A SPECTROSCOPIC SMARTPHONE BIOSENSOR FOR USE IN POINT-OF-CARE DIAGNOSTIC APPLICATIONS

BY

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THESIS

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ABSTRACT

This thesis serves to introduce a new point-of-care testing device capable of providing spectrometric data for solid and liquid phase samples. A custom cradle was developed to maintain optical alignment allowing for the diffraction, and therefore spectrometric analysis, of light transmitted either directly through a sample or via an intermediary optical biosensor. Demonstration of the instrument is provided with both an enzyme-linked immunosorbent assay (ELISA) and a photonic-crystal (PC)-based investigation of biomolecular adsorption. ELISA analysis is completed on both human interleukin-6, an important diagnostic cancer biomarker, and Ara h 1, one of the principle peanut proteins responsible for allergic reactions. PC-based sensing was completed with a Protein A-immunoglobulin G interaction with a .009 nm accuracy. All of these demonstrations were completed at physiologically-relevant concentrations, and as such, serve as proof-of-concept for two separate modalities of a smartphone-based spectrometric optical biosensor. In the future, such a sensor has the potential to provide a low-cost, point-of-care diagnostic device that is inherently robust, user-friendly, cloud-connected, and suited for use in remote sensing applications.
ACKNOWLEDGEMENTS

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CHAPTER 1: INTRODUCTION

Medical diagnostics has grown into a burgeoning $48.5$ billion dollar market in the United States alone, with significant growth expected due to the implementation of the Affordable Care Act.\(^1\) Combined with the anticipated shortage in clinical physicians,\(^2\) the importance of providing accessible point-of-care testing (POCT) technologies is rapidly increasing so as to facilitate the dissemination of diagnostic capabilities from the traditional hospital laboratory setting to the community clinic.

On a global scale, there is an echoing of this need for simple POCT technologies. The existing medical infrastructure present in the US is one of the most developed in the world; in many other, especially less developed, nations, a majority of clinical testing is completed at the secondary and tertiary levels.\(^3\) The importance of medical laboratory testing has been neglected in many of the funding initiatives for healthcare systems on a global scale,\(^4,5\) and POCT provides a unique answer to this critical need for diagnostics that is implementable in tandem with the slow process of building out national laboratory infrastructure.

Recent developments in POCT have been widely demonstrated across multiple clinical fields, and in certain cases such as diabetes testing, have quickly moved into the realm of every-day life. POCT is expected to extend to important applications beyond human health diagnostics, including food safety, food processing, quality control, water quality monitoring, animal diagnostics, and pathogen detection.\(^6-9\)

An important prerequisite for the widespread adoption of POCT is the availability of detection instruments that are inexpensive, portable, and able to share data wirelessly over the internet. Due to the rapid development of computational, communicational, and sensing
capabilities of smartphones since the introduction of the iPhone in 2007, these devices have become more like personal computers with integrated cameras, geolocation capabilities, and access to the internet. Since 2011, over 478 million smartphones are sold annually, with that number expected to double in the next 4 years, making them a nearly ubiquitous tool that can be adapted to performing POCT.

A variety of input methods exist on contemporary smartphones: microphones, cameras, accelerometers, touchscreens, GPS, and even fingerprint scanners. While designed for specific usage cases, many of these input methods have been successfully utilized to sense a variety of interesting phenomena: fall detection in the elderly via accelerometer, aggressive driving behavior via GPS, and a variety of fitness metrics catalogued by a variety of downloadable applications, including Gymskill, Spirosmart, and Balance. One of the most rapidly improved and consumer-visible sensors present is the rear-facing camera, which has similarly been used for a variety of diagnostic purposes. Recent examples include attachments that enable smartphones to serve as stethoscopes, ultrasound probes, as well as optical and fluorescent microscopes.

1.1 Scope

The following studies were completed as a proof-of-concept for two different optical sensing modalities. There is significant work that must be done to transfer the developed technology to a truly point-of-care device that can be of use in a clinical setting. This work is meant to compliment other work that has been completed in the realms of microfluidics and sample collection. Additionally, the instruments created as part of this work were fabricated as prototypes with off-the-shelf components. A custom-fabricated system would provide several advantages, but was beyond the cost and time considerations of this work. As a proof-of-concept, this is believed to be the first demonstration of spectroscopic assays being performed on a smartphone.
platform. As a mainstay of diagnostic medicine, spectroscopy poses an important measurement technique to be translated to a point-of-care device. Finally it should be noted that the data processing and analysis was completed off-device via Matlab. As a more user-friendly and broadly applicable software package, it has provided significant advantages over iOS or Android programming environments for the development of the technology and the demonstration of proof-of-concept. While future iterations of these sensing systems will likely be completed via onboard software, this was not a focus of this work.

1.2 Overview

This thesis aims to investigate two fundamental optical biosensing modalities: the absorption-based measurement of enzyme-linked immunosorbent assays (ELISAs) and the label-free measurement of biomolecular adsorption on photonic crystal (PC) sensors. First, an overview of ELISA testing will be given, and a survey of contemporary attempts to bring the technology out of the traditional laboratory setting will be completed. Then, using the developed biosensing platform, a demonstration of the system will be completed using a cancer biomarker, human interleukin 6 (IL-6), and a peanut allergen, Ara h 1. Then, the underpinnings of photonic crystal-based detection will be discussed, followed by a summary of the practical experiments that have been demonstrated thus far on a smartphone-based platform. A brief discussion of other optical sensing modalities amenable with the current platform will be completed, followed by a look forward toward a single platform capable of performing all of these basic optical techniques without augmentation. Finally future directions and anticipated challenges will be discussed.
CHAPTER 2: DESIGN OF A SMARTPHONE BIOSENSOR

2.1 Instrument Design and Fabrication

The instrument cradle was designed to interface with an iPhone 4 (800 MHz ARM Cortex A8, Omnivison OV5653 5MP BSI CMOS sensor with f/2.4, 5 element lens; Apple Inc.) by using the onboard, rear-facing camera as a digital light detector. A custom cradle fabricated from anodized aluminum was designed to align the necessary optics with the phone camera.\textsuperscript{18} This cradle allows for the precise alignment of the optical path with the aperture of the smartphone camera, while maintaining full utility of the screen, enabling the camera to function as a spectrometer. The main optical path is comprised of a square channel with two rubber bands along its length so that optical components can be moved along the optical axis, but will maintain proper centering and not move freely on their own. For these experiments, two configurations were used, one for solid-phase PC-based measurements and the other for liquid-phase immunoassays, as shown schematically in Figure 1.

