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Diagnostic smartphone application for anemia in developing countries

Team Members:

Leader: Colin Dunn

BSAC: Allison Benna

Communicator: Timothy Abbott

BWIG: Scott Schulz

Under the Supervision of:

Dr. Philip A. Bain • Kris Saha

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Abstract

Anemia is the most common nutritional disorder in the world affecting approximately 30% of the world's population (WHO, 2012, p. 1). However, this disorder can be easily treated if diagnosed. In developing countries such as Ghana, screening is largely unavailable due to limited resources leaving many cases of anemia left undiagnosed thus untreated. The development of a point of care diagnostic tool could assist in the diagnostic process of this disorder decreasing the number of undiagnosed and subsequently untreated anemia cases.

The overall goal of this project is to develop a cost effective, accessible point of care diagnostic tool to diagnose treatable forms of anemia in developing countries. This tool will have magnification and resolution capabilities to measure erythrocyte size and shape as well as interface with a device to measure hemoglobin concentration. Anemia is defined by having a reduced level of hemoglobin. The tool will allow mean corpuscular volume (MCV) to be measured, which is a common method of differentiating types of anemia. Accurate measurements if RBCs requires that, the magnification and resolution be adequate to differentiate the shape and size of erythrocytes from a peripheral blood smear. The gold standard for measuring HGB and MCV is the Coulter counter, a ubiquitous device found in all hematology laboratories in developed countries.

The first phase to achieve this goal is to determine the adequate magnification and resolution needed and how to reach those requirements with a cost effective alternative.

Introduction

Problem statement

The goal of this semester was to determine the magnification and resolution capabilities adequate to measure erythrocyte size to determine mean corpuscular volume from a peripheral

blood smear. Once the necessary magnification and resolution requirements were determined, the data would act as an input to portable diagnostic computing device for anemia.

This focused goal comes from the solution framework to the overarching aim of the project to develop a cost effective, intuitive, point of care application for a portable device to diagnose anemia using a multistep process:

Phase 1. To determine the minimum requirements of magnification and resolution to accurately analyze peripheral blood smears to diagnose anemia.

Phase 2. To develop a software application interface and magnification hardware that can detect anemia based on two inputs - the hemoglobin concentration by a pulse oximeter and the MCV calculated from the magnified peripheral blood smear.

Phase 3. To determine and classify anemia based on MCV

Phase 4. To classify and differentiate the types of anemia based on the cell morphology or shape observed in images by comparing them to an archived library of erythrocyte morphological abnormalities.

Phase 5. To introduce a treatment recommendation feature for the treatable types of anemia currently observed in developing countries based on the information gathered by the device.

Background

The Biology behind Anemia

Anemia is a disorder due to insufficient quantity of hemoglobin or improper binding of oxygen to hemoglobin. Tissues in the body require oxygen to carry out vital cellular functions. When an inadequate amount of oxygen is delivered to the tissues (either from a lack of hemoglobin or excessively bound oxygen), cellular dysfunction occurs. Common causes of anemia include inherited abnormalities in erythrocyte shape, lowered concentration due to

internal blood loss, decreased or abnormal erythrocyte production or low levels of folic acid, vitamin B12 or iron. Symptom associated with anemia can range from mild – e.g. fatigue and difficulty concentration, to severe – e.g. heart failure and death.

How Anemia is Diagnosed in the United States

The diagnosis of anemia is based on analysis of a blood sample. In a United States clinic, a physician would order a complete blood count (CBC) to test a person for anemia. A CBC is a panel of tests that evaluate white blood cells, red blood cells, and platelets. When testing for anemia, the most important results from this test are the red blood cell count (RBC), hemoglobin (Hb) and hematocrit (Hct). These levels are used to calculate the mean corpuscular volume (MCV). The accepted normal range of MCV is from 80 to 100 fL (DeBakey, Graham, & Johnson-Wimbley, 2011, p. 6). From this calculation, the size of the red blood cells in the blood sample can be determined to be greater than, less than, or equal to their accepted normal (American Association for Clinical Chemistry, 2012).

The most common laboratory devices that are used to measure RBC, Hb/Hct and MCV in developed countries are the Coulter counter and CASY (NHANES, 2004, pg. 3). A second diagnostic procedure is the interpretation of a peripheral smear by a trained clinician to evaluate erythrocyte morphology (Bain, 2012).

Coulter Counter

The Coulter counter is the gold standard for diagnosing and classifying types of anemia. Wallace H. Coulter patented this device in 1953, which utilizes a method known as "electric sensing zone" (US Patent 2656508, 1953). This method simply measures the difference in resistance between particles and their surrounding fluid (Mechanical Engineering – Duke University, 2010, p. 8).

A Coulter counter can count particles, such as red blood cells, when they are present in an electrolytic solution in a test tube as shown in Figure 1. The test tube should contain a tube with an electrode and a second electrode both connected to a battery to charge the system. When this system is pressurized, the solution can flow through an aperture of a tube containing an electrode. The resistance between the two electrodes increases each time a non-conducting particle passes through the aperture. The number of particles is observed based on the number of current drops between the two electrode as this is directly related to number of times the resistance increases Mechanical Engineering – Duke University, 2010, p. 9).

The invention of the Coulter counter changed the world of hematology. In the past, blood cells were previously counted by an individual viewing a slide under a microscope (MIT School of Engineering, 2000).

Automation of this process has resulted in the ability to analyze a much greater volume of specimens per unit time and reduced variation in results due to subjective human interpretation.

