Pathogen Detection with Brewster's Angle Straddle Interferometer

Product Requirements Document

Engineering Team

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Customer

Professor Lewis Rothberg, Chemical Engineering at University of Rochester

Document 00003

Revision	Date
C	Dec. 7, 2015

Authentication Block

Revision History

Rev	Description	Date	Authorization
A	Initial PRD after meeting with customer	10/30/2015	GG
B	Including new information from primary laboratory tests and research, verifications from customer, adding Hardware and Software Specs, fixed errors.	11/15/2015	GG
C	Revisions for final PRD review in fall. Updates on the optical design, additional sections: Appendices, Project Scope and Team Responsibilities, and Required Resources.	12/7/2015	GG
D	Post final customer meeting of fall	12/10/2015	GG

Contents

The Pathogen Detection project is a senior design driven instrument to detect pathogens in biological samples. As such its design inputs were derived from our interactions with our project advisers Lewis Rothberg and Wayne Knox.

Product Vision

Inexpensive, portable BASI for "third world diagnostics." Expose to blood, saliva, etc.; rinse; system records difference, reports if sample contains specific pathogen.

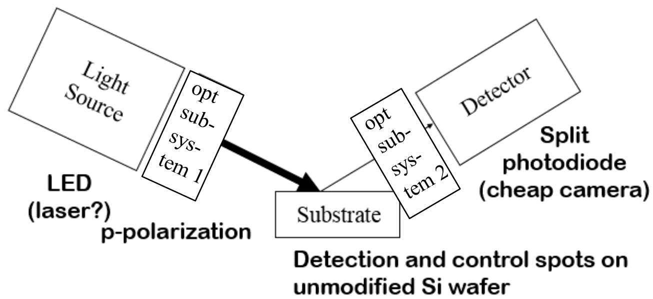


Figure 1: A theoretical set up of this product's optical components.

The theory has been tested and proven [1] and now the design is contingent on condensing the set-up such that the final product is rugged and portable with laboratory accuracy. See *Appendix A* for information on Brewster's Angle and incident light. See *Appendix B* for procedure to find/verify Brewster's Angle in the lab set up. Below is the block diagram of all parts of the system.

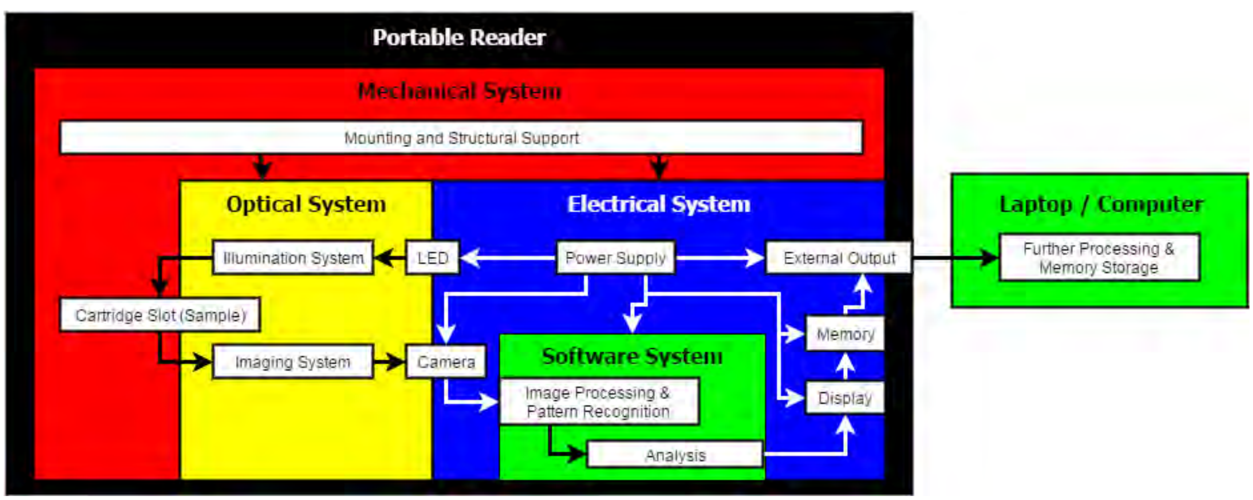


Figure 2: Block diagram of deliverable including mechanical system, optical system, electrical system, software system and the external functions of the laptop.

Project Scope

Optical Engineering Senior Design Team (OPT 311) is responsible for the following deliverables.

Compact, operational instrument with the following components:

- Illumination and imaging optics lens design
  - Illumination system:
    - 635 nm LED
    - Optical diffuser
    - Pinhole or Iris
  - Imaging system:
    - Plano-convex lenses (2)
    - CCD Camera
  - For both systems, we will deliver
    - Dimensions of the optics (diameters, thicknesses, radii)
    - Spacing between optics (vertex – vertex)
    - Tolerances on any optical elements (Decenters, Tilts, etc)
    - Location/tolerances of aperture stop
    - Diameter/tolerances of any apertures
    - Preferred method of optic mounting, as starting point
    - Possibly help vendors

- Electronics and Software (as required for ECE Senior Design)
  - C++ GUI (to interface with camera, capture images, perform image processing and pattern recognition algorithms, analyze the data, and display the results)
  - Circuit Board (that has the same functionality as the GUI, but performs all operations onboard; no laptop or computer is needed, but can be interfaced with external output)
  - Power and Regulation Circuit

Not responsible for:

- Chemical Engineering department will provide engineered silicon wafers
- Mechanical Engineering Senior Design Team will provide:
  - CAD, FE analysis, machined parts
  - See Appendix C for all requirements

Team Responsibilities

Gary Ge	team leader, ECE consultant, optical consultant
Lauren Brownlee	document manager, optical consultant
Sean Reid	scribe, MechE consultant, optical consultant
Pedro Vallejo-Ramirez	customer liaison, optical consultant

Environment

As a portable in-field instrument, it needs to operate in the following environment:

Temperature
-10 º – 50º C

Humidity
up to 100%

It will operate with replaceable batteries.

