Development of a Point-Of-Care Lensless Birefringent Molecule Detection System

by
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# Approval

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Abstract

This thesis is a proof-of-concept exploration of an optical birefringent Point-Of-Care (POC) detection device. Birefringent detection can be useful for monitoring glucose and cholesterol, as well as diagnoses of diseases such as malaria, Ebola, bacterial infection and AIDS. Many diseases cause optical birefringent materials to precipitate in blood. These precipitates can be used as a biomarker to diagnose the disease. In this thesis, we will focus on the development of a device for detection of a birefringent phantom, called Tetrabutylammonium (TA), suspend in deionized water. We will show a method for, a low cost, Point-Of-Care, and easy to use birefringent detection platform. This thesis builds on the concepts of flow cytometry for detection of depolarized light and uses these concepts for the development of a miniaturized optical birefringent detection setup, utilizing a lensless design, for a sample flowing through a microchannel. A microfluidic channel with a serpentine shape was developed in order to increase the volume of sample present within the detection area, while also decreasing the total volume used per measurement by reducing the cross sectional area of the channel. To demonstrate the concept of birefringent detection, a bulk optic setup was developed which used two detection channels. The two detection channels were a 2 dimensional (2D) Charged Coupled Device (CCD) and a 1 dimensional (1D) Avalanche Photodiode (APD). Using the bulk optic setup, we compared 2D imaging with 1D sensing and compared the ability of the two detectors to identify detection events. We identify detection events at a concentration of 1 µg/mL of TA using both 1D sensing and 2D imaging in the bulk optic setup, before using a 1D APD detector for the miniaturized optical setup. In the miniaturized optical setup, we detected events at the same concentration limit.

Keywords: Birefringent bio molecules, disease bio markers, Point-Of-Care, Malaria diagnosis, lensless optical detection
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## List of Acronyms

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<th>Description</th>
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<tbody>
<tr>
<td>ATC</td>
<td>Artemisinin Combination Therapies</td>
</tr>
<tr>
<td>BS</td>
<td>Beam Splitter</td>
</tr>
<tr>
<td>CCD</td>
<td>Charge Coupled Device</td>
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<tr>
<td>CDC</td>
<td>Center for Disease Control</td>
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<tr>
<td>CMOS</td>
<td>Complementary Metal Oxide Semiconductor</td>
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<tr>
<td>DI</td>
<td>Deionized water</td>
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<tr>
<td>ELISA</td>
<td>Enzyme Linked Immunosorbent Assay</td>
</tr>
<tr>
<td>iRBCs</td>
<td>Infected Red Blood Cells</td>
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<tr>
<td>LED</td>
<td>Light Emitting Diode</td>
</tr>
<tr>
<td>MOPID</td>
<td>Mobile Optical Polarized Imaging Device</td>
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<tr>
<td>NAA</td>
<td>Nucleic Acid Amplification</td>
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<tr>
<td>NI-DAQ</td>
<td>National Instruments Data Acquisition Card</td>
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<tr>
<td>Op-Amp</td>
<td>Operational Amplifier</td>
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<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
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<tr>
<td>PMT</td>
<td>Photomultiplier Tube</td>
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<tr>
<td>POC</td>
<td>Point-Of-Care</td>
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<tr>
<td>PS-OCT</td>
<td>Polarisation Sensitive Optical Coherence Tomography</td>
</tr>
<tr>
<td>RBCs</td>
<td>Red Blood Cells</td>
</tr>
<tr>
<td>RDTs</td>
<td>Rapid Diagnostic Tests</td>
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<tr>
<td>USB</td>
<td>Universal Serial Bus</td>
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<td>WHO</td>
<td>World Health Organization</td>
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Chapter 1: Introduction

Most current medical imaging modalities such as x-rays or investigating immunohistochemically stained tissue under a microscopic are used by specialist in radiology or pathology. Many of these techniques require specialized laboratory equipment and a specialist to diagnose the illness, therefore there is a need for all health care providers to be able to identify and diagnose various diseases at the Point-Of-Care (POC) level. POC technologies offer a low cost detection alternative that can provide useful data to health care professionals that can aid screening and diagnostics in all resource settings. In low resource environments, trained health care workers are not always available and therefore patients may have to travel a long distance to get the necessary care. Even in many high resource environments, there is often a delay between the laboratory where the tests are done and the clinic where the illness is diagnosed. With POC technologies, global health care could be transformed to help physicians in low and high resource settings provide accurate and fast disease screening and diagnosis [7]. Optical imaging technologies have several advantages for POC diagnostics, such as portability, low cost, and real time diagnosis [7]. Boppart et al. [7] states that POC technology could significantly improve screening and detection in both low resource and high resource settings.

The purpose of this thesis is to develop a novel POC system for birefringent detection. The approach developed in this thesis is a lensless detection system based on flow cytometry. The approach is designed around the detection of depolarization of light due to birefringent material. There are several medical applications that can be exploited by detecting birefringent properties of a crystalline product including glucose and cholesterol monitoring, detection of pathogenic bacteria and viruses, and detection of the malaria parasite [2]. Vallooran et al. have shown real time detection of different analytes for monitoring health and detecting diseases, by utilizing a birefringent based Enzyme Linked Immunosorbent Assay (Birefringent-ELISA) [2]. While most of the analytes require the use of Birefringent-ELISA, malaria has a birefringent by-product called hemozoin [2]. Therefore, the approach in this thesis can be utilized to detect hemozoin without the use birefringent-ELISA. In this thesis, we demonstrate that we can detect a birefringent phantom. This technique could be applied for a variety of medical applications. The main application that we will focus on, as our motivation, is the detection of malaria because malaria is a global endemic, developing a sensitive POC detection for low resource settings is a prudent exercise for applied research.
In 2015, The World Health Organization (WHO) reported 214 million cases of malaria and 438,000 deaths globally [3]. In endemic areas, less than 50% of suspected malaria infected patients are actually infected with malaria, and therefore, it is important that the diagnosis is confirmed [3]. The misdiagnosis of malaria has become a widespread problem and new drug resistant strains of malaria have emerged in recent years [4]. Therefore, there is a need for early and highly sensitive diagnosis. However, the current diagnostic modalities have several shortcomings. Many currently used techniques, such as a bright field microscopy, are restricted to a laboratory setting and require the blood smear to dry overnight before staining to prepare the sample [5]. In addition to long sample preparation time, the bright field microscope test can take up to 60 min to complete depending on how fast and skilled the technician is [6]. Novel systems and approaches for malaria diagnosis are needed for use in limited resource settings at the POC level.

While some POC malaria diagnostic technologies have been developed, effective treatment is limited by the accuracy of the diagnostic techniques. Developing portable diagnostic modalities for malaria detection with high sensitivity has been the focus of recent research [7], however effective treatment is difficult to control because of a lack of access to rapid, affordable, and accurate diagnostic techniques.

Several malaria detection approaches have been developed, as an alternative to the current techniques. Many of the new detection methods are based on detecting the birefringent properties of a malaria by-product, called hemozoin, in the blood of infected individuals. These methods have been shown to have high sensitivity and have the advantage of being able to easily distinguish whether a sample is infected with malaria. In the next section, the birefringent detection approaches are briefly explained.

1.1. Review of Birefringent Malaria Biomarker Detection

A dark field polarizing light microscope has been developed to detect the optical anisotropy of a blood smear for malaria infected blood [18]. The sample preparation is done by placing a drop of blood on a microscope slide with a glass coverslip place on top of the blood. This method for detecting malaria doesn’t require any staining. This method was tested with the use of rodent blood infected with the Plasmodium Yoelii strain [18]. A high power blue Light Emitting Diode (LED), with a peak wavelength of 470 nm, was used to illuminate the sample and a variable
dark-field stop was used. The microscope was configured in epi-illumination. Wilson et al. [18] reported a dark field spectrum of hemozoin with a peak at wavelength 670 nm and a smaller peak at 425-450 nm. This system has reduced the sample preparation time, by removing the need for staining, and shown indications that it has potential for highly sensitive detection, however this method was not tested for a detection limit. The dark field polarizing microscope would require some modification to make it a compact POC device.

Another method that was developed was the crossed polarized smart phone camera microscope which was used to detect hemozoin on a glass slide [17]. Pirnstill et al. [17] developed a mobile optical polarized imaging device (MOPID) and it was configured in trans-illumination. The MOPID was a lens attachment designed for the iPhone 5s, which used the concepts of a polarized light microscope and applied it to a portable platform. The lens attachment had an integrated white light LED source and a removable polarizer sheet to allow both bright field and cross polarized imaging modes. This allowed the group to do a comparison between Giesma stained blood slides and unstained blood slides. The smart phone based concept led to a portable detection device that can be used with minimum sample preparation.

A modified flow cytometer has been shown to be able to detect hemozoin in a malaria blood sample [27]. The flow cytometer was modified to include a depolarized side scatter channel. The flow cytometer used a 488 nm blue light excitation. The group modified the flow cytometer by replace a dichroic mirror with a 50/50 beam splitter (BS). This change allowed for 2 side scatter channels, where one had a polarizer placed before the detector in order to detect the depolarized light. Rebelo reports a detection limit of 1500 parasites/ µL (0.3 % parasitemia) [28]. The blood sample was centrifuged and only Red Blood Cells were observed in the detection channel.

There have been various approaches shown to detect the birefringent property of the malarial by-product hemozoin. Several of these approaches used an imaging technique which would require the user to interpret the results. While the another method used a sensing approach. The sample was either a blood smear or a flowing blood sample. The blood smears used for hemozoin detection, do not require the sample to be stained and therefore have a reduced sample preparation time. The flow cytometer required the sample to be centrifuged before use.
1.2. Overview of thesis

The goals of this thesis are to develop a compact, portable, POC device that detects birefringence. This thesis is a proof-of-concept exploration, where a birefringent phantom was used for experiments instead of malaria infected blood. Instead, the results based on detecting a birefringent material which simulates the properties of malaria are presented. As mention previously this approach can be utilized for a variety of applications. By varying the sample and using birefringent-ELISA this approach can be used to detect different analytes for monitoring health and detecting disease [2]. Some of the analytes that can be detected using birefringent-ELISA are glucose and cholesterol, pathogenic bacteria such as Escherichia coli, and viruses including Ebola and HIV [2]. The application that we focused on was malaria because a birefringent crystal, called hemozoin, is present within infected malaria blood. However, obtaining malaria infected blood was considered a biohazard and therefore avoided during the proof-of-concept exploration.

Before describing our approach, a background on the malaria parasite and the properties that make hemozoin an excellent candidate as a biomarker are discussed in Chapter 2. This chapter also includes a description of the birefringent sample that was used to demonstrate the ability to detect depolarization of light. Chapter 3 provides a discussion of the proof-of-concept methodology for a birefringent detection system based on flow cytometry that we designed and built. Chapter 4 contains an evaluation of the proof-of-concept design and the results for a glass capillary, before a new serpentine microchannel was developed in order to reduce the size of the channel and increase the total area that is exposed to the detector. The results of the serpentine channel are shown on the proof-of-concept design in chapter 5, along with the results for the compact design. Chapter 6 concludes the thesis with a summary of the experiments and a future works section.
Chapter 2: Malaria Parasite and Diagnostic needs

In this chapter, the motivation for malaria research is discussed. A background on the disease and the use of hemozoin as a biomarker are provided. The chapter also includes a description of tetrabutylammonium, the sample used to simulate the birefringent properties of hemozoin. Since the device described in this thesis is based on flow cytometry, a description of a conventional flow cytometer is also included in this chapter.

