The Role of The Nucleus in Collective Cell Migration

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Specific Aims

Cell migration is a fundamental process in normal physiology and pathology. The cell nucleus is emerging as a key mediator in this process. Being the largest and stiffest organelle in eukaryotic cells, the nucleus acts as a limiting factor during cell migration. The role of nuclear mechanics in cell migration is therefore a topic of recent interest to both the basic science and clinical research communities. The objective of the proposed work is to study how nuclear mechanical properties affect collective cell migration using a model system of the scratch wound assay. Quantitative understanding of collective cell migration will be obtained using live time lapse imaging of cells with modified nuclear stiffness and altered chromatin remodeling abilities.

Specific Aim #1: Establish a scratch wound assay of cell migration using NIH-3T3 cells. NIH-3T3 mouse embryonic fibroblast cells will be used in this study because they are an excellent model system for this assay and easy to culture. Establishment of a scratch wound assay will involve culturing of the cells in a confluent manner and applying the scratch in a consistent manner. Migration parameters such as speed and persistence will then be quantified using the image processing software Fiji. Determining these image processing methods will also be needed for Aim 2. The assay technique will then be validated using one or two migration inhibitors such as cyto-D to inhibit F-actin or Y27 to inhibit actomyosin contraction.

Specific Aim #2: Observe how changes to nuclear mechanics affect migration parameters. After establishing the control, drugs such as Trichostatin A and GSK126 will be applied to the NIH-3T3 cells which will modify their nuclear stiffness and chromatin remodeling ability. These cells will then undergo the scratch wound assay

established in Aim 1. Subsequently, migration parameters will be quantified using image processing and will be compared to the results found in the control group.

Specific Aim #3: Perform live imaging of the cytoskeleton and nucleus at high resolution to quantify a strain map in the cell monolayer. The scratch wound assay will be repeated for both the control and modified groups with visualization of the cell cytoskeleton and nucleus using live staining of actin and DNA, respectively. Live imaging at a high resolution will then be performed on these cells during migration. Using image processing, a strain map will be generated at the cell monolayer providing detailed mechanical insight of subcellular mechanics in the cell migration process.

Determining the effects of nuclear mechanical properties on cell migration, and how these effects pertain to the cell monolayer, will help increase our understanding of this fundamental physiological process. If the aims of this project are successfully met, it would help lay groundwork for further studies in understanding the synergistic role of nuclear mechanics with cell crowding effects and differing substrate mechanics applicable in tissue engineering applications.

Significance

Abstract

Cell migration is a fundamental physiological process in normal physiology and pathology. The cell nucleus is emerging as a key player in this process [1], [2]. Being the largest and stiffest organelle, in the eukaryotic cells, the nucleus acts as a limiting factor during cell migration. The role of nuclear mechanics in the cell migration is

therefore a topic of recent interest to both the basic science and clinical research communities.

This research proposes to study how nuclear mechanical properties affect collective cell migration using a model system of the scratch wound assay. Through live time lapse imaging, cell migration parameters will be quantified. High resolution live imaging of the nucleus and cytoskeleton will be performed which will allow us to develop strain maps at the cell monolayer, thus providing detailed mechanical insight of the process. Further studies might be performed to understand the synergistic role of nuclear mechanics with cell crowding effects and differing substrate mechanics.

Background

In order to quantitatively assess the role of the nucleus in collective cell migration, it is important to understand the trajectory of single cells in the context of collective cell migration, and to decide on some parameters to quantify the trajectory of migration.

Cellular Movement

In the motility mode of cell crawling, cells generally move in response to external chemical or physical signals in the environment detected by focal adhesions connected to the cytoskeleton [3]. Although the cytoskeleton is composed of actin filaments, microtubules, and intermediate filaments, it is the actin cytoskeleton that is generally referred to as the engine of motility. This is due to the actin network driving the first step of cell movement through protrusion using forces generated from asymmetric polymerization. After protrusion of the leading edge, the cell membrane adheres to the

substrate on the leading edge and retracts the adhesion from the tailing edge. Finally, the cell body is then pulled forward by forces generated by myosin motor proteins sliding on the actin network [4].

Collective Migration

When a group, sheet, or chain of cells move together, there are additional forces due to cell-cell adhesion effects via adherens-junction proteins coupled to the cytoskeleton increasing complexity compared to single cell migration [5]. These junctions connect cells to their neighbors allowing for both chemical and mechanical communication and is attributed to why collective migration is observed to be faster and more efficient than cells migrating individually [6].

