Image Analysis Based Hematocrit measurement on a Centrifugal Microfluidic Point-of- Care Platform using a mobile phone

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Abstract

The Objective of this Work is to develop a centrifugal microfluidic system for determining haematocrit fully automatically within in a few minutes, only. Measuring of the haematocrit is performed using a mobile phone, by an app developed during this thesis.

In order to achieve this, a fully integrated cartridge where blood can be loaded from a finger prick, separated into a plasma and a cellular fraction and analysed by a mobile phone was developed. All fluidic operations such as metering and blood separation were performed by a custom built setup to spin the microfluidic cartridge. Initially, different bonding methods were evaluated to determine the one most suitable for this application. Furthermore, bonding methods were evaluated regarding suitability for large scale production. Following this, microfluidic structures for sample metering and blood separation were designed, manufactured and tested.

Next, an android app was developed for analysing the blood samples and calculating the hematocrit value. The algorithms of the application were developed in Java® and run on the Android® platforms. In order to measure the hematocrit, an image of the cartridge was acquired following separation of the cellular components from the plasma. Using Image analysis and Image recognition, the hematocrit was calculated. Finally, the same algorithm was used to determine whether pre-stored reagents in the microfluidic cartridge were dissolved according to protocol, thus allowing for a built in quality control using image recognition.

In summary, this work presents a fully integrated and automated system for measuring the hematocrit of a blood sample, using a mobile phone for image analysis.

Keywords

POC — Hematocrit — Mobile Phone Measurement

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Contents

Introduction 2
1 Fabrication and experimental Setup 2
1.1 Disc Design 2
1.2 Micro milling 2
1.3 Hot Embossing 2
1.4 Laser machining 3
Alignment and disc assembling
1.5 Centrifugal Stand 3
1.6 Smartphone Setup 3
2 Bonding methods 3
2.1 Tape characterization 3
Assay compatibility • Peeling test • Contact angle
2.2 Results and Conclusion 4
3 Blood Loading 5
3.1 Coatings 5
3.2 Results 5
Experiments with hydrophilic surface coating in a new design
3.3 Conclusions 5
4 Hematocrit measurement 6
4.1 Structure for blood loading separation 6
4.2 Structural algorithms 6
4.3 First results 7
4.4 First application 7
4.5 Second application - “Left-and-Right algorithm” 8
4.6 Algorithm improvement - “Area” algorithm 8
4.7 Results 8
Error in blood loading • Results from error in blood loading
5 Optical analysis of bead resuspension 9
5.1 Results 10
Introduction

Microfluidics is the science and technology of manipulating and controlling fluids, typically in the microliter range of volume, in networks of microchannels with very small dimensions. Recently, it has been developing a lot due to the increasing microscale analytical chemistry techniques, as well as the development of microelectronic technologies. Microfluidics hardware requires different construction and design from macroscale devices. It is not possible to just downscale macro devices and then except them to work, at the microscale fluids flow behaviour is different from the macroscale. There are other forces and variables involved, such as microscale heat transfer and mass transfer. [1] [2] The Lab-on-a-disc (LOD) is a centrifugal microfluidics platform, that uses the forces in a spinning system to move and manipulate liquids. LOD rotationally controlled to integrate and automate analysis and synthesis protocols in life sciences [3]. The way the platform works offers a wide range of available operations and a flexible device technology, that allows a rapid development of new diagnostic methods.[4]. LOD is particularly well suited for Point-of-Care (POC) applications since it is very user-friendly, does not need any failure prone components such as pumps or tubing, and can perform a wide range of operations. In this type of fabrication, it is also advantageous particle handling, in case it is wanted particle sedimentation. This type of centrifugal actuation is also independent of pH and viscosity, which can be an advantage, depending on the type of sample being handled. Finally, each device being in contact with the sample is later discharged, hence the possibility of contamination is reduced. [5] On LOD, Real-time PCR [6], viruses detection [7] or Biochemical assays [8], as well as immunoaassay experiments [9] have been performed.

This increasing interest in these platforms is due to the advantages that microfluidics has to offer. The time consumption per sample, is reduced, when compared with the traditional way of analysing it. The sample volume needed is also smaller, hence the quantity of reagents is also lower. This results in a reduced final pricing of the technology. Besides, it is possible to perform task parallelization, that is, achieve different activities at the same time on the device. With this, the risk of user error is decreased, leading to the possibility of a user unfamiliar with the technology being able to perform the testing. At a reproducibility level, this technology has also an advantage towards the traditional way, as there are not so many intermediates evolved on the handling and analysis of the sample, making it an easier and quicker performance.

