Analysis system for characterisation of simple, low-cost microfluidic components

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ABSTRACT

There is an inherent trade-off between cost and operational integrity of microfluidic components, especially when intended for use in point-of-care devices. We present an analysis system developed to characterise microfluidic components for performing blood cell counting, enabling the balance between function and cost to be established quantitatively.

Microfluidic components for sample and reagent introduction, mixing and dispensing of fluids were investigated. A simple inlet port plugging mechanism is used to introduce and dispense a sample of blood, while a reagent is released into the microfluidic system through compression and bursting of a blister pack. Mixing and dispensing of the sample and reagent are facilitated via air actuation.

For these microfluidic components to be implemented successfully, a number of aspects need to be characterised for development of an integrated point-of-care device design. The functional components were measured using a microfluidic component analysis system established in-house. Experiments were carried out to determine:

1. the force and speed requirements for sample inlet port plugging and blister pack compression and release using two linear actuators and load cells for plugging the inlet port, compressing the blister pack, and subsequently measuring the resulting forces exerted,
2. the accuracy and repeatability of total volumes of sample and reagent dispensed, and
3. the degree of mixing and dispensing uniformity of the sample and reagent for cell counting analysis.

A programmable syringe pump was used for air actuation to facilitate mixing and dispensing of the sample and reagent. Two high speed cameras formed part of the analysis system and allowed for visualisation of the fluidic operations within the microfluidic device.

Additional quantitative measures such as microscopy were also used to assess mixing and dilution accuracy, as well as uniformity of fluid dispensing - all of which are important requirements towards the successful implementation of a blood cell counting system.

Keywords: Microfluidics, blood cell counting, microscopy, point-of-care (POC)

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1. INTRODUCTION

Low-cost point-of-care diagnostic systems have gained increasing attention as a solution to improve health care, particularly in under-resourced or rural communities[1] where the need for disease diagnosis is often greatest.

The technology of microfluidics, which encompasses the manipulation of tiny volumes of fluid in compact devices, has and continues to be applied to the development of point-of-care diagnostics[2]. Although a number of advances in microfluidics have enabled the successful implementation of point-of-care devices, there is often still a trade-off between cost and operational integrity of the device. As a result, point-of-care diagnostic solutions remain expensive, with specialized requirements and/or trained operators needed for successful functioning of the device.

Comparisons between different microfluidic technologies have been explored in terms of important parameters - such as cost and complexity - that are required to produce a successful low-cost point-of-care diagnostic system[3]. Some technologies, including paper-based microfluidics, rank higher in terms of cost but not as well in terms of sensitivity, while other technologies are very accurate, but much higher in cost.

It is clear that the strong points of these technologies need to be utilized collectively to develop microfluidic components that meet the criteria for an ideal low-cost point-of-care diagnostic solution. This work presents an analysis system to enable the balance between function and cost of microfluidic components to be established quantitatively. The developed system allows for microfluidic components for performing blood cell counting to be characterised, and thus for the minimum requirements of the complexity of these components to be established to enable results equivalent to those of accepted standard techniques for blood cell counting applications to be produced.

Important aspects such as force and speed requirements for introducing blood and reagent samples into the device by means of a sample inlet port plugging mechanism and blister pack compression mechanism, respectively, were explored and characterised. Additionally, dispensing of the sample and reagent volumes was analysed, as these aspects are all important for accurate cell counting analysis. The success of the implementation of these components was investigated in this work towards the development of a final device for blood cell counting.

2. MICROFLUIDIC DEVICE COMPONENTS

A number of microfluidic device components were investigated to prepare a sample of blood for visual analysis using a dilution factor of 1:20, as shown in figure 1. These components were deemed important for correct functionality of the complete microfluidic device and can be categorized as follows (with numbering referring to figure 1):

1. Blood sample introduction into the device through a plugging mechanism,
2. Reagent introduction and propulsion through the device via a blister pack,
3. Mixing of blood sample and reagent,
4. Air actuation to assist with mixing and fluidic transportation, and
5. Dispersion of the mixed blood sample and reagent into a visualisation area on the microfluidic device.