Regulatory Issues

Light source will be enclosed, no eye safety regulations.

There will be a power safety specification.

Eye safety and other regulations regarding LED's are covered in *IEC 62471:2006 Photobiological safety of lamps and lamp systems* [2]

Fitness for use

The system will:

Detect if the sample contains a specified pathogen.

Be smaller than a shoebox.

Be as accurate as laboratory set-up used for proof of concept.

Contain an LED source to illuminate the sample, it will be incoherent, it will be p-polarized.

It will use a silicon wafer with silicon dioxide monolayer as the sample substrate.

It is desirable that:

Small enough to be hand-held.

Connect to laptop for transfer of data.

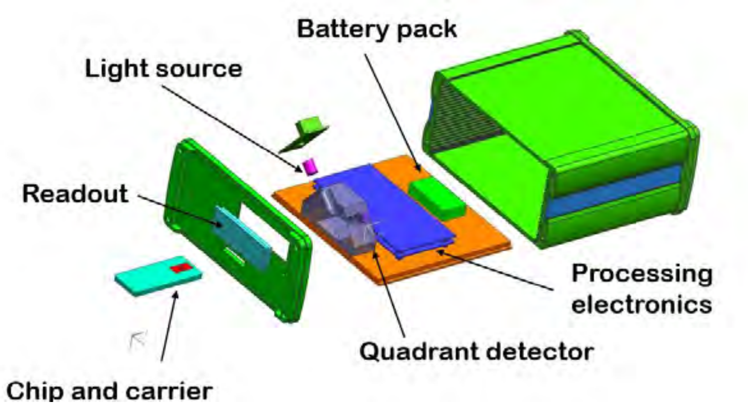


Figure 3: The desired deliverable with components and casing. Sydor Instrument Design. [3]

Specifications

Source Specifications	
Type	Light Emitting Diode
Incidence angle θ	74.9° ±0.5°
Beam divergence Δθ	± 0.5°
Wavelength λ	634nm center 16 nm FWHM
Polarizer extinction ratio	9000:1
Power	5mW total

Detector Specifications (Aptina MT9V022)	
Size	752x480pix
Frame Rate	60 FPS
Megapixels	0.3MP
Chroma	Mono
Sensor Type	CMOS
Pixel Size	6.0 µm
Exposure Range	0.031 ms to 512 ms
Interface	USB 2.0
Power Requirements	4.75 to 5.25 V
Power Consumption Max	>1W
Dimensions	44 mm x 34 mm x 24.4 mm

System Specifications	
Weight	0.5 kg
Cost	< $100
Size	< size of shoebox
Sensitivity	95% *
Specificity	95% *
Lifetime	3 years

*Sensitivity and Specificity should be above 95% in order to be a marketable product approved by the FDA, however this team will not have the means to test this specification.

Software Specifications

The off-board software will be in the form of a very simple GUI such that a user can take images and process them on a laptop, interface can be seen in *Appendix D* This will act as the preliminary mode of hardware-software integration, and will provide a means of assessing and designing future hardware.

System Interface	USB 2.0
User Interface	Computer (Laptop)
Software Application	GUI
Platform	Cross Platform
Development	Qt
Libraries	OpenCV
Means of Pattern Recognition	- BLOB detection - Contour matching - Feature extraction
Language	C++
Features	- Obtain/upload images (single or averaged) - User Control Panel - Look for circular or elliptical patterns - Output image with highlighted "matches"

The on-board software will consist of the processing capabilities mentioned above, but will translate the system to an electronic board which will output to an LCD screen as well as store the data in memory (i.e. hardware-software integration in later development).

Overview:

System Interface	6 pin IEEE 1394a; 7 pin JST GPIO connector; Board TBD
User Interface	LCD screen and Push Buttons, Indicator LEDs
External Interface	USB 2.0
Software Application	Compiled on board
Development	TBD
Libraries	OpenCV, Native libraries
Means of Pattern Recognition	- BLOB detection - Contour matching - Feature extraction
Language	C/C++
Features	- Obtain images (single or averaged) - User Control Panel - Look for circular or elliptical patterns - Output image with highlighted "matches" - Interpret output (statistics)

Hardware Specifications

The software specifications as well as cost will drive much of the hardware requirements.

Operating Voltage	5V (Batteries) with on-board regulators
Power Consumption	TBD
Main Board	TBD (PIC32, PCduino, etc.)
Memory	TBD
A/D Converter	10-bit on sensor chip
Interface to sensor	GPIO
Interface to LCD	ZEBRA Elastomeric Connector
External Interface	USB 2.0
External Buttons	TBD (Power on/off, Capture, Adjust, Output)

Resources Needed

General Resources

Optical design consultant will be identified by spring.

Optical elements will be ordered from optical catalog.

