micropipette aspiration involves aspirating a cell into a micropipette to determine its cellular mechanical properties. Let's investigate how this process works.

The illustration on the right depicts a single cell (dark gray) being sucked into a pipette (light gray) in suspension (white) during the process of micropipette aspiration. The protrusion of the cell into the pipette is equal to the radius of the pipette and creates a hemispherical dome.



The illustration includes the following labels: pressure inside of the cell P_c, pressure inside of the pipette P_p, and pressure in the surrounding liquid P_L.

Rank the pressures in terms of smallest to largest.

5. In class you were given that for a sphere $\Delta P = 2\gamma/r$. Show how this is possible (true).

6. (i) Now that you are familiar with the Laplace Pressure equation, consider the following scenario: Two attached bubbles are in an environment of constant atmospheric pressure. The internal pressure of each of the attached bubbles remains constant, as does the surface tension. What happens to the curvature of the bubbles as atmospheric pressure increases? What happened to the radius?

(ii) Now holding atmospheric pressure, and the internal pressure of the bubbles constant, what happens to the curvature when surface tension increases?

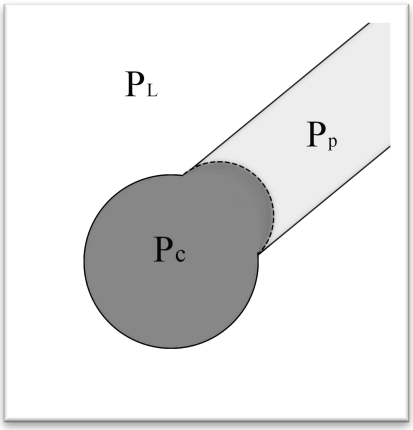
(iii) Now holding atmospheric pressure and surface tension constant, what happens as the internal pressure of the bubbles decrease?

(iv) Now let's examine the interface of the two bubbles. If the pressure of the first bubble is greater than the pressure in the second bubble, what does the interface of the two bubbles look like?

7. Consider the simulation found via the link on your course website or by visiting: http://ggbtu.be/m2118515. Use the simulation to create each of the scenarios in question 6 and test your answers. For each of the above parts (i-iv) state whether your answers agree or disagree.

8. If any parts of your answers to questions 6 and 7 did not agree, what happened? If they did agree, why do you think that is?

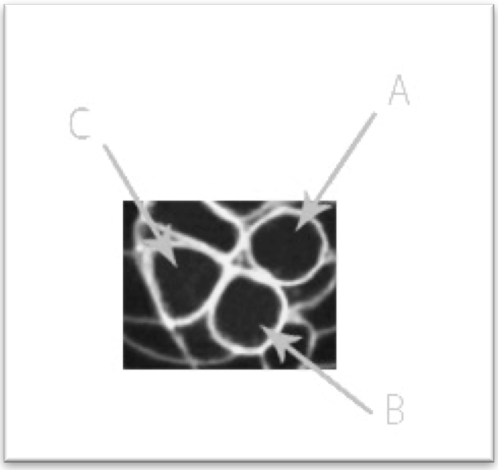
9. Consider the micropipette system introduced in question 4, pictured at right, and the equation for Laplace Pressure from question 5.



(i) What is the surface tension of the cell in terms of the radii and the given pressures?

(ii) If the pressure in the pipette is 1 kPa less than the pressure in the surrounding liquid, calculate the surface tension of the cell.

10. Now that we have investigated these different scenarios, lets see how these principles apply to an *in vivo* biological scenario. During zebrafish development, a group of migrating cells deposits neuromasts, which are clusters of cells that develop into sensory organs.



(i) Some cells have irregular geometry. This could occur for a number of reasons. What are some biological or physical reasons that could contribute to a cell's irregular geometry?

(ii) When the cells do have irregular geometry, which parts of the Laplace Pressure formula are affected and in what way?

(iii) What can you say about the pressure difference of cells A, B, and C?

(iv) If the surface tension of these cells ≈ 0.2 mNm^{-1}, estimate the pressure difference between cell A and its surroundings.

11. Describe qualitatively the role that pressure has in determining cell shape, citing biological examples.

Bibliography

1. Lemon WC, Pulver SR, Höckendorf B, et al (2015) Whole-central nervous system functional imaging in larval Drosophila. Nat Commun 6: . doi: 10.1038/ncomms8924
2. Amat F, Lemon W, Mossing DP, et al (2014) Fast, accurate reconstruction of cell lineages from large-scale fluorescence microscopy data. Nat Methods 11:951–958 . doi: 10.1038/nmeth.3036
3. Lämmermann T, Afonso P V, Angermann BR, et al (2013) Neutrophil swarms require LTB4 and integrins at sites of cell death in vivo. Nature 498:371–5 . doi: 10.1038/nature12175
4. Alexander S, Koehl GE, Hirschberg M, et al (2008) Dynamic imaging of cancer growth and invasion: A modified skin-fold chamber model. Histochem Cell Biol 130:1147–1154 . doi: 10.1007/s00418-008-0529-1
5. Weijer CJ (2009) Collective cell migration in development. J Cell Sci 122:3215–23 . doi: 10.1242/jcs.036517
6. Scarpa E, Mayor R (2016) Collective cell migration in development. J Cell Biol 212:143–55 . doi: 10.1083/jcb.201508047
7. Friedl P, Weigelin B (2008) Interstitial leukocyte migration and immune function. Nat. Immunol. 9:960–969
8. Moser B, Wolf M, Walz A, Loetscher P (2004) Chemokines: Multiple levels of leukocyte migration control. Trends Immunol. 25:75–84
9. Rørth P (2009) Collective Cell Migration. Annu Rev Cell Dev Biol 25:407–429 . doi: 10.1146/annurev.cellbio.042308.113231
10. Friedl P, Wolf K (2003) Tumour-cell invasion and migration: Diversity and escape mechanisms. Nat. Rev. Cancer 3:362–374
11. Lee RM, Stuelten CH, Parent CA, Losert W (2016) Collective cell migration over long time scales reveals distinct phenotypes. Converg Sci Phys Oncol 2:025001 . doi: 10.1088/2057-1739/2/2/025001
12. Friedl P, Gilmour D (2009) Collective cell migration in morphogenesis, regeneration and cancer. Nat. Rev. Mol. Cell Biol. 10:445–457
13. Ridley AJ, Schwartz MA, Burridge K, et al (2003) Cell Migration: Integrating Signals from Front to Back. Science (80-) 302:1704–1709 . doi: 10.1126/science.1092053
14. Gilbert SF (2000) Developmental Biology. 6th Edition. Sunderland (MA): Sinauer Associates
15. Lee RM, Kelley DH, Nordstrom KN, et al (2013) Quantifying stretching and rearrangement in epithelial sheet migration. New J Phys 15: . doi:

10.1088/1367-2630/15/2/025036

16. Wang C, Chowdhury S, Driscoll M, et al (2014) The interplay of cell-cell and cell-substrate adhesion in collective cell migration. J R Soc Interface 11:20140684–20140684 . doi: 10.1098/rsif.2014.0684
17. Friedl P, Hegerfeldt Y, Tusch M (2004) Collective cell migration in morphogenesis and cancer. Int. J. Dev. Biol.
18. Chitnis AB, Dalle Nogare D, Matsuda M (2012) Building the posterior lateral line system in zebrafish. Dev Neurobiol 72:234–255 . doi: 10.1002/dneu.20962
19. Pollard TD, Earnshaw WC (2008) Cell biology
20. Gilbert S (2007) Developmental Biology. Dev Biol 311:691 . doi: 10.1016/j.ydbio.2007.08.033
21. Karp G (2008) Cell and Molecular Biology: Concepts and Experiments. Mendeley Deskt. doi: 10.1002/bmb.2002.494030049993
22. Alberts B, Johnson A, Lewis J, et al (2014) Molecular Biology of the Cell 6e
23. Uzman A, Lodish H, Berk A, et al (2000) Molecular Cell Biology (4th edition) New York, NY, 2000, ISBN 0-7167-3136-3. Biochem Mol Biol Educ 29:Section 1.2The Molecules of Life . doi: 10.1016/S1470-8175(01)00023-6
24. Lodish HF, Berk A, Zipursky SL, et al (2013) Molecular Cell Biology, 7th edition
25. Dalle Nogare D, Chitnis AB (2017) A framework for understanding morphogenesis and migration of the zebrafish posterior Lateral Line primordium. Mech. Dev.
26. Knutsdottir H, Zmurchok C, Bhaskar D, et al (2017) Polarization and migration in the zebrafish posterior lateral line system. PLoS Comput Biol. doi: 10.1371/journal.pcbi.1005451
27. Metcalfe WK, Kimmel CB, Schabtach E (1985) Anatomy of the posterior lateral line system in young larvae of the zebrafish. J Comp Neurol 233:377–389 . doi: 10.1002/cne.902330307
28. Bailey JM, Singh PK, Hollingsworth MA (2007) Cancer metastasis facilitated by developmental pathways: Sonic hedgehog, notch, and bone morphogenic proteins. J. Cell. Biochem.
29. Autzen P, Robson CN, Bjartell A, et al (1998) Bone morphogenetic protein 6 in skeletal metastases from prostate cancer and other common human malignancies. Br J Cancer
30. Hamdy FC, Autzen P, Robinson MC, et al (1997) Immunolocalization and messenger RNA expression of bone morphogenetic protein-6 in human benign and malignant prostatic tissue. Cancer Res
31. Masuda H, Fukabori Y, Nakano K, et al (2003) Increased expression of

bone morphogenetic protein-7 in bone metastatic prostate cancer. Prostate 54:268–274 . doi: 10.1002/pros.10193

32. Ananthakrishnan R, Ehrlicher A (2007) The forces behind cell movement. Int. J. Biol. Sci.
33. Paluch EK, Raz E (2013) The role and regulation of blebs in cell migration. Curr. Opin. Cell Biol.
34. Cooper GM, Hausman RE (2007) The Cell: A Molecular Approach 2nd Edition
35. The Mathworks Inc. (2016) MATLAB - MathWorks. In: www.mathworks.com/products/matlab.
36. Adrian RJ, Westerweel J (2011) Particle Image Velocimetry
37. Sveen JK (2004) An introduction to MatPIV v. 1.6.1. World Wide Web Internet Web Inf Syst 27 pp . doi: 10.1038/nature12618
38. Zhang Y, Xu G, Lee RM, et al (2017) Collective cell migration has distinct directionality and speed dynamics. Cell Mol Life Sci 74:3841–3850 . doi: 10.1007/s00018-017-2553-6
39. Lee RM, Yue H, Rappel WJ, Losert W (2017) Inferring single-cell behaviour from largescale epithelial sheet migration patterns. J R Soc Interface. doi: 10.1098/rsif.2017.0147
40. Stuelten CH, Lee RM, Losert W, Parent CA (2018) Lysophosphatidic acid regulates the motility of MCF10CA1a breast cancer cell sheets via two opposing signaling pathways. Cell Signal. doi: 10.1016/j.cellsig.2018.01.005
41. Lee K, Losert W (2005) Controlled dynamics of grain boundaries in binary alloys. Acta Mater 53:3503–3510 . doi: 10.1016/j.actamat.2005.03.049
42. Brewer C, Smith D (2011) Vision and change in undergraduate biology education: a call to action
43. AAMC (2009) Scientific foundations for future physicians. Assoc Am Med Coll
44. Ramaley J a. (2004) Bio2010: Transforming Undergraduate Education for Future Research Biologists (review)
45. AAMC (2010) Ratings of the Importance of Topics in the Natural Sciences , Research Methods , Statistics , and Behavioral Sciences to Success in Medical School
46. Woodin T, Carter VC, Fletcher L (2010) Vision and Change in Biology Undergraduate Education, A Call for Action - Initial Responses. CBE Life Sci Educ 9:71–73 . doi: 10.1187/cbe.10
47. National Research Council (2003) BIO 2010: Transforming Undergraduate Education for Future Research Biologists. Education 208 . doi: ISBN 0-309-08535-7

