Design Description Document

Pathogen Detector

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Product Requirements Document
(refer to previous document)
Overview

This project is an automated, more efficient, smaller implementation of Brewster Angle Straddle Interferometry (BASI) for the detection of pathogen samples on silicon wafers used for testing. A thin film of silicon dioxide (SiO\textsubscript{2}) grows spontaneously on Silicon (Si), forming an optically flat native oxide layer with a consistent thickness of approximately 2nm. This presents a convenient and reliable three-layer interface where complete destructive interference of incident, p-polarized light can be achieved; by illuminating a test slide with collimated, p-polarized light at a specific angle between the two Brewster’s angles of the air/oxide interface and the oxide/silicon interface accommodating BASI, half of the incident light experiences a \( \pi \) -phase shift while half undergoes no phase shift, resulting in complete destructive interference upon exiting the slide. Anything that is on the surface, such as an additional layer of other material reflects light. As such, slides are engineered with an array of spots of binding chemistry on the surface that may receive specified pathogens. The user drops potentially-pathogen-containing fluid on the surface, then inserts the slide into the system for testing. The measured increase in brightness of these spots compared to a blank slide can be used to quantitatively characterize the presence of bound pathogens on the slide.

Customer and Objectives

The customer is Professor Lewis Rothberg of the University of Rochester Chemistry Department. The objective of the project is to provide the customer with:

1) An improved optical design to the current model in terms of size, optical quality, and power consumption;

2) automated calibration/measurement functionality.

First Order Design

This system presents a three-part design: optics for illuminating a slide with collimated p-polarized light and imaging the reflected light; a motorized Arduino-servo subsystem that rotates the slide angle and calibrates to the BASI destructive interference condition using MATLAB software.

System Block Diagram

1) Optical system for illuminating the test slide at the BASI angle and imaging the reflected light

2) Arduino board and servo motor system to calibrate angle of incoming light to BASI angle

3) Software that interacts with Arduino/motor system to calibrate angle of incoming light to BASI angle
Figure 1 – Overall layout of system
Optical Design: 4-lens system
A 625nm wavelength (red) LED behind a diffuser is the illumination source, placed at optical infinity (~10*EFL of first lens). Light passes through illumination optics comprised of a 2f double telecentric system (with an aperture at the focal point) and a polarizer to provide collimated uniform illumination of p-polarized light. The slide is oriented at the BASI angle with respect to the optical axis. Reflected light from the slide passes through imaging optics comprised of a 2f double telecentric system (with an aperture at the focal point) and is imaged onto a detector.

Figure 2 – First-order layout of optical design

Theory: Brewster Angle Straddle Interferometry
A ~2nm layer of SiO2 grows natively on Silicon. This presents a three layer—Si, SiO2, air—thin film interface. For collimated light entering an interface below the interface’s Brewster’s angle, both s-polarized and p-polarized light are reflected. For collimated light entering an interface above the interface’s Brewster’s angle, s-polarized light is reflected, while p-polarized light experiences a phase shift of $\pi$ on reflection. By illuminating the Si/SiO2/air interface at an angle so as to “straddle” the separate Brewster’s angles for the two interfaces (Si/SiO2 and SiO2/air), destructive interference occurs for reflectance amplitudes of the same magnitude, but opposite signs.
The wafer is a three layer interface, so we can use the airy equation for thin films to compute the reflected power. The Fresnel equations for an interface between two optical media is:

**Equation 1:**

\[
\begin{align*}
(a) \ r_{s12} &= \frac{n_1 \cos \theta_1 - n_2 \cos \theta_2}{n_1 \cos \theta_1 + n_2 \cos \theta_2} \\
(b) \ r_{p12} &= \frac{n_1 \cos \theta_2 - n_2 \cos \theta_1}{n_1 \cos \theta_2 + n_2 \cos \theta_1}
\end{align*}
\]

There are two interfaces on the wafer, and the Fresnel equations apply to both. The airy equation can be used to find the combined reflectance, for p-polarization, for the two interface (three layer) system. In the following equations (1) refers to air, (2) refers to SiO$_2$, and (3) refers to Si.

**Equation 2:**

\[
\begin{align*}
\text{r}_{p, \text{total}} &= r_{p,1,2} + r_{p,2,3} e^{-2i \beta} \\
&= \frac{r_{p,1,2} + r_{p,2,3} e^{-2i \beta}}{1 + r_{p,1,2} r_{p,2,3} e^{-2i \beta}}, \text{ where } \beta = d_2 \frac{2\pi n_2 \cos \theta_2}{\lambda}.
\end{align*}
\]

$\beta$ is the phase thickness of the SiO$_2$ layer, $d$ is the thickness of the SiO$_2$ layer, $\lambda$ is the wavelength.

