# Detection of Malaria Using Magnetic Fields

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Undergraduate Honors Thesis

Daniel Roll

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Department of Mechanical & Aerospace Engineering
The Ohio State University

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Dr. Vish Subramaniam, Advisor
Dr. Mark Drew, Co-Advisor

# Abstract

Malaria is one of the most deadly diseases in the world, infecting approximately 200 million people annually. While malaria is a curable disease, the fact that it is endemic to some of the most poverty stricken and remote regions of the world make it a uniquely challenging disease to combat, especially considering that modern diagnostic techniques require skilled pathologists, cumbersome equipment, and are very time-consuming. A major hurdle in effectively administering treatment and combatting the spread of malaria lies in the development of a novel diagnostic technique that is both fast and inexpensive. The focus of this thesis is the development of an electromagnetic probe to noninvasively detect hemozoin, a paramagnetic byproduct of a malaria infection, in an infected person's bloodstream.

While such a probe would represent significant advantages over existing diagnostic techniques, the probe design in this paper was found to be unsuitable for the noninvasive detection of hemozoin due to its sensitivity to capacitive effects in fluids. This thesis outlines the design, tuning, testing, and ultimate shortcomings of this probe in detail. Because of the extreme difficulties encountered in developing an *in vivo* detection technique, it is concluded that the development of an improved technique for *ex vivo* malaria detection via a blood draw is a more realistic short term goal. The last section of this thesis proposes several such techniques. Such a detection method would still represent vast improvements over current diagnostic methods, provided that it eliminates the need for need for a pathologist, and can handle a large quantity of tests in a timely manner.

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Many thanks to my co-advisor, Dr. Mark Drew, for his contributions as well. Dr. Drew provided a wealth of knowledge on malaria infections and critical background information on the current malaria detection industry. Thanks to Dr. Drew and his lab for providing the purified malaria food vacuole samples used in this research

Joe West was also integral in the completion of this thesis. Joe was always available to assist with building test setups and designing any electronic systems needed . Furthermore, Joe's wide range of experience and ingenuity was invaluable for brainstorming new ideas and finding ways around the many technical hurdles encountered in this research.

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based upon in the completion of his Undergraduate Honor's Thesis on the electromagnetic detection of malaria [11].

Lastly, I would like to extend a huge thank you to The Ohio State University College of Engineering and the Department of Mechanical and Aerospace Engineering for providing financial support and creating an accessible program for undergraduates like myself to get access to real, challenging research experience.

# Chapter 1: Introduction

## 1.1 Background

Every year, an estimated 200 million people globally become infected with malaria, resulting in approximately 627,000 deaths [1]. Furthermore, 3.4 billion people globally are considered at risk for malaria infection; that is, they do not exhibit immunity and live in an area where the parasite is present [1]. These statistics make it clear that malaria is a serious epidemic in need of immense efforts to eradicate. Compounding the problem is that fact that the spread of the disease is facilitated by the presence of anopheles mosquitoes and infected individuals who are asymptomatic [2]. This is a significant problem on offshore oil platforms and mines in remote locations where either a significant portion of a workforce or the entire workforce may be afflicted, resulting in significant loss of productivity [3].

In spite of these statistics, malaria is a curable disease. The major obstacle to the eradication of malaria is the fact that the parasite is endemic to some of the most remote regions of the world, including Southeast Asia and Africa. In these regions, pathologists and microscopes are not readily available to analyze blood smears for a definitive diagnosis and there is no other relatively inexpensive method for detecting a malaria infection, even *ex vivo,* that can be effectively implemented in these areas and handle the immense numbers of tests needed annually. It is estimated that one billion tests would be needed annually to fulfill the global demand for malaria testing, and no existing diagnostic technology is capable of handling this

vast quantity [1]. The ultimate goal of any research on malaria detection would be to detect the disease without a blood draw and in a non-invasive and continuous manner.

## 1.2 Existing Detection Technology

The current standard for malaria diagnosis is blood draw microscopy. In the state-of-the-art procedure for blood smear microscopy, blood is drawn from a patient with a suspected malaria infection, a blood smear is created from this sample, and the blood smear is sent to a lab for microscopic analysis by a pathologist [4]. The pathologist analyzes the blood smear under a microscope in an attempt to identify malaria food vacuoles, a byproduct of malaria infection that will be described in more detail in the following section [4]. Figure 1 shows a typical blood smear of an infected patient, where the small black spots indicated by the arrows are food vacuoles formed by the malaria parasite after digestion of the hemoglobin from red blood cells [4]. The accurate identification of these food vacuoles in a blood smear requires a highly skilled pathologist, along with a microscope [4].

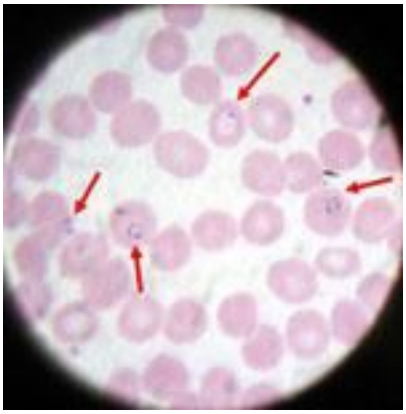

Figure 1: Infected Blood Smear [4]

While this detection method can very accurately identify a malaria infection when deployed correctly, it has a litany of issues that keep it from being a reasonable means for meeting all of the testing needs required to effectively combat malaria. Foremost among these issues is that blood smear microscopy testing cannot keep up with the massive global demand for malaria tests. It is both expensive and difficult to make highly skilled pathologists and microscopes commonly available in the vast, remote areas of the world where malaria is endemic. This typically results in blood testing stations being few and far between, which means that blood smears must be sent significant distances to be analyzed. This process can take several days, and such a time delay can make the difference between life and death for someone at a critical stage of a malaria infection. For a working person, this time delay can also cause significant loss in productivity due to the debilitating symptoms. Furthermore, this detection method is invasive, in that it requires a blood draw. This means that disposable medical equipment must be used during testing, and a sterile environment must be made available where the blood is to be drawn - yet another difficult task in remote areas. The fact that this testing

method is invasive and cannot be conducted repeatedly or continuously on a patient poses another unique problem. The malaria parasite infected red blood cells can sequester inside of a patient at times, depending on the stage of infection. During these sequestration periods, malaria food vacuoles are not freely flowing in an infected person's bloodstream. If blood is drawn while the food vacuoles are sequestered, they may not be present in the blood smear in spite of the fact that the patient has an infection. The best remedy to this issue would be to repeatedly test a patient with a suspected infection over a period of time, but the logistical issues that come with blood smear microscopy mean that this cannot be done on a wide scale. In summation, while accurate, blood draw microscopy is not capable of the high throughput required to fulfill all global malaria testing needs, and a testing method that is noninvasive, portable, continuous, and easy to use is essential if significant progress it to be made in combatting malaria and its spread.

In response to the shortcomings of blood draw microscopy, a method of detection known as the Rapid Diagnostic Test (RDT) has been and is being developed. An image of a RDT can be seen in Figure 2. A RDT is a disposable method for detecting malaria in which a patient applies a small sample of blood to a testing pad, and horizontal bars appear in the RDT window indicative of whether the patient is infected with malaria or not [5]. The RDT in Figure 2 tests for two different species of malaria, *Plasmodium Vivax* and *Plasmodium Falciparum* [5]. The RDT addresses many shortcomings of blood microscopy in that it requires no highly trained personnel or cumbersome equipment. The RDT also provides quick results - the time from application of a blood sample to the display of results is approximately 15 minutes [5]. However, the RDT lacks the accuracy necessary to be a standalone method of malaria diagnosis, and blood smear microscopy is still needed to confirm negative results [5]. The RDT is also only able to

accurately detect malaria infections at relatively high levels of parasitemia when compared to blood smear microscopy, meaning that it is only accurate in late stage infections [5]. Finally, the RDT lacks reusability and is an invasive detection technique, meaning that false negative results are still possible if the test is administered at a time when the parasite is sequestered within a patient [5].

