# A COMPACT, OPTOFLUIDIC SYSTEM FOR MEASURING RED BLOOD CELL CONCENTRATION

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## ABSTRACT

We developed a compact, single-cell imaging system for measuring red blood cell (RBC) concentration from diluted, whole blood samples. Key element of the design is the integration of a microfabricated lens array with a commercial haemocytometer. To image large areas and therefore a larger number of cells, the field of view (FOV) can be adjusted by simply increasing the thickness of the haemocytometer coverslip. We used a FOV = 200 μm, corresponding to a resolution of 0.53 μm, to demonstrate RBC counting: counts obtained from our system were in excellent agreement with counts obtained from standard microscopy and flow cytometry over a range of dilution factors.

## KEYWORDS

Red blood cell counting, optical imaging system, optofluidics, microfabricated lens array, haemocytometer.

## INTRODUCTION

Red blood cell (RBC) count, quantified as the number of cells per unit of blood volume, is an important clinical test that provides critical information about a patient's health status [1]. RBC in adults should be on the average of 4.2-6 million/μL. High RBC count (>6 million RBCs/μL) can be the result of a chronic condition such as lung (pulmonary) disease, kidney cancer, or even smoking. Low RBC count is a typical sign of anemia which can be the result of internal bleeding, leukemia, bone marrow failure or even iron deficiency [1-2].

The most common methods for measuring concentration of RBCs in blood are based on flow cytometry and light microscopy. Flow cytometers, although extremely accurate, they are expensive, bulky instruments that require regular maintenance. On the other hand, light microscopy is an easy-to-use and cheap alternative that relies on the use a reusable device, called the haemocytometer that allows direct cell visualization. Haemocytometers cannot provide reliable counts as they suffer from human errors during the RBC counting process which is performed manually. Therefore a portable and accurate imaging system could make a significant contribution in monitoring pathological conditions that require RBC or other cell type counting, especially in resource-limited settings [3-5] .

Several groups have been developing compact imagers for visualizing/counting biological samples over the last decade. Zhu et al. [6] demonstrated a cell phone-based fluorescence and dark field imager. The resolution of their system was in the range of 10-20 microns. Heng et al. [7] demonstrated a compact optofluidic microscope with high resolution that requires the sample to be scanned over a specially fabricated mask. Demirci et al. [8-9] developed a lensless shadow imaging system for cell counting.

Here, we demonstrate a compact, high-resolution, optofluidic imaging system for visualizing and counting RBCs (Fig. 1). We call it 'optofluidic' because the core imaging unit is an array of microfabricated oil-immersion ball lenses. Its use is not limited to RBC counting: it can potentially be an ideal tool for imaging biological specimens in a resource limited setting or at the doctor's office, replacing bulky microscopes. Our portable system has a footprint of 7 cm x 7 cm and a total height of 6 cm.

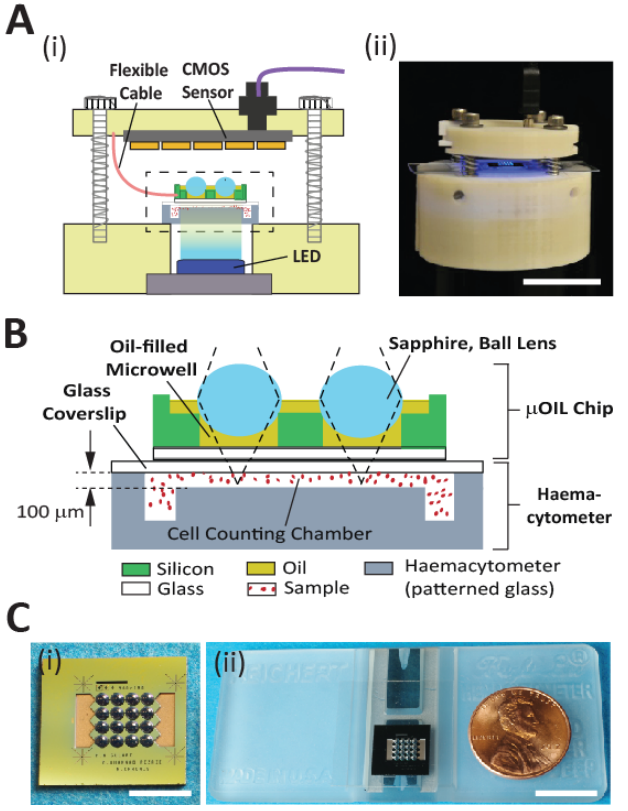


*Figure 1. (A) The compact imaging system. (B) Schematic cross-section of the μOIL chip on top of a haemocytometer. Scale bar in (ii), 3 cm. (C) Pictures of the μOIL chip (i) and the μOIL chip/ haemocytometer assembly (ii). Scale bars, 5 mm in (i), 1 cm in (ii).*