To find the reflected power, the modulus squared is taken.

**Equation 3:**

\[
\begin{align*}
(a) \ R_{s,1,2} &= |r_{s,1,2}|^2 \\
(b) \ R_{p,1,2} &= |r_{p,1,2}|^2
\end{align*}
\]

For the following calculations the refractive index of air is 1.0003, the refractive index of SiO$_2$ is 1.4573, and the refractive index of Silicon is 3.895+0.22*i, where i is the imaginary number.
Figure 3: Reflected power for three layer interface. At BASI angle, theoretical reflected light is \( R_p = 8.21 \times 10^{-05} \), \( R_s = .77 \).

**LightTools/CodeV Design**

Initial designs were laid out in CodeV optical design software and then imported into LightTools for analysis. The wafer is modeled as a mirror oriented at the BASI angle (LightTools does not model thin film interference). Using this simplified model of the slide, we also removed the polarizer from the system, as its effects would not be useful to simulate in the LightTools model.
Figure 4 – LightTools design of optical system, both without traced rays (top) and with traced rays (bottom).

In this iteration of the setup, it is assumed that the CCD would have an imaging lens of capable diameter for imaging the entire spot.

**Photon Budget**

The lenses in the designed system (3x18mm EFL, diameter plano-convex, 1x18mm EFL, diameter biconvex) have a reflectivity spec of less than 1.75% reflectivity between 400nm and 700nm at normal incidence.\(^1\) We consider the ratio of the transmission of p-polarized light to s-polarized light throughout the system in order to assess collimation. Assuming the test slide is correctly oriented at BASI angle, we can expect .00003% transmission of p-polarized light (since the reflected light will go on to the rest of the system instead of destructively interfering) and 76.93% transmission of s-polarized light as derived from Fresnel equations. Transmission of the various elements in the system are outlined below:

<table>
<thead>
<tr>
<th>Element</th>
<th>Affected surfaces</th>
<th>Transmission of p-polarized (at 625nm)</th>
<th>Transmission of s-polarized (at 625nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lens 1</td>
<td>2</td>
<td>98.25%</td>
<td>98.25%</td>
</tr>
<tr>
<td>Lens 2</td>
<td>2</td>
<td>98.25%</td>
<td>98.25%</td>
</tr>
<tr>
<td>Slide (assuming orientation at Brewster’s angle)</td>
<td>1</td>
<td>.008%</td>
<td>76.93%</td>
</tr>
<tr>
<td>Lens 3</td>
<td>2</td>
<td>98.25%</td>
<td>98.25%</td>
</tr>
<tr>
<td>Lens 4</td>
<td>2</td>
<td>98.25%</td>
<td>98.25%</td>
</tr>
<tr>
<td>Overall total transmission</td>
<td></td>
<td>.003%</td>
<td>71.28%</td>
</tr>
</tbody>
</table>

Theoretically, this shows a contrast ratio between s-polarized light and p-polarized light to be around 23760:1 for perfect alignment. Realistically, there will be some alignment issues and our contrast ratio is expected to be much lower; data from previous BASI designs shows that a contrast ratio of around 2000:1 is achievable\(^2\). Measurements of completely s-polarized light and completely p-polarized light on a clean slide can be used to confirm alignment of the system based on intensity ratios.

Based on the dimensions of the designed slide and the currently designed system, we expect an image height of 5.75mm x 13mm, representing 1:1 magnification (no magnification) of the slide image. The pathogen spot size test areas on the slide are designated at a 2mm diameter separated by 3mm spacing between spots, which is very manageable.

A PointGrey Firefly MV camera containing a sensor chip Aptina MT9V022 is used. It has dimensions of 752px x 480px and a pixel pitch of 6µm x 6µm, for a sensor size of 4.512mm x 2.88mm\(^3\). The amount of the image we will be able to see on the camera will depend on the imaging optics for the camera (independent of the relay system following the slide). The sensor has a dynamic range of >55dB, translating to a power ratio of 316227:1, which is much larger than the theoretical max limit of the s-polarized/p-polarized light contrast ratio; the camera is more than sensitive enough to make the required measurements.

\(^2\) Gregory McKay’s thesis paper, provided by Prof. Lewis Rothberg

Mechanical Design

Slide Design

Figure 5 – Layout of test slide; top down view. Circles represent possible locations of sample placement on slide.

Figure 6 – Layout of test slide; side view.

