Blood Count on a Smartphone Microscope: Challenges

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ABSTRACT

Low-cost lenses with magnifications of 150-200x are being sold in the market today as accessories for mobile smartphones. Attaching these lenses to a smartphone camera creates low-cost, ultra-portable digital microscopes, with a potential for significant impact on applications in a variety of fields such as healthcare, agriculture, education etc.

In this paper, we consider a low-cost do-it-yourself Complete Blood Count (CBC) application using a smartphone microscope. We discuss several unique challenges that come up in implementing this application that include preparing the blood sample, correcting the small field of view and blur of the lens, and automating the cell counting procedure. We present our approach to overcome these challenges and report early promising results on counting red blood cells.

CCS Concepts

•**Applied computing** → **Health informatics;**

1. INTRODUCTION

The number of smartphones worldwide is approaching 2 billion today [6]. The mobile accessory market, riding on the coattails of the growth in smartphones, is expected to grow to $100 billion by 2020 [5]. One such accessory, the smartphone camera microscope lens, has made tremendous progress recently in both cost and functionality. For example, in the commerical market today, one can obtain smartphone lens attachments with 150-200X magnification for $15-$30 [4, 11]. Further, the Foldscope project promises a lens with up to 2000X magnification for less than a dollar [2].

These advances in smartphone microscopy can open up a variety of applications with significant impact in healthcare, agriculture, education, etc. In this paper, we focus on challenges in implementing one such application, namely, blood cell counting using a smartphone microscope.

Human blood is composed of blood cells or corpuscles suspended in blood plasma. Plasma constitutes about 55% of the volume and is mostly (up to 95% by volume) water. There are three kinds of blood cells: (a) red blood cells (RBCs) or erythrocytes, (b) white blood cells (WBCs) or leukolytes, (c) platelets or thrombocytes.

Complete blood count (CBC) test is one of the most widely ordered laboratory tests. A complete blood count (CBC) test report helps confirm the diagnoses for several medical conditions, for example, anemia (low RBC count), leukemia (low RBC count), dehydration (high RBC count), renal cell carcinoma (high RBC count), bone marrow failure (low WBC count), lupus (low WBC count), stress (high WBC count), infection (high WBC count), and dengue (low platelet count).

As we discuss in Section 2, CBC can be performed either by manual count using a microscope or through an automated flow cytometry machine. However, the manual count is tedious and can be error-prone. Thus, most diagnostic labs today use the automated approach which increases cost and also requires a visit to the diagnostic lab. In this paper, we investigate the feasibility of a do-it-yourself blood test that automates the manual count approach using a smartphone microscope. This allows the CBC test to be done anywhere and at low cost, thereby addressing the needs of users in both developed and developing nations.

While smartphone lens attachments are inexpensive and provide good magnification, a key drawback of these lenses is their small field-of-view. For example, the practical field-of-view of one of the lenses [4] is only an area of about 0.2 mm$\times$ 0.2 mm while manual blood cell counting protocol requires observation of an area of few square mm – an area that is up to two orders of magnitude larger. This introduces a number of challenges in blood sample imaging and the vision algorithms used in their processing. We describe these and other challenges that arise in handling blood in Section 4.

We discuss our approach for addressing these challenges and our preliminary results in computing red blood cell count in Sections 5 and 6. As part of future work, we plan to enhance our processing to support other blood cell types as well as scale our evaluation. If successful, we believe that a low-cost do-it-yourself CBC test using a smartphone microscope can complement conventional lab blood testing in situations where convenience or cost considerations prevent/delay users from visiting a conventional lab.

2. BACKGROUND

In this section, we describe the essential parameters measured by a CBC test and the laboratory procedure for it.

The complete blood count (CBC) test procedure starts with a phlebotomist drawing approximately 0.5 ml of the

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patient's blood by venipuncture into a test tube containing an anticoagulant such as EDTA or sodium citrate. The cell counting can be either manual or automated. The important parameters measured in a CBC test and their normal ranges are as follows [22].