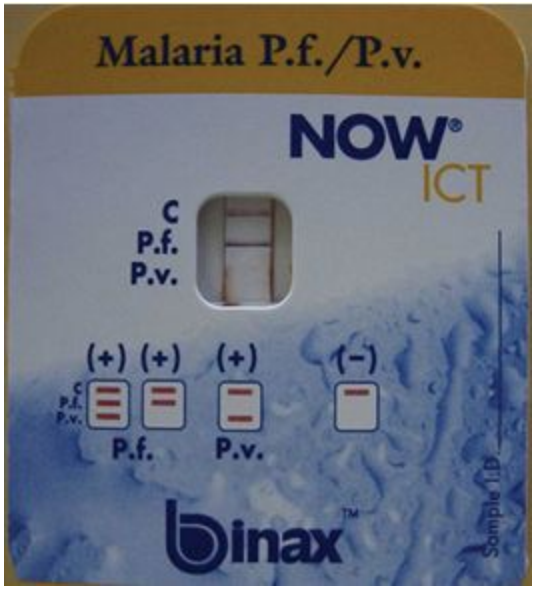


Figure 2: Rapid Diagnostic Test (RDT) Indicating a *Plasmodium Falciparum* Malaria Infection [5]

## 1.3 A Novel Detection Method

To best understand the theory behind the research discussed in this thesis, some background information on the malaria parasite life cycle is necessary. *Plasmodium falciparum* (PF) is by far the most prevalent and deadly form of malaria, accounting for a vast majority of

deaths and infections worldwide. Therefore, the focus of this paper will be on PF detection. As such, the malaria lifecycle described herein is that of PF. Malaria is a parasitic infection that is transmitted to a human host by a bite from an infected mosquito. After a bite from an infected mosquito introduces the parasite sporozoites in a human host's bloodstream, the parasite replicates in the host's liver cells [6]. Once this stage is complete, the parasite re-enters the host's bloodstream and invades the host's red blood cells (RBCs), where the parasite metabolizes the protein component of the hemoglobin therein for nutrients [7]. This releases free heme moeities, which are toxic to the parasite [7]. The parasite then chemically changes these moeities into hemozoin, also known as malaria pigment [7]. Next, the parasite seals the hemozoin inside of vacuoles, often referred to as "food vacuoles" [7]. Hemozoin has a number of unique characteristics that can be utilized for noninvasive detection, and detecting these hemozoin-filled food vacuoles as an indication of a malaria infection is the basis of the research in this thesis.

Hemozoin has a crystalline structure with an aspect ratio of (1:1:8) and crystals of up to 1000 nm in length [8]. Many of these crystals are sealed in each food vacuole, which begin floating freely in a patient's blood stream once the parasite replicates to the point that the RBC it has invaded bursts. Significantly, hemozoin crystals exhibit superparamagnetic properties, while hemoglobin does not [8]. Paramagnetic substances can be magnetized temporarily by an external magnetic field, but exhibit a no average magnetization otherwise. Hemozoin is classified as superparamagnetic because the crystals have significantly higher magnetic permeability than other paramagnetic substances [8]. The goal of this research is to utilize the fact that a patient infected with malaria has superparamagnetic particles flowing through his/her bloodstream, and detect these particles externally my magnetizing them and using advanced detection techniques.

## 1.4 Review of Relevant Literature

Magnetic fields are regularly used to isolate and separate infected erythrocytes in blood. In this procedure, a purified sample of infected erythrocytes is used [9]. This sample is flowed through a chamber containing densely packed ferromagnetic material, commonly steel wool or ferrite beads [9]. The chamber is subjected to a homogeneous magnetic field produced by a solid-state magnet [9]. While this magnetic field is applied to the chamber, the infected erythrocytes that are flowed through the chamber adhere to the ferromagnetic material [9]. Upon the removal of the magnetic field, the infected erythrocytes are released and can be rinsed out of the chamber [9]. This technique has been demonstrated successful with homogeneous field strengths of .7 T, resulting in magnetic field gradients of up to 100 Tesla/cm at the surface of the ferromagnetic material [9]. Because erythrocytes in a later stage of infection contain more paramagnetic hemozoin than those in an earlier stage, weaker homogeneous field strengths can be used to exclusively separate erythrocytes in a later stage of infection [9].

A similar procedure has been used to separate food vacuoles from blood, as opposed to infected erythrocytes. As with the separation of infected erythrocytes, a sample of purified erythrocytes is used [10]. The key difference in this procedure is that erythrocytes are lysed or broken by grinding prior to flowing the sample through the chamber [10]. This releases the food vacuoles from the erythrocytes. The remainder of the procedure is identical to the magnetic separation of erythrocytes [10]. Separation of hemozoin via this procedure has been accomplished with a homogeneous field strength of .7 Tesla [10]. In spite of the fact that these methods of separation take advantage of the paramagnetic properties of hemozoin, there appears

to be no existing technology that utilizes this property for the detection of a malaria infection, as is the intent of this research.

# Chapter 2: Motivation and Objectives

## 2.1 Motivation

The foremost objective in developing an improved method of diagnosing malaria infections is to save lives. Given that malaria is curable, a test that can address and improve upon the shortcomings of existing diagnostic technologies such as blood smear microscopy and the RDT would have an immediate impact globally with regards to getting infected patients adequate treatment faster. Beyond saving lives and contributing significantly to the cause of eradicating malaria, an improved diagnostic method could also have significant financial benefits. In fact, if all suspected malaria cases globally could be accurately diagnosed, the *World Health Organization* estimates that the need for artemisinen-based combination therapy (ACT) treatments, which are the standard method of treatment of *Plasmodium Falciparum* infections, would decrease by up to 60% [1]. This change would result in the saving of many millions of dollars.

Improved testing frequency, quantity, and accuracy could also provide a much more accurate picture of exactly where in the world malaria poses the greatest risk, so that efforts to battle the disease could be more accurately aimed. Increased testing rates would not only provide valuable data on where the disease is currently a major issue, but could also provide predictive data on where the disease is spreading. This could lead to far more accurate administrations of preventative measures, allowing for not only more effective treatment of the disease, but far more effective prevention of the spread of the disease as well.

Lastly, many large oil and mining companies have sizeable operations in areas of the world where malaria poses a significant risk to the workforce because of the pool of infected, asymptomatic individuals [2, 3]. For these industries, there is an immense safety and financial motivation behind the development of an improved diagnostic technique. Ideally, these companies would be able to test their workers quickly and on a daily basis for malaria infections, allowing for prompt treatment of any infections, as well as mitigating the risk of a false negative result in the case that the food vacuoles in an infected worker's body are sequestered in such a way that a blood draw would not yield a positive result.

## 2.2 Objective

The objective of this research is to utilize the paramagnetic properties of hemozoin, a byproduct of malaria infection that is present in microscopic food vacuoles in an infected person's bloodstream, to effectively diagnose malaria. An electromagnetic probe sensitive to the presence of small quantities of hemozoin is to be developed. Ideally this probe will exhibit the following properties:

- Non-invasive
    - The probe is able to detect a malaria infection in a patient externally, without a peripheral blood draw.
- Portable
    - The probe has small form-factor, and is easily made available in remote regions of the world where malaria poses the greatest risk.
- High-throughput

    - The probe can handle large quantities of tests quickly, likely made possible by the probe exhibiting reusability.
- Easy-to-Use
    - The probe will not require highly trained personnel or pathologists to operate, nor will it require the use of microscopes.

If all of these characteristics can be implemented, the probe will represent vast improvements over current detection methods, and allow for great strides to be made in combatting malaria.

# Chapter 3: Experimental Design and Setup

## 3.1 Experimental Setup

This research builds on earlier work on electromagnetic detection of malaria [11]. In addressing the shortcomings of existing malaria detection methods, the probe must be portable, inexpensive, and capable of handling the vast quantities of malaria tests needed globally. Initial probe designs, consisting of mutually inductive electromagnetic coils, will be outlined in the following sections. A diagram of the experimental setup for these probes can be seen in Figure 3. The following are descriptions of the major components in this setup.