48. National Research Council (2009) A new biology for the 21st century. Natl Acad Press 98

49. AAAS (2009) Vision and Change in Undergraduate Biology Education: A Call to Action

50. Nielsen N (2011) Promising Practices in Undergraduate Science, Technology, Engineering, and Mathematics Education: Summary of Two Workshops

51. AAMC (2011) MCAT 2015: A Better Test for Tomorrow's Doctors

52. AAMC (2012) MCAT 2015: A Better Test for Tomorrow's Doctors 2nd Edition. Foundations

53. Cuff PAR (2015) Envisioning the Future of Health Professional Education: Workshop Summary

54. AAPT (2014) Conference on Introductory Physics for the Life Sciences Report

55. AAAS (2015) Vision and Change in Undergraduate Biology Education: Chronicling Change, Inspiring the Future

56. Alberts B (1998) The cell as a collection of protein machines: Preparing the next generation of molecular biologists. Cell 92:291–294

57. Adams WK, Perkins KK, Podolefsky NS, et al (2006) New instrument for measuring student beliefs about physics and learning physics: The Colorado Learning Attitudes about Science Survey. Phys Rev Spec Top - Phys Educ Res 2: . doi: 10.1103/PhysRevSTPER.2.010101

58. Harold Varmus (1999) The Impact of Physics on Biology and Medicine. APS News

59. Labov JB, Reid AH, Yamamoto KR (2010) Integrated biology and undergraduate science education: A new biology education for the twenty-first century? CBE Life Sci Educ 9:10–16 . doi: 10.1187/cbe.09-12-0092

60. May RM (2004) Uses and Abuses of Mathematics in Biology. Science (80-) 790:790–793 . doi: 10.1126/science.1094442

61. Wolgemuth C (2011) Does cell biology need physicists? Physics (College Park Md) 4:4 . doi: 10.1103/Physics.4.4

62. M. Klymkowsky (2011) Getting Serious About Science Education. JBC

63. C. H. Crouch, R. Hilborn, S. A. Kane, T. McKay MR (2010) Physics for Future Physicians and Life Scientists: a moment of opportunity. APS News

64. Mylott E, Kutschera E, Dunlap JC, et al (2016) Using Biomedically Relevant Multimedia Content in an Introductory Physics Course for Life Science and Pre-health Students. J Sci Educ Technol 25:222–231 . doi: 10.1007/s10956-015-9588-y

65. Meredith DC, Redish EF (2013) Reinventing physics for life-sciences

majors. Phys Today 66:38–43 . doi: 10.1063/PT.3.2046

66. Redish EF, Hammer D (2009) Reinventing college physics for biologists: Explicating an epistemological curriculum. Am J Phys 77:629 . doi: 10.1119/1.3119150

67. Smith DP, Mcneil LE, Guynn DT, et al Transforming the content, pedagogy and structure of an introductory physics course for life sciences majors David P. Smith 1 , Laurie E. McNeil, David T. Guynn, Alice D. Churukian, Duane L. Deardorff, Colin S. Wallace. 1–26

68. Crouch CH, Heller K (2014) Introductory physics in biological context: An approach to improve introductory physics for life science students. Am J Phys 82:378–386 . doi: 10.1119/1.4870079

69. Mochrie SGJ (2016) Vision and change in introductory physics for the life sciences. Am J Phys 85:542 . doi: 10.1119/1.4947003

70. Redish EF (2010) Introducing students to the culture of physics: Explicating elements of the hidden curriculum. In: AIP Conference Proceedings. pp 49–52

71. Watkins J, Hall K, Redish EF, Cooke TJ (2010) Understanding How Students Use Physical Ideas in Introductory Biology Courses. 2010 PER Conf Proc - AIP Conf Proc 1289 333–336 . doi: 10.1063/1.3515237

72. Hall KL, Watkins JE, Coffey JE, et al (2011) Examining the Impact of Student Expectations on Undergraduate Biology Education Reform. arXiv, 11056349 1–10

73. Dreyfus BW, Redish EF, Watkins J (2011) Students' Views of Macroscopic and Microscopic Energy in Physics and Biology. Phys Educ Res Conf 1413:179–182

74. Svoboda J, Passmore C (2013) The Strategies of Modeling in Biology Education. Sci Educ 22:119–142 . doi: 10.1007/s11191-011-9425-5

75. Watkins J, Coffey JE, Redish EF, Cooke TJ (2012) Disciplinary authenticity: Enriching the reforms of introductory physics courses for life-science students. Phys Rev Spec Top - Phys Educ Res 8: . doi: 10.1103/PhysRevSTPER.8.010112

76. Redish EF (2012) The role of context and culture in teaching physics: The implication of disciplinary differences. Proc World Conf Phys Educ 1–22

77. Thompson K V., Chmielewski J, Gaines MS, et al (2013) Competency-based reforms of the undergraduate biology curriculum: Integrating the physical and biological sciences. CBE Life Sci Educ 12:162–169 . doi: 10.1187/cbe.12-09-0143

78. Redish EF, Cooke TJ (2013) Learning each other's ropes: Negotiating interdisciplinary authenticity. CBE Life Sci Educ 12:175–186 . doi: 10.1187/cbe.12-09-0147

79. Gouvea JS, Sawtelle V, Geller BD, Turpen C (2013) A framework for

analyzing interdisciplinary tasks: Implications for student learning and curricular design. CBE Life Sci Educ 12:187–205 . doi: 10.1187/cbe.12-08-0135