Measuring Migration

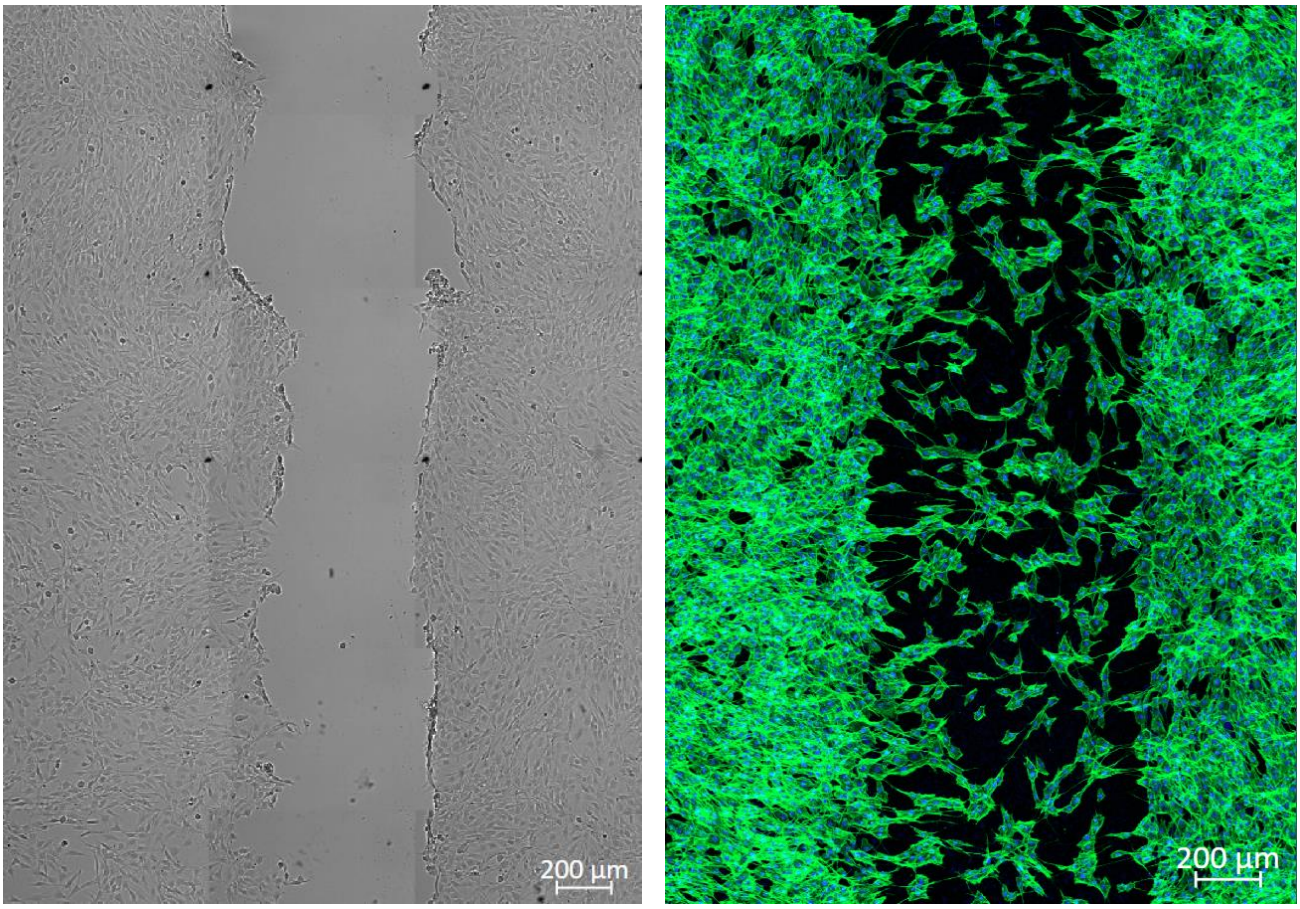
The scratch wound assay is a simple technique to quantify some standard collective cell migration parameters such as speed and persistence. In the scratch wound assay, a sheet of cells is scratched with a pipette tip causing the cells to move perpendicular to the wound to seal it. This begins the migration process from a defined starting point and allows the use of time-lapse imaging and image processing to capture speed and directional persistence data. While cell speed can be thought of simply by the distance traveled over time, persistence is defined as a ratio of start to end point distance compared to the total path length. Speed and persistence calculations are reported in equations 1 and 2 respectively where V is velocity, P is persistence, t is time, s is traversed path length, and d is the start to end point distance over time [7].