The microfluidic components were integrated into a device manufactured from injection moulded polycarbonate. The same microfluidic device design was used for different blood cell counting tests, with only the blister pack reagent contents differing for each of the tests. Different volumes of blood could then be introduced to the sample introduction port depending on the requirements of the blood cell counting test to be performed.

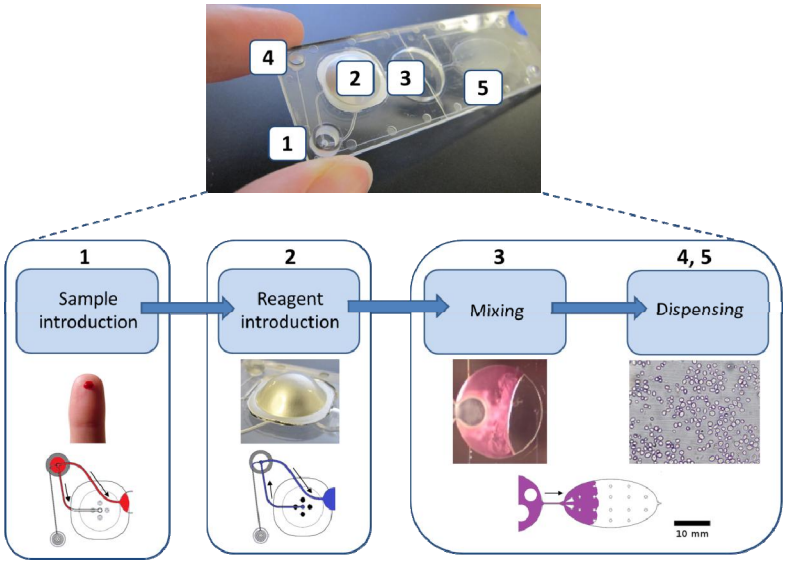

Figure 1. Microfluidic device with various components numbered (top), and corresponding descriptions and schematic illustrations of fluid flow through the device (bottom). 1: inlet port for sample introduction, 2: blister pack for reagent storage and introduction, 3: mixing chamber, 4: air actuation port for fluid movement in the device, 5: visualisation chamber into which mixed blood sample and reagent are dispensed.

Schematic illustrations of the various functional components of the microfluidic device with fluidic flow indicated, are shown in figure 2, and can be divided into three main parts with numbering as indicated on the left side of the figure:

1. Blood sample introduction via a plugging mechanism that pushes the blood sample along channels (a – d) towards a mixing chamber,
2. Blister pack compression (a) and release of reagent (b, c) into the channels, with initial mixing of the blood and reagent, as well as propulsion of the blood and reagent into a mixing chamber (d), and
3. Dispensing of the mixed blood sample and reagent via air propulsion into a shallow visualisation area (a – c) for cell counting to be performed via microscopy once the chamber is completely filled.

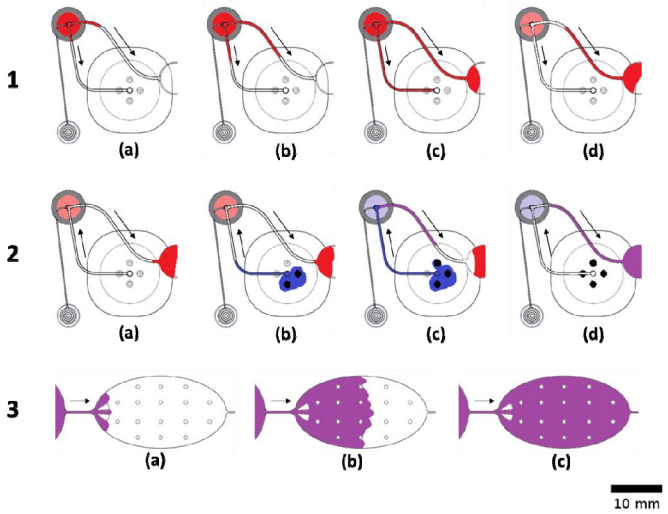

Figure 2. Schematic illustrations of microfluidic device components, showing the fluidic movement through the device in a number of steps for (1) blood sample introduction via a plugging mechanism, (2) blister pack compression and release of reagent to initiate mixing of the blood and reagent in a mixing chamber, and (3) dispensing of the mixed blood sample and reagent into a visualisation chamber.

