Boston University
Electrical & Computer Engineering
EC463 Capstone Senior Design Project

Problem Definition and Requirements Review

Calibration Device for Microarray Slides

Submitted to

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Executive Summary

Fluorescence based testing in microarrays is a viable and efficient method of detecting target proteins, allergens, or diseases with only microliters of sample. Currently, cost-effective fluorescence testing can give no more than a qualitative diagnosis: yes, the target exists, or no, it does not. In most experiments, the probe density of the microarray spots is assumed, and these assumptions are not always accurate. Without knowing the probe density, the efficiency of target-probe binding cannot be quantified from fluorescence measurements alone. The task of this project is to build a microarray calibration system, which includes assembling an Interferometric Reflectance Imaging Sensor (IRIS), fabricating compatible microarray slides, and composing robust software for automated data acquisition and analysis of the microarrays. The development of this system will enable laboratories and clinics to test samples quickly for rapid diagnoses, as well as accelerate the pace of diagnostics research.

1 Introduction

Microarrays allow for multiple simultaneous interactions with only microliters of sample solutions. A microarray is a collection of biological substances adhered to a solid surface (See Figure 1.1). In research laboratories, as well as clinical settings, microarrays are used for fast, high throughput testing, a result of the ability to pattern numerous samples on a single slide [1].

For target detection in experimentation and diagnostics, fluorescence based microarray tests are often used. Fluorescence is a 3-step process, typically called a sandwich assay. Capture probes are attached to the surface. Next, the target molecules are first bound to the capture probes. Finally, secondary probes tagged with fluorophores are introduced to bind to the target molecules. Once the binding process completes, the microarray is placed under incident light, and the tagged targets fluoresce. The fluorescence intensity can then be measured and used to make diagnoses or analyses.

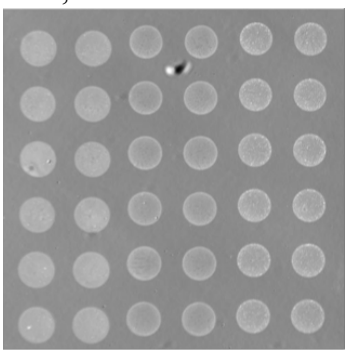


Figure 1.1. *Top view of a microarray*. The lighter spots contain the biological substance to be tested. [1]

Currently, there is one major debilitating factor to florescence testing accuracy. When spotting the slide with the probe solution, the density is assumed to within a particular constant volume from spot-to-spot and chip-to-chip. Optimum spotting conditions are found by what gives the maximum fluorescence signal. Assuming the density of the immobilized probe on the slide can provide an unreliable analysis, because the fluorescence is dependent on the amount of probe. For example: in detecting a solution with 50 targets, if the sensor has 100 probes and all 50 targets are captured, 50% of the total possible signal will be observed. However, if it is assumed that the sensor has 100 probes but actually has only 20, the probes would saturate and only 20% of the total possible signal will be observed. Measuring the probes can prevent this misconception. When probe density is assumed, it

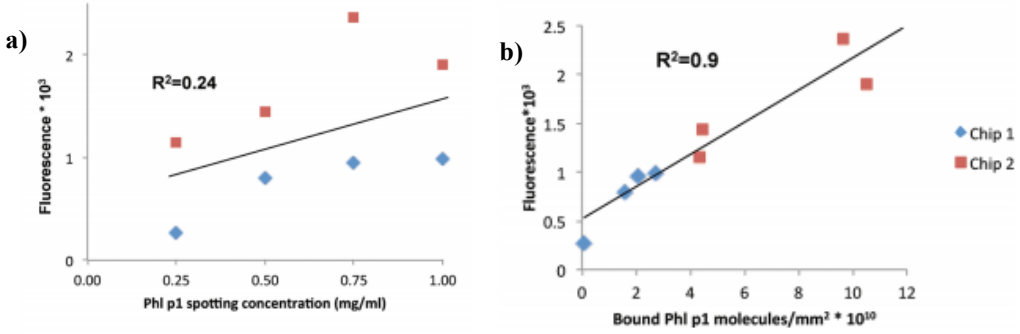


Figure 1.2. *Fluorescence vs. Spotting Concentration*. (a) Subtle correlation between fluorescence measurement and probe concentration when the probe density is assumed. (b) Clear correlation between fluorescence measurement and probe concentration with the probe density is measured [2].

is difficult to see the relationship of probe to fluorescence (Figure 1.2.a). However, as seen in Figure 1.2.b, if the probe density is measured, a clear correlation exists between fluorescence and probe density [2].

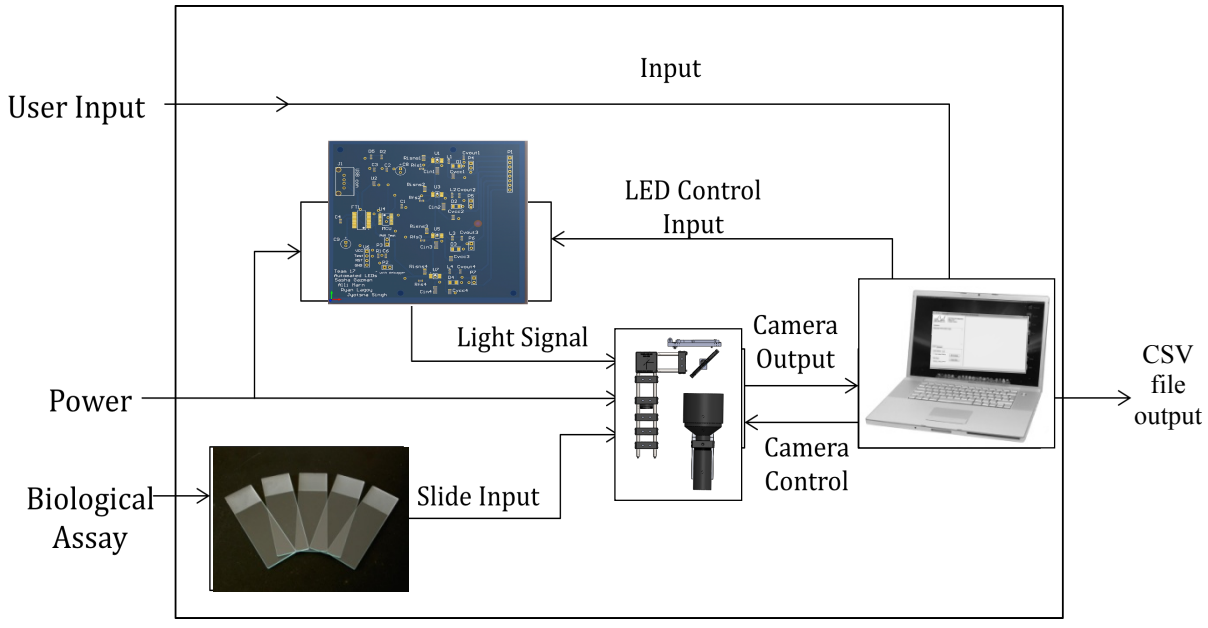
There is a need for an inexpensive, robust device that will quantify the amount of probe to provide a better basis for microarray measurements such as fluorescence.

