Rapid Malaria Detection using the Magneto-Optical Properties of the Malaria Pigment

Gregory Stephen
Advisors: Robert Deissler and Robert Brown

May 2014
Abstract

A major challenge in malaria diagnosis is the need for a cheap, portable device capable of detecting low concentrations of the malaria pigment, hemozoin. The malaria parasite converts hemoglobin in the blood into heme, a ferrous compound that is toxic to the parasite. The parasite then processes the heme into the crystalline hemozoin. Hemozoin crystals are dichroic and paramagnetic; Thus, an applied magnetic field will align the crystals in a blood sample such that light passing through the crystal is attenuated differently depending on its polarization. This effect has previously been used to build a device using rotating permanent magnets. We improved on this by building a low cost device that detects low levels of hemozoin in solution without the use of continuously moving parts. To do this, we designed and built a device that measures the relative attenuation of two orthogonally polarized laser beams after passing through a sample. The difference in the attenuation between the two polarizations is related to the concentration of hemozoin. This difference is measured with and without a magnetic field, removing any differences between the two lasers. The device is able to measure concentrations of hemozoin as low as 4.9 ng/mL.
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1 Introduction

In 2012, there were an estimated 660,000 deaths resulting from malaria [7]. One of the difficulties with treating the parasite is accurately detecting its presence in blood. Currently, the most accurate methods are either time consuming, require expertise or need laboratory grade equipment [4, 5]. For example, the current standard is a blood smear, where the patient’s blood is smeared on a slide and then stained [2]. A technician then manually looks for the parasite, which has a distinctive appearance due to the stain. This method requires both a high quality microscope and a technician who knows what to look for, making it impractical for field diagnosis. Thus, a low cost, compact alternative is needed.

The malaria parasite converts hemoglobin in the blood into heme, a compound that is toxic to the virus. The parasite neutralizes the heme by converting it into crystalline hemozoin, which has paramagnetic and dichroic properties. When a magnetic field is applied to a suspension of hemozoin, a magnetic moment is induced in the crystals. In solution, the crystals are free to rotate until the induced magnetic moment aligns with the field. Once the crystals are aligned, the dichroic properties become useful. A dichroic crystal is characterized by an index of refraction that is dependent on the polarization direction of incoming light [1]. The amount of induced dichroism is dependent on the concentration of hemozoin.

Thus, by measuring the amount of magnetically induced dichroism, the
concentration of hemozoin, and by extension the amount of parasitic infection, is measured. Continuing off the work of Jones et al. we aim to build a device capable of detecting malaria infection at least as low as 0.005%, the capability of Jones’ device [3].

Their device utilized rotating magnets, giving the time averaging needed for a sensitive measurement. We aim to build a device capable of the same or better sensitivity as Jones et al., but without continuously moving parts. We will measure the concentration of $\beta$-hematin, the synthetic analog of hemozoin which is chemically identical to hemozoin [6].

2 Background

Malaria is a blood-borne parasite carried primarily by mosquitoes. Once infected, the parasite consumes globin from hemoglobin, leaving the heme compound. Heme is toxic to the parasite, so it is further processed into crystalline hemozoin. The hemozoin crystal consists of an iron atom surrounded by a cyclic ring of nitrogen and carbon atoms with an oxygen atom bonded to the iron atom out of plane. These molecules form long needle shaped crystals with a magnetic moment along the iron-oxygen bond (figure 1). These crystals are paramagnetic, dichroic and birefringent.

Dichroism and birefringence are properties resulting from an axially-dependent index of refraction for the crystal. The crystal has a preferred optical axis and the attenuation of index of refraction for light passing through
Figure 1: Hemozoin Crystal Structure
An iron atom (yellow) is surrounded by a ring nitrogen atoms (blue) with an oxygen atom (red) out of plane. This heme compound is surrounded by carbon atoms (grey). These structures are bonded together as shown, creating long, needle-like crystals. The picture on the right shows a TEM micrograph of the hemozoin crystals [1].

the crystal depends on the polarization direction. For a dichroic material, the index of refraction depends on the linear polarization of the incoming light. When linearly polarized light is incident on a dichroic material, the crystal will absorb light differently depending on the orientation of the polarization vector with respect to the materials optical axis. Thus, by measuring the attenuation of light when polarized along the length and width of the crystal, we can measure the net dichroism by the difference between the two signals.

