ND*nano* Undergraduate Research Fellowship (NURF)
2012 Project Summary

1) **Student name:** Michael Hunckler
2) **Faculty mentor name:** Dr. Marya Lieberman
3) **Project title:** PAD Fabrication and Image Analysis

4) **Briefly describe any new skills you acquired during your summer research:**
I have greatly increased my knowledge of programming in various languages. Coming into the summer, I had only learned MatLAB. Due to some of the capabilities needed in the image analysis portion of the project, I had to work with programming of various tasks done in in C++, Python, and Java. I have learned to write both macros and plugins in ImageJ as well. I also worked with Adobe Illustrator to design many different versions of the PAD. The entire fabrication process of printing and spotting hundreds of PADs allowed me to experience a small part of manufacturing processes that occur with anything that is produced on a large scale. I also learned how to use and program the tasks for the Biomek liquid handling machine to spot the PADs.

5) **Please briefly share a practical application/end use of your research:**
The practical applications of this research involve creating a low-cost and dependable verification of the components of a drug. Many countries are experiencing a substantial prevalence of counterfeit and low quality pharmaceuticals. PADs use colorimetric analysis of multiple chemical reactions to detect active pharmaceutical ingredients as well as binders and fillers in pharmaceutical tablets. My portion of the research involved image analysis of the PADs to give a dependable identification of colors and inform the user of validity of the drug.

Project summary:

In countries all over the world, especially many developing countries, low quality and counterfeit pharmaceuticals have prompted a need for low-cost detection methods. Paper analytical devices (PADs) are one possible solution to this problem. PADs use colorimetric analysis of multiple chemical reactions to detect active pharmaceutical ingredients as well as binders and fillers in pharmaceutical tablets. A dependable analysis method of the colors produced will provide the user with information of the components in the drug that is being tested. The first part of my project involved designing a PAD that could accommodate more chemical tests. The second portion focused on designing an image analysis software to identify the colors produced on the PADs.

PADs with seven lanes (Figure 1.A) for reactions were being used, but more lanes were needed to accommodate additional chemical tests. I developed a 12-lane PAD (Figure 1.B) and designed a specific program for a liquid handling robot so it could spot the test reagents into the lanes.