1. Fabrication and experimental Setup

1.1 Disc Design

The first step in designing a microfluidic device, is to define which functions it should fulfill and in which order. This is then broken down in individual steps and microfluidic structures are designed to fulfill the individual tasks.

Microfluidic depends a lot on how the design is made, since this will determine how the fluids are guided throughout the device.

All microfluidic chips are designed in a 3D computer-aided design (CAD) software (Solidworks (SW), Dassault Systemes®, France). Designing the full fluidic network in 3D allows already at an early stage to visualize how the final chip will turn out.

Several disc designs were made, either for fluidic testing, sample loading or for testing the bonding strength of different materials.

The materials of the discs containing the designs are usually made of Poly (methyl methacrylate) (PMMA), which is a strong and lightweight transparent thermoplastic. It also has a good impact strength, higher than glass.

1.2 Micro milling

The first step towards turning the microfluidic design into a microfluidic chip is to produce an aluminium master which then is used to transfer the microfluidic features into polymer. A Mini Mill 3 (Minitech®, USA) machine is used for this step. [10]

First, the designs previously made in SW are saved in a specific file format, which are files that extend to a 3-D graphic file, that is, files that are used to store 3D image data. Then, a program is responsible for creating the tool paths for the milling process and enables the simulation of the milling process using different types of tools. It simulates how the milling will end-up being, the duration of 3 mm end mill usage (usually used for roughing), or a 1 mm end mill (used for smaller and more complex designs). Usually, the 3 mm end mill is used firstly, with the primary objective of removing great amounts of unnecessary material, that is not needed for the final design. After this degradation, a thinner end mill of 1 mm is used to mill the final design, as this tip is more sensitive and precise. This final polishing enables the surface to be smoother and flatter, enhancing the performance of liquid flowing, when tested. All masters are milled in 3 mm thick aluminium plates. This aluminium alloy plate (AlCuMg1) is a solid basic material, with good machinability, good corrosion resistance and high tension, which makes it a good base for the milling process. The milling process takes several hours, and in the final step, the surface is polished by removing a very thin layer of material at a low feed rate. Finally, after milling the surface is cleaned with acetone and an ultrasonic bath, and after that it is ready for using.

1.3 Hot Embossing

After all the procedures of milling the new design, the aluminium master is used to produce chips containing the microfluidic network via hot embossing. The embosser is a hydraulic press that consists of two pre-heated plates aligned vertically. A digital temperature controller controls the temperature of both plates separately, up to 250°C. Initially, a
When designing a disc, usually alignment circles are made whenever the user wants, with steps that can equally differ in well as the other discs that will be assembled together with. The Centrifugal Stand is a fundamental tool for developing platform. The phone setup, with known conditions (distance to cartridge, Korea) was used as the platform, which runs the Android R.

For the main goal of this project, the hematocrit detection and count, a smartphone (Samsung Galaxy Grand Prime, South Korea) was used as the platform, which runs the Android R platform.

2. Bonding methods

Once the microfluidic structures are transferred to the polymer substrate (e.g. via hot embossing, 1.3), the channels and chambers need to be sealed. This is typically done by bonding the structured substrate to a flat polymer substrate using an adhesive layer. Bonding strength and bonding quality are always decisive factors when choosing a bonding method. It is important not to have any leaking when running tests, especially when processing potentially infectious biological samples. The bonding surface that interacts with the microfluidic surface, should be chemically stable and compatible with the substances that will interact with it, otherwise it can degrade or change some of the sample proprieties and compromise the assay. Optical properties of the bonding material are also crucial in microfluidics, mainly when using assays which rely on optical readout (e.g. fluorescence or absorbance).

2.1 Tape characterization

This project focuses on indirect bonding, especially using pressure-sensitive adhesives and thermally activated adhesives.

The main goal of the tests in this chapter was to assess which tape would fit best for a microfluidic cartridge device.

The tapes were characterized regarding their contact angle, assay compatibility, and on their bonding strength.