This microarray calibration device created by Microarray Calibration, Inc. uses interferometry to measure the density of microarray spots. The compact device has an acquisition and analysis time of less than two minutes, and takes up only 0.128 ft^2 of counter space. The automated acquisition and analysis requires only four user clicks before obtaining results. A custom transparent substrate was designed to accompany the device, designed to be compatible with interferometric imaging, fluorescence imaging, and other imaging modalities.

With this device, more conclusive and quantitative diagnoses can be made in numerous research laboratories and clinical practices. It will also increase the pace of research where microarray testing is involved, by reducing the margin of error and increasing the accuracy of analysis. The following pages describe the setup, operation, and maintenance of Microarray Calibration Inc.'s device.

2 System Overview and Installation

2.1 Overview block diagram

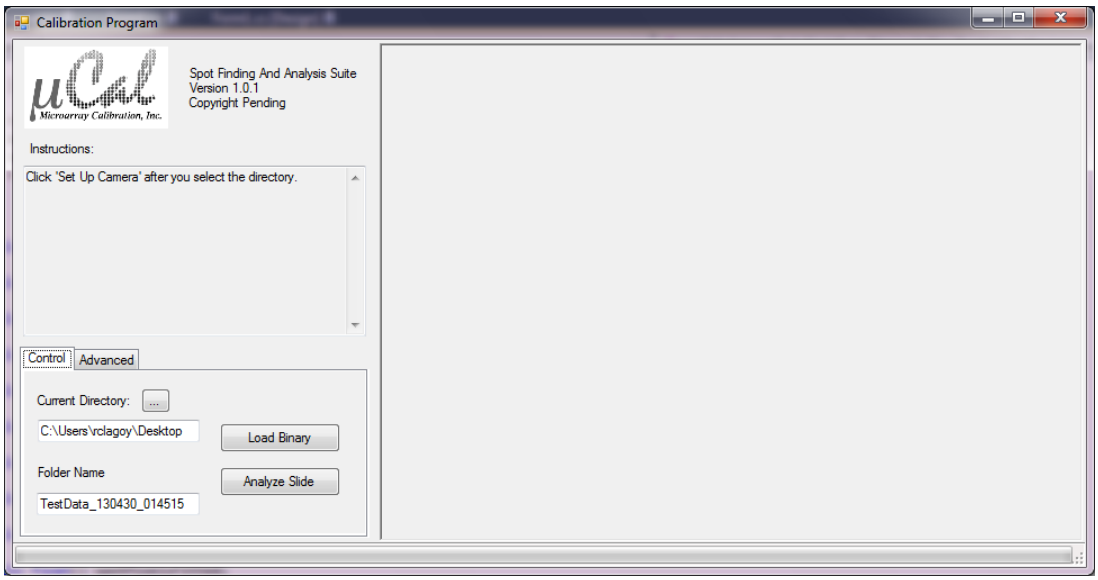


The system accepts inputs in the form of a microarray slide, power from a single USB connection to a PCB, and user mouse-clicks to set up and initiate the acquisition and analysis. The software communicates with the PCB to instruct when to light which LED. The PCB is wired to the LEDs since it was designed to deliver constant current to the

LEDs. The software and camera communicate consistently: the software tells the camera when and how to take images and the camera communicates back the captured images. After thesoftware analyzes the captured images, the software outputs a user-friendly CSV file containing the important information

2.2 User interface.

The main view of the user interface is pictured below. The image will appear in the box in the right, and the instructions and buttons are located in the left panel.



2.3 Physical description.

The device has a length, width, and height of 7.38 in, 4.19 in, and 12.50 in respectively. The entirety of the enclosure occupies a volume of 370.6 in^3. Figure 2.1 A shows the front view of the enclosed device, and Figure 2.1 B shows the back view. The internal components can be visible in the cutout view shown in Figure 2.2.

A) B)

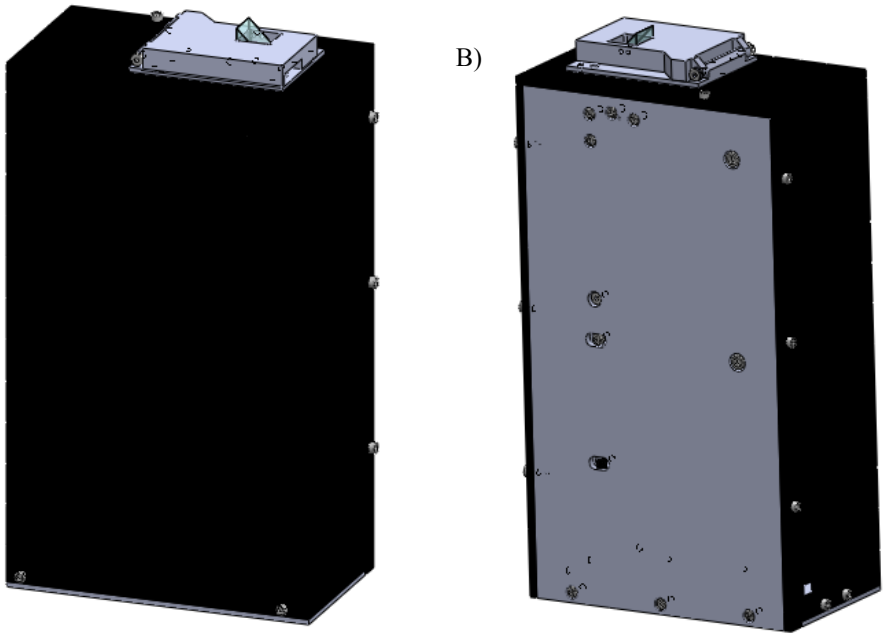


Figure 2.1. *Outer appearance of the device*. A) The front view of the enclosure showing the black acrylic walls. B) The back view of the enclosure showing the aluminum backing.