80. Cooper MM, Klymkowsky MW (2013) The trouble with chemical energy: Why understanding bond energies requires an interdisciplinary systems approach. CBE Life Sci Educ 12:306–312 . doi: 10.1187/cbe.12-10-0170

81. Watkins J, Elby A (2013) Context dependence of students' views about the role of equations in understanding biology. CBE Life Sci Educ 12:274–286 . doi: 10.1187/cbe.12-11-0185

82. Geller BD, Dreyfus BW, Sawtelle V, et al (2013) Students' reasoning about interdisciplinarity. AIP Conf Proc 1513:146–149 . doi: 10.1063/1.4789673

83. Dreyfus BW, Sawtelle V, Turpen C, et al (2014) Students' reasoning about "high-energy bonds" and ATP: A vision of interdisciplinary education. Phys Rev Spec Top - Phys Educ Res 10:010115 . doi: 10.1103/PhysRevSTPER.10.010115

84. Sawtelle V, Sikorski T, Turpen C, Redish EF (2012) Examining the positioning of ideas in the disciplines. Phys Educ … 366–369 . doi: 10.1063/1.4789728

85. Thompson K V., Cooke TJ, Fagan WF, et al (2013) Infusing quantitative approaches throughout the biological sciences curriculum. Int J Math Educ Sci Technol 44:817–833 . doi: 10.1080/0020739X.2013.812754

86. Dreyfus BW, Geller BD, Gouvea JS (2014) Negative Energy: Why Interdisciplinary Physics Requires Multiple Ontologies. PERC 2013 Proc 129–132 . doi: 10.1119/perc.2013.pr.020

87. Geller BD, Dreyfus BW, Gouvea JS, et al (2013) "Like Dissolves Like": Unpacking Student Reasoning About Thermodynamic Heuristics. PERC Proc 157–160 . doi: 10.1119/perc.2013.pr.027

88. Redish EF, Bauer C, Carleton KL, et al (2014) NEXUS / Physics : An interdisciplinary repurposing of physics for biologists. AJP 82:368

89. Dreyfus BW, Gouvea J, Geller BD, et al (2014) Chemical energy in an introductory physics course for the life sciences. Am J Phys 82:403–411 . doi: 10.1119/1.4870391

90. Geller BD, Dreyfus BW, Gouvea J, et al (2014) Entropy and spontaneity in an introductory physics course for life science students. Am J Phys 82:394–402 . doi: 10.1119/1.4870389

91. Moore K, Giannini J, Losert W (2014) Toward Better Physics Labs for Future Biologists

92. Geller BD, Gouvea JS, Sawtelle V, Turpen C (2014) Sources of affect around interdisciplinary sense making. Proc Int Conf Learn Sci ICLS 2:1142–1146

93. Dreyfus BW, Geller BD, Gouvea J, et al (2014) Ontological metaphors for negative energy in an interdisciplinary context. Phys Rev Spec Top - Phys Educ Res 10: . doi: 10.1103/PhysRevSTPER.10.020108

94. Dreyfus BW, Geller BD, Meltzer DE, Sawtelle V (2015) Resource Letter TTSM-1: Teaching Thermodynamics and Statistical Mechanics in Introductory Physics, Chemistry, and Biology. Am J Phys 83:5–21 . doi: doi:http://dx.doi.org/10.1119/1.4891673

95. Dreyfus BW, Gupta A, Redish EF (2015) Applying Conceptual Blending to Model Coordinated Use of Multiple Ontological Metaphors. Int J Sci Educ 37:812–838 . doi: 10.1080/09500693.2015.1025306

96. Redish EF, Kuo E (2015) Language of Physics, Language of Math: Disciplinary Culture and Dynamic Epistemology. Sci Educ 24:561–590 . doi: 10.1007/s11191-015-9749-7

97. Sawtelle V, Turpen C (2015) Leveraging a Relationship with Biology to Expand a Relationship with Physics

98. Redish EF (2017) Analysing the competency of mathematical modelling in physics. In: Springer Proceedings in Physics. pp 25–40

99. Brown JS, Collins A, Duguid P (1989) Situated Cognition and the Culture of Learning. Educ Res 18:32–42 . doi: 10.2307/1176008

100. Vosniadou S, Mason L (2012) Conceptual change induced by instruction: A complex interplay of multiple factors. APA handbooks Psychol 2:221–246 . doi: http://dx.doi.org/10.1037/13274-009

101. Kaplan A, Katz I, Flum H (2012) Motivation theory in educational practice: Knowledge claims, challenges, and future directions. APA Educ Psychol handbook, Vol 2 Individ Differ Cult Context factors 2:165–194 . doi: 10.1037/13274-007

102. Alexander PA, Dinsmore DL, Fox E, et al (2011) Higher order thinking and knowledge: Domain-general and domain-specific trends and future directions. Assess High order Think Ski 47–88

103. Chmelir L (1998) Handbook of the undergraduate curriculum: A comprehensive guide to purposes, structures, practices, and change. J Acad Librariansh 24:176 . doi: 10.1016/S0099-1333(98)90186-0

104. Knight DB, Lattuca LR, Kimball EW, Reason RD (2013) Understanding Interdisciplinarity: Curricular and Organizational Features of Undergraduate Interdisciplinary Programs. Innov High Educ 38:143–158 . doi: 10.1007/s10755-012-9232-1

105. Clement J (1993) Using bridging analogies and anchoring intuitions to deal with students' preconceptions in physics. J Res Sci Teach 30:1241–1257 . doi: 10.1002/tea.3660301007

106. Li L, He Y, Zhao M, Jiang J (2013) Collective cell migration: Implications for wound healing and cancer invasion. Burn Trauma. doi: 10.4103/2321-3868.113331

107. Szabó A, Mayor R (2016) Modelling collective cell migration of neural crest. Curr. Opin. Cell Biol.

108. Theveneau E, Mayor R (2012) Neural crest delamination and migration: From epithelium-to-mesenchyme transition to collective cell migration. Dev. Biol.