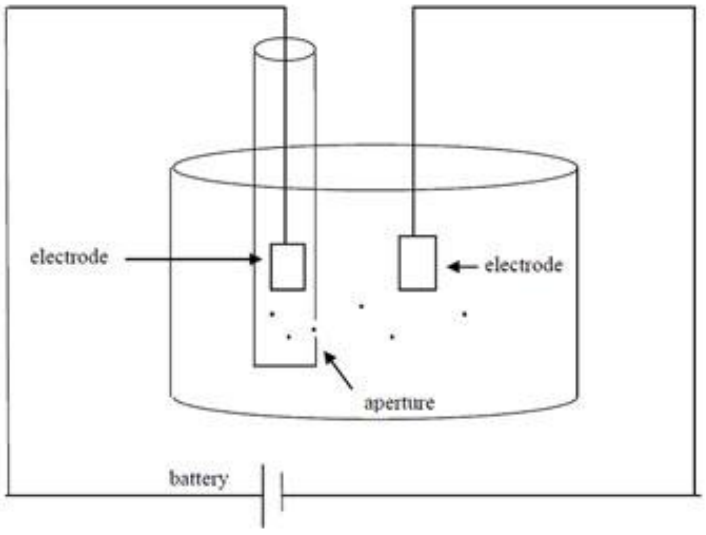


Figure 1. A voltage is applied between the two electrodes over the aperture. When a red blood cell travels through the aperture, the resistance in the aperture increases. The resulting current drop can be measured to determine the number and volume of the red blood cell (Mechanical Engineering – Duke University, 2010, p. 8).

How Anemia is Diagnosed in Developing Countries

Developing countries often lack adequate resources and manpower to process high volumes of blood samples. Point of care testing is lacking and samples usually have to be sent to large metropolitan laboratories. This inefficiency leads to a significant under diagnosis of patients with anemia. For example, approximately 50% of pregnant women and 40% of preschool children are considered to be anemic in developing countries. Assuming that many of these patients may have treatable anemia, significant opportunities exist to improve the health of developing country residents.

Currently, the diagnosis of anemia in developing countries "is made purely on clinical grounds" using symptoms such as fatigue and ice cravings (Bain, 2012). Patients found to be clinically anemic are given iron pills primarily because iron deficiency anemia is so prevalent. Limited resources, such as iron fortified foods, in developing countries causes lower hemoglobin concentrations to be very common in countries such as Palestine compared to the United States. Statistics such as those observed in Figure 1 support the idea of prescribing iron pills on the regular (Yip and Ramakrishnan, 2002). However, the types of treatable anemia that affect developing countries include many other causes than iron deficiency anemia – e.g. Sickle Cell anemia, G6 PH deficiency, Septicemia, B12 deficiency, folate deficiency, and vitamin A deficiency (ADAM Medical Encyclopedia, 2012).

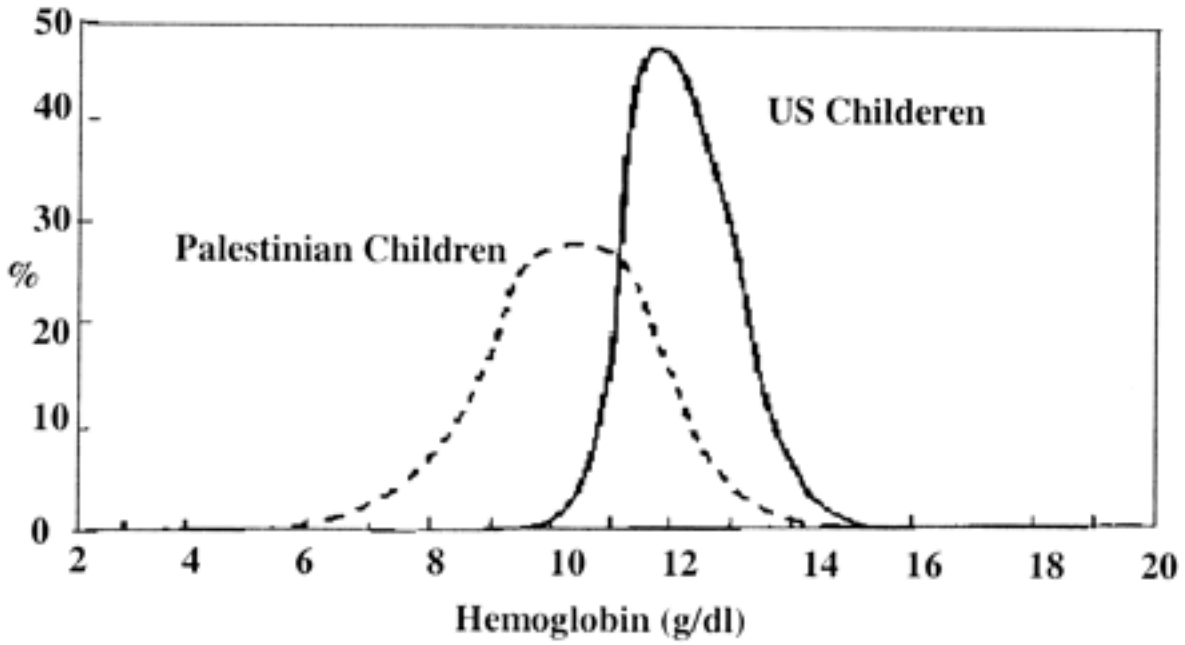


Figure 2. The solid line represents the level of hemoglobin of US children affected with iron deficient anemia. The dotted line represents Palestinian children with iron deficient anemia (Yip and Ramakrishnan, 2002).

