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$E = mc^2$ High School Science Journal Submission

Personal Section

When I was in sixth grade, I first became aware of my family history of heart disease. My dad battling chronic hypertension, close relatives passing away from heart failure, and me knowing that I could be next in line inspired me to act: I was curious to learn exactly what caused heart disease and how doctors treated it. When I read that over 8 million people, nearly 6.5 million of whom live in developing nations, die of myocardial infarctions (MIs), heart failure, and cardiomyopathy each year, I was appalled. What surprised me more, however, was the fact that conventional treatments fail to remediate the lethal loss of cardiac function experienced by these patients, particularly those who suffer from post-MI cardiac scarring.

During my freshman year of high school, my curiosity to investigate better heart disease therapies drove me to take honors biology. I was especially intrigued by our discussion of the incredible healing potential of pluripotent stem cells. Eager to learn more, I decided to take a summer class in biotechnology at the University of Pennsylvania, through the Summer Academy of Applied Science and Technology. Through this program, I was introduced to the exciting world of cardiac tissue engineering: regenerating the scarred hearts of MI and heart failure patients using stem cells and therapeutic hydrogels, bolstering their cardiac capacities and giving them a second chance at life. I was committed to exploring this field, and became fascinated by the idea of engineering a biomaterial platform to support the differentiation of cardiomyocytes from patient-specific induced pluripotent stem cells (iPSCs). During my sophomore year, I was honored to meet Dr. Oscar Abilez, my mentor, and learn about his pioneering work in this field.

After completing a deep and comprehensive literature review of this field, I was eager to

make my own contributions to the goal of precision cardiovascular medicine. In the summer of 2016, through the SIMR Research Program at Stanford, I was excited to have the opportunity to work with Dr. Abilez and Dr. Huaxiao Yang, both researchers in the Stanford Cardiovascular Institute & Joseph Wu Lab. One of the foremost goals of my research was to devise a method for the rapid analysis of beating heart tissues that are engineered in the laboratory or procured in the clinic. Today, in the lab, researchers engineer patient-specific tissues for drug testing studies, to identify the best drug therapy for a specific patient's cardiac disease phenotype. I envisioned my algorithm as an easy-to-use, rapid mechanism by which researchers and doctors could analyze whether treated tissues exhibit healthy or diseased phenotypes, helping them make decisions on the best course of action for a patient. Furthermore, I envisioned my algorithm being used for cardiac diagnostics, to diagnose a patient's cardiac disease using either a biopsy or heart tissue engineered from the patient's own stem cells. I aspired to make my algorithm robust, automated, and non-invasive, requiring only a video of the tissue sample to produce meaningful and accurate results over multiple time points. Overall, I hoped to present my algorithm as a cost-effective and accurate alternative to current diagnostic systems that require specialized equipment, sample sacrifice, and other costly hassles that render them inaccessible for clinics in developing nations.

As I began to code my algorithm, I found myself exploring the exciting interface between biology and vector calculus. I realized that, by thinking of a tissue contracting as a point being displaced in the cartesian coordinate system, I could make the problem of identifying contractile force magnitude and direction much simpler. Thinking about displacement immediately called to mind vectors, which I had just learned about in my junior year multivariable calculus class. As I reviewed the literature for previous attempts at modeling tissue contractions using vectors, I encountered a paper from UCSF that employed vector fields to represent tissue displacement. I

immediately downloaded their source code and started playing with it[1]. Recalling the various vector field operations from multivariable calculus, I began to apply some of them to my field to see where they would take me. Calculating the flux of the field across different surfaces around the tissue's center of beating gave me some interesting results, prompting me to dig deeper. It was then I realized that field divergence, which I could compute from flux using the divergence theorem, could serve as a highly useful indicator of contractile motion: it could tell me relatively how forcefully the tissue was contracting/relaxing about its center. As I computed and graphed the divergence values, I was struck by how closely they matched the tissue's contractile patterns. It was this moment that the mathematics truly came alive for me: beyond being mere equations on a piece of paper, these incredible relationships truly governed everything in the natural world, from the smallest cellular behaviors to the most powerful and intriguing galactic phenomena.

