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Low-Cost Disposable Cartridge for Performing a White Blood Cell Count and Partial Differential at the Point-of-Care*

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Abstract

Being able to perform a white blood cell (WBC) count and differential is a crucial laboratory test for basic diagnostic practices. In this paper, we demonstrate proof of concept results for a disposable cartridge that could be used to perform a WBC count and 3-part differential at the point-of-care. The cartridge is composed of a glass slide, a layer of transfer tape, and a glass cover slip and incorporates acridine orange for cell staining and sub-type differentiation; the stained blood is then imaged, and image analysis techniques return a WBC count and 3-part differential. The cartridge was tested on a laboratory microscope with 3 normal samples, and had promising results with 85.7% of images resulting in a WBC count with $\pm 15\%$ of the true value. Further, the 3-part differential concentrations determined using the disposable cartridge had strong correlations with the true concentrations (R^2 values of 0.9986, 0.9421, and 0.6942 for granulocytes, lymphocytes, and monocytes, respectively). Preliminary designs for a low-cost, portable microscope have been created and are currently being prototyped.

I. Introduction

White blood cells (WBCs or leukocytes) are crucial players in the body's immune response to protect tissues against infectious and toxic agents [1]. Being able to count cells and differentiate between WBC subtypes has become a critical laboratory test for screening and diagnosing various disorders and diseases. For example, a WBC differential can be used to diagnose bacterial or viral infections, assess allergic conditions, and stage HIV infection via a CD4 count. Further, a WBC count and differential can be used to monitor the effectiveness of a treatment for a given patient [2].

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Automated hematology analyzers are used to perform WBC counts and differentials in most clinical settings in developed regions today. Automated blood counters are based on flow-based analysis systems in which cells are flown through small diameter tubing, and a combination of electrical impedance sensing, light scattering measurement, and immunostaining followed by optical detection are used to count and differentiate between WBC subtypes [2,3]. Depending on the sophistication of the system, 2-part (lymphocytes vs. granulocytes), 3-part (lymphocytes, neutrophils, and other leukocytes), and 5-part differentials can be achieved [3,4]. However, these devices are often large, bulky, expensive, and complex, creating difficulty in employing these methods at the point-of-care. Typically these devices are only found at central hospitals or research facilities, creating a barrier for the rapid return of results to patients.

Before the advent of automated cell counters or in areas where these technologies are unavailable, WBC counts and differentials were performed using manual counting with microscopy. In this method, the WBC count is performed by loading a small amount of diluted blood into a hemocytometer. The WBCs are then counted microscopically, using the gridlines on the hemocytometer as a guide [2]. For a WBC differential, cells are spread on a glass slide, dried, and stained with a Romanofsky stain, such as the Wright or Giemsa stain; these stains allow a trained user to differentiate between the various WBC sub-types due to different staining patterns [5]. This technique is time-consuming, labor intensive, and requires high levels of training to perform [2].

Due to the high cost and associated infrastructure required for automated hematology analyzers and the time and labor required to perform manual WBC counts and differentials, a new solution is required for performing these tests at the point-of-care. Therefore, we have developed a disposable cartridge and image analysis algorithm that can be used with a single drop of blood and returns a WBC count and differential in under 5 minutes. Our device employs a staining agent, Acridine Orange, and image analysis methods to determine a 3-part differential and WBC count capable of being used at the point-of-care. A portable microscope has been designed and is currently in the prototyping phase.

II. Methods

A. Disposable Cartridge Design

The disposable WBC cartridge is designed from three components: a standard glass slide (VWR), transfer tape (3M 8153LE), and a no. 2 glass cover slip (VWR); these components were layered as shown in Fig. 1a. A single 9.25 mm × 19.3 mm rectangle is cut from the center of the transfer tape, which is then applied to a clean glass slide. This creates a shallow well with a depth of 88µm. The glass cover slip is cut with two holes with diameters of 2.88 mm placed at locations corresponding to the top and bottom of the well, as shown in Fig. 1b. These holes create inlet and outlet ports, shown in Fig. 1b, which allow the well to be filled by capillary action with a drop of 20µL whole blood with air escaping through the outlet port. The sample was imaged in the region between the inlet and outlet ports.