The device is concealed behind shiny black acrylic walls. A cutaway view of the device can be seen in Figure 2.2, which shows the many components and how they are mounted in the device. The PCB is mounted to the front of the enclosure's aluminum base, and the optical components are attached through the back wall of the device, which is also milled aluminum. The aluminum slide holder is mounted at the top of the device for easy user access, and contains a clamping arm system to hold a microarray slide (Figure 2.3). The specific components are isolated and labeled in Figure 2.4. The microarray slide that fits into the slide holder is pictured in Figure 2.5.

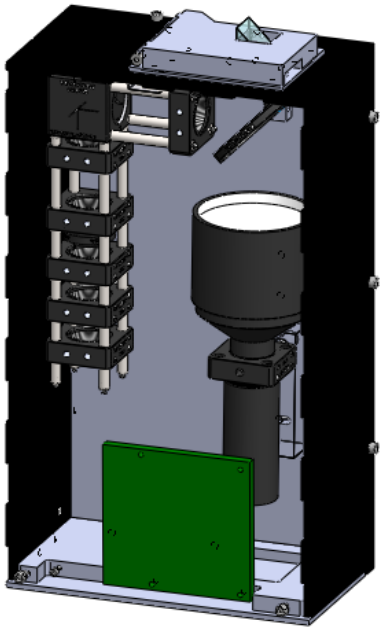


Figure 2.2. *Cutaway view of the device*. The front wall was removed to display the inner compenents

The PCB is shown to scale with the instrument in Figure 2.2. An isolated view of the PCB can be seen in Figure 2.6.

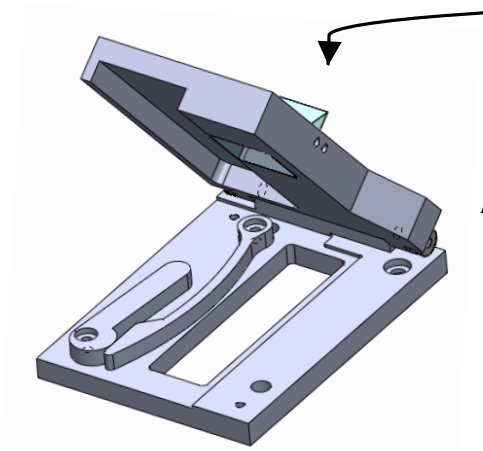


Figure 2.3. *Slide holder*. The aluminum component that allows the user to secure in their slide to be tested.

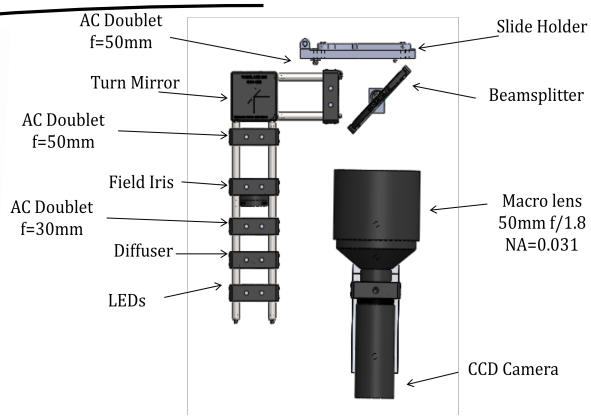


Figure 2.4. *Inner components of the device*. The components inside the device are show and labeled.

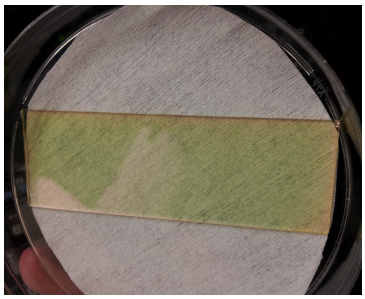


Figure 2.5. *Inner components of the device*. The microarray slide is shown above.

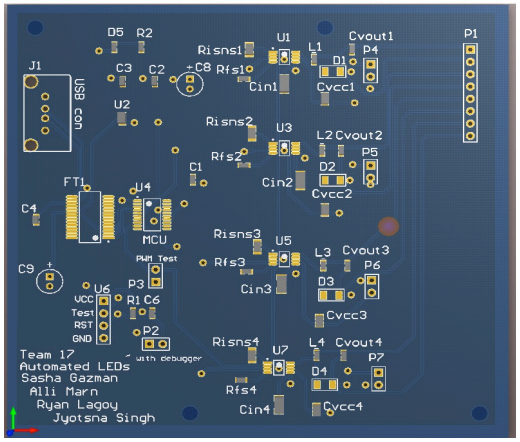


Figure 2.6. *Inner components of the device*. The PCB is shown unpopulated to the above. The dimension of the PCB are 3.583 x 3.379 in^2

2.4 Installation, setup, and support

System requirements include a CUDA capable Nvidia GPU with at least 512 MB of space, Microsoft Visual Studios, and Windows 7 32-bit or higher.

There are four main steps for installation and support of this software.

(1) **CUDA Toolkit and Driver Installation:** If the CUDA toolkit is not installed, go to Nvidia's website, and locate the appropriate drivers for your GPU model. Once the drivers are located, download them, and follow the on-screen instructions that come with the installation files.

(2) **Camera Driver Installation:** Second the camera software needs to be installed using the Starlight Autoguider Installation disc. View the README.txt file on the camera installation disk before connecting the camera or beginning the installation process. Follow the on screen instructions, until the software and drivers are installed.

(3) **Locate and Download Utility Directory:** Navigate to Software Documentation->Source Code->uCal_bin folder. Copy the folder to a location that you would like to keep the files. These files will never be viewed by the user, and should be kept in the background when the GUI is running. Record the entire directory path for this Utility folder (This will be added to the source code in the next step).

(4) **Compile Software:** Navigate to the Software Documentation->Source Code->ImageCaptureCS folder in the Resource CD. This contains all of the source code for the graphical user interface. Copy entire folder locally, and open the Microsoft Visual Studios solution. When the software IDE opens, right click on the Form1.cs filename in the Solution Explorer (You may need to expand a few folders) and choose "View Code". Once this opens, search for the utilDir variable in the global variable list. Change the variable name to the directory that you had recorded in the previous step. When this is complete, choose "Release" in the drop down menu on the top toolbar next to the green play button to build the project. Then press the green play button. When the build is complete, and if there are no errors, the GUI will appear. You may exit the GUI.

