SmartScope: Using Mobile Microscopy to Characterize Anemia
Disha Kumar, Sunny Kim, Kyle Bailey, Marco Tocchetto
ELEC 419, Innovation Lab for Mobile Health

Anemia is a Widespread Condition
According to the World Health Organization, anemia affects about 2 billion people worldwide including over 50% of pregnant women.¹ Most of these affected persons reside in developing countries.¹ Anemia is a condition characterized by low normal red blood cell (RBC) count or low hemoglobin count. Hemoglobin is a protein inside RBCs that carries oxygen in the blood to tissues. Oxygen is essential for the production of energy among other vital cell functions. Without treatment, an anemic condition can cause fatigue, shortness of breath, and rapid heartbeat. There are several types of anemia, such as iron deficiency, pernicious, aplastic, sickle cell, and hemolytic anemia. The most common types result from nutritional deficiencies such as iron, folic acid, vitamin C, and vitamin B₁₂ deficiencies. Cost presents the most significant barrier to diagnosing and correctly treating anemia in low-resource settings. These costs include fixed costs of diagnostic tools and operational costs of requiring trained healthcare experts, network connection in some instances, and time.

Current Methods of Anemia Diagnosis
The standard definition of an anemic condition is a hemoglobin count of less than 13 mg/dL for males and less than 11 mg/dL for females.² Anemia is currently diagnosed in hospitals through a Complete Blood Count (CBC). Automated machines use the Coulter principle to measure various blood cell characteristics. However these machines cost upwards of $10,000 USD and are not feasible for low-resource health care settings. Standard methods to diagnose anemia in developing regions include the HemoCue Analyzer, the World Health Organization (WHO) color scale, and the hematocrit test. However, these methods only measure hemoglobin concentration and do not diagnose the type of anemia. See Appendix for potential competitors to SmartScope.

The Blood Smear: A Traditional Method of Anemia Diagnosis
The blood smear is the traditional method of diagnosing anemia, among other blood diseases. A blood smear is inexpensive and consists of a drop of blood on a glass slide covered by a coverslip. A trained person can observe the smear under a microscope and diagnose the severity and type of anemia. Important indicators for anemia include a below average number of RBCs or abnormal RBCs. Blood smears remain a valuable diagnostic tool especially in low-resource settings. Additionally, blood smears are used to validate a CBC or to more accurately differentiate a diagnosis of anemia even in U.S. health care settings.⁴ Despite their usefulness, blood smears are labor- and time- intensive. We seek to develop a device that can be easily and rapidly used to diagnose anemia type by healthcare workers in low-resource settings. See Figure 1 for a normal blood smear image.

SmartScope: Diagnosing Anemia using Blood Smears
Our device will consist of a microscope attachment to a smartphone camera that uses simple microlens technology to magnify an image -- in our case, a blood smear. An app on the smartphone will then process the image to determine presence of abnormal cells, diagnose the type of anemia, and send the image to a hematologist for confirmation. The image processing will occur on the phone itself, thus eliminating the need for a network connection. Abnormal morphology and color distribution cell characteristics will be used to identify the type of anemia. For the prototype model, we have focused on diagnosing iron-deficiency and sickle
anemia. Iron deficiency anemia can be identified by the presence of RBCs that are microcytic, or small (<7μm diameter), and hypochromic, or with a large central white pallor. The presence of 20-35% hypochromic cells is a diagnostic feature of iron-deficiency anemia. See Figure 2 for an iron-deficiency anemia blood smear image. Sickle cell anemia is diagnosed by the presence of at least 25% sickle red blood cells, or elongated cells that are tapered at opposite ends. See Figure 3 for a sickle cell anemia blood smear image. Thus this device will establish a valuable proof-of-concept to automatically diagnose types of anemia using a smartphone device.