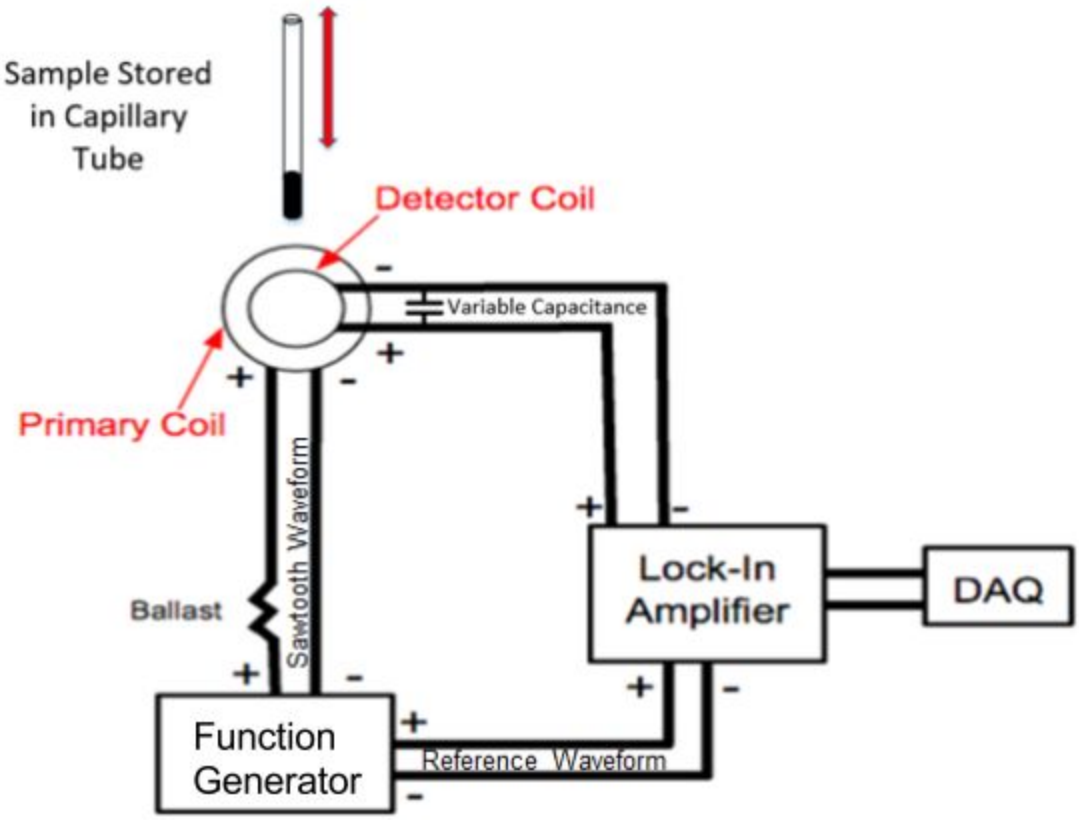


Figure 3: Electromagnetic Probe Test Setup

***Function Generator***

This is a Hewlett Packard 33120a function generator, which generates a sawtooth waveform used to drive the primary coil through a ballast resistance of 1 $k\Omega$. The frequency of the signal is 100 kHz. The amplitude of this signal is 7 Volts peak-to-peak. The output impedance of the function generator is set to "Hi-Z".

***Electromagnetic Probe: Primary and Detector Coils***

The primary and detector coils shown in Figure 3 represent what will be called the electromagnetic (EM) probe. The EM probe is the where a sample is introduced to the circuit, whether it be a sample of pure malaria food vacuoles for testing or eventually a body part of a patient with a suspected malaria infection. The EM probe consists of two concentric, mutually inductive coils. The outer coil is called the primary coil, and the inner coil is called the detector coil. Previous work conducted by Brad Smith used coaxial cylindrical coils for the EM probe, but due to multiple shortcomings of this design, a planar coil design was developed. A varying voltage applied across the primary coil will cause an electrical current through the primary coil. The current through the primary coil generates a magnetic field in the core of the primary coil. This behavior can be shown mathematically by Equation 1, where $B_{primary}$ is the magnetic flux density through a coil of $N_{primary}$ turns carrying current $I_{primary}$, and $\mu_{primary}$ is the magnetic permeability of the coil's core [12].

$$B_{primary} = \frac{\mu_{primary} N_{primary} I_{primary}}{L_{primary}}$$

**Equation 1: Magnetic Flux Density Through a Current Carrying Coil [12]**

If the voltage across the primary coil varies with time, then the current through the coil will also vary with time. This will result in the magnetic flux density, $B_{primary}$, through the primary coil varying with time.

Because the primary coil is inductively coupled to the detector coil, the varying magnetic field generated by the primary coil is present in the core of the detector coil as well. This results in varying magnetic flux in the core of the detector coil. This varying magnetic flux generates a voltage across the detector coil, and therefore a current through the detector coil. This behavior can be shown in Equation 2, where the voltage $\varepsilon_{detector}$ is the voltage generated across the detector coil of $N_{detector}$ turns with $\phi_{detector}$ magnetic flux through its core. The voltage across the detector will also vary in time, so long at the voltage applied across the primary is not linear with time.

$$\varepsilon_{detector} = -N_{detector}\frac{d\phi_{detector}}{dt}$$

**Equation 2: Induced Voltage due to Changing Flux in a Coil**

Figure 4 illustrates the function of the mutually inductive coils governed by Equation 1 and Equation 2. It is important to note that as long that the coils are coaxial and close enough to one another to be strongly inductively coupled, the general behavior described by these equations will hold true, and a voltage applied across the primary coil can be used to excite the detector coil.

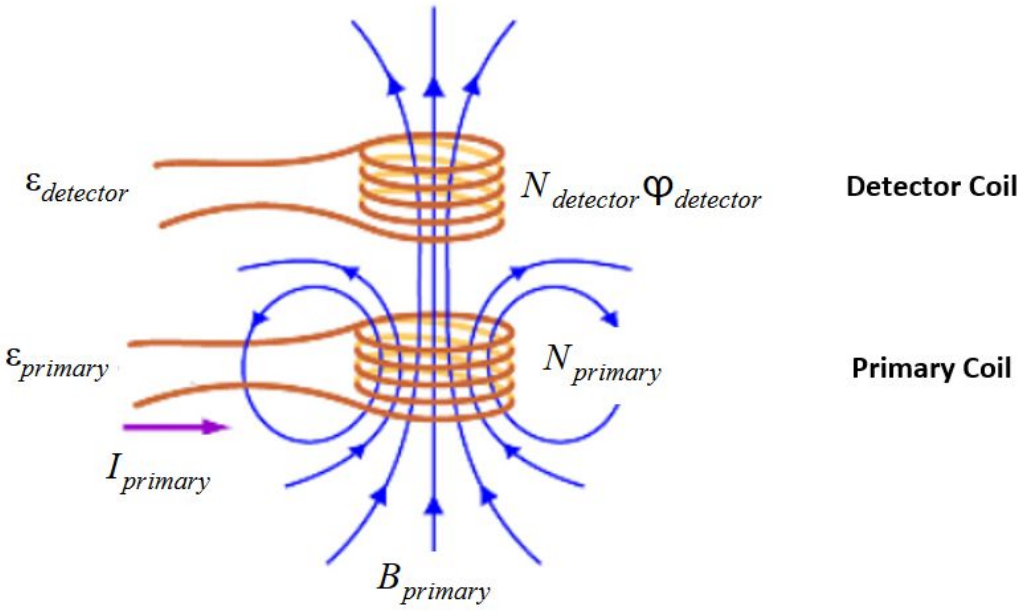


Figure 4: Mutually Inductive Coil Operation [12]

In this experiment, the primary coil is excited with a 100 kHz, 7 Volt peak-to-peak sawtooth waveform. Because this waveform has a fast voltage transient, it will causes a voltage ringing on the detector coil at each transient point. The frequency of this ringing is the resonant frequency of the detector coil, which is strongly dependent on the inductance of the detector coil. Because the inductance of a coil is strongly dependent on the magnetic permeability of the core about which the coil was wound, the introduction of a paramagnetic substance to the core of the detector coil will cause a change in the voltage ringing waveform across the terminals of the detector coil. If small changes can be detected in this waveform, the presence of a material with a high magnetic permeability, which includes paramagnetic substances, can be detected. However, hemozoin is generally only present in small amounts and must be detected through a skin barrier to create an effective noninvasive probe, so changes in the voltage ringing across the detector

coil due to the presence of small amounts of hemozoin in its core will be very small. Advanced signal conditioning techniques are necessary to extract this small change in signal from noise.

***Variable Capacitor***

A variable capacitor is connected in parallel with the detector coil of the EM probe. This capacitor can be adjusted to capacitances from 10pF to 100pF continuously using a dial. An image of the variable capacitor can be seen in Figure 5. This is the same variable capacitor that was used in previous work [11].