$$V = \frac{s}{t - t_0} \quad \text{Eq. 1}$$

$$P = \frac{d}{s}$$ Eq. 2

Preliminary Work

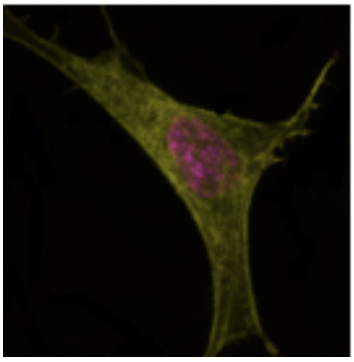
The bulk of preliminary work to date has focused on establishing the scratch wound assay that will be used to run the experiments. The first step of this process was determining the culturing time to reach confluence in the 8-well plates, observed to be between one and three days. Next, a repeatable method was needed for applying the scratch wound with edges as straight as possible. This was accomplished using a pipette tip to apply the scratch guided by a straight edge. The difference made with this technique can be seen in figure 1 below with a freehand scratch made on the left and a guided scratch on the right with much straighter edges.



(Figure 1: Freehand scratch (left) in brightfield view and guided scratch (right) with fixed cell staining)

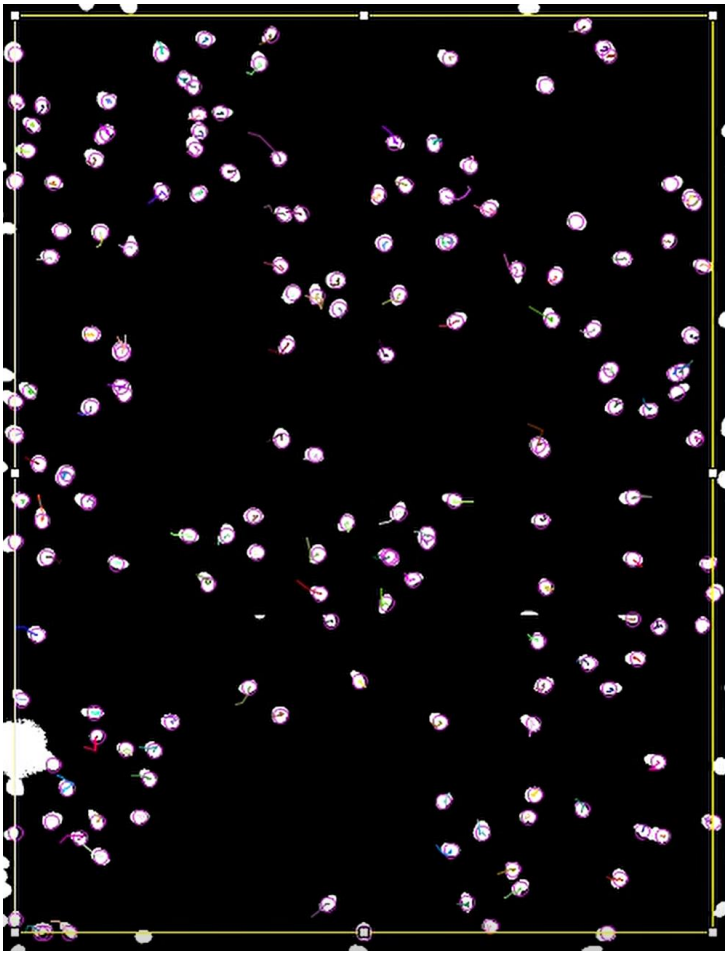
From preliminary studies, it has been determined that an optimal timeframe for migration imaging after scratch application is around 12 hours. Identifying the timeframe was time consuming as medium must be added to the wells throughout the imaging process to avoid having the cells dry out. This factor limits the feasible duration of the imaging process as someone must be present throughout it.

