HIGH SPEED PLATELET COUNTING BY MICROFLUIDIC IMPEDANCE MEASUREMENT IN DILUTED WHOLE BLOOD

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ABSTRACT

We show results from a microfluidic system for rapid (<300s) platelet measurement in diluted whole blood by flow-through impedance spectroscopy (FTIS). We compare the device performance across a clinical range of human blood samples (60-700 platelets nl^{-1}, normal healthy range 200-300 platelets nl^{-1}), to a hospital central lab haematology analyser. Excellent agreement is obtained with an R^2 of 99.8%, and offset of 4 platelets nl^{-1}.

KEYWORDS: Impedance Spectroscopy, platelets, blood, cell counting

INTRODUCTION

The Full Blood Count (FBC), also termed the Complete Blood Count, is a diagnostic test that measures the cellular composition of whole blood. It is a very fundamental test that is often used as an initial "general purpose" diagnostic tool or as a more targeted monitoring solution. Currently, large scale automated laboratory instruments known as haematology analysers are used to perform all the measurements that comprise the FBC. The high cost and complexity of the instrumentation, together with the need for venous blood and rigorous quality control, means that the haematology analysers described above are mostly restricted to a large scale, centralised laboratory setting. There is a clinical need for Point of Care (PoC) Full Blood Count (FBC) [1, 2]. We have reported on 3-part differential WBC measurements in an identical microfluidic system previously [3]. Several companies have developed microfluidic PoC devices capable of measuring individual components of the FBC [4]. However, the accurate enumeration of platelets in a microfluidic format over a wide clinical range remains a challenge. Platelets determine blood clotting ability, and are an important component of the FBC.

In this paper, we demonstrate that a system with transverse electric fields is capable of accurate platelet measurements in a microfluidic channel wide enough to allow the passage of the largest blood constituents. Figure 1(a) shows a schematic of the system, and figure 1(b) shows photographs of the microfluidic chip.

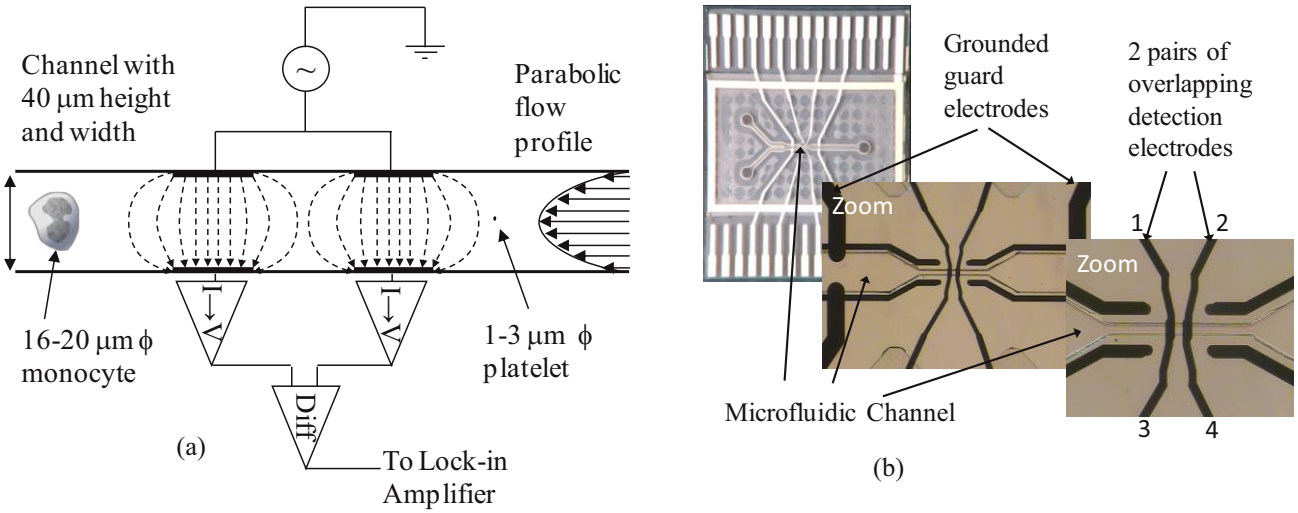

Figure 1: (a) Schematic showing the microfluidic impedance sensor, and the configuration of the detection electronics. The comparative size of the 40µm channel height, the largest common blood cell (monocytes, ~16-20µm diameter) and the platelet size (1-3µm diameter), are shown. (b) Microfluidic FTIS chip in top-down view. Two further zooms are shown on the central sensing zone consisting of four electrodes, of which signal input are on the top (1,2), and two (3,4) for differential virtual ground current sensing are on the bottom of the microfluidic channel.