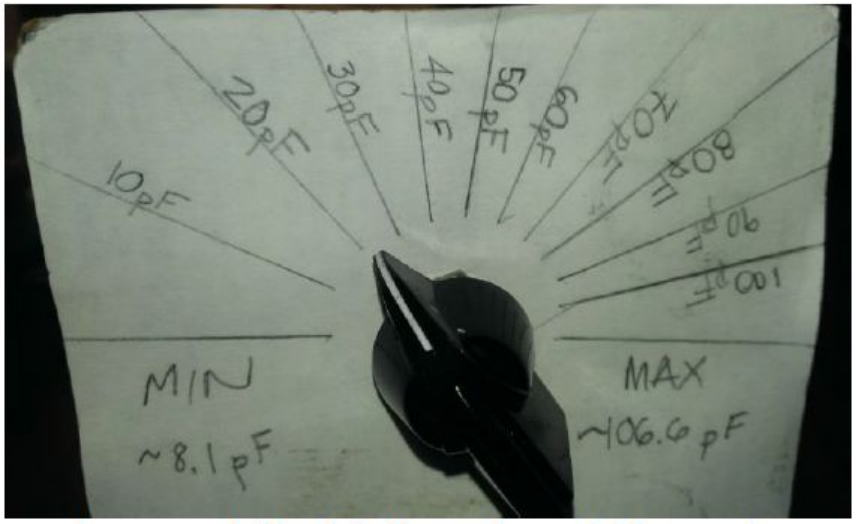


Figure 5: Variable Capacitor Adjustment Knob [11]

***Lock-in Amplifier***

The Lock-in amplifier is a critical component in the measurement, as well as many of the additional experiments that will be suggested in the later parts of this thesis. The lock-in amplifier used in this research is the Stanford Research Systems Model SR510. The lock-in amplifier is capable of detecting very small AC signals and extracting them from noise by

operating as a bandpass filter with an extremely narrow band tuned to a frequency of interest [13]. The lock-in amplifier's behavior is outlined in the block diagram in Figure 6. The lock-in amplifier requires two inputs: one input from the experiment, and one input that provides the lock-in amplifier with a reference frequency about which to center its band-pass filter behavior. In Figure 6, the signal fed into the differential amplifier is the signal from the experiment, and the signal that is used as an input to the Phase Locked Loop (PLL) is the reference signal. The lock-in amplifier mixes these two signals, then then filters out the higher frequency components, leaving the differential signal to be fed through a DC amplifier. As such, if the experiment is driven with a signal of the same frequency as the reference signal, then small changes in the signal from the experiment will be seen as changes in a DC voltage output from the lock-in amplifier.

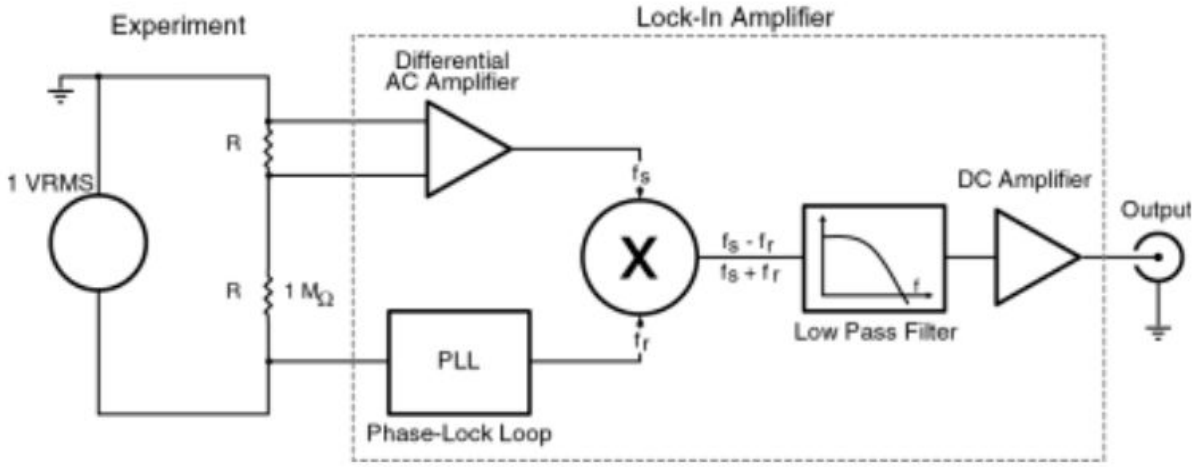


Figure 6: Lock-In Amplifier Block Diagram [13]

There are three main settings than can be adjusted on the lock-in amplifier: the sensitivity, the time constant, and the dynamic reserve. For all data presented in this report, the sensitivity was set to 100 $\mu V$ , the time constant was set to 100 ms, and the dynamic reserve was set to LOW.

As can be seen in Figure 3, the lock-in amplifier is given a reference frequency input in the form of the SYNC output of the function generator that is driving the primary coil. This is a 100 kHz square wave. As the introduction of a paramagnetic substance to the core of the detector coil occurs, the voltage across the detector coil will change minutely, which will result in a DC shift in the output of the lock-in amplifier.

***Sample***

Two main samples were used in the research: an iron powder sample and a food vacuole sample. The iron powder sample consisted of a small amount of -325 mesh iron powder at the end of a glass capillary tube. Due to this mesh size, maximum particle size is limited to 44 microns. The sample of iron particles used to create the iron powder data in this work had a mass of less than 1 $\mu g$ , and the glass capillary tube the sample was stored in was 1.8 mm in diameter and 95 mm long. The sample was placed at the end of the capillary tube, which was capped using clear nail polish. A magnified image of this sample can be seen in Figure 7. This nail polish was shown have no effect when introduced to electromagnetic coils in previous work [11].

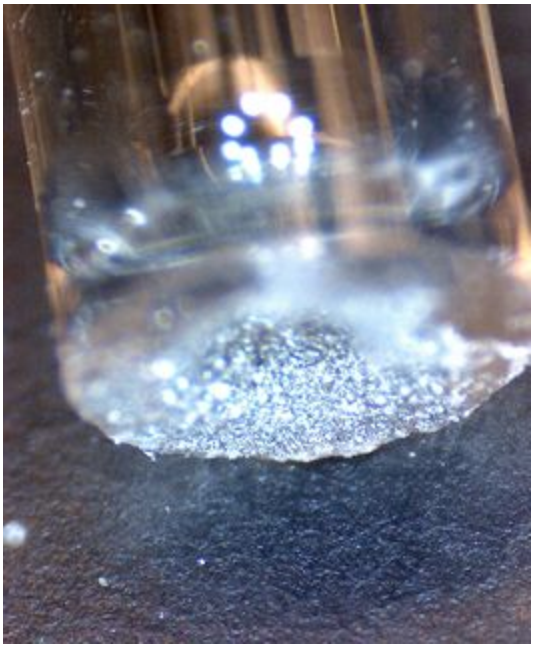

**Figure 7: 1 μg Iron Powder Sample Stored in Capillary Tube**

Dr. Mark Drew of Ohio State University provided all the malaria food vacuoles used in this research. The food vacuoles came from Dr. Drew's lab suspended in a Phosphate Buffered Saline (PBS). In order to obtain dried, isolated food vacuole samples in a capillary tube, the capillary tubes were necked by holding them above a propane torch until the glass began to melt and then pulling the tube while removing it from the flame. These capillary tubes were of the same dimensions as those the iron powder was stored in initially (95 mm long, 1.8mm diameter), but after the necking process a narrow region was created in the center of the capillary tube on the order of 100-200 microns in diameter. The fluid suspension of food vacuoles was then pumped through the necked capillary tube using a syringe. As the fluid flowed through the necked region, the vacuoles would clog the tube, leaving a small cluster of vacuoles trapped in the necked region as can be seen in Figure 8. The capillary tube was then snapped just below the

point where the sample was captured. The sample shown in Figure 8 is in a glass region of 22 microns in diameter, and is estimated to have a mass of approximately 0.2 $\mu g$.