The test slides have dimensions of 23mm x 13mm, with pathogen “spot” sizes of 2mm diameter spaced 3mm edge to edge. With these dimensions, there will be 8 spots on each slide, 4 spots in 2 rows. From the point of view of the optical system, the projected area of the slide from BASI angle is 5.75mm x 13mm. In order to ensure that the slide is completely illuminated by the illumination system, the minimum necessary diameter of all optics in the system is 15.8mm.

Angular Tolerancing

Though BASI is convenient in its lack of too significant sensitivity due to changes in illumination angle, an analysis of angular precision of the servo motor is necessary. Servo motors are feedback control systems with perfect angular resolution. The angular resolution is limited by the signal that the Arduino can send to the servo.
The Arduino controls the servo angle by sending a pulse to the servo that is high voltage for the first part of the pulse, and low voltage for the second part of the pulse. The length of the high voltage part of the pulse determines the servo angle—the Arduino will keep sending this pulse to the servo until the value is changed, at which point the high voltage length is changed.

The Arduino output pin is 8 bits, which means 256 possible high voltage pulse widths. The maximum pulse width is 1.020e-03 s. The smallest pulse width is the maximum pulse width divided by the number of possible pulse widths, 256. The result is 3.986e-06 seconds as the smallest pulse width. This servo ranges from 0 - 1800e-06 seconds to rotate through an angle range of 180 degrees. With this conversion, the smallest possible angle is \(0.3986\) degrees, or \(0.007\) radians, which is suitable for the application (Figure 3).

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4 http://www.me.berkeley.edu/ME102B/Lab4/images/servo-pwm_w400.jpg
Software Design
In order to ensure correct BASI illumination, an auto-calibrating system is implemented with MATLAB and Arduino. MATLAB software is written to communicate over a virtual serial port with an Arduino that controls the servo. The pseudo code is as follows:

1. User places slide in system (w pathogen)
2. For loop (4-6 iterations):
   a. Arduino moves angle to next near-BASI point
   b. Capture image
   c. Measure average irradiance of image; store
3. Irradiance vs angle plot. This will be a positive parabola. The ideal BASI angle will be at the minimum of this parabola.
4. Move angle to plot minimum
5. Capture image of wafer.
6. Remove slide; wash, put back in
7. Capture background image
8. Subtract background, image process
Data and Results

Lab Setup

Figure 8 – Picture of working lab setup.

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Additional comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lenses</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Illumination system:</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 x 18mm EFL, diameter plano-convex lens</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 x 18mm EFL, diameter biconvex lens</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Imaging system:</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 x 18mm EFL, diameter plano-convex lens</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 x 18mm EFL, diameter biconvex lens</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Imaging lens for CCD:</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 x 18mm EFL, diameter biconvex lens</td>
<td></td>
</tr>
<tr>
<td>All lenses were purchased from Edmund Optics. Ultimately, the camera we received for the project did not have an imaging lens; an additional lens was used to image the light onto the detector.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polarizer</td>
<td>1 x glass polarizer</td>
<td>Polarizer received from Prof. Lewis Rothberg.</td>
</tr>
<tr>
<td>Diffuser</td>
<td>1 x ground-glass diffuser</td>
<td>A small ground-glass diffuser was used to improve uniformity of light source.</td>
</tr>
<tr>
<td>Apertures</td>
<td>3</td>
<td>Two apertures are used in the optical system to reduce stray light effects.</td>
</tr>
<tr>
<td>LED</td>
<td>1 x OSRAM LR W5SN</td>
<td>A red LED at 625nm is used as a light source.</td>
</tr>
<tr>
<td>Camera</td>
<td>1 x Point Grey Firefly MV 0.3 Mono USB 2.0</td>
<td>A camera was purchased to take images for testing. This</td>
</tr>
</tbody>
</table>
Various difficulties were encountered while constructing the lab setup. The initial mounting equipment proved to be too large for our design; the diameter of the mounting posts were much too large to fit mounted apertures between two mounted lenses. The theoretical design could not be assembled without some workarounds. Ultimately, apertures were mounted “sideways” using an off-axis rail mount whose angle could be adjusted such that the apertures would be aligned. Using this method, mounts did not collide with each other when attempting to align elements to the specified distances as designed, but were inconvenient as they were not fixed in place.

Additionally, the camera we purchased did not have its own imaging lens. A fifth lens was introduced as an imaging lens in order to use the sensor for data collection. The imaging lens used was biconvex and had a short focal length; this introduced spherical aberration into the system output, which limited the imaging quality.