(5) **Add Shortcut To Desktop:** Navigate to the ImageCaptureCS->ImageCaptureCS->bin->Release folder. Inside the release folder there is an executable that is called *ImageCaptureCS.exe*. Right click on this executable, and send a shortcut to Desktop . From this point on, the user will be able to click on the shortcut to run the software.

(6) **To Begin Calibration:** Connect hardware to the USB port in the computer. Begin the application using the shortcut to the application on the Desktop, and follow the on screen instructions.

(7) **Support:** For all software support contact Ryan Lagoy at rclagoy@bu.edu.

3 Operation of the Project

3.1 Operating Mode 1: Normal Operation

3.1.1 Microarray Slide Setup:

The microarray slide to be analyzed is inserted, with its top facing downward, and then locked into position with the clamping arm as shown in Figure 3.1 below. The lid of the slide holder is closed, and the slide is now ready for imaging.

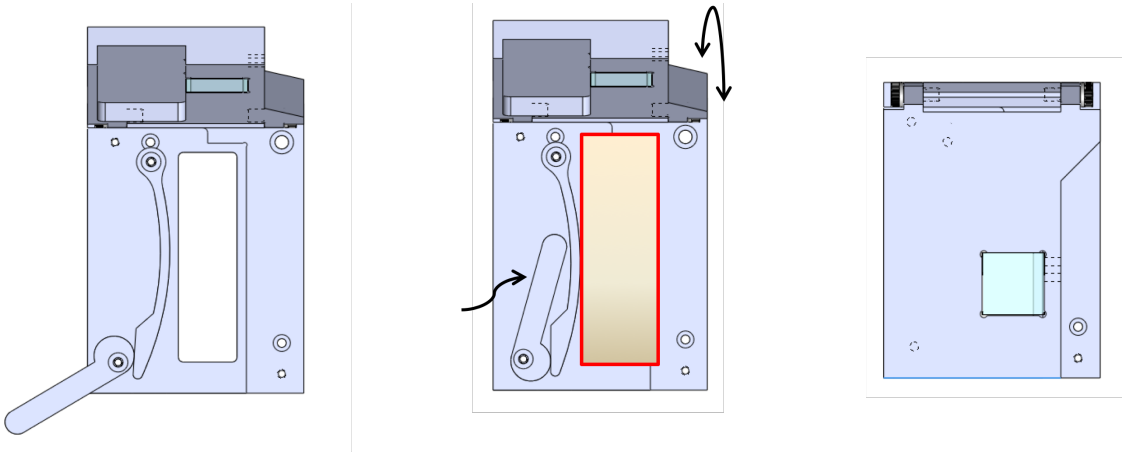


Figure 3.2: *Inserting the microarray slide*. The microarray slide (outlined in red above) is inserted with the three steps above. The slide is inserted, clamped in, and the lid is closed

3.1.2 Software Startup:

The user opens the executable by double clicking the GUI icon. Once the GUI window opens, a text box labeled "Instructions" is visible in the left side of the GUI as seen in Figure 3.2. This text box walks the user through the set up procedure.

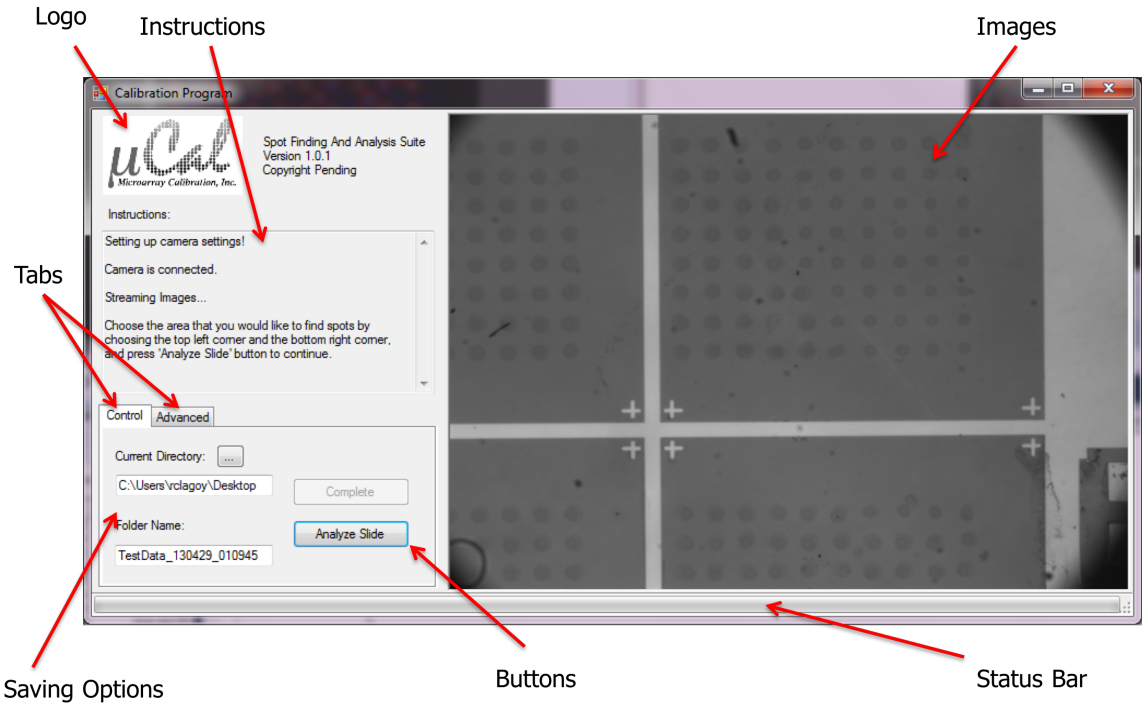


Figure 3.2: *GUI view during Setup*. The GUI is labeled to highlight all of the current features.

3.1.3 Choosing a Directory:

The user can select where to save their file by changing the "Current Directory in the Saving Options (Figure 3.2). The user can type a name for their folder into the "Folder Name" textbox, which will create a new folder to save the data in during analysis.

3.1.4 Camera Set-up:

The user then presses the "Set Up" button in the "Control" tab, which creates the folder to deposit the data, setups the camera, and checks to see if the LEDs are connected.