2.1. Motivation for Malaria Diagnostics

Malaria is a global endemic that spreads the plasmodium parasite through the bite of a female mosquito. Since 2000, a global effort has been made to reduce the risk of being infected with malaria by 37% and decrease the risk of mortality by 60% [4]. Even though the risk of infection and mortality have decreased significantly, the disease is still prevalent in African and South-East Asian countries. However, it is also found to a lesser extent in the Americas, Western Pacific, and Eastern Mediterranean regions. In high endemic areas, malaria is often misdiagnosed, which has led to the emergence of drug resistant strains of malaria [8]. During clinical diagnosis, if malaria infection is suspected, it should be confirmed with a light microscope or a Rapid Diagnostic Test (RDTs) [4]. However, in high endemic areas light microscopy is often not available and requires the sample to be prepared overnight [5]. While RDTs can provide a diagnosis around 15 minutes, they struggle to detect low levels of infection [6]. The current diagnostic modalities are Bright Field Microscopy, Rapid Diagnostic tests (RDTs) and to lesser extend molecular tests [12]. The current diagnostic modalities all have areas that could be improved on and therefore, there is a need for early and highly sensitive diagnosis. Before explaining the current imaging modalities, a background on malaria and the biomarker properties of hemozoin are provided.

2.2. Malaria Background

There are five species of the plasmodium parasite that affect humans P. falciparum, P. vivax, P. malariae, P. ovale, and P. knowlesi [4]. P. falciparum and P. vivax are the most prevalent and have the largest health risk for humans. P. vivax covers a wider geographical region, since it can survive at lower temperatures and higher altitudes. However, P. falciparum is most prevalent in Africa and is responsible for most of the malaria related deaths [4]. P. falciparum
and *P. vivax* pose the largest health hazard for humans and are the main focus of malaria research to date.

The five strains of malaria that affect humans all have similar behavior. The parasite goes through three different cycles. Figure 2-1 shows the life cycle of the malaria parasite. The first cycle is within the mosquito, during this cycle the parasite develops from gametocytes into sporozoites [1]. The sporozoites live in the salivary gland of the mosquito and they are then transferred to humans through the bite of a female mosquito. Once the parasite enters the human body the sporozoites travel within the blood stream and enter the liver. While within the liver the sporozoites infect hepatocytes and develop into schizonts, which eventually burst releasing merozoites into the bloodstream. This cycle is referred to as the pre-erythrocytic cycle [10]. During this cycle the patient is asymptomatic, therefore it is not the focus on malaria diagnosis and treatment. The erythrocytic cycle is where the merozoites enter red blood cells (RBCs) and the patient starts to experience symptoms [10].
Figure 2-1: The life cycle of the malaria parasite including the mosquito, the pre-erythrocytic, and the erythrocytic cycle.

In humans, symptoms start to occur when the parasite enters RBCs and it is the focus of diagnostics and treatment [1]. During the erythrocytic cycle the patient experience symptoms periodically. The first stage of the cycle is referred to as the immature trophozoite stage, or more commonly referred to as the ring stage. Hemozoin starts to form during the ring stage and has been reported to be detectable after approximately 6 hours [3]. Hemozoin has important optical and magnetic properties that can be used for detection, thus making hemozoin a good candidate as a biomarker. As the parasite matures further into the trophozoite stage, hemozoin continues to develop in size. The parasite itself continues to grow and forms new merozoites and then hemozoin is released into the blood stream when a schizont bursts [1]. Figure 2-2
shows the erythrocytic cycle. The figure shows a new merozoite entering a RBC and developing until it reaches the schizont stage where it bursts and releases new merozoites into the bloodstream.

Figure 2-2: Erythrocytic cycle where a new merozoite enter a RBC and develops into a schizont, which eventually burst.

The degree of malaria infection is reported as either percent parasitemia or in parasites/µL. Table 1 shows parasitemia levels and the equivalent parasites/µL with a description of how severe the symptoms are. The target level for a new detection device, which is set by the WHO, is 0.002 % parasitemia. Figure 2-3 shows a graph of percent parasitemia and parasites/µL. The plot also shows the points for the WHO target level, the point where an immune patient shows symptoms and the point where hyperparasitemia occurs.
### Degrees of Infection

<table>
<thead>
<tr>
<th>Parasitemia (%)</th>
<th>Parasites/µL</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0001-0.0004</td>
<td>5-20</td>
<td>No symptoms</td>
</tr>
<tr>
<td>0.002</td>
<td>100</td>
<td>May be symptomatic, WHO target detection level</td>
</tr>
<tr>
<td>0.2</td>
<td>10,000</td>
<td>Partially immune patients show symptoms</td>
</tr>
<tr>
<td>2</td>
<td>100,000</td>
<td>Max parasitemia for P. Vivax/P. Ovale</td>
</tr>
<tr>
<td>5</td>
<td>250,000</td>
<td>Hyperparasitemia, severe malaria, increased mortality</td>
</tr>
<tr>
<td>10</td>
<td>500,000</td>
<td>High mortality</td>
</tr>
</tbody>
</table>

*Table 1: Parasitemia levels and a description of symptoms experienced from the malaria parasite [20].*
The symptoms for malaria include fever, headache, nausea, vomiting, diarrhea, and muscle pain. Since these symptoms are non-specific it is important to rule out other illnesses when diagnosing malaria [19]. Once malaria reaches 5% parasitemia or hyperparasitemia, symptoms become more severe and include severe anemia due to hemolysis, abnormalities in blood coagulation, acute kidney failure, cerebral malaria with abnormal behavior and death if malaria is left untreated [19].

A detection device that is portable, POC, and affordable can increase the number of patients screened for malaria and improve diagnosis. The WHO has outlined a target detection limit of 0.002 percent parasitemia or 100 parasites/µL [20]. At 100 parasites/µL (0.05 µg/mL), patients may start to experience symptoms, therefore this is a reasonable target for a detection limit. The WHO reported that in 2015, 44% of malaria patients were treated without a diagnostic test to confirm the illness [4], which suggests that many patients were treated for malaria without having the disease. Physicians often diagnosed malaria based only on general symptoms because diagnostic tests were not available to confirm the illness, which resulted in a high number of false positives. The WHO reported that the P. Falciparum strain developed drug
resistance to Artemisinin Combination Therapies (ATCs) in five countries in the Greater Mekong subregion [4], therefore to prevent drug resistance it is important to treat only patients that have the illness. A diagnostic device with high specificity that can be distributed to more health care providers can improve malaria detection by increasing the number of patients being treated with a diagnostic test.

2.3. Hemozoin as a Biomarker

Recent research has focused on the detection of hemozoin as a way of determining whether a patient is infected with malaria [37]. Hemozoin has several properties that make it a good candidate for detection. The birefringence property is the focus of hemozoin detection.

Once the malaria parasite enters a RBC it begins a process that creates a by-product called hemozoin. Hemozoin has a micro-crystalline structure that is produced when the parasite detoxifies free heme, which comes from hemoglobin. The malaria parasite transforms accumulated monomeric heme into an insoluble crystallized form. Hemozoin continues to develop in size as the parasite matures into the trophozoite stage. This continues to happen until the parasite releases the newly formed merozoites into the blood stream as the schizonts burst [1]. Blood that is infected with a clone of P. Falciparum was reported to form hemozoin particles with a cross section of 100 nm by 100 nm and a length of 300-500 nm [9]. Hemozoin is formed in a triclinic crystal structure [23].

All plasmodium species of malaria produce hemozoin, which is a brown birefringent crystal [9]. The birefringent component of the crystal is an important property that can be exploited for detection purposes. A material that is birefringent will have a different index of refraction depending on the polarization angle of the light that is illuminating the material. Since hemozoin demonstrates a high degree of optical anisotropy the depolarization of light can be detected using polarization optics. The birefringent property allows for automatic detection of malaria as opposed to manual counting the infected cells.

The birefringent property of hemozoin can be used to detect malaria in the blood, however it is important to consider other birefringent materials that could contaminate the sample and lead to false positives. There are other components in human anatomy that have depolarization properties, such as melanin from skin. Polarization Sensitive Optical Coherence Tomography
(PS-OCT) was reported to measure the birefringent nature of human skin [24]. Baumann et al. used PS-OCT system to measure depolarization of light from the melanin phantom and concluded that higher concentrations of melanin produce more depolarization of light [25]. Hemozoin based detection systems use a human blood sample, which is doesn’t contain melanin. To prevent false positive measurements, it is important to ensure that human skin does not contaminate a blood sample.

### 2.4. Malaria diagnostic approaches

The most commonly used method for detecting malaria is still the use of a bright field microscope. This method has the capabilities of detecting low levels of the malaria parasite in red blood cells (RBCs). Zimmerman reports the lower detection limit to be between 5 and 200 parasites per µL (0.0001-0.004 % parasitemia) however this method depends heavily on the ability of the technician [13]. Before performing the test, the technician is required to prepare a stained blood smear, most commonly stained with a Giemsa stain [15]. After preparing the sample the technician manually counts the number of infected red blood cells (iRBCs).

According to the Center for Disease Control (CDC) a minimum of 500 RBCs should be counted to accurately diagnose a patient [16]. Since the technician needs to manually count the RBCs, the detection limit is dependent on the skill of the operator and there is potential for human error in this time consuming technique. Sample preparation and requirements to meet ISO 9000 microscope standards, means that this technique is limited to a laboratory setting [15]. Since this technique is limited to a laboratory setting it is often difficult to diagnose people in high endemic areas, which are far away from any hospitals [17].

The limitations of the bright field microscope have led to the development of the rapid diagnostic tests (RDTs). The RDTs were developed as a POC alternative and offer benefits such as no sample preparation, portability, and fast diagnostic speed. RDTs use an antigen based detection system that takes a finger prick of blood [18]. An RDT is self-administrable and easily field deployable, which is beneficial for high endemic areas. Another benefit of RDTs is that they are able to diagnose a patient quickly. However, RDTs have some limitations, such as different antigen requirements for different strains of the disease [15]. Therefore, a combination of antigens is required to detect and identify the malaria strain. It is also important to note that in tropical areas, where malaria is most prevalent, RDTs experience degradation in performance [17]. Zimmerman et al. reports the detection limit of 200 parasites per µL (0.004 % parasitemia)
A less commonly used method for malaria detection is Nucleic Acid Amplification Tests (NAA), the most commonly used NAA is the Polymerase chain reaction test (PCR). NAA tests have increased sensitivity over microscope and RDTs [21]. The lower detection limit is reported to be 5 parasites per µL (0.0001 % parasitemia) [13]. A benefit for NAA test is that they are capable of detecting mixed infections and can differentiate between different strains of malaria [21]. Molecular tests are mostly used as a complementary test to the bright field microscopy because of the ability to determine different strains of the disease [21]. However, NAA test are not able to identify the stage of the malaria cycle [13]. Sample preparation is also required unlike RDTs. DNA is extracted from a dried blood sample and stored in a buffer solution at -20 °C until use [21]. Storing the sample at such a low temperature is difficult without a laboratory setting.

The current technologies all have different areas that could be improved upon. The bright field microscope is not portable, requires a skilled technician, and has complicated sample preparation. RDTs require different antigens for different strains of the disease, have a poor detection limit, and have lower performance in tropical environments. Molecular tests have problems differentiating between different stages within the malaria cycle, have complicated sample preparation, long test times, and are limited to a laboratory setting. These limitations offer an opportunity to design a novel diagnostic system for portable cost-effective rapid malaria diagnosis.

A new system should have the following requirements: the device should have high sensitivity and specificity. It should also be a POC rapid diagnostic system, portable and cost effective. The system should not require special training or skill sets.

2.5. Conventional Microfluidics

A representative model for the state of the art flow cytometer is the SL CyFlow Blue. For the purposes of this thesis, we will use the SL CyFlow Blue as a representative benchmark for our research. Rebelo et al. [27] demonstrated that the SL CyFlow Blue can be modified to include a
depolarized side scatter channel. The SL CyFlow Blue uses a single solid state laser at a wavelength of 588 nm. There are five detection channels available on this flow cytometer, these channels are forward scatter, side scatter, and three fluorescent channels (green, orange, and red). Each detection channel uses a Photomultiplier Tube (PMT). A Charged Coupled Device (CCD) camera is used to monitor the flow. The SL CyFlow Blue is large with a weight of 17 Kg, a cross-section of 430 mm by 370 mm and a height of 160 mm. It also comes equipped with a syringe pump that can pump with speeds up to 1200 µL/min through a channel with a cross-section of 200 µm by 350 µm. To analyze RBCs, 200 µL of centrifuged blood is used with the standard flow cytometer [28].