B. Acridine Orange Staining

Acridine orange (3,6-dimethylaminoacridine) is a pH-sensitive fluorescent cationic dye. It binds to DNA by electrostatic interactions and intercalation of the acridine nucleus between base pairs. When this binding occurs, the dye fluoresces green (excitation maximum = 502 nm, emission maximum = 525 nm). In addition to binding to double stranded DNA, acridine orange binds to single stranded DNA and RNA, shifting the emitted fluorescence to red (excitation maximum = 460 nm, emission maximum = 650 nm). When in a neutral pH, acridine orange is hydrophobic, allowing it to diffuse through membranes into the nucleus and cytoplasm of cells. However, in acidic environments, such as those in the lysosomes of granulocytes, the dye is protonated, making it cationic and preventing it from diffusing across the lysosome membrane. In these acidic environments, the dye fluoresces red and allows lysosome labeling [6,7]. These interactions cause the nuclei of WBCs to have a strong green fluorescence with weak red fluorescence due to the presence of DNA and RNA and the cytoplasm to fluoresce red due to RNA and lysosomes [8]. Therefore, as illustrated in Fig. 1c, agranulocytes will fluoresce nearly completely green due to their lack of cytoplasm while granulocytes will have both green and red fluorescence due to the acidic lysosomes in their cytoplasm.

Acridine orange was one of the first fluorescent dyes used in the supra vital staining of WBCs in the 1960s [8]. In the 1970s, Adams et al. demonstrated a 3-part differential of leukocytes using the red and green signals of each acridine orange-stained WBC in a flow chamber [9].

Initially, acridine orange (Life Technologies) was mixed with whole blood to achieve a final concentration of 10 µg/mL. After validating the staining and cartridge design using this method, 0.5µL of acridine orange [400µg/mL] was dried over the inlet port of the cartridge to mix with the sample upon application. By drying the acridine orange into the cartridge, the need for any sample preparation is eliminated, and the user places a drop of whole blood on the inlet port to achieve staining.

C. Optical Detection System and Image Analysis

Images analyzed here were captured using a Zeiss AxioVision microscope with a 10× 0.45NA objective and Zeiss AxioCam MRc camera. A custom filter block was designed with a band pass excitation filter (450 nm – 590 nm, Chroma) and a long pass emission filter (>532 nm, Semrock). This design decreases the strong green fluorescence while optimizing the red fluorescence.

Image analysis was performed by a custom, automated program in Matlab (Mathworks). In the program, each WBC in the field of view (FOV) is segmented, and the mean green and red pixel values for each individual WBC are calculated. The ratio of red signal to green signal is calculated for each cell, and these values were used for WBC classification.

D. Blood Collection, Handling, and Testing

Fresh blood was obtained from four normal volunteers via venous draw into EDTA coated vacutainer tubes. The WBC count and differential were immediately measured using a

Beckman Coulter AcT Diff2 hematology analyzer. 20 μL of the sample was then pipetted into the inlet port of the disposable cartridge and allowed to flow into the cartridge well via capillary force. Samples were incubated at room temperature for 5 minutes prior to imaging. For each sample, one image was collected for analysis ($n = 4$) in the development of an algorithm to convert WBCs in the FOV to the concentration of WBCs in the sample.

Following this, blood was collected from three normal volunteers and imaged in the same manner as above. At least three images were taken for each sample ($n = 11$) and were used for determining the accuracy in counting WBCs and differentiating between sub-types.

III. Results

A. White Blood Cell Count

Segmented cells in the FOV of an image are initially counted in the automated Matlab program. Results (shown in Fig. 2) show an accurate fit ($R^2 = 0.948$) between the number of cells in the FOV and the true concentration of WBCs as determined by the hematology analyzer. Using this fit,

$$35.962 * FOV + 884.87 = C \quad (1)$$

was generated for converting the cells in the FOV to the concentration of WBCs in the sample (C). This fit was then validated using separate samples ($n = 11$), and 81.8% of the images fell within $\pm 15\%$ of the true value and therefore met the requirements for CLIA (Clinical Laboratory Improvement Amendments) waived devices.

B. Three-Part Partial Differential

Image analysis and plotting of the red-to-green ratio values of the validation data set on a histogram revealed three distinct peaks and groupings of the WBCs, as shown in Fig. 3a. These peaks correspond to the fluorescence signal from lymphocytes, monocytes, and granulocytes; the first peak is composed of the signal from lymphocytes, as these cells have strong green fluorescence and weak red fluorescence, creating a low red-to-green ratio. The third wide grouping is composed of the granulocytes, as these cells have a large presence of cytoplasm causing red fluorescence; the ratio values from this population of cells have a wider range due to the variation in nuclear and cytoplasmic size in granulocytes. The middle peak corresponds to monocytes as these cells are primarily composed of a nucleus but have a small cytoplasmic area which fluoresces red. By detecting the minimum values between these peaks for each individual image, cells were classified into the corresponding WBC sub-type. The percentage for each group was used with the determined WBC count to determine the concentration of each subtype. Fig. 3b illustrates that granulocytes had the best fit with true value ($R^2=0.9732$), and lymphocytes also had an accurate correlation with the true value ($R^2=0.9319$). However, monocytes had the worst fit ($R^2=0.6165$); this is theorized to be due to the limited range of monocyte concentrations in normal blood.