To other high schoolers who want to undertake a research project bridging science and mathematics, my biggest piece of advice would be to not be afraid to think audaciously and try out daring new ideas. You do not need to be an expert in order to make a breakthrough—even something that you learn in your high school math class, if applied in the right way, can have the potential to impact real scientific research. For me, it began by playing around with some simple vector calculus concepts, not knowing what I would find in the end or even if these concepts would apply at all to the problem of tissue contractility evaluation. The pure joy and excitement of making an unexpected discovery by putting together simple concepts is unmatched and incredible. Overall, with the right mindset, you have the ability to make a substantial impact.

Research Section

In my tissue contractility research study, I sought to develop a robust computational tool to rapidly and non-invasively evaluate the phenotypes of cardiac tissue samples from patients

and laboratory drug testing studies. To begin, I worked with Dr. Yang to engineer 3 different cardiac tissues from stem cells, each tissue differentiated using a different concentration of the molecule CHIR—3 μM, 4 μM, and 5 μM. These tissues served as my test samples, as I wanted my algorithm to identify whether the tissues exhibited any difference in phenotype due to their CHIR concentration. Once I had beating samples, I took bright field videos of all 3 tissues using

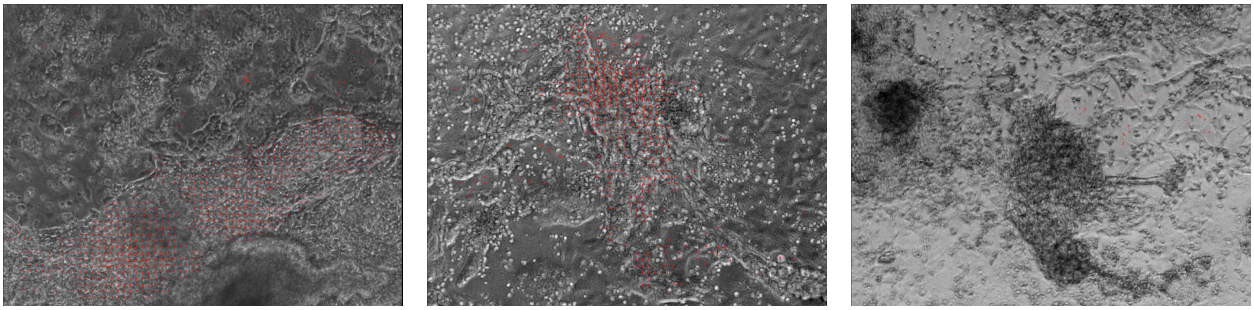

Figure 1: "Beating Videos of Tissue Patches with Overlaid Vectors." **Fig. 1a** (left) comes from the CHIR(3μM) sample, **Fig. 1b** (center) from the CHIR(4μM) sample, **Fig. 1c** (right) from CHIR(5μM).

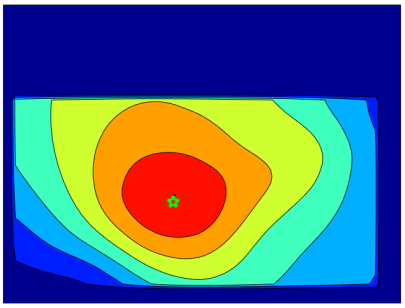

Figure 2: "Heatmap generated by the Matlab algorithm in finding the COB (green marker) for the CHIR(3) tissue patch."