The work presented here focusses on total white blood cell count and total red blood cell count tests. Reagents for each of these tests were prepared according to standard haematology protocols for manual visual cell counting tests using microscopy[4]. The white blood cell count test reagent was made up of 100 mg crystal violet, 10 ml glacial acetic acid, and 490 ml de-ionised water to make up a 500 ml volume of reagent. The red blood cell count test reagent consisted of a 2 g phosphate buffered tablet in 200 ml of de-ionised water. Blister packs were filled with the respective reagents as part of the manufacturing process.

For initial tests, a 10 µl blood sample was used, while the blister packs mounted on the microfluidic devices contained 190 µl of reagent. For the white blood cell count test, a dilution of blood sample to reagent of 1:20 was implemented on the microfluidic device, while for the red blood cell count test, a dilution of blood sample to reagent of 1:200 was implemented by introducing a 1:10 pre-diluted sample of blood in the phosphate buffer solution to the sample inlet port of the microfluidic device, with a further 1:20 dilution taking place in the device. Fresh blood samples were provided on a weekly basis to ensure repeatability.

3. FLUID CONTROL AND MONITORING SYSTEM

To measure the functional aspects of the microfluidic in a quantitative manner, a microfluidic component analysis system was established, as shown in figure 3. The microfluidic device forms the central part of the analysis system. The system consists of two linear actuators; one for plugging the blood sample inlet port (Z825B actuator with TDC001 controller, Thorlabs, Germany), and a larger actuator for compressing and bursting the blister pack (DRV014 actuator with BSC201 controller, Thorlabs, Germany). A load cell (HB1/R/0010K with TDC 550 indicator, Transducer Developments Company, South Africa) is attached to each of the actuators to allow for the forces exerted through the plugging mechanism and the blister back compression to be measured over time and further analysed.

A programmable syringe pump (NE 500, New Era Pump Systems, USA) was used for air actuation to facilitate mixing of the blood sample and reagent and dispensing of the mixed sample into a shallow visualisation chamber for blood cell counting to be microscopically performed. Two high speed Gigabit Ethernet (GigE) colour cameras (DFK23GM021 with H0514-MP2 lens, The Imaging Source, Germany) formed part of the analysis system and allowed for image processing to be performed by providing side and bottom views of the microfluidic device.

A graphical user interface (GUI) enabled parameters to be set as required for the various analysis system components, and enabled the user to monitor the progress of the experiment, including live views from both the cameras. For each microfluidic device tested, a complete set of data was recorded, enabling volumes and flow rates of different fluids within the microfluidic device to be calculated, as well as allowing for the analysis of positions and times of contact of the actuators with the device components.