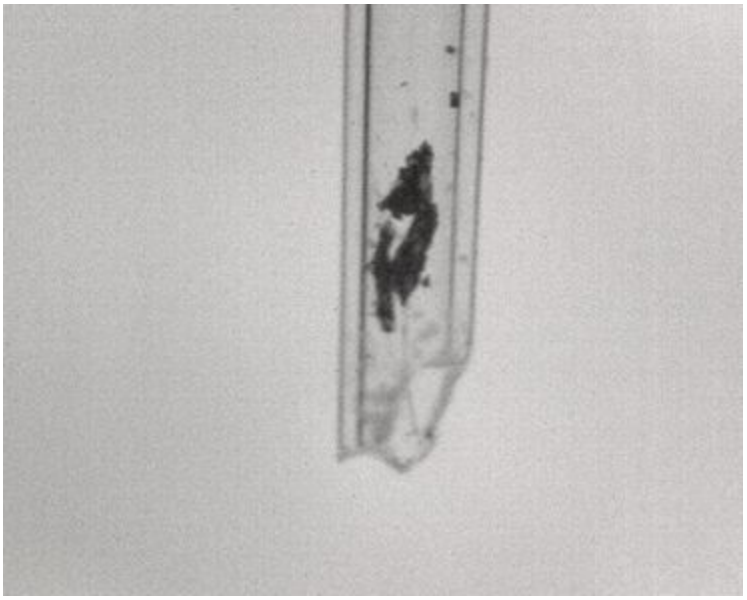

Figure 8: Malaria Food Vacuole Sample in Necked Capillary Tube

***DAQ***

Most data acquisition for this project was in the form of an oscilloscope monitoring the output of the lock-in amplifier, although on occasion the data was sent to a PC and plotted using MATLAB.

## 3.2 Detection Circuit Function

Now that all major system components have been described, the overall system function can be understood as follows:

1. A function generator generates a sawtooth wave that excites the primary coil, which causes voltage ringing across the terminals of the inductively coupled detector coil.
2. A sample (food vacuoles or iron powder) is introduced to the core of detector coil, resulting in a minute shift in amplitude or phase of the voltage across the detector coil.
3. The lock-in amplifier, which is tuned to a reference frequency provided by the function generator, detects and amplifies any small changes in the voltage across detector coil, outputting a DC signal proportional to this change.

## 3.3 Previous Work

In the earlier experiments upon which this research is based, the EM probe was comprised of two coaxial cylindrical coils, with the end goal of designing a cylindrical probe that could be worn around a patient's fingertip [11]. It was initially reported that food vacuoles were successfully detected with these coils, but results could not be repeated because of strong capacitive coupling which could not be reproduced. However, the cylindrical probe showed significant sensitivity to very minute amounts of iron particles, and it was believed that with an increase in probe sensitivity, food vacuoles could be detected.

It was apparent from re-examination of the earlier work that a key way to increase probe sensitivity to small amounts of paramagnetic substances was to decrease the core size of the

probe coils. However, the EM probe constructed of cylindrical coils was only able to detect particles that were fully inserted into its core, and the probe was already less than 5 mm in inner diameter. This meant that a cylindrical probe with the adequate sensitivity to detect small amounts of hemozoin would not be able to fit around a patient's fingertip, and there was no non-invasive way that such a probe could be applied *in vivo*.

## 3.4 Planar Coil EM Probe Design and Construction

To address the shortcomings of the cylindrical probe design, a probe consisting of concentric planar coils was constructed. The probe can be seen in Figure 9. The outer coil is the primary coil and the inner coil is the detector coil. By using a planar design, the inner diameter of the probe could be kept small, maximizing sensitivity, and the probe could be easily placed in anywhere on a patient.

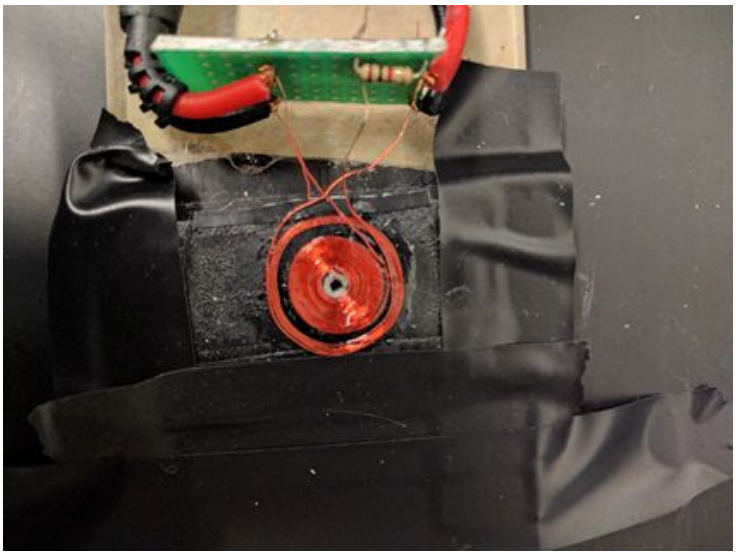

Figure 9: Planar Coil Electromagnetic Probe

The probe was constructed out of 32 gauge magnet wire. First, a hole was melted into a 2 mm thick piece of plexiglass with a fine point soldering iron to create a core around which the coil was to be wrapped. The size of this core was approximately 1 mm. The plexiglass was then wrapped in two-sided tape, and a hole was cut out of the tape around the hole in the plexiglass. The detector coil was then wound by sticking the wire down to the tape starting at the core and winding counterclockwise outward. Once the inner detector coil was wound, the outer primary coil was wound in the same manner, starting at a point approximately 1 mm from the outside of the detector coil. The leads of the coils were then soldered to a small section of perfboard, where leads from BNC connector cables were also soldered to complete the connections to the function generator and lock-in amplifier. The positive leads of both coils were chosen to match so that the path from positive terminal to negative terminal through each coil was clockwise, with the positive lead on the outer side of each coil and the negative lead on the inside. The 1 kΩ ballast resistor was connected to the positive lead of the primary coil, by soldering it to the perfboard between the positive lead from the BNC that connected to the function generator output and the positive lead of the primary coil. A thin layer of clear nail polish was then applied to the coils to improve integrity. The physical and electrical characteristics of the completed probe can be found in the table in Figure 10.

| | Primary Coil | Detector Coil |
|---|---|---|
| Turns | 5 | 18 |
| Layers | 1 | 1 |
| R (Ohms) | .23 | .36 |
| L (uH) | 2.63 | 4.92 |
| C (pF) | 44.5 | 76.6 |

Figure 10: Planar EM Probe Characteristics

# Chapter 4: Experimental Procedure

## 4.1 Introducing Samples to the EM Probe

A motorized stage was used to introduce and remove samples from the planar coil EM probe. It was important that no other substances with magnetic properties be inadvertently introduced to the EM probe during testing, so the capillary tube containing the sample was taped to the end of a long wooden rod, which was connected to the motor at the other end. The stage was programmed to start with the sample 4 inches above the core of the detector coil, then lower the sample to a position 1 mm directly above the core of the detector probe. The stage would hold the sample in this position for several seconds, then pull the sample back away from the probe to its original position 4 inches above the probe. A view of this setup can be seen in Figure 11.

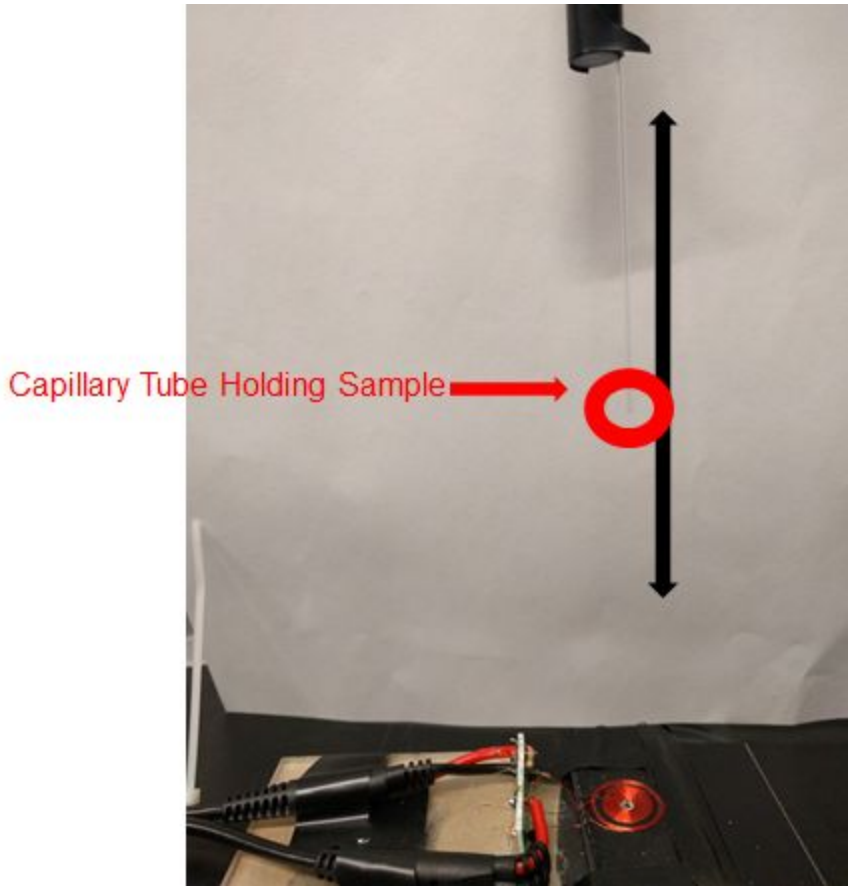


Figure 11: Sample Introduction Apparatus

## 4.2 Tuning

As reported in earlier work, the electromagnetic probe could be tuned to maximize sensitivity by correctly setting a variable capacitance placed in parallel with the detector coil [11]. The intent of using an iron powder sample initially was that it would create a larger shift in the ringing voltage waveform across the detector coil than the food vacuole sample, being the iron powder is ferromagnetic. The iron powder sample could therefore be used to optimize the

parallel capacitance value before introducing the malaria food vacuole sample, which requires much higher probe sensitivity to detect. The process of adjusting this parallel capacitance to maximize coil sensitivity is as follows.