3.1.5 Selection of Area to Analyze:

The user then selects the region that he or she would like to have analyzed after acquisition. The user clicks the top left corner and the bottom right corner of area to be analyzed, and a red square appears around the region as seen in Figure 3.3.

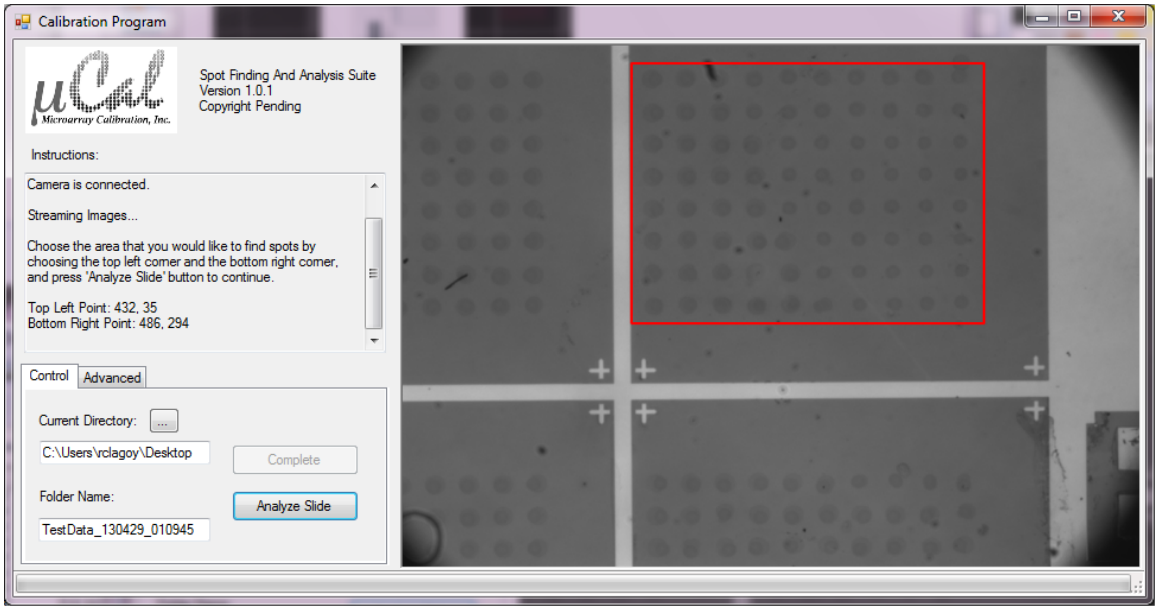


Figure 3.3: *Selecting the area to analyze*. The user chooses the area to analyze, and a red square appears to mark the region.

3.1.6 Analysis:

The user then presses the "Analyze Slide" button to begin the data acquisition and analysis. The camera will capture the specified number of frames for each wavelength sweeping through all four LEDs, and display an averaged image.

The image processing begins automatically once the images are captured. The image processing software detects the spots and displays a labeled image of the spots that were detected in the chosen area on the microarray.

The detected spots are then fit using a nonlinear fitting algorithm to determine the heights of the spots. The progress can be monitored via the status bar located at the bottom of the GUI window.

3.1.7 Data Output:

A CSV file is save in the user-specified file and directory, providing useful data such as spot height, radius, and location.

3.1.8 Advanced:

Additional functionality in the "Advanced" tab (Figure 3.4) allows the user to customize settings such as: the number of frames to average, and which COM port to use. The tab also allows the user to toggle and calibrate the LEDs. The location of the advanced tab can be seen in Figure 3.2.

3.1.8.1 Toggle LEDs

The "Toggle LEDs" button sweeps through each of the four LEDs to verify their functionality to the user.

3.1.8.2 Calibrate LEDs

The "Calibrate LEDs" button allows the user to calibrate the intensity of the LEDs, which is described in "Abnormal Operations."

3.1.8.3 Number to Average

A textbox in the advanced tab labeled "# to Average" allows the user to choose the number of images to acquire and average together for analysis.

3.1.8.4 Reset Form

The "Reset Form" button resets the GUI if ever the user desires to start over.

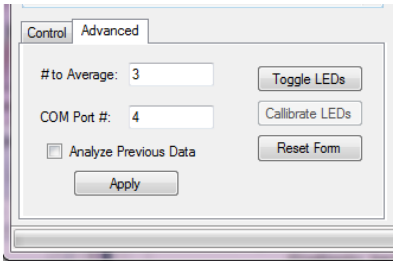


Figure 3.4: *Advanced tab*. This tab includes additional functionality that the everyday user may not need

3.2 Operating Mode 2: Abnormal Operations

3.2.1 LED Calibration

For optimum functioning of the imaging device, each LEDs must illuminate the CCD sensor of the camera at 70% of its full well capacity. Modulating each LED will optimize the signal to noise ratio, allow the exposure time to remain constant between LEDs, and reduce the chances of pixel saturation at the CCD sensor.

The microarray calibration device provides an automated calibration process that allows the user to ensure optimum intensity level of the LEDs, by following these simple steps:

3.2.1.1 Mirror Insertion

Insert a mirror (thing you look into to check your make-up every morning) with its face down in the slide holder, and lock into position using the clamping arm (as described in microarray set up in previous section).

3.2.1.2 Calibrate LEDs

Select the “Calibrate LEDs” button in the “Advanced” tab in the GUI window. The GUI will set up the camera, and then provide a proportionality feedback to the microprocessor to change the duty cycle of the signal to the PCB, which in turn alters the illumination level of the LED.

3.2.1.3 Calibration completion

The instructional text box will notify the user when the calibration is complete (Figure 3.5). The mirror can then be removed and normal operation resumed.