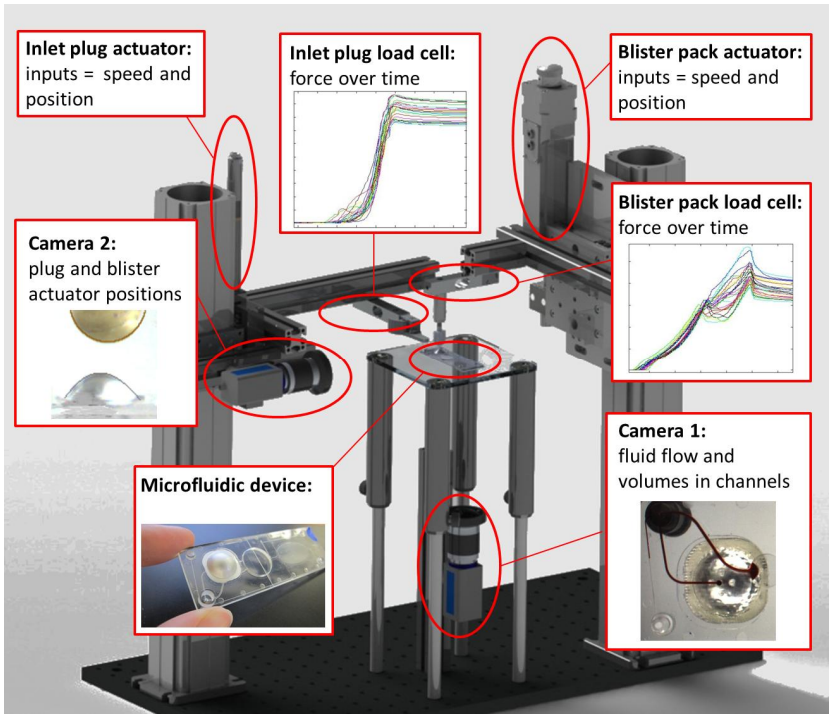

Figure 3. Analysis system for microfluidic device component characterisation. Various pieces of equipment and corresponding information that can be extracted are illustrated.

4. RESULTS

4.1 Load cell data and fluidic actuation

As a first step, the forces required to manually plug the blood sample inlet port and compress and burst the blister pack were determined in order to provide a range of input parameters to use for automated actuation of the microfluidic devices. Forces for plugging the sample inlet port manually were found to range between 10 and 20 N, and thus the linear actuator was set to travel a distance that would result in a force of approximately 15 N when plugging of the inlet port was complete. For blister pack compression and bursting, the forces required were much higher, and ranged from 40 N to 55 N. The linear actuator for blister pack compression was set to travel a distance that would result in an approximate peak force of 45 to 50 N when the blister pack started to burst. Actuation speeds were set at 1 mm/s for the inlet port actuator, and 0.25 mm/s for the blister pack actuator, speeds which were similar to those achieved when performing manual actuation. For air actuation, a flow rate of 500 µl/min was used for propelling the blood and reagent from the mixing chamber into the visualisation chamber.

Figure 4 shows the results of the load cell data recorded, with plots of forces exerted over time for (a) inlet port plugging, and (b) blister pack compression over 25 experiments. The mean values over time are plotted as a solid line, with the standard deviations indicated in grey shading. It can be seen that repeatable inlet port plugging and blister pack compression was achieved using automated actuation. The mean peak force for inlet port plugging was found to be 17.26 N (SD = 1.39) and the mean peak force for the blister pack compression was found to be 44.96 (SD = 4.73).

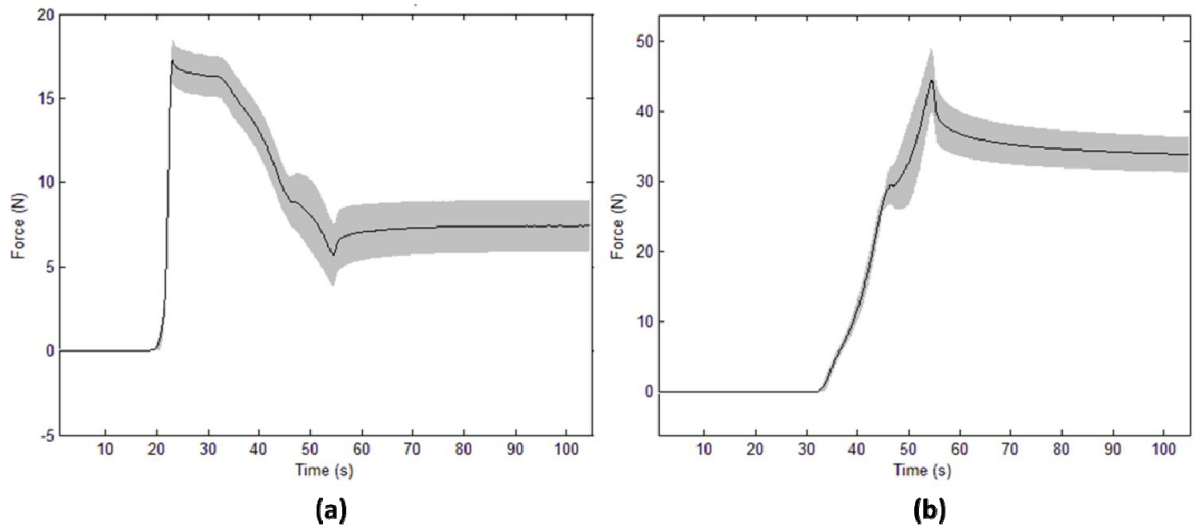

Figure 4. Load cell data results of force over time for (a) inlet port plugging, and (b) blister pack compression over 25 experiments, with mean values over time indicated by a solid line, and standard deviations across experiments shown in grey shading.

