

NDnano 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 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.

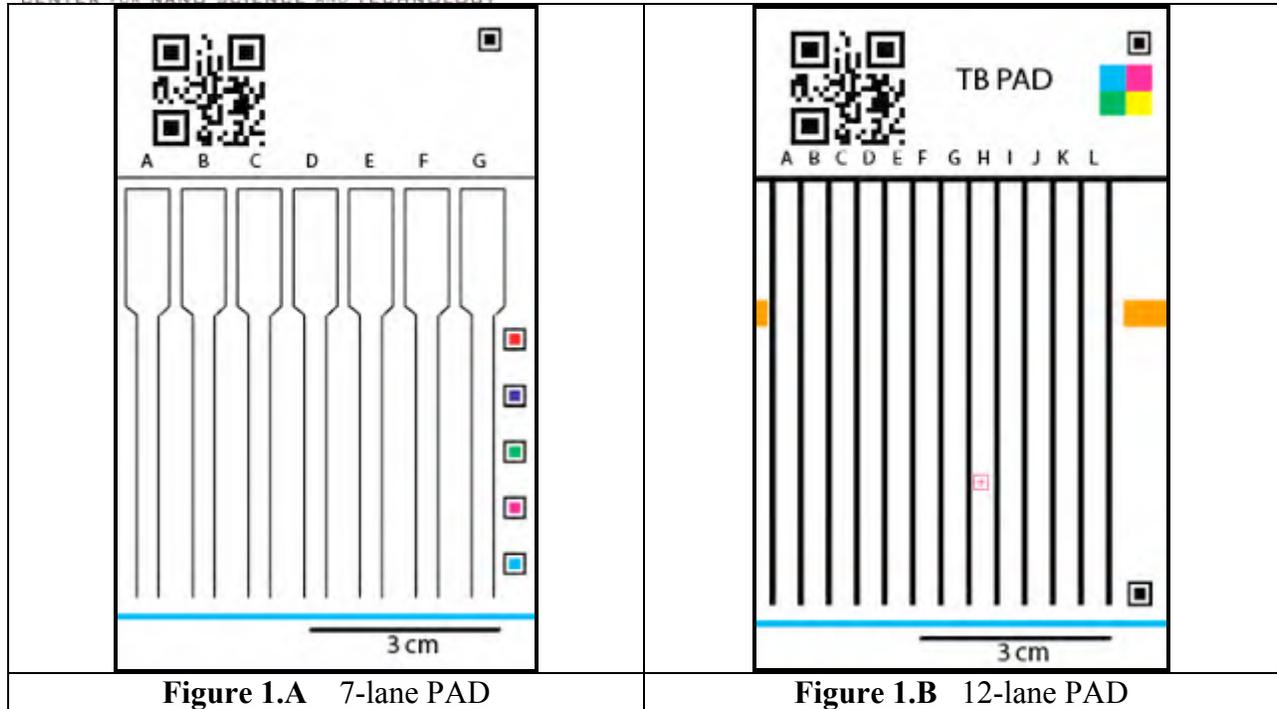
5) **Please briefly share a practical application/end use of your research:**

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.