Project Motivation

Developing countries often lack resources and manpower to diagnose common medical conditions such as anemia. Many types of anemia are amenable to treatment using inexpensive, readily available medications. Because anemia is one of the most common causes of preventive illnesses worldwide, an accurate, accessible, point of care tool to diagnose anemia could revolutionize clinics in developing countries by allowing health care staff to better serve the population with more accurate diagnosis, specifically anemia. The accuracy of this tool would be dependent on its magnification and resolution capabilities to differentiate the shapes of red blood cells. This device would drastically improve the diagnostic procedures currently being utilized in developing countries. Hematologists studying developing countries have further requested such a device due to the fact only basic hematology test are currently used to diagnose anemia as it is caused by diverse and complexly interrelated causes (Phiri, 2008, p. 2).

Magnification and Resolution

The magnification and resolution of the designed diagnostic device must be adequate to view external cell shape. When cells are typically analyzed, internal characteristics are the primary area of interest. However, the scope of this project requires the number and size of red blood cells, which can be observed with the ability to view only the external shape. To view this external shape, the magnification ratio be equivalent to the length of the original red blood cell over the image of the red blood cell, equaling approximately 400X (Thomas, 2007; Michigan Technological University, 2012).

While magnification enlarges the image, magnification alone cannot create a detailed, clear image. Resolution allows for two closely positions objects to be differentiated. High resolution refers to the smallest distance between two objects that can be distinguished. Because red blood cells are so small, magnification and resolution will play an integral role in the design process. However, the resolution of a microscope is defined by its numerical aperture as proved in the equation R = /(2NA), where R is the resolution and NA is the numerical aperture (Weeks, 2012; Abramowitz & Davidson, 2012). The numerical aperture is a measure of the ability of the microscope to gather light and resolve fine object details at a fixed object distance. The higher the numerical aperture, the better the resolution (Abramowitz & Davidson, 2012).

Project Design Specification (PDS)

Physical Requirements

The primary focus of the designed diagnostic tool is that it must be easy to use to ensure an efficient workflow by users that may have different levels of medical experience. The purpose

of this device is to screen as many people as possible for anemia. Because clinics in developing countries do not have the resources to support advanced medical equipment, the solution itself must be cost effective and it must be compatible with a cost effective, portable imaging device. To ensure portability and prevent damage, this tool must be made cost effectively of durable, lightweight material such as high-density polyethylene, polycarbonate, and aluminum.

The three main specifications that will need to be met in the design of this tool include adequate magnification, adequate resolution, and glass slide ready. Glass slide ready refers to the ability of the device to insert a glass slide and to lock the slide in place for analysis. There should also be the ability for 360-degree movement of the slide to ensure the correct area is analyzed. The magnification and resolution must be comparable to clinical grade analysis. However, the specific characteristic of erythrocytes, neutraphils, and lympocytes are their external shapes which requires magnification of 400X and resolution no greater that 3300 nanometers. The area will then be analyzed by a program called ImageJ.

ImageJ is a public domain, Java-based image-processing program developed at the National Institutes of Health (NIH, 2004). This program will be utilized by the developed application to count erythrocytes and analyze erythrocyte size based on neutrophils and lymphocytes found on the slide. These “landmarks” will also require analysis.

Safety

This diagnostic tool proposed three major safety concerns: misdiagnosis, water exposure, and sterility.

Misdiagnosis may occur if the user fails to to accurately position the slide within the tool. The two types of misplacement are poor positioning of area of interest on the slide or failing to

lock the slide in position. This may result in ImageJ not accurately analyzing the erythrocytes in the area of interest leading to misdiagnosis.

There are two water exposure concerns: to the slide or to the device. If the slide is accidentally exposed water, it can leak into the peripheral blood smear. This may cause lysis to occur destroying the erythrocytes and the slide will not be able to be analyzed. If the tool is exposed to water, the tool may become unusable because the water could potentially seep into the slide. Also, the portable imaging device being utilized will require an electrical source which puts the user in danger of electrical shocks.

Sterility is a major concern for user. Because blood borne illnesses are prevalent in developing countries, it is important that the tool can be easily disassembled for thorough cleaning to protect the user from contracting bacteria/viruses that may be in blood sample.

Also, the procurement of the slides requires that blood be taken from the patient and usual blood borne pathogen precautions need to be followed.

Approach

In the initial stages of this project, the team was presented with a multifaceted problem: creating a portable, cost effective application to diagnose anemia for use in developing countries. This required extensive background research to fully understand the problem before being able to propose a solution. This started with becoming experts on the disorder itself, the causes, the diagnostic process and tools, and the course of treatment. Once this was understood, a more exhaustive search on the diagnostically relevant values, how they were calculated, and the clinical and commercial tools available to gather these indicators and differentiators of anemia. Clinically relevant tools, such as the Coulter counter and CASY, were researched for an understanding of functioning and output values. Commercial devices to accomplish a solitary

diagnostic output were also researched, with an abundance of devices found. The Hemocue could determine hemoglobin concentration using spectrophotometry and a reaction cuvette. The Masimo Pronto-7 was a device that utilized pulse oximetry to determine hemoglobin concentration non-invasively. After researching these devices coupled with the knowledge of the diagnostic process, the necessary values to calculate became clear.

With this understanding, an initial process to solve this complex problem could be presented. This procedure was to measure hemoglobin concentration to determine whether a patient was anemic or not, calculate the MCV to differentiate anemias, investigate morphological abnormalities of erythrocytes, and provide clinical decision support based upon these values. The first step of this process was to attempt to creatively determine a cost effective means of measuring hemoglobin concentration.