For the solid-phase system, a broadband light source (150 W halogen fiber optic high intensity illuminator; Cole Parmer) is used to illuminate a 100 micron pinhole at the end of the channel. Upon entry, light is collimated (focal length, f=75mm) and linearly polarized to align the electric field with the PC grating. At the PC surface, the characteristic narrowband reflection occurs, and the remaining transmitted light is focused to a line via a cylindrical lens (f=50 mm) through the smartphone aperture and onto the CMOS sensor. Between the cylindrical lens and the smartphone is a diffraction grating (1200 lines/mm),
which disperses light in one direction generating a spectral dimension on the resulting captured image.

![Diagram of Optical Cradle](image)

Figure 1. Schematic Drawings of Smartphone Biosensing Attachments for A) Absorption measurements for ELISA analysis and B) for PC-based label-free detection of biomolecular attachment. To align the first order diffraction pattern with the CMOS sensor of the smartphone, the optical path is at an angle of approximately 47°. In both systems, the pinhole serves to optically isolate the internal optical path and to maximize spectral resolution, as the diffracted spectrum is convoluted with the apparent light source, just as a slit does on a traditional spectrometer. Inset: Photograph of smartphone cradle in use.
For the liquid-phase system, the same broadband light source is used to illuminate the sample cuvette. Light that is not absorbed by the sample is collected by a 1 mm diameter optical fiber and relayed to a 0.1 mm diameter pinhole at the end of the optical channel. Light that enters the optical channel is similarly collimated (f=75mm), focused to a line by a cylindrical lens (f = 50 mm), and diffracted (1200 grooves/mm) as in the solid-phase instrument.

In both cases, the wavelength components of the light are separated by the diffraction grating, which disperses the light onto the pixels of the camera. Illumination from the lamp is observed in the resulting camera image as a bright, rainbow band appearing near the center of the screen on the smartphone. Although the iPhone4 camera image sensor has a total of 5 megapixels (2592 x 1936), the spectrum band covers approximately 750 pixels in the dispersive direction and it is roughly 100 pixels wide. Assuming a focal length of the iPhone4 lens of 3.85 mm, the wavelength separation between adjacent pixels in the spectral direction will be 0.334 nm/pixel. Due to the spectral responsivity of the Si-based sensor and internal infrared cutoff filters within the camera optics, the range of wavelengths which may be observed by the system extends from approximately 400 to 700 nm.

2.2 Use of the Smartphone Instrument

Before introduction to the cradle, the iPhone was prepared by first locking the focus and exposure of the native camera application by focusing on a point sufficiently far away in a dark room or hallway to focus the camera at an infinite distance in a low-light setting. The phone was then placed in the cradle, and a broadband illumination source was turned on at the end of the optical path. The phone was aligned using adjustment knobs to achieve
an x-y positioning providing a centered image of a broadband spectrum on the smartphone display. The broadband light source used allowed for the manual manipulation of intensity, which was adjusted to maximize the dynamic range of the sensor for each of the two modalities. For every measurement step, five consecutive images were captured using the native iPhone camera application and averaged to account for variations arising from the camera hardware and sensor noise. Red and green laser pointers (λ = 656.26 nm and λ = 532.10 nm, respectively) measured with a spectrometer (USB2000+; Ocean Optics) were imaged using the smartphone system to provide reference wavelengths for calibration of the pixel to wavelength conversion.

2.3 Image Processing and Wavelength Calibration

Post-processing scripts were written in Matlab to convert raw images into absorption spectra. Once images are uploaded to a PC, auto-thresholding is performed to crop non-data pixels from analysis by removing pixels with zero intensity, which yields a reduction of the image to a ~ 1100 x 100 block of pixels. The intensity of pixels in the non-spectral direction is averaged to yield a one-dimensional vector representation of the spectra.

For both label-based and label-free modalities the data of interest lies in the non-transmitted light: either the light absorbed by the liquid sample or the resonantly reflected resonant peak of the PC. The non-uniformity of both the red, green, and blue (RGB) color filters and the broadband illumination source across the visible wavelengths requires a broadband response be defined (Figure 2). For each assay run, a blank consisting of aqueous solution for liquid-phase or air for solid phase is measured. The resulting RGB responses are recorded, and all other analyte samples are subtracted from the blanked RGB channels. To adjust for differences in RGB channel
intensity, each channel is individually normalized to unity to provide a near-uniform spectral response.

Pixel values are converted to wavelength values using a linear approximation via known pixel indices calculated by illuminating the pinhole with red and green laser pointers of known wavelength. The laser emission profiles are fit with a 4th order polynomial curve to determine the precise pixel index of each laser, and are then used to calculate the pixel-to-wavelength conversion, found in this case to be 0.334 nm/pixel.

Figure 2. Background spectra and calibration lasers. Nonuniform transmission is observed for the brightfield light source when imaged by the iPhone as a result of the RGB filter responses. While broadband transmission must be corrected for via normalization, laser peaks display a high similarity between the spectrometer and the smartphone setup. Using the known wavelengths of these lasers, the rest of the spectrum is interpolated linearly.
CHAPTER 3: QUANTIFICATION OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY WITH A SMARTPHONE BIOSENSOR

3.1 Rationale

Since its introduction 40 years ago, ELISA has become one of the most widely adaptable tools for biological assays, allowing for the rapid quantification of proteins and antibodies for diseases ranging from HIV to cancer, yielding over 40,000 new articles involving the technology annually.\textsuperscript{19}

An ELISA test is completed by immobilizing primary antibodies that possess an affinity for a specific biomolecule of interest onto a surface (usually a 96-well microplate). Next, the biomolecule of interest is introduced to the sensor surface where it will be bound by the immobilized antibody. A secondary antibody, also complimentary to the molecule of interest, is conjugated to an enzyme and introduced to the sensor surface after a thorough washing to remove unbound molecules of interest. These secondary antibodies will only bind to successfully captured molecules of interest. Finally after another series of washes, a chromogen, or color-generating compound, is introduced. The enzyme present on the secondary antibody cleaves a peptide on the chromogen, which causes it to undergo a shift in color.

![Figure 3. Schematic of an ELISA Procedure.](image) On a biosensor, immobilized antibody binds to a molecule of interest, which is then targeted by a secondary antibody. The enzyme bound to this antibody catalyzes a color change of the chromogen, yielding a visible color in the solution which can then be quantified via spectroscopy.
in its absorption spectrum to the visible range. After the optical absorptions of standards at known concentrations are used to obtain a calibration curve, the concentrations of an analyte within a test sample may be accurately determined via interpolation.

