Optical Design Description Document

Pathogen Detection
with Brewster's Angle Straddling Interferometer

Customer: Professor Lewis Rothberg, Department of Chemistry at University of Rochester

Engineers: Lauren Brownlee, Gary Ge, Sean Reid, Pedro Vallejo-Ramirez

Advisor: Professor Wayne Knox of the Institute of Optics, University of Rochester

Document0001

Revision	Date
E	April 27, 2016

Authentication Block

The Pathogen Detection project is a senior design driven instrument that will be inexpensive, portable and use Brewster's Angle Straddling Interferometry technology to detect pathogens in biological samples.

Revision History

Rev	Description	Date	Authorization
A	Initial DDD	2/2/16	GG
B	Collimating optics update	2/12/16	GG
C	Updates to SW/HW, optical designs, progress from lab work	2/24/16	GG
D	Changes to reflect updated design and lab work. New plan for integration with mechanical engineering team. Some updates to SW/HW	4/20/16	GG
E	Final updates, edits to appendices, and more information in response to advisor's questions	4/27/16	GG

Contents

Product Requirement Document

See digital document 0003.

System Block Diagram

Overview

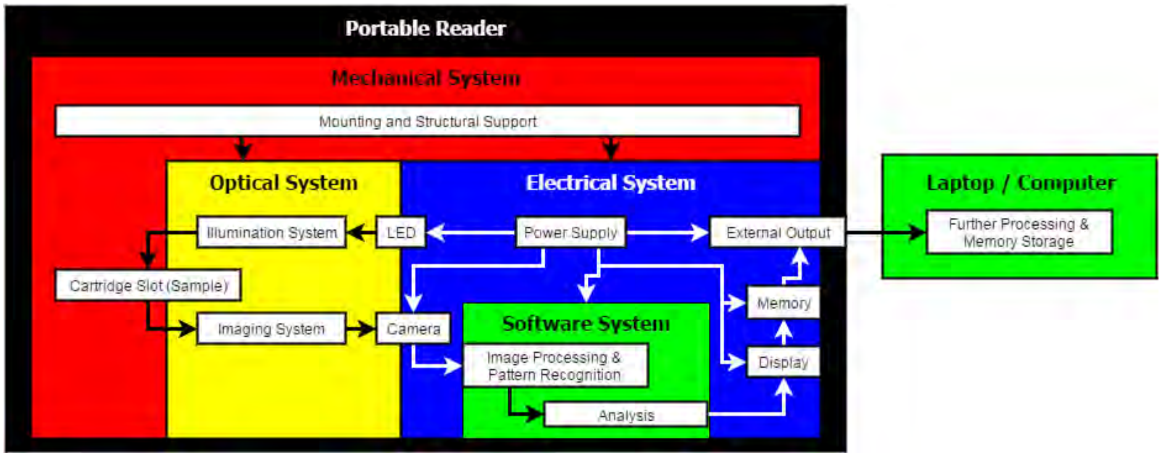


Figure 1: Block diagram of deliverable including mechanical system, optical system, electrical system, software system and the external functions of the laptop.

Optical System

Description of Optical System

The optical system consists of an illumination system and an imaging optic. The purpose of the illumination system is to uniformly illuminate the silicon sample with p-polarized light at the minimum reflectance angle (75.5 degrees). We placed the polarizer before the sample to deliver p-polarized light and to avoid birefringence effects from the silicon wafer on the detector. The contrast of the image is improved with better collimation. The purpose of the imaging system is to map the silicon sample to the size of the detector. We found that longer focal length lenses decrease distortions in the image.

The illumination system uses the following components:

Part	Price
633nm LED, 1.5mW power	~$0.50
Adjustable Iris, 5mm diameter	$77.50 each, <$69.00 in higher quantities
Thin Film Polarizer	$22.50 each, $18 in higher quantities
25mm diameter, 100mm efl , coated, plano-convex lens	$42.00 each, < $33.00 in higher quantities
Firefly MV 0.3 MP Mono USB camera	$300

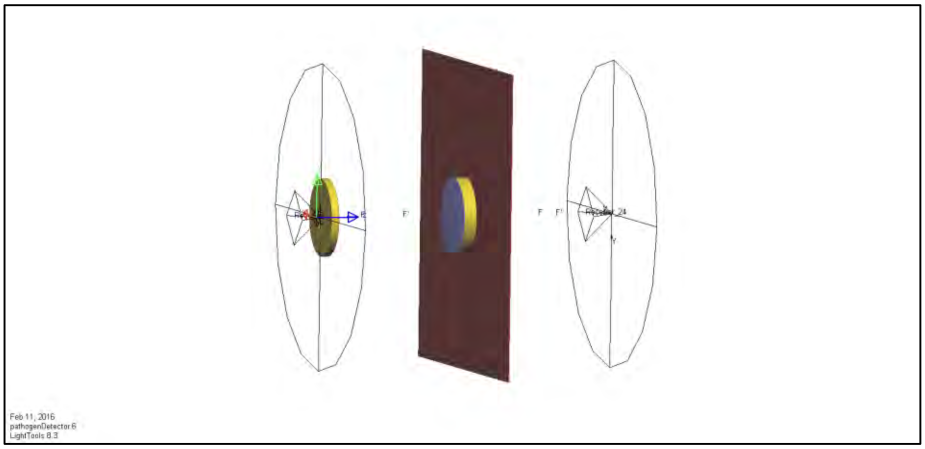


Figure 2: Theoretical model created in the LightTools software.

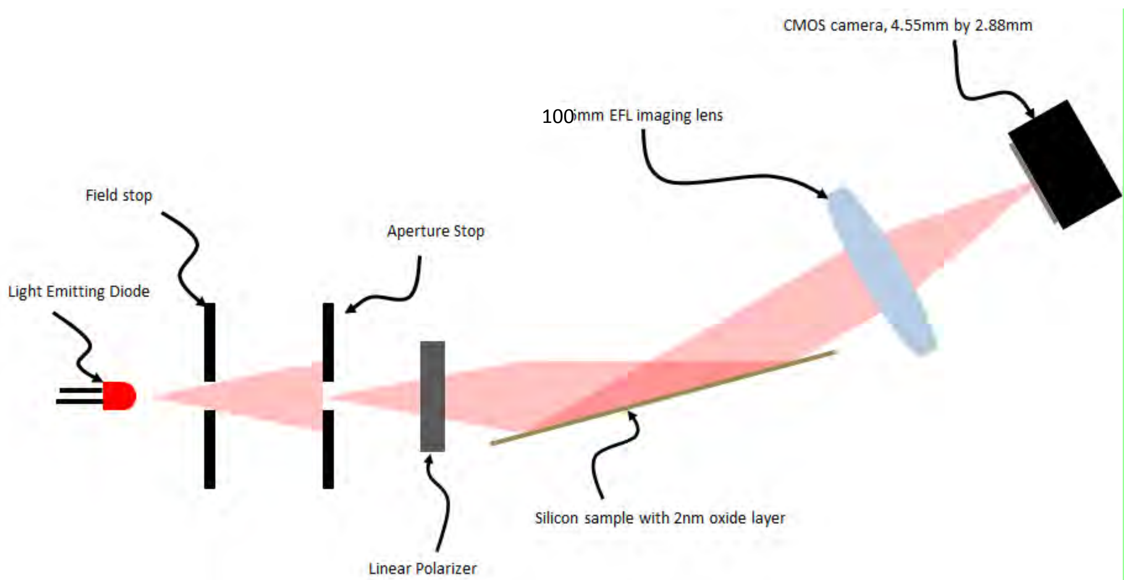


Figure 3: Schematic of the optical system.