## METHODS

### The μOIL System

Our imaging system operates in bright field (transmission) mode and it consists of (Fig. 1A): (i) an array of Microfluidic-based Oil Immersion Lenses (termed 'the μOIL chip') [10], (ii) a commercially available haemocytometer, (iii) a low cost 5MP CMOS sensor (2.2 x 2.2 μm pixel size) and, (iv) a blue LED. The haemocytometer is thick glass slide which forms a 100

μm deep cell counting chamber (known as the Neubauer chamber) when a glass coverslip is placed on top of it. A custom-made plastic housing was manufactured to secure all those components in place except the haemocytometer that is manually inserted into the housing from the side.

The haemocytometer containing the blood sample is illuminated by the LED (centered at 470 nm) from below. The transmitted light is collected by the high numerical aperture (NA) lenses of the μOIL chip and is focused on the CMOS sensor that is placed 3-4 mm above the μOIL chip (Fig. 1B). To achieve a sharp, well-focused image, the exact distance between the μOIL chip and the CMOS sensor is manually adjusted using a set of screws. Finally, the CMOS sensor is connected to a computer that acquires and analyzes the image.

The μOIL chip (described in [10]) consists of 16 sapphire, ball lenses (1 mm in diameter, refractive index of 1.77) integrated on top of a 4x4 array of oil-filled microwells (Fig. 1C (i)). The oil/ball lens assembly forms a doublet lens and resembles the hemispherical front lens of an oil-immersion objective. The μOIL chip is placed directly on top of the haemocytometer (Fig. 1C (ii)). To achieve a good contact between the μOIL chip and the haemocytometer coverslip (and therefore eliminate any gap between them), a flexible cable is used as a spring to push the μOIL chip against the coverslip. As a result, the distance between the μOIL chip and the sample in the Neubauer chamber is always fixed.

The microfabrication of the μOIL chip is based on a two-step DRIE process for creating the microfluidic channels and the microwell array. A 100 μm thick glass wafer is anodically bonded to the DRIE etched silicon. This extra microfbarication step has been added to the original microfabrication process [10] to eliminate chip to chip variations in the total thickness of the μOIL chip.

## Counting RBCs

We performed RBC counts with fresh whole, diluted blood samples on a commercial haemocytometer (Hausser Scientific) covered with a 150 μm thick glass coverslip (Fig. 2A). The Neubauer chamber of the haemocytometer has a total volume of 6-7 μl and it has a square grid pattern to facilitate the cell counting (Fig. 2B).

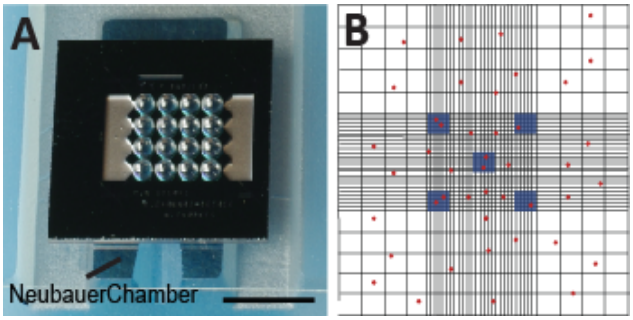


*Figure 2. (A) The μOIL chip on top of the Neubauer chamber. Scale bar, 4 mm. (B) Schematic of the grid pattern that sits beneath the μOIL chip . The dark-colored boxes are 200 μm x 200 μm and represent the 5 regions where cells (red dots) are counted.*

To image the haemocytometer and count the RBCs, we used: (i) a microscope (Olympus-BX51WI) in epi-illumination mode with a 50x objective (NA=0.5), connected to a 5 MP digital camera and, (ii) our compact imaging system with a field of view (FOV) of 200 μm and a NA of 0.54. In this case, the distance between the cells and bottom surface of the μOIL chip was ~250 μm (RBCs tend to settle at the bottom of the Neubauer chamber). In all our imaging experiments, we used a single lens from the μOIL chip for consistency in the image quality.