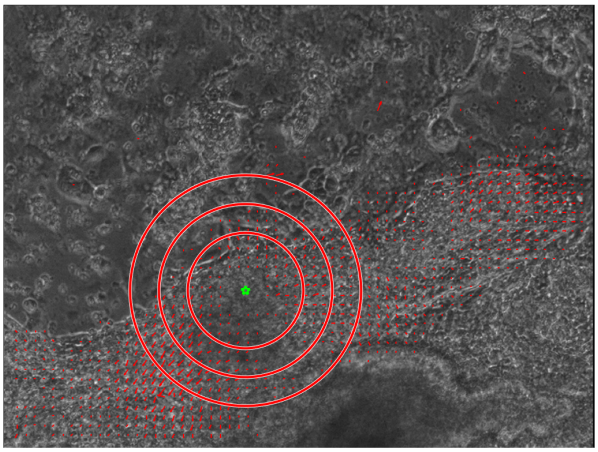

Figure 3: "Vector-overlaid image frame of the CHIR(3) tissue patch." Vectors emanate out from the COB (green marker), crossing the plotted flux circles (100, 150, 200 pixel radii centered at COB).

a standard microscope and imported the videos into Matlab. I first used the UCSF algorithm to overlay contraction vector fields atop my beating videos, to represent tissue displacement over time (Fig. 1)[1]. Using these vector fields, the centers of beating (COBs) for each tissue construct were calculated; Figure 2 presents the heatmap-based COB acquisition process for the CHIR(3) sample. The hotter layers on the heatmap delineate areas on the tissue patch with a higher chance of containing the COB; thus, the algorithm used gradient descent to identify the COB's true

location. Figure 3 shows one frame from the vector-overlaid video of the CHIR(3) sample with the calculated COB incorporated as a marker. As shown in the image, contraction vectors seem to clearly emanate from the COB, indicating the accuracy of the COB identification algorithm.

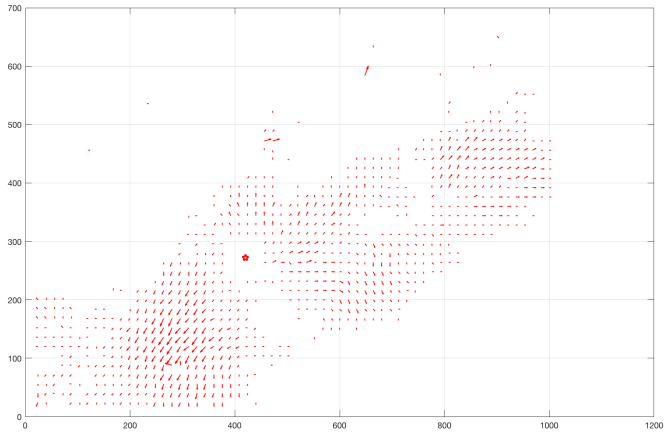

Figure 4: "Isolated CVF from the CHIR(3) video frame shown in Fig. 3." Red marker indicates COB. At this time point, the vectors clearly emanate outwards from the COB, characteristic of the relaxation phase of tissue contraction.

Using Matlab, I isolated the contraction vector field (CVF) for CHIR(3) and plotted the field along with the COB (Figure 4). Subsequently, I plotted 3 "flux circles" (Figure 3) of radii 100, 150, and 200 pixels centered around the COB; these were the surfaces through which I could calculate the flux of the contraction vector field about the COB. In order to accomplish this, I computed the components of each vector perpendicular to the flux circles. Then, because flux is a property of vector fields and surfaces in 3-dimensions and not 2-dimensions, I extended my flux circles to become "flux cylinders" in 3-space, having a height of 1 pixel (to simplify calculations). These flux cylinders can be seen in Figure 5: the top and bottom faces of the cylinder are not shown because they play