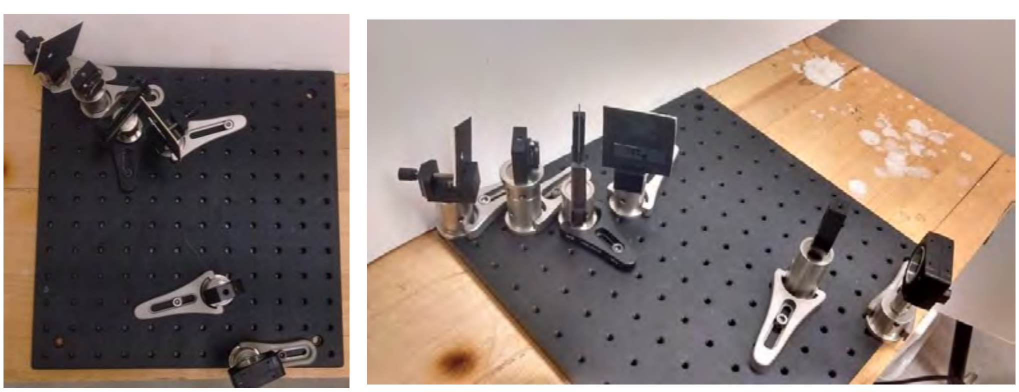


Figure 4: BASI system breadboard. The footprint of the mounting plate is 1ft x 1ft.

Evaluation of S/P ratio

An important specification for our system is the ratio of S-polarized light reflected off of the sample to the P-polarized light reflected off of the sample. This value indicates the level of contrast that the system is able to achieve.

The Firefly MV 0.3 MP Mono USB camera used in the setup does not have a dynamic range that is high enough to image S and P polarized reflected light with the same settings. When S-polarized light is imaged at the minimum reflectance angle such that the camera does not saturate, P-polarized light imaged with the same settings results in an image with all pixels zero-valued.Therefore we added a neutral density filter for the S-polarized light and we took it out for the P-polarized light.

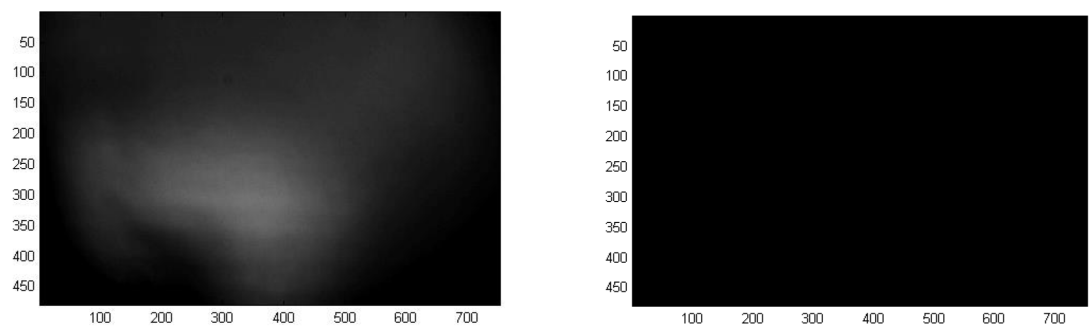


Figure 5: Left: the reflected S-polarized light has a mean value of 31.4 Right: the reflected P-polarized light has an average of 0. Therefore, the camera does not have the dynamic range necessary to detect the proper S/P ratio.

In the lab, we estimated our S/P ratio to be approximately 2000. This value is satisfactory toour customer. Professor Rothberg's experimental value was 10,000. Some errors in our setup may include:

- Purity of polarizer too low (solution: We bought a polarizer with higher extinction ratio)
- Degree of collimation too low (solution: collimate with shear plate, put pinhole back in)
- Angle of incidence on sample is incorrect (solution: try several angles)

The experiment was repeated with two aligned polarizers in place of one. The S/P ratio doubled, indicating that polarization purity may be an important issue.

The Expected Sample

The samples to be used are designed by Professor Rothberg and produced by Professor Shestopalov. There is some flexibility with the size and location of the spots and we are proposing the sample be designed as seen below in order to minimize the size of the entire system. The optical design and dimensions are optimized for such a sample.

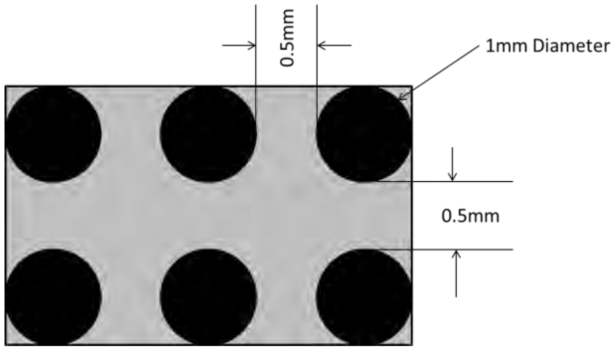


Figure 6: The expected sample that our optical system is optimized for.

The Delivered Sample

February

Professor Rothberg gave us two samples of Silicon chips. These samples have a 2nm thick oxide layer and chemically treated areas, referred to as "spots." Each chip has four spots in a linear array.

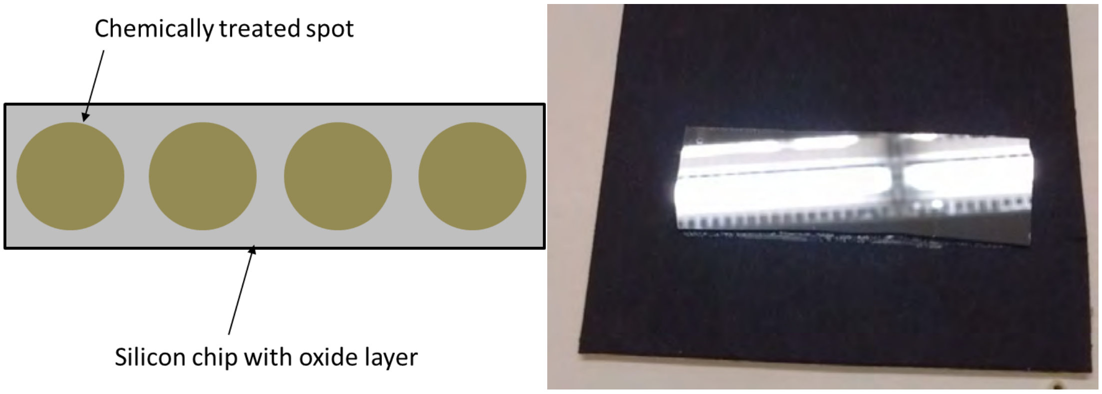


Figure 7: Left: illustration of silicon chip. Right: actual chip mounted on cardstock paper with double-sided tape. The cardstock is clamped to an optical filter mount.

We expected the spots to exhibit a lower S/P contrast ratio than the untreated silicon. Our collimated beam is smaller in diameter than the entire sample, so the sample was scanned across the beam. We expected to see regions of high and low reflectance moving past the camera as the beam crossed a spot. However, when multiple images were captured in this manner, the intensity of the imaged beam remained constant.

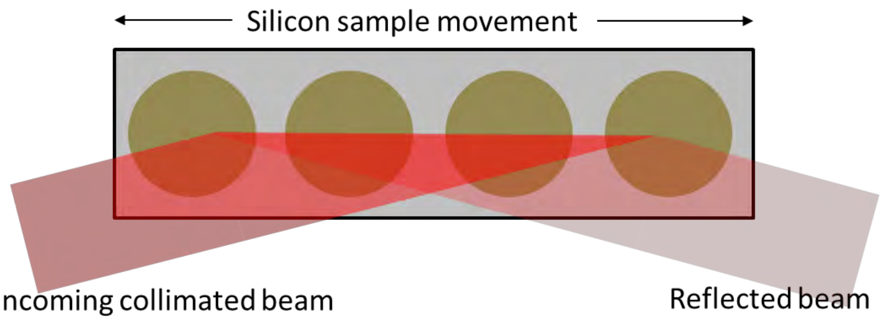
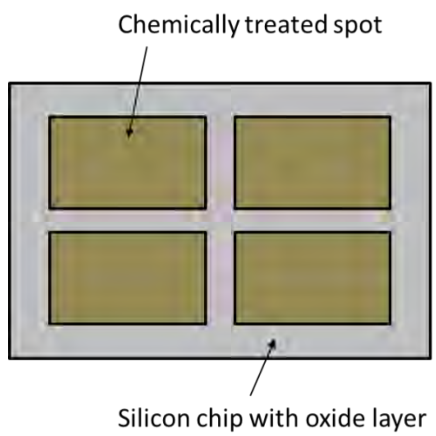


Figure 8: Schematic illustrating the raster-scanning technique used to image the entire sample. No edges were detected on the silicon chip.