1. Set lock-in amplifier to LOW dynamic reserve, 100 $\mu V$ sensitivity, 100 ms time constant.
2. Set function generator to Hi-Z output impedance. Set function generator output to 100 kHz, 7 V peak-to-peak sawtooth wave.
3. Set parallel capacitance to 10pF (minimum value or variable capacitor).
4. Lower iron powder sample to 1 mm above the detector coil, observe DC signal shift from lock-in amplifier.
5. Remove sample, increase variable capacitor capacitance value by 5pF.
6. Repeat Steps 2 and 3 until all capacitance values have been tested up to the maximum 100pF of the variable capacitor.
7. The capacitance value that results in the largest DC signal change from the lock-in amplifier when the iron powder sample is introduced is the optimum parallel capacitance.

After implementing these steps, it was found that the optimum parallel capacitance value was 80pF. The output of the lock-in when the 1 $\mu g$ iron powder sample was introduced with a parallel capacitance value of 80 pF can be seen in Figure 12, showing a signal change of approximately .5 V. No other parallel capacitance value led to a larger signal change.

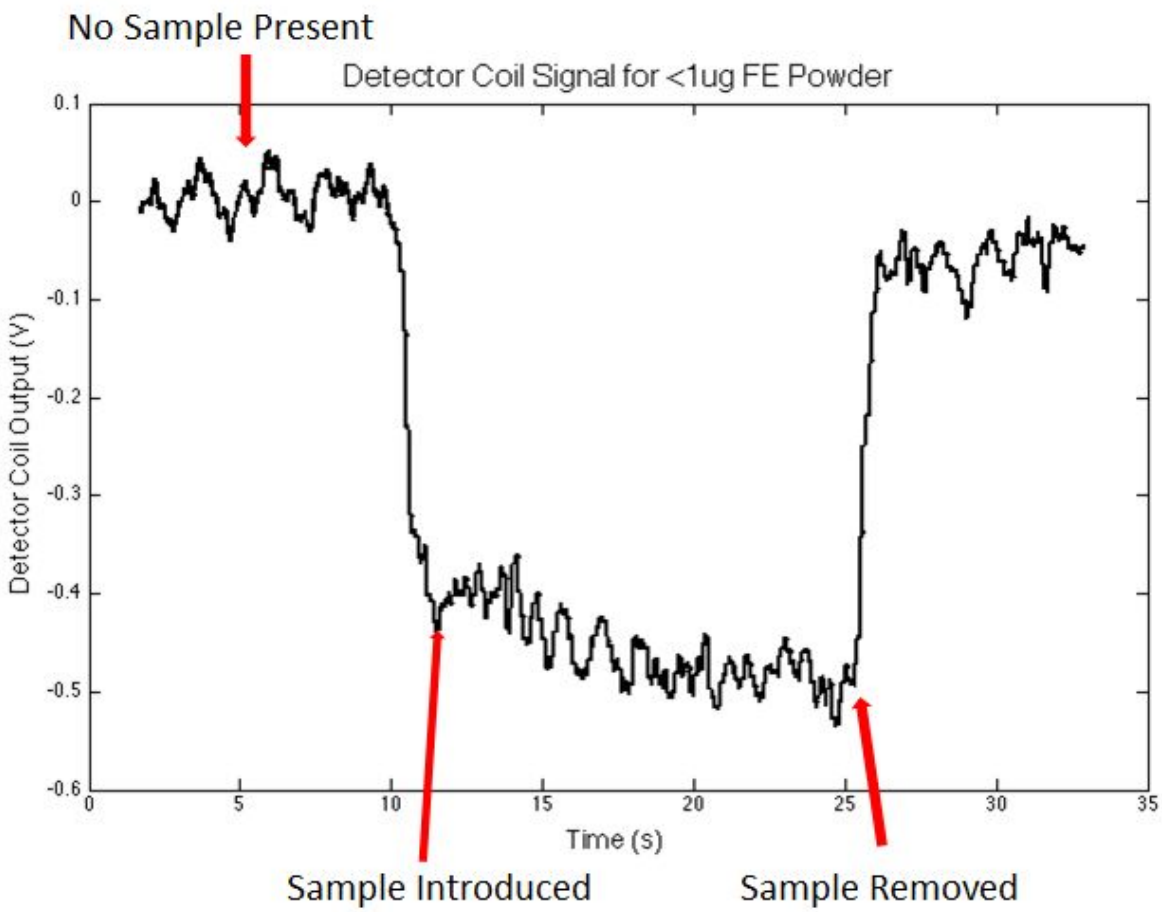


Figure 12: Lock-in Amplifier Output for 1 ug Iron Powder Sample with 80 pF Parallel Capacitance

# Chapter 5: Results and Conclusions

## 5.1 Results with Food Vacuoles and PBS Solution

Now that the parallel capacitance for the probe had been optimized, the food vacuole sample was introduced to the probe. The resulting lock-in amplifier output signal can be observed in Figure 13. As can be seen, no signal shift occurs upon the introduction of the dry food vacuole sample. This was likely due to the weaker magnetic properties of hemozoin compared to iron particles, as well as the fact that the food vacuole sample was smaller than the iron powder sample. This means the probe was not sensitive enough to detect the food vacuoles sample.

Next, a solution of food vacuoles suspended in Phosphate Buffered Saline contained in a capillary tube was introduced to the EM probe. The results for this sample can also be seen in Figure 13, indicated by the orange trace. As can be seen, the lock-in amplifier output a significant DC shift upon the introduction of this solution, meaning that the solution was being detected.

Finally, a sample of PBS only was introduced to the probe, represented by the yellow trace in Figure 13. As can be seen, the lock-in outputs a significant DC offset upon the introduction of the PBS sample. It should be noted that the time shift in Figure 13 between the PBS sample and PBS-plus-food vacuole sample was due to slightly different times at which the samples were introduced and not a result of the sample type. It should also be noted that this signal is very similar to the output signal shown in Figure 12 for the iron powder sample.