This presented a very difficult challenge because almost all devices and associated technologies were cost prohibitive. Further research confirmed that no cost effective options were available to us to diagnose a decreased level of hemoglobin. For several weeks, the reverse engineering of existing technologies done at a far lower cost was explored. Unfortunately, the technology associated with existing devices was determined to be too costly or too sophisticated to create in the time frame of the project. A microfluidic option currently under development by the film DFA (Diagnostics for all), non profit research group in Cambridge, MA was reviewed and the researchers were contacted by the client as to the possibility of collaboration. They are developing a microfluidic filter paper option that can diagnose anemia using qualitative inspection of the paper. After a discussion with Professor John Webster of the UW-Madison Biomedical Engineering Department, this option was discouraged as he believed it was far beyond the scope of the project. After weeks of research and brainstorming, it was concluded

that the measurement of hemoglobin concentration needed to be outsourced to an existing device. This was attempted by seeking a donation from a company that manufactured a handheld pulse oximeter called Masimo.

After that section of the project was concluded, the next challenge was determining how to calculate MCV from a blood sample. Initially, the creation of a 'mini' Coulter Counter of CASY system at a lower cost was explored. As with the first phase of our solution, this was deemed to be too costly, time consuming, and out of the scope of the project. Professor Webster was approached for novel ideas on the calculation of MCV.

He presented the idea of using a Neubauer hemocytometer, and also suggested setting up a meeting with Professor David Beebe to discuss the use of a microfluidic device in the calculation of MCV. He presented two possible ways that would make the utilization of a microfluidic device useful: differences in erythrocyte membrane proteins or size differentiation. Research was again conducted to determine the feasibility of these ideas. No biochemical differences based on MCV was found, and erythrocytes were too small to differentiate by size. This led to the conclusion that magnification of a blood sample was the most cost effective, feasible solution.

Attention was the focused on better understanding the magnification and resolution requirements of the project. The least costly option was derived from a Do It Yourself project found involving retrofitting a common android telephone with a tiny 1 mm microscopic lens with duct tape and a rubber shim. While this option was said to be usable and cost less than $25, it turned out to be unable to allow for adequate magnification and resolution. After attempting to use this with no success, it became clear that an expert on optics was necessary. Professor Kevin Elicieri was contacted and invited to share his expertise on a regular basis. He explained that

micro imaging did not only require the power to magnify, but also the power to resolve at a given magnification. He suggested that we test different quality of microscopic tools to determine the necessary specifications to view erythrocytes and calculate MCV. He also suggested amending the problem statement to reflect what was accomplished during the semester. This change specifically entailed a change in focus from the idea that a device would be created to showing a framework of a solution of a complex problem was attained, with specific work done in the area of optics and imaging to move towards a prototype that represented that solution.

The primary focus of the project became defining the magnification and resolution necessary for a microscopic tool to calculate MCV. In this meeting, the tangible product for this semester was determined to be defining our microscope and using this to calculate MCV for blood samples utilizing a program called ImageJ. Before this was accomplished, Dr. Bain reached out to pathology resident at St. Mary's hospital who has an expertise in hematology. Team members went to the laboratory to review a variety of peripheral smears with the physician. He reviewed how he approached diagnosing anemia based on visual inspection of the slide. This served as the starting point for the development of an algorithm to scan slides and assess RBCs for anemia. This concept was utilized to examine images with Dr. Elicieri. The cartoon in Figure 3 shows what is expected to see in these images under the adequate magnification need to calculate MCV. The next step will be to determine a cost effective alternative to reach this magnification.

