Point of Care Platelet Counter

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Abstract

To answer the demand for faster turnaround times between sample collection and results in medical testing, haematological point of care devices have been rising in popularity. In this work, a proof of concept for an automated visual method of detecting platelets in images as a basis for a point of care device is presented. This work presents the design and development of the optical system needed to acquire the images, the sample treatment necessary to prepare the glass slides, the template-matching-based algorithm for object detection, and a preliminary analysis of the correlation, precision and bias of the two methods.

The first method, Manual counting, has the technician manually count each acquired image for platelets, while the second method, Algorithm counting, makes use of the template-matching algorithm to perform the object detection step. Both these methods will be compared to reference values obtained through traditional blood analysis methods.

Both methods showed a very strong positive correlation with the reference, as well as acceptable imprecision and bias values. The developed optical system performed up to standard and the acquired images had high enough quality to be used in both assessments. This work has proven to be a positive first step towards the development of the first point of care platelet counter.

1. Introduction

To answer the demand for faster turnaround times between sample collection and results in medical testing, haematological point of care (POC) devices have been rising in popularity. POC devices usually require low power consumption, since they're low in weight and small in size [1, 2]. They're also more patient-friendly and cost-friendly, as they require a smaller amount of samples and reagents to deliver the same test results as traditional alternatives. Furthermore, their short turnaround time makes them especially useful in cases where the patient needs constant monitoring since they more accurately reflect the stat of a patient at the moment.

Most POCT devices related to haematology are related to haemoglobin concentration measurements, with some being used for red blood cell indices, as well as white blood cell count [2]. Haematology assays are of the utmost importance when it comes to evaluating one's health state, as they are one of the main resources used by medical professionals to detect health anomalies. However, the typical blood analysis procedure can both be time and labour consuming, and prone to the same errors as traditional laboratory testing, not to mention it requires the existence of such a facility, to begin with [3].

In this work, a proof of concept for an automated visual method of detecting platelets in images as a basis for a point of care device is presented.

Platelets, or thrombocytes, are small, non-nucleated blood cells, whose functions are essential to the normal process of hemostasis. They are small biconvex oval cells with diameters varying between 1.5 μm to 3.5 μm, with numbers in circulating blood varying between 150 and 450 Giga platelets per litre of whole blood. In a blood spread, platelets tend to partially clump together, making their shape

hard to distinguish, even with a proper staining technique. Most image-based systems use brightfield microscopy to identify the specimen.

As the name suggests, brightfield microscopy is based on the fact that a bright viewing field will contrast with the specimen placed between the microscope's light source and objective lens. This type of microscope is simple to use, however, most specimens will require staining to be properly identified since brightfield microscopy doesn't alter the colour of the specimen. Furthermore, brightfield microscopy can be coupled with digital imaging to obtain high-resolution images of the stained specimen. The main disadvantages of the brightfield technique are the inherently low contrast without staining, the need for a strong light source which may cause heat damage to the specimen, and the fact that the user needs to be knowledgeable in proper staining techniques.

2. Methodology

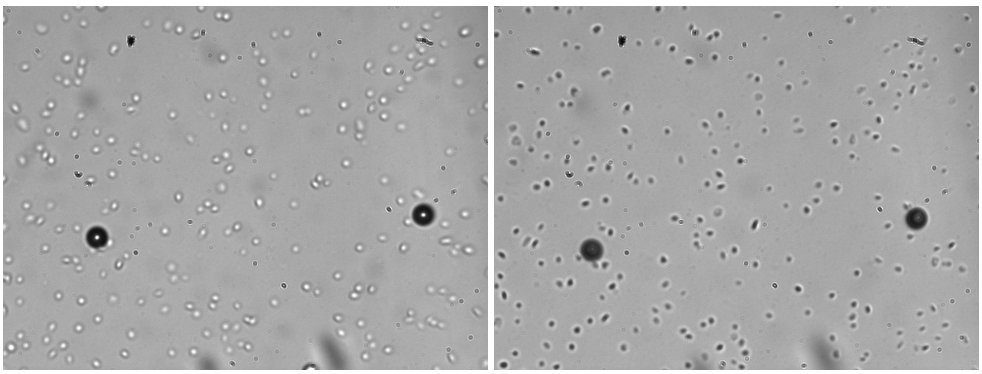
For this project, the optical brightfield microscope had a magnification of 20x. The eyepiece will consist of a singular achromatic aspheric lens, with a focal length, f, equal to 4.5 mm and a 3 mm diameter; the illumination source is planned to be either green, violet, or red light emission diode (LED), and to focus the light in a singular beam, two condenser lenses were placed between the specimen and the LED. All images will be recorded using a Chameleon USB2 (ICX445 Mono). The initial simulation analysis using the OSLO EDU software proved that the lens performance would be as expected and that there were no optical aberrations present in the planned lens design. For the colour of the LED, originally there were 3 options of colour for the LED light: red, green, or violet. In this work, there's a clear improvement in image quality as the LED's emitting wavelength decreases. The shutter speed of the camera increases as the wavelength of the LED increased, meaning the required time for an image acquisition also increases. Thus, the LED chosen for the optical system was the violet one.