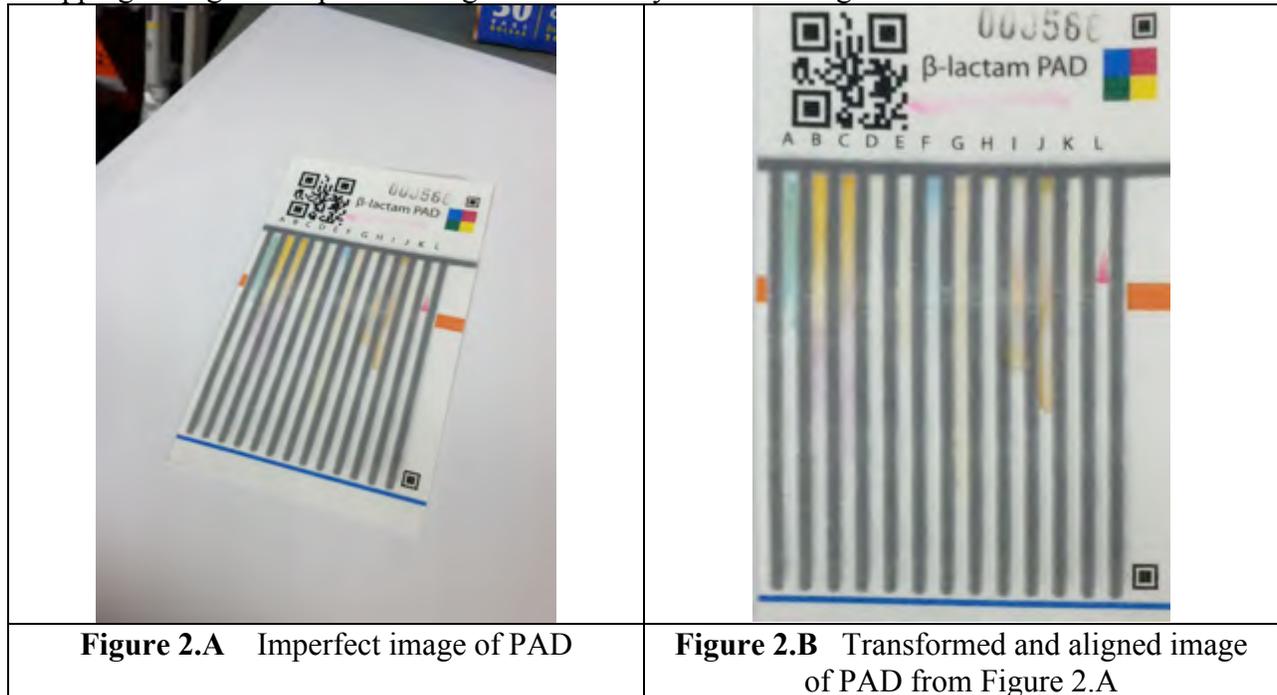
Project summary:

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.



4 μ l 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.



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.

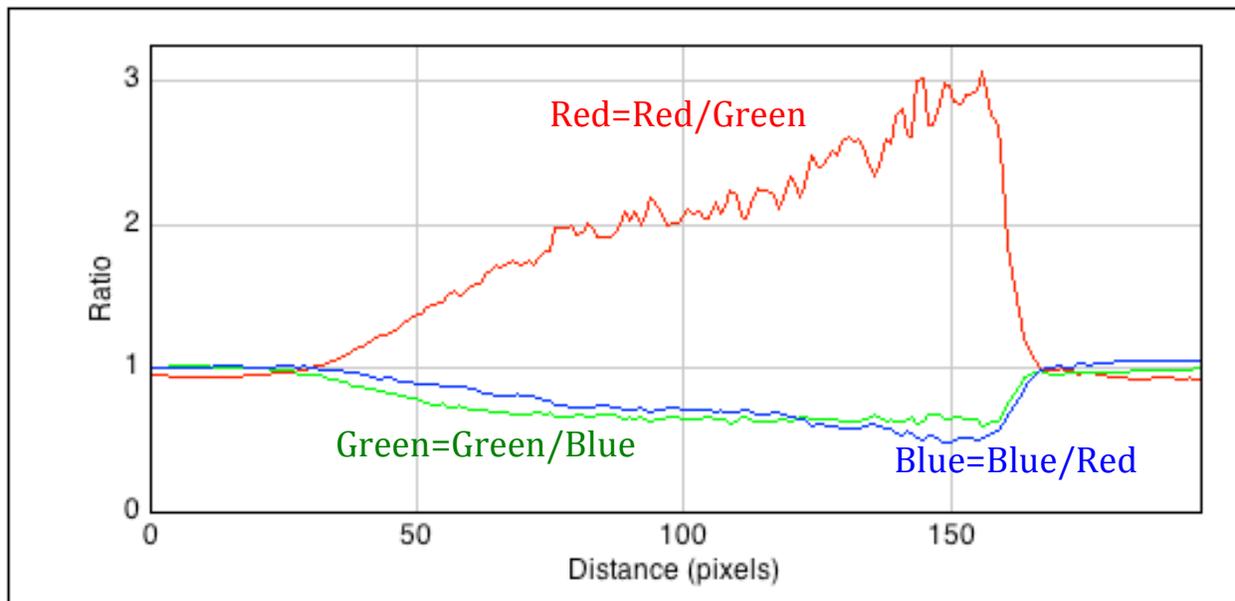


Figure 3. RGB Ratios along a single line of pixels

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.