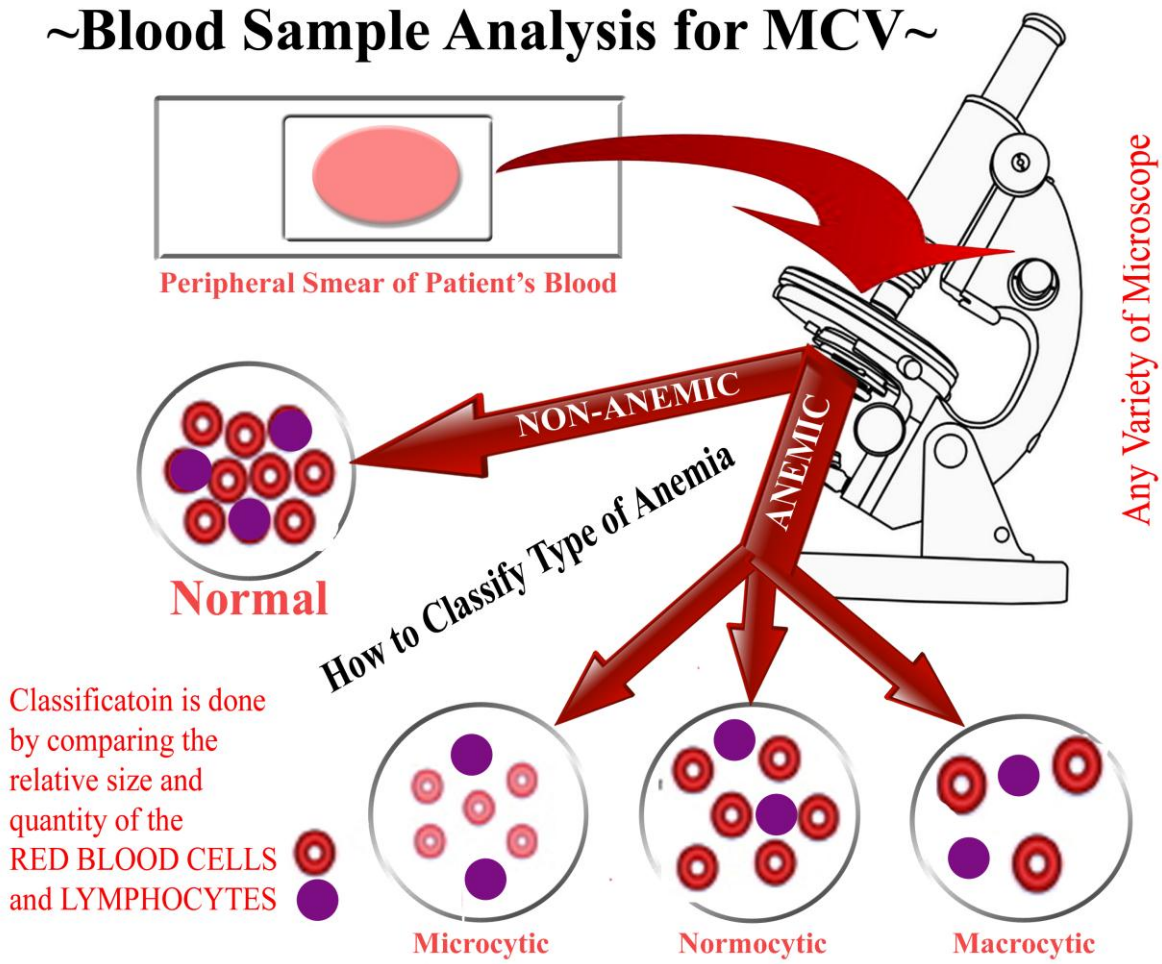


Figure 3. This cartoon provides an example of what should be observed when examining anemic slides.

Data in the form of images at different magnifications as well as the computation of MCV were then conducted to conclude the semester.

Design

The project currently consists of a central computing device with peripheral attachments for data gathering. The attachments include a pulse oximeter-like device capable of measuring blood hemoglobin concentration non-invasively. It will be accomplished using donated device. This will provide the computing device with a hemoglobin concentration input to determine

whether or not the patient is anemic. For the purposes of the project, anemia will be defined as a HBG of less than or equal to 11gms/DL

The next device will be the magnification apparatus. The device will have a lens with 400x magnification and a numerical aperture of .65 that will attach to a camera on the computing device or contain a camera. It will also have an apparatus similar to the one contained in Appendix C. Our images gathered by the magnification apparatus will then be analyzed by the Image J software to determine the MCV. At this point, only a manual viewing of cells is available for determining morphological abnormalities. A decision support tool suggesting interventions for various types of anemia based on hemoglobin concentration, MCV, and cell morphology will be written by the client.

This design required multiple decisions to be made for each step of the proposed design. The first was the determination of a device (shown in Figure 4a). The portable imaging device was chosen because it requires no programming, any source files are readily available, the cost is unknown but appears to be relatively similar to each phone, and received a low score for target audience because it will be a completely new device. The second decision was for the determination of hemoglobin concentration (shown in Figure 4b). The pulse oximeter was the preferred option because it was non-invasive, easy to use, portable and accurate. The third decision was for the cell counting procedure (shown in Figure 4c). ImageJ was the best design because it requires no programming, works quickly and accurately, and is free. The final decision was the data gathering technique (shown in Figure 4d). The magnified blood smear was chosen due to feasibility to manufacture cost, and ease of use with only a slight reduction in accuracy.

Design Matrices

Figure 4. Decision matrices for design consideration (a-d). The parameters for each matrix were weighed in order of importance on a scale of 1-10. Values for each design alternative were assigned for each parameter. The total was determined by finding the sum of the value times the weight of the parameter, and then dividing by the sum of the parameter weights.

(a) Device Consideration					
	Programming Difficulty (10)	Source Files (8)	Cost (5)	Target Audience (5)	**Total**
iPhone	4	3	3	3	3.36
Andriod	8	7	3	4	6.11
Portable Imaging Device	10	8	3	2	6.75

(b) How to Determine Hemoglobin Concentration						
	Safety (8)	Efficacy in diagnosis (10)	Training (7)	Cost (8)	Mobility (7)	**Total**
Hemocue	5	8	3	2	6	4.98
Pulse Oximeter	8	7	6	6	7	6.83
Chemical Indicator	?	?	?	?	?	?

(c) Cell Counting Decision Matrix					
	Feasibility (8)	Accuracy (7)	Cost(10)	Time Cost (6)	**Total**
Manual Count	8	4	10	1	6.39
Design Program Algorithm	3	5	10	4	5.90
ImageJ	8	6	10	6	7.81
Coulter counter	2	7	1	5	3.39

(d) Method to gather necessary data to calculate MCV					
	Feasibility (9)	Accuracy (8)	Cost (8)	Ease of Use (10)	**Total**

Coulter counter	3	8	2	5	4.49
Magnified Peripheral Smear	8	5	7	9	7.37
Microfluidics	0	0	6	3	2.23
Magnified Blood Sample in Hemocytometer	6	4	6	8	5.94

Testing

The main goal of testing for the semester was to determine the most cost effective microscope that could give the magnification and resolution needed for ImageJ to accurately count cells in the determination of an MCV value. An Olympus Bx41 microscope, Proscope, student microscope, and a 2.5 mm diameter Edmund Optics ball lens mounted on the camera of an android citrus cell phone were tested. The Olympus microscope, due its cost of around 30,000 dollars, is not feasible for use in our project, but can be used as a benchmark for the other microscopes.

First, each microscope was used to take a picture of a copper SPI supergrid. This grid has bars of width 20μm and hole size of width 105μm. These pictures displayed a qualitative comparison of resolution between the different microscopes. Different microscopes would resolve the grid lines with varying capability. While one microscope might show a solid chip or very blurry lines, another could resolve the grid to very distinct clear lines. Because the width of the grid lines are relatively similar to the diameter of a erythrocyte(6-8μm), this provides us with a good qualitative comparison of each microscopes ability to resolve images of the size needed (Persons, 1929).