Software packages: MATLAB, Code V, Open CV, Eagle, Light Tools

Software License: GNU LGPL

ANSI Documents as per section *Regulatory Issues*

Budget

Optics				
Part	**Qty**	**Price**	**Vendor**	**PN**
25.4 mm, MgF2 coated Plano-Convex Singlet	4	$30.50	Edmund Optics	Stock No. #49-857
High Contrast Linear Polarizing Filter, Extinction Ratio 9000:1	2	$22.50	Edmund Optics	Stock No. #86-178
Optical Iris	2	Readily available	Wilmot Teaching Labs	
635 nm LED	2	Readily available	Wilmot Teaching Labs	
Optical diffuser	1	Readily available	Wilmot Teaching Labs	
CCD Camera	1	Readily available	Wilmot Teaching Labs	
Electronics				
Part	**Qty**	**Price**	**Vendor**	**PN**
Wiring (Solid Core)	N/A	Readily Available	ECE Lab (HPN 202)	
AA Batteries	3	Readily Available	ECE Lab (HPN 202) CS Lab (CSB 628)	
9V Batteries	1	Readily Available	ECE Lab (HPN 202) CS Lab (CSB 628)	
LEDs	5+	Readily Available	ECE Lab (HPN 202) CS Lab (CSB 628)	
Regulators	4	Readily Available	ECE Lab (HPN 202)	
Camera	1	Already Obtained		
Circuit Perfboard	2+	Readily Available	ECE Lab (HPN 202) CS Lab (CSB 628)	
pcDuino 3B	1	$60	Sparkfun	DEV 13707
LCD Screen	1	$60	Sparkfun	SEN-13101 ROHS
Mini-USB to USB Cable	1	Readily Available	CS Lab (CSB 628)	
Micro-USB to USB Cable	1	Readily Available	CS Lab (CSB 628)	
TOTAL		$173 for prototype		

References

[1] T. Gao and L. J. Rothberg, "Label-Free Sensing of Binding to Microarrays Using," *Analytical Chemistry*, pp. 79, 7589-7595, 2007.

[2] International Electrotechnical Commission, *IEC 62471:2006 Photobiological safety of lamps and lamp systems*, 26 07 2006. https://webstore.iec.ch/publication/7076#additionalinfo.

[3] "ISO 14971," 15 10 2007. http://www.iso.org/iso/catalogue_detail?csnumber=38193.

[4] Sydor Instrument Design. *Hypothetical portable reader*, 2014.

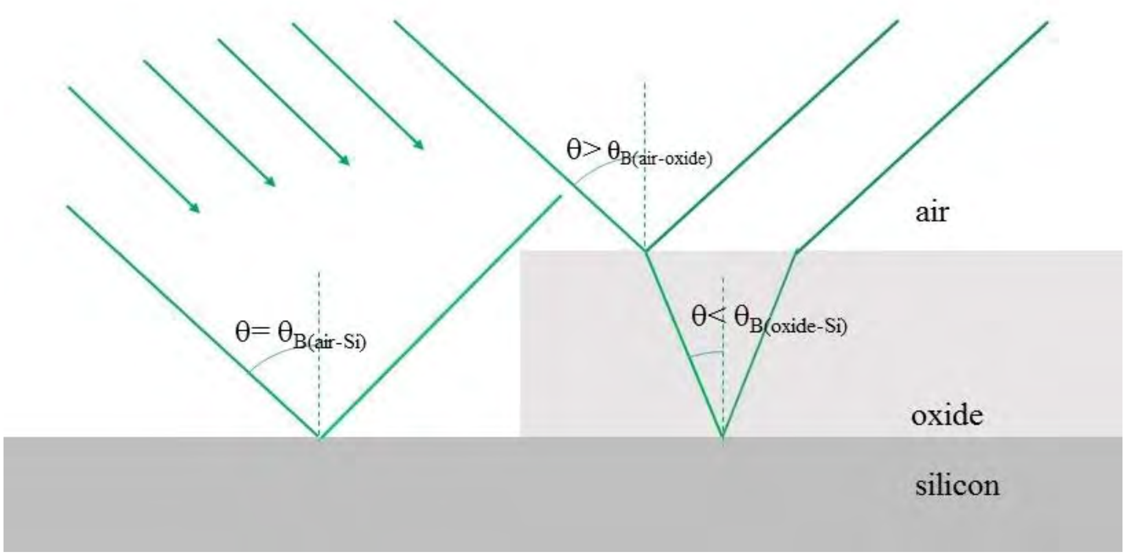
Timeline

Week	Goals	Meetings
10/26-10/30	*Initial PRD Rev A, present 10/30*	*Initial meeting with customer, meet with previous group member, M,W,F*
11/2-11/6		*MF*
11/9-11/13	*Working lab set-up for testing (all)*	*MF, customer*
11/16-11/20	*Preliminary CAD (Sean), Preliminary board design (Gary), PRD V00002, working lab set up*	*MF*
11/30-12/4	*CAD, Board design, all optical components ordered/present*	*MF*
12/7-12/11	*Accurate way to measure Brewster's Angle, images taken show minimum, PRD V00003*	*MF, customer*
12/14-12/18	*Planning to minimize lab set up, goals for break*	*MF*
January	GUI done, final optical design	MWF, customer meetings, MechE team/adviser, Optics professors, ECE adviser,
February	Electronics board ordered, optical parts ordered, assembly/testing on breadboard, redesign if necessary, send requirements to MechE Design Team (tolerances, spacing, dimensions, etc.),	MWF, customer meetings, MechE team/adviser
March	Board programmed, redesign if necessary, requirements to MechE Design Team (tolerances, spacing, dimensions, etc.),	MWF, customer meetings, MechE team/adviser
April	Testing, evaluation of final design	MWF, customer meetings, MechE team/adviser
May	Final product, presentation, design day	MWF, customer meetings, MechE team/adviser

Ongoing: brainstorming; testing; updating PRD, BOM; design plans

Appendix

Appendix A: Theoretical Brewster's Angle Substrate



The schematic of the incoming light (collimated ±0.5°). From air, it will be incident upon the native oxide layer at the Brewster's Angle for an air-silicon interface. This angle is greater than the Brewster's Angle for an air-oxide interface and the transmitted light through the air-oxide interface is refracted such that the incidence angle upon the silicon is less than the Brewster's Angle for an oxide-silicon interface. The oxide layer's thickness is engineered such that there will be destructive interference between the reflected and transmitted light at the air-oxide interface.