Here we demonstrate the use of a smartphone to perform the readout and quantification of any ELISA assay and compare results with those obtained from a conventional laboratory ELISA microplate reader for two representative biomarkers. First, we demonstrate smartphone-based detection of a clinical biomolecule used for human disease diagnostics, IL-6. Second, we demonstrate detection of a peanut allergen, Ara h 1, in the context of a food safety application. Together, these biomarkers illustrate both the immediate utility and broad applicability of smartphone-based ELISA.

While there have been successful demonstrations of simplifying liquid handling steps\textsuperscript{20-22} and both macro- and microfluidic sample collection techniques amenable to POCT,\textsuperscript{23-26} high quality spectroscopic instruments have yet to be developed for on-site analysis. By providing a spectroscopic reader for ELISA tests that is integrated with a mobile communication device, this technology can be used with the myriad of existing, well-studied ELISA procedures that have been well characterized for wavelength-specific analysis. Combined with approaches for microfluidic-based automation of sample preparation procedures, the detection instrument presented here opens up the possibility of transferring ELISA-type tests to other fields requiring point of care analysis, such as quality control validation and agricultural monitoring. In this chapter we will demonstrate the comparative robustness and sensitivity of a smartphone-based ELISA reader, and pose a method for further increased sensitivity via use of integrative spectral measurement.
3.2 Comparison to Other Research

3.2.1 Other POCT Immuoassays

The Cunningham lab is not unique in identifying the potential impact that a POCT device capable of performing immunoassays might have. POCT immunoassay have been around for many years, and are readily identifiable as part of everyday life. The most basic and prevalent form of POCT ELISA procedures would likely be a standard pregnancy test for human chorionic gonadotropin (hCG). These tests are completed on a paper membrane, and the colorimetric output is observed as a binary readout via visual comparison with a control band. More advanced versions of ELISA as POCT have been demonstrated for a variety of conditions including tuberculosis,\(^{27}\) HIV,\(^{28}\) and Giardia infection.\(^{29}\) While positive/negative results are acceptable for some types of diagnostic situations, often quantitative characterization of the amount of biological analyte is important within a specified range.

To this end, several different approaches have been taken. Paper-based microfluidic ELISA tests were demonstrated by the Whitesides group in 2004 that provide a quantitative measurement system based upon single-wavelength absorption.\(^ {30}\) This set of experiments is widely credited as the birth of paper microfluidics, a growing area of research for low-cost diagnostics. Aside from the custom-built illumination/sensor system used in that work, the most prevalent method for quantitative POCT ELISA measurement uses either a CMOS or CCD to directly image solutions, which has recently been shown to be successful on a smartphone platform.\(^ {31-34}\)

3.2.2 The Importance of Spectroscopy

Systems such as these use various RGB color image weighting techniques to analyze concentrations of biological molecules of interest and therefore do not provide the spectrometric data required to perform a wavelength-based ELISA test. CMOS sensors, like those found in
most smartphone devices, are wavelength-independent photon collectors that convert photons into RGB pixels via physical filters. These filters are comprised of colored dyes that each have both their own spectral response\textsuperscript{35} and an additional infrared cutoff filter with a response that provides a sharp cutoff that can vary among batches of identical cameras by 10 nm.\textsuperscript{36} In such a system, light intensity and color are intrinsically intertwined as they both rely on the number of photons captured by the RGB pixels compared with the number captured by those adjacent to them.

Spectrometers have been a cornerstone of modern analytical chemistry. The Beckman DU Spectrophotometer surpassed its contemporaries in popularity rapidly upon its invention, due largely to its increased spectral resolution across the entire range of measured wavelengths. They present the standard laboratory tool for the measurement of a variety of experiments as they can provide wavelength-specific absorption information. The peaks at which a substance absorbs light the most correspond to chemical bonds and interactions at the molecular level, and can be used to identify specific components of a mixture. By dispersing the spectrum of absorbed light along one of the CMOS camera dimensions, we are able to decouple the wavelength from intensity and thereby determine the wavelength-specific absorbance of samples. This enables the accurate analysis of ELISA tests in a way that is immune to polychromatic spectral absorption variation.

3.3 Quantification of Absorption-Based Measurements

For this experiment, as the entirety of chromogenic spectral data for 3,3′,5,5′ trimethylbenzidine (TMB) tests is captured solely in the blue channel of the RGB data, red and green channels are omitted from further calculation for ELISA-based measurements. To generate absorption spectra, converted vector forms of the data were subtracted from those of a blank comprised of deionized (DI) water, and normalized to the RGB response of the sensor when illuminated without sample or cuvette present in the optical path.
To best compare the smartphone measurement sensitivity with that of a commercially available microplate reader, absorption values at $\lambda = 450$ nm are interpolated from calibrated spectra. In addition, recognizing the potential value of the full spectral data for each sample, we calculate the integrative absorption from $410 < \lambda < 500$ nm (Figure 4). Finally, the ELISA microplate reader data is imported from the instrument, appropriately normalized with respect to the blank, and averaged across replicates.

For each measurement method, the absorption as a function of concentration for the calibration standards is fit to a four parameter logistic regression model based upon a sigmoidal response curve governed by

$$F(x) = D + \frac{A-D}{1+(\frac{x}{C})^B}$$  \hspace{1cm} (Eq. 3.1)

![Figure 4. Example of Wavelength-Normalized Spectra Generated from Camera Images with Peanut Allergen Ara h 1.](image)

Five independent smartphone images were averaged, then normalized to a blank. Using lasers of known wavelength, images were converted to wavelength-dependent spectral absorbances. Five concentrations of peanut allergen Ara h 1 are present to illustrate the difference in absorption characteristics. The dashed line at $\lambda=450$ nm represents the single wavelength at which conventional ELISA measurements are taken using a microplate reader.
where A is the minimum asymptote, B is the Hill’s slope, C is the inflection point, and D is the maximum asymptote. While Beer-Lambert relations are often used to characterize absorption-based measurements, this logistic model was selected both on the recommendations of the assay protocols and to account for sensor saturation effects and polychromaticity for integrative absorption measurements. Limits of detection are calculated as three standard deviations above the average zero-concentration value for each measurement method.

3.4 Human Interleukin 6 Quantification

Human Interleukin-6 (IL-6) is an important cytokine involved in the signaling cascade for the pro-inflammatory activation of B-cells following T-cell activation due to an antigen-mediated immune response. Elevated expression of IL-6 in serum samples has been demonstrated as an important indicator in conditions ranging from diabetes to postoperative surgical stress. Additionally, IL-6 has been shown to be a relevant cancer biomarker important for the tracking, classification, and prognosis of various cancers including breast, hepatic, prostate, and renal varieties.

A commercially available ELISA kit was purchased (Invitrogen) for the quantification of human IL-6. Lyophilized IL-6 was reconstituted in standard diluent buffer, and serially diluted to concentrations of 512, 128, 32, 8, 2, 0.5 and 0.125 pg/mL. Standards were added to pre-prepared wells with immobilized anti-IL-6 antibodies in replicate (n=6). A blank well was incubated with only diluent buffer. Biotinylated anti-IL-6 antibody was added to each well, and the solutions were allowed to incubate, covered, for 2 hours at room temperature on an orbital shaker (100 rpm). Solutions were removed, and the wells were rinsed (4x) with the provided wash solution, per assay instructions.
Streptavidin-HRP solution was added to each well, and the plate was returned to the shaker for another 30 minutes. Another wash step was completed as before, and 100 µL chromogen solution (TMB) was added to each well. The plate was then incubated for 10 minutes in the dark, before applying 100 µL of the provided stop solution.

Using a commercially available ELISA microplate reader (BioTek Synergy HT), the completed assay was measured for absorbance at 450 nm, and blanked against the control well. Afterward, samples were individually transferred to polystyrene cuvettes that were placed in a cuvette holder fixed permanently in the optical path to minimize variation due to geometric inconsistencies. A cuvette filled with 200 µL deionized water was used to normalize the RGB intensity values during image post-processing.

After data processing, the captured images were converted to absorption spectra that show clear concentration dependence across a range of wavelengths from 400 to 550 nm. From these spectra, either single wavelength measurements, akin to a microplate reader, or integrative absorption measurements can be calculated. After digital data analysis, IL-6 concentrations were observed to follow a sigmoidal dose-response curve, as expected (Figure 5). From the data collected, it can be observed that the smartphone-based absorption at 450 nm replicates the microplate reader measurements throughout the entire range of concentrations, validating our instrument as a portable, low-cost alternative to traditional benchtop apparatus. The integrative absorption has the same characteristic shape, and has a comparable Limit of Detection (LOD) lower than the minimum assayed
concentration of .25 pg/ml. Validation of these techniques using the results from the traditional microplate reader via Bland-Altman analysis are discussed in Section 3.6.

3.5 Peanut Allergy Detection

The Ara group of peanut proteins consist of those that elicit allergic responses. While the specific proteins that an individual might be allergic to might vary, between 70 % and 90 % of individuals allergic to peanut products produce an immune response to the
proteins Ara h 1, Ara h 2, and Ara h 6, a homolog for Ara h 2. In the US, over 1% of the population has a self-reported peanut allergy, and this rate seems to be increasing in children. While many precautions exist to assist those with severe allergies to avoid peanut-based products, there is an increasing trend in the number of peanut allergy-related hospitalizations each year. There is a high potential value in a portable smartphone-based test for peanut allergens present in food, but careful quantification is absolutely essential. To our knowledge, this work presents the first proof-of-concept of a portable peanut assay that is capable of measuring peanut at concentrations less than the smallest measured No Observed Adverse Effect Levels (NOAELs).

A commercially available ELISA kit was purchased (R-Biopharm) for the quantification of peanut allergens Ara h1. Samples were taken from cookies purchased at two local bakeries, Insomnia Cookies and Schnuck’s Grocer, as well as from pre-packaged vanilla Oreo® cookies. Samples were extracted per kit instructions: 1 gram of each cookie sample was ground and placed in a solution of extraction buffer diluted from the provided 10x concentrate. Extraction was completed at 60°C for 10 minutes, followed by a 10 minute centrifugation at 2,500 g. The supernatant was then incubated, along with calibrated concentrations of peanut solution, in 400 µL wells with immobilized anti-Ara h 1 antibodies in triplicate. Enzyme conjugate, chromogen, and stop solutions were then added for 10 minutes each, with a 3x wash step in between. The entire process can be completed in under an hour.

The same preparation protocol developed for the IL-6 assay was again used for the Ara h 1 assay. The spectra were normalized to a water-filled cuvette during post-processing, and positioning was repeated. Lasers were used to recalibrate, and samples
were measured in a similar fashion. After digital data analysis, standard Ara h 1
concentrations were also observed to follow a sigmoidal dose-response curve (Figures 6, 7). The limits of detection for the reported peanut assays were between .09 and .56 ppm.

Once again, comparative validation was completed with a microplate reader via Bland-Altman analysis (Section 3.6). Samples from peanut-containing cookies were found to have absorbances over that of the maximally assayed standard concentration (20 ppm), and so
could not be quantitatively measured without further dilution. While neither Insomnia nor Schnuck’s claimed that their sugar cookies were safe for consumption by people with severe peanut allergies, the sugar cookie from Schnuck’s did, in fact, have an absorbance less than the assayed limit of detection (Figure 8). As expected, prepackaged sandwich crackers without a label indicating a risk of nut contamination tested below the assay LOD.
Figure 8. Quantification of Ara h 1 in Cookie Samples from Two Local Bakeries. Sample cookies were collected and contained separately in plastic to avoid cross-contamination. Samples were measured, ground, and proteins were extracted following manufacturer protocol. Vanilla Oreo® samples (n=3) and Schnuck’s cookie samples (n=2) were assayed with data in Figure 5, and Insomnia cookies were assayed with data in Figure 6 (n=3). As the cookies were run on two different assays, only the higher LOD is shown.

* Peanut Butter cookies had concentrations of Ara h 1 higher than the maximum assayed standard concentration, so significant dilution would be required for accurate quantification.
3.6 Comparison of Smartphone Sensitivity and ELISA Microplate Reader

3.6.1 Comparing Capabilities

For each sample measured in the conventional microplate reader, a spectrum was generated from a series of 5 collected images. Both the absorption at $\lambda=450$ nm and the integrative absorption from $\lambda=410$ to $500$ nm were calculated. All data points were fit with the same four-parameter logistic model described above. From the data collected, the absorptive LOD from either sensing modality appear to be in rough concordance, whereas the LOD obtained using the integrative absorption method is consistently 2-3x lower (Table 1) than that obtained by considering the absorption of only a single wavelength. This suggests that by taking advantage of the full spectral profile of the absorption peak resulting from the chromogen, it is possible to achieve a higher sensitivity than that obtained by observing a single wavelength. The availability of the full spectral absorption information also enables the system to easily function with a broad range of chromogens that generate different colored solutions.

<table>
<thead>
<tr>
<th></th>
<th>ELISA Microplate reader</th>
<th>Smartphone Absorption at 450nm</th>
<th>Smartphone Integrative Absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td>0.314 ppm</td>
<td>0.268 ppm</td>
<td>0.107 ppm</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td>0.529 ppm</td>
<td>0.560 ppm</td>
<td>0.269 ppm</td>
</tr>
</tbody>
</table>

Table 1. Comparison of detection limits of the Ara h 1 ELISA microplate reader, smartphone absorption at $\lambda=450$nm, and smartphone integrative absorption

3.6.2 Analyzing Agreement of Measurement Techniques

In the realm of clinical medicine, the appearance of agreement between measurement techniques is not sufficient to support the adoption of a new instrument. In
the field of diagnostics, understanding the agreement between the new device and the current gold-standard is both important and non-trivial. Recognizing that even gold-standard techniques have inherent measurement uncertainty, a simple correlation analysis will not accurately reflect the combined uncertainties. To address this issue, Bland and Altman developed a methodology that has become widely accepted as a viable technique, based upon the average measurements of the two techniques and the absolute differences at those averages.$^{48}$

As ELISA absorbance measurements often have a lognormal distribution, a variation on the standard method using the geometric mean and log difference was applied to all non-zero values for each assay (Figure 9).$^{48-50}$ The log differences showed a

![Graphs A, B, and C showing validation](image)

**Figure 9. Validation of Smartphone Measurement Technique versus Microplate Reader via Bland-Altman Analysis.** The lognormal quality of ELISA absorbances dictated the use of the geometric mean vs. log difference plot shown. All nonzero points are plotted, with the dashed line being the mean of the primary linear regression. Solid lines represent the 95% confidence limits of agreement, which contain all but one data point across the three separate experiments.
dependence on the magnitude of the measurement, so a regressive approach was used to model the relation so appropriate limits of agreement could be attained.\textsuperscript{51} The regression was modeled as

\[ \hat{y} = b_0 + b_1 \hat{x} \]  \hspace{1cm} (Eq. 3.2)

where \( \hat{x} \) is the vector of the geometric means of the two measurement techniques, \( \hat{y} \) is the vector of absolute log differences, and \( b_0 \) and \( b_1 \) are the intercept and slope, respectively. For all three experiments, this provided a viable fit for the data; however, it was still necessary to see if the dependence on measurement magnitude continues into the residuals which are given by

\[ r = c_0 + c_1 x \]  \hspace{1cm} (Eq. 3.3)

where \( r \) is the vector of residuals, and \( c_0 \) and \( c_1 \) are the slope and intercept for the residual regression. The R\(^2\) value for the IL-6 assay residuals was significant at .224, while the values for the two Ara h 1 experiments were between .02 and .06. As the relationship between the residuals and magnitude of the measurement was found to be significant in at least one of the experiments, the model of the residuals was combined with the first model to provide the 95\% limits of agreement. Assuming that these residuals follow a normal distribution, the limits of agreement can be calculated by combining Equations 3.2 and 3.3 as discussed by Altman:\textsuperscript{52}

\[ \bar{a}_1, \bar{a}_2 = b_0 + b_1 \hat{x} \pm \sqrt{\pi/2} \times (c_0 + c_1 \hat{x}) \]  \hspace{1cm} (Eq. 3.4)

where \( \bar{a}_1 \) and \( \bar{a}_2 \) are the 95\% limits of agreement. Of all data points assayed, only one point was found to be outside the \( \pm 1.96 \) S. D. limits of agreement (solid lines). These data demonstrate a high level of consistency between the conventional ELISA microplate reader and the smartphone-based spectroscopic absorbance measurements.
3.7 Discussion

The diagnostic potential for biomarkers such as IL-6 is significant. Tracking the post-operative serum levels of biomolecules to assess the chances of post-operative complications could potentially increase positive outcomes for those patients via early-detection,\textsuperscript{40} and decrease required post-operative in-hospital recovery time. While long-term monitoring of biomarkers could likely provide valuable prognostic information, the associated load on hospitals and diagnostic laboratory facilities make any such testing at best expensive, and at worst, unrealistic. Similarly, the measurement of cancer-related biomarkers might prove a useful tracking mechanism for potential relapse or metastatic risk.\textsuperscript{53} The development of a cheap yet reliable ELISA reader could facilitate the development of a broadly available test in clinic-type environments.

In contrast with medical diagnostic assays, allergen detection provides a consumer-based testing opportunity. While existing microplate readers are prohibitively expensive for individuals and designed specifically for laboratory settings, a handheld smartphone attachment is naturally amenable to an in-home testing environment. Though ELISA assays require multiple liquid-holding steps, there have already been several successful demonstrations of ELISA performed as a lab-on-a-chip technique that could be integrated into a low cost, disposable cartridge, readily read by an instrument such as the one described here. While significant work has gone into simplifying both the sample collection process and the liquid handling steps used in a traditional ELISA test, there is still a need for an instrument that can replicate the accuracy and repeatability of a microplate reader in a point-of-care system.
Current alternatives for assay readout are based on either visual inspection or non-spectral image analysis, neither of which provide the spectral resolution of traditional microplate readers. ELISA testing is dependent on this spectral resolution, as the chromogens that are being measured are characterized by non-uniform absorption spectra in the visible range. Non-spectral methods of color analysis are subject to the variations in intensity present across various wavelengths, usually binned into three RGB channels via a Bayer filter. By diffracting the wavelength of transmitted light along one of the physical dimensions of the CMOS sensor, we are able to separate the intensity information of the absorbed light from its wavelength, thereby allowing for the wavelength-specific quantification of chromogenic absorption. Furthermore, our technique is broadly applicable across all visible wavelengths of the spectrum, even in regions where RGB channels sometimes overlap, including both 450 and 492 nm, wavelengths where the important ELISA chromogens TMB and OPD maximally absorb, and where RGB analysis cannot suffice.

While there is general consensus on the importance of minimizing peanut material in non-peanut-containing foods, surprisingly little work has been done to quantify the amount of peanut protein required to produce an allergic reaction in affected individuals. Literature review-based statistical modeling has produced a starting point for such efforts, but variations in testing modalities as well as an abundance of situations where NOAELs could not be determined due to observed allergic responses at the lowest assayed dosage have resulted in a large number of patient results being excluded.\textsuperscript{54, 55} One of the most promising studies of existing data was collected from clinical records of patients being tested, rather than from specific patient-recruited studies, which yielded an eliciting dose
in 5% of the population (ED₅) of 4.2 mg peanut protein.⁵⁶ While there is uncertainty in some of the numbers prevalent in the literature, the overall range of subjective NOAELs reported (or estimated) is typically between .4 and 10 mg protein,⁵⁶⁻⁵⁹ with objective ED₅ have been estimated as low as 1.5 mg.⁶⁰ For a reasonable maximal daily intake of 500g of food based upon a 2000-3000 calorie diet, this would translate to .8 and 3 ppm peanut protein for minimum reported NOAEL and ED₅, respectively, which are higher than the LOD for each of the testing methodologies used. Thus we have successfully demonstrated a point-of-use testing modality that can plausibly be used to measure clinically relevant amounts of peanut.

While this work has focused upon the demonstration of a liquid-based color absorption assay in the context of an ELISA, the approach demonstrated here may be applied to any assay that results in the color change of a liquid. Colorimetric tests are relatively common due to their ability to be qualitatively measured via visual inspection. Such tests are commonly used in testing liquids for pH, chlorination, and contaminants ranging from metals to organic material. While qualitative information is useful, spectroscopic analysis is able to provide quantitative information that is consistent across phone manufacturers and independent of operator or hardware-based differences, a significant improvement over both visually comparative methods and non-spectrally resolved imaging methods. Broadly, this instrument can perform any such test, assuming the absorption spectra exists within the visible range of wavelengths.
CHAPTER 4: LABEL-FREE BIODETECTION USING PHOTONIC CRYSTAL SENSORS

4.1 Rationale

While spectroscopic biological assays such as ELISA can be used for the detection of many biological analytes, their widespread adoption into scenarios outside the laboratory is hindered by the complexity of the assay protocol which often involves multiple liquid handling steps and conjugated antibodies.\textsuperscript{61-64} Detection of an analyte via one of its intrinsic physical properties (dielectric permittivity, mass, conductivity, or Raman scattering spectrum – for example), called “label-free” detection,\textsuperscript{65-67} is preferable for assay simplicity in terms of the number of reagents required, washing steps needed, and assay time.

Of all the label-free detection approaches that have been demonstrated, those based upon optical phenomena have been commercially preferred due to a combination of high sensitivity, low sensor cost, detection system robustness, and high throughput capacity. Adsorption of biomolecules, viral particles, bacteria, or cells onto the surface of an optical biosensor transducer results in a shift in the conditions of optimal optical coupling, which can be measured by illuminating the transducer surface and measuring a property of the reflected or transmitted light. Such a detection approach is robust, and has become economically advantageous due to the advent of low cost light emitting diodes and semiconductor lasers. Surface plasmon resonance (SPR) based biosensors\textsuperscript{68-72} and photonic crystal (PC) optical biosensors\textsuperscript{73-77} are capable of detecting broad classes of biological analytes using their intrinsic dielectric permittivity. Each approach has been demonstrated using both large laboratory instruments and miniaturized (shoebox-sized)
systems. However, no prior label-free optical biosensor instrument has been fully integrated with a smartphone, using the onboard camera itself as the detection instrument.

Here, we demonstrate the use of a smartphone to detect shifts in the resonant wavelength of a PC-based label-free biosensor. PC biosensors are amenable to readout by a smartphone, as they function as narrow bandwidth reflection filters that can be measured by illuminating them at normal incidence with collimated white light. The PC used in this work was designed to produce a high efficiency resonant reflection at a wavelength in the visible spectrum (λ= 565 nm) in air. Adsorption of biomolecules on the PC surface results in a red-shift of the resonant peak. To enable the rear-facing smartphone camera to measure this resonant peak, we use a setup very similar to that used for absorption measurements. While the phone and the optical components are held in position, the PC sensor itself may be inserted and removed from a narrow slot within the cradle. Importantly, the system incorporates the ability to compare wavelength shifts from adjacent “active” and “reference” regions of the PC as a means for incorporating an accurate experimental control. To demonstrate operation of this sensor and detection system, we will analyse the concentration dependence of the Protein A-porcine IgG interaction.

4.2 Background on Photonic Crystals

Photonic crystals (PCs) comprise a broad class of optical structures that are composed of a subwavelength periodic modulation of refractive index. While these modulations have been demonstrated in two and three-dimensional structures, this thesis will focus on specific applications of the simplest case: the 1-dimensional periodic grating.

As described in previous publications, these gratings can be easily manufactured via nanoreplica molding in a UV-curable polymer on a disposable plastic substrate. Upon curing, this then provides a grating structure of the desired height, width, and period over which subsequent
depositions of specific thicknesses of dielectrics can be made. For these experiments, SiO$_2$ and TiO$_2$ were used for low- and high-refractive index materials, respectively.

When collimated light is incident on the grating structure, a portion of the light is diffracted by the high-refractive index TiO$_2$ layer in a wavelength- and angle-dependent manner, according to the grating equation:

$$\Lambda (\sin(\theta_i) - \sin(\theta_d)) = n\lambda$$  \hspace{1cm} (Eq. 4.1)

where $\Lambda$ is the grating period, $n$ is the diffraction order, $\lambda$ is the wavelength of light, and $\theta_i$ and $\theta_d$ are the incident and diffracted angles, respectively. Much of the light passes through without diffraction ($n = 0$), but some of the light undergoes first-order diffraction, which at normal incidence conveniently simplifies to

$$\sin(\theta_d) = -\frac{\lambda}{\Lambda}$$  \hspace{1cm} (Eq. 4.2).

Periodic waveguides such as this have been shown to have a specific resonance condition where the first-order diffracted light propagates as a guided mode due to total internal reflection when

$$2k_3d + 2\Phi_{1,2} + 2\Phi_{2,3} = 2\pi m$$  \hspace{1cm} (Eq. 4.3)

where $k_3 = 2\pi/\lambda$ is the wavevector of the light perpendicular to the grating in the SiO$_2$, $d$ is the thickness of the grating, $m$ is the order of diffraction and $\Phi_{1,2}$ and $\Phi_{2,3}$ are the Fresnel coefficients due to differences in refractive index between the waveguide (SiO$_2$) and surrounding layers. At each interface of the waveguide that the internally refracted light hits, a portion of the light is refracted back out of the grating. Light that undergoes two instances of internal refraction before escaping the grating is angularly aligned with light that simply passed through the grating, but it
has undergone a phase shift relative to its non-diffracted counterpart, resultant from the Fresnel coefficients of reflection and diffraction and the difference in path length, which is given by

\[ \Phi = 2k_3d + 2\Phi_{1,2} + 2\Phi_{2,3} + \pi \]  

(Eq. 4.4)

It is important to note that this path length difference results in the twice-reflectd and non-reflectd light being exactly \( \pi \) out of phase, yielding complete destructive interference.\(^{81}\)

Similarly, light that undergoes a single internal refraction before escaping the waveguide can be shown to completely constructively interfere, yielding effectively 100\% reflection.

Assuming constant perpendicular illumination, the only factors that affect this resonant wavelength are the Fresnel coefficients, which are dependent on the relative refractive indices of the grating and waveguide layers. As biomolecules attach to the exposed surface of the PC, the effective refractive index of the TiO\(_2\) is slowly increased in a quantifiable manner, which serves as the foundation for label-free quantification of biomolecular attachment (Figure 10). The Cunningham group has utilized this technique for a variety of analytes, including pancreatic cancer cytotoxicity screenings,\(^{82}\) the presence of porcine rotavirus in biological samples,\(^{83}\) and HIV viral load.\(^{84}\)

### 4.3 PC Characteristics for Smartphone Biosensing

For these experiments, a one-dimensional grating structure was created on a disposable plastic substrate in UV-curable polymer via nanoreplica molding. As depicted in Figure 10, the PC grating period was 360 nm and the depth was 60 nm. The polymer grating was subsequently over-coated with sputtered thin films of SiO\(_2\) (\( t = 200 \text{ nm}, \text{refractive index} = 1.54 \)) and TiO\(_2\) (\( t = 60 \text{ nm}, \text{refractive index} = 2.35 \)). After fabrication, the flexible plastic substrates were attached to standard 25 by 75 mm glass slides using double-sided, pressure-activated adhesive film. The
Grating dimensions and thin film thicknesses were designed to provide a 95% efficient narrowband reflectance with a central wavelength of $\lambda = 565$ nm and a resonance bandwidth (full width at half-maximum: FWHM) of $\lambda \sim 5$ nm when the surface of the PC is dry. Adsorption of biochemical molecules onto the surface of the PC results in an increase of the effective refractive index of the high refractive index waveguide layer, yielding a positive shift in the peak wavelength value (PWV) of the narrowband maximum of reflection efficiency (measured as a minimum in transmission efficiency by the smartphone). The magnitude of this shift can be directly correlated to the quantity of IgG present on the PC surface.

Figure 10. A. The nanopatterned PC biosensor surface is functionalized via PVA and gluteraldehyde surface chemistry to facilitate the capture of IgG molecules. B. The associated PWV undergoes a red-shift when antibodies attach to the surface and increase the effective refractive index of the TiO$_2$ layer.
4.4 Measurement Protocol

To perform a label-free biological assay, the PC is initially prepared by immobilizing a capture molecule (such as an antibody, aptamer, or single-strand DNA sequence) that selectively recognizes the analyte in a test sample. The sensor surface is further prepared with a “blocking” step that prevents nonspecific adsorption of other molecules. The PWV of the sensor is measured prior to the addition of the target analyte to establish a baseline reading. Exposure of the sensor to the test sample results in adsorption of the analyte on the PC, which is followed by rinsing/drying the sensor to remove unbound molecules. A second PWV measurement is then taken and the difference in PWV is recorded as the shift due to the adsorption. The basic approach outlined in this protocol can be augmented by the incorporation of positive and negative experimental controls, including the use of a “reference” sensor that is prepared with an unmatched capture molecule, but still exposed to the test sample. Although all measurements reported here were taken with the PC surface in a dry state, the system could be further modified via the incorporation of a static liquid chamber or flow cell with the PC, so that the PWV shifts can be monitored kinetically.

4.5 PC-based Biodetection with a Smartphone

To demonstrate the ability of the sensor and detection instrument to perform a biodetection assay in which a layer of immobilized capture molecule is able to selectively capture a specific biomolecule of interest, we captured porcine immunoglobulin G (IgG) using an immobilized layer of Protein A. While not in the realm of the biotin-streptavidin interaction, Protein A/G-antibody reactions are some of the stronger interactions observed in physiological conditions.

To begin, the PC sensor was functionalized with an aldehyde-based surface chemistry to facilitate the attachment of Protein A. To prepare the surface for functionalization, the PC slide was thoroughly cleaned using an acetone/IPA/DI-H₂O wash, and then functionalized with a 10%
solution of polyvinylamine in water at 40ºC for two hours. It was then washed 3x in ultrapure water, and incubated in a 25% solution of glutaraldehyde in water (Sigma Aldrich) for 4 hours at room temperature. After a subsequent 3x wash in ultrapure water and drying of the sensor surface, the PWV from 14 distinct locations on the functionalized PC surface were recorded by the smartphone detection instrument to establish the baseline PWV upon which subsequent wavelength shifts caused by the attachment of capture and target molecules would be compared.

Next, a custom-made polydimethylsiloxane (PDMS) gasket was adhered to the PC sensor surface to form 14 separate assay wells (diameter = 6 mm) over the 14 measurement locations. Each well was incubated with 60 μL of 0.5 mg/mL Protein A in .01M phosphate buffered solution (PBS; pH= 7.4) for 20 minutes. After Protein A immobilization, the wells were washed with PBS, the gasket was removed, and the PC sensor was dried. A second PWV reading was gathered from each location, in order to establish the PWV shift due solely to the attachment of the Protein A layer. Figure 11 A shows the initial PWV associated with each of the 14 wells on a single PC sensor, and the PWV shift induced at each location by Protein A deposition, demonstrating the initial variability induced by the PC functionalization (average PWV = 561.99 nm, stdev = 0.464

![Figure 11](image-url)

**Figure 11. Concentration dependent PWV shift of porcine IgG antibody.** A. Variation in slide functionalization versus Protein A deposition. B. Protein A was immobilized on two PC sensors to detect concentrations of porcine IgG antibody present in quantities between 1 nM and 5000 nM. N = 3 for assay wells; N = 2 for control wells.
nm), and the PWV shift induced by the Protein A deposition process (average \( \Delta \text{PWV} = 1.46 \text{ nm, stdev } = 0.288 \text{ nm} \)). These results demonstrate that the surface chemistry for protein attachment used here introduces a greater degree of variability than the variability of the subsequently observed sensor measurements from the smartphone instrument.

Prior to the exposure of the PC sensor to the IgG analyte, the gasket was replaced, and the PC was rehydrated with 60 \( \mu \text{L} \) PBS in each well for 10 minutes. After aspiration, 60 \( \mu \text{L} \) of porcine IgG solutions were added to each well at concentrations ranging from 4.25 nM to 3.4 \( \mu \text{M} \) (Figure 11 B) and incubated for 10 minutes. After a rinse with 0.01 M PBS to remove unbound antibody, the PC sensor was dried and measured using the smartphone to calculate the \( \Delta \text{PWV} \) induced by IgG attachment. Concentration-dependent PWV shifts were observed, with each IgG concentration being measured in triplicate. Intra-sensor variability is low (average stdev for triplicate measurements was 0.335 nm). The lowest IgG concentration tested (4.25 nM) was observable above the negative control (0 nM IgG), indicating the ability to clearly measure wavelength shifts of 0.16 nm above sensor-specific background noise on that sensor (data not shown) that is limited by assay-related variability, rather than by the wavelength shift resolution of the detection instrument. Despite its compact size and simplicity, the detection instrument is capable of detecting the presence of analytes in the nM concentration range with a “direct” assay. The limits of detection of the system can be reduced further through the use of a variety of tags, such as secondary antibodies\(^86\) or nanoparticles\(^87, 88\) that can amplify the wavelength shift by factors of 5 - 1000 x.

4.6 Discussion

For the practical utility of the apparatus, it is important for future versions of the smartphone biosensor to have the ability to use wet samples by allowing for the use of thicker
cartridges comprised of 3D wells, as opposed to the current methodology of current limitation of working with planar slides. The decision to take measurements of the PC surface in a dry state was dictated by the desire to measure sensors in as simple of a manner as possible, without the possibility for bulk refractive index effects, or the incorporation of fluid flow (with associated pumps and valves) into the instrument. Detection in a dry state also allows for the assay steps to be performed separately from the detection step in both time and physical location, which would be desirable for several of the real-world detection scenarios being considered at that time. Incorporation of a microfluidic channel into the sensor through the fabrication of a more sophisticated cartridge would enable liquid-based detection and the potential for measuring the kinetics of analyte binding without altering the fundamental measurement approach, but was not addressed as a part of this thesis.

The experiments used to characterize the smartphone detection instrument presented in this paper serve to demonstrate the feasibility of measuring PWV shifts from a PC biosensor, rather than focusing on the application of the system to complex test samples that contain many potentially interfering compounds. In order to address this issue for such applications, it will be necessary to incorporate reference sensors that can serve as negative controls to the effects of nonspecific binding. Such methods, commonly reported in the biosensor literature, should include multiple types of negative controls. First, as shown in this work, a detection experiment should include a sensor that is prepared with the capture molecule, but that is exposed to buffer rather than to the test sample. Secondly, the experiment should also include a sensor that is prepared with a capture molecule that does not recognize the analyte (such as an antibody for a nonrelated antigen), and exposed to the test sample. The combination of these two experimental controls can determine the mass density of analyte molecules that are bound nonspecifically to the sensor.
surface from a complex media, while at the same time taking into account common mode error sources such as temperature variability or bulk refractive index variability.
CHAPTER 5: CONCLUSION

In this manuscript, we have demonstrated two usage cases for a novel smartphone-based spectrometer: absorption measurements for use with standard ELISA procedures, and PWV measurements for use with photonic crystal based label-free assays. The need for POCT that can transfer traditional laboratory assays to primary care settings is significant both domestically and globally.

In the first part of this thesis we describe the successful detection of human IL-6, an important cancer biomarker, and Ara h 1, one of the principle peanut proteins responsible for causing a reaction. Limits of detection were found to be as low as those from the standard microplate reader, and the high level of agreement between the two sensing methods was demonstrated via Bland-Altman analysis. Finally, cookies from two local bakeries were tested for cross-contamination with peanut ingredients, and the instrument was demonstrated as a practical tool for detecting clinically relevant concentrations of allergen.

In the latter half of the work, the instrument was modified to provide polarized light which could be used to illuminate a photonic crystal at its resonance condition, providing a narrowband reflection phenomenon that redshifts as biomolecular adsorption takes place on the PC surface. As demonstration of its biological utility, the concentration dependence of the Protein A-IgG interaction was investigated. Concentrations of less than 1 µg/mL were successfully quantified.

5.1 Future Directions

The experiments described in this manuscript are primarily point-of-concept exercises. Significant improvement can be made upon the techniques used in areas ranging from sample preparation to on-board automation. Many of these ancillary improvements have been investigated
in the literature, as mentioned previously, and compliment the sensing device we have developed. Eventually a microfluidic device will be created that will take advantage of these advances, providing an assay experience much closer to that which a clinician is looking for, in comparison with these first-generation proof-of-concept experiments.

5.1.1 Developing Other Modalities

Absorption measurements comprise a large portion of the canonical optical diagnostic methodologies. PC-based techniques are significantly further behind in development, but offer several key benefits, namely the simplification of assay protocol due to their label-free nature. In between the two, though, sits an important body of work within the realm of optical biosensing that should be mentioned: fluorescence-based assays. There are a handful of interesting and useful diagnostic tests that can be completed with the use of fluorescent reporter probes including Förster resonant energy transfer, fluorescent lifetime, molecular beacon, and fluorescent polarization assays. This modality is readily integrated with our prototype system via augmentation with a few external pieces on an optical bench. Initial tests have already been run showing that such a system is feasible on a smartphone platform, but additional work needs to be done to characterize the sensitivity of the instrument. One of the principle requirements of any fluorescent assay is the orthogonal illumination of the sample to minimize noise due to the light source in the fluorescent signal. With this modification, new types of assays can be added to those demonstrated here, further expanding the capabilities that the instrument can provide in a point-of-care environment.

5.1.2 Combination of Modalities into a Single Instrument

While expanding capabilities is important, ease-of-use and simplicity of design are two of the most important attributes for a POCT device. If a single instrument could be used without significant modification for absorbance, fluorescence, and PC-based measurements, this would
provide an even greater benefit for clinicians. In addition to simplicity of use, it would decrease the costs associated with the testing and decrease the amount of equipment that must be transported.

To this end, a second generation prototype is currently being designed as the first step toward a multi-modal optical attachment. This device will be able to accommodate both fluorescent and absorption measurements by using fiber optics to direct light paths internally, and will be created via stereolithography (SLA) to allow for continued optimization of optical distances once the prototype is functional. The flexibility provided by SLA technique will also make it possible to empirically test the feasibility of a PC-based measurement on the same setup and investigate the tradeoff between spectral resolution and illumination strength.

5.2 Anticipated Challenges

Providing a unified system that is capable of performing absorbance, PWV, and fluorescent-based measurements without modification presents a significant technical challenge. Each of the individual modalities possess its own optimal operational conditions. Absorption measurements require high-intensity illumination, while PWV shifts require high spectral resolution. These two characteristics are both dependent on the diameter of the pinhole used in the design, and so careful consideration must be attributed to this design choice.

Similarly, developing a system that is broadly applicable across smartphone handsets will prove difficult. While there is some observational evidence that handset manufacturers are converging on standard camera placement, the current variety, as well as dimensional variation, poses a significant challenge to large-scale adoption of the technology. While SLA provides a simple solution for low production volumes, high-volumes are often created via injection molding. To address this, the current approach is to focus on a single handset to develop the technology.
Additional options, such as the inclusion of an off-board CMOS chip connected via USB, will be investigated with future work.
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