After acquiring the information from the grids, the microscopes were then used to take pictures of blood smears. The ability of ImageJ to analyze the picture obtained from each

microscope was tested. ImageJ was used to count the number of erythrocytes. Using this data and the area captured in the image, an MCV value can be determined. If the value given by this calculation is more than 2g/dL different than the value given from the coulter counter, the resolution given by this microscope is not suitable for our device.

For ImageJ to count cells in an image, the 8-bit image must first be converted to black and white and thresholded. Thresholding the image turns objects above a specified light intensity to black, and below to white. This is shown in Figure 5. This helps ImageJ distinguish between objects and their background. An established scale is used to measured neutrophils that are approximately 13 micrometers in diameter. Finally, a range of areas to be counted as cells was established to eliminate specks in the picture and other non-cell interference.

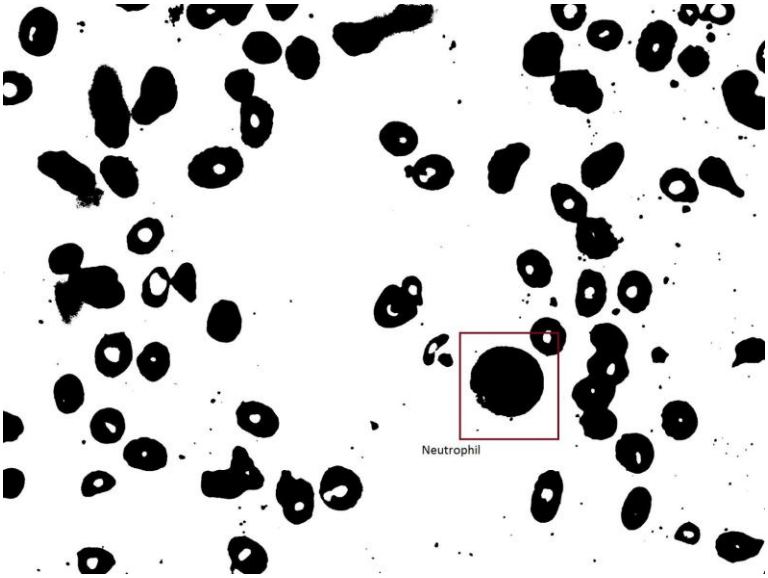


Figure 5. Threshold Image

Once a suitable microscope has been determined and a device has been fabricated large scale testing can begin. Our device will be field tested using actual peripheral smears from de-identified patients compared with the gold standard Coulter Counter results. If the diagnosis given by our device agrees with that of the coulter counter 95% of the time, our device can be considered adequate for the point of care diagnosis of anemia in developing countries.

Results

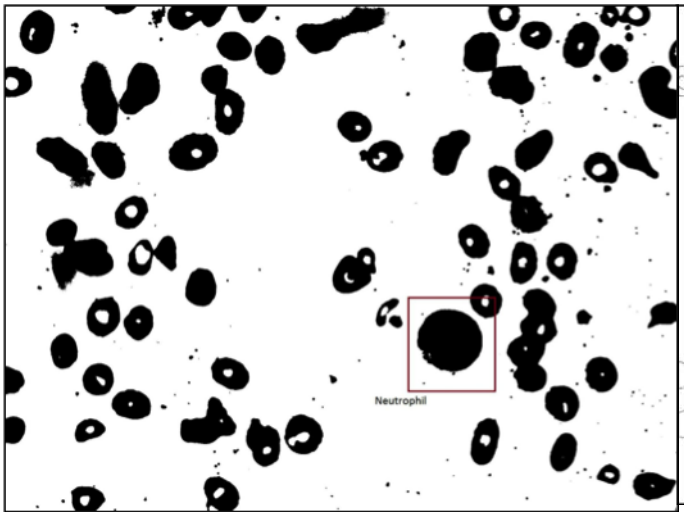


Figure 6. Threshold Image

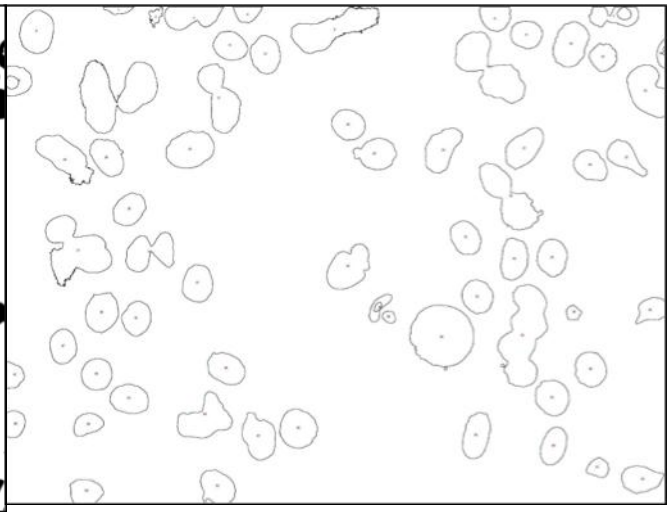
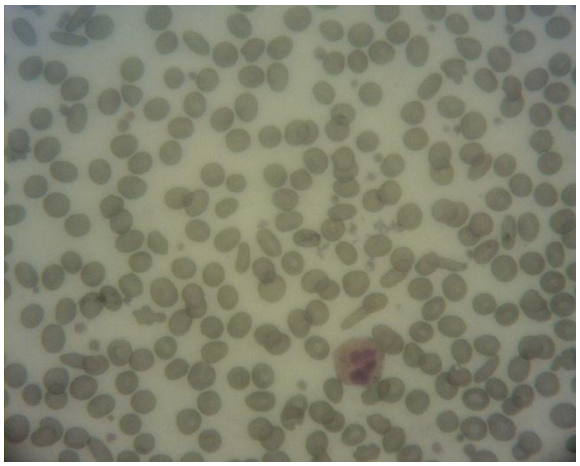


Figure 7. Cells that were counted

ImageJ count**:
64 cells

Area of Interest:
14867.040micrometer^2 area

Area of Cells**:
Mean 44.025
SD 31.170
Min 5.405
Max 148.492
**All measurements are rough estimates based on the scaled determined by the diameter of neutrophils.