1. Red blood cell (RBC) count: 4.7 to 6.1 million cells per microliter[1] in males and 4.2 to 5.4 million cells per microliter in females,
2. White blood cell (WBC) count: 4,500 to 10,000 cells per microliter,
3. Platelet (PLT) count: 150,000 to 450,000 cells per microliter,
4. Hematocrit (HCT) or the percentage volume of RBCs in the blood: 40.7 to 50.3 % in males and 36.1 to 44.3 % in females,
5. Hemoglobin (Hgb): 13.8 to 17.2 grams per deciliter in males and 12.1 to 15.1 grams per deciliter in females.

A typical CBC test report may also include additional information such as the following.

1. WBC differential count or the separate counts of different types of WBCs – neutrophils, band neutrophils, lymphocytes, monocytes, eosinophils, and basophils,
2. Mean corpuscular volume (MCV) or the average volume of a red blood cell,
3. Mean corpuscular hemoglobin (MCH) or the average mass of Hemoglobin per red blood cell.

For manual count, the blood sample is diluted (1:100 for RBC count, 1:20 for WBC count), stained, and loaded into a hemocytometer or a counting chamber using a micropipette. Figure 1 shows a Neubauer hemocytometer [7], which is a reusable, glass counting chamber of fixed depth (0.01 mm) with a laser-etched 3 mm $\times$ 3 mm grid. The middle 1 mm $\times$ 1 mm square is divided into a 5×5 grid, which has a further sub-division into a 4×4 grid. Thus, the smallest square in the middle has dimensions 0.05 mm $\times$ 0.05 mm, and at 0.1 mm depth, it corresponds to 0.25 nanoliter (or equivalently, 0.00025 microliter) volume.

Figure 1 shows the regions used for RBC and WBC counts marked by R and W, respectively. To get an idea of how tedious a manual count is, the normal range for RBC count at 1:100 dilution corresponds to an average count of 10-15 cells per each of the smallest 0.05 mm $\times$ 0.05 mm squares, and one needs to count this over $5 \times (4 \times 4) = 80$ such squares. For statistical and laboratory studies on the error of counting with a hemocytometer, see [20, 13].

Some automatic cell counters based on image cytometry mimic the manual count procedure described above, and oftentimes provide image processing tools on a digital microscope to assist lab technicians doing the manual count. However, majority of the automatic cell counters are based on flow cytometry instead. The blood sample is passed through a microfluidic channel and a fast but indirect count is obtained by laser-based or impedance-based counters such as Coulter counters. Flow cytometry is faster but cannot provide additional details about the cells that image cytometry can. Recent research on imaging flow cytometry tries to combine the best of both approaches [12, 14]. All of these automated hematology analyzers have a steep price of more than $1000, are far from portable, and have their coefficient of variation of about 3-5% [24].

[1]One microliter is equal to one cubic millimeter volume.

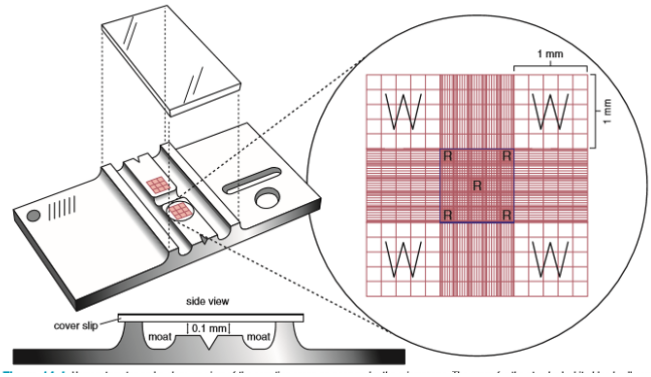

Figure 1: Neubauer glass hemocytometer

3. RELATED WORK

There are three important directions related to our work (a) mobile microscopy for digital pathology, (b) the resolution vs. field of view tradeoff, and (c) the approaches to build low-cost portable microscopes, in general.