The paramagnetic property allows the crystals to align with an applied magnetic field. Butykai et al. demonstrated that in a suspension, hemozoin crystals align along a common axis when a magnetic field is applied. This axis corresponds to the iron-oxygen bond in figure 1. With the crystals aligned,
Figure 2: Change in transmission versus magnetic field [1].

A net dichroism is induced in the sample that is linearly proportional to the concentration of the crystal in solution. Figure 2 shows the induced dichroism with respect to the applied magnetic field. As the presence of hemozoin in a person’s blood is indicative of malaria infection, this effect can be used for early diagnosis of infection [1].

Jones et al. used this effect to construct a device as laid out in figure 3. The device used a single red diode laser passing through a linear polarizer. The beam then passes through a beam-splitter, with one path hitting a photodiode (monitor diode) and the other passing through the sample and onto a second photodiode (cuvette diode). The sample is held between two wheels, each containing a pair of permanent magnets producing a field of 0.5 T. The wheels constantly rotate at a frequency of several Hertz. By constantly turning the field on and off a control value is constantly measured. The cuvette diode output is divided by the monitor diode output to remove
Figure 3: Schematic of Jones’ Device

The device uses a single red diode laser. The sample is held between two continuously rotating wheels, each of which houses a pair of permanent magnets. This provides a constant control measurement. The final measurement is the difference in the signal with and without the magnetic field.

Laser drift and the difference between the output with the field on and off is measured.

The device built by Jones et al. has a few drawbacks. The rotating magnet causes vibrations, which can affect the signal. There is also significant signal drift when used with blood samples. This limits the sensitivity of the device. Our design improves on this by measuring both polarizations of light passing through the same part of the sample. By rapidly switching between the polarizations, we should be able to eliminate this drift.
3 The Device

Our design is laid out in the schematic in figure 4. We use two 650 nm red diode lasers focused with a single plastic lens in an aluminum housing. The driving circuit for each laser is laid out in figure 5. The voltage regulators and resistors produce a constant current of approximately 30 mA. The transistor shorts the current to ground when the clock input is off. When the clock is on all 30 mA pass through the laser diode. The transistor switch is run by a TTL clock input. The clock for each laser is set such that only one laser is on at any given time, switching at about 50 Hz. The laser modules and photodiodes are mounted on a solid aluminum block with the beam-splitter mounted inside the block. The laser diodes are held in aluminum clamps which are screwed into the primary aluminum block, with a plastic linear polarizer between the clamp and the block. By mounting everything on a single aluminum block, we ensure that the diodes are at thermal equilibrium, removing drift due to temperature differences between the lasers. The circuits for driving and modulating the lasers, as well as the reverse-bias photodiode circuits (as shown in figure 5) are soldered onto a single through-hole breadboard, which is contained within an aluminum box to electrically shield the circuits.

The lasers then pass through a non-polarizing beam-splitting cube. One path from the beam-splitter hits a photodiode (reference diode), which monitors the output power of the laser. The other path passes through the sample, which is held between two wheels, each containing a pair of permanent mag-
The Side view shows the device with the magnetic field on. A pair of magnets is housed on a rotating wheel so that the sample can easily be moved into and out of the field. A control run with zero magnetic field is required for the current device. This accounts for any difference between the output power of the individual laser diodes.
Figure 5: Laser Driver and Modulation Circuit
The circuit drives and modulates the red laser diodes. The LM7809 stabilized the voltage input and produces a constant voltage that, when dropped across the resistor, creates a constant current. When the clock input is high, the transistor is saturated and all the current runs through the laser diode. When the clock is off, the current is shorted to ground.

The beam then hits a mirror reflecting down at a small angle, returning through the sample and hitting a second photodiode (the signal diode). The photodiodes have a 9V reverse bias. The lasers and mirror are aligned such that the beam is always in the plane perpendicular to the field vectors of the magnets. This is to ensure that the measured signal is entirely from the dichroism of the hemozoin crystals.