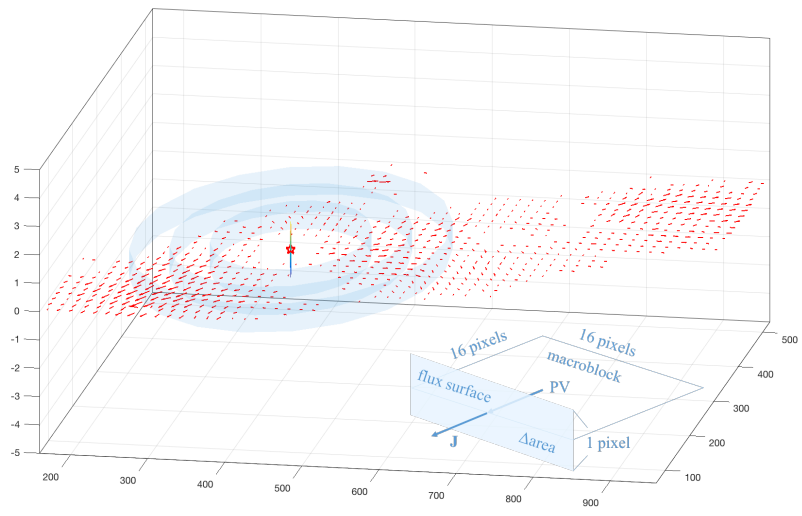

Figure 5: "3-dimensional representation of the vector field from the frame shown in Fig. 3." The vectors, which lie on the x-y plane, are punctuated by the 3 cylindrical surfaces through which the flux of the CVF can be calculated. While the tops of the cylindrical surfaces are not shown, they are all considered closed surfaces for the purposes of flux calculation. The bottom-right schematic shows the dimensions of Δarea, 16 pixels^2, and enables visualization of one flux calculation (PV*Δarea).

no part in accumulating flux (since the contraction vector field is only on the x-y plane). Flux calculations were performed using the equation **J** = Σ(PV*Δarea), total flux **J** equaling the sum of the fluxes from all perpendicular vector components PV across Δarea of 16 pixels squared. This was a novel mathematical model to calculate the flux of the contraction vector field across circular surfaces situated at various radii about the COB, for all 3 CHIR concentration samples.

Once I computed the flux, the divergence (div) of the contraction vector field at a specific radius about the COB was easily retrieved using the divergence theorem, which in my case was:

$$\boldsymbol{J}\,(flux) = \iiint div(\boldsymbol{F})\,dV = \int_{0\,|\,0\,|\,-0.5}^{2\pi\,|\,radius\,|\,0.5} div(\boldsymbol{F})\,rdzdrd\theta \qquad \boldsymbol{F} = contraction\,vector\,field\,(CVF)$$

Bounding and evaluating the right-hand triple integral with the parameters that define the cylindrical flux surfaces (radii 100-200 pixels), it can be concluded that **J** = div*π(radius)2. I discovered that the divergence can serve as a powerful tool for modeling tissue contractility over time: when the tissue contracts, the divergence of the contraction vector field will be negative, indicating that the COB serves as a sink for the field. When the tissue then relaxes, the COB serves as a source for the field, and the divergence is positive. I also found that the magnitude of the divergence during contraction and relaxation, along with the rate of change of the divergence during a single beat, can reveal insights into tissue contractility. Using these divergence values, I was able to identify the CHIR(3) tissue sample as having longer, more powerful beats, while the CHIR(4) tissue demonstrated shorter, less forceful contractions[2]. This disparity could indicate that each tissue might be better suited to support a different region of the adult heart[2]. Overall, this algorithm could pave the way towards facile, automated, non-invasive analysis of engineered cardiac tissues *in vitro*, obviating the need to perform expensive procedures or sacrifice cultures. Feel free to contact me at arvindsridhar2010@gmail.com if you are interested in learning more!

References

[1] Huebsch, Nathaniel et al. “Automated Video-Based Analysis of Contractility and Calcium Flux in Human-Induced Pluripotent Stem Cell-Derived Cardiomyocytes Cultured over Different Spatial Scales.” *Tissue Engineering Part C* 21.5 (May 2015). Web. 2 Jun. 2016.

[2] Bootman, Martin D. et al. “Calcium signaling during excitation-contraction coupling in mammalian atrial myocytes.” *Journal of Cell Science* 119 (Aug. 2006). Web. 6 Oct. 2016.