Appendix B: Characterizing the Brewster Angle of Silicon

Mission Statement

The purpose of this document is to detail the procedures used to characterize the Brewster's angle of silicon. At the Brewster's angle, zero p-polarized light is reflected, theoretically. The goal of this experiment is to determine, given the geometry of our setup, what combination of orientations of the polarizing filter and the silicon substrate yield a minimum amount of reflected 635nm light.

Setup Geometry

An image of the setup is annotated below.

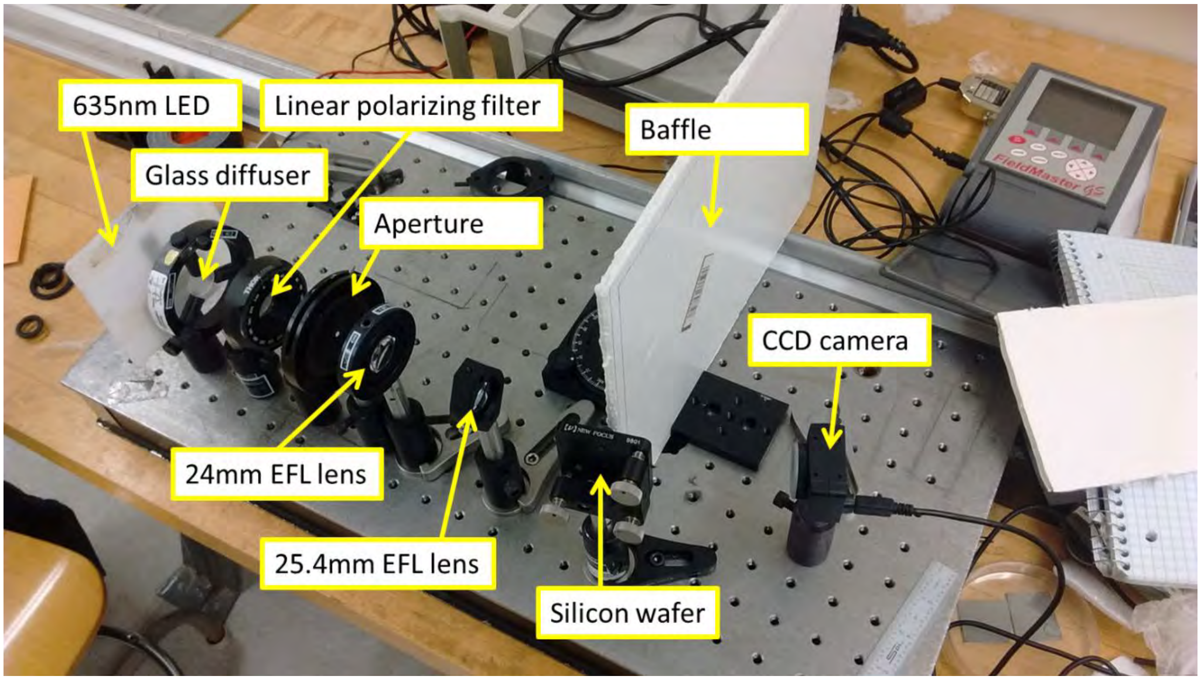


Figure 1: Annotated optical setup of pathogen detector. The aperture is placed at the front focal length of the 24mm EFL lens, so the system is telecentric. The baffle is very important in stopping extra light from reaching the CCD. The breadboard that the components are fixed to has ¼"-20 threaded holes arrayed in x and y, separated by 1.000" ± 0.001". The diffuser is centered on one of these holes, as is the CCD camera. The silicon wafer mount is clamped but is not centered on a hole. Therefore the relative position of the CCD is known to within 0.001", whereas the silicon wafer mount's position is less accurately known.

Geometric Calculation of Incident Angle

The system is modeled as shown in the annotated schematic below.

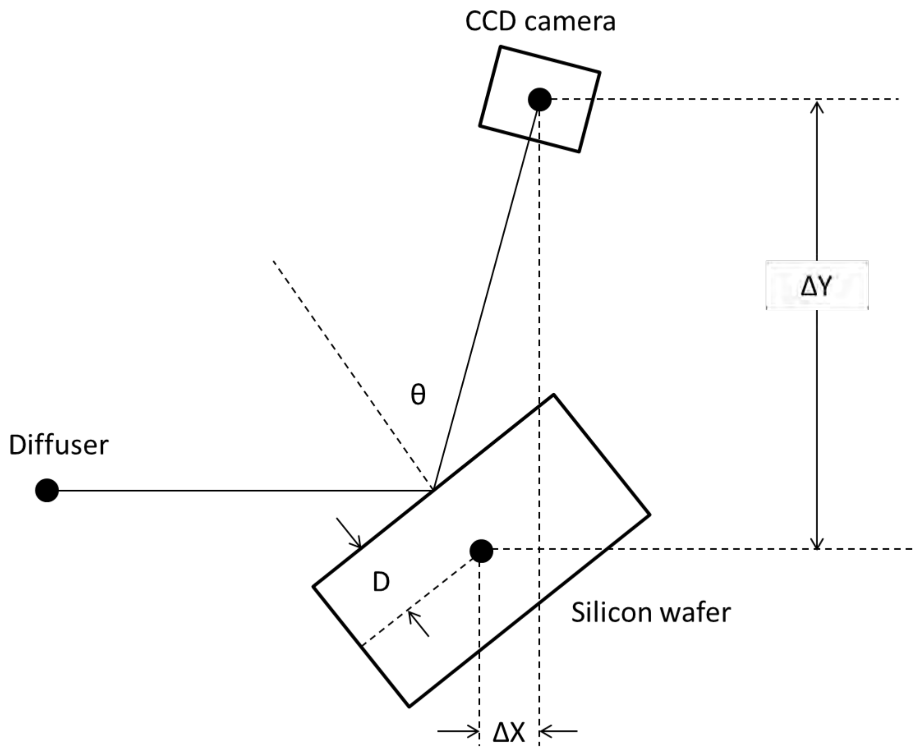


Figure 2: Annotated schematic of setup geometry. The desired calculated value is theta. The measured values are the x and y displacement of the silicon wafer mount from the diffuser, and the displacement D of the axis of rotation of the silicon wafer mount to the wafer surface.