**Device Prototype**

**Hardware: Imaging Cells**

The hardware design is based on Single Lens Off-Chip Cellphone Microscopy by the MIT Media Lab. The mobile microscope attachment will consist of a microlens held in place from the image at a distance equal to its focal length. This setup will magnify the image based on the ratio of the camera focal length to the objective lens focal length $M = \frac{f_{\text{camera}}}{f_{\text{lens}}} = 3.97x$. The lens we used has a 1mm diameter, 1mm effective focal length, and 0.57mm back focal length (Figure 1). We calculated the lens to object distance to be 0.311mm based on the equation $x = BFL - n*d$ where BFL is the back focal length, n is the refractive index of the coverslip (1.53) and d is the thickness of the coverslip (0.17mm). Using these calculations, there would be 17 pixels per cell (6μm diameter) on the Android camera – adequate for image analysis. The microlens will be held in a 3D-printed base attached to the smartphone. The lens will be positioned at the exact fixed distance from the blood smear slide (0.311mm). The base also holds the blood smear slide and an LED light for backlight illumination of the slide. See Figure 4 for an image of the base that holds the microlens and Figure 5 for an image of the device taking a photo of a blood smear slide.

**Software: Using an Android Application to Diagnose Iron-Deficiency and Sickle Cell Anemia**

Using the hardware as specified above, the user will obtain blood smear images. This can be done through the app, or the app can retrieve images saved onto the phone. The user will select an algorithm to test for a type of anemia, select the image to be analyzed, and the app will process the image to output if the type of anemia is present. Furthermore, the app will provide functionality to share these images with a hematologist for further diagnosis. The Hough transform is used to automate normal RBC counting, taking advantage of normal RBCs being roughly circular in shape. The counting process includes conversion of the image to greyscale, smoothing of the image with Gaussian blur, and then employing the Hough transform algorithm to count cells. To identify iron-deficiency anemia, K-means clustering algorithm is used to differentiate morphology differences between hypochromic and normal RBCs. We classified iron-deficiency anemia as a blood smear having at least 30% of hypochromic cells. To identify sickle cells, we used an algorithm that extracts the Histogram of Oriented Gradients (HOG) descriptors and trains a Support Vector Machine (SVM) to recognize sickle cells from normal cells. The algorithm can identify sickle cells within a blood smear of normal and sickle RBCs. See figure 6 for sickle cell anemia image processing.

**Software: Reliability of Image Analysis**

The software is able to process blood smear images with accuracy and precision. We first tested the accuracy and precision of the software in processing images taken by a gold-standard laboratory microscope. The software was able to count cells in a normal blood smear with at least 95% accuracy 60% of the time (n=5) in comparison to hand counts. Second, we tested the software on counting hypochromic cells of iron-deficiency anemia. In a sample of 9 normal and 7 iron-deficiency anemia blood smear images, the algorithm was 94% accurate (n=16) in identifying iron-deficiency anemia blood smears. The software was also able to identify
sickle cells; however, further software development with more data is necessary to accurately and consistently diagnose sickle cell anemia from normal blood smears. Third, we first tested the accuracy of counting cells in the image taken by the microlens setup (Figure 7). The image is of low quality compared to a blood smear image taken by laboratory microscope. However, the algorithm counted cells with an 87% accuracy (n=1) compared to a hand count of the same image. Finally, to identify the minimum magnification of the blood smear necessary to count cells, we tested accuracy at different magnifications. The software can count normal RBCs with 97% accuracy at 400x, 88% accuracy at 100x, and 84% at 50x magnification. See Figure 8 for a screenshot of the app analyzing a normal blood smear for iron-deficiency anemia.

**Future Work**

Our current device represents a valuable prototype that can be used to diagnose iron-deficiency and sickle cell anemia in low-resource settings. In three months we have identified a healthcare issue and designed and developed a prototype device to facilitate diagnosis and thus treatment and better health. Our device could be further developed to diagnose other types of anemias, such as pernicious anemia and thalassemia. This is dependent on identifying cell size among other diagnostic features. Our current device is not specific for the hardware attachment, and thus cannot differentiate cell sizes of micrometer differences.