The photocurrent from each of the photodiodes passes through a 20 kΩ resistor. The voltage drop across the resistor is measured by a National Instruments NI 9238 DAQ module, which measures the voltage on four channels simultaneously at 24 bit precision. The sample rate is 50,000 Hz. The signal from each diode is stored in a 1-D array. The signals are normalized,
removing the difference between the output power of the photodiodes. The diodes are normalized by the average signal, so some “normalized” values are greater than one. The arrays are averaged in sets of 50 points to remove high-frequency noise. This averaging, combined with the sample rate of the DAQ, limits the laser switching frequency to 50 Hz, which gives about 10 points for each polarization per cycle. The cuvette diode signal is then divided by the reference diode signal, creating the “division” signal. This removes signal noise resulting from drift in laser output power. These signals are shown in figure 6. The clock signal is then used to split the divided signal into values corresponding to the vertically and horizontally polarized lasers. Values where the clock is between the HIGH and LOW states are discarded. This removes data where the lasers may both be on as well as some artifacts of the lasers switching on and off. The averaging also helps to remove these effects. The values for each polarization are then averaged, giving a normalized value for the attenuation of that polarization. The difference between this averaged value for the two polarizations gives the degree of dichroism in the sample.

Because we are using two diode lasers that are not locked into the same output power, the above procedure gives a signal with no magnetic field applied. Thus, a control value must be measured without the magnetic field and subtracted from the signal with a magnetic field applied.
4 Results and Conclusions

The device was used to test samples of $\beta$-hematin in a saline solution. Each sample was measured 5 times and averaged to get a value for each sample. The error bars were calculated from the standard deviation of these measurements. These data are shown in figure 7. The signal measured by the device is linear with concentration. This agrees with the measurements by Butykai et al. [1], which showed that the change in transmission is linear with concentration of hemozoin in solution. Down to the lowest measured concentration, the measured signal is non-zero to within the error bars of the measurement. Although the error bars for low concentrations are comparable to the magnitude of the signal, the signals are still non-zero to within
Figure 7: Signal vs Concentration

Samples were tested with concentrations from 10 $\mu$g/mL down to 4.9 ng/mL in dilutions of 2. The right plot shows a zoomed-in view of the lowest concentrations, with the solid black line denoting the zero-value.

The data suggest that the lower limit of concentrations that the device can detect is around 4.9 ng/mL. The results show that the new device is more sensitive than Jones et al.’s device. However, the process of taking a measurement is more difficult. For Jones’ device, the user turns on the laser, photodiode and hall probe circuits, and the motor. A measurement can then be taken with a single button in the software. The new design requires the operator to take a control run, input that value back into the software, turning on the magnetic field, and doing a second run. This process takes more time than Jones et al.’s device, but the data suggest that it is more sensitive at low concentration. The improved sensitivity along with the elimination of continuously moving parts make this a very promising device.
for use in the field.

This is, of course, a prototype. Our device was built around the backbone of Jones’ device. Since there are no continuously moving parts, vibrations are not a source of noise. Thus, a future iteration of the device much of the support material could be eliminated, significantly reducing the size of the device. Currently, the footprint of the device is about 1’ × 1.5’, with the beam path along the long direction. The limiting factor on the laser path length, and thus the size of the device, is the small angle reflection which must pass back through the sample. The beam must drop low enough to hit the photodiode below the beam-splitter and also pass through the sample. Shortening the support structure would require a larger angle through the sample. This could be avoided by using a right angle prism. The light reflects twice and returns parallel to the incoming beam, but lower.

The sensitivity of the device can also be improved with a higher sampling rate on the DAQ, or by reducing the number of points that are averaged. This would allow the lasers to be switched faster while still having enough averaged points per cycle to get an accurate reading.

5 Future Work

The device has not yet been used to test blood samples, so the first step is testing blood sample to check if the new device avoids the drift present in the old device. Past experience suggests that the results with $\beta$-hematin
should be a good guide to the effectiveness with sampling blood. Using what we learned from the new design, we have every expectation that significant further improvements can be made. The new device would use a liquid crystal shutter to rotate the polarization of a single diode laser. Ideally, this would eliminate the need for the control run. The primary reason for the control run on the current device is the difference in the output power of the two lasers. By using a single laser and rotating the polarization, the device should give zero signal for a sample without hemozoin. This should eliminate the need for a control run, making the device as easy to operate as Jones’ device. We will be collaborating with our colleagues at the Center for Global Health and Diseases in implementing the device for field applications.

6 Acknowledgements

I thank Richard Bihary for machining the various parts of the device, Edwin Burwell for help with circuit design, D’Arbara Blankenship and Brian Grimberg for help with sample preparation, and Eric Abenojar and Anna Samia for synthesizing the β-Hematin crystals used in the samples.
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