Table 1: Measured Values and corresponding error

Measurement name	Measurement Uncertainty Value	Reason for uncertainty
Relative position of breadboard holes	0.001”	CNC milling process
Position of wafer mount to nearest hole	0.016”	Smallest increment on ruler
Offset of wafer from mount axis	0.016”	Smallest increment on ruler

To calculate the incident angle based on the above geometry, the following equation was solved for theta:

$$\tan(\pi - 2\theta) = \frac{\Delta Y - D\sin\theta}{\Delta X + D\cos\theta}$$

An upper and lower bound for theta was calculated for each instance of dx and dy, to determine the uncertainty in theta. This is typically about a half a degree for the setup geometry shown.

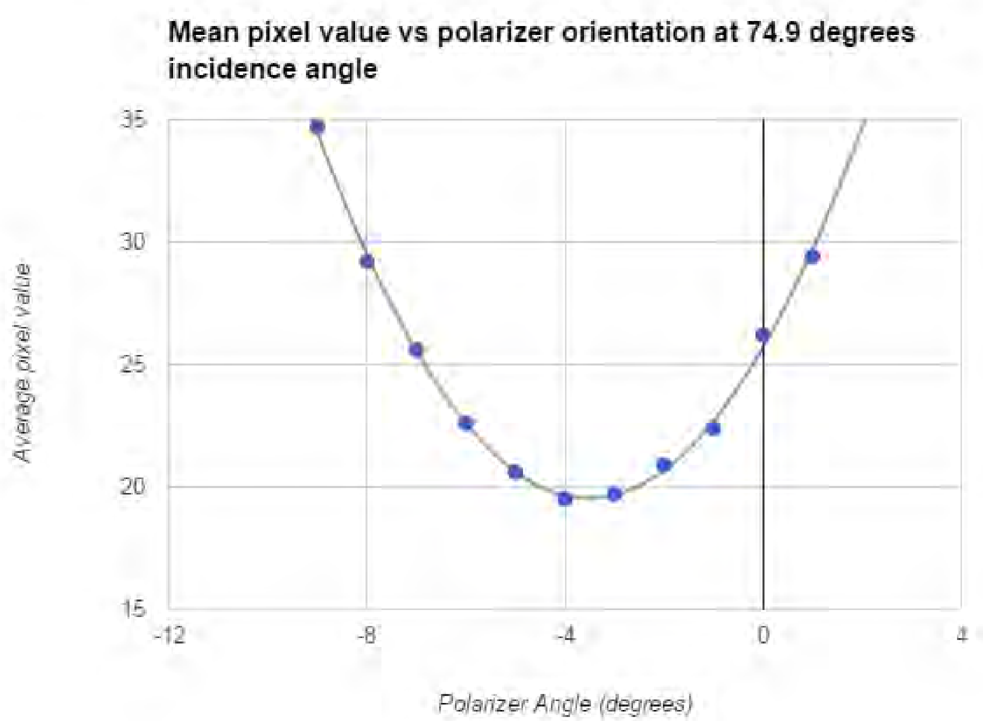
Experimental Procedure

1.) The lights were turned off, and all computer monitors dimmed, to eliminate extra light which the measurements are very sensitive to.

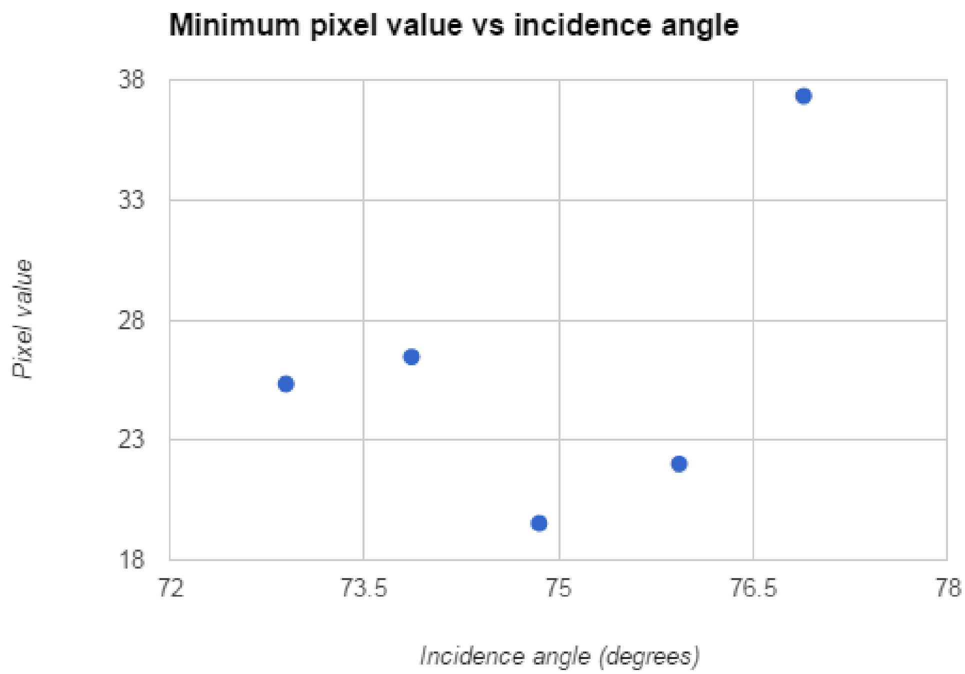
2.) The silicon wafer mount is positioned such that the beam of light is reflected onto the CCD.
3.) The relative x and y positions of the silicon wafer mount to a reference hole are measured with the ruler.
4.) The relative coordinates from the silicon wafer mount to the camera are calculated. The camera is fixed to a hole.
5.) The above equation is solved for an upper and lower bound on theta. The average of these bounds is recorded as the incident angle to the surface.
6.) The polarizer is set to -9 degrees. The mean pixel value from the CCD is recorded.
7.) Increment the polarizer by 1 degree (to -8 degrees) and repeat the pixel-recording until 11 datapoints are collected.
8.) Repeat these procedures for 5 different incidence angles, all close to 75 degrees.