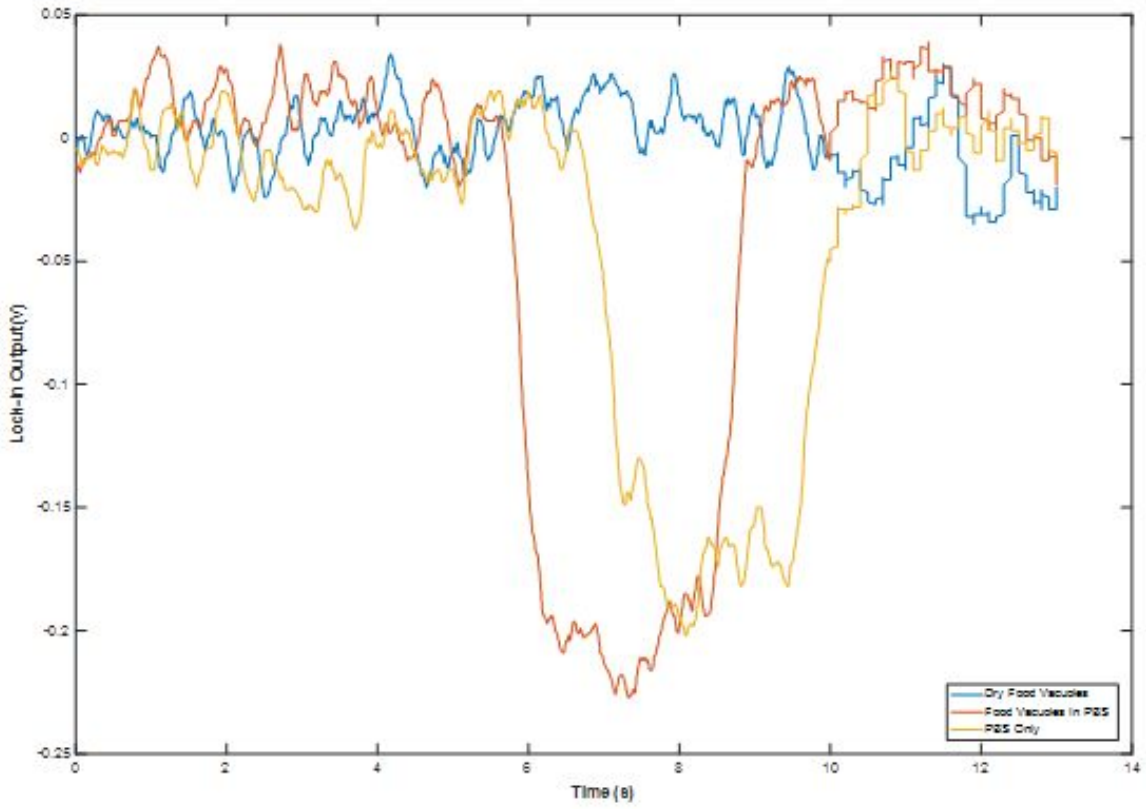


Figure 13: Dry Food Vacuoles vs. Food Vacuoles Suspended in PBS vs. PBS Only Results

This highlights a major shortcoming of the concentric coil EM probe design. This detection technique relies on the fact that malaria food vacuoles have strong paramagnetic properties, and no other substances in the human bloodstream do. However, many components of a human's blood, and even a human's skin, could have effects similar to PBS, and it is clear that these effects result in EM probe outputs indistinguishable from those due to ferromagnetic particles. The inability of the probe to differentiate between PBS and magnetic substances means

that even if the concentric coil EM probe design could be further optimized to detect small amounts of food vacuoles, the probe would not be useful in identifying the presence of hemozoin.

## 5.2 Conclusions

The concentric coil EM probe design - both in cylindrical and planar forms - has been shown to exhibit a number of significant shortcomings. One major shortcoming is that significant efforts must be made, even with the assistance of the lock-in amplifier for signal conditioning, to create a probe sensitive enough to detect small amounts of food vacuoles. These efforts could be more easily justified but for the fact that these EM probe designs show extreme sensitivity to capacitive effects in fluids in a fashion that is indistinguishable from the detection of magnetic substances. This first and foremost eliminates the possibility of the practical application of such a probe *in vivo*, as many fluids present in the human body will cause signals indistinguishable from those due to magnetic substances. Beyond this, the probe cannot even be used *ex vivo* on a blood sample, as capacitive effects due to fluids will be present in both infected and uninfected blood, obscuring any effects due to food vacuoles and rendering the two samples indistinguishable to the probe. Due to these issues, such a probe design has been deemed unsuitable for malaria detection, and suggested future research should focus on other methodologies. Two such methodologies will be described in the following section.

# Chapter 6: Future Work

## 6.1 Motivation for Alternative Detection Techniques

Because of the extreme difficulties encountered in detecting small amounts of malaria food vacuoles *ex vivo*, much less *in vivo*, the future work that will be proposed in the following sections will focus on developing a method for detecting malaria infections invasively that still addresses the major shortcomings of blood smear microscopy. These methods will not require cumbersome equipment or pathologists. Such a detection method, while invasive, would still result in significant improvements over current detection methods and significantly aid efforts in malaria treatment.

## 6.2 Atomic Vapor Magnetometry

Due to its superparamagnetic properties, a small amount of hemozoin moving through a non-homogeneous magnetic field will cause a small disturbance in the magnetic field. If this disturbance could be detected, it could be used to verify the presence of the hemozoin. While this is a theoretically accurate way to detect hemozoin, the small quantities of hemozoin that would need to be detected would cause extremely small magnetic field disturbances. To measure these disturbances, an extremely sensitive magnetometer would need to be used. Until recently, the industry standard in extremely sensitive magnetometer technology was the superconducting quantum interference device (SQUID). While these magnetometers exhibit the high sensitivity

necessary to detect extremely small magnetic field disturbances, SQUIDs must be supercooled during operation. In regards to creating a portable and practical device for detecting malaria in remote regions of the world, the requirement of SQUIDs to be supercooled makes them an impractical tool.

The recent advent of atomic vapor magnetometers (AVMs) addresses this issue. AVMs are magnetometers capable of sensitivity on the same order of magnitude as SQUIDs (sensitivity down to 1 pT), but AVMs require no supercooling [14]. To detect magnetic fields with such precision, AVMs detect small changes in valence electron spin resonance in vaporized alkali atoms [14]. Typically, vaporized Rubidium atoms are used [14]. Small changes in the magnetic field applied to these atoms cause small changes in the atoms' valence electron spin, which AVMs detect optically [14].

An example of a test setup that could utilize AVM technology can be seen in Figure 14. In this test setup, all measurements take place in a zero-gauss chamber, so as to reduce the undesirable effect of external electromagnetic interference on measurements, as well as the effects of the Earth's magnetic field. This is especially crucial given the extreme sensitivity of the AVM to small magnetic fields. Within this zero-gauss chamber, a non-homogenous magnetic field in generated by using a power supply to supply a constant current in an electromagnetic coil wrapped around a pointed core. Opposite the electromagnetic coil, the AVM will be placed in the chamber. Between the coil and the AVM, a malaria food vacuole sample will be moved back and forth in an oscillating lateral motion. The periodic motion of the paramagnetic food vacuoles will cause a periodic variation in the magnetic field that the AVM senses. If it is found that the AVM lacks adequate sensitivity to detect these small changes, the sensitivity of the setup can be

significantly boosted by using the output of the AVM as an input to the lock-in amplifier along with a reference signal from the actuator that moves the sample.

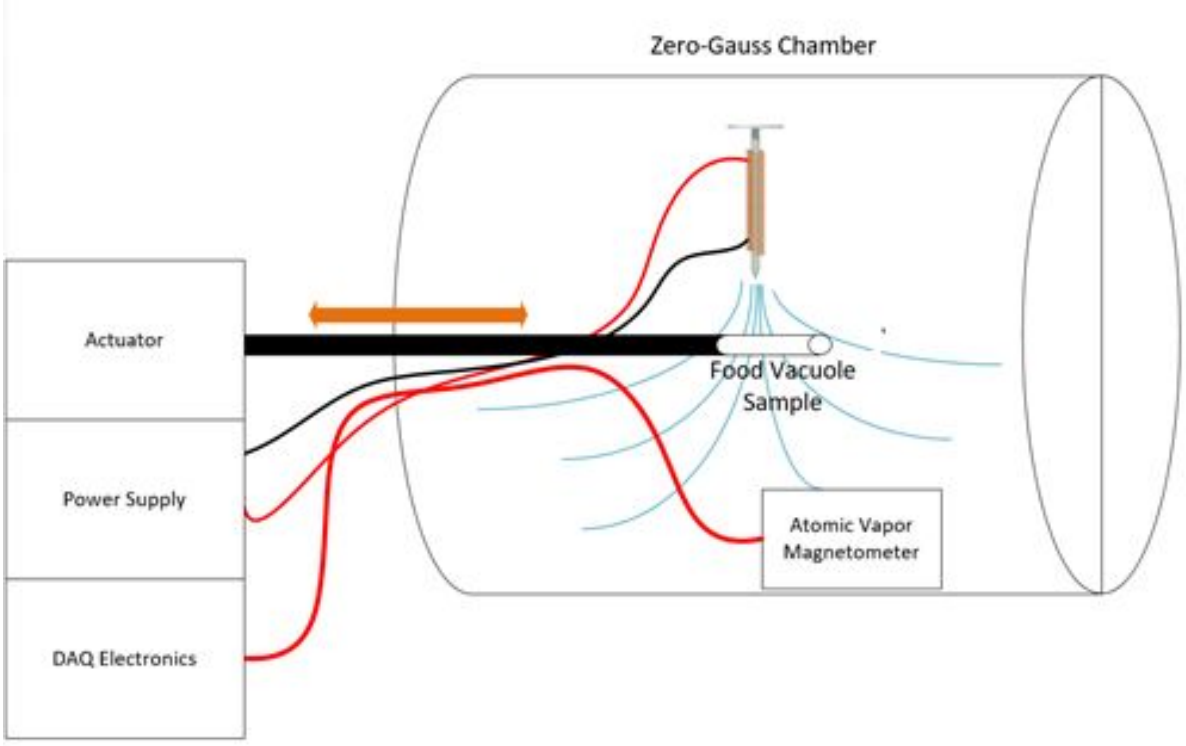


Figure 14: Potential AVM Detection Setup

This setup will exhibit extreme sensitivity to paramagnetic substances, but would not be susceptible to capacitive effects in fluids. Because of this, blood could be used as the sample. In the field, operation would simply require a blood draw and software that could analyze the AVM output, eliminating the need for a pathologist.