109. Friedl P, Locker J, Sahai E, Segall JE (2012) Classifying collective cancer cell invasion. Nat. Cell Biol.

110. Gaind N (2016) US grants for zebrafish studies on the rise. Nature. doi: 10.1038/nature.2016.20391

111. United Kingdom Statistics Authority (2016) Annual Statistics of Scientific Procedures on Living Animals Great Britain 2016. London

112. Langheinrich U, Hennen E, Stott G, Vacun G (2002) Zebrafish as a model organism for the identification and characterization of drugs and genes affecting p53 signaling. Curr Biol. doi: 10.1016/S0960-9822(02)01319-2

113. Feitsma H, Cuppen E (2008) Zebrafish as a Cancer Model Cancer Research in Zebrafish. Mol Cancer Res. doi: 10.1158/1541-7786.MCR-07-2167

114. Pereira TCB, Rico EP, Rosemberg DB, et al (2011) Zebrafish as a Model Organism to Evaluate Drugs Potentially Able to Modulate Sirtuin Expression. Zebrafish. doi: 10.1089/zeb.2010.0677

115. Itoh M, Chitnis AB (2001) Expression of proneural and neurogenic genes in the zebrafish lateral line primordium correlates with selection of hair cell fate in neuromasts. Mech Dev. doi: 10.1016/S0925-4773(01)00308-2

116. Hisaoka KK, Battle HI (1958) The normal developmental stages of the zebrafish, Brachydanio rerio (Hamilton-Buchanan). J Morphol. doi: 10.1002/jmor.1051020205

117. Fishman MC (2001) GENOMICS: Zebrafish--the Canonical Vertebrate. Science (80-). doi: 10.1126/science.1066652

118. Kimmel CB, Ballard WW, Kimmel SR (1995) Stages of embryonic development of the zebrafish. Am J …

119. Haas P, Gilmour D (2006) Chemokine Signaling Mediates Self-Organizing Tissue Migration in the Zebrafish Lateral Line. Dev Cell 10:673–680 . doi: 10.1016/j.devcel.2006.02.019

120. Tsien RY (1998) The green fluorescent protein. Annu Rev Biochem 67:509–544 . doi: 10.1146/annurev.biochem.67.1.509

121. Kollmar R, Nakamura SK, Kappler JA, Hudspeth AJ (2001) Expression and phylogeny of claudins in vertebrate primordia. Proc Natl Acad Sci U S A 98:10196–201 . doi: 10.1073/pnas.171325898

122. David NB, Sapède D, Saint-Etienne L, et al (2002) Molecular basis of cell migration in the fish lateral line: role of the chemokine receptor CXCR4 and of its ligand, SDF1. Proc Natl Acad Sci U S A 99:16297–302 . doi: 10.1073/pnas.252339399

123. Montgomery J, Carton G, Voigt R, et al (2000) Sensory processing of water currents by fishes. Philos Trans R Soc B Biol Sci 355:1325–1327 . doi: 10.1098/rstb.2000.0693

124. Bleckmann H, Zelick R (2009) Lateral line system of fish. Integr. Zool. 4:13–25

125. Rouse GW, Pickles JO (1991) Paired development of hair cells in neuromasts of the teleost lateral line. Proc Biol Sci 246:123–8 . doi: 10.1098/rspb.1991.0133

126. Kumar V, Abbas AK, Fausto N, Aster JC (2009) Kumar: Robbins and Cotran Pathologic Basis of Disease, Professional Edition, 8th ed.

127. Christopher R. Jacobs, Hayden Huang RYK (2012) Introduction to Cell Mechanics and Mechanobiology

128. Trepat X, Wasserman MR, Angelini TE, et al (2009) Physical forces during collective cell migration. Nat Phys. doi: 10.1038/nphys1269

129. Hochmuth RM (2000) Micropipette aspiration of living cells. J Biomech 33:15–22 . doi: 10.1016/S0021-9290(99)00175-X

130. Lecuit T, Lenne P-F (2007) Cell surface mechanics and the control of cell shape, tissue patterns and morphogenesis. Nat Rev Mol Cell Biol 8:633–644 . doi: 10.1038/nrm2222

131. Venugopalan G, Tanner K, Orellana W, et al (2012) Externally applied forces can phenotypically revert malignant breast epithelial structures. Mol Biol Cell 23:

132. Scarcelli G, Polacheck WJ, Nia HT, et al (2015) Noncontact three-dimensional mapping of intracellular hydromechanical properties by Brillouin microscopy. Nat Methods. doi: 10.1038/nmeth.3616

133. Scarcelli G, Yun SH (2008) Confocal Brillouin microscopy for three-dimensional mechanical imaging. Nat Photonics. doi: 10.1038/nphoton.2007.250

134. Dil JG (1982) Brillouin scattering in condensed matter. Reports Prog. Phys.

135. Scarcelli G, Kim P, Yun SH (2011) In vivo measurement of age-related stiffening in the crystalline lens by Brillouin optical microscopy. Biophys J. doi: 10.1016/j.bpj.2011.08.008

136. Thompson RE, Larson DR, Webb WW (2002) Precise nanometer localization analysis for individual fluorescent probes. Biophys J. doi: 10.1016/S0006-3495(02)75618-X

137. Darling EM, Zauscher S, Block JA, Guilak F (2007) A thin-layer model for viscoelastic, stress-relaxation testing of cells using atomic force microscopy: Do cell properties reflect metastatic potential? Biophys J. doi: 10.1529/biophysj.106.083097

138. Ng L, Hung HH, Sprunt A, et al (2007) Nanomechanical properties of individual chondrocytes and their developing growth factor-stimulated

pericellular matrix. J Biomech. doi: 10.1016/j.jbiomech.2006.04.004

139. Oh M-J, Kuhr F, Byfield F, Levitan I (2012) Micropipette Aspiration of Substrate-attached Cells to Estimate Cell Stiffness. J Vis Exp e3886 . doi: 10.3791/3886