Testing also experienced a delay due to a mixup in the samples provided by Prof. Lewis Rothberg. The slides given appeared to show the opposite of the expected image when under test; after proper alignment, the images of the slide showed dark spots on a brighter background instead of bright spots on a darker background. After some time it was concluded that the slides given may have been for a separate experiment where spots were blocked and the slide background unblocked such that the spots would resemble “holes” instead of “raised spots” as would be expected. Though that is not to say we could not evaluate our test setup; while a proper analysis of pathogen spot images could not be obtained, some initial theory could be tested in the lab setup as mentioned using clean slides. The slides used do not match those defined in the slide design section but proved to be adequate for testing.

Figure 9 – Test image of alternate samples in lab setup (left) compared with test images in previous iterations of BASI design provided by Prof. Lewis Rothberg (right). Lab images are distorted due to spherical at full field.
Data Analysis
The theoretically ideal design has perfectly uniform illumination such that all spots receive equal amounts of light; and perfect collimation (zero beam divergence) so that all light hits the wafer at the correct BASI angle. These must be measured and characterized, and evaluated by the theoretical s- to p-polarization contrast ratio.

Divergence of Illumination
This is tested by measuring the beam diameter at various distances after the last illumination lens and plotting versus distance.

![Illumination Beam Divergence](image)

Figure 10: Illumination beam divergence. Measured beam radius is given as a function of distance from last lens surface. Slope of curve is equal to tangent of divergence angle. Divergence angle = .03032 rads = 1.737 degrees

Illumination Uniformity
An image of the beam after the illumination optics was taken and irradiance was averaged over multiple beam ‘cross-sections’.
Figure 11: Illumination uniformity. Wafer width for reference--100% uniformity desired across entire wafer width.

The uniformity varies by, at most, .06 relative power over the wafer width.

Contrast Ratio
Images of the beam after the illumination optics were taken for s- and p- polarization. The s-polarization required an ND2.0 filter so as to not over expose the camera.

Contrast ratio = s-pol:p-pol = (100.8033/ND2.0 attenuation factor):11.5792 = 10080.33:11.5792 = 935:1
Test Plan: Optical system and prototype

1. Further testing

Further testing will involve setting up the basic system (no moving parts) and validating that it works with a test slide (containing ‘dummy’ pathogen of some chemically added layer that behaves optically analogously to pathogen).

2. Test prototype (with mechanics)

Fully testing the prototype will involve designing a CAD model and constructing the full design (optical design in 3D-printed case with movable arm controlled by Arduino/servo-motor system) and verifying that the calibration and alignment methods work. Following the algorithm of the MATLAB program, the prototype will be validated if the system calibrates to Brewster’s angle correctly after several iterations and the MATLAB program appropriately calculates the thickness of the pathogen-containing film on the test slide.
Risk Assessment

In testing our design, there will be some significant risks in alignment and image quality of the system. Our design is based on an older design for BASI interferometry that uses larger lenses. Using the calculated minimum size needed for the optics in order to resolve the slide, our system images the slide with smaller optics (18mm), which have shown some issues with regard to spherical aberration (figure 9). Additionally, the precision required to align the lenses may prove to be infeasible in a manually-aligned lab setup; typical mounting posts for optics are larger than some of the air spacing thicknesses required in the theoretical design and may not be achievable without purchasing smaller mounts or devising some sort of workaround. Though these present risks for testing, they are nullified upon the introduction of a mechanical system with perfect (within tolerances) alignment.

Once in use, the only risk of use is that of pathogen contact, which is beyond the scope of this design.
Summary and Future Plans

Despite some setbacks in creating and testing our full design, including acquiring proper samples, technical difficulties and alignment issues, we were able to produce a functioning lab setup capable of implementing BASI technique for pathogen detection. Given more time, we would have liked to construct a mechanical design and test the full system with proper angle calibration using the servo-motor/Arduino.

Design development will continue in summer 2015, and image processing development will continue upon receipt of test slides from the customer.

As the system is made smaller, alignment becomes more and more of an issue, especially for lab testing involving manual alignment. With an ideal mechanical design, the angle made by the two “arms” of the system are fixed to some tolerance and lens alignment can amended with built in mounting; issues due to alignment of the lenses and the system with a proper mechanical design become trivial. The design then becomes limited by uniformity of the light source and illumination angle.

Cost was a relevant target in this design; stock lenses and standard mounting equipment were used to make the system affordable. For an even smaller system, higher quality lenses can be used with an engineered diffuser to improve collimation of the light source. A laser source may even be considered to further improve collimation. Aberrations at the image can also be reduced with customized optics more suited for this design.
References


- Gregory McKay’s Senior Thesis, 2013


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We thank Professor Wayne Knox for teaching the OPT 310/311 senior design course. We thank our peers for their help and collaboration. We thank Professor Rothberg for providing advice, guidance, and funding for the project. We thank Doug Schwarz for help with Arduino programming.