The output of this experiment is 55 data points, representing mean pixel values at a variety of incidence angles and polarizer orientations. The goal, as stated, is to find the combination of angles that results in the minimum pixel value.

To do this, a quadratic curve was fit to the data points at each incidence angle as shown in figure 3.



The minimum pixel value could then be extracted for each incidence angle, as shown in figure 4.



Conclusion

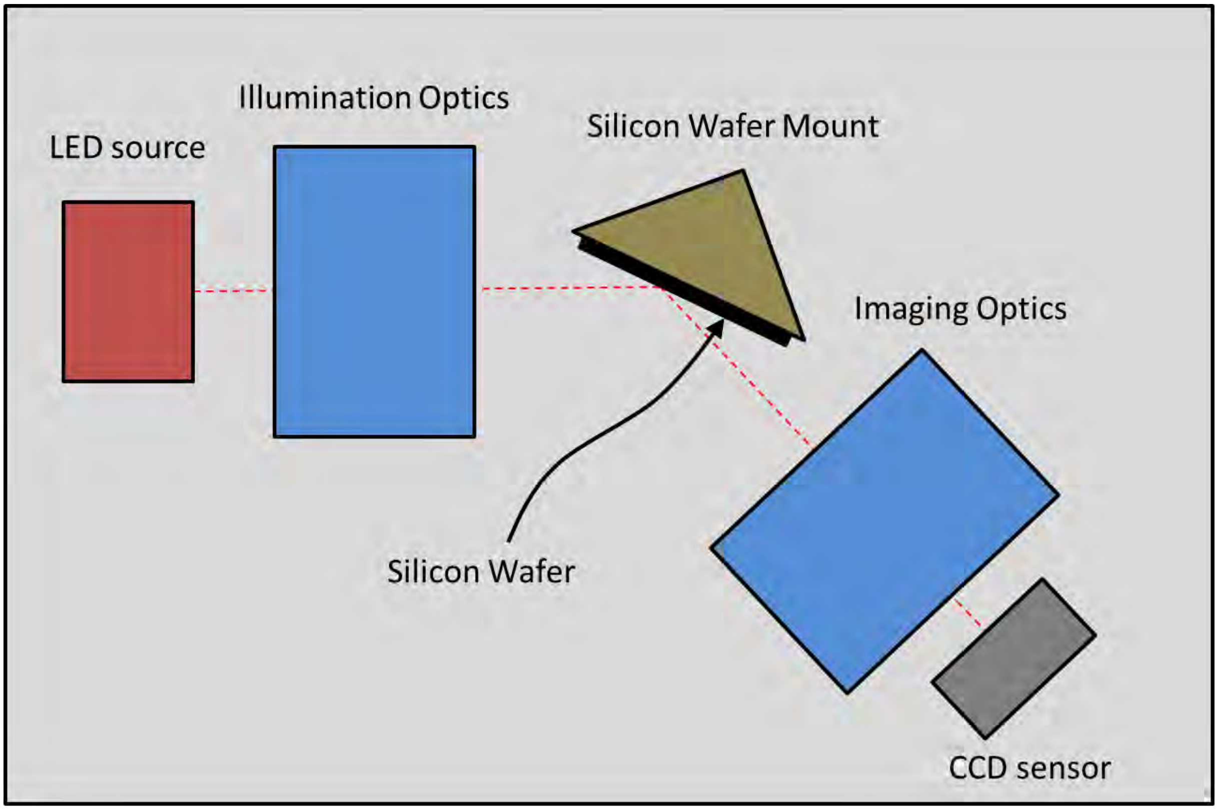
The incidence angle that minimizes pixel value is 74.9±0.5 degrees. The best polarization orientation for this incidence angle is -3.5±0.5 degrees. The minimum pixel value of the reflected spot that can be achieved with this setup is a 19.50±0.04 average.

Appendix C: Requirements for Mechanical Engineering Team

A four person team of students from Chris Muir's ME205 class will be accompanying the OPT310 students in this project. The ME students are responsible for the following custom machined components:

- Mount for LED source
- Mount for Illumination Optics
- Mount for silicon wafer
- Mount for imaging optics
- Mount for CCD sensor

The general sequence of these subsystems in the BASI device is shown in the following schematic.