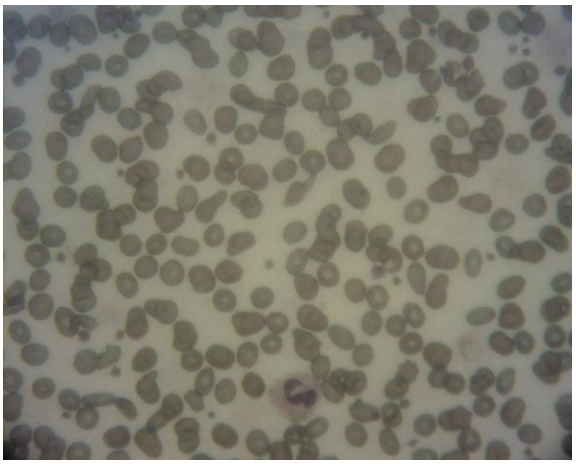


Figure 8. Both images are of the same anemic blood sample at 400X before ImageJ analysis.

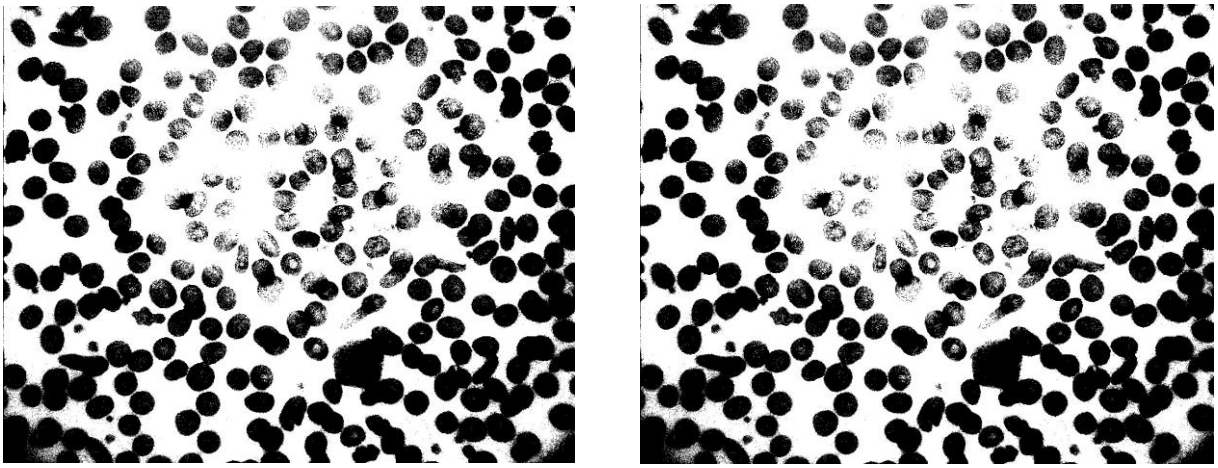


Figure 9. Both images are of the same anemic blood sample at 400X. However, the densities are drastically different.

ImageJ count: 117 cells	ImageJ count: 178 cells
Area of Interest: 24035.671 micrometer^2 area .005 cells/micrometer^2	Area of Interest: 16441.958 micrometer^2 area .011 cells/micrometer^2

Different images of same slide result in large differences in cell density.

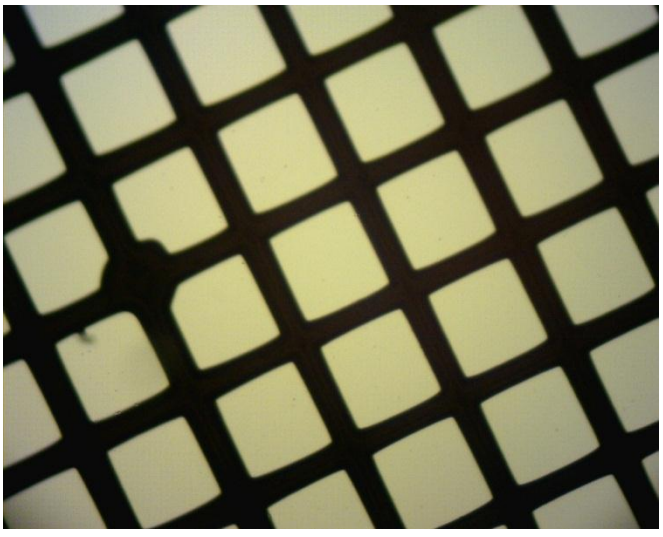


Figure 10. Copper SPI super mesh grid at 100x magnification.

The results show that while the images taken with the Olympus microscope and student microscope looks similar, when thresholded, the cells in the Olympus taken picture are more distinct and there is less "fuzz" in the picture. This is most likely do to the condenser in the Olympus Bx41 microscope that focuses light, eliminating stray light that shows up in the threshold image.

It was also revealed that pictures taken of a different section on the same peripheral smear produced a cell density vastly different. This demonstrated that a peripheral smear would not be adequate for our analysis.

Discussion

A major source of error in our image analysis include neutrophils and lymphocytes being recognized as erythrocytes instead of relative counting landmarks leading to a higher cell count than observed. Also, the inability of ImageJ to discern between overlapping cells may cause the cell count to be lower than observed. This could be mitigated through careful consideration of appropriate sample areas and through the use of the watershed command provided by ImageJ. This feature splits cells where overlap is projected. However, due to the hollow shape of the red blood cells, the watershed feature appears to split the cells significantly more than necessary.