Work has also been done establishing cell staining methods for actin and the nucleus in both live and fixed cells. Staining of fixed cells, using DAPI and Phalloidin, allowed for observation of cell morphology and density in the wound more clearly at a given time point. Meanwhile, staining of live cells using NucBlue and Lifeact allows for easier tracking during the wound healing process. Different live and fixed stains are required due to live stains needing to be small enough to fit through pores in live cells whereas fixed cells may be permeabilized to apply the stains. Figure 2 below shows an example of the live staining where NucBlue is shown in purple and Lifeact is shown in yellow.



(Figure 2: NIH-3T3 cell live stained with NucBlue (purple) and Lifeact (yellow))

The most recent work has focused on processing collected data and quantifying baseline parameters for the study. This is being done using ImageJ software and a plugin known as TrackMate [8]. This plugin allows for automated cell tracking of cells with DAPI stained nuclei. Individual nuclei are recognized with the software and paths are generated connecting them between frames. From here, parameters such as cell speed and persistence can be obtained by applying known scaling of the images and the time intervals between them. A visualization of this is provided in figure 3 showing migration within the wound where cell nuclei are presented in white.



(Figure 3: TrackMate path visualization for cells migrating within the wound. Cell nuclei are shown in white with spots detected shown as purple rings. Paths are colorized to show distinction between each other.)

After locating spots and determining their paths, filters may be to ensure quality of data. One such example would be filtering out data for paths with cells not present in a majority of frames. An initial mean data set for migrating cells in the wound can be seen in table 1 below using such a filter over two hours of images yielding 958 tracks. Distribution charts of these parameters are also included in appendix A of this report as figures A1-4.

Table 1: Mean and (stdev) parameters for cells migrating within the wound of a single well

Track Displacement (micron)	Total Track Distance (micron)	Mean Speed (micron/sec)	Persistence
11.004 (6.699)	16.403 (7.023)	0.00238 (0.00099)	0.661 (0.225)

Approach

Specific Aim 1: Establish Scratch Wound Assay

To investigate effects of nuclear mechanical properties on cell migration, an established and validated test setup is required. The remainder of the fall 2021 semester will be used to complete this aim. The assay has been established as shown in the previous section with remaining tasks being validation and establishment of base parameters. Validation will be completed by repeating the experiment and treating cells with one or two migration blockers such as cyto-D to inhibit actin or Y27 to inhibit actomyosin contraction. Base parameters may then be established for control NIH-3T3 cells using the assay and image processing techniques that have been established.

Specific Aim 2: Modify Nuclear Mechanical Properties

Observing how modified nuclear mechanical properties affects migration will begin in the spring 2022 semester. Trichostatin A and GSK126 will be applied to the cells to alter the nucleus via HDAC inhibition and halting chromatin remodeling respectively. These cells will then be used in the scratch wound assay and have their migration parameters compared to that of the control NIH-3T3 cells.

Specific Aim 3: Generation of Monolayer Strain Map

Soon following completion of Aim 2, the scratch wound assay will be repeated for both control and modified NIH-3T3 cells. The live stains Nucblue and Lifeact will be used to visualize the nucleus and actin respectively during the healing process. This will allow for the development of a strain map at the cell monolayer level providing detailed mechanical insight into the cell migration process both in the wound and the surrounding areas.

Potential Complications

All the techniques I have established are robust. Still, a few pitfalls might arise, and the following risk mitigation strategies are proposed as an alternative approach. (1) In Aim 2, the dosage of Trichostatin A ad GSK 126 for this specific experiment might need to be optimized. Although Dr. Ghosh's lab already found the non-lethal degree of dosage of these drugs in NIH 3T3 cells, for a confluent monolayer that dosage may not work. In that case I will perform the experiment at lower dose. (2) in Aim 3, the strain mapping will require high resolution imaging. Because we can only image a few cells at a time in that resolution, the experiment may not be high throughput. In that case, we

may sacrifice the resolution to some degree so that we have a large enough number of datapoints for executing the statistical analysis.

Timeline

GANTT Chart

Table 2: Project GANTT Chart

	December	January	February	March	April
1 – Aim 1 Validation					
2 – Aim 1 Baseline					
3 – Aim 2 Data Collection & Analysis					
4 – Aim 3 Experiment Replication					
5 – Aim 3 Strain Mapping					
6 – E-days Preparation					

Aim 1 focuses on establishing a validated and well defined test setup necessary for Aims 2 and 3 that should be completed during the remainder of the fall 2021 semester. Data collection for Aim 2 will begin at the start of the spring 2022 semester while analysis may carry to February. This analysis may be conducted at the same time that experiments are being replicated for Aim 3. Development of the strain map for Aim 3 will then be the final step before preparations for E-days may begin.