March

The most recent sample delivered by Professor Rothberg has the layout as seen on the right. This sample has the same 2nm thick oxide layer on a silicon substrate with chemically engineered spots.

This sample comes with a solution to treat the spots. When the solution is applied to the sample then washed off and dried with compressed air, the intention is that the spots profiles should change size/shape to mimic the changes we would see in testing if a pathogen was present.



Dummy Samples

In order to test our system independently of the samples being delivered by our customer, we have imitated the presence of spots on a sample by lightly applying a sharpie to a silicon substrate with the 2nm thick oxide layer. This layer of sharpie ink is thick enough to destroy the negative interference and show up on our detector as bright spots. For this dummy sample, we recreated the 2x3 array of spots. Image taken with a camera is on the right.

Questions arose regarding whether the spot due to absorption or interference effects. We confirmed that it was due to interference by tilting the sample away from Brewster's angle and observing a steep drop in contrast. Similarly, the oil from a fingerprint showed that the spots are due to interference effects, not absorption.

Figure 9: Dummy sample with six sharpie spots.

Lab Results

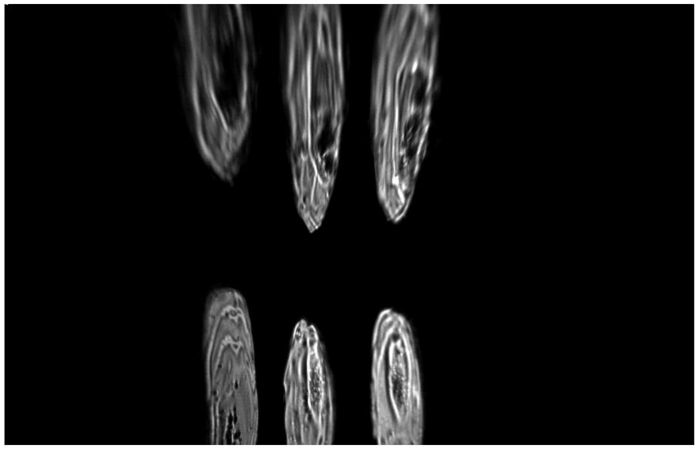


Figure 10: Image of the dummy sample.

Important metrics for the spot-finding algorithm include contrast and distortion. Contrast is defined as:

$$C = \frac{(max - min)}{(max + min)}$$

and represents how easily bright and dark features in an image can be discerned. Distortion represents warping in an image and increases with numerical aperture. The following figure compares images with varying levels of these metrics.

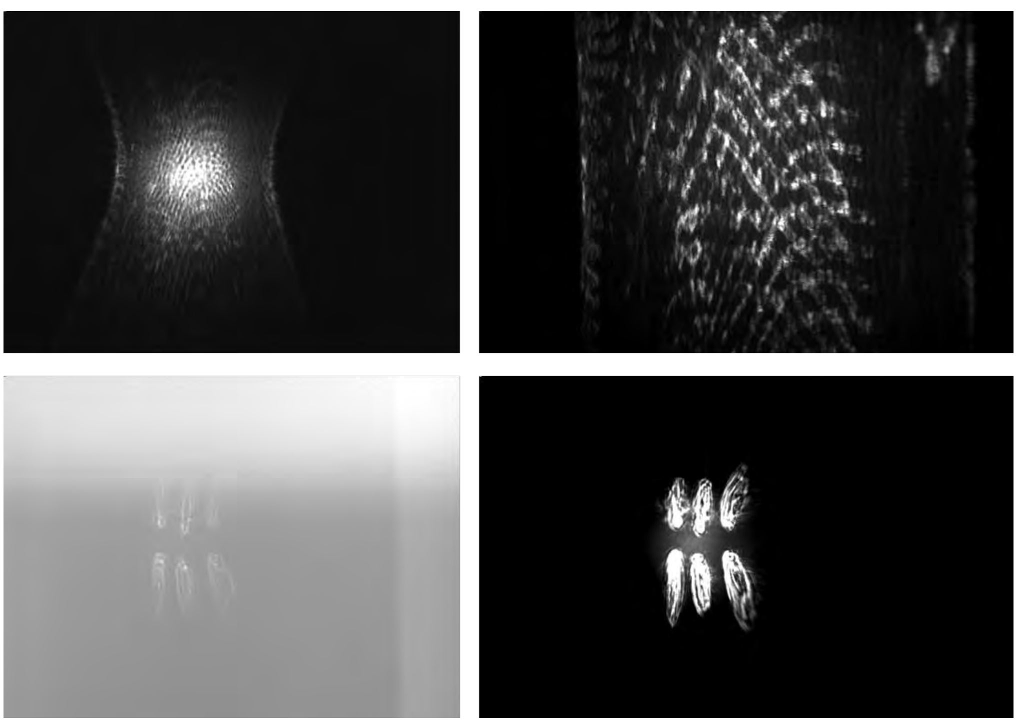


Figure 11: Top left: distorted image. Top right: less distorted image. Bottom left: low contrast image. Bottom right: high contrast image.

Trade-offs in the system:

- Our customer wants a compact, shoe-box sized system. We can achieve this using a shorter focal length imaging lens; however this introduced more distortion into our spots (which compromises the software's ability to detect them.
- Contrast and uniformity are a function of the collimation angle of the incoming light. In the current system, we are not using collimating optics therefore the contrast is lower for some samples, and we are currently exploring how much we can trade off the collimation aspect for a more compact system.

Mechanical System

The mechanical engineering team presented the following deliverable proposals:

1. Mounts for all of the elements in the optical system
2. A mechanical enclosure for the optical system and the electronics
3. A sample holder that can be easily removed and re-inserted with a degree of stability repeatable withina ±0.25 degree tolerance.
4. A mock-up (sketch, or physical) of how the system will look like.

The mechanical engineering team decided to move forward with a prior optical design we had given in March due to time constraints. They will be delivering this housing which could potentially be modified for future iterations of our system.

Electrical and Software System

Software:
A graphical user interface (GUI) application in C++ will be created. The user will be able to:

- Analyze an image:
    - Upload a previous image
    - [Optional] Capture a real-time image (with or without averaging)
- [Optional] Identify a region of interest (ROI) in which the spots are located
- [Optional] Identify a second region representative of the native oxide layer (reference)
- Process the images and identify the spots using modified OpenCV libraries and functions
    - Spots will be outlined and displayed
- Obtain the average 2-D size and intensity (grayscale 0-255) of each identified spot
- Convert the image into a topology map that shows the heights of the spots
    - Based on Professor Rothberg's topology2.m file
- Save the image so it can be further analyzed with other software (e.g. Python, MATLAB)
- [Optional] Obtain difference data that indicates growth of spots (with uncertainties)
    - Create a likelihood distribution that assigns a statistical probability to whether something was detected (on the basis of comparing active versus control spots)

The GUI is capable of communicating with the camera detector via USB 2.0 (however, functionality is limited due to lack of settings). Image segmentation algorithms are combined with graph cuts to estimate the spot sizes. A general methodology is described:

- Defining a rectangular region of interest (ROI) in which all objects are within the ROI and everything else is the background (this can be defined by the user)
- Each pixel is connected to its neighboring pixels and has a label so that it can be described as a Markov Random Field (MRF)
- Optimal labeling can be completed using a variety of methods including graph cuts (similar to max-flow min-cut algorithms)
- By weighting the pixels and edges, a well labeled image will result in meaningful segmentation (foreground from background)
- A standard weighting is using the Gaussian Mixture Model (GMM), and OpenCV uses Expectation-Maximization (EM) to estimate this
- Usually results in four regimes: positively background, probably background, probably foreground, and positively foreground
- The process is iterative and repeated until convergence (or a maximum number of iterations is reached)
- A maximum of 6 spots can be detected at a time (but <4 is optimal). Spots that are either too close to background in intensity (dark) or too small are rejected

Upon identifying the spots, a topology map is created from the image. Further analysis of the image will allow for characterization of each spots and estimated thickness.