Mobile microscopy can provide low-cost, portable digital pathology solutions to improve healthcare accessibility in underserved and low-resource areas. Breslauer et al. [15] use a mobile phone-mounted light microscope and demonstrate its potential for clinical use by imaging P. falciparum-infected and sickle red blood cells in brightfield and M. tuberculosis-infected sputum samples in fluorescence with LED excitation.

Switz et al. [21] present a simple and low-cost mobile microscope by adding a reversed mobile phone camera lens to a mobile phone camera, which enables high quality imaging over a large field of view, and demonstrate its use in imaging red and white blood cells in blood smears and soil-transmitted helminth eggs in stool samples.

Skandarajah et al. [19] show that quantitative microscopy with micron-scale spatial resolution can be carried out with multiple phones and that image linearity, distortion, and color can be corrected as needed. They observe that phones with greater than 5 MP are capable of nearly diffraction-limited resolution over a broad magnification range. They find that automatic focus, exposure, and color gain standard on mobile phones can degrade image resolution and reduce accuracy of color capture if uncorrected, and devise procedures to circumvent them.

The tradeoff between resolution and field-of-view is inherent to most optical systems but it becomes even more critical in mobile phone microscopy for digital pathology applications. It is important to mention the following recent works in this regard, even though their focus is not specifically mobile microscopy or digital pathology.

Fourier Ptychographic Microscopy (FPM) introduced by Zheng et al. [25] is a method that takes a number of low-resolution images under variable-angle, coded illumination using an LED array, and then iteratively stitches them in the Fourier space to produce a wide-field, high-resolution complex sample image. Adopting a wavefront correction, it can also correct for aberrations and digitally extend a microscope's depth of focus beyond the physical limitations of its optics. Horstmeyer et al. [18] discuss applications of FPM to digital pathology.

Another important direction related to our work is ap-

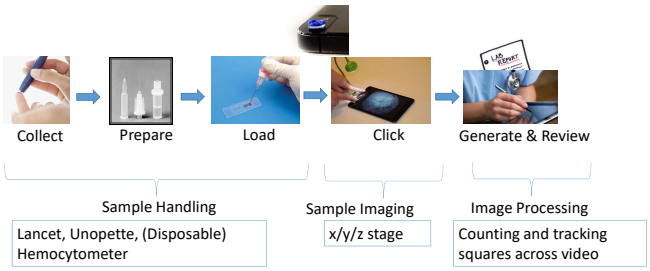

Figure 2: Do-it-yourself Blood Count Pipeline

proaches to built low-cost portable microscopes in science and education using simple lenses. Foldscope by Cybulski et al. [16] is a simple, origami-based, portable, ultra-low-cost microscope with a spherical ball lens that is easy to manufacture at large scale. The diagnostic variants of Foldscopes are more suited for manual detection of micro-organism.

It is important to note that unlike the blood cell count, a smartphone application HemaApp by Wang et al. [23] uses chromatic analysis to measure hemoglobin level with a precision of 76.5% and compares favorably with an FDA-approved noninvasive hemoglobin measurement device.

Zhu et al. [17] have looked at imaging cytometry attachments for RBC and WBC counts on a smartphone but require many processing steps with large-sized attachments (different ones for RBC, WBC etc.) meant for a lab technician. To our knowledge, ours is the first attempt at a low-cost do-it-yourself smartphone-based blood counts using video scans and minimal processing steps.

4. CHALLENGES

A low cost do-it-yourself blood count pipeline is shown in Figure 2. As shown in the figure, the challenges in analysing blood can be divided up into three parts: (a) Sample handling, (b) Sample imaging and (c) Image processing. We now discuss each of these below.

4.1 Sample Handling

The challenges here pertain to dealing with blood, i.e., how to collect and prepare the blood sample so that analysis can be performed using a smartphone.