Paper Analytical Device (PAD) Fabrication and Image Analysis

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Introduction

A 15-20% improvement in low quality and counterfeit pharmaceuticals, especially in developing countries, has 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 seen by Kelley and others in pharmaceutical papers. The device consists of a strip of absorbent (paper) strips in the paper that react with the drug or precursor producing a specific color. Image analysis of the PAD is necessary for fast, accurate, automatic and accurate analysis of the color on the PAD, and for a thorough evaluation of the accuracy of counterfeit pharmaceuticals seen through cross-bordering of the data. Image analysis will be accomplished using a pipeline to take a picture, send to the picture for analysis, and the user will be provided with the results of the test.

Image Analysis

Image analysis of the absorbent components of color is currently being developed to give a probabilistic identification of quality and inform the user of validity of the drug. Images are analyzed using processing programs created by the National Institute of Health, is being used to perform the necessary steps of the analysis. Because it is open source, the user can write plugins to perform particular tasks that the original software is incapable of performing. These tasks that I focused on were the following:

1. Orienting the PAD into a vertical alignment using the fiducial marks and the QR code.
2. Selecting the regions of interest in the strip test.
3. Analyzing the colors of each region of interest (ROI) analysis.

Conclusions

By writing a custom image analysis program to analyze the colors in the test, separable identification of the drug components will be available to the user. Implementation of the image analysis into a software will allow for large cross-bordering of data about the accuracy of the counterfeit drugs. Combining all three necessary parts of image analysis into one functioning program for efficient use is my current endeavor.

Further work:

- Place fiducial marks in all four corners of PAD; precise mapping works best with a rectangle that covers the entire PAD.
- Analyze large amounts of data from PADs about R&B distribution in the regions of interest.
- Define exact regions of interest for each drug test.
- Standardize length of time to take picture after PAD is run; Figure 1 below shows the variation of color and difference in the size of a region for the same PAD (image taken 24 hours apart).

Orienting the PAD Image

For the PAD to be correctly analyzed, it is necessary that the program knows where to look when analyzing the colors. For the program to consistently identify the regions of interest, a robust method of alignment needed to be corrected. I wrote a plugin for ImageJ to use perspective mapping to align the image into a correct orientation.

Currently, the user clicks on four fiducial marks from the far left and near on the far right. The program may perform interpolation to standardize and align the image; Figure 1 shows the program in action. It takes Figure 2.A and aligns/perspective it into Figure 2.B.




Figure 2.A: Unaligned image of PAD. Figure 2.B: Transformed and aligned image for test Figure 2.A.

RGB Analysis

After reducing each a sample image of the color in a region of interest was not sufficient because multiple colors may exist in the same region, I investigated two other approaches:

1. Analyze the relationship of RGB values in a single line of pixels; this allows for uniform across the different colors to become apparent. I wrote an ImageJ macro to graph the various ratios of RGB values along a selected region (see line in figure 3).
2. Histogram distribution of RGB values in a selected region; similarly, one peak for each color would reveal the relation of the color in the region. A program was written to extract the RGB values from a selected region and put them on a histogram (figure 4).

PAD Fabrication

The PAD is fabricated using the following steps:

1. Using software, 2D codes, print off first layer (QR code, fiducial marks, letters, etc.) using laser printer.
2. Print lines on the back and back of absorbent substrate (MSI) was chosen.
3. Make in steps at 100°C for 15 minutes to allow the wax to cure through the paper using hydrophobic wax.

PADs with seven lines (Figure 1.A) for reactions were being used, but more lines were needed to accommodate additional chemical tests. In response, I developed a 12-lane PAD (Figure 1.B). The bottom board featuring (plastic) paper (the chemical reagents) only the lines had I had to redesign the software in the program to accommodate for the narrower lanes (4.5 mm).




Figure 1.A: 7-lane PAD. Figure 1.B: 12-lane PAD.

References

Bullis-Meyer-Function, ImageJ, National Institute of Health, 14 July 2012. <http://rsbweb.nih.gov/ij/developer/macro/function.html>

ImageJ, William, and Mark Suggs. *Introduction to Digital Image Processing: Core Algorithms*. London: Springer, 2000. Print.

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Acknowledgements

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• Eddie Kozubal and Nick Myers for help with PAD fabrication and images.

• Dr. Tom Flavin and the Staff for the college PAD team.



Figure 3: Selected 12-lane image of 12-lane PAD demonstrating the usage of region of interest (ROI)

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