THEORY

Performing the test in microfluidics reduces the volume of blood required. This will enable finger-prick blood to be used as opposed to the larger volumes of venous blood used by conventional analyzers. We use minimal sample preparation (10,000:1 dilution) that can later be integrated in the microfluidic platform. Dilution enables reliable analysis by ensuring the probability of multiple cells in our detection volume to be <5%. We measure diluted whole blood, so the microfluidic channel (40µm width and height) must accommodate the largest circulating blood cells (WBCs) without clogging (illustrated in figure 1(a)).

Our detection electronics follows the same essential design as that used previously [3], and is shown schematically in figure 1(a). Briefly, the sensing electrodes on top of the microfluidic channel are driven in parallel by an AC voltage

source. The bottom electrodes are connected to virtual-ground current-to-voltage converters, so that the output signal depends on the impedance of the channel. These two signals are then connected to a differential amplifier, so that only the mismatch of the impedance is amplified. This mismatch is due to either the presence of a cell in one of the sensing electrodes, or due to the residual mismatch due to non-idealities in the fabrication process. As a cell travels down the microfluidic channel, first it will cause a peak in one polarity as it passes the first electrode pair, and then it will cause an identical peak in the opposite polarity as it passes the second electrode pair. It is essential to this sensing system that the cells are dilute enough that the probability of two cells entering the sensing volume at the same time, which would cause overlapping peaks, is very low. The output signal is then detected using a custom built lock-in amplifier and a standard data acquisition card (NI-6259-pcx; National Instruments, USA) in a desktop computer. The experiment was controlled by custom written software (LabView; National Instruments, USA). The data was analysed in a custom written computer program (MatLab, The Mathworks, USA). The sample is measured rapidly, so the detection electronics bandwidth must be wide, which increases the thermal noise level. To suppress noise, we correlated the captured signal with a model function, which was chosen to fit large events well (figure 2(a)). This analysis rejects noise, and so small events near the noise level can be detected reliably (figure 2(b)). The volume scale was determined by polymer beads of known size [3].

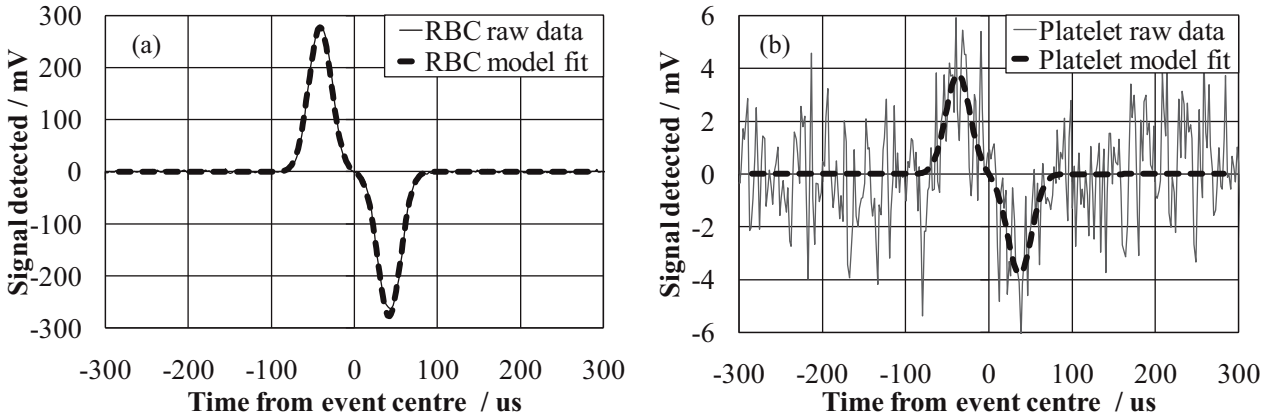

Figure 2: (a) red blood cell (RBC) (~70μm^3) event raw data and fitted model, showing that the model function, which is composed of a pair of anti-symmetric Gaussian peaks, is a good fit to the event data, with a correlation coefficient of typically> 98%. (b) Small (~1μm^3) platelet event raw data and fitted model, showing that the use of a correlation based analysis can reliably extract events even when they are comparable to the thermal noise level, as the noise does not correlate well to the model function.

EXPERIMENTAL

To demonstrate that the impedance spectroscopy system was capable of resolving platelets, they were purified from fresh (<2h old) whole blood obtained from a donor participating in the Philips Research UK donation programme. 2 × 4ml samples of EDTA-anticoagulated, fresh whole blood were transferred to two 15ml centrifuge tubes. The tubes were transferred to a centrifuge and spun at 200rcf for 20mins at room temperature. The sample separated out into three distinct zones, with the red blood cells comprising the deep bottom later, a narrow white band in the middle consisting of white blood cells and finally the platelet rich plasma on the top. The platelet rich plasma (PRP) fraction at the top of the gradient was carefully and slowly pipetted off and transferred to another tube. A stock suspension of platelets at < 500 platelets nl^{-1} was prepared by diluting 40µl of the PRP sample into 10ml of pocH-pack D diluent (Sysmex, Japan). Light scattering flow cytometry analysis confirmed that the purified platelet preparation was 93% pure (data not shown). The FTIS system was cleaned in 4M NaOH, 10% v/v bleach, flushed, and primed in pocH-pack D diluent before each sample run. The excitation signal was 500.3kHz and 8Vpp. At least 200µl of the sample was flowed through the chip at 1.5µl s^{-1} with computer data acquisition and analysis. Blank controls were always performed before each measurement run to ensure the system was free of debris.

