ND*nano* Undergraduate Research Fellowship (NURF)
2014 Project Summary

1. Student name: Steve Penny

2. Faculty mentor name: Professor Greg Timp and Professor Tetsuya Tanaka

3. Project title: Artificial Tissue Formation Using Laser-Based Optical Tweezers

4. Briefly describe any new skills you acquired during your summer research:

- Operating and calibrating optical tweezers
- Forming and using photopolymerizable hydrogels to fix cells
- Using microfluidic devices to pump endothelial and blood cells through a desired region
- Creating an artificial tissue on a cell by cell and layer by layer basis
- Performing live/dead stains to assess viability after certain experimentation on cells
- Using Fiji Image software to track red blood cell velocity through artificial capillaries and to use mathematical processes to extract data from images.
- Using Imaris 3D Imaging software to render capillaries and find cross sectional areas
- Seeding, culturing/sub-culturing, and trypsinizing several types of cell lines

5. Briefly share a practical application/end use of your research:

The goal of this project is to create an artificial tissue that can mimic in vivo human capillaries. Once completed, these tissues can serve a variety of purposes for medical advancements. One effective application of our capillaries would be seen in metastasis studies. Instead of pumping solely blood through the tissues, cancer cells would be added as well. If our capillaries behave similar to in vivo tissue, we should observe the cancer cells burrowing into the endothelial wall, through pericytes and fibroblasts and exit to the surrounding. Quantifiable data such as average metastasis time and percentage of cells that metastasize could be measured and used for diagnostic tools. In a similar manner, our capillaries could be used to study drug delivery through the blood stream and even diseases such as systemic capillary leak syndrome (SCLS) without having to do in vivo testing.

Begin two-paragraph project summary here (~ one type-written page) to describe problem and project goal and your activities / results:

The goal of this synthetic capillary project is to create an in vitro environment using “live cell lithography” that mimics true capillary tissue in humans. To achieve this, cells were inserted into the microfluidic in a mixture consisting of photopolymerizable liquid hydrogel and PBS. Optical tweezers were then used to capture flowing cells and transport them to a desired x, y, and z position. In order to fix the cells in place, UV light was used to activate the hydrogel and semi-encapsulate cells in either PEGDA or GelMA hydrogels. These now solid gels mimic the

structural support and porosity of the extra-cellular matrix. In order to analyze whether or not these capillaries were similar to in vivo tissues, blood flow and oxygen gradient data was recorded.

This ND NURF project was focused on analyzing the blood flow portion of this analysis. Using image sequenced data of blood flow from a confocal microscope, each frame was subtracted from the previous (in Fiji Image Processing Software) to generate a video of solely changing pixels over a certain period of time. After this operation, only the outline of the flowing red blood cells and some noise, which was eliminated using a Fast Fourier Transformation filter, remained in the image sequence. Using Fiji's MTrackJ plugin, each blood cell was tracked throughout the capillary by clicking the center of the cell in each consecutive frame. The tool then returns the x and y coordinates, time and velocity between each frame for each track point. The velocities of each cell were averaged and a standard deviation was found based on their x-position in the capillary at 1 micrometer intervals.

Fluorescent nanospheres were also pumped through the capillary to line the cell walls for cross-sectional area measurements. After taking a series of z-height images on a confocal microscope, the capillary was 3D rendered using Imaris Software and then sliced at 1 micrometer intervals in the y-z plane. Since these synthetic capillary lumens are not perfectly circular, diameter was not a good estimate for its area. Fiji was used again to trace the sections of highest fluorescence and measured for area in micrometers^2. These areas and velocities were matched by x-position and then plotted for several capillaries at a similar pressure differential across the capillary.

Another side project that was tackled during this program was increasing the viability of our capillaries. In vivo capillaries are 100% viable, so in order to properly mimic true human tissue one of the goals for the group was to optimize our procedure to get as close to perfect viability as possible. This involved using different sources of UV light for gelling (lamp and laser), different wavelengths of infrared laser light for the tweezing process, and time spent under the tweezers. Several experiments were run at different combinations of values, in an attempt to find an optimal scenario, but 100% viability still has not been achieved. More data will continue to be taken on these processes in order to gain that goal of perfection.