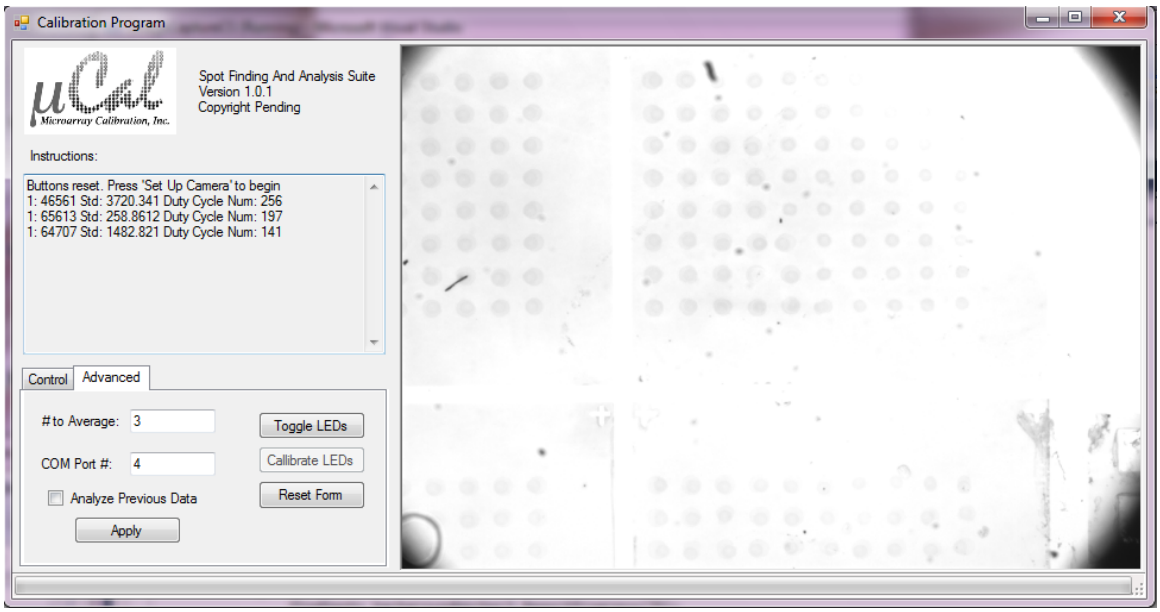


Figure 3.5: *GUI view during LED Calibration*. The "Instructions" text box gives the status of the calibration

3.2.2 Back Analyzing

If there is an error with analysis, or if a user wants to reanalyze data that was acquired earlier, there is a feature in the "Advanced Tab" that allows a user to load an already saved binary file to analyze (Figure 3.6). To do this:

1. Go to the "Advanced Tab" and check the box marked "Analyze Previous Data," (Figure 3.4) and then press "Load Binary" to select the desired binary file (Figure 3.6). Analyze the data with the "Analyze Data Set" button.

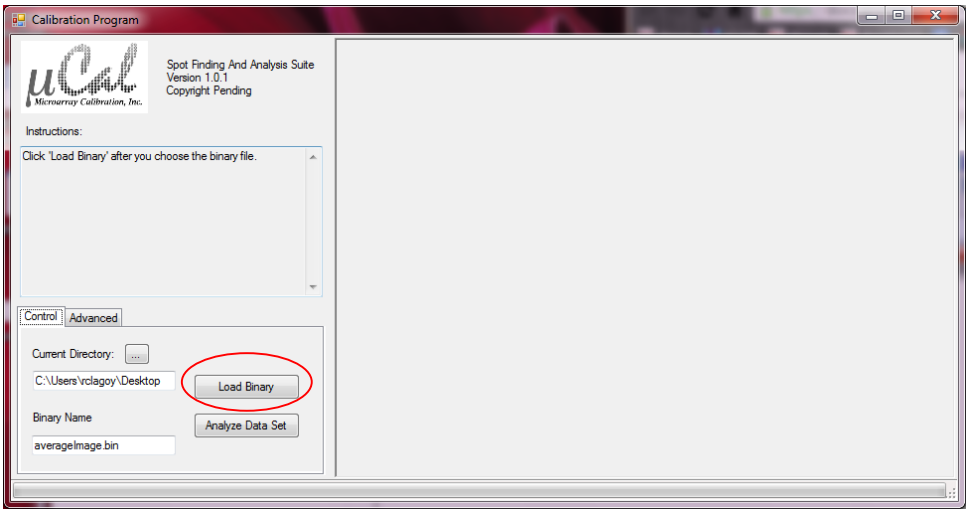


Figure 3.6: *GUI view during Back Analysis*. Use the "Load Binary" button, circled in red to load a previous dataset.

3.3 Safety Issues

3.3.1 Exercise caution while inserting and removing the microarray slide from the slide holder. Dropping the slide through the holder would damage expensive optical components, which would require replacement, installation of the damaged components, and realignment of the optical set up.

3.3.2 The device weighs about 12 pounds and breakable; keep on a sturdy tabletop and transport carefully.

3.3.3 The microarray slide consists of hazardous chemicals and biological solutions and is not fit for consumption.

3.3.4 The microarray slide must be carefully handled with gloves. When using biological materials, avoid contact with skin and eyes, and dispose of the slide safely after use.

4 Technical Background

The microarray calibration device created by Microarray Calibration, Inc. was built using the principles of the Interferometric Reflectance Imaging Sensor (IRIS) developed in the laboratory of Professor Selim Ünlü. Using the information from an interference pattern, measurements can be made of objects much smaller than an optical systems resolution.

This is done by illuminating a reflective dual layer substrate with normal incident light, allowing the reflection from each layer to interfere with each other. The resulting intensities are collected for four discrete wavelengths and then these data points are fit to a characteristic reflectance curve using the reflectance equation below (Equation 1) to achieve a value for ø. The index of refraction, n, and the wavelength, λ, are known, and the light is assumed to be normal incidence, θ=0. Using this information, the height top layer, d, can be determined from Equation 2 below where r_1 and r_2 are the reflection coefficients of the two boundaries, d is the thickness of the top layer, λ is the wavelength of the incident light, n is the refractive index of the top layer, and θ is the angle of incidence..

Equation 1 $$R = \frac{r_1 + r_2 e^{-2j\emptyset}}{1 + r_1 r_2 e^{-2j\emptyset}}$$

Equation 2 $$\emptyset = \frac{2\pi}{\lambda} nd \cos\theta$$

When a biological material is added to the substrate, for example in a microarray spot, the top layer appears to grow. This causes a phase dependent shift resulting in a new value for ø, and a new height. A characteristic reflectance curve is fit to the new shifted data points, and a new value for d is achieved. The height of the added material can be determined by subtracting the original height from the new height. This height can be directly correlated to the density, since 1 nm of height corresponds to 1 ng/mm^2 for most biological substances [3].