Figure 5 shows both the inlet port load cell data (red) and blister pack load cell data (blue) for an individual test with trends similar to the mean load cell data as shown in figure 4 above. A number of time points are marked and numbered, with corresponding images extracted from the high speed video data. The snapshots illustrate the correspondence between the load cell data and the functional steps of the microfluidic device, showing the progression of the sample being dispensed as the plug is inserted (1 – 4) and sample movement into the mixing area as a result of blister pack compression and release (5 – 8). The example given here is for a white blood cell count test, with a 10 µl whole blood sample introduced at the sample inlet port, and a blister pack filled with 190 µl of white blood cell count reagent, the contents of which are described in section 2.

Once the blister pack has been fully compressed, allowing the reagent and blood sample to be pushed through into the mixing chamber, air is applied to the air actuation port to propel the fluid from the mixing chamber through to the visualisation chamber for blood cell counting to be performed. An air flow rate of 500 µl/min was used throughout the experiments. Figure 6 shows an experimental example extracted from the high speed video data, where the air first fills the remainder of the mixing chamber (a), after which the sample begins to dispense into the visualisation chamber (b), taking approximately 2 seconds to fill the 20 µl chamber (c). Once the blood sample and reagent mixture has been dispensed into the visualisation chamber, cell counts were performed using microscopy and automated cell counting software developed in-house.

4.2 Cell counts and distributions

Figure 7 presents an example of results obtained for both a white blood cell count test (left) and a red blood cell count test (right). In each case the tests carried out for the cartridge are identical, only the reagent in the blister pack differs for each of the tests. For the white blood cell count tests, a total magnification of 100X is used for the microscopy images that are captured and analysed. For the red blood cell count tests, a total magnification of 400X is used. As can be seen from figure 7, for the white blood cell count test (left), the reagent causes lysing of the red blood cells, while the white blood cells stain a dark purple colour. For the red blood cell count test the red blood cells can clearly be seen as small pink cells.