Fortunately, these are well-known issues that have already been addressed by the market today. A lancet (cost $0.06 [3]) can be used to prick the finger and extract a small amount of blood. The blood is then loaded into a disposable device, called a unopette. This device is simply a calibrated capillary tube with a reservoir pre-filled with the right type and amount of dilution, lysing and staining fluids. Unopettes are available for WBC [10], RBC [9] and platelets [8] for about $1.5 each. Finally, the diluted blood can be placed onto a disposable hemoctyometer, which has 2 counting chambers and costs less than $3 each [1] (a reusable hemocytometer costs about $35). Thus, the materials required for a CBC test is under $10, and the preparation for loading the processed blood sample onto a hemocytometer is straightforward.

4.2 Sample Imaging

Small field of view. The challenge in imaging the blood sample in the hemocytometer arises primarily due to the small field-of-view (FOV) of the smartphone microscope. The inexpensive microscope lens attachment [4] has a significantly smaller FOV than a conventional microscrope. Furthermore, as shown in Figure 3, there is severe distortion around the edges of the image from the lens. Thus, the practical FOV is only the central 4×4 grid in Figure 3 of size 0.2 mm $\times$ 0.2 mm, which is at least an order of magnitude smaller area than a conventional microscope.

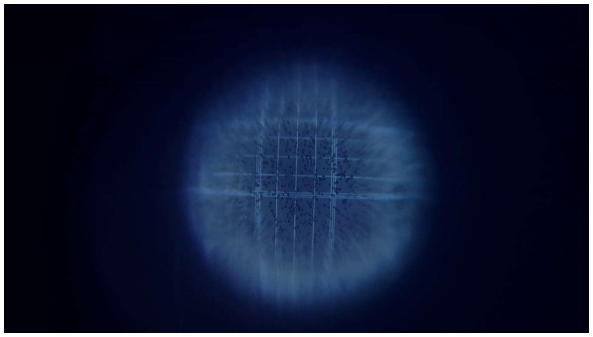

Figure 3: Lens Edge Distortion

Multiple photos vs. video. A red blood cell count for the hemocytometer (Section 2) requires counting cells from an area of 1 mm $\times$ 1 mm or 25 frames (since the practical FOV is only 0.2 mm $\times$ 0.2 mm), whereas a white blood cell count requires counting cells from 4 such 1 mm $\times$ 1 mm squares or 100 frames in total. If we require the user to manually center and focus on each of the 100 such squares and capture a photograph, it would be very tedious. A more practical alternative is for the user to simply perform a (guided) video scan of the region of interest and capture it in a single video.

XYZ stage accessory. The key challenge then is how we can help the user to scan the smartphone over the entire 3 mm $\times$ 3 mm region of interest in the hemocytometer. This requires movement of the hemocytometer in small steps of 0.1 mm in the X and Y directions while capturing the video and a one-time adjustment in the Z-direction so that the hemocytometer image is in focus. Besides, a manual XYZ stage is an essential part of every microscope. The adjustment in XY direction is required to scan the slide whereas the adjustment in Z direction is required to put the region of interest in focus. Replicating this in a cost-effective manner using origami was the key part of the Foldscope design too [16]. However, the diagnostic variants of Foldscopes are more suited for identification of micro-organisms and a manually adjusted XYZ stage based origami does not provide a smooth enough translation for a video scan. To address this challenge, in Section 5, we describe a 3D-printed XYZ stage that we developed as an accessory to the smartphone. This accessory allows the user to easily scan the region of interest in a hemocytometer by rotating a few dials and producing a composite video.

4.3 Image processing

Once the video of the blood sample in a hemocytometer is acquired, one can use computer vision techniques to perform cell counting. We now discuss a number of challenges that arise in this context.

Panorama. We first tried to convert the video into a panorama image by using standard photo-stitching software.