**Device Limitations**

The blood smear must be prepared by the healthcare professional. A capillary tube can be used to non-invasively obtain a drop of blood from the patient. Fixing and staining the blood smears will allow for better cell visualization. The blood smear should be immersed first in methanol and then either a Giemsa staining solution or Wright’s staining solution. These solvents are typically under $100 per jar and are commonly used in clinic laboratories. We do not foresee blood smear preparation as being a significant limitation as blood smears are already commonly prepared in clinical and the supplies are low-cost and widely available. WHO biosafety guidelines should be followed to properly dispose of blood smears.

**Device Value**

Anemia is a global health concern that negatively affects the health of those in developing regions. Current diagnostic methods are time-consuming or costly and many only determine hemoglobin concentration. Thus these methods only diagnose the presence of an anemic condition and not the type of anemia, yielding little insight for treatment. SmartScope is able to diagnose the type of anemia (currently focused on iron-deficiency and sickle cell anemia) without a network connection. Thus SmartScope has much potential in low-resource settings and could achieve several UN Millennium Development Goals by reducing the burden of anemia.

![Figure 1] Normal blood smear

![Figure 2] Iron-deficiency anemia. Red blood cells in iron-deficiency anemia are hypochromic, or characterized by a large

![Figure 3] Sickle cell anemia
Appendix

Competition

Given the reach and convenience of mobile phones, they are ideal platforms upon which to build diagnostic tools. Several simple devices have been created to determine hemoglobin concentration such as TouchHb\textsuperscript{6} and Hemoglobe.\textsuperscript{7} As noted, these devices do not differentiate the type of anemia – a critical factor in determining treatment. However, they could be used in conjunction with SmartScope to further validate the presence of an anemic condition. No U.S. patents currently exist to specifically diagnose iron-deficiency or sickle cell anemia in blood smears using an Android platform. However, patent US2011/052038 has developed a method for distinguishing iron-deficiency anemia based on automated readings of hematology analyzers.\textsuperscript{8}

\textit{The Ozcan Lab}\textsuperscript{9,10}

The Ozcan Lab at the University of California Los Angeles has developed a holographic lensfree on-chip microscope termed LUCAS that can be attached to a mobile phone camera. In their device a simple light
emitting diode (LED) is used to create a hologram of cells in a blood sample. The hologram is then digitally reconstructed at a central computer to form the microscope image. Several costly staining solutions are used to identify different cell types.

The Fletcher Lab

Also notable is the Fletcher Lab at the University of California Berkeley. They have developed a brightfield microscope attachment (CellScope) to a mobile phone that can image sickle RBCs. The device employs a microscope eyepiece and objective attached to a mobile phone camera. However, image processing was accomplished using a central computer.

The Prakash Lab

Another innovative mobile microscopy device is Foldscope from the Prakash Lab at Stanford University. Foldscope is based on paper that uses origami to fold into a low-cost, portable microscope. The Prakash Lab has developed brightfield, darkfield, and fluorescence origami microscopes. Foldscope contains microlenses, an LED with diffuser or condenser lenses, and other components embedded into the paper that enable it to take quality microscopic images. The goal of FoldScope is to provide microscopes for science education and fieldwork. However, it is not attached to a mobile phone camera and does not suit rapid diagnostic purposes.

Barriers to Implementation in Low-Resource Settings

These systems are innovative and adaptable to several diagnostic purposes. However, they are not specific to anemia and pose two critical problems in low-resource settings to diagnosing anemia: cost and network connectivity. Low-resource healthcare settings cater to large numbers of patients and are poorly staffed. Thus expensive stains may not be attainable. The settings furthermore have inadequate network connectivity. Images taken by these devices need to be sent from a mobile phone to a central computer, processed, and then the diagnostic report sent back to the mobile phone. Additionally, this extra time spent in processing images presents a key barrier against the usability of these devices.

References