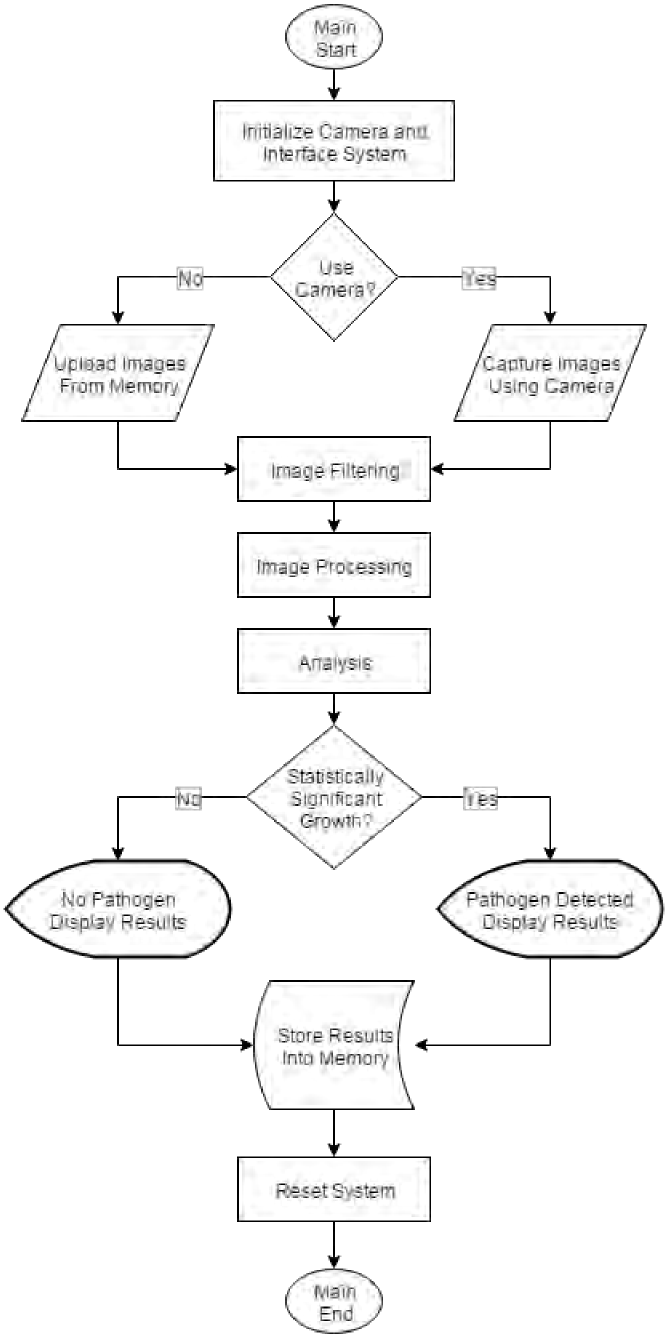


Figure 12: Generalized flowchart of the software system.

Screenshots of GUI application with verifications from MATLAB (3 Examples):

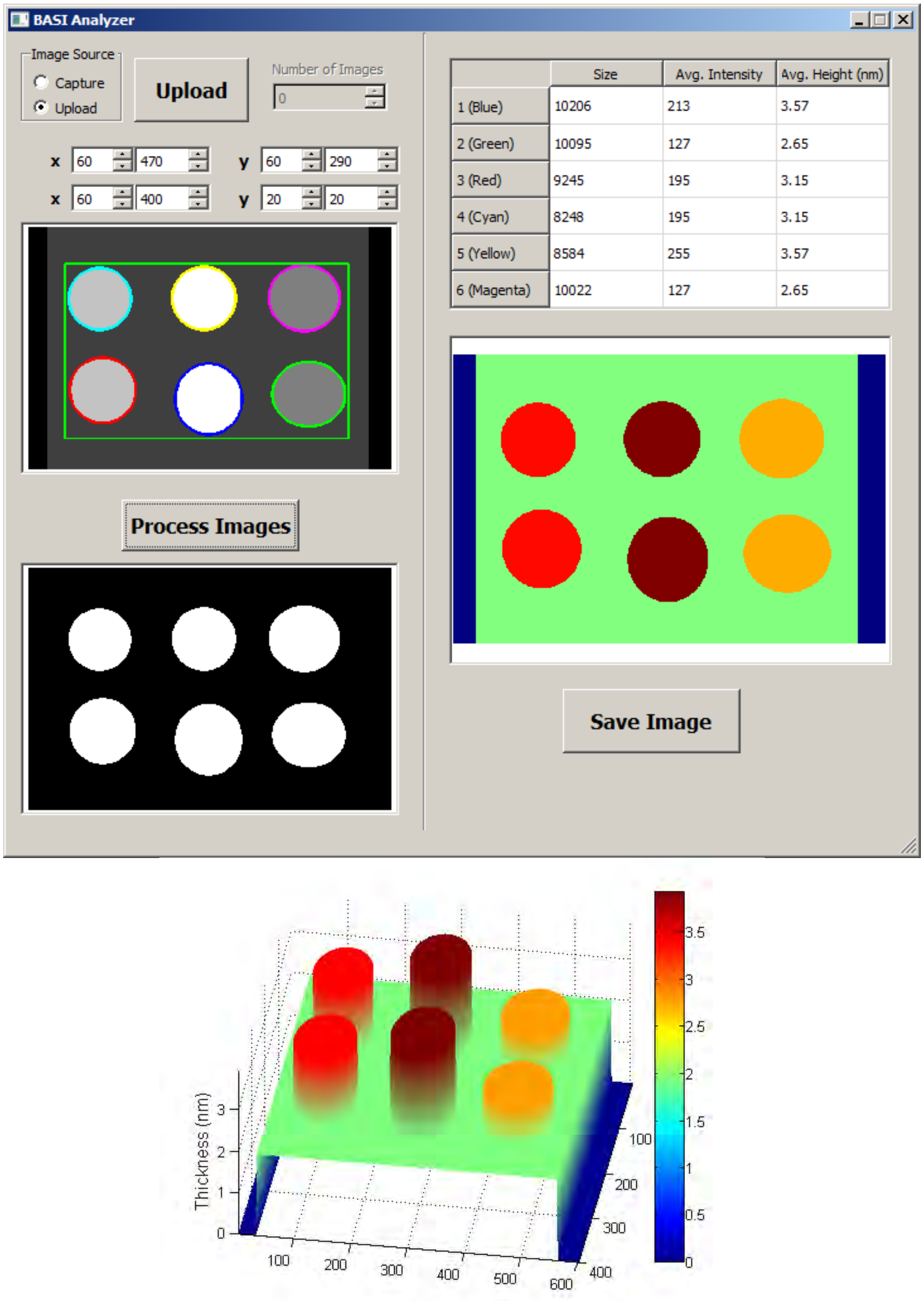


Figure 13: The ideal image. Top: GUI program after processing image. Bottom: MATLAB verification.