140. Engström KG, Meiselman HJ (1997) Combined use of micropipette aspiration and perifusion for studying red blood. Cell volume regulation. Cytometry 27:345–352 . doi: 10.1002/(SICI)1097-0320(19970401)27:4<345::AID-CYTO5>3.0.CO;2-B

141. Young T (1805) An Essay on the Cohesion of Fluids. Philos Trans R Soc London. doi: 10.1098/rstl.1805.0005

142. Laplace PS marquis de (1805) Traité de Mécanique Céleste

143. Lee LM, Liu AP (2014) The Application of Micropipette Aspiration in Molecular Mechanics of Single Cells. J Nanotechnol Eng Med. doi: 10.1115/1.4029936

144. Ranft J, Prost J, Jülicher F, Joanny JF (2012) Tissue dynamics with permeation. Eur Phys J E Soft Matter 35:46 . doi: 10.1140/epje/i2012-12046-5

145. Malicki J, Jo H, Pujic Z (2003) Zebrafish N-cadherin, encoded by the glass onion locus, plays an essential role in retinal patterning. Dev Biol. doi: 10.1016/S0012-1606(03)00181-7

146. Lele Z, Folchert A, Concha M, et al (2002) Parachute/N-Cadherin Is Required for Morphogenesis and Maintained Integrity of the Zebrafish Neural Tube. Development. doi: 10.1242/dev.00567

147. Hong E (2006) N-cadherin is required for the polarized cell behaviors that drive neurulation in the zebrafish. Development. doi: 10.1242/dev.02560

148. Shimizu T, Yabe T, Muraoka O, et al (2005) E-cadherin is required for gastrulation cell movements in zebrafish. Mech Dev. doi: 10.1016/j.mod.2005.03.008

149. Hava D, Forster U, Matsuda M, et al (2009) Apical membrane maturation and cellular rosette formation during morphogenesis of the zebrafish lateral line. J Cell Sci. doi: 10.1242/jcs.032102

150. Montell DJ (2008) Morphogenetic cell movements: Diversity from modular mechanical properties. Science (80-.).

151. Theveneau E, Mayor R (2012) Cadherins in collective cell migration of mesenchymal cells. Curr. Opin. Cell Biol.

152. Slanchev K, Carney TJ, Stemmler MP, et al (2009) The epithelial cell adhesion molecule EpCAM is required for epithelial morphogenesis and integrity during zebrafish epiboly and skin development. PLoS Genet. doi: 10.1371/journal.pgen.1000563

153. Gallardo VE, Liang J, Behra M, et al (2010) Molecular dissection of the migrating posterior lateral line primordium during early development in

zebrafish. BMC Dev Biol. doi: 10.1186/1471-213X-10-120

154. Vannier C, Mock K, Brabletz T, Driever W (2013) Zeb1 regulates E-cadherin and Epcam (epithelial cell adhesion molecule) expression to control cell behavior in early zebrafish development. J Biol Chem. doi: 10.1074/jbc.M113.467787

155. Chalasani K, Brewster RM (2011) N-cadherin-mediated cell adhesion restricts cell proliferation in the dorsal neural tube. Mol Biol Cell. doi: 10.1091/mbc.E10-08-0675

156. Dejana E, Corada M, Lampugnani MG (1995) Endothelial cell-to-cell junctions. FASEB J. doi: 10.1101/cshperspect.a006528

157. McCann CP, Kriebel PW, Parent CA, Losert W (2010) Cell speed, persistence and information transmission during signal relay and collective migration. J Cell Sci 123:1724–1731 . doi: 10.1242/jcs.060137

158. Schwartz MA, DeSimone DW (2008) Cell adhesion receptors in mechanotransduction. Curr. Opin. Cell Biol.

159. Takeichi M (1988) The cadherins: cell-cell adhesion molecules controlling animal morphogenesis. Development

160. Edelman GM, Crossin KL (1991) Cell adhesion molecules: implications for a molecular histology. Annu Rev Biochem. doi: 10.1146/annurev.bi.60.070191.001103

161. Ma EY, Raible DW (2009) Signaling Pathways Regulating Zebrafish Lateral Line Development. Curr. Biol. 19

162. Aman A, Nguyen M, Piotrowski T (2011) Wnt/β-catenin dependent cell proliferation underlies segmented lateral line morphogenesis. Dev Biol 349:470–482 . doi: 10.1016/j.ydbio.2010.10.022

163. Romero-Carvajal A, Navajas Acedo J, Jiang L, et al (2015) Regeneration of Sensory Hair Cells Requires Localized Interactions between the Notch and Wnt Pathways. Dev Cell 34:267–282 . doi: 10.1016/j.devcel.2015.05.025

164. Venero Galanternik M, Navajas Acedo J, Romero-Carvajal A, Piotrowski T (2016) Imaging collective cell migration and hair cell regeneration in the sensory lateral line. In: Methods in Cell Biology. pp 211–256

165. Niehrs C, Meinhardt H (2002) Developmental biology: Modular feedback. Nature 417:35–36 . doi: 10.1038/417035a

166. Tuazon FB, Mullins MC (2015) Temporally coordinated signals progressively pattern the anteroposterior and dorsoventral body axes. Semin. Cell Dev. Biol. 42:118–133

167. Salazar VS, Gamer LW, Rosen V (2016) BMP signalling in skeletal development, disease and repair. Nat Rev Endocrinol 12:203–221 . doi: 10.1038/nrendo.2016.12

168. Zhang H, Bradley A (1996) Mice deficient for BMP2 are nonviable and

have defects in amnion/chorion and cardiac development. Development 122:2977–2986 . doi: 10.1016/J.YDBIO.2004.12.037

169. Ying Y, Zhao G-Q (2001) Cooperation of Endoderm-Derived BMP2 and Extraembryonic Ectoderm-Derived BMP4 in Primordial Germ Cell Generation in the Mouse. Dev Biol 232:484–492 . doi: 10.1006/dbio.2001.0173