As mentioned, the conventional flow cytometer has a large volume, therefore making it impractical as a portable device. In this thesis, the concepts of the flow cytometer are built on to develop a miniaturized design of the optics for the detection of depolarization of light. The conventional flow cytometer also uses 200 µL of centrifuged blood. In chapter 5 we will present a miniaturized microfluidic channel designed to reduced the volume to a 11.5 µL, which is equivalent to a finger-prick of blood and therefore making the product minimally invasive.

2.6. Tetrabutylammonium

Hemozoin is a by-product of the malaria parasite and therefore the risk of infection when working with malaria infected blood presents a significant biohazard. Although hemozoin is a by-product of the malaria parasite there can be synthetically grown versions of hemozoin, which demonstrate similar chemical compositions, crystal structure, optical properties that make the crystal an ideal alternative for detection of hemozoin [7]. However, an experienced chemist is needed to synthetically grow hemozoin and since we didn’t have the expertise or the resources we chose to use a birefringent crystal that shared similar properties to hemozoin.

For testing we chose a sample that shared similar properties as the malaria pigment hemozoin. Since the objective was to detect the birefringent property associated with hemozoin, a crystal that shared this property was the priority for the experiment. Ideally a crystal that had the same size and crystal structure was also desired.

The sample used is called tetrabutylammonium (TA), because it is birefringent like hemozoin. TA also has the same triclinic crystal structure as hemozoin [22]. The sample was suspended in
deionized water (DI). The sample concentration was recorded in terms of micrograms of TA per milliliter of DI (µg/mL). Orbán et al. provided a conversion between µg/mL of hemozoin to parasites/µL [26]. Since the sample was TA and not hemozoin, we assumed that 1 g of TA has the same conversion factor as 1 g of hemozoin. Figure 2-4 below shows a graph for conversion between concentration from µg/mL to parasites/µL. The red line shows the reported detection limit for the flow cytometry setup used by Rebelo et al. [28]. The detection limit of 1 µg/mL (1500 parasites/µL), reported by Rebelo, is used as a reference point for the experimental results.

![Conversion graph for parasites/µL to µg/mL](image)

Figure 2-4: Conversion graph for parasites/µL to µg/mL. The red line indicates the detection limit reported by Rebelo [28] and the green line is the WHO target limit of 100 parasites/µg (50 ng/mL).

TA was used a substitute for hemozoin because of the birefringent properties [29]. Utilizing TA as the birefringent sample instead of hemozoin had some limitation because there wasn’t a comparison between the two crystal and their birefringent properties. We don’t know if TA exhibits more birefringent properties than hemozoin and therefore the system we built could be less sensitive to detecting hemozoin crystals.
2.7. Summary

In this section, we provided a background on the malaria parasite. We also discussed the motivation for malaria diagnostic. We also discussed the use of hemozoin as a biomarker, due to the birefringent properties. Since there are high safety precautions required and difficulties obtaining the malaria infected blood an alternative sample were utilized. We found a sample that shares similar properties in terms of size and birefringence to hemozoin. Also, a summary of a conventional flow cytometer was explained in this chapter. In the next chapter, the bulk optic proof-of-concept experiments are described.
Chapter 3: Methods for Bulk Optic Birefringence Detection

In this chapter, our design of a birefringent detection system is discussed. For our proof-of-concept, we designed a flow cytometer that detects depolarization of light. In this chapter we will show the optical design for the flow cytometer system that was designed with two detection channels. One detection channel was based on a 2D image of the sample. The images captured by the 2D imaging channel were captured by first a Complementary Metal Oxide Semiconductor (CMOS) camera and then a CCD camera. In addition to a signal comparison, a noise comparison between the CMOS camera and CCD camera was done, before the CCD camera was chosen for further experiments. The other channel was setup to detect a 1D light intensity signal and was captured with an Avalanche photodiode (APD). The CMOS/CCD imaging channel offered a visual representation of the data and was used to understand the APD sensing signal. The CMOS/CCD signal was also summed to approximate the sensing data from the APD channel. By doing the comparison between CMOS/CCD signal and the APD signal, we could correlate the two detection channels. The proof-of-concept shows that we can detect birefringent particles in flow using this method. Validation of the approach with this system leads towards the compact design of the optics and microfluidics that is presented in chapter 5.

The birefringence detection system, that we developed, can be considered in four main components: the optics, the electronics, the data processing, and the microfluidics. The first component that will be discussed is the optics, where the overall schematic will be presented and an explanation of how the polarizers are used to detect birefringence.

3.1. Bulk Optic Setup

Figure 3-1 shows the optical setup that demonstrated the proof-of-concept. A white light LED is used to illuminate the sample. A linear polarizer is placed before the light is incident on the sample. The light that scatters off the sample is then collected by the objective lens and passes through a second linear polarizer, referred to as the analyzer. The analyzer is rotated 90 degrees from the polarizer. The purpose of the analyzer is to allow only the birefringent light to transmit through and extinguish all other light. That means if no birefringent material is present within the sample then no light will be detected. After the analyzer there is a 50:50 beam splitter
(BS), were half the light goes to the camera and the other half goes to the avalanche photodiode (APD). Both a CMOS camera and a CCD camera were used to acquire data.

<table>
<thead>
<tr>
<th>Distances</th>
<th>(mm)</th>
<th>Focal Lengths</th>
<th>(mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>d1</td>
<td>50</td>
<td>$f_1$</td>
<td>50</td>
</tr>
<tr>
<td>d2</td>
<td>11</td>
<td>$f_{obj}$</td>
<td>11</td>
</tr>
<tr>
<td>d3</td>
<td>131</td>
<td>$f_{tube1}$</td>
<td>120</td>
</tr>
<tr>
<td>d4</td>
<td>120</td>
<td>$f_{tube2}$</td>
<td>4</td>
</tr>
<tr>
<td>d5</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d6</td>
<td>4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: List of distances and focal lengths for the schematic in Figure 3-1.

3.1.1. Imaging and Sensing

In the bulk optical setup, both a camera for imaging and an APD for sensing were used. The camera allowed us to visually see particles as they appeared and allowed us to better understand the APD signal. The pixels in each frame of the camera were summed into a single value, this provided a 1-dimensional (1D) signal representation of the camera images that could be used as a comparison with the APD signal. We could identify what an event on the APD signal represents by comparing it with the camera images. The detectors used in this...
experiment didn't have a trigger and therefore a time delay may have been introduced to either detector signal. To compensate for the time delay the APD signal was time shifted during data processing after the experiment.

3.1.2. Polarization Sensitive Detection

In this section we will examine the role of the polarizers in the schematic shown in Figure 3-1. In this schematic there are 2 polarizing elements present, they are labeled as polarizer and analyzer. By definition when unpolarized light passes through a dichroic and the transmitted light becomes polarized the device is called a polarizer [34]. However, when the light is already polarized and transmits through a dichroic medium then the device is called an analyzer. The LED light source produces unpolarized light, which passes through optical component called a linear polarizer and transmits polarized light, therefore this element is called the polarizer. The role of the polarizer is to confine the polarization of the light source to a single plane. It also orients the polarization of the light source to be parallel to the plane of incidence (p-polarized). The analyzer is placed after the sample, the analyzer is oriented perpendicular to the plane of incidence (s-polarized). Figure 3-5(A) shows, unpolarized light with random polarization, while Figure 3-5(B) shows linearly polarized light in the vertical direction.

![Figure 3-2: A) is unpolarized light, where the plane of polarization is randomly oriented. B) linearly polarized light in the vertical direction.](image)
The angle of orientation between the polarizer and the analyzer needs to be 90 degrees. This is important because this will extinguish light after the analyzer, unless a birefringent sample is present. Polarized light can be represented mathematically with Jones Calculus. Polarized light can be represented with a Jones Vector $\begin{bmatrix} E_1 \\ E_2 \end{bmatrix}$, where $E_1$ is the field in the horizontal direction and $E_2$ is the field in the vertical direction. A linear polarizer can be represented by a Jones matrix, where the matrix $\begin{bmatrix} 1 & 0 \\ 0 & 0 \end{bmatrix}$ represents horizontally polarized light and $\begin{bmatrix} 0 & 0 \\ 0 & 1 \end{bmatrix}$ represents vertically polarized light. Equation 1 shows, a mathematical representation of the effect of 2 linear polarizers, oriented horizontally and vertically, on unpolarized light. The resulting Jones vector is zero, indicating that all light has been extinct. When 2 polarizers are mutually orthogonal, as shown in equation 1, then they are said to be in a cross-polarized configuration [34]. In the setup in Figure 3-1, this indicates that no birefringent sample is present.

$$\begin{bmatrix} 1 & 0 \\ 0 & 0 \end{bmatrix} \begin{bmatrix} E_1 \\ E_2 \end{bmatrix} = \begin{bmatrix} 0 \end{bmatrix}$$  (Equation 1)

$$\begin{bmatrix} 1 & 0 \\ 0 & 0 \end{bmatrix} \begin{bmatrix} \varepsilon_{xx} & \varepsilon_{xy} \\ \varepsilon_{yx} & \varepsilon_{yy} \end{bmatrix} \begin{bmatrix} 0 & 0 \\ 0 & 1 \end{bmatrix} \begin{bmatrix} E_1 \\ E_2 \end{bmatrix} = \begin{bmatrix} \varepsilon_{xy} E_2 \\ 0 \end{bmatrix}$$  (Equation 2)

Birefringence is when a transparent crystalline material creates optical anisotropy for the light that propagates through it [34]. A birefringent material has a different index of refraction for different polarization angles of light incident on the material. In Equation 2, a model is presented of a birefringent sample present between the polarizer and the analyzer. The birefringent material is modeled by the matrix $\begin{bmatrix} \varepsilon_{xx} & \varepsilon_{xy} \\ \varepsilon_{yx} & \varepsilon_{yy} \end{bmatrix}$. Since the light incident on the sample is affected by the birefringent material that is present some light will reach the detector. The principle of cross-polarized detection allows only material that have birefringent properties, such as the TA sample used for the experiments, to be detected.
3.2. Design of Analog Circuitry for Birefringent Detection

For the experiment, a white light LED was used to illuminate the sample. The LED was pulsed at 800 hertz. The light source was pulsed to modulate the signal and then a bandpass filter was used to suppress noise that appeared on the APD channel. By introducing a carrier wave at a single frequency (800 Hz), a type of lock-in detection could be performed because the signal will only be present at this frequency. Figure 3-3 shows the circuit schematic used for the detection of the APD signal. The circuit is split into four blocks which are: the light detection, bandpass filter, rectifier, and input into a computer. An APD module was used for this experiment. The output of the APD module went into a breadboard, where a bandpass filter and a half wave rectifier circuits were implemented. After the half wave rectifier, the signal was read into a laptop with a National Instruments data acquisition card (NI-DAQ). The NI-DAQ (USB-6215) connected to the computer through a USB connection. The output of the half-wave rectifier went into the NI-DAQ board where the analog signal was read by Matlab on the acquisition computer. While some additional circuitry was required for the APD signal, the cameras were read directly into Matlab. The CMOS camera used a USB connection, while the CCD camera was connected through gigabit Ethernet (gigE) connection.