However, we discovered that the distortion caused by the lens and the movement resulted in numerous artifacts (e.g., blurring, skipping) in the panorama image that rendered it useless for our needs. Since our goal was to simply count the cells and not the generation of a panorama image, we decided to process the video directly.

Cell counting. The first challenge is given a frame of the video, how do we count the number of cells in it? A number of vision algorithms for counting objects are available such as countour detection, template matching, and Haar cascade. Further, these algorithms have different characteristics in terms of accuracy, speed, computational needs, etc. Thus, we evaluate a suite of such algorithms in Section 5 to better understand the trade-offs and determine if they provide sufficient accuracy for our needs.

Avoiding double counting. The second challenge is to avoid double counting as we process multiple frames of the video. In order to do this, we need to uniquely identify *squares* that are shown in Figure 3 so that we count cells in each square exactly once. This requires us to first identify the squares and then uniquely label each square.

The identification of squares is fairly straightforward since we know the existence and spacing of the various grid lines. However, uniquely labeling each square turned out to be a harder problem. While there are standard vision techniques like SIFT (scale-invariant feature transform) to identify unique features of a cell, we found that simply applying these techniques is not robust enough for our needs as it resulted in a large number of double counts. Instead, in Section 5, we find that using the location of cells (relative to the grid) to create a unique feature for a square and further applying special location and neighbor tracking filters is necessary to avoid double counting of cells in a video.

Aggregating the final count. The third challenge is in consolidating the count values and producing a final density of the cells in blood. This requires making sure that the user has performed a complete sweep of the required area in the hemocytometer. Once all the unique squares have been processed, the calculation of the final density of different blood cells is straightforward since the volume of blood in hemocytometer and dilution used is standardized.

5. OUR APPROACH

In this section, we describe our approach to overcome the challenges mentioned in Section 4, mainly, the XYZ stage needed for getting the video scan right and the computer vision algorithms for an automated cell count.

3D-printed XYZ stage. Figure 4 shows a simple 3D-printed XYZ stage accessory based on adjustment screws that gives a smooth translation in X and Y directions in steps smaller than 0.1 mm each and can also cover the entire 3 mm $\times$ 3 mm grid on a hemocytometer. It attaches to a smartphone (Samsung S6 or iPhone 6) and has a slot for a disposable hemocytometer. We do not require the exact precision of a commercially available XYZ stage for our purpose, only small-step movements with a smooth enough translation that we get a good video scan with overlapping frames. Our XYZ stage is 3D-printed, so it can be replicated easily and at a relatively lower cost.

Cell detection. Contour detection provides a simple approach for detecting and counting cells in microscope images. However, it cannot distinguish overlapping or adjacent cells, has too many parameters that need to be manually fine-tuned, and is not robust to changes in images. Therefore, we opt for a more statistical and robust approach such as template matching. The key idea is to look for a certain template in an image or a frame by sliding the template across the frame, compute correlation, and then threshold to find contours. We manually crop 100 images of 12×12 pixels containing cells and generate the following templates: (1) The mean template (2) The weighted eigenvalue template (3) The eigen cells (similar to the eigenfaces used in face detection), and (4) An artificial template (disc-shaped like an RBC).We also compare these against a Haar feature cascade popularly used for face detection. A Haar feature cascade is a cascade of classifiers trained to extract a set of Haar features (such as edge features, line features, rectangular features), and its final result is a weighted sum of weak classifiers. We create training data using positive samples of 100 manually cropped 12×12-pixel images of RBCs. We generate negative images using frames from the scan of an empty hemocytometer and positive images using overlays of positive samples over negative images, and create a 20-stage Haar cascade classifier. See Figure 5 for a visual comparison of these cell detection algorithms on a few sample frame. A detailed comparison of their accuracy is given in Section 6.