170. Logan M, Martin JF, Nagy A, et al (2002) Expression of Cre Recombinase in the developing mouse limb bud driven by a Prxl enhancer. Genesis 33:77–80 . doi: 10.1002/gene.10092

171. Tsuji K, Bandyopadhyay A, Harfe BD, et al (2006) BMP2 activity, although dispensable for bone formation, is required for the initiation of fracture healing. Nat Genet 38:1424–1429 . doi: 10.1038/ng1916

172. Wang RN, Green J, Wang Z, et al (2014) Bone Morphogenetic Protein (BMP) signaling in development and human diseases. Genes Dis 1:87–105 . doi: 10.1016/j.gendis.2014.07.005

173. Hemmati-Brivanlou A, Thomsen GH (1995) Ventral mesodermal patterning in Xenopus embryos: Expression patterns and activities of BMP-2 and BMP-4. Dev. Genet. 17:78–89

174. Zou H, Niswander L (1996) Requirement for BMP signaling in interdigital apoptosis and scale formation. Science 272:738–41 . doi: 10.1126/science.272.5262.738

175. Stewart A, Guan H, Yang K (2010) BMP-3 promotes mesenchymal stem cell proliferation through the TGF-beta/activin signaling pathway. J Cell Physiol 223:658–66 . doi: 10.1002/jcp.22064

176. Kobayashi T, Lyons KM, McMahon AP, Kronenberg HM (2005) BMP signaling stimulates cellular differentiation at multiple steps during cartilage development. Proc Natl Acad Sci 102:18023–18027 . doi: 10.1073/pnas.0503617102

177. Bobacz K, Gruber R, Soleiman A, et al (2003) Expression of bone morphogenetic protein 6 in healthy and osteoarthritic human articular chondrocytes and stimulation of matrix synthesis in vitro. Arthritis Rheum 48:2501–2508 . doi: 10.1002/art.11248

178. Huang Z, Wang D, Ihida-Stansbury K, et al (2009) Defective pulmonary vascular remodeling in Smad8 mutant mice. Hum Mol Genet 18:2791–2801 . doi: 10.1093/hmg/ddp214

179. Gamell C, Osses N, Bartrons R, et al (2008) BMP2 induction of actin cytoskeleton reorganization and cell migration requires PI3-kinase and Cdc42 activity. J Cell Sci 121:3960–3970 . doi: 10.1242/jcs.031286

180. Gamell C, Susperregui AG, Bernard O, et al (2011) The p38/MK2/Hsp25 pathway is required for BMP-2-induced cell migration. PLoS One 6: . doi: 10.1371/journal.pone.0016477

181. Inai K, Norris RA, Hoffman S, et al (2008) BMP-2 induces cell migration

and periostin expression during atrioventricular valvulogenesis. Dev Biol 315:383–396 . doi: 10.1016/j.ydbio.2007.12.028

182. Sanvitale CE, Kerr G, Chaikuad A, et al (2013) A new class of small molecule inhibitor of BMP signaling. PLoS One 8:e62721 . doi: 10.1371/journal.pone.0062721

183. Mohammadi M, McMahon G, Sun L, et al (1997) Structures of the tyrosine kinase domain of fibroblast growth factor receptor in complex with inhibitors. Science (80-) 276:955–960 . doi: 10.1126/science.276.5314.955

184. Lecaudey V, Cakan-Akdogan G, Norton WHJ, Gilmour D (2008) Dynamic Fgf signaling couples morphogenesis and migration in the zebrafish lateral line primordium. Development. doi: 10.1242/dev.025981

185. Poss KD, Shen J, Nechiporuk A, et al (2000) Roles for Fgf signaling during zebrafish fin regeneration. Dev Biol. doi: 10.1006/dbio.2000.9722

186. Jackman WR, Draper BW, Stock DW (2004) Fgf signaling is required for zebrafish tooth development. Dev Biol. doi: 10.1016/j.ydbio.2004.07.003

187. Zhang J, Li L (2005) BMP signaling and stem cell regulation. Dev. Biol. 284:1–11

188. Sanvitale CE, Kerr G, Chaikuad A, et al (2013) A New Class of Small Molecule Inhibitor of BMP Signaling. PLoS One 8:e62721 . doi: 10.1371/journal.pone.0062721

189. Windhausen T, Squifflet S, Renn J, Muller M (2015) BMP signaling regulates bone morphogenesis in zebrafish through promoting osteoblast function as assessed by their nitric oxide production. Molecules. doi: 10.3390/molecules20057586

190. Sakamoto Y, Prudhomme S, Zaman MH (2014) Modeling of adhesion, protrusion, and contraction coordination for cell migration simulations. J Math Biol 68:267–302 . doi: 10.1007/s00285-012-0634-6

191. Schwartz MA, Horwitz AR (2006) Integrating Adhesion, Protrusion, and Contraction during Cell Migration. Cell 125:1223–1225

192. Vicente-Manzanares M, Zareno J, Whitmore L, et al (2007) Regulation of protrusion, adhesion dynamics, and polarity by myosins IIA and IIB in migrating cells. J Cell Biol 176:573–580 . doi: 10.1083/jcb.200612043

193. Straight AF, Cheung A, Limouze J, et al (2003) Dissecting temporal and spatial control of cytokinesis with a myosin II Inhibitor. Science 299:1743–7 . doi: 10.1126/science.1081412

194. Kovács M, Tóth J, Hetényi C, et al (2004) Mechanism of blebbistatin inhibition of myosin II. J Biol Chem 279:35557–35563 . doi: 10.1074/jbc.M405319200

195. Allingham JS, Smith R, Rayment I (2005) The structural basis of blebbistatin inhibition and specificity for myosin II. Nat Struct Mol Biol 12:378–9 . doi: 10.1038/nsmb908

196. Brack KE, Narang R, Winter J, Ng GA (2013) The mechanical uncoupler blebbistatin is associated with significant electrophysiological effects in the isolated rabbit heart. Exp Physiol. doi: 10.1113/expphysiol.2012.069369