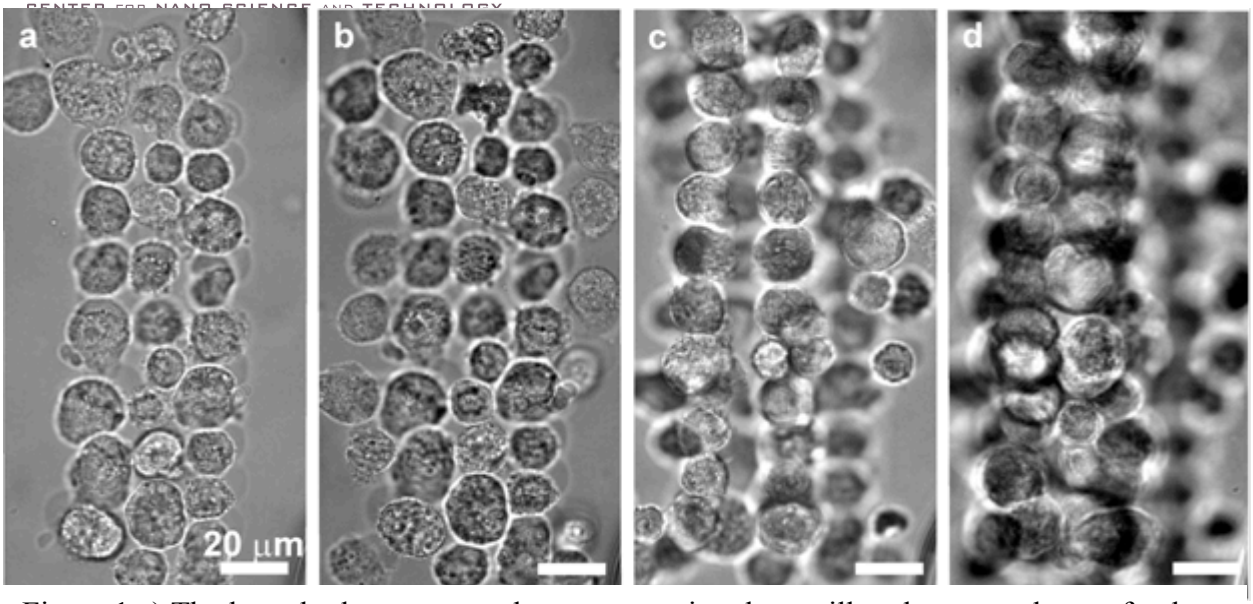

Figure 1 a) The layer by layer approach to constructing the capillary basement layer of only endothelial cells. b) The pericytes and fibroblasts have been added to surround capillary and regulate lumen area under the heavy shear stress by the blood on capillary walls. c) Second layer has been added and lumen is now visible. d) Third layer has been added closing off lumen.