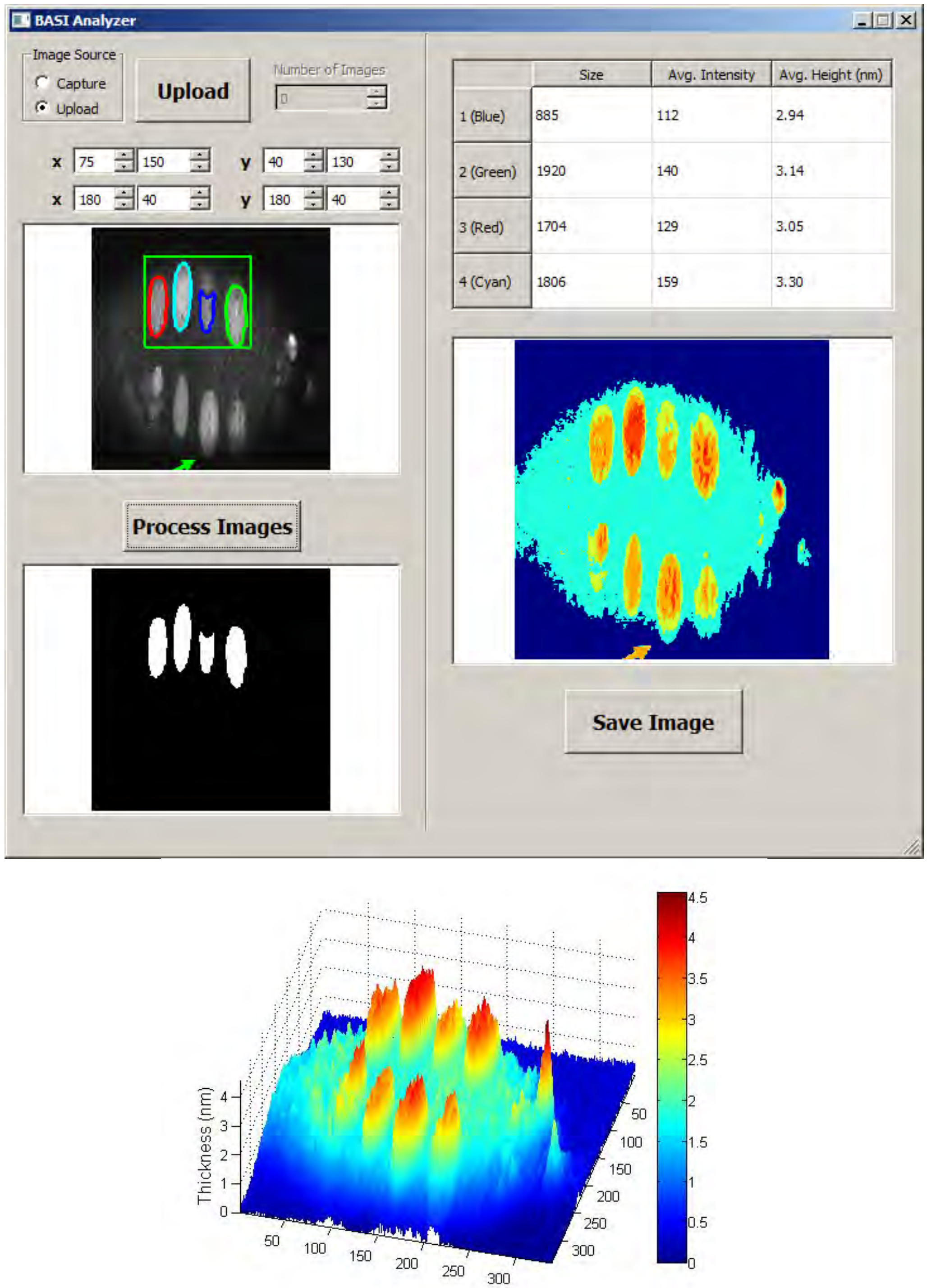


Figure 14: A sample from Professor Rothberg's previous setup. Top: GUI program after processing image. Bottom: MATLAB verification.

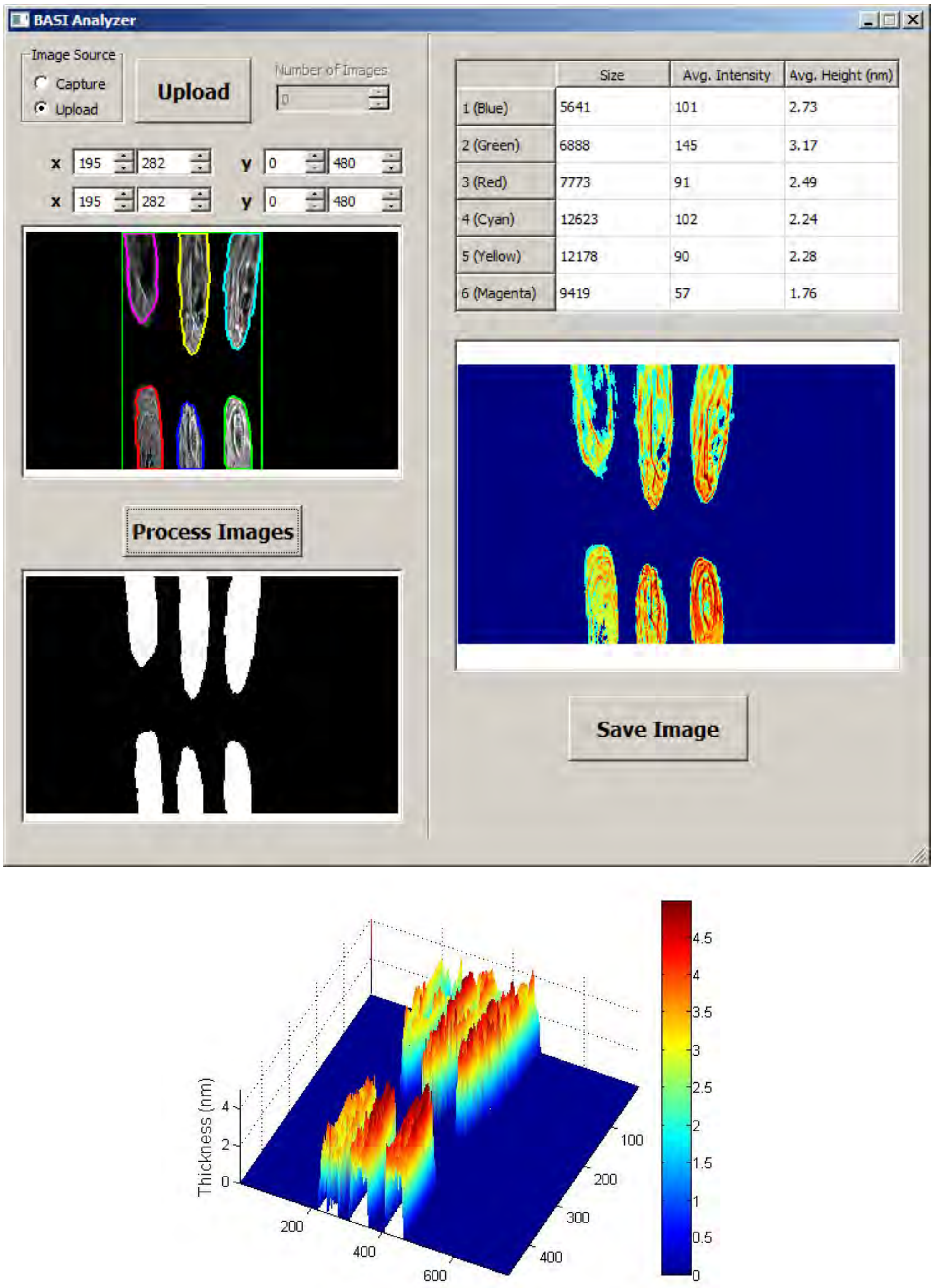


Figure 15: Image of our dummy sample with six sharpie spots. Top: GUI program after processing image. Bottom: MATLAB verification.

Hardware System:

Power circuits can be built for the LED, the camera detector, and supporting electronics.

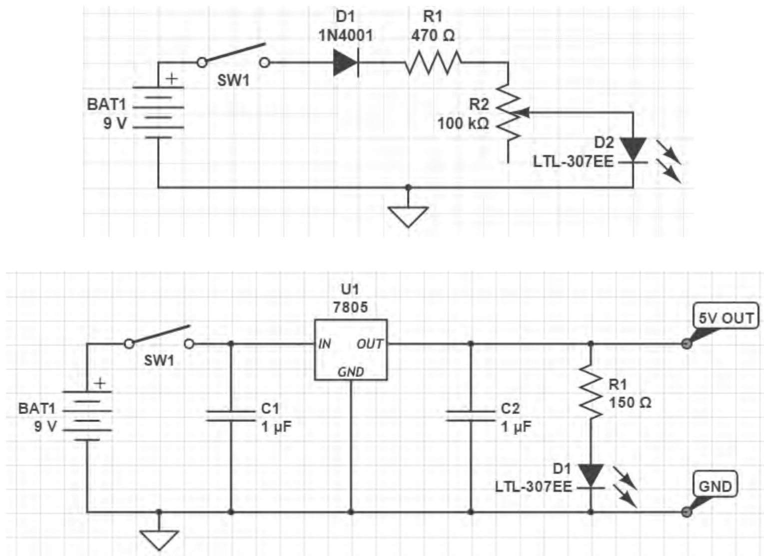


Figure 16: Top: circuit for LED. Bottom: circuit for camera and supporting electronics.