References

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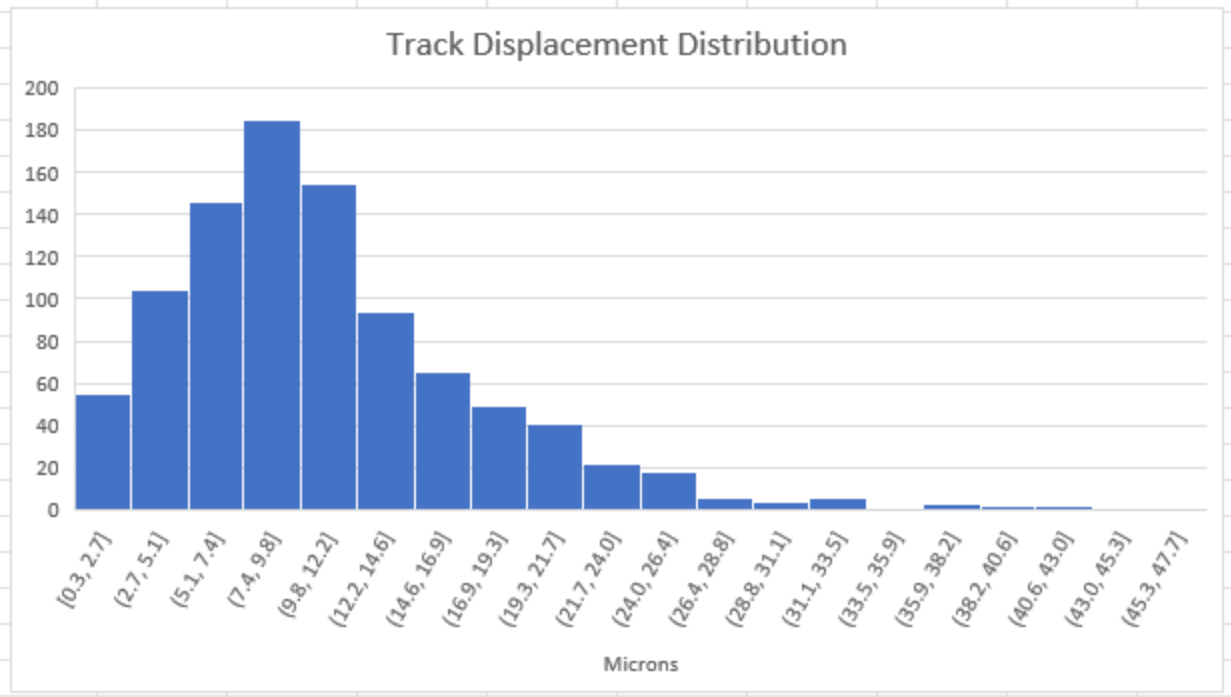