IV. Conclusion

In conclusion, we have demonstrated proof-of-concept results for a disposable cartridge for performing a WBC count and 3-part partial differential at the point of care. The cartridge is composed of a glass slide, 3M transfer tape, and a glass cover slip and automatically stains blood with acridine orange; at low production volumes (i.e., less than 50), this cartridge will cost less than \$0.50. Preliminary results show an accurate fit with true WBC counts with 85.7% of measured WBC counts falling within $\pm 15\%$ of the true value. Further, the 3-part differential results are accurate with R^2 values of 0.9986, 0.9421, and 0.6942 for granulocytes, lymphocytes, and monocytes, respectively.

The optical system of a low-cost portable microscope has been designed and is shown in Fig. 4. The design incorporates a three-lens system and a 532nm long-pass emission filter. Illumination is achieved with a 473nm narrow band laser source. The system has a numerical aperture of 0.35, a field-of-view of 1 mm in diameter and a working distance of 1.6mm. In addition, the system exhibits diffraction limited nominal performance in 92% of the FOV. The presented system will image an estimated 129 WBSs in a FOV for a normal blood sample, which is sufficient for performing a WBC differential. This device is currently in prototyping. The optical system, together with a CMOS image detector, will be incorporated with a self-contained single board computer to perform all image analysis, resulting in a portable device capable of performing a WBC count and 3-part differential at the point-of-care.

This device, in its final form, has the potential to radically affect clinical outcomes for those suffering from a range of clinical conditions, including infections, allergies, or HIV. Because of the small volume of blood required for use, our design is particularly well suited for tracking patient changes over time. Further, because the sample requires no preparation by the user and can be imaged and analyzed with a small, portable microscope and single board computer design, this method has applications for low-resource regions that do not have access to trained technicians or expensive hematology analyzers. This will allow clinicians in these regions to perform a WBC count and partial differential in order to assess a patient's condition accurately, rapidly, and at the bedside. This decrease in per test cost and decentralization of health care infrastructure has, therefore, the ability to strongly impact regions of the world with little access to centralized health care.

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Figure 1.

The disposable cartridge is composed of three layers: a glass slide, 3M transfer tape, and a glass cover slip (A). The inlet and outlet ports allow blood to fill the well by capillary action, creating an even layer of blood in the imaging region (B). Acridine orange is dried at the inlet port, automatically staining WBC nuclei green and cytoplasm red, allowing for the differentiation of WBC sub-types (C).

Figure 2.

Preliminary results of the WBC count performed in the disposable cartridge with image analysis show a strong correlation ($n=4$). This data was used as a training set to create an equation for converting the number of WBCs in the FOV to the concentration of WBCs in the sample.

Figure 3.

A histogram of the red-to-green ratio shows three distinct groups of WBCs: two sharp peaks corresponding to lymphocytes and monocytes and a larger spread of WBCs corresponding to granulocytes (A). When the minimums of the histogram are used to classify the WBCs based on these peaks, an accurate correlation is found between the concentration of each of the subtypes as determined by image analysis of the disposable cartridge with the true value determined by the hematology analyzer (B). The error bars represent the standard deviation between the results of individual images of a sample (n=11).

Figure 4.

The optical system consists of three lenses and various other components: from left to right, image surface, coverslip glass, lens 1, aperture stop, glued achromatic doublet, emission filter, camera IR filter, and the image surface. Our system was achromatized for two wavelengths, 525 nm and 650 nm, corresponding to the emission peaks of acridine orange bound to DNA and RNA, respectively. In order to simplify optical layout, our system was designed with a curved image surface (radii of curvature: 40 mm). Image surface sag at the end of the field of view equals 63 μm and is below depth-of-focus of our system (DOF = 107 μm). A DOF larger than an image surface sag guarantees that sample will be sharply imaged on the plane surface of image detector. The entire optical system length is 77 mm.