197. Wang X, Chong M, Wang X, et al (2015) Block the function of nonmuscle myosin II by blebbistatin induces zebrafish embryo cardia bifida. Vitr Cell Dev Biol - Anim. doi: 10.1007/s11626-014-9836-0

198. Kolega J (2004) Phototoxicity and photoinactivation of blebbistatin in UV and visible light. Biochem Biophys Res Commun. doi: 10.1016/j.bbrc.2004.06.045

199. Sakamoto T, Limouze J, Combs CA, et al (2005) Blebbistatin, a myosin II inhibitor, is photoinactivated by blue light. Biochemistry. doi: 10.1021/bi0483357

200. Hemingway D, Sawtelle V, Turpen C (2015) How an educator characterizes scientific domains and disciplinary relationships : A case of change

201. Rasband WS (1997) ImageJ. Natl Institutes Heal Bethesda, Maryland, USA http://imagej.nih.gov/ij/

202. Huang LK, Wang MJJ (1995) Image thresholding by minimizing the measures of fuzziness. Pattern Recognit 28:41–51 . doi: 10.1016/0031-3203(94)E0043-K

203. Vicente-Manzanares M, Ma X, Adelstein RS, Horwitz AR (2009) Non-muscle myosin II takes centre stage in cell adhesion and migration. Nat. Rev. Mol. Cell Biol. 10:778–790

204. Teven CM, Farina EM, Rivas J, Reid RR (2014) Fibroblast growth factor (FGF) signaling in development and skeletal diseases. Genes Dis 1:199–213 . doi: 10.1016/j.gendis.2014.09.005

205. Repko AF, Szostak R, Buchberger MP (2017) Introduction to Interdisciplinary Studies, 2nd ed. SAGE Publications

206. LeDoux JE (1998) The emotional brain: The mysterious underpinnings of emotional life

207. Ledoux JE (2000) Emotion Circuits in the Brain. Ann Rev Neurosci 23:155–184 . doi: 10.1146/annurev.neuro.23.1.155

208. Damasio AR (1995) Descartes' Error: Emotion, Reason, and the Human Brain

209. Damasio a R (2001) Descartes' error revisited. J Hist Neurosci 10:192–194 . doi: 10.1076/jhin.10.2.192.7250

210. Danielak BA, Gupta A, Elby A (2014) Marginalized Identities of Sense-Makers: Reframing Engineering Student Retention. J Eng Educ 103:8–44 . doi: 10.1002/jee.20035

211. Tipler P a., Mosca G (2009) Physics for Scientists and Engineers

212. Walker J (2007) Physics, 3rd ed. Benjamin Cummings

213. Freedman R, Ruskell T, Kesten PR, Tauck DL (2014) College Physics, 1st ed

214. Dreyfus B, Sawtelle V, Geller B, et al (2012) Insane in the membrane, part 2: Lipid bilayers. http://umdberg.pbworks.com/w/page/50354961/Insane in the membrane%2C part 2%3A Lipid bilayers

215. Coombs S, Van Netten S (2005) The Hydrodynamics and Structural Mechanics of the Lateral Line System. Fish Physiol. 23:103–139

216. Russ RS, Coffey JE, Hammer D, Hutchison P (2009) Making classroom assessment more accountable to scientific reasoning: A case for attending to mechanistic thinking. Sci Educ 93:875–891 . doi: 10.1002/sce.20320

217. Hutchison P, Hammer D (2010) Attending to student epistemological framing in a science classroom. Sci Educ 94:506–524 . doi: 10.1002/sce.20373

218. Hestenes D (1987) Toward a modeling theory of physics instruction. Am J Phys 55:440–454 . doi: 10.1119/1.15129

219. Brewe E (2008) Modeling theory applied: Modeling Instruction in introductory physics. Am J Phys 76:1155–1160 . doi: 10.1119/1.2983148

220. Wenning CJ (2011) Physics Courses. J Phys Teach Eduacation Online 6:1–20

221. Wenning CJ (2009) Scientific epistemology : How scientists know what they know. J Phys Teach Educ Online 5:3–15

222. Von Glasersfeld E (1995) Radical Constructivism: A Way of Knowing and Learning

223. Donovan MS, Bransford JD (2008) How Students Learn: History, Mathematics, and Science in the Classroom

224. Davis EA, Krajcik JS (2005) Designing Educative Curriculum Materials to Promote Teacher Learning. Educ Res 34:3–14 . doi: 10.3102/0013189X034003003

225. Quintana C, Reiser BJ, Davis EA, et al (2004) A scaffolding design framework for software to support science inquiry. In: Journal of the Learning Sciences. pp 337–386

226. Docktor JL, Mestre JP (2014) Synthesis of discipline-based education research in physics. Phys Rev Spec Top - Phys Educ Res 10: . doi: 10.1103/PhysRevSTPER.10.020119

227. diSessa AA (1993) Toward an Epistemology of Physics. Cogn Instr 10:105–225 . doi: 10.1080/07370008.1985.9649008

228. Hammer D (2000) Student resources for learning introductory physics. Am J Phys 68:S52–S59 . doi: 10.1119/1.19520

229. Hammer D, Elby A, Scherr RE, Redish EF (2005) Resources, framing, and transfer. In: Transfer of learning from a modern multidisciplinary perspective. pp 89–120

230. Hammer D, Elby A (2002) On the form of a personal epistemology. In 169–190 . doi: 10.4324/9780203424964

231. Hammer D, Elby A (2012) On the form of a personal epistemology. In: Personal Epistemology: The Psychology of Beliefs about Knowledge and Knowing. pp 169–190

232. Hofer BK, Pintrich PR (2012) Personal epistemology: The psychology of beliefs about knowledge and knowing

233. Cuban L (1995) The hidden variable: How organizations influence teacher responses to secondary science curriculum reform. Theory Pract. 34:4–11