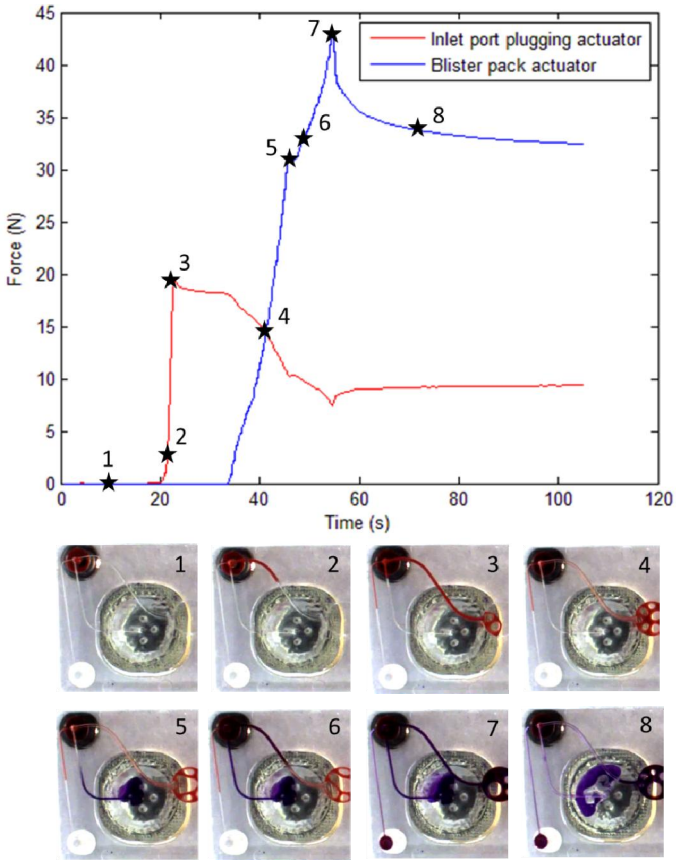

Figure 5. Example load cell data for a white blood cell count experiment, showing sample inlet port force data (red) and blister pack load cell data (blue), with corresponding fluidic operation photos for times indicated (1) to (8). (1) t = 10 sec, before the plug makes contact with cartridge device, (2) t = 20 sec, plugging commences, (3) t = 22 sec, plugging complete, (4) t = 42 sec, blister compression begins, air is released, (5) t = 45 sec, blister ruptures, reagent is released, (6) t = 50 sec, blister further compressed, (7) t = 54 sec, all fluid from blister has been released, and (8) t = 70 sec, blister completely compressed, releases small volume of air from blister.

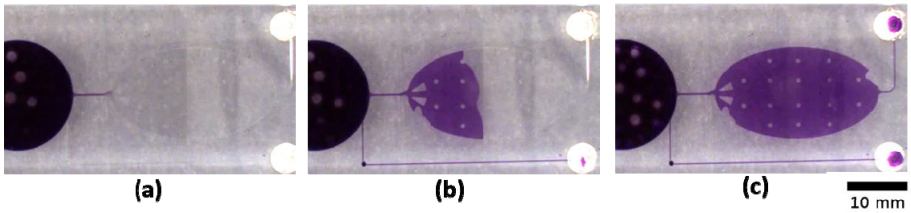

Figure 6. Mixed blood sample and white blood cell count reagent dispensed into visualisation chamber via air actuation at 500 µl/min. (a) t = 15 sec after air begins to be introduced to the system, (b) t = 16 sec, and (c) t = 17 seconds.

For each cartridge test device, cell counts were performed across the visualisation area by selecting sample areas within the chamber at which to capture microscope images for further analysis. A map of the visualisation area with numbered regions indicating the selected areas was drawn up for this purpose as illustrated in figure 8.

The distribution of cells across the visualisation chamber area for each of the microfluidic devices was compared to a reference sample where a pre-mixed and accurately diluted blood sample with the corresponding reagent was manually and directly dispensed into the visualisation chamber of a microfluidic device. No distinct distribution patterns could be noted, and even in the case of the pre-mixed and diluted sample that was manually dispensed into a viewing chamber, the distribution of cells across the visualisation chamber was not uniform. The only noticeable trend in the distribution

results was that the cell counts obtained from each sample image across the visualisation chamber for each test, both in the case of white blood cell counts and red blood cell counts, were much lower than the cell counts in each image across the visualisation chamber for the manually dispensed sample.

For each of the images captured, the volume was calculated using the area of the microscope image captured, and the depth of the chamber as 100 μm. For each image, this enabled the number of cells per unit volume to be counted, which is the standard representation of blood cell counts. From the cell counts carried out for each image, a concentration of cells across each visualisation chamber for each cartridge could be determined. This gives an indication of the accuracy of the cartridge device test and the components of the microfluidic device.

Figure 9 shows the results of cell counts obtained for both the white blood cell test devices (left), and the red blood cell tests devices (right). In each case, the microfluidic device results are shown in blue with a standard deviation indicated across all the cartridges tested (25 white blood cell test devices, 10 red blood cell test devices). These results are compared to a manually dispensed sample of blood and reagent (red bar graph), with precise measured volumes and complete mixing having occurred before the sample is dispensed into the visualisation chamber of the microfluidic device. This is then also compared to haemocytometer results - the gold standard of microscopy to perform manual cell counts (black bar graph). It can be seen that the sample that was manually dispensed into the microfluidic device compares well to the haemocytometer gold standard method, and produces cell counts that are within the normal expected healthy ranges. However, the cell counts obtained from the automated microfluidic device testing are much lower than the standard and reference counts for the same sample.