## 6.3 Gouy Balance

Figure 15 depicts a Gouy Balance. The theory of operation is that, if a substance with magnetic properties is placed on a very finely tuned balance and then subjected to a magnetic field as shown, the substance will undergo a weight change and disturb the balance of the scale. The small weight change could be detected in a number of ways. One such method would be to connect the opposite side of the scale to an extremely light capacitive plate, and measure the capacitance between this plate an external plate.

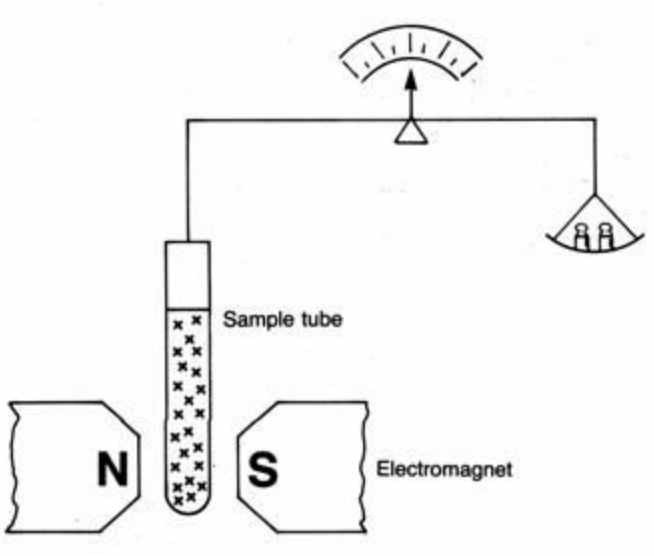


Figure 15: Gouy Balance Diagram [15]

It is important to note that, in detecting paramagnetic substances, the Gouy balance will only function properly if the sample has a magnetic field gradient across it, so that the the dipole forces on the substance do not cancel one another. This first means that the sample must be

slightly above the magnets used in the balance, where there there is a magnetic field gradient. Secondly, the sample must have sufficient volume to be spread out across the gradient of the magnetic field.

To illustrate this point, let the sample being used in the Gouy balance be a small glass vial containing a paramagnetic sample. If the sample is purely food vacuoles that are not suspended in a fluid, the sample will lack sufficient volume for there to be a significant field gradient across it. If the sample is the PBS suspension of food vacuoles there will be sufficient volume for a significant field gradient across the sample, but the food the vacuoles can move around within the fluid, resulting no net weight change of the sample for the balance to sense. A way to combat both of these issues would be to "freeze" the sample into a solid suspension. If such a frozen sample is used, all net forces that would contribute to translative movement of the particles within the fluid will instead contribute to disturbing the balance of the scale, and the paramagnetic particles will still have enough volume that a significant magnetic field gradient will be present across the sample.

The main challenge with such a detection method is that the forces due to the magnetic field on the sample will be extremely small, so extremely sensitive measurements will need to be made, and as large of a magnetic field as possible should be used. Like the AVM experiment outlined in the previous section, this detection method would not be susceptible to interference from capacitive effects, so a blood sample could be used, so long as the sample were solidified.

## 6.4 Revised Gouy Balance

The principle upon which the Gouy balance relies is that a substance with magnetic properties will undergo a force when a magnetic field is applied to it. In the case of a paramagnetic substance, there must be a magnetic field gradient across the sample. The Gouy balance shown in Figure 15 is simply a means of detecting this force. In this section an alternative means of detecting this force will be proposed. A diagram of the test setup can be found in Figure 16.

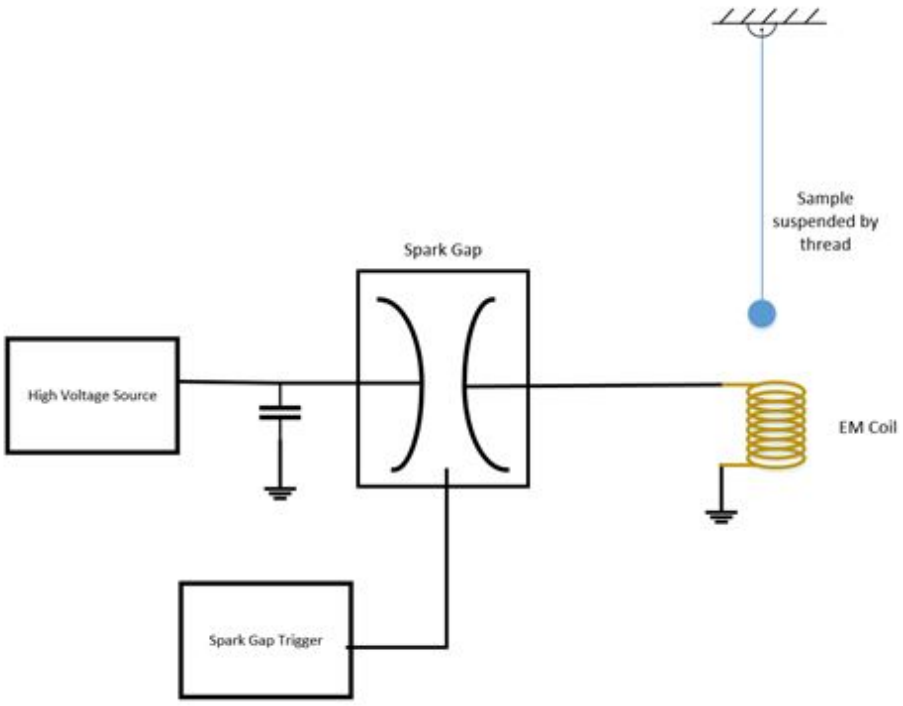


Figure 16: Proposed Method for Detecting Small Forces Due to a Magnetic Field on a Paramagnetic Sample

In the setup shown in Figure 16, first a high voltage source charges a large capacitor. Once the capacitor is charged, the spark gap trigger triggers the spark gap, which creates an electrical arc between the terminals of the spark gap. This allows the capacitor to rapidly discharge through the spark gap and through the EM coil. This high current will generate a large magnetic field in the core of the EM coil. This field will have a gradient in the radial direction of the coil, and will therefore exert a horizontal force on a paramagnetic sample. This will cause the sample to move slightly, and this motion can be detected in many ways, with one possibility being the use of optical techniques.

The orientation and creation of the sample in this setup are critical. As mentioned in the previous section, the sample must have a field gradient across it, so it must be placed just outside the coil core, where there is a strong field, but the edge effects of the coil also create a significant field gradient. There must be a field gradient in the radial direction, as the pendulum allows for horizontal deflection of the sample. Secondly, the sample must be solid, and of a size such that a significant field gradient can be present across it. In the case of detecting a solution of food vacuoles, or even infected blood, the best way to generate such a sample would be to first evaporate any fluid from the sample, then mix the remaining sample into an epoxy, which can then be stuck to the end of the thread. It is critical to make sure that neither the epoxy or the thread exhibit any magnetic properties, else the force exerted by the magnetic field on them could be mistaken for a force exerted on the sample.

If this can be achieved, and a large enough field can be generated to move the sample adequately, this would represent a simple and inexpensive way to detect a malaria infection. Furthermore, because the triggering of the spark gap results in a time varying periodic field

through the magnetic coil, the lock-in amplifier could be synchronized to this frequency and used to enhance measurement sensitivity. This method could be implemented on a dried blood sample as well, and would require no pathologist to conduct, giving it significant advantages over existing diagnostic techniques.

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