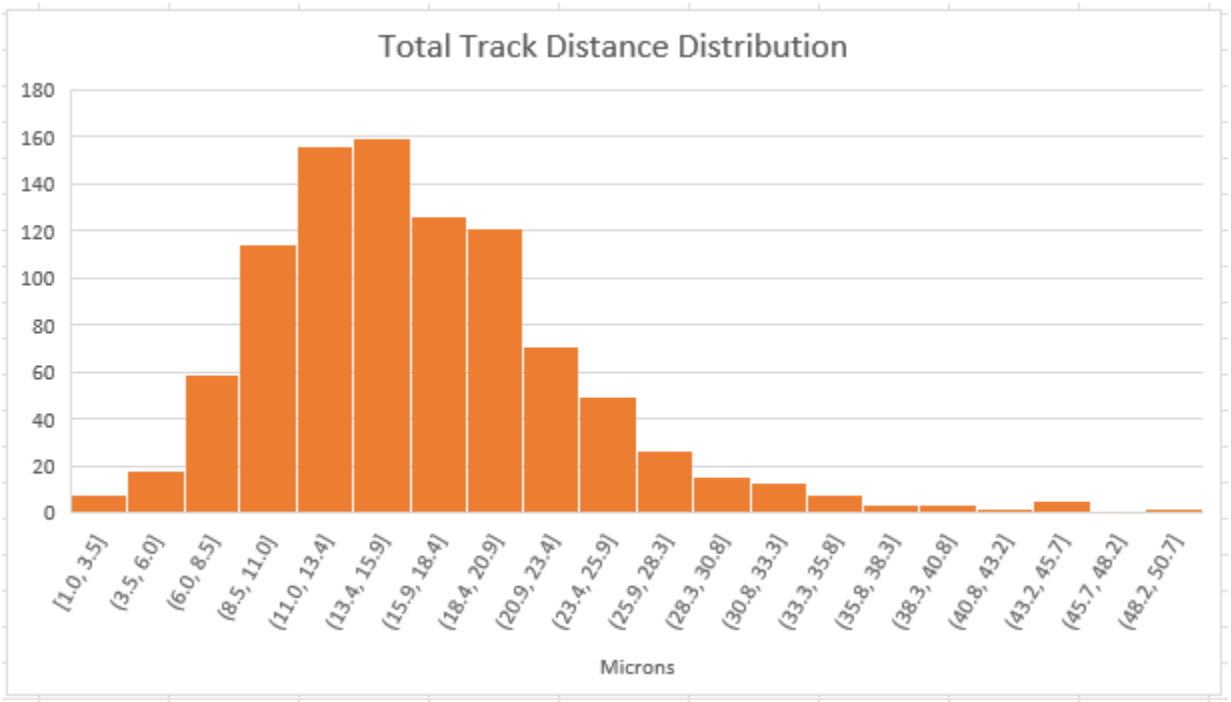
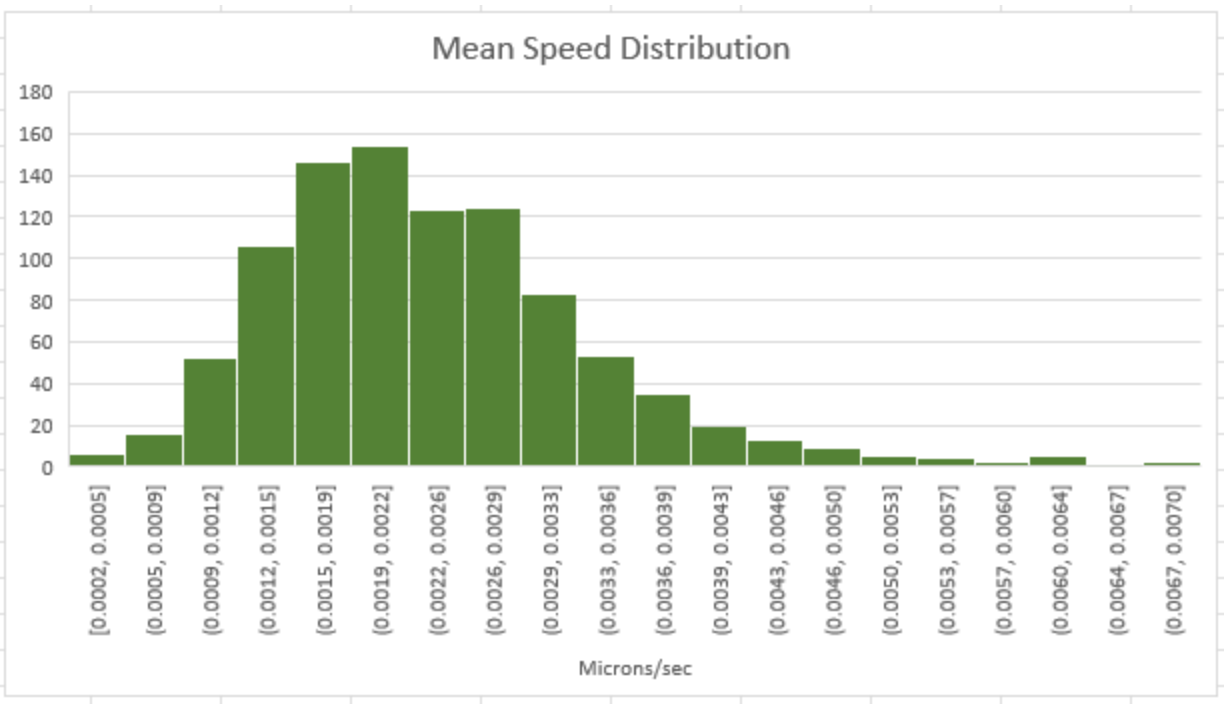
Appendix A