Blood samples (~10 μL in volume, diluted by a factor of 50, 100 and 200) were pipetted into the haemocytometer and driven in the Neubauer chamber by capillary action. Images of the blood samples were captured from 5 different 200 μm x 200 μm square areas in the grid pattern of the Neubauer chamber (marked as dark boxes in Fig. 2B). The dilution and imaging procedures used in our experiments are the recommended ones for haemocytometer-based RBC counting [11-12].

# RESULTS AND DISCUSSION

## FOV, Resolution of the μOIL System

For cell counting applications, the FOV of each lens in our system is an important feature as it determines how much blood volume (and therefore how many cells) can be imaged.

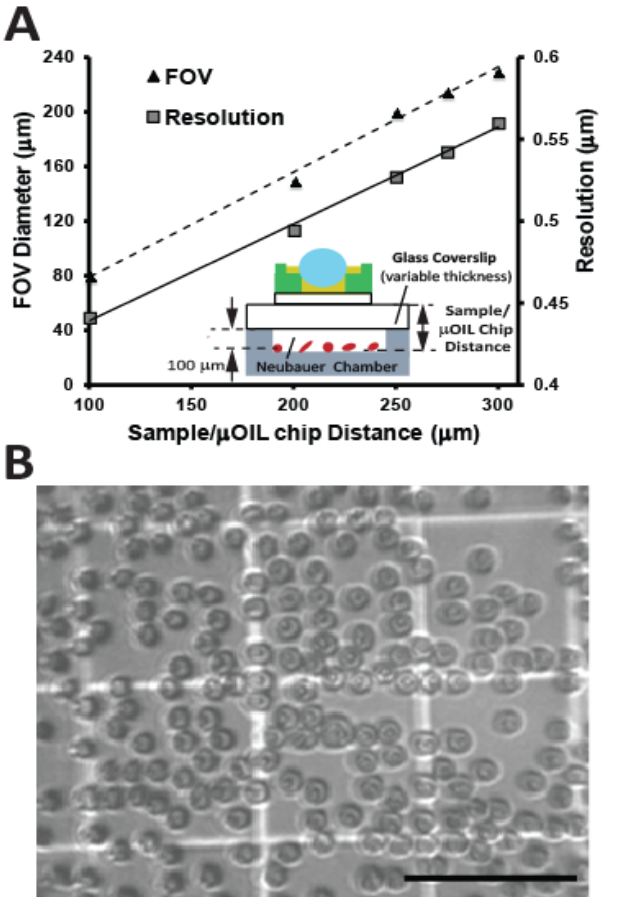


*Figure 3. (A) FOV and resolution versus sample/μOIL chip distance. The FOV is the diameter of the area that a single sapphire lens can visualize. (B) An image of RBCs as captured through our system for a sample/μOIL chip distance of 200 μm. Scale bar, 50 μm.*

The FOV in our system can be modified by changing the distance between the bottom surface of the µOIL chip and the sample. To estimate that dependence, we used the grid pattern of the Neubauer chamber as a reference sample, placed it at 100, 150, 175 and 200 µm below the bottom surface of the µOIL chip and measured the corresponding FOV. We used glass coverslips of different thicknesses to vary the sample/µOIL chip distance. We also performed optical simulations in OSLO software to estimate the corresponding resolution (Fig. 3).

As expected, the larger the sample/µOIL chip distance the larger the FOV and lower the resolution. For our RBC counting experiments as explained earlier, we selected a FOV of ~200 µm as it matches the standardized, 200 µm x 200 µm imaging area of the Neubauer chamber (depicted in Fig. 2B). The resolution and NA in this case were estimated to be 0.53 µm and 0.54 respectively.

## Comparing RBC counts

We obtained images from diluted whole blood samples with our system and with a microscope (Fig. 4) as described earlier. In both cases, we used a haemocytometer with a 150 µm thick glass coverslip.