The venous blood samples used had a complete blood count report associated with each of them, which had been performed with traditional blood analysis techniques. Blood samples were prepared using a solution containing 90μL of sedimented blood plasma and 10μL of a microbead solution, then 10μL of the final solution were placed on a glass slide to be observed under the optical system. Two replicants were created for each sample used.

The algorithm chosen for this proof of concept is a simple template matching algorithm. It was implemented in python used the open-sourced computer vision library, *OpenCV*. The algorithm is based on the premise that a template is compared to a base image in every pixel in the image. The output of this comparison is a map of similarities, where the higher the score, the higher the likelihood of your template being in that pixel. For this work, 5 templates created from 5 platelets were used and each template was compared against the image twice: once at full size, and once at 95% of the size. To be considered a match, the similarity score had to be above the predefined threshold, 0.85.

3. Results and Discussion

After the introduction of the microbeads into the sample to be observed under the microscope, there were two possible planes of focus: one where the focal points are the platelets, which will be referred to as the focus plane, and one where the focal points are the microbeads, which will be referred to as Off Focus, due to the fact that the platelets are off focus. For the case of Focus type images, all the objects that were small, dark, and of circular shape, were considered to be platelets. On the other hand, for the Off Focus type images, the objects that had a bright centre, a round shape, were small, as well as had a distinguishable border were classified as platelets. The main differences between the two types of images were since platelets were still in motion inside the glass slide, plus the fact that debris could be easily mistaken for a platelet for the case of Focus images. Furthermore, the use of microbeads as a reference focal point allowed the plane of focus to remain constant throughout the different replicants. Thus, the Off Focus images were used for the remainder of the project.



(a) Example of an Off Focus Image **(b)** Example of a Focus Image

Figure 1: An Off Focus and Focus Image captured in the same FOV

To evaluate the correlation between the reference value and the number of platelets seen in each field of view, 9 samples with reference values ranging from 152 to 397 Giga platelets per litre of blood were used. The average of all counts was used to infer a correlation between the average number of platelets per field of view and the reference value. For this purpose, objects that were classified as platelets had to be small in size, circular, and with a bright centre surrounded by a darker border; they couldn't be located beneath any of the debris present in the image, and they couldn't be located in shaded areas.

Table 1: Average platelet count per field of view manually determined, compared to the reference value.

Sample	Reference (x10^9PLT/L)	Average (PLT/FOV)
1	152	108.7
2	182	119.6
3	246	163.7
4	268	181
5	280	198
6	306	213
7	338	232.9
8	360	261.9
9	397	263.3

With these criteria clearly defined, each FOV for each sample was counted and recorded in a table for further analysis. The results obtained have a Pearson's correlation value, R, equal to 0.9915, which implies that there's a near-perfect positive correlation between the average number of platelets seen per FOV and the platelet reference value of the original sample, for the manual method.

For the algorithm method, the images and samples used were the same, the difference lies in the fact that each image was processed by the template matching algorithm, instead of being analysed by someone. Almost all objects identified as platelets through manual counting have been identified as platelets by the algorithm. However, the more noticeable exceptions were the microbead added to help find the right focus plane was also classified as a platelet by the algorithm; objects that were too close together, or overlapped partially, were not classified as platelets, and objects present in shaded areas were classified as platelets by the algorithm. Nonetheless, there was a strong positive correlation

between the average number of platelets seen per FOV and the platelet reference value of the original sample.