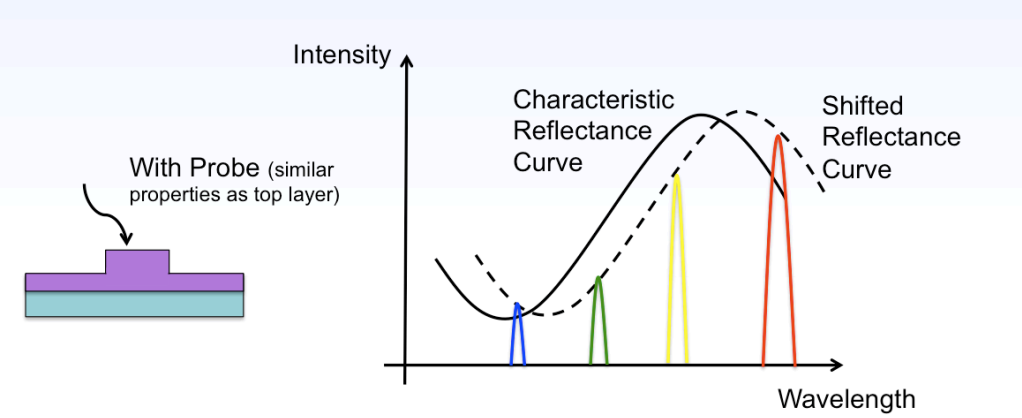


Figure 4.1: *Reflectance curve fitting*. The characteristic reflectance curve can be seen by the solid line, and the shifted reflectance curve can be seen by the dotted line

For Microarray Calibration, Inc.'s device, the development of a transparent microarray slide design was done based on the electromagnetic modeling of the reflectance equations shown in Equation 1 and 2. The top layer was chosen such that its index of refraction would match that of the biological material being spotted on the substrate, so that the phase dependent shift could be quantified, and the other layers were chosen based on reflectivity as well as other properties in order to provoke the interference and reflectance needed for measurements.

The optical configuration used to implement the IRIS technology uses a diffuser, Köhler Illumination, and an additional achromatic doublet to collimate the light into a uniform beam for illuminating the substrate. The light emitted from the LEDs first enters a diffuser to randomize the light and destroy the LEDs spatial coherence. Next Köhler Illumination is used to collimate the light. Köhler Illumination is a lens configuration that uses an initial lens to collect the light, an aperture to filter out the high frequencies, and a second lens to condense the light into a beam. An additional achromatic lens was added to correct for any stray rays that remained uncollimated.

A 2" pellicle beamsplitter is used to direct the collimated beam to the substrate and back to the camera. A larger beamsplitter was used to eliminate clipping in the image, and a pellicle was used to prevent aberrations and ghosting in the image. After the light reflected off the substrate, a macro lens with an adjustable aperture and focus was used to collect the light, which was beneficial due to its adjustable aperture and focus.

In order to optimize signal to noise ratio in the device, each of the four LEDs were calibrated to 70% of the full well capacity of the camera. This was done through a feedback loop in the software that adjusted the pulse width modulation of each LED. When calibrating, the camera captures images with a single LED wavelength. The images are averaged and the mean pixel value is generated. In a closed loop, the duty-cycle is changed till the meaned pixel value generated reaches 70% of the full well capacity. The system switches to the next LED when the same duty cycle sent to the PCB three times.

With calibrated LEDs, a good image was acquired, and various technical approaches were applied for image processing in order to detect the spots. After the images were acquired, a series of filtering steps were used to improve spot detection.

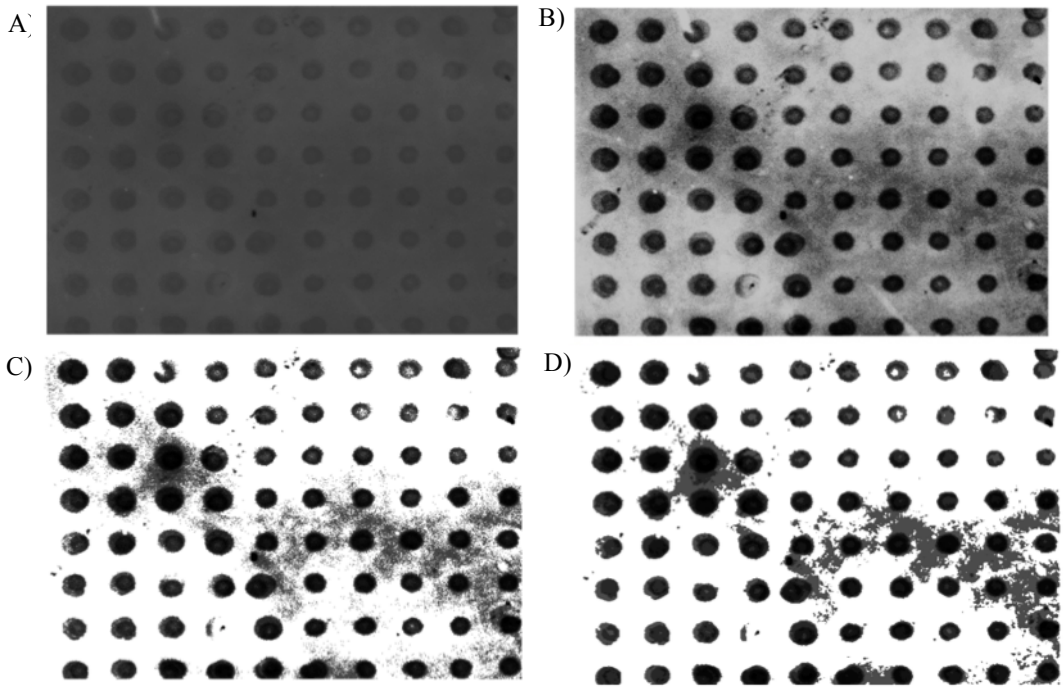


Figure 4.2: *Filtering*. A) The unfiltered image. B) The image after equalizing the histogram. C) The image after background subtraction B) The image after using a median filter

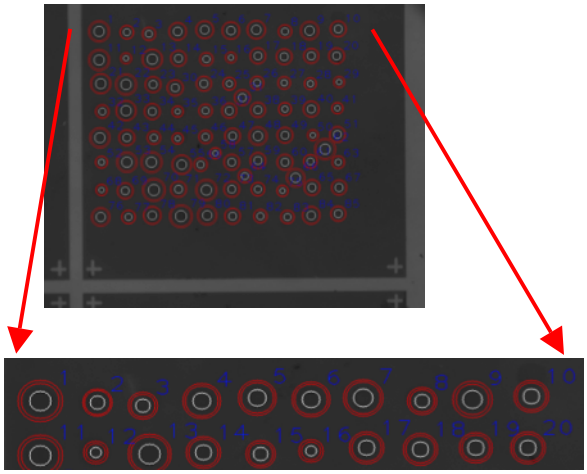


Figure 4.3: *Spot Detection*. The spots found are displayed to the user.