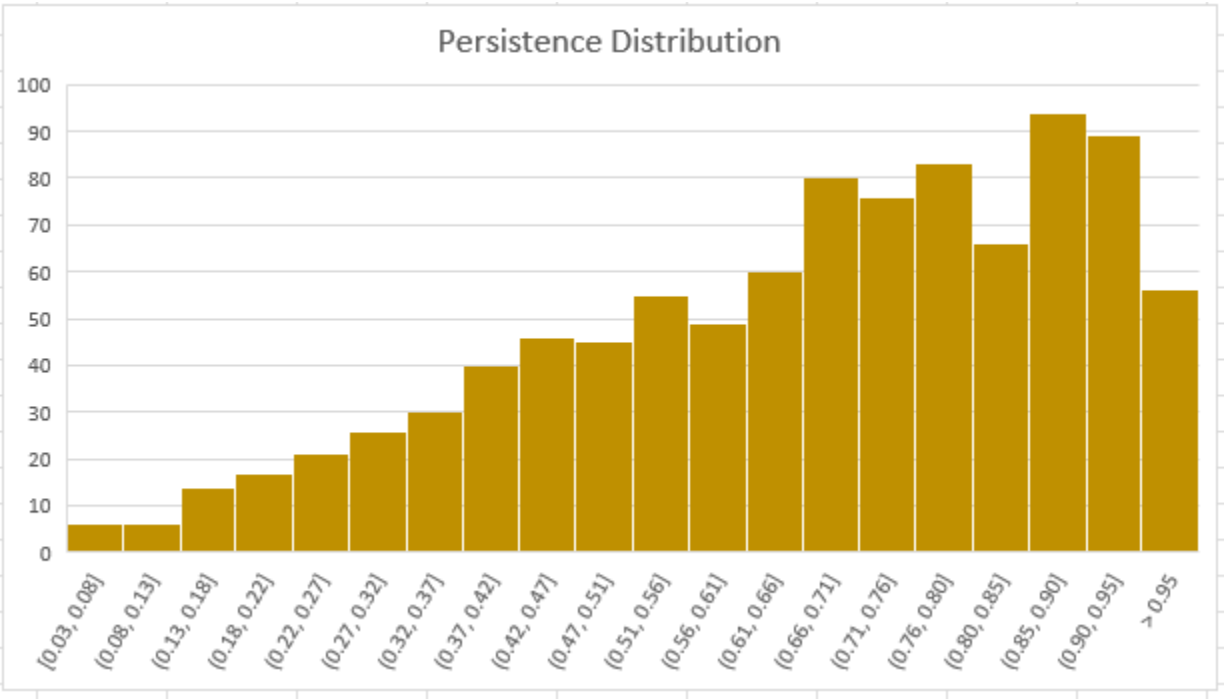
(Figure A1: Distribution of track displacements in microns)



(Figure A2: Distribution of total track distances in microns)



(Figure A3: Distribution of mean speeds in microns/second)



(Figure A4: Distribution of track persistences)

Appendix B

Introduction

There are various accreditation requirement experiences that students in their capstone experience must have in order for their degree to meet the bar for ABET accreditation. One of these is to design and conduct engineering experiments, as well as analyze and interpret data. Another is to design a system, component, or process to meet desired needs within realistic constraints. This research project meets these requirements as outlined below.

Design and Conduct Engineering Experiments

This research project involves designing an experiment to observe and quantify migration parameters of cells and understand the role of the cell nucleus in this process. This involves the establishment of a scratch wound assay model and validation of the experimental setup using selected migration blockers. Data will be acquired using time lapse microscopy which will then be analyzed using the image processing software ImageJ. Interpretations of the analyzed data will then come from comparing control and experimental groups as well as drawing conclusions from literature. The project involves finding a scientific question, creating a hypothesis based on rationale and designing experimental strategies to test the hypothesis.

Design of a System, Component, or Process

This research project requires the development of multiple processes or techniques to successfully achieve the specific aims. The first of which was to design a process of applying a scratch reliably with edges as straight as possible for the scratch

wound assay. The design of a more complex process arose from the challenge of processing collected images. This involved specifying a repeatable method for quantifying data including determining detectors, trackers, filters, and pre-processing steps needed. Developing the monolayer strain map will also involve designing another process to complete while executing Specific Aim 3 of this project.