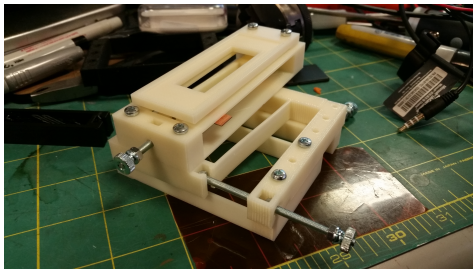

Figure 4: 3D-printed XYZ stage with adjustment screws and a hemocytometer slot

Square detection. Once the cells are detected accurately, it is also important to detect the grid lines and squares of the hemocytometer. The algorithmic pipeline for this is shown in Figure 6. We first do grayscale and histogram equalization followed by morphological opening, and use Hough line detection algorithm to detect lines. As Figure 6 shows, this also finds some slanted lines erroneously, which need to be filtered out to get the horizontal and vertical lines of the hemocytometer grid. The intersection points of these horizontal and vertical lines define the squares we are interested in.

Avoiding double counting. To get an accurate cell count using a video scan, avoiding double counting in squares across frames is as important as counting cells accurately in each square or a frame. To match and detect similarity of two squares in the hemocytometer grid, there are two types of features that we could use. SIFT (Scale Invariant Feature Transform) features are widely used in computer vision, which detect and describe local features, and the similarity of two squares can be measured using Euclidean distance between their feature vectors. However, we find that SIFT features lead to a lot of double counting and also many mistakes. A better approach is to exploit the fact that we already have a reasonably good cell detection algorithm for detecting cells in each square at our disposal. Thus, we define *cell location features* that create features based on the