2.1.1 Assay compatibility

Assay compatibility is the ability of an external material to perform with an appropriate response from the host in a specific application. Microfluidic devices are used for testing biological samples, usually blood. In this context, biocompatibility can be applied to materials that are in contact with it. Concerning the bonding material, it is also in contact with biological material, on one side, so it also needs to be compatible with it, in order not to change properties of the sample, so that the analysis in the end are not altered.

The assay compatibility (biocompatibility) of the tapes was assessed by performing an experiment, in which the concentration of HbA1c, glycated haemoglobin, is measured.
protocol was performed initially using a tape already in use, and that is known to be very biocompatible, and then the other experiments using the other tapes were made, and compared with the known tape, as a control. For each concentration of HbA1c, three tests were made for each tape, so in each agglomerate on the graph, there are six different data points, three for the control tape and three for the tape being tested. The assay compatibility of a tape is assessed by measuring how much the measured results for each tape deviate from the results obtained using the reference tape.

![Image](image1.png)

**Figure 2.** Example of a result of a biocompatibility test

After performing all tests, the next step was to compare the obtained results with the measurements using the reference tape.

**2.1.2 Peeling test**

The strength of the bond an adhesive forms with the substrate is one of the most important feature when evaluating tapes for bonding. Peeling tests examine those adhesion properties, usually by tensile.

In our experiments, the testing was performed at 90° (Figure 3), and with weights being added in fixed balance, due to the lack of a driven motor, usually used. The weights were weighed before the experiment, and were added in fixed time intervals, during the testing of the different tapes, so that all the tapes were subjected to the same conditions.

![Image](image2.png)

**Figure 3.** Tape being tested, using the system described

Performing a peeling test can provide the user some conclusions: firstly, when performing the test, the user can conclude about the uniformity of the bonding film, realizing how the bonding interface interacts. It also provides, after delimiting a threshold that suits the propose of the experiment, if the film has the desirable strength to perform the desired tasks.

**2.1.3 Contact angle**

To properly study the behaviour of materials in microfluidics, it is necessary to analyse different properties and features that can define them and anticipate how they will act. One of those important features is the wetting behaviour of the tapes. This attribute is responsible for the interaction between solid and fluid, and dictates how the fluids flow, inside a microfluidic design. After the image is taken, the contact angle of the drops in each tape are analysed (Figure 4).

![Image](image3.png)

**Figure 4.** Image analysis of a drop. Measurements of the contact angle in a drop are made with the software ImageJ.

Concerning the contact angle analysis, an open source plugin for ImageJ (Contact Angle, [11]) was installed on ImageJ, that was made specifically to measure contact angles. Basically, when run, the plugin asks first to set a reference, that is mark two dots on the image that is the baseline of the drop. Next, it asks to add three more points on the outline of the drop. With this set-up, the algorithm is able to calculate the contact angle of the drop, with the surface

**2.2 Results and Conclusion**

Testing the different properties of the tapes allowed us to have an idea of which ones to use for future assays and experiments. All the tests were made for the tapes being considered. After acknowledging every result from all the tapes, it was possible to reach a final outcome.

In conclusion, and considering all the results from the experiments, tape 92804 was the one which came closer to the actual used tape, PSA. It performed well in the peeling test, holding as much as PSA. It also was very close to the PSA results for compatibility assay. On the other hand, the tape 92804 demonstrated in the contact angle measurements that it is very hydrophilic, with a different of almost 40°, when in comparison with PSA, which can be a problem when working with certain designs.
3. Blood Loading

In microdevices, specially the ones dedicated to analysing biological samples, the way a sample is loaded is very important. Not only for contamination purposes, but also to have the right volume loaded, as well as how user-friendly the device will be. If it is difficult to load the sample, it is more difficult for the consumer to use it and the likelihood of errors increases. Having that in mind, a part of the project was to find ways to help loading the sample, in this case blood, into the microfluidic cartridge device. For these experiments, different blood loading chamber designs were tested to reduce variability, and investigate if any shape or size would have better loading behaviour.

3.1 Coatings

Since the polymer used to manufacture the microfluidic cartridges (PMMA) is not very hydrophilic, capillary loading required the application of a hydrophilic coating. During the course of this work, P100 (Jonsman Innovation ApS®, Denmark) has been tested. The coating is dispensed as a liquid on the polymer surface and after evaporation of the solvent, a permanent hydrophilic layer remains. The contact angle of the coated surface is about $10^\circ$, making the material very hydrophilic. It also has a very long functional life, which is very important when taking into account the shelf-life of the diagnostic cartridges.