![Circuit Schematic](image-url)

*Figure 3-3: Circuit Schematic overview. The circuit was four main blocks, the light detection, the bandpass filter, the half wave rectifier, and the computer input. In the bandpass filter stage, the resistors R1, R2, and R3 are equal and have a value of 28 kΩ. The Resistors R4 and R5 are used to adjust the gain and have value of 3.9 kΩ and 1.4 kΩ. The resistor in the half wave rectifier stage has a value of 82 kΩ, while the capacitor has a value of 10 nF.*
3.2.1. Modulation

Noise on the analog signal can be reduced by modulating the signal. We used a Single Side Band modulation method called Frequency Discrimination to suppress the noise [40]. In this method, the carrier signal is produced by pulsing the LED light source and the message signal is produced by the scattered light from the birefringent particles. In Equation 3 we have the carrier signal which was a sinusoidal signal at a frequency, $f_c$, of 800 Hz. Equation 4 was the modulated signal in time domain, which was the product between the message signal $m(t)$ and the carrier signal $c(t)$. The modulated signal produces an upper and a lower sideband which were centered around the carrier frequency ($\pm f_c$). The two sidebands are expressed in Fourier space by Equation 5 before a bandpass filter was used to suppress the lower sideband.

\[
c(t) = \cos(2\pi f_c t) \quad \text{(Equation 3)}
\]
\[
s(t) = m(t) \cos(2\pi f_c t) \quad \text{(Equation 4)}
\]
\[
S(f) = \frac{1}{2} A_c [M(f-f_c) + M(f + f_c)] \quad \text{(Equation 5)}
\]

3.2.2. Light Detection

The light detection was done with an APD module. The APD module used was the Laser Components LCSA500-01. The module output a voltage, therefore there was no need to build a trans-impedance amplifier. The APD module had a bandwidth of DC-1 MHz and was more than adequate for the frequency at which particles were detected. The module had an APD detector with an active area of 0.5 mm. A different APD module was used for the compact design.

3.2.3. KRC Bandpass Filter

After the APD module, a bandpass filter was used. The purpose of the filter was to remove noise by suppressing the frequency information that is outside of the modulation center frequency. This bandpass filter was chosen to pass the frequencies within the frequency range
of the light source and attenuate frequencies outside the frequency range. The center frequency of the filter was chosen to match the frequency of light source. The center frequency of the filter was 800 Hz and the bandwidth (BW) of the filter was 100 Hz. The bandpass filter was designed according to the rules from the textbook “Design with Operational Amplifiers and Analog Integrated Circuits” by Sergio Franco [32].

The filter bandpass stage is set by an R-C stage followed by a C-R stage. A gain stage is provided by the positive feedback from via $R_3$. As mentioned before the BW was chosen to be 100 Hz and the center frequency was chosen as 800 Hz. Given the center frequency and BW, a selectivity factor, $Q$, can be calculate by Equation 6. Using Equation 6 the selectivity factor, $Q$, is equal to 8.

$$Q = \frac{\omega_0}{BW}, \quad \text{(Equation 6)}$$

A more selective filter has a narrower BW. A convenient choice is to set $R_1 = R_2 = R_3 = R$ and $C_1 = C_2 = C$. In the case of $Q > \sqrt{2}/3$, the gain of the bandpass circuit can be described by Equation 7.

$$H_{OBP} = \frac{K}{4 - K}, \quad \text{(Equation 7)}$$

The center frequency is defined by Equation 8.

$$\omega_o = \frac{\sqrt{1 + R_1/R_2}}{\sqrt{R_1C_1R_2C_2}}, \quad \text{(Equation 8)}$$
and a selectivity is given by Equation 9.

\[ Q = \frac{\sqrt{2}}{4 - K} \]  \hspace{1cm} (Equation 9)

The design equations can be simplified are setting \( R_1 = R_2 = R_3 = R \) and \( C_1 = C_2 = C \). Equation 8 can be simplified towards Equation 10 and then it can be used to calculate the resistor and capacitor values that are needed to get the desired center frequency. Equation 9 can be rearranged to Equation 11, which provides the gain of the circuit for the desired selectivity factor \( Q \).

\[ RC = \frac{\sqrt{2}}{\omega_0} = \frac{\sqrt{2}}{2\pi f} \]  \hspace{1cm} (Equation 10)

\[ K = 4 - \frac{\sqrt{2}}{Q} \]  \hspace{1cm} (Equation 11)

\[ R_4 = (K - 1)R_5, \]  \hspace{1cm} (Equation 12)

Since the desired frequency was 800 Hz, Equation 10 can be used to calculate an RC value. Choosing a capacitor value of 10 nF, the resulting resistor value of is 28 kΩ. In order to critically damp the circuit, the resistor ratios of \( R_4 \) and \( R_5 \) should be defined by Equation 12. Using Equation 11, the gain \( K \) is found equal to 3.82 and therefore \( R_4 = 2.82R_5 \). Figure 3-4 shows the KRC bandpass filter, with the resistor and capacitor values that were used to implement the filter.
3.2.4. Half-wave Rectifier

The next stage of the circuit, was the half wave rectifier. A rectifier was used for converting an AC signal into a DC signal. The purpose of the rectifier circuit, in birefringent detection, was to detect peaks. Since the light source was pulsed, the response signal from the bandpass filter was a sinusoidal wave. When a birefringent crystal was present within the flow chamber and detected, then the amplitude of the sinusoidal wave increases. However, it was difficult to notice a change in amplitude, therefore the sinusoidal signal was passed through a half-wave rectifier. The half wave rectifier then had a constant signal value as if no birefringence particles were present and spikes appear if birefringent particles were detected.

The half wave rectifier used a diode to remove any negative voltage. Figure 3-5(A) shows the effect of a diode on a sinusoidal signal. The diode allows only the positive voltage to pass through and blocks and negative voltage. By doing this, the output signal had only the positive peaks and not the negative peaks of the sinusoidal wave. Figure 3-5(B) had a capacitor added in parallel to the resistor from Figure 3-5(A), the capacitor was charged when the voltage was positive and slowly discharges before becoming fully charged during the next period. The output of the rectifier was defined by the time constant, $\tau = RC$. It was important to pick the time constant appropriately, so that the capacitor didn’t discharge too quickly. However, the time constant cannot be too long either, since the signal would be integrated over time. The time constant that was chosen for the half-wave rectifier was $82 \mu s$ or approximately four times larger than time constant for the 800 Hz light source signal. The resistor value was 82 KΩ and the capacitor value was 10 nF.
Figure 3-5: A) shows the effect of the diode on an AC signal. B) shows the half-wave rectifier circuit. The resistor value was 82 kΩ and the capacitor was 10 nF.

The half wave rectifier used was a passive circuit. The input for the half wave rectifier comes directly from an operational amplifier (Op-Amp) and therefore doesn't require a buffer. Since the next stage was the NI-DAQ card, which had high input impedance, there was no need to buffer the output of the half wave rectifier.

3.2.5. Data Processing

In this section, the methods used for data processing are shown. The video data was used to create a 2-dimensional video representation. The video data was also used to create a 1D signal that was compared with the APD signal. All data processing was done using Matlab.

A 2D representation of the video data was developed by colour coding the frames of video. Figure 3-6 shows the colour coding method used to create the 2D representation of the data. The first step was done by multiplying the video data with a colour coded matrix. Then all the frames of the colour coded matrix were summed into a 2D image. Since the cross polarized configuration removed all light that wasn’t birefringent, the background wasn’t affected by the colour coding method. Only birefringent particles produced a signal that was detected by the camera and therefore only the birefringent particles were colour coded. Since each frame has a different colour, each colour represents a different moment in time.
Figure 3-6: Colour coding method for 2 dimensional video representation. The video data was multiplied by a colour coded matrix.

The video data was also used to create a 1D signal that could be compared with the APD signal. Figure 3-7 shows the summation of each pixel within each frame that created a 1D signal of intensity vs time, which can be compared with the APD signal.

Figure 3-7: Summation of video data to create an approximation of the APD signal.

The APD signal was processed using Matlab. A peak detection filter was a software implemented rectifier and was used to improve on the output signal from the half wave rectifier. The peak detection filter finds local peaks and returns a matrix that consists of only the data points that were larger than the two neighboring data points. After the peak detection filter was
implemented, an averaging filter with a window size of five was used. The same averaging filter was also applied to the summed camera signal. The size of the window on the averaging filter remained the same size for all data sets. Figure 3-5(A) shows unprocessed data of a DI water sample. Figure 3-5(B) shows the signal after the peak detection filter was applied. Figure 3-5(C) shows the signal after peak detection and with an averaging filter applied.

![Figure 3-8: A) shows the unprocessed data. B) shows the peak detection of unprocessed data. C) shows the peaked detected data with an averaging filter applied.](image)

### 3.3. Microfluidics

In the optical setup in Figure 3-1, a microchannel was placed on the sample plane. As mentioned in section 2.4, the TA sample was suspended in DI water. Figure 3-9 is a picture of the microchannel and all if its components. The microchannel consists of a syringe to pump the sample through the channel, a glass capillary, and some tubing to connect the capillary to the syringe. For the initial proof-of-concept test, a square glass capillary was used. The square shape of the capillary was important, since a round capillary introduces a lensing affect. The size of the channel was a 1 mm x 1 mm square capillary. The total volume used per test was approximately 500 µL.
The glass capillary was able to provide an excellent test for how a conventional flow cytometer works, however they contained a large volume of liquid. In chapter 5, the methods for how a new microchannel was fabricated to reduce the total volume of sample, while also maximizing the volume of sample exposed to the detector.

### 3.4. Summary

In this chapter, an overview of the proof-of-concept design was presented. The proof-of-concept consisted of a crossed polarized optical setup that compares imaging and sensing. From the imaging side of the detection a CMOS and a CCD camera were used and could be plugged into a laptop directly. While the sensing required some additional analog circuitry before the signal went to the laptop through an NI-DAQ card. In the next chapter, an evaluation of the proof-of-concept design will be shown.
Chapter 4: Bulk Optics Proof-of-Concept Evaluation

The purpose of this chapter is to compare imaging (2D) and sensing (1D) for detecting birefringent material within a flow channel. Before performing the experiment, a comparison between the performance of a CMOS camera and a CCD camera was done to identify which camera was best for detection of birefringent particles. The CMOS camera was more cost effective and had a faster readout time than the CCD, but the CCD had less noise and higher sensitivity. In this chapter we present a representation of video data from the 2D detector in comparison with a 1D signal. The 2D image provides a visual representation of birefringent particles that were present within the detection channel. The 2D image was summed to create an equivalent of the 1D signal from the APD, which was used to compare and map the data. Results are shown for the APD and CCD camera for three different samples. The samples used were: DI water, TA suspended in DI water, and borosilicate beads suspended in DI water. The DI water was the first control sample. We introduced borosilicate beads to the control sample to simulate the scattering effect of non-birefringent particles that would exist within human blood. The sample with TA suspend in DI water was measured at different concentrations.

4.1. Camera comparison

In this section the CMOS and CCD cameras are compared. The CMOS camera that was used for the experiment was the Thorlabs DC1545M camera. The Thorlabs DC1545M camera has a pixel dimension of 1280Hx1024V which corresponds to an active area of 6.66mm x 5.32 mm. The CCD camera that was used was the Basler Scout SCA1390-17gm camera. The Basler Scout SCA1390-17gm camera has a pixel dimension of 1392Hx1024V which corresponds to 6.46 mm x 4.76 mm. The noise value of both cameras was measured and images were compared between the two cameras.

Two experiments were performed to compare the cameras. To measure the dark noise for each detector, 10 seconds of video was acquired with the sensor covered. Each frame of the video was summed into a single value and then plotted against time. Both cameras are 8-bit, so the maximum pixel intensity was 255. For this experiment the camera parameters were set to identical values, to do a fair comparison. The frame rate was 15 frames/sec, the exposure was 40 ms, and the gain was set to 50. In Figure 4-1, the plot of the dark noise of the two cameras are shown. From Figure 4-1(A), the total noise for the CMOS was much higher than the CCD.
From Figure 4-1(B), the total noise of all the pixels for the CCD camera was in the range of 19 to 23. While in Figure 4-1(C), the total noise for the CMOS camera was on the order of $10^5$.

![Camera Noise Comparison](image)

**Figure 4-1**: Noise comparison between CMOS and CCD camera. Where A) shows the comparison on one plot, while B) and C) show the noise for the CCD and CMOS cameras respectively. In A) the orange line represents the noise of the CMOS camera, while the blue line represents the noise of the CCD camera.

A second experiment was used to compare the two cameras. In this experiment images of a flowing sample, in a microchannel, were captured. Figure 4-2 shows a comparison between two sequentially acquired data sets obtained with the CMOS camera and then with the CCD camera.