The top circuit is designed so that one can vary the intensity of the LED by turning the potentiometer. A switch and protection diode are included.

The bottom uses a voltage regulator to output 5V that would power the camera and the BeagleBone Black (if not using a USB). Decoupling capacitors and a status LED are included.

The BeagleBone Black is capable of running simplified algorithms onboard. It will output to a LCD screen that indicates the presence of spots. Functionally, it will operate similar to the GUI, except it will not provide images. A block diagram of the system is shown below.

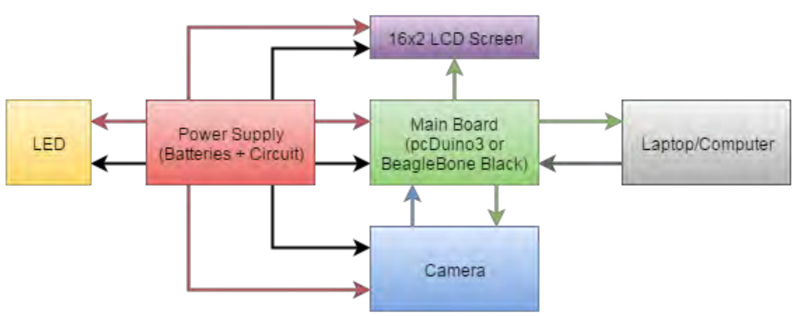


Figure 17: Simplified block diagram of the hardware system.

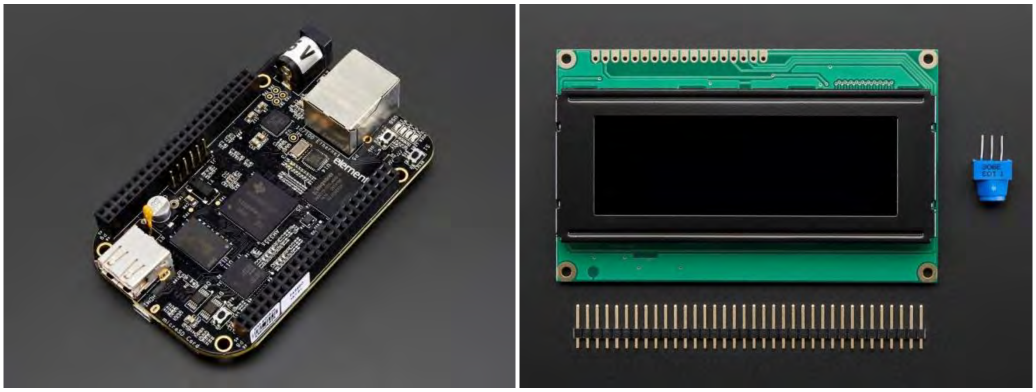


Figure 18: Supporting electronics. Left: BeagleBone Black. Right: 20x4 LCD character display.

The hardware is meant to act as proof of concept in that the reader can indeed be small and portable (since custom circuits can be designed to be much smaller than commercial boards).

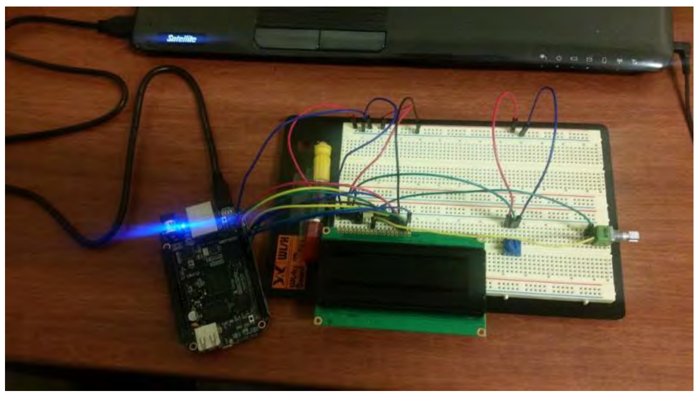


Figure 19: Example setup of the hardware system.

Test Plan and Validation

1. Using the new breadboard BASI system to test the remaining samples (4 silicon wafers with the different patterns of spots) to determine the balance of distortion and compactness. We will also measure contrast and uniformity as a function of collimation angle of incident light to determine if the final system will need a collimating lens or not

2. Determine tradeoff of optical and digital complexity
    a. Make the system as compact as possible without compromising the ability of the software to detect the spots and build the topography map

3. For demonstration of our prototype (design day) we will have:
    a. A dark enclosure
    b. Power supply
    c. Additional samples, possibility of blank silicon wafers to display fingerprints (contingent on delivery of samples from customer)
    d. Final breadboard to show compactness and portability of final design

Risk Assessment

The success of this project is dependent directly upon how compact we can make the system without compromising the effectiveness of the software to detect pathogens. This means achieving a high enough level of contrast and uniformity, and low degree of distortion.

Overall

Objectives Assessment

- "The project involves design, fabrication and testing of a compact Brewster Angle Straddling Interferometry Pathogen Sensing System, as defined by the Customer."
- We have developed an optical system that can be utilized for BASI
- Software has been created to analyze and process the resulting images

Future Steps

- Incorporate the mechanical engineering senior design team build into the system
- Add statistical analysis of the difference images to the software
    - This is dependent on a concrete sample manufacturing process (i.e. the same sample dimensions for each chip)
- Load the software into custom electronics for a fully functional device

Appendix

A: Adjustable Iris

http://www.edmundoptics.com/optomechanics/irises-apertures/iris-diaphragms/standard-series-iris-diaphragms/54352/

Edmund optics | worldwide 1-800-363-1992 | www.edmundoptics.com

10mm Outer Diameter, Iris Diaphragm

Stock No. #54-352

Availability: IN STOCK

$77.50

1 - 4 for $77.50 each.
5 - 9 for $69.75 each.

Specifications

Outer Diameter (mm)	10.00
Maximum Aperture (mm)	5.0
Minimum Aperture (mm)	0.70
Thickness (mm)	4.50
Number of Leaves	6
Type of Lever	Pin
Construction	Brass Housing
Lever Diameter (mm)	1.50
Lever Length (mm)	7.50
RoHS	C

B: Polarizer

http://www.edmundoptics.com/optics/polarizers/linear-polarizers/high-contrast-linear-polarizing-film/86186/

Edmund optics | worldwide

WORLD'S LARGEST INVENTORY OF OPTICAL COMPONENTS
1-800-363-1992 | www.edmundoptics.com

50 x 50mm, 0.75mm Thickness, Polarizing Laminated Film

Stock No. #86-186

Availability: IN STOCK

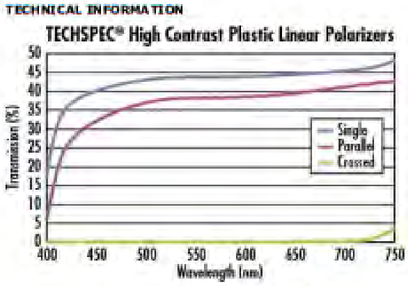
$22.50

1 - 5 for $22.50 each.
6 or more for $18.00.

SPECIFICATIONS

Dimensions (mm)	50.0 x 50.0
Dimensional Tolerance (mm)	+1.0/-1.0
Thickness (mm)	0.75
Thickness Tolerance (mm)	±10
Transmission (%)	Single: 42 Parallel: 36 Crossed: <0.004
Wavelength Range (nm)	400 - 700
Polarization	>99 Efficiency (%)
Extinction Ratio	9000:1
Operating Temperature (°C)	-40 to +80
Construction	Polarizing Film
Type	Linear Polarizer
Substrate	Film
RoHS	C

TECHNICAL INFORMATION



C: 100mm efl Lens

http://www.edmundoptics.com/optics/optical-lenses/plano-convex-pcx-spherical-singlet-lenses/vis-0-coated-plano-convex-pcx-lenses/47350/

Edmund optics | worldwide

1-800-363-1992 | www.edmundoptics.com

TECHSPEC 25.0mm Dia. x 100.0mm FL, VIS 0° Coated, Plano-Convex Lens

Stock No. #47-350

Availability: IN STOCK

$42.00

1 - 5 for $42.00 each.
6 - 25 for $33.60 each.