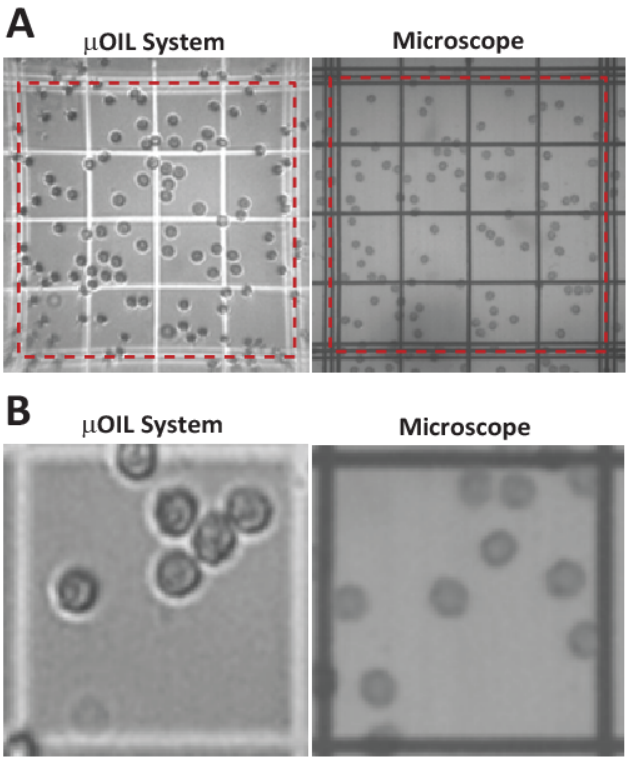


*Figure 4. (A) Diluted blood samples as imaged through our system (NA=0.54) (left) and a microscope with 50x (NA=0.5) objective (right). (B) Magnified images from a single 50 µm x 50 µm square of the grid containing RBCs. The dimple of the RBCs is visible with the µOIL system.*

We noticed that, although the NA of the µOIL system and the microscope are similar (0.54 versus 0.5), the µOIL system could resolve details of the cell geometry/size much better than the microscope (Fig. 4B). The biconcave RBC shape with the characteristic dimple in the middle was clearly visible. We believe that the main reason for such a difference on the image quality is due to the high contrast that is generated by the bright-field illumination of our system. We also noticed that the sapphire lens of µOIL system distorts the edges of the viewing area. That effect did not alter the RBC counting results as cells are still identifiable.

We furthermore performed imaging experiments with blood samples diluted by three different factors (50, 100 and 200) (Fig. 5). For all 3 dilution factors, we did not observe any significant differences between the RBC counts obtained with the microscope and our system.

We also compared our system with a flow cytometer which is considered the gold standard for cell counting applications. The flow cytometer count was 4.57 million cells/µL (see dotted line in Fig. 5, no dilution). The RBC count from our system is in good agreement with the flow cytometer results within one standard deviation for dilution factors of 50 and 100. RBC counts with larger (e.g. >200) dilution factors are not recommended as there is a significant difference (~10% error) between the flow cytometry and µOIL system results. However, standard haemocytometer-based RBC counting procedures recommend a dilution factor of 200 to facilitate the manual counting process. We should also note that despite the fact that we used whole blood, the presence of white blood cells (WBCs) does not affect the RBC count as WBCs make up only 0.1% of the total number of cells in blood and as a result they rarely appear in the images.

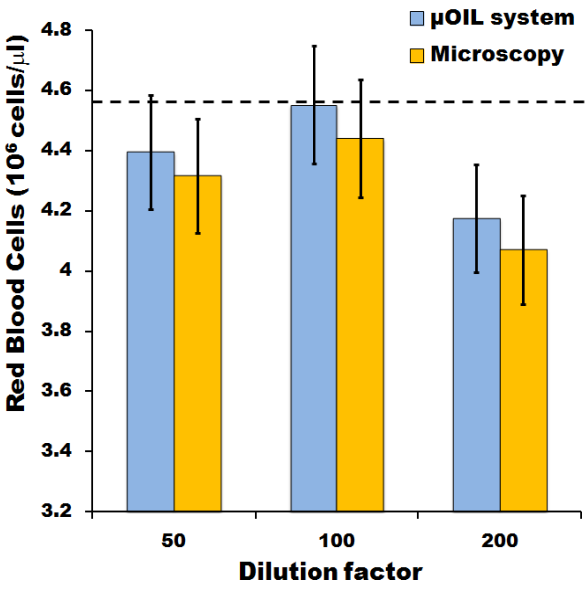


*Figure 5. Comparing RBC counts. The dotted line is the RBC count (4.57x10$^6$ cell/mL) as measured by flow cytometry. The error bars represent one standard deviation.*

## CONCLUSION

We developed a high resolution, compact, single-cell imaging system for counting cells. The imaging capabilities of the system can be attributed to the use of a high NA microfabricated lens array. Although we demonstrated counting results from a single lens, we anticipate that the use of the entire lens array will automate the counting process and eliminate statistical variations as the sample size (number of cells counted)

will significantly increase. We believe that the use of our system is not limited to cell imaging/counting but it can be expanded to imaging other biological samples such as bacteria, microbes, microorganisms and tissue samples as it provides excellent image quality that is comparable to the one obtained from high end microscopes.

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