The OPT310 students will give the ME205 students the following as input:

Regarding the entire system

Dimensions of device

Maximum weight of device

Positions of each subsystem

Tolerance for incident angle of light on silicon wafer

Subsystem misalignment tolerance (tip/tilt, translation in X, Y, Z)

Regarding the LED source

CAD file showing the dimensions and shape of the LED

How the LED will be connected to the accompanying electronics

Dimensions of the diffuser to be used

Position of LED, diffuser, and polarizing filter

Dimensions of the polarizing filter to be used

Polarizer orientation (horizontal/vertical)

Rotational tolerance on polarizer orientation

Tolerances regarding source component placement (translation in X, Y, Z)

Regarding the Illumination Optics

Dimensions of the optics (diameters, thicknesses, radii)

Spacing between optics (vertex – vertex)

Tolerances regarding optics placement (tip/tilt, translation in X, Y, Z)

Location/tolerances of aperture stop

Diameter/tolerances of aperture

Preferred method of optic mounting, as starting point

Possibly helpful vendors

Regarding the silicon wafer mount

Dimensions of silicon wafer (thickness, width, length)

Ease of removal

Incident angle and tolerance on this angle

Degrees of freedom

Regarding the imaging optics

Same as illumination optics

Regarding the CCD sensor

CAD drawing showing dimensions of camera, and threaded mounting hole position/type

Tolerances on centering the sensor (X/Y)

Position of sensor relative to imaging optics

The ME students will execute each deliverable in the following sequence (from Chris Muir’s notes on the 205 project):

Document requirements and specifications

Brainstorm multiple design concepts

Present concept selection matrix indicating most promising design alternative

Create CAD of proposal, including associated drawing package

Prototype the best design

Machine a working, tested breadboard/engineering model

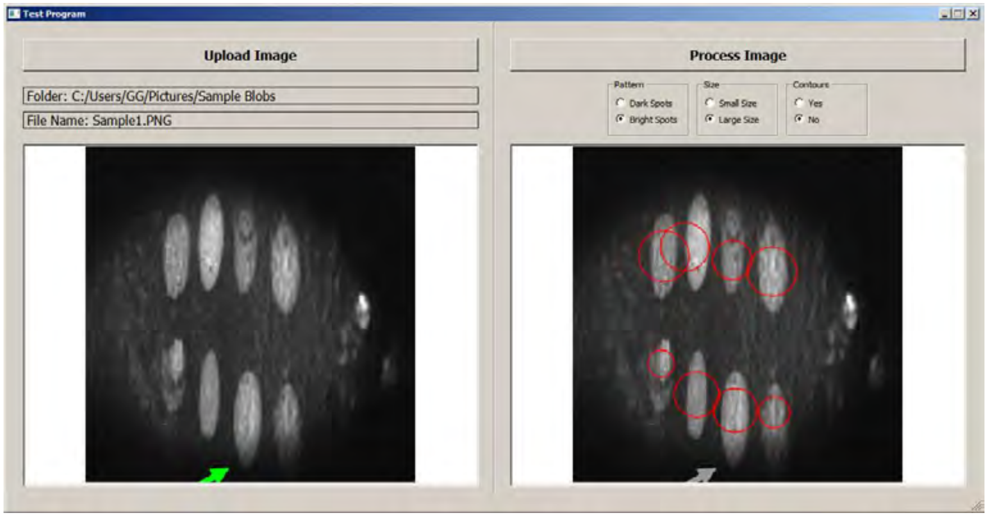
Appendix D: GUI

The GUI was created in Qt using native libraries and OpenCV libraries (compiler: MinGW-32bit 4.9.2 for Windows). OpenCV provides powerful feature extraction and detection libraries, which include BLOB detection and contour matching functionalities.

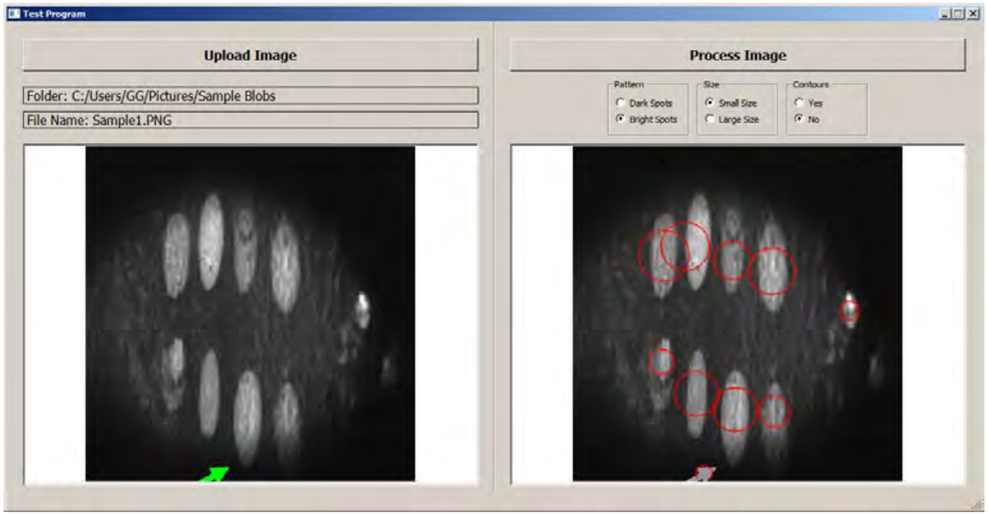
For now, an image can be uploaded and displayed in the left hand side. Later, the ability to take an image with the desired camera will be incorporated.

The image is then processed on the right according to user inputs (detect dark or bright spots, look for small or large spots, and to display contours).