The histogram of the intensities was first equalized, which stretched the intensity to fill the full spectrum available. This provides better contrast in the image for detection (Figure 4.2 B). The background is then subtracted in an attempt to isolate the spots (Figure 4.2 C), and then a median filter is then applied (Figure 4.2 D). A median helps to remove extraneous pixels by replacing each pixel with the median of the surrounding pixels.

In the final step, after the image has been filtered by each of the three filtering steps, a Hough transform is preformed to detect blobs in the image that resemble spots. The detected spots

are then sorted, labeled and displayed to the user as seen in Figure 4.3.

The detected spots are then analyzed using a non-linear fitting algorithm to fit the characteristic reflectance curve as described above. The results determine the height of the spots, which are output in a table format by the software.

5 Cost Breakdown

Project Costs for Production of Beta Version				
Item	Quantity	Description	Unit Cost	Extended Cost
1	1	LED control PCB	$55.84	$55.84
2	1	Starlight Xpress Superstar Autoguider Camera	$630.00	$630.00
3	1	AF Nikkor 50mm f/1.8D	$134.95	$134.95
4	7	Custom Mechanical Components	~$200	$1,244.00
5	27	Optical Components (lenses, aperture, beam splitter)	~$40	$973.47
			Beta Version-Total Cost	$3,038.26

In order to simplify the price breakdown, the cost was broken down into components. Item #1 shows the cost for fabrication and components for a single PCB. Item #2 is the CCD camera used, the Superstar Autoguider, and Item #3 is the Nikon Macro lens used. The custom mechanical components cost $1,786 for the alpha prototype; however, by ordering 50 sets versus one, the cost of a single lowered drastically as seen in Item #4. Additionally, there are 27 Thorlabs optical components including lenses, a diffuser, an aperture, a beamsplitter, and the optical cage system that were grouped together under Item #5. These pieces range in cost from $10 to $200; however, the totality of the optics cost $973.47as shown above.

6 Appendices

References:

[1] G. G. Daaboul, R. S. Vedula, S. Ahn, C. A. Lopez, A. Reddington, E. Ozkumur, and M. S. Ünlü, "LED-based Interferometric Reflectance Imaging Sensor for quantitative dynamic monitoring of biomolecular interactions," *Biosensors and Bioelectronics*, Vol. 26, January 2011, pp. 2221-2227

[2] Monroe MR, Reddington AP, Collins AD *et al*. "Multiplexed Method to Calibrate and Quantitate Fluorescence Signal for Allergen Specific IgE." Anal Chem 2011; 83:9485–91.

[3] E. Ozkumur, C.A. Lopez, A. Yalcin, J. Connor, "Spectral Reflectance Imaging for a Multiplexed, High-Throughput, Label-Free, and Dynamic Biosensing Platform." IEEE Jour OF SELECTED TOPICS IN QUANTUM ELECTRONICS; 10.1109/JSTQE.2009.2037438

6.1 Appendix A - Specifications

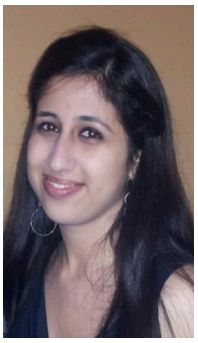
Category	Specification
Device Volume	0.128 ft^2
User input	Maximum of 4 clicks
Computer connection inputs	Single USB connection
Illumination standard deviation	1.5% of the full well capacity
Field of View	7.8 x 5.8 mm
Spatial Resolution	9.96 µm
Camera Specs	16 bit depth, full well capacity of 65K
Spot Detection	Over 95% accuracy
Microarray slide properties	Reflective, transparent, biologically compatible
Slide insertion time	1.5 seconds
Acquisition and analysis time	Under 5 minutes

6.2 Appendix B – Team Information

Team Biographies

Ryan Lagoy is currently a senior in electrical engineering at Boston University. Ryan was born and raised in central Massachusetts, and grew up with an eye for technology and engineering. Today, his focuses are primarily on electromagnetics, wave optics, and software engineering. His diverse research background, ranging from echoic memory studies in neuroscience to electric field simulations of human tissue, allows for integration of the biomedical sciences with the electrical engineering discipline. His hobbies include surfing, running, and playing guitar. He is entering the Leadership Development Program at BAE Systems. He can be reached at (978) 660-4037 or rclagoy@bu.edu.

Allison Marn is a senior at Boston University, double majoring in biomedical and electrical engineering. Allison grew up in the suburbs of Rhode Island, but decided to come to Boston for the local surplus of innovation. She began her research experience in BU's Biological Sensing and Imaging Lab where she worked with label-free pathogen detection and optical design. In her spare time she enjoys running, painting, and baking. After graduation, she is joining InterSystems as a Support Advisor. She can be reached at (401) 439-4033 or ammarn@bu.edu.



Jyotsna Singh is a senior majoring in Electrical Engineering at Boston University. She was born in Maryland, but spent most of her childhood years in India. Her diverse research experience includes the development of a portable microfluidic lab-on-a-chip model for purification of human plasma. Her current research focuses on the application of biosensors for pathogen detection and nanoparticle size characterization. This project has enabled her to apply the knowledge and skills gained from the Electrical Engineering curriculum to biological experimentation. Jyotsna's hobbies include traveling, photography, and running. Following graduation she will be starting a career at Accenture as a Systems Integration Consultant. She can be reached at (857) 241-6432 or sjyotsna@bu.edu.

Sasha Gazman is a senior at Boston University. Sasha is majoring in Electrical Engineering and concentrating in nano-technology. Sasha was born in Russia, and most of his life lived in Israel. He speaks three human and one animal (sea horse) language, and has experience in computer languages too. Sasha has worked on drones communication components and their maintenance. He has research experience on graphene micro-device fabrication and characterization. Other non-engineering interests include sports, travelling and reading. Following graduation he will be continuing working at MC10 as an electrical engineer. Sasha can be reached at sashagn@bu.edu or by phone (857)284-6206.