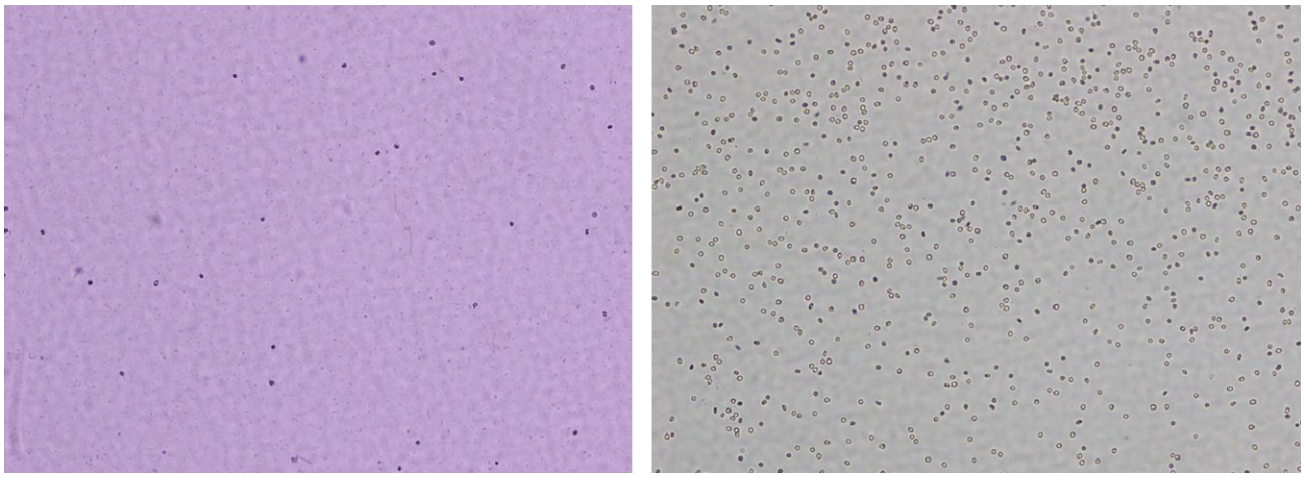

Figure 7. Blood cell microscope images captured for white blood cell count tests (left) and red blood cell tests (right) which can be used to identify and count the number of cells in each image.

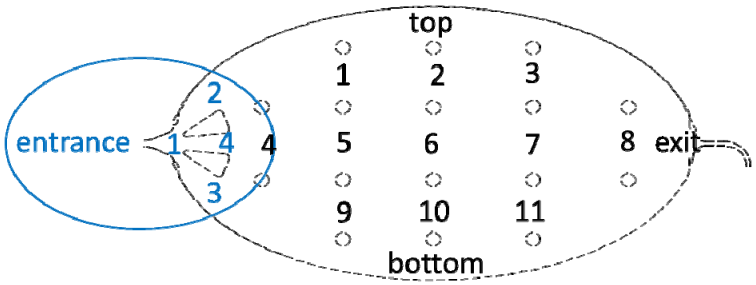

Figure 8. A map of the microfluidic device visualisation chamber, with numbering indicating different selected areas at which sample images were captured.