H100 (Jonsman Innovation ApS®, Denmark) is another hydrophilic coating, and has the same characteristics as the P100. The difference is that H100 uses water as a solvent as compared to isopropanol in the case of P100. Using water result in longer drying times, however H100 can be applied to materials which are not compatible with isopropanol.

3.2 Results

The first studies were made using PSA for bonding the cartridges. PSA is hydrophilic, which means that it can aid in loading the sample, even having a considerable contact angle ($\simeq 77^\circ$).

The results brought some concerns. As anticipated, the sample was loaded through capillary force, but it would take a considerable amount of time (Table 1). This would be too long for a point of care product.

<table>
<thead>
<tr>
<th>Channel</th>
<th>Average loading time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2,5,6</td>
<td>32.3</td>
</tr>
<tr>
<td>3,4,7,8</td>
<td>67.6</td>
</tr>
</tbody>
</table>

Table 1. Time results when loading the sample on the cartridges, depending on the chamber shapes

3.2.1 Experiments with hydrophilic surface coating in a new design

The first experiments conducted in this new design (Figure 5) research were done also using the PSA tape (90106, ARcare®, USA). First of all, the chambers were tested without any coating:

<table>
<thead>
<tr>
<th>Chamber</th>
<th>Tape and coating</th>
<th>Loading</th>
<th>Time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PSA</td>
<td>Partially</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>PSA</td>
<td>Yes</td>
<td>$\simeq 45$</td>
</tr>
<tr>
<td>3</td>
<td>PSA</td>
<td>Partially</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>PSA</td>
<td>Yes</td>
<td>$&gt; 60$</td>
</tr>
<tr>
<td>5</td>
<td>PSA</td>
<td>Yes</td>
<td>$&gt; 60$</td>
</tr>
<tr>
<td>6</td>
<td>PSA</td>
<td>Partially</td>
<td>—</td>
</tr>
</tbody>
</table>

Table 2. Results of the cartridge loading, using PSA only

After the results obtained, it was clear that a coating would be necessary to make it easier and quicker to load the sample, so P100 was used with PSA:

<table>
<thead>
<tr>
<th>Chamber</th>
<th>Loading</th>
<th>Sample in next chamber</th>
<th>Time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Partially</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>Yes</td>
<td>No</td>
<td>$\simeq 20$</td>
</tr>
<tr>
<td>3</td>
<td>Partially</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>Yes</td>
<td>Yes</td>
<td>$\simeq 34$</td>
</tr>
<tr>
<td>5</td>
<td>Yes</td>
<td>Yes</td>
<td>$\simeq 37$</td>
</tr>
<tr>
<td>6</td>
<td>Partially</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Table 3. Results of the cartridge loading, using PSA and P100

As it can be seen from this first results, with only PSA, the sample would take too much time to load, or would not load at all the entire chamber. With the use of P100, the loading was more effective.

3.3 Conclusions

Starting with the experiments done from using PSA only, loading the samples would take a lot of time, which is against the principles that want to be applied: quick loading, for better and easier use for the users. In order to try to improve loading, two different coatings were tried out: P100 and H100. The overall results were better, in terms of time, although some other issues appeared, such as the sample advancing to other fluidic structures before spinning the cartridge.

In conclusion, after considering all the results, the chosen way of loading the sample was with PSA plus P100, and using chamber 2 as the standard structure (Figure 6).
Hematocrit measurement is a vital part of any blood analysis. The Hematocrit value is defined as the ratio of volume of red blood cells to total blood volume. The percentage of hematocrit can tell a lot of a patient's health, like possible diseases concerning blood, such as anemia.

The goal of this chapter is to develop a simple and user friendly method to integrate hematocrit measurement on a centrifugal microfluidic platform. To this end a mobile phone app has been developed which captures a picture of the blood following centrifugal separation and automatically calculates the hematocrit value.

4.1 Structure for blood loading separation
A blood sample has a lot of different components. Each component has distinct density, which can be very useful when concerning the calculus of the hematocrit.

Firstly, when the blood is loaded, it has homogeneous colour, just like when it is taken from an individual.

When the microfluidic cartridge is run in the microfluidic stand, the blood starts to separate, as the denser particles tend to migrate more, due to their heavier weight.