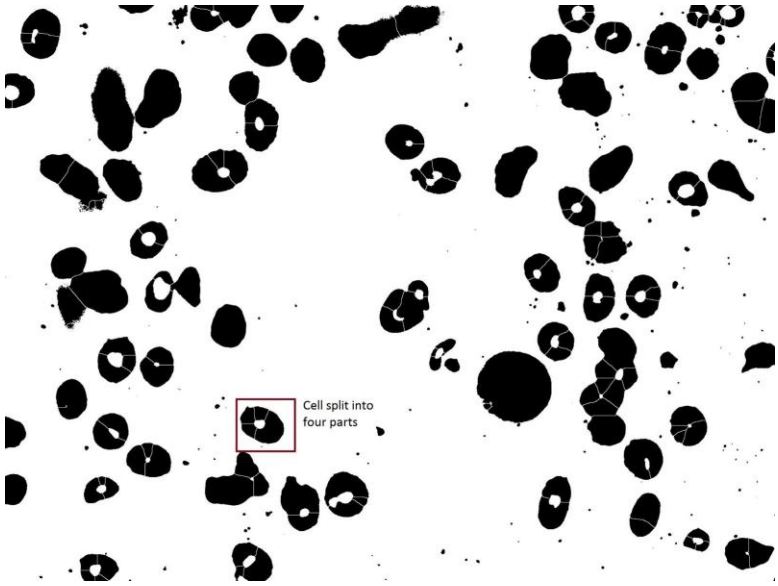


Figure 11.Watershed image

We unfortunately cannot get an accurate estimate for the MCV based on an image of a peripheral smear. Due to the uneven distribution of cells along the slide and the inconsistency in preparing a peripheral smear, the cell density within the image is not an accurate representation of that within the human body. However, images of peripheral smears allow one to identify the magnification and resolution needed to count cells. A hemocytometer would dilute the blood by a known factor but keep the blood at an even distribution representative of what you would find in the body. This would allow for the calculation of an accurate MCV.

Conclusion

The initial problem statement called for the creation of a cost efficient portable smartphone application capable of determining hemoglobin concentration, calculate MCV, and determine erythrocyte abnormalities to diagnose and offer treatment suggestions for anemia. A fully functional, innovative prototype was the expected outcome of the semester. After weeks of research, meetings with Professors and field experts, it became clear that the scope of our project was far too broad to complete in a semester. The result of this was narrowing the focus after

deciding to outsource the determination of hemoglobin concentration due to our inability to create a breakthrough technology.

In the early stages of the semester, focus was placed on finding a creative way to measure hemoglobin concentration. After extensive market research, multiple methods were identified. These were highly sophisticated devices that were cost prohibitive for our project. For the sake of progress, the best choice was deemed to be outsourcing the first step of our initial problem statement.

The next step was then to develop a tool and method for measuring MCV. Due to correlation with the investigation of cell abnormalities, a magnifying device capable of measuring MCV was determined. At this stage in the semester and after meeting Professor Elicieri, the scope was determined to be too broad to produce any tangible results. We were guided by Professor Elicieri into amending the problem statement to focus on finding the necessary magnification and resolution to view cells, and begin the calculation of MCV using our most cost effective microscopy device capable of reproducible results.

MCV will be determined with a hemocytometer because it is the most efficient way to obtain an erythrocyte count. After diluted blood has entered a hymocytometer, it will settle over the lined grid. These grid lines represent known distances, and coupling this with a known height, volume of the sample can be calculated. The designed diagnostic tool will be used to enlarge this area. ImageJ, via the portable image device, can then count the number of cells per unit volume leading o the magnified image, which can be used to calculate MCV.

After initial testing of magnification devices, it appears a better apparatus with the determined lens fitted to a camera, a hemocytometer for the blood sample, and further work with ImageJ will be necessary to create a working prototype. Future mircroscopy testing will allow

prototyping to begin.

Future Work

The initial problem statement had phases that were not addressed in this project due to time and feasibility concerns. More importantly, the magnification and resolution characteristics of the device need to first be determined before a algorithm was produced as these characters would directly affect the input values.

To continue this project, the accomplishments made this semester will be built upon with the following phases:

Phase 1. To design a smartphone application interface and magnification hardware that can detect anemia based on two inputs - the hemoglobin concentration obtained from a pulse oximeter and the MCV derived by ImageJ from a hemocytometer image.

Phase 2. To classify and differentiate the types of anemia based on cell morphology or shape observed in the ImageJ images by comparing the images to an archived library of images of erythrocytes with potentially similar abnormalities.

Phase 3. To develop a customized treatment plan for specific anemias that could assist health care employees at the point of care.

While the scope of this project focuses on anemia, the future of this project could lead to the development of a tool that could be used to diagnose other common conditions. A tool utilizing similar technologies and processes to those created in this project, would be a step in further eliminating non-anemia related preventable deaths in developing countries such as malaria and skin cancer. While both of these conditions are more difficult to treat than anemia, both are easier to treat if they are diagnosed early. This creates a need for a low cost, point of care diagnostic tool.

Going further, an application that would not only diagnose but treat preventable death diseases would create a stronger need for such a device as one does not currently exist. This impedes the treatment process in developing countries causes most of the population to never receive proper treatment. Such a powerful tool, combined with low cost and high efficiency would without question lead to a dramatic decrease in preventable premature mortality worldwide.

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Appendix

Appendix A - Product Design Specification (11/24/12)
Appendix B – Product Design Specification (12/12/12)
Appendix C - Device Sketches