Specifications

Diameter (mm)	25.0
Diameter Tolerance (mm)	+0.00/-0.10
Clear Aperture CA (mm)	24.00
Effective Focal Length EFL (mm)	100.0
Back Focal Length BFL (mm)	97.17
Focal Length Tolerance (%)	±1
Radius R_1 (mm)	51.68
Edge Thickness ET (mm)	2.77
Center Thickness CT (mm)	4.30
Center Thickness Tolerance (mm)	±0.1
Centering (arcmin)	<1
Surface Quality	40-20
Bevel	Protective bevel as needed
Substrate	N-BK7
Coating	VIS 0°
Coating Specification	R_{avg} ≤0.4% @ 425 - 675nm
f/#	4
Numerical Aperture NA	0.13
Wavelength Range (nm)	425 - 675
Focal Length Specification Wavelength (nm)	587.6
Typical Energy Density Limit	5 J/cm² @ 532nm, 10ns
Type	Plano-Convex Lens
RoHS	C

D: Firefly USB camera

https://www.ptgrey.com/firefly-mv-03-mp-mono-usb-20-micron-mt9v022

HOME / CAMERAS / IEEE1394 (FIREWIRE) / FIREFLY MV 1394A / FIREFLY MV 0.3 MP MONO USB 2.0 (MICRON MT9V022)

Part Number

FMVU-03MTM-CS

Technical Specifications

- 752 x 480 at 60 FPS
- Micron MT9V022 CMOS
- Global shutter
- Mono
- CS-mount

Availability: In-stock

Please Log-In or Register to see pricing and access store.

Model Specifications | Documents | Downloads

Resolution	752 x 480
Frame Rate	60 FPS
Megapixels	0.3 MP
Chroma	Mono
Sensor Name	Micron MT9V022
Sensor Type	CMOS
Readout Method	Global shutter
Sensor Format	1/3"
Pixel Size	6.0 µm
Lens Mount	CS-mount
ADC	10-bit
Gain Range	0 dB to 12 dB
Exposure Range	0.031 ms to 512 ms
Trigger Modes	Standard, skip frames
Partial Image Modes	Pixel binning, ROI
Image Processing	Gamma, lookup table, hue, saturation, and sharpness
User Sets	2 memory channels for custom camera settings
Non-isolated I/O Ports	2 bi-directional
Serial Port	1 (over non-isolated I/O)
Auxiliary Output	3.3 V, 150 mA maximum
Interface	USB 2.0
Power Requirements	4.75 to 5.25 V
Power Consumption (Maximum)	> 1 W
Dimensions	44 mm x 34 mm x 24.4 mm
Mass	37 grams
Machine Vision Standard	IIDC v1.31
Compliance	CE, FCC, KCC, RoHS
Temperature (Operating)	0° to 40°C
Temperature (Storage)	-30° to 60°C
Humidity (Operating)	20 to 80% (no condensation)
Humidity (Storage)	20 to 95% (no condensation)
Warranty	1 year

Accessories:

Required:

- Cables
- Camera Lens
- Host Adapters
- Tripod Mount Adapter (included)

Optional:

- GPIO Cable

Recommended System Configuration:

- Windows or Linux (32-bit or 64-bit)
- 3.1 GHz or equivalent CPU
- 2 GB RAM or more
- 200 MB hard drive space
- USB 2.0 Port

E: Former Design Data

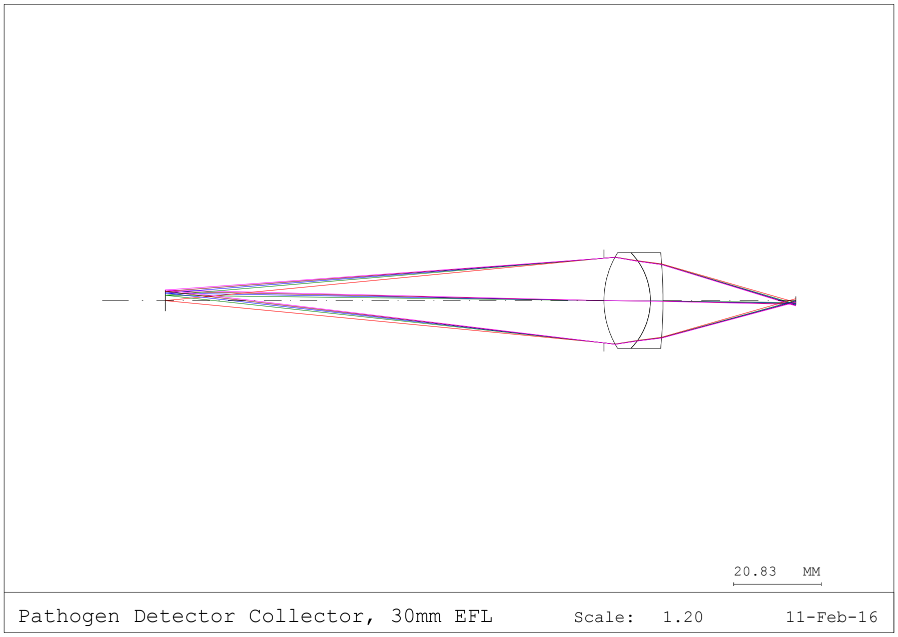


Figure i: CodeV rendering of the 30mm lens that puts light through the pinhole. The same lens is used after the pinhole to collimate the light.

```
    Pathogen Detector Collector, 30mm EFL
              RDY              THI      RMD       GLA           CCY    THC    GLC
> OBJ:        INFINITY      104.349772                          100      0
  STO:        INFINITY        0.000000                          100    100
    2:        21.17000       11.040000       NBAF10_SCHOTT      100    100
    3:       -16.08000        3.000000       NSF10_SCHOTT       100    100
    4:      -118.66000       34.309823                          100    PIM
  IMG:        INFINITY       -2.699635                          100      0

SPECIFICATION DATA
   EPD       20.00000
   DIM             MM
   WL          635.00
   REF              1
   WTW              1
   XOB        0.00000       0.00000       0.00000       0.00000       0.00000
   YOB        0.00000       1.25000       1.76780       2.16510       2.50000
   WTF        1.00000       1.00000       1.00000       1.00000       1.00000
   VUY        0.00000       0.00000       0.00000       0.00000       0.00000
   VLY        0.00000       0.00000       0.00000       0.00000       0.00000
   POL              N

REFRACTIVE INDICES
    GLASS CODE                    635.00
   NBAF10_SCHOTT                1.666952
   NSF10_SCHOTT                 1.722917

SOLVES
   PIM

No pickups defined in system


INFINITE CONJUGATES
   EFL        30.0603
```

```
   BFL         22.2833
   FFL        -29.2140
   FNO          1.5030
AT USED CONJUGATES
   RED          0.4001
   FNO          2.0970
   OBJ DIS    104.3498
   TT         150.0000
   IMG DIS     31.6102
   OAL         14.0400
   PARAXIAL IMAGE
    HT          1.0002
    THI        34.3098
   ANG          1.3724
   ENTRANCE PUPIL
    DIA        20.0000
    THI         0.0000
   EXIT PUPIL
    DIA        20.5793
    THI        -8.6477
```

Design 1: 0.05% throughput, collimates all rays to within ±0.2 degrees, and is 200mm long