Finally, after an stipulated amount of time, the blood is completely separated. On the outer layer lies the red blood component of the blood (the layer that will be read), while the remaining of the readout chamber is filled with plasma.

With this separation, it is possible to have a more concrete RGB analysis.

As it will be shown, a waste chamber was also designed, in order to make sure that the readout chamber is always completely filled.

4.2 Structural algorithms
In Java®, and later in Android®, the image analysis makes use of RGB values.

For better understanding this type of image analysis, an image must be considered as a matrix. Each pixel is one element of the matrix, with a pair of coordinates:

\[
\begin{bmatrix}
(i-1, j-1) & (i, j-1) & (i+1, j-1) \\
(i-1, j) & (i, j) & (i+1, j) \\
(i-1, j+1) & (i, j+1) & (i+1, j+1)
\end{bmatrix}
\]

With these coordinates, it is possible to access all the pixels of an image, by knowing their location. Starting with this approach, the first algorithm developed used this concept: first read the red cell pixels, and then the plasma pixels. With known values of the red, green and blue channels, the algorithm finds the respective pixels, searching the whole image for each of the regions, blood and plasma. The cycle starts searching the image horizontally, line by line.

One of the main issues was that, with this RGB thresholding, some pixels in the target areas would not be recognized and therefore would not be counted.

As it can be seen in Figure 7, there are some non-counted "holes" in the middle of the recognized region. These pixels are not counted for the final ratio, but they are part of the target area, hence the result would not be accurate. In order to solve this problem, the algorithm was developed further.

The algorithm was developed and is introduced in the analysis, after the recognition and colouration of the target areas. What the algorithm does is, runs the image again, but this time is not looking for an interval of RGB values, but for a certain RGB value, red value (255, 0, 0), which was the value given on the algorithm before for the found pixels. After running the image, some conditions were added, to solve the holes that sometimes appear. The first approach was to imagine the above matrix with the middle pixel not being red, and all the surroundings being red. Immediately the algorithm turns that pixel red and adds it to the final count.

The main problem with this part of the algorithm was that it would take a lot of time, as the number of cycles would be too high, for a mobile phone based platform. A new version of the algorithm consisted of the image being run in squares. That is, the image was run in squares each time, and after doing the whole square, again in lines in the horizontal, it would go to the next one, horizontally too. The goal of this approach was to realize how many coloured pixels were in the squares.

On the same algorithm, different sizes of squares were performed, as the holes may take different sizes. When a square was run, and for instance, if it was a 20x20 pixel square, the total number of pixels is 400. Then the algorithm states that if at least 350 of those pixels is the target red, then the remaining 50 are also turned into red, and added to the final count (Figure 8).
With the different sized-squares, all the holes are eventually filled, and the previously non-counted pixels are added to the final count, making it more accurate. Concerning the plasma count, it was performed the same way, but with different RGB values. As plasma is neither white nor yellow, the RGB arranged values were a range of values between both colours. Then, the same squared-algorithm was performed. It was also observed that sometimes pixels were counted outside of the readout chamber (contamination on the cartridge, painting, leaked sample, obstructed sample), which would change the final percentage. To solve the problem, a modified version of the previously described algorithm was used. After running the squares algorithm, another version of it was ran, where it searched again for red pixels, but using different settings. If there were less than a certain number of red pixels inside that square, then those pixels would return to their original colour, and would be discounted on the final count.

4.3 First results
Having the structural algorithms ready and functional, a first android application was created.

To first test the accuracy of it, a printed calibration disc, with known ratios of red and yellow areas was used in order to test the quality of the application (Figure 9).

As it can be seen, the results obtained by the algorithm from the printed test disc are very close to the real ones ($R^2 \approx 0.99$). The difference between percentages might be due to lightning, distance to the chamber, or the angle.

With this calibration, a first android application was created using the Java code.

4.4 First application
The first application created allowed the user to take a photo or load a previous photo, and then analyse it. After the image analyses, the user can also save the photo.

With the application ready to be tested, several cartridges with different concentrations of hematocrit were tested. In order to have different concentrations, and as blood samples were not always available, a blood sample was centrifuged, and part of the plasma was then used to create a dilution series of the original blood sample. Samples of each dilution were loaded into the microfluidic disc and the test was run.

Several cartridges were made, and different measurements were made for each dilution. The results are as follows:