![Noise for CCD Camera](image)

![Noise for CMOS Camera](image)

A 2D video representation was developed and presented in Section 3.2.4. Each frame of the video was colour coded with time and then a pixel-wise summation of all the frames was performed. The resulting image, show a black image if there was no signal present and has coloured spots, which correspond to a temporal legend, at any location where a birefringent detection event occurred. The temporal legend remains black unless a birefringent detection event was recorded on the camera. 2D video representative image, that was acquired by the
CMOS camera for and a DI water sample and a TA sample concentration of 10 mg/mL are shown in Figure 4-2(A) and (C), respectively. Figure 4-2(B) and (D) shows 2D video representative image acquired by the CCD camera for a DI water sample and a TA sample concentration of 10 mg/mL. The 2D video representative images acquired from the CMOS camera have more noise, while the CCD images show only the birefringent particles. The CCD images also have higher contrast than the CMOS images. After performing the two experiments, it was clear that the CCD camera was the better option for comparing with the APD signal, since it had less noise and higher sensitivity to detect birefringent particles.
Figure 4-2: Measurements of DI water (A and B) and 10 mg/mL sample of TA (C and D) measured with a CMOS detector (A and C) in comparison to a CCD detector (B and D). There are two blue lines annotated on each figure, which represent the boundary of the channel.

In addition to our experiment, we compared the detector specifications for noise performance. The noise sources of interest were the dark noise and the readout noise, since the experiment was conducted in a low light setting. The Thorlabs DC1545M CMOS camera had a reported dark noise value of 10 e⁻/pixel and a reported readout noise value of 20 e⁻/pixel. The Basler
Scout SCA1390-17gm CCD camera had a reported dark noise value of 10 e/pixel and a reported readout noise value of 3.1 e/pixel. The readout noise reported was significantly lower for the CCD camera and was the likely cause of the noise measured in our experiment. A smartphone based detection system could be developed provided that it performed similar to the CCD camera used in our experiment. Smartphone camera manufactures do not commonly present dark noise or readout noise values, however, Holland did a qualitative comparison of camera performances in low light settings [42]. Holland concluded that in low light settings, high quality Digital Single Lens Reflex (DSLR) cameras such as Nikon D800 outperformed smartphone cameras in terms of noise and preserving the details of an image. Mid-range DSLR cameras produced sharper, but noisy images than smartphone cameras [42]. The smartphone camera images benefited from software based noise reduction techniques to improve the image quality. It is difficult to determine whether a smartphone camera would be effective for birefringent detection without measuring the dark noise and readout noise characteristics, however based on the qualitative analysis high end smartphone cameras are worth further investigation. A key point to also consider is that the frame rate and integration time of the smartphone based cameras may also not be well suited for this application.

4.2. Results comparing CCD and APD signal

In this section, results are presented to show that the CCD and APD are both capable of detecting birefringent particles. All the measurements within this section were done with the same camera parameters. The frame rate was set to 21.7 frames/sec, the exposure was 40 ms, and the gain was set to 50. Three different samples were experimented with and measured by the optical setup. There were two control samples experimented with, the first control sample was DI water and the second sample was DI water with 10 µm diameter borosilicate beads mixed within the sample. The addition of the borosilicate beads provided non-birefringent scattering, which was utilized to simulate the scattering properties of other particles or cells that exist within human blood. To simulate the birefringent properties of hemozoin, experiments were done with TA. The initially concentration was mixed at a concentration of 10 mg of TA/mL of DI water. After doing test at the highly concentrated sample of TA (10 mg/mL), the sample was diluted with DI water to produce lower concentrations until a detection limit was reached.
4.2.1. Control Sample

The control sample was measured to confirm that no signal was observed when no birefringent material was in the sample. The control sample was first measured with a DI water sample. A 1 mL sample of DI water was mixed with a drop of soap (0.05 mL). The purpose of the soap was to act as a detergent and prevent the formation of large particles and prevent the build-up of particles on the walls of the channel. The soap was added to the control sample to ensure that it didn’t affect the signal. Figure 4-3(A) shows the 2D representation of the CCD video data recorded for the DI water sample. In Figure 4-3(B), the 1D CCD signal is compared to the 1D APD signal and the two signals are displayed in a percentage variation from the minimum value recorded. The DI water control sample has no birefringent material, therefore the variation in the 1D signals is the noise floor of the detectors. In Figure 4-3(B) the APD signal has a maximum variation of 0.78 %, while the CCD has a maximum variation of 0.29 %.

![Figure 4-3: Measurements of a DI water sample are shown in (A and B), where A is the 2D video representation and B is the 1D signal comparison between the APD and the CCD signals. In B the APD signal is shown in and the 1D CCD signal is in red.](image)

The second control sample was done with a mixture of DI water, soap, and borosilicate beads at a concentration of 10 mg/mL. The beads represent a non-birefringent scattering within human blood. Since the beads are not birefringent they are expected to exhibit similar results as shown in Figure 4-3. Figure 4-4(A) shows the CCD video results for the sample containing borosilicate beads, which are not visible in the cross polarized optical setup, as expected since they are not
birefringent. In Figure 4-4(B) the APD had a maximum signal variation of 0.82 %, while the CCD had a maximum signal variation of 0.33 %. The noise floor of the DI water sample is comparable to the noise floor of the sample containing the beads, therefore the addition of beads did not have a significant impact on the signal.

4.2.2. Birefringent Sample

The birefringent sample, TA, was mixed with DI water at a high concentration of 10 mg/mL of TA and then diluted to measure different concentrations. The solution was diluted in steps of 10x up to a target concentration of 1 µg/mL, as demonstrated in [28]. For each measurement 1 mL sample was prepared and mixed with approximately 0.05 mL of soap. Figure 4-5(A) shows the 2D video representation of the CCD measurements for a 10 mg/mL sample of TA, and Figure 4-5(B) shows a comparison between the APD signal and the 1D CCD signal. From Figure 4-5(B), a similar trend was observed between the two signals. Comparing the APD signal from Figure 4-5(B) to the image from Figure 4-5(A) was difficult because the sample was so highly concentrated that it was difficult to map the events that occurred on the image to the events that appear on the signal. However, the 1D CCD signal could be used to compare the APD and the CCD. The APD signal appeared to have more spikes than the CCD. The reason that the APD signal had more spikes was because the sampling rate for the APD was higher.
than the sample rate for the CCD. The APD sample rate was 10,000 samples/second and the CCD had a maximum frame rate of 21.7 frames/second. While the APD was measured instantaneously, the CCD signal was dependent on the exposure time of the camera which was set to 40ms. Due to the comparatively long integration time of the CCD, the motion of the particles was recorded as a streak within a particular frame and creates a comparatively larger 1D signal, but had less variation than the APD.

![Figure 4-5: Measurements of a 10 mg/mL sample are shown in (A and B), where A is the 2D video representation and B is the 1D signal comparison between the APD and the CCD signals. In B the APD signal is shown in and the 1D CCD signal is in red.](image)

The concentration of TA was reduced and the measurements were repeated. The lower concentrations that were experimented with were 1 mg/mL, 100 µg/mL, 10 µg/mL, and 1 µg/mL. Figure 4-6 shows the measurement results for 1 mg/mL of TA sample. In Figure 4-6(A) there were less particles than the images of the higher concentration, since there are more black spots on the legend. From the legend of Figure 4-6(A), we can map the events from the image to the graphs of Figure 4-6(B). Between 4 and 5 seconds, shown in the green box, there was a dark spot on the temporal legend which corresponds to a lower signal value on the graph. Similarly, between 5 and 6 seconds, where there were two spikes, which are annotated by blue boxes. These spikes correspond to the bright spots in the legend at that instant in time. In
Figure 4-6(B), both the CCD and APD signals have a similar pattern and the APD signal had more spikes as expected from the higher sample rate.

Figure 4-6: Measurements of a 1 mg/mL sample are shown in (A and B), where A is the 2D video representation and B is the 1D signal comparison between the APD and the CCD signals. In B the APD signal is shown in and the 1D CCD signal is in red. The green box shows an instant in time where no birefringent signals are present and the blue box shows a detection event that is recorded on both detectors.

Figure 4-7 shows a measurement result for a sample of TA where the concentration was 100 µg/mL. Figure 4-7(A) shows the 2D video representation of the CCD camera. There was a detection event that occurs at 9 seconds, colour coded in pink, that produced a 28 % change in the APD and a 30 % change in the CCD. The event at 9 seconds was shown within the purple box and when comparing to the 2D image there were significantly more particles present than any other time during the measurement. In Figure 4-7(A), the signal between 7-8 seconds that was shown in the blue box, produced a 10 % change for the 1D CCD signal. There were more particles present for the spike at 9 seconds and therefore the spike was larger. The APD identified one spike at 7.4 seconds with a 2.5 % change and a second event at 7.6 seconds, however it recorded a small spike of 1.2 % change which was close to the noise floor.
Figure 4-7: Measurements of a 100 µg/mL TA sample are shown in (A and B), where A is the 2D video representation and B is the 1D signal comparison between the APD and the CCD signals. In B the APD signal is shown in and the 1D CCD signal is in red. The blue box shows a window in time where the CCD camera detects two events but the APD detects only one event and the purple box shows a detection event that is recorded on both detectors.

Figure 4-8 shows the measurement results for a 10 µg/mL sample of TA. In Figure 4-8(A), there were less particles present in the sample and less detection events occurred. Figure 4-8(B), shows the comparison between the APD signal and CCD signal. Both APD and CCD signals still have events occurring in the same time frame, as expected. The CCD signal had a spike of 48 % variation at 6 seconds, this event was shown in the blue box and the APD detected an event at 5.5 seconds with 30 % variation. These two spikes occur at different time frames on the detector, but represent the same detection event, the difference in time occurred because the two detectors were not triggered and therefore a time lag was recorded. Between 9 and 10 seconds the APD detected two spikes at 21 and 27 % variation, while the CCD only detects one event with 20 % variation. The events between 9 and 10 seconds are shown within the yellow box, where there are two separate particles present. These two particles could have appeared as separate events on the APD, but not the CCD because of the different sample rates.
Figure 4-8: Measurements of a 10 µg/mL TA sample are shown in (A and B), where A is the 2D video representation and B is the 1D signal comparison between the APD and the CCD signals. In B the APD signal is shown in and the 1D CCD signal is in red. The blue box shows a window in time where the CCD camera detects one event but the events do not occur at the exact same time and the yellow box shows the APD detecting two events while the CCD detects only one.

Figure 4-9 shows the video representation and APD signal for a sample of 1 µg/mL of TA, which was the same as the limit reported by Rebelo et al. [28]. At this concentration only a few detection events occurred and both the APD and the CCD were able to detect this amount. A detection event, shown in the green box, was recorded at 2.5 seconds and two green streaks were recorded on the CCD, however these two streaks correspond to just one spike on the 1D CCD signal with 7 % deviation from the minimum value. During this time the APD signal detected multiple spikes with a maximum signal spike of 25 % change.
Both the CCD and the APD were able to detect the birefringent properties of TA at the same concentration that was reported by Rebelo et al. [28]. The APD signal was sampled by the NI-DAQ at a rate that was much higher than the frame rate of the CCD, therefore the APD could pick up more spikes from the signal variation. However, the CCD had a lower noise floor than the APD.

4.3. Comparison of 2D Imaging and 1D Sensing for Compact Design

The CCD camera and the APD were able to distinguish between the control sample and the birefringent sample. As observed from the graphs presented in Figure 4-5 to Figure 4-9, the 1D CCD signal had a lower noise floor and a larger percent variation than the APD, especially for particles larger than 50 µm. However, for particles smaller than 50 µm, the APD produced a similar percent change to the CCD. Even though the CCD had a lower noise floor and a higher percent change, the APD was able to identify signal variations at the same instances in time, which suggest that both detectors were able to identify the same events. The 1D CCD signal was compared with the APD signal and they produced similar trends, however the APD produced more spikes than the CCD due to a higher sampling rate. The CCD integrated several events that occurred because of the relatively higher exposure time. There were some
instances were a detection event from the APD was leading/lagging the CCD signal, but still occurred within 0.5 seconds of the CCD event and therefore it was considered to be the same event, this occurred because there was no trigger to ensure that the two signals were synchronized at all times. From comparing the APD signal with the 1D CCD signal we were able to conclude that the CCD was not significantly more sensitive than the APD.