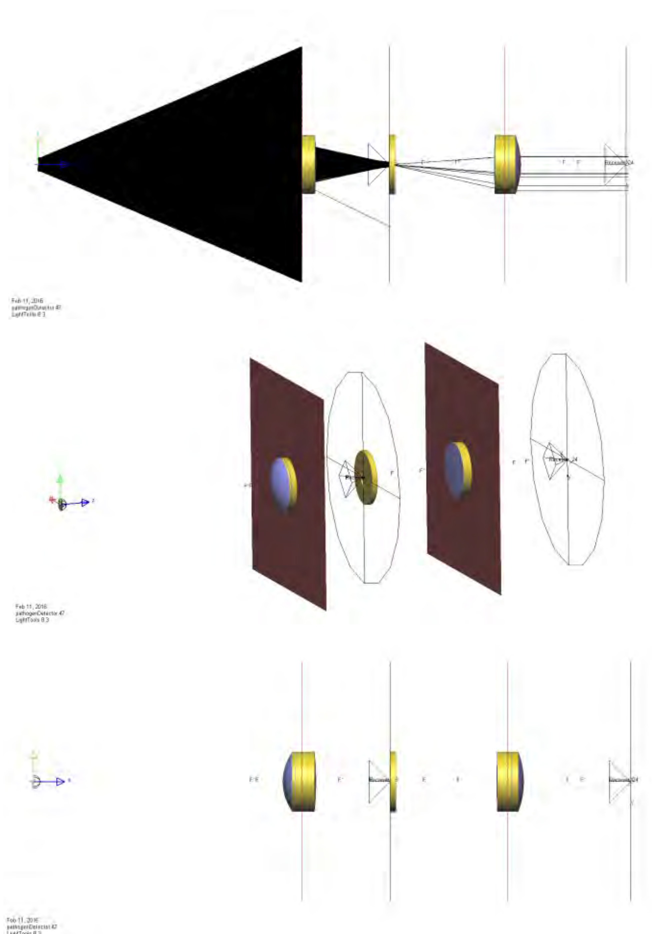
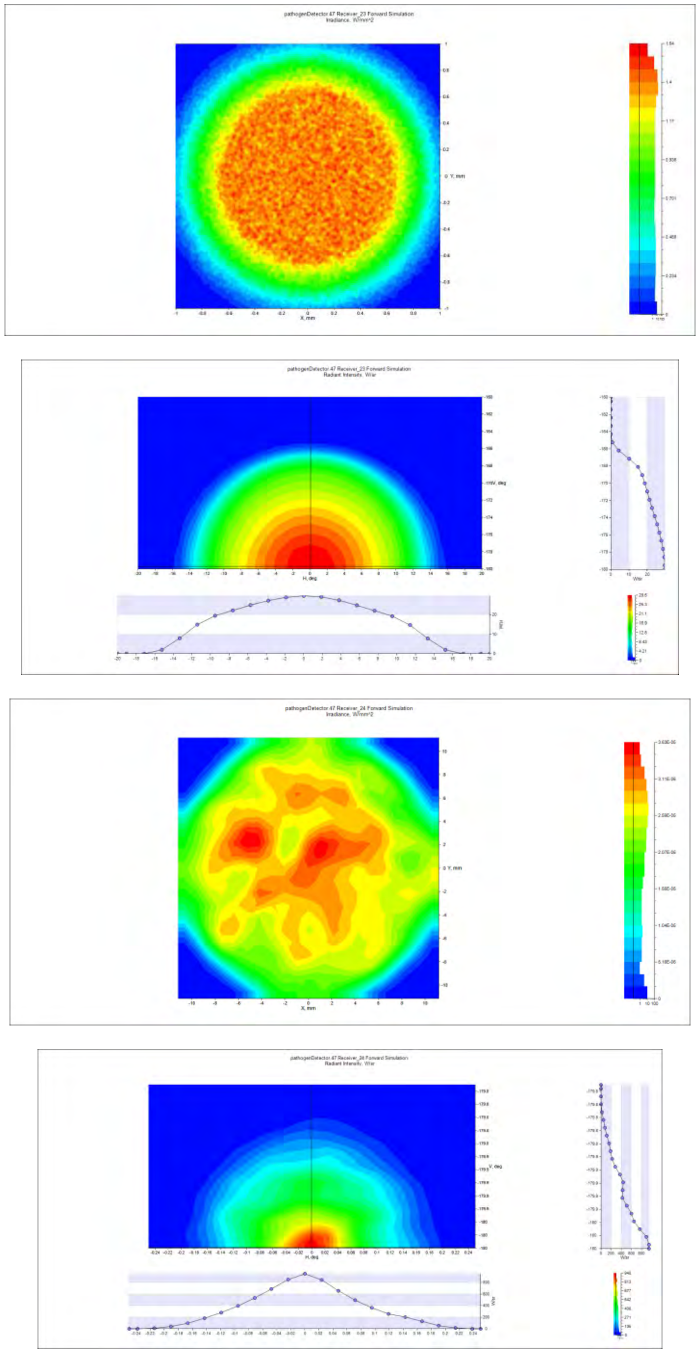


Figure ii: LightTools model of Design 1.



Intensity

Figure iii: LightTools simulations of Design 1. Top: spot on pinhole (irradiance). Upper middle: intensity distribution of pinhole. Lower middle: collimated light after lens B (irradiance). Bottom: intensity distribution after lens B.

Design 2: 0.023% throughput, LED is 2mm from pinhole

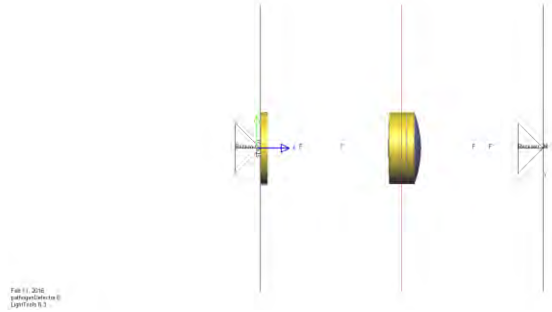


Figure iv: LightTools model of Design 2.

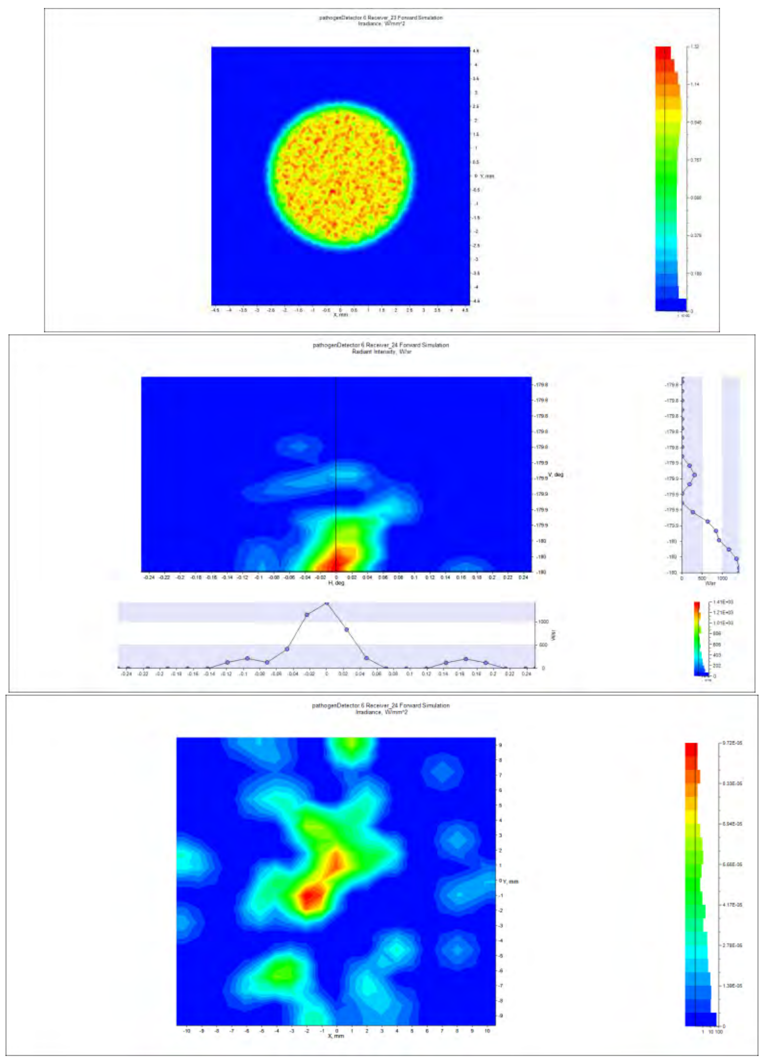


Figure v: LightTools simulations of Design 2. Top: intensity at pinhole. Middle: intensity after lens. Bottom: irradiance after lens.