Table 2: Results obtained for images after using an object detection through template matching algorithm.

Sample	Reference (x10^9PLT/L)	Average Algorithm
1	152	104.7
2	182	123.3
3	246	142.6
4	268	191.9
5	280	213.8
6	306	197
7	338	225.6
8	360	254.3
9	397	241.9

Both methods showed promising correlations, as well as acceptable values of precision and bias. Although 2 replicants were used per sample and the samples covered a clinically meaningful range, the number of samples used was too low. The Australasian Association of Clinical Biochemistry [4] recommends the use of at least 40 samples between both methods and, even including the replicants, this proof of concept out contained 9 samples. Furthermore, the discrepancy between the manual method and algorithm method is also due to the fact that there was no outlier treatment performed. As mentioned previously, the algorithm couldn't properly identify platelets in scenarios where the microbead was too close to the edge of the FOV. If these instances were to be removed from the analysis, then it is highly likely that the correlation values, standard deviation, as well as coefficient of variation would improve and, perhaps, make this difference between the two methods smaller.

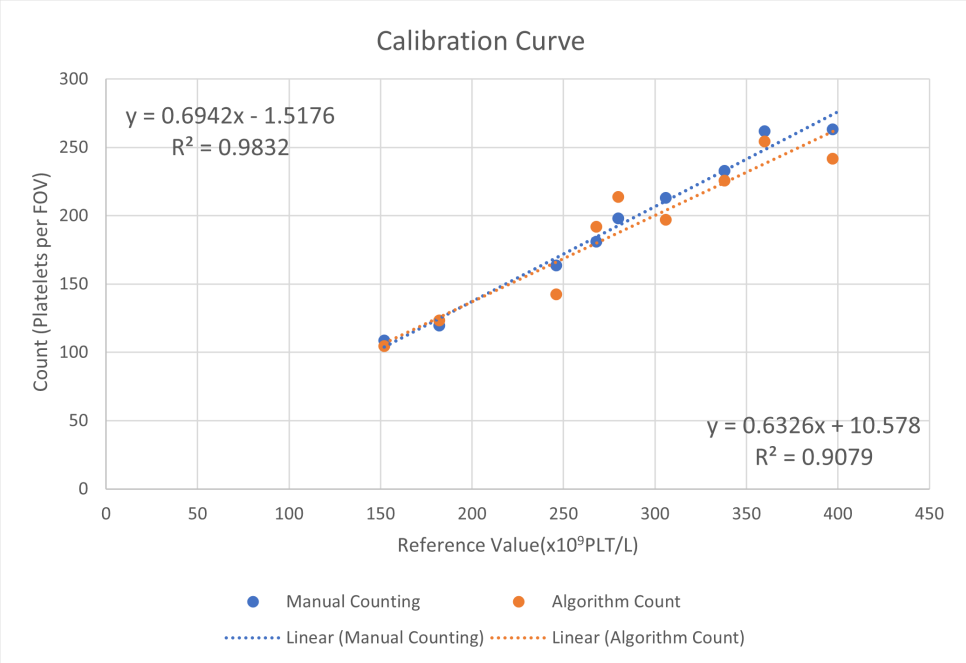


Figure 2: Calibration curve obtained for both manual and algorithm methods. On the top right corner is the estimated equation for the manual method. On the bottom left corner is the estimated equation for the algorithm method.

4. Conclusion

In conclusion, the optical system is small, cheap, and easily recreated in other environments. The optical aberrations noticed in the image analysis didn't change the fact that both through manual object detection and template matching object detection there was a positive correlation between the number of platelets detected and the original sample value. This work was also demonstrated to be precise and unbiased in its results, as well as easily reproducible. Furthermore, it fits the criteria of a PoC device: it is small and only requires a small sample to perform its work, making it very cost and patient-friendly. Although this particular device still has a long journey ahead of itself, these are positive first steps towards a brighter future.

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