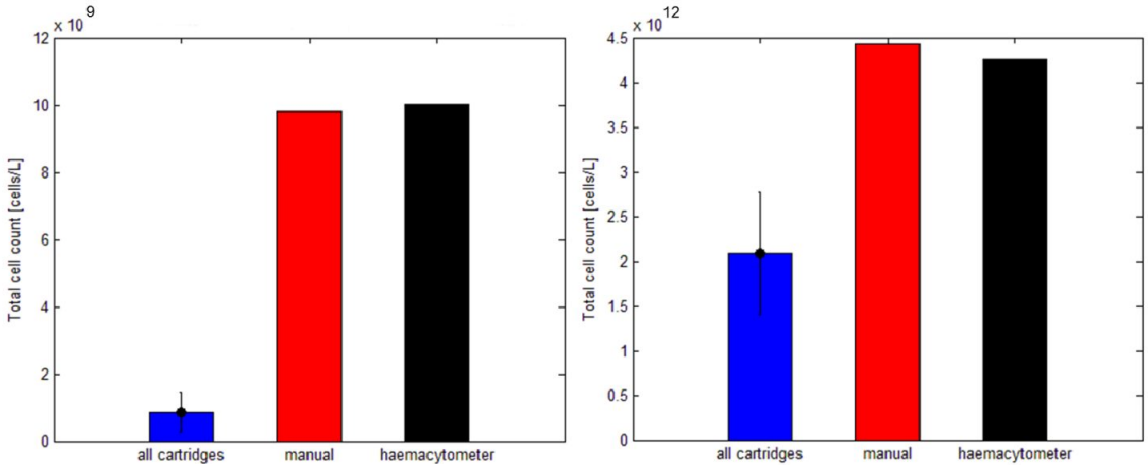

Figure 9. Calculated cell count results for white blood cell counts (left) and red blood cell counts (right) for automated microfluidic devices (blue), manually dispensing of pre-mixed, accurately metered samples into the visualisation chamber of a microfluidic device (red), and comparison to the gold standard haemocytometer results using manually dispensing of pre-mixed, accurately metered samples into a glass haemocytometer device.

DISCUSSION

Microfluidic devices consisting of components for sample introduction, reagent storage and introduction, mixing and dispensing were designed using simple implementations for each component. A number of microfluidic devices for both white blood cell and red blood cell counting were successfully tested using the analysis system that was developed (figure 3). A graphical user interface (GUI) allowed for ease of interaction with the various components and enabled repeatable tests with set parameters to be carried out effectively. Forces and speeds of actuation for sample introduction and blister pack compression over the duration of the experiment were successfully recorded (figures 4 and 5), with corresponding high speed videos of the fluidic operations occurring in the microfluidic devices which could easily be analysed (figures 5 and 6).

The linear actuators provided repeatable actuation of inlet plugging and blister pack release, producing consistent trends in the forces exerted over the duration of the experiments, with small standard deviations across the experiments, as shown in figure 4.

Figures 5 and 6 show that the general principles of the microfluidic device function as expected: the blood is successfully introduced, the blister pack reagent is released, both the sample and the reagent are combined in a mixing chamber, and then dispensed successfully in a viewing chamber. Visualisation and counting of cells via bright field microscopy was effectively carried out.

The reagents for both the white blood cell and the red blood cell count tests were found to be implemented effectively and reacted as expected with the blood samples tested. This can be seen clearly from figure 7 where the white blood cells are stained a dark purple while the red blood cells are not visible for the white blood cell count test. For the red blood cell count test the red blood cells can clearly be seen as pink discs. For each test, the blood sample and reagent were dispensed into the visualisation chamber with no substantial irregularities in the dispersion of the sample in the viewing chamber when compared to the manually dispensed sample.

Figure 9 shows that the cell counts obtained from the microscope images for the microfluidic cartridge devices were much lower than those obtained from pre-mixed and diluted samples that were manually dispensed into a microfluidic device or from a haemocytometer reference. This indicates that too few cells are passing through to the visualisation chamber in the case of the automated microfluidic devices. This could be as a result of either 1) the volumes of blood and reagent that are dispensed are not accurate (dilution factor incorrect), or 2) mixing of the blood sample with the blister pack reagent is not effective or complete.

Basic microfluidic components were used for the initial microfluidic device development to understand the minimum requirements for the device in terms of component complexity and cost. This work shows that these simple components are inadequate for achieving accurate cell counting results, and thus more complex and refined components will need to be pursued to improve the functionality and accuracy of the microfluidic cell counting devices.

CONCLUSION

The initial results show simple fluidic operations from sample introduction to sample analysis for blood cell counting applications, utilizing an experimental set-up to allow for a range of parameters to be investigated. The outcomes of the first microfluidic device design and testing process have shown that the most basic microfluidic components, although effective in terms of complexity and cost, are not sufficient to produce accurate blood cell count results. A next iteration device will be required to address these issues. These components will facilitate the implementation of accurate dilution factors and sufficient mixing of sample and reagent - important requirements towards the successful implementation of a blood cell counting system.

This work provides a foundation for accurate and low-cost point-of-care blood count diagnostic systems such as a full blood count to be realized, with particular relevance and benefit to rural clinics and hospitals, where the optimal balance between function and cost is crucial.

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