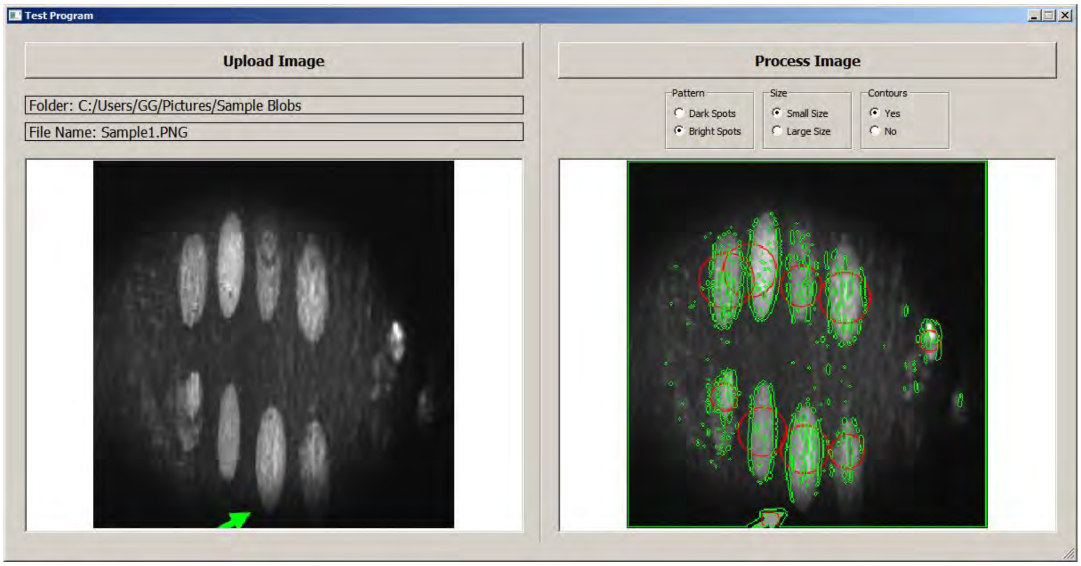
Images used below come from Rothberg's research paper [1].



Options specified: bright Spots, large size, not observing contours

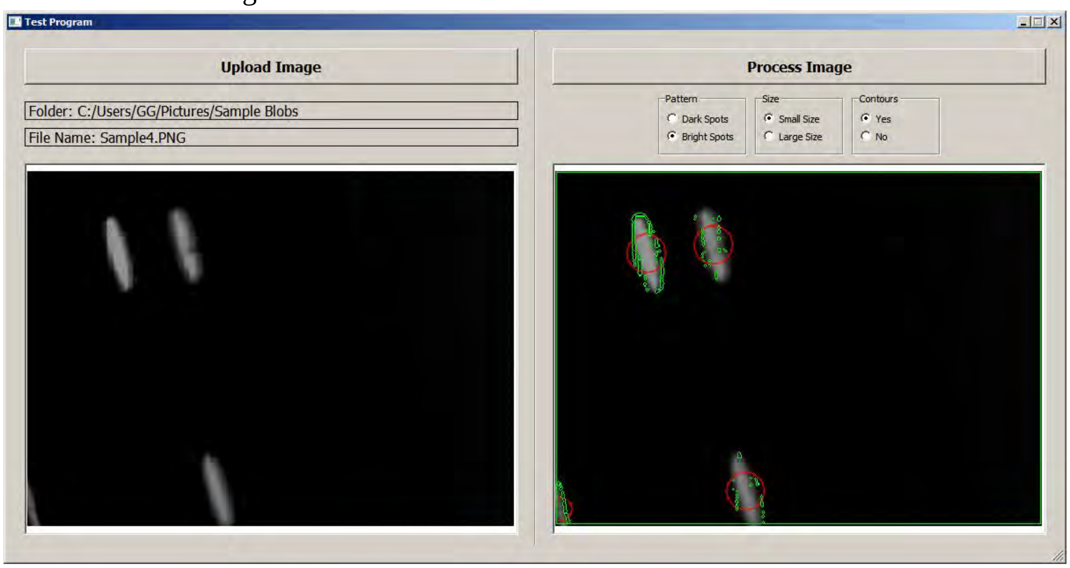


Options specified: bright spots, small size, not observing contours



Options specified: bright spots, small size, observing contours (green)

With a different image:



Options specified: bright spots, small size, observing contours (green)

Appendix E: Sensitivity of Setup on Stray Light, Effect of Background Subtraction

The purpose of this appendix is to characterize the sensitivity of the BASI prototype setup to background counts on the CCD. Background counts can occur from the following:

- Stray light (light outside of the room leaking underneath the door, or light from the LED scattering towards the detector from an unintended optical path)
- Dark noise (statistical variation of electron counts, dependent on temperature)

To calibrate the detector, standard procedure is to take an image with the source turned off, called the background. When the source is turned on, the background is then subtracted on a pixel-by-pixel basis from every successive image.

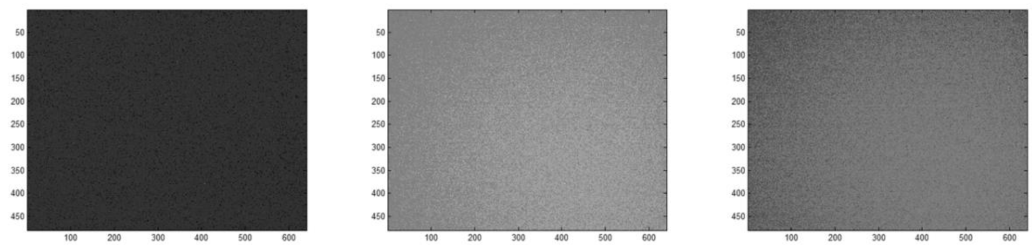


Figure 1. The leftmost figure corresponds to the background counts, with the LED source turned off. The average pixel value of this image is 8.8 counts. The middle figure is the raw image of reflected light on CCD sensor, with 74.9±0.5 degrees angle of incidence at silicon wafer. The average pixel value of this image is 34.9 counts. The rightmost figure is the middle image, calibrated for background counts. The average pixel value of this image is 26.0 counts.

The following table summarizes the three images in figure XX.

Image Type	Average Pixel Value (Counts)
Background	8.8
Raw image with LED source on	34.9
Calibrated image with LED source on	26.0

Subtracting the background decreased the average pixel count by 8.8 counts, or 25%. Therefore, the reflectivity measurement is quite sensitive to background counts.

Appendix F: Preliminary Design Sketches