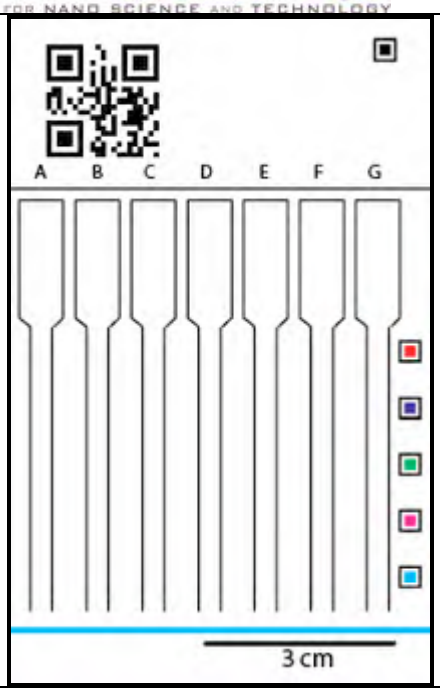


Figure 1.A 7-lane PAD

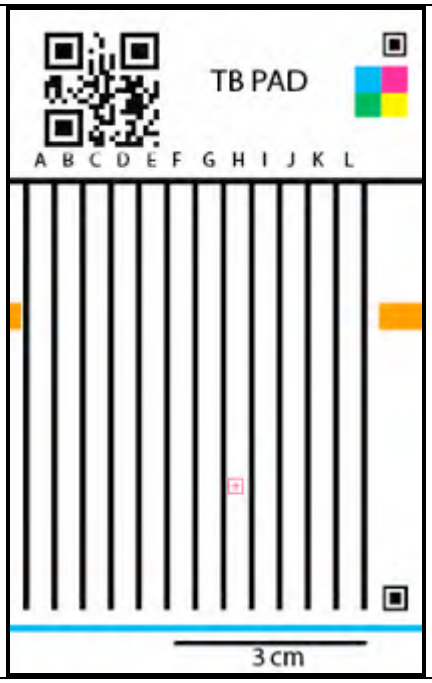


Figure 1.B 12-lane PAD

4 µl chemical spots were found to need a minimum length of 18 mm between the drop centers in order to keep the reagents separated on the lanes. The fabrication procedure (PAD creation and spotting) yielded hundreds of reliable PADs for field tests and lab use. I have been using ImageJ (Java-based image processing program) to perform three separate tasks to analyze the PAD: orienting the PAD into a correct alignment, selecting the regions of interest, and analyzing the colors in the regions. Figure 2 is an example from a plugin that I created that uses projective mapping to align an imperfect image to a correctly oriented image.

Figure 2.A Imperfect image of PAD

Figure 2.B Transformed and aligned image of PAD from Figure 2.A

I have created an ImageJ macro that extracts the RGB values along a single line and plots the ratios between those three values (Figure 3). This allows for patterns among the different colors to become apparent. A program that generates a histogram of RGB values in a selected region has also been created to analyze the entire selected region in a lane.