To test the system with whole blood, we obtained five clinical EDTA-anticoagulated venous whole blood samples with platelet counts over a larger concentration range (60-700 platelets nl^{-1} compared to normal range 200-300 platelets nl^{-1}). These were sourced from University College London Hospital (60 Whitfield Street, London W1T4EU) and measured using FTIS. We pipetted 1µl of whole blood into 10ml of pocH-pack D diluent, to obtain a stable, debris-free sample. Note that serial dilution could not be used as repeated pipetting steps resulted in very unstable platelets (data not shown). This was measured in the same way as previously, except that the sample was flowed through the chip at 0.67µl s^{-1}

RESULTS AND DISCUSSION

Figure 3(a) summarizes the results of the platelet enrichment experiment. The number of FTIS platelet events scaled linearly with relative concentration. This data confirmed that; (1) it was possible to resolve platelet events using impedance spectroscopy (2) the FTIS instrument was capable of producing meaningful absolute platelet counts over a clinically relevant range and (3) the ability to discriminate platelets from noise and background debris in the system was sufficient to give a very low offset.

For the clinically sourced whole blood samples, shown in figure 3(b), comparison between the absolute platelet counts obtained from the FTIS system and the haematology analyzer revealed excellent linearity with R^2 =99.8%, and an offset of 4 platelets nl^{-1}. Blank control runs showed a background count of (2.9±0.3) platelets nl^{-1}. The small offset indicates that the diluent was largely free of platelet-sized debris, and that our ability to discriminate the platelets from the noise is good. However, the fit revealed that the FTIS setup was consistently under-counting the platelets by 12% relative to the reference method. A similar undercounting is present for the RBCs also (data not shown). As both the platelets and red blood cells are undercounted, and both show such good linearity we believe that a systematic error is the main cause of error. A possible source of this systematic error could be the single step dilution step (used to obtain platelet stability).

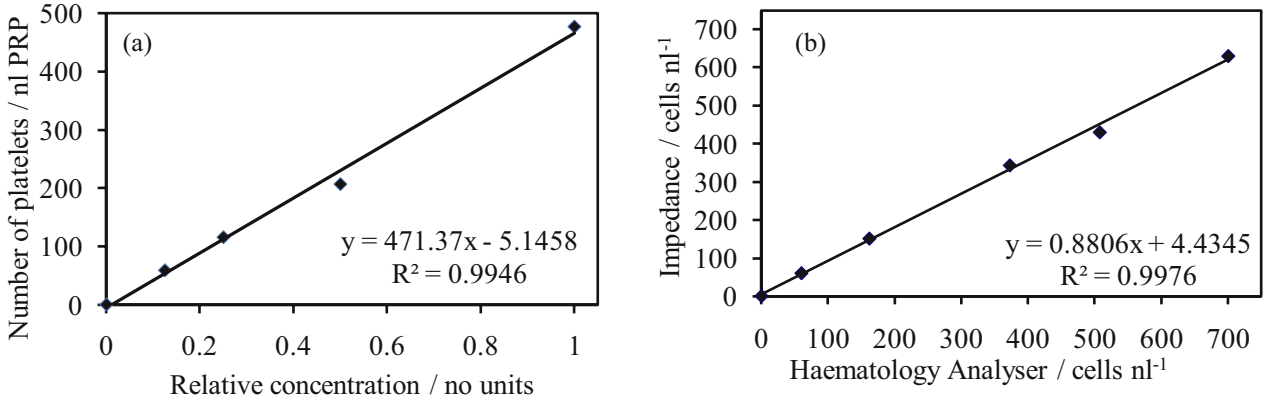

Figure 3: (a) Number of platelets detected by FTIS for a series of diluted platelet rich plasma samples. (b) A series of clinically sourced, diluted human whole blood samples, showing excellent linearity between the absolute platelet counts obtained from the FTIS system and the hospital central lab haematology analyzer.

CONCLUSION

We have described a system which uses flow-through impedance spectroscopy to measure platelets in diluted whole blood, in a few minutes. We have shown that the results are in excellent agreement with those from an automated haematology analyser for samples covering a clinical range with a R^2 value of 99.7%, and only a small offset of 4 platelets nl^{-1}. Work on white blood cell differentiation using a nearly identical FTIS system has been presented elsewhere [3]. Together, these represent the most important parameters of the FBC test, enabling further work to integrate them into an automated microfluidic cartridge. This is a significant step forwards in developing a Point of Care haematology analyser.

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