In order to make a compact camera setup, a similar approach to the cellphone camera microscope created by Pirnstill et. al., can be evaluated [17]. The cellphone based design would be convenient, since the cellphone could power the LED light source and the additional circuitry. However, a cellphone uses a CMOS camera and therefore would have more noise and would be less sensitive than the CCD camera that was used in the bulk optic setup. Our experimental results suggest that the APD was capable of detecting a birefringent sample with similar sensitivity as the CCD camera. By doing a comparison between the 1D CCD and APD signals, we were able to show that a 1D sensing system was as sensitive as a 2D imaging system. After evaluating the bulk optic design, we decided to pursue a miniaturized 1D sensing design that was based on the flow cytometry work presented by Frita et al. [27]. The flow cytometer utilizes multiple detectors, several lenses, and consumes a large volume of 200 µL of centrifuged blood per measurement. The approach we developed was a lensless sensing design that uses one APD detector. Our design includes a serpentine microchannel that reduces the total sample volume and increases the volume of the sample that was exposed to the detector at any instant in time. A smaller sample volume was beneficial when considering the use human blood, which would therefore be less invasive. By reducing the number of detectors and removing the lenses, we not only provide a low cost alternative, but also a more compact optical path that would therefore be beneficial when considering portability. For this method to work, an APD with a larger active area was necessary since no lenses are used to focus on the detector. This approach has the benefit of being very compact and would still be capable of highly sensitive detection.

4.4. Summary

In this chapter, two different comparisons between CMOS and CCD cameras were presented. The CMOS camera had significantly more noise than the CCD and therefore had too much signal variation to accurately distinguish between signal and noise especially at lower concentrations. Consequently, the CCD and APD were compared in the proof-of-concept
experiments presented in this chapter. Results were shown for two different control samples as well as TA suspended in DI water with varying concentrations from a highly concentrated sample (10 mg/mL) to a low concentrated sample (1µg/mL). A comparison between the 1D CCD signal and APD signal was shown and provided a platform for comparing the APD to the CCD. The CCD signal and the APD signal shared a similar trend, but the APD had more spikes and had larger signal variation when a birefringent sample was in the channel. After evaluating the results, a comparison between two different approaches to miniaturize the detection system were presented. The APD and the CCD had similar sensitivity and the APD offered a better solution for a compact design because it offers a lower cost solution. In the next chapter a compact design for the optics and the fluidics is described. The miniaturized fluidic design was first tested in the proof-of-concept setup, before measurements of the miniaturized flow channel was done on the lensless optical setup.
Chapter 5: Miniaturized Optics and Fluidics

The goal of this thesis was to demonstrate that a polarized flow cytometer can be made compact and portable. This chapter will focus on miniaturization of the optics and the microfluidics from the bulk optics design. The optics are the part of the system that occupies the most physical space and therefore by miniaturizing the optics the device can be made compact. A lensless design for the optics is presented in this chapter, which provides a more compact alternative to the conventional flow cytometer. The microfluidic channel was reduced to contain a smaller volume of sample. With a conventional flow cytometer, a sample of 200 µL of centrifuged blood is used. This is a large volume and would be relatively invasive. By reducing the volume required for a measurement to a finger prick of blood (11.5 µL), the test would become minimally invasive.

For the miniaturization of the microfluidics a method will be presented that will reduce the total volume to a finger prick of human blood. A microchannel was fabricated to not only reduce the total volume used, but the channel was also fabricated to maximize the volume of the sample that is exposed to the detector at any instant in time. This was done by creating a serpentine pattern in the channel. This chapter will explain how the channel was fabricated and show results for the fabricated channel, within the bulk optic setup. Before the channel is placed into the miniaturized optical setup, the fabricated channel was verified within the bulk optic setup.

A lensless sensing design was developed to miniaturize the optical setup. The advantage of a lensless system is that it requires a short optical path and therefore the physical dimensions can be minimized. In this chapter the methods for design of the lensless optical setup are shown and the results for the fabricated microfluidic channel are shown.

5.1. Methods for Design and Fabrication of Miniaturized Microfluidic Channel

The square capillaries provided great optical quality, which make them suitable for initial experiments of the bulk optic setup. However, they required a large volume of the sample and therefore a new flow channel was needed and designed. For the miniaturized version of the microfluidics, the microchannel was designed to contain a smaller volume per measurement. The total volume was reduced to less than the equivalent volume from a prick of blood used by a glucose monitor. Pacaud et al. state that a standard finger prick, from a glucose monitor,
removes 11.5 µL [36]. A reduction in volume of blood allows for a less invasive test. We also wanted to design the channel in such a way that maximized the sample that was exposed to the detector at any instant in time.

The goal of using the microchannel was to minimize the size of the channel while maximizing the detectable area. Figure 5-1 shows the design for the microchannel. The channel was designed with a serpentine pattern within the detection region. The cross sectional area of the channel interacting with the beam reduced the amount of fluid required for a measurement (i.e. a smaller channel diameter). The addition of the serpentine pattern for a smaller channel allowed a larger volume of the sample to be exposed to the detector at one time and created a higher probability of detecting a birefringent signal. Table 3 shows a comparison of the length required for a channel to hold a finger prick of blood. The channels with a cross section of 1 mm² and 0.5 mm² squared would be too short and therefore not a good choice. While the channel with a cross section of 0.25 mm² would be long enough to contain such a small volume.

Figure 5-1: Design for microchannel.
The serpentine shaped channel was fabricated at SFU, since no off the shelf components for the channel exist. Figure 5-2 shows the process flow diagram used to fabricate the microchannel. The first step was to use an opaque plastic that can be cut with a laser cutter as the base material. We used an acrylic sheet as our base material. The reason the plastic needed to be opaque was because most transparent plastic have birefringent properties and therefore could result in false positive measurements. The thickness of the base material was important since we are using a subtractive process and the width of the channel was defined by the thickness of the material. Before cutting the material a piece of double sided tape was applied to the top and bottom surfaces. The laser cutter at the SFU facility was used to cut through the acrylic sheet in the shape of the desired channel. At the end of the channel a larger bonding pad was added. Once the channel was cut, a glass cover slip was placed on double sided tape on both the top and bottom surfaces of the channel. The glass cover slip did not cover the bonding pads, which were covered by two additional pieces of plastic. The additional piece of plastic had a hole cut into it, this was done to attach the plastic tubing. The hole was only on the plastic that covers the top surface of the channel. The final step was to seal the tubing and the edges of the channel with epoxy, this prevented the tubing from leaking.

<table>
<thead>
<tr>
<th>Cross section (mm x mm)</th>
<th>Cross sectional Area (mm²)</th>
<th>Maximum length of channel (mm) for a finger prick of blood (11.5 µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 x 1</td>
<td>1</td>
<td>11.5</td>
</tr>
<tr>
<td>0.7 x 0.7</td>
<td>0.5</td>
<td>23</td>
</tr>
<tr>
<td>0.5 x 0.5</td>
<td>0.25</td>
<td>46</td>
</tr>
</tbody>
</table>

Table 3: Comparison of different sized channels and what length is required to make a channel that contains a finger prick of blood.
To verify the serpentine channel, the channel was evaluated using the bulk optics setup before proceeding with the lensless design. The microscope setup shown in Chapter 3 was modified for a wider Field Of View (FOV) by decreasing the magnification of both detection channels. The focal lengths and distances in Figure 5-3 were adjusted, to the values shown in Table 4, for a
wider FOV. After adjusting the FOV the experiment was done with a control sample of DI water and a second control sample of DI water mixed with beads. Experiments were also done with TA at varying concentrations until a lower detection limit was found. The frame rate was set to 21.7 frames/sec, the exposure was 13 ms, and the gain was set to 50 for the CCD. The exposure time was decreased because more background light reached the detector with the lower magnification. Increasing the exposure time would result in background light leaking through and affecting the CCD signal.

Figure 5-3: Optical schematic.
Table 4: List of distances and focal lengths for the schematic in Figure 5-3.

5.2.1. Control Sample

For the control sample two types of samples were measured, the first control sample was mixed with 1 mL of DI water and a drop of soap (0.05 mL). The second control sample was with DI water mixed with borosilicate beads, which are non birefringent. The borosilicate beads were mixed at a concentration of 10 mg/mL. Through the addition of borosilicate beads we were able to confirm that non birefringent scattering particles also did not affect the signal. We were also able to identify the noise floor of the signal. Figure 5-4(A) shows the 2D video representation of a DI water sample captured by the CCD camera and the corresponding percent change of the APD and CCD signal was shown in Figure 5-4(C). The noise floor on the APD signal was 0.83 %, while the noise floor on the CCD was 0.56 % signal variation. Figure 5-4(B) shows the 2D video representation of a DI water sample mixed with borosilicate beads. Figure 5-4(D) shows the corresponding percentage change of the APD and CCD signals. The addition of beads in the control sample did not affect the noise floor of the signal. The APD signal had a noise floor of 0.9 % signal variation and the CCD was 0.48 % signal variation.
5.2.2. Birefringent Sample

After identifying the noise floor of the signal for the control sample, a birefringent sample was experimented with. For the birefringent sample, TA was mixed with DI water at an initial concentration of 1 mg/mL. Serial dilution was utilized to reduce the concentration of TA down to 100 µg/mL, 10 µg/mL, and 1 µg/mL. The tests were done starting from the lowest concentration to highest, this was done to prevent contamination of the experimental results. After each measurement the channel was rinsed with DI water and if any particles were stuck to the walls of the channel then the channel was discarded.
Figure 5-5(A) shows results captured by the CCD camera of a sample mixed at a concentration of 1 mg/mL. Figure 5-5(C) shows the comparison between the percent change in the APD signal and the CCD signal for the sample in Figure 5-5(A). Both the APD and CCD signals in Figure 5-5(C) identify more signal variation than the noise floor presented for the control sample. The majority of the signal has approximately 5 % change from the minimum recorded value. This was reasonable because the concentration was high and the channel was constantly full of particles. A larger particle, approximately 100 μm in diameter, was present at ~1 second which caused a large spike on both signals. The 100 μm particle was shown within the green boxes as it passes through the channel. The APD spike was 9.8 % change, while the CCD had 16 % change. The majority of the particles in the sample were approximately 10-20 μm in diameter, and cause smaller signal variation. From Figure 5-5(A) there are only a few spots where the legend doesn’t record a signal, this occurred because the sample had a high concentration and there was a higher probability that a particle was present because of the wider FOV.

Figure 5-5(B) and (D) show the results for a repeated measurement at the same concentration. In this dataset a large spike occurred at 0.5 seconds into the measurement, this spike corresponds to a red coloured particle that was approximately 200 μm in diameter. The 200 μm particle was shown within the yellow boxes as it flows through the channel. In this measurement there was more signal variation, which was caused by a less uniform distribution of birefringent particles. From the legend of Figure 5-5(B), we can see more spots where there are no particles present, which causes the larger signal change, but the majority of the APD signal was approximately 5 % change from the minimum value during the measurement.
Figure 5-5: A) 2-Dimensional video representation of sample 1 at a concentration of 1 mg/mL of TA. B) 2-Dimensional video representation of sample 2 at a concentration of 1 mg/mL. C) A comparison between the percentage change of the APD signal and CCD signal for sample 1. D) A comparison between the percentage change of the APD signal and CCD signal for sample 2. In A and B the channel is outlined with the grey colour.

Experiments were done at a lower concentration of 100 µg/mL of TA. Figure 5-6(A) shows the CCD video data for a sample at a concentration of 100 µg/mL of TA and Figure 5-6(C) shows the corresponding percentage change of the APD and CCD signals. In this sample the signal variation was much smaller on the APD and there was a clear spike at 5 seconds which corresponds to a cyan coloured particle for which the path was highlighted within the green box. There are sections of the APD signal that are just above the 0.9 % deviation line from the minimum recorded value, however the APD signal was much closer to the noise floor than the CCD signal.
Figure 5-6(B) shows another CCD video dataset for a concentration of 100 µg/mL of TA and Figure 5-6(D) shows the corresponding percent change of the APD and CCD signals. A clear spike was identified at 1 second, which corresponds to a dark yellow coloured particle shown within the yellow box, which was identified by both the APD and CCD signals. The dark yellow particle has a higher spike on the CCD signal with 10 % change compared to 2.5 % change for the APD. Another detection event occurred around 6 and 7 seconds, the CCD and APD signals identified this as two spikes, which correspond to the blue coloured particles. The detection event was shown within the blue box. The second blue spike had a higher percent change on the CCD with 5 % change and only 2.5 % change on the APD.