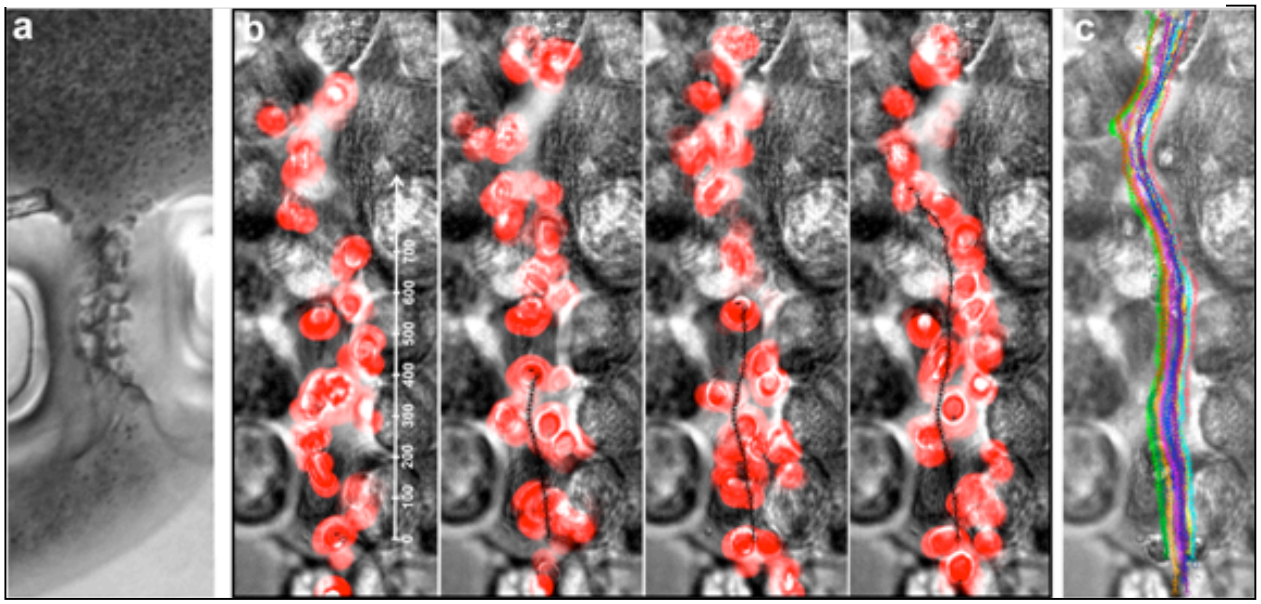

Figure 2 a) Blood being pumped through a capillary. b) Still shots of blood traveling through the capillary lumen and being tracked with Fiji Image Processing Software (artificially colored for clarity). c) Summary of a series of blood cells traveling through lumen.

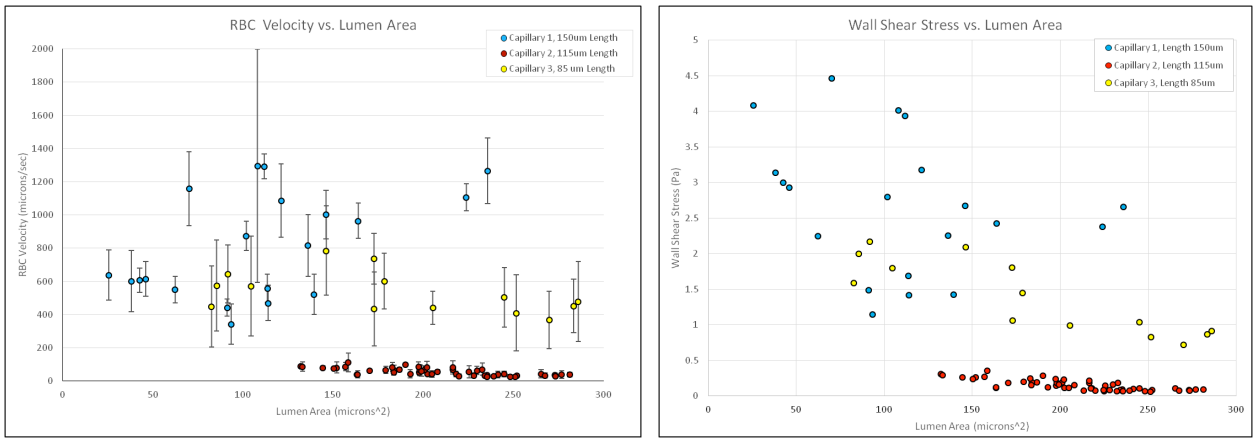

Figure 3. Plots of the velocities and wall shear stress as a function of area in the capillary. The pressure differential across all capillaries were 1 ± .5 mbar. Capillary 1 and 2 were pumped with 10% blood, where as Capillary 3 was pumped with 6x concentrated blood. The higher density resulted in a thicker fluid with lower velocity and stress on average.