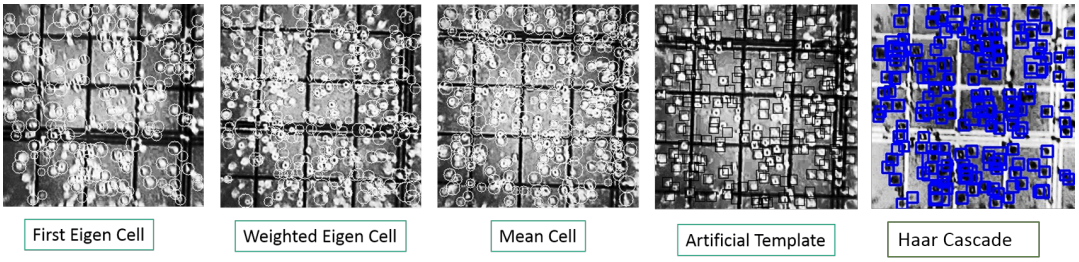

Figure 5: Cell detection

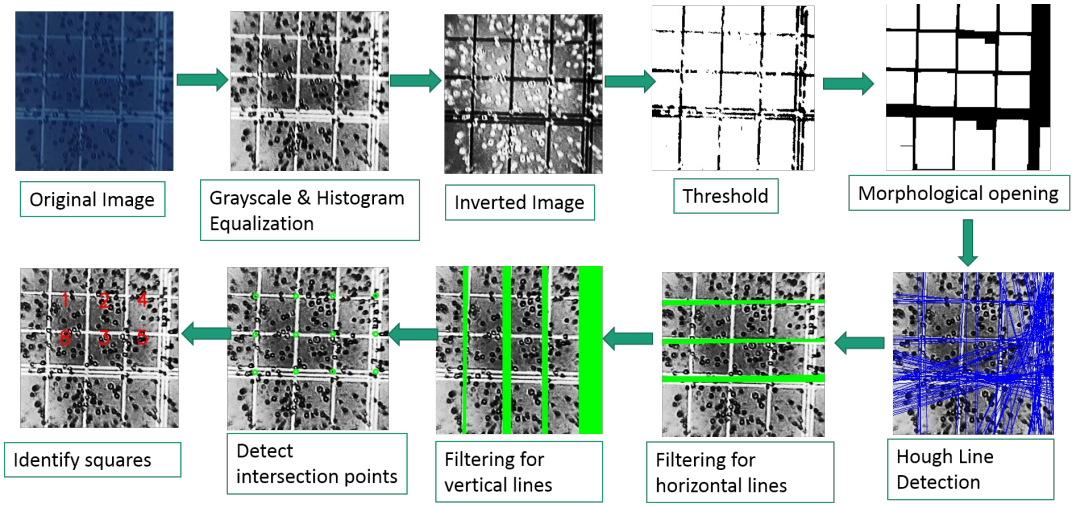

Figure 6: Square detection

locations of cells within each square. These features are simply the distances of each cell to all the four borders of the square that contains it. Once we have these, we can match two squares if their distance in this feature space is less than a threshold. However, this is not sufficient on its own and we apply two filters – (a) *location match filter* - Based on the intuition that the location of a square should not change drastically across consecutive frames, we design this filter to match squares that are similar in features but have a large separation across consecutive video frames and (b) *neighbor tracking filter* - Given that squares having the same neighbors in different frames must be the same, this filter matches not just the squares but their neighbors as well. Upon using these two filters, we observed that we could uniquely identify and track all squares in all frames of a video. We then use the cell counts within these unique squares along with the number of unique squares to determine the density of blood cells. See Section 6 for a detailed discussion on the performance of these feature matching algorithms and filters.

6. PRELIMINARY RESULTS

Our preliminary set of experiments that we present here were done on a blood sample that we diluted and loaded into a reusable, improved Neubauer hemocytometer using a micropipette. We obtained a video scan by mounting a Samsung S6 phone on a manual stage of a microscope. The phone camera had an acrylic lens [4] attached to it with a magnification ratio of 150x. We wanted to automate the manual counting procedure without making many drastic changes for sanity check.

For RBC count, one needs to look at $5 \times (4 \times 4) = 80$ smaller squares of the hemocytometer grid, as mentioned in Section 2. Algorithmically one needs to find these 80 unique squares from a video scan. Our feature based similarity measures are not sufficient to find all the duplicates. In our experiments, applying the location match filter brought the number of unique squares down to 171 and applying the neighbor tracking filter brought it further down to 79.

A visual comparison of various cell detection algorithms described in Section 5 can be seen in Figure 5. We plot the accuracy of these algorithms on our smartphone microscope against a careful manual counting on an actual microscope in Figure 7. The template matching algorithm using an artificial template (white circle shaped like an RBC on a black background) performs better than all other methods with about 7% error and 5% standard deviation. The corresponding RBC count we obtain is 5.36 million cells per microliter, compared to the ground truth of 5.22 million cells per microliter. Our results are reasonable in comparison with the clinically acceptable 5% coefficient of variation on automated hematology analyzers [24].

7. DISCUSSION

Most of our algorithms are not computationally intensive (except the Haar classifier) and can be implemented

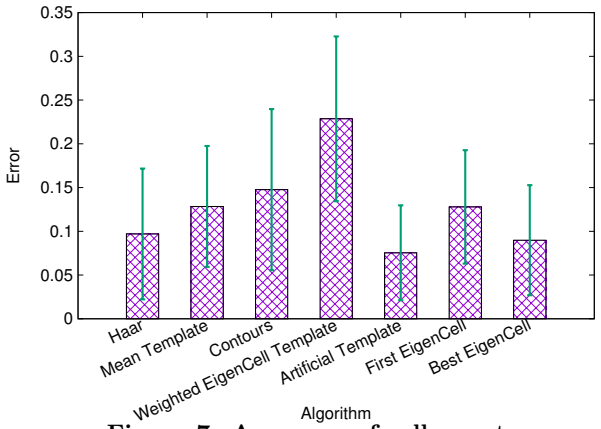

Figure 7: Accuracy of cell counts

on a smartphone. However, more complex classifiers for WBC differential counts or micro-organism detection could be computationally challenging open problems for the mobile vs. cloud trade-off.

Similar to cameras and accelerometers, we expect the magnifying lenses to get better with time or even get integrated into the smartphone camera, making mobile microscopy a promising area for future research.

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