Figure 5-6: A) 2-Dimensional video representation of sample 1 at a concentration of 100 µg/mL of TA. B) 2-Dimensional video representation of sample 2 at a concentration of 100 µg/mL. C) A comparison between the
percentage change of the APD signal and CCD signal for signal for sample 1. D) A comparison between the percentage change of the APD signal and CCD signal for sample 2. In A and B the channel is outlined with the grey colour.

At a sample concentration to 10 µg/mL of TA, the APD and CCD signals both identified birefringent particles. Figure 5-7(A) shows the 2D video representation for a sample concentration at 10 µg/mL and Figure 5-7(C) shows the corresponding 1D APD and CCD signals. Two green coloured particles were identified at 2 and 3.5 seconds. The detection event at 2 seconds appears inside the green box and had a 2.5 % change on the APD and a 2.3 % change on the CCD. The detection event at 3.5 seconds was shown inside the yellow box and has a 2.5 % variation on the APD and a 2.4 % change on the CCD. A few other small signal spikes were identified, but with the smaller variation it became difficult to distinguish these results from the noise floor.

Figure 5-7(B) shows 2D video representation of the CCD data for a repeat measurement of a sample concentration of 10 µg/mL and Figure 5-7(D) shows the corresponding 1D APD and CCD signals. A detection event occurred at 1 second, where a dark orange coloured particle was shown inside the yellow boxes. This event corresponds to a 7 % signal variation in the CCD signal and 4 % variation in the APD signal. Two additional spikes were observed around 2 seconds, these spikes correspond to a green coloured particle inside the green boxes. On the CCD signal these spikes had a 5 % variation and a 2.5 % variation on the APD signal. From the legend on Figure 5-7(B) more events are detected, but these events correspond to a particle that became stuck to the walls of the channel. This particle shows up as white spot inside the purple box on Figure 5-7(B).
Figure 5-7: A) 2D video representation of sample 1 at a concentration of 10 µg/mL of TA. B) 2-Dimensional video representation of sample 2 at a concentration of 10 µg/mL. C) A comparison between the percentage change of the APD signal and CCD signal for sample 1. D) A comparison between the percentage change of the APD signal and CCD signal for sample 2. In A and B the channel is outlined with the grey colour.

Measurements were performed at a concentration of 1 µg/mL of TA. Figure 5-8(A) shows the 2D representation of the CCD video data for a sample at a concentration of 1 µg/mL of TA. Figure 5-8(C) shows the corresponding APD and CCD 1D signals. There was a green particle, shown inside the green box, present at 2 seconds that created a 2.5 % variation for the CCD and APD signals. From 7-9 seconds there was a particle that was coloured from blue to pink, shown inside the purple box. This particle created a 2.5 % signal change for the CCD and a 2 percent signal change for the APD.
Figure 5-8(B) shows the 2D representation of the CCD video data for a repeat measurement at a sample concentration of 1 µg/mL of TA. Figure 5-8(D) shows the 1D signals corresponding to the values for the APD and CCD for this sample. There was a signal spike at 2.5 seconds that was identified by both APD and CCD signals and corresponds to a green coloured particle, which was annotated with a green box. There was a spike at 6.5 seconds that corresponds to a blue coloured particle, which was annotated with a blue box. There was a spike at 7.5 seconds that corresponds to a purple coloured particle and was shown inside a purple box. This spike appeared at 7 seconds for the APD channel. The event inside the purple box doesn’t occur at the same exact time on both detectors, however it was likely that the two spikes correspond to the same event because they occur within 0.5 seconds and there are no other detection events that are observed. The two detectors were not perfectly synchronized because the CCD camera couldn’t be triggered and therefore the acquisition could have introduced a time lag on either signal. All the spikes produce a 2.5 % signal variation.
5.3. Miniaturized Optical Setup Methods

In this section the lensless design for the optical setup is shown. The lensless optical approach has been demonstrated for bright field microscopy, by placing a microchannel directly on a detector [35]. The lensless approach allowed us to make the bulk optics system more compact. By removing the lenses and placing all the components as close together as physically possible, we were able to demonstrate a compact sensing system.
By removing the lenses, there was no longer a component to focus the light onto the detector. Therefore, the detector needed to be as close to the sample as possible so that the majority of the light that scatters off of the sample was incident on the active area of the detector. Figure 5-9 shows the optical setup for the lensless design. In this setup, the key components from the bulk optics design were used and only the lenses were removed. A linear polarizer was placed after the white LED to illuminate the sample with linearly polarized light. The analyzer was oriented 90 degrees from the polarizer in order to extinct all the light that wasn’t birefringent.

The spacing between any two components in the schematic was minimized. The detector was still an APD, however a different APD module was used because it had a larger active area and therefore captures more of the incident light. The module used was the Hamamatsu C12703-01, where the diameter of the active area was 3 mm. The light source was pulsed at 800 Hertz and the detection electronics was the same as presented in Section 3.2.

![Figure 5-9](image)

**Figure 5-9:** A) Schematic of lensless optical setup. B) Photograph of implemented setup.

### 5.4. Results for the Miniaturized Optical Setup

In this section the results for the miniaturized optical setup are presented. The setup was experimented with two control samples, the first was DI water and the second was DI water mixed with borosilicate beads. The setup was then experimented with the birefringent sample, TA, at varying concentrations. There was no video data for the compact optical setup, only the APD data was available to determine whether birefringent material was within the sample.
5.4.1. Control Sample

The first sample data sets that were collected were the control samples. There were two different control samples measured with the miniaturized setup. The first control sample was DI water and the second control sample was DI water mixed with borosilicate beads at a concentration of 10 mg/mL. Both control samples were mixed with a drop of soap (0.05 mL). Figure 5-10(A) and Figure 5-10(B) are two separate measurements done with the first control sample. In both cases the noise floor was less than 1 % change within the signal. The maximum noise floor in Figure 5-10(A) was 0.84 % with a mean (μ) value of 0.42 % and a standard deviation (σ) of 0.24 %. The maximum noise floor in Figure 5-10(B) was 0.87 % with mean value of 0.40% and a standard deviation of 0.25 %. The noise floor measurements were similar to that of the bulk optic setup, therefore removing the lenses did not introduce more noise into the system. Figure 5-10(C) and Figure 5-10(D) are two separate tests for a sample of DI water mixed with beads at a concentration of 10 mg/mL. Figure 5-10(C) has a noise floor of 0.86 % with mean value of 0.46 % and a standard deviation of 0.25 %. The repeat measurement in Figure 5-10(D) has a noise floor of 0.83 % with mean value of 0.45 % and a standard deviation of 0.26 %. In both cases the beads do not affect the measurements and the noise floor was similar to noise floor of the bulk optics setup.
Figure 5-10: Results from the compact setup for the control samples. Test A) and B) were done with only DI water, while tests C) and D) were done with DI water and beads.

By evaluating the control sample measurements, we can determine the probability that a measurement was signal or noise. The noise variation was a random variable that can be treated as normal distribution and the confidence level was determined by how close a data point was to the mean value ($\mu$) [41]. Table 5-5 shows confidence values for the control sample measurements. By assessing the control samples, we can determine the confidence that the noise will fall below a certain value. If we had a three-sigma confidence level, that means 99.7 % of the sample was expected to be below this value. For example, in measurement Figure 5-10(D), there was a 99.7 % percent confidence that any noise variation will be below 1.23 % change. Extending this to a birefringent sample any measurement value that was higher than 1.23 % change results in a three-sigma confidence (99.7 % confidence) that the measurement was signal and not noise.
<table>
<thead>
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<th>Measurement</th>
<th>Figure 5-10(A)</th>
<th>Figure 5-10(B)</th>
<th>Figure 5-10(C)</th>
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<td>0.45</td>
</tr>
<tr>
<td>σ</td>
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<td>0.25</td>
<td>0.25</td>
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<td>1.65</td>
<td>1.71</td>
<td>1.75</td>
</tr>
</tbody>
</table>

Table 5-5: Maximum, mean, standard deviation values for the measurements of the control samples. There are also two-sigma to five-sigma confidence measurements for the detection of an event based on the noise for each case.

### 5.4.2. Birefringent Sample for the Miniaturized Setup

For the birefringent sample, TA mixed with DI water was used and the concentrations were varied from 1 mg/mL down to 1 µg/mL. The experiments performed on the miniaturized optical setup were done with the same sample preparation and concentrations as the bulk optic setup, which allows for a fair comparison between the two optical setups. Figure 5-11 includes four different measurements at a concentration of 1 mg/mL and all repeat measurements were able to detect a birefringent signal. Figure 5-11(A) had several spikes corresponding to birefringent particles, which varied from 2.4 % variation to 9 % variation. Figure 5-11(B) contained several spikes ranging from 2 to 5 % change within the first 2 seconds of the measurement and a large particle from 8-10 seconds with 16.7 % change. Figure 5-11(C) had many small signal changes
that range from 2.1 to 2.5 % change. The smaller signal changes refer to smaller particles. Figure 5-11(D) has some signal changes from 2.2 to 2.5 % change.

Figure 5-11: Four separate measurements done at a concentration of 1 mg/mL.

Figure 5-12(A) shows a measurement at a concentration of 100 µg/mL. A clear spike was identified at 2 seconds with 3.3 % variation. Repeat measurements were also carried out at 100 µg/mL to show the results are repeatable. Figure 5-12(B) contained a large spike at 4.2 seconds and at 9 seconds with 5.8 % and 12 % variation. Smaller signal variation exists throughout the measurement that are above the noise floor. Figure 5-12(C) had a spike with 7.9 % variation at 2 seconds. Figure 5-12(D) contained large spikes and some smaller signal variation. From 0 to 4 seconds the baseline was higher than 5 to 8 seconds, this was because there were more particles in the channel during this time.
Figure 5-12: A, B, C, and D are four separate tests done at a concentration of 100 µg/mL.

Figure 5-13(A) was a measurement done with a concentration of 10 µg/mL of TA. This measurement had two clear detection events at 1.7 seconds and 7 seconds with a value of 5.7 and 3.2 % change. Figure 5-13(B) was a repeat measurement done at a concentration of 10 µg/mL of TA and had one clear detection event at 2.1 seconds with 3.3 % variation. Smaller signal variation exists before 2 seconds that were above the noise floor, but were visually difficult to distinguish. Figure 5-13(C) had two clear detection events. The first detection event occurred at 2.5 seconds and had a 3.9 % change from the minimum value. The second detection event occurred at 8.2 seconds which spiked up to 3.2 % change and decreased to 2 % change at 10 seconds. Figure 5-13(D) had a clear detection event at 3 seconds with 2.5 % change. Overall the 10 µg/mL repeat measurements were consistent with the number of detection events and the size of the detection events. The confidence of the smaller detection events was determined using the confidence measurements that was established for the control
samples in Section 5.4.1. In Figure 5-13(D) there were some small spikes ranging from 1.5 % to 1.7 % change, annotated in the figure, which had a four-sigma confidence (99.99 %) that the spikes were not noise.