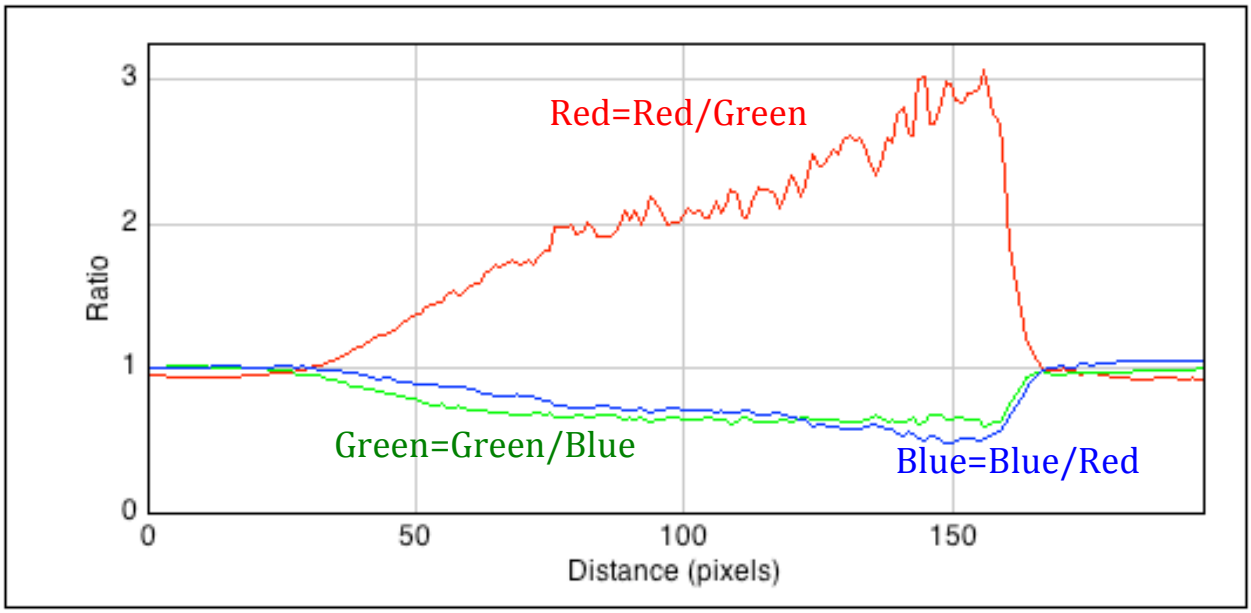
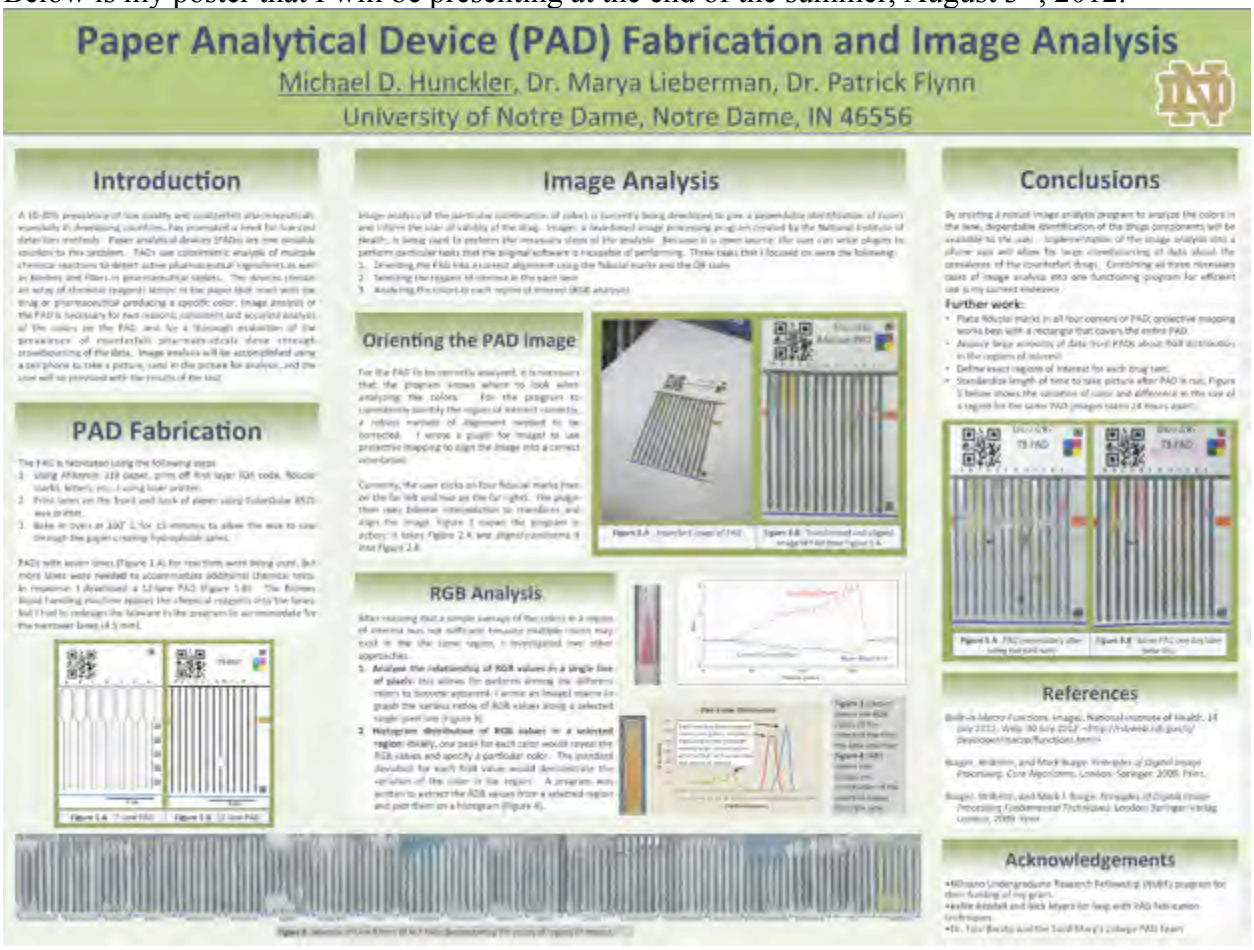


Figure 3. RGB Ratios along a single line of pixels

By creating a robust image analysis program to analyze the colors in the lane, dependable identification of the drugs components will be available to the user. Implementation of the image analysis into a phone app will allow for large crowdsourcing of data about the prevalence of the counterfeit drugs. Combining all three necessary tasks of image analysis into one functioning program for efficient use is my current endeavor.

Publications (papers/posters/presentations):

Below is my poster that I will be presenting at the end of the summer, August 3rd, 2012.



The design of the 12-lane PAD (Figure 1.B) has been used in a current patent application.