![Graphs showing APD (% Change) over time for TA 10 µg/mL (10 s)](image)

*Figure 5-13: A, B, C, and D are four separate tests done at a concentration of 10 µg/mL.*

Figure 5-14 contains four different measurements for a concentration of 1 µg/mL. Figure 5-14(A) and (B) recorded a clear detection event. Figure 5-14(A) had a detection event at 1.8 seconds with a 4.2 % change in signal. Figure 5-14(B) had a detection event at 6.7 seconds with at 2.6 % change in signal. Figure 5-14(C) and (D) had some small events that appear above the noise floor. In Figure 5-14(C) there was a spike with 1.6 % change, which would give four-sigma (99.99 %) confidence that the spike was a detection event. In Figure 5-14(D) the largest
detection events recorded were 1.4 % change, which gave a three-sigma (99.7%) confidence that this value was not noise. Similarly, in Figure 5-14(A) the 4.2 % change detection event was above the five-sigma (99.999 %) confidence level, but smaller events were also recorded with 1.4 % change that correspond to a three-sigma (99.7 %) confidence level that it was a signal and not a random event.

Figure 5-14: A, B, C, and D are four separate tests done at a concentration of 1 µg/mL.

5.5. Discussion

The results presented in Section 5.2 show that the fabricated microfluidic channel doesn't provide a false positive when there was a control sample present. When experiments with birefringent particles were performed on the microfluidic channel in the bulk optic setup, both detectors were able to detect the birefringent signal from a concentration of 1 mg/mL down to 1 µg/mL. A comparison between the 1D CCD signals and the 2D CCD images show that larger
birefringent particles will produce a larger signal change than multiple smaller birefringent particles. A birefringent particle always created an increase in the signal (a positive signed variation) and never a decrease. As the concentration was decreased the percent change became smaller and less detection events occurred per measurement. Comparing the 1D signal and the 2D signal from the same detector showed that they have similar birefringence detection capabilities. The 1D APD signal detected the same events as the 1D CCD signal, however the 1D CCD signal had a lower noise floor. The APD signal had more spikes than the 1D CCD signal, which was consistent with the results from Chapter 4. The experimental results showed that the APD had a similar sensitivity to the CCD.

The experimental results for the miniaturized setup, shown in Section 5.4, were done with the same sample preparation so that the results for the APD could be compared with the bulk optic setup. The noise floor for the APD signal in the miniaturized optical setup had a maximum value of 0.87 % change which was similar to the maximum noise floor of 0.9 % change for the bulk optic setup. There were clear detection events record on the miniaturized setup with similar amplitudes as record from the bulk optic setup, for example the maximum percent change at a sample of 1 mg/mL in the miniaturized setup was 16.7 % change and in the bulk optic setup was 9.8 % change. A repeat measurement for the miniaturized setup had a maximum of 9 % change, while a repeat measurement for the bulk optic setup had an 8 % change. The birefringent particles were identified from a sample concentration of 1 mg/mL down to a sample concentration of 1 µg/mL. At a TA concentration of 1 µg/mL, the small signal variation was determined to have at least a three-sigma confidence (99.7%) that the spikes were detection events and not noise. The miniaturized optical setup was able to detect birefringent particles with similar amplitudes and reached the same lower detection limit and therefore the miniaturized setup was as sensitive as the bulk optics setup.

In this chapter, we demonstrated a reduction in the volume of sample required for a measurement. by using a single APD detector we were able to demonstrate that a low cost, compact design could detect the same concentration of a birefringent sample as the flow cytometer reported by Rebelo et al. [28]. However, we were not able exceed the detection limit and increase the sensitivity of the polarized flow cytometer.
5.6. Summary

In this section, we showed the method for developing a serpentine microfluidic channel. This channel reduced the total volume used per measurement and it increased the volume of sample that was exposed to the detector. The section also showed measurements for the bulk optic setup with the serpentine microfluidic channel. The noise floor was identified before the birefringent sample was measured. The birefringent sample was measured from a concentration of 1 mg/mL down to 1 µg/mL, where a comparison between the two detectors were made before proceeding to the miniaturized optical setup.

This section also included the methods and measurements for the miniaturized optical setup. The noise floor was identified for that miniaturized optical setup and it was similar to the noise floor of the bulk optic setup. The birefringent sample preparation was the same as for the bulk optic setup and measurements were performed on the miniaturized optical setup, from a concentration of 1 mg/mL down to 1 µg/mL. The miniaturized optical setup reached the same detection limit as the bulk optic setup. Additional measurements were done at each concentration to show that the results were repeatable.
Chapter 6: Conclusion and Future Work

In this thesis, we demonstrated that we could detect birefringent material inside a microchannel using polarization optics for POC detection. This method of detection has many different medical applications including monitoring glucose and cholesterol, detection of a wide variety of bacteria and viruses, and detection of the malaria parasite. Through the additional method of Birefringent-ELISA, birefringent particles can be linked to biomarkers of various diseases. Malaria produces a unique birefringent signature in blood and therefore doesn’t require birefringent particles to be linked to enzymes through Birefringent-ELISA. There are many applications that can use birefringent molecule detection for POC devices. In this thesis we have demonstrated that a birefringent phantom can be detected effectively with our prototype device.

To develop our detection device, we first implemented a bulk optic configuration. As shown in Chapter 3, the bulk optic setup could be considered in the following four components: the optics, the electronics, the software, and the microfluidics. The bulk optic setup was based on using a polarizer and an analyzer rotated at 90 degrees in order to extinct non-birefringent light. The bulk optical setup was implemented to include two detection channels for a comparison between a 1D and a 2D detector. The 2D detector provide a visual representation of birefringent particles flowing through a microchannel. A summation of all the pixel from each frame of the camera provided a 1D signal from the 2D detector, which was used to identify and map events that occurred on the 1D signal to the 2D detector. Having an APD detector also provided a 1D signal that was compared with the 1D CCD signal. The CCD provided 3D video data, were a pixel-wise colour coded summation for each frame was done to create a 2D image that displayed temporal data from the camera.

The bulk optic setup was explored with two different microchannel designs: the first channel was a square glass capillary, and the second was a fabricated serpentine channel. The glass capillaries provided great optical quality and were off the shelf components, while the serpentine microchannel was developed to reduce the total volume of sample per measurement and increase the volume of sample that was exposed to the detector at any instant in time. The measurements of the bulk optic setup were initially done with the glass capillaries and the results were shown in Chapter 4. While the serpentine channel was tested under the bulk optic setup in Chapter 5, where the optics were modified to have a wider FOV.
After experimentally validating the serpentine channel, the channel was measured under the miniaturized optical setup. The miniaturized optical setup had a lensless design, which reduced the optical path and provided a compact alternative. The miniaturized optical setup relied only on a single APD detector, which reduces the cost of the design. In Chapter 5, we presented results for the serpentine channel in the miniaturized optical setup. We were able to conclude that the miniaturized optical setup was able to detect birefringent particles as well as the bulk optic setup and as well as the reported detection limit from the birefringent flow cytometer, reported by Rebelo et al. [28].

This thesis was a proof-of-concept demonstration that a compact optical setup was capable of detecting birefringent particles in a microfluidic channel. We were successful in demonstrating the miniaturization of the optics and microfluidics, however there were some areas that the device could be improved upon and some challenges that could be addressed. The challenges that could be improved upon are: making the electronics compact, introducing borosilicate beads into a mixture of TA, and measuring a biological sample.

6.1. Improve Detector Comparison for the Bulk Optic Setup

In the bulk optic setup, the CCD and APD detectors acquire data simultaneously, however the two detectors were not synchronized. The bulk optics setup was used for a comparison between the two detectors and we were able to do an accurate comparison, however there were minor lag issues during acquisition that could be resolved by synchronizing the detectors. There was a situation where the APD detection event occurred approximately 0.5 seconds before the 1D CCD detection event and it was likely that the two signals detected the same event, however through the addition of a trigger the uncertainty from any lag events could be removed. By triggering the two detectors, synchronous detection would allow the two signals to be simultaneously detected and compared without any lag related issues. Adding a trigger to the bulk optic setup would be a nice feature for a more accurate comparison, but it is not necessary because the bulk optic setup was used for comparison of birefringent detection before the miniaturized optical setup was developed.

6.2. Compact Electronics

During the experiments performed in this thesis, a laptop was used to acquire data through a USB-NIDAIQ card. To make the device portable the laptop is not a suitable option. In this section
we will explain what can be done to replace the laptop and we will discuss the use of Printed Circuit Board (PCB) to improve the analog circuitry. These two changes provide a way to make the electronics compact and portable.

The analog circuit that was presented in section 3.2 can be implemented on a PCB. The same electronic components would be used and soldered onto the PCB. By implementing a PCB design the components are connected with printed copper wiring that can be placed in close proximity without creating a short circuit and therefore would reduce the size of the analog circuitry. A PCB circuit is also more reliable than a circuit implemented on a breadboard. The circuit can be designed and implemented on a PCB with the circuit schematic without any knowledge of project.

To replace the laptop and NIDAIQ card an Arduino can be utilized to read the information and display it on a Liquid Crystal Display (LCD) screen. An Arduino can be programmed to read in the APD data from the PCB circuit and MATLAB support packages can be used to program the Arduino. The processing code would not be changed and can be run automatically once a signal is acquired through the Arduino. Once the code is loaded on the Arduino device it doesn't require a computer to run the program. The results of each measurement can be displayed on a LCD screen. Programing the Arduino is a simple task and doesn't require high level of knowledge of the project.

Implementing the analog circuits on PCB and programming the Arduino are two things that can be done to improve and make the electronics compact and more reliable. The PCB would make the circuit more reliable, and the Arduino would remove the dependence on a laptop to make measurements. The task for making the electronics compact could be done by an engineering student.

6.3. Borosilicate Beads with Birefringent Phantom

The samples experimented with in the thesis, consisted of DI water, DI water with borosilicate beads, and TA with DI water. No experiments were done with the combination of borosilicate beads, TA, and DI water. By mixing the beads with DI water we were able to confirm that the beads do not create a false positive measurement. We did not mix the beads with the birefringent sample because we verified that the beads do not appear in the cross polarized
configuration, however we did not confirm that the beads do not interfere with the birefringent sample. It is possible that, at a high concentration, borosilicate beads can block the light that scatters from the birefringent particles and therefore reduce the detection limit of the birefringent signal.

The ultimate goal is for the device to measure biological samples, such as malaria infected blood, however we do not know how much non-birefringent scattering exist within a blood sample. An experiment with varying concentrations of borosilicate beads could identify the concentration of non-birefringent scattering that can affect the detection limit and could be a helpful guide to understanding how other components in a biological sample will affect the signal.

To verify that the beads do not interfere with the birefringent sample the measurements can be repeated with different concentrations of TA mixed with borosilicate beads. Figure 6-1 shows a measurement done under the bulk optic setup with a sample consisting of 1 mg/mL of TA and 10 mg/mL of borosilicate beads. The initial test indicates that the beads do not interfere with the birefringent signal. Performing the experiment with varying the concentration of TA would be able to show whether the detection limit is impacted by the addition of a non-birefringent scattering sample. The concentration of beads could be varied to determine if higher concentrations of borosilicate beads interfere with the birefringent signal.
The addition of beads to a birefringent sample adds non-birefringent scattering that could potentially interfere with the birefringent signal. Experiments at varying concentrations of beads can determine how much non-birefringent scattering can be present within a sample before the birefringent signal decreases. A measurement indicates that the beads do not interfere with the birefringent signal, but further investigation needs to be done at varying concentrations of both TA and beads to determine the impact of non-birefringent scattering.

6.4. Biological Samples

A phantom sample was used to perform the experiments presented in this thesis as a proof-of-concept exploration of birefringent detection that can be utilized to detect a variety of different diseases. A biological sample would be the next step in demonstrating the ability of the birefringent detection device for diagnosing disease.

The main application that we choose to focus on in this thesis was malaria, therefore experimenting with malaria infected blood would be useful in demonstrating the ability of the device to detect hemozoin for disease diagnostics. During the thesis we used a phantom sample, because malaria infected human blood is a biohazard and dangerous to work with.
However, mouse blood is often infected with the parasite and used as an alternative to human blood [9], but this still requires biohazard clearance. The biohazard clearance would be required for the laboratory as well as the people working with the sample. Creating and maintaining a malaria blood culture requires expertise that we are unable to provide and contracting this service is expensive, therefore we performed our experiments with a birefringent phantom. Having demonstrated the method for detecting a birefringent sample in our proof-of-concept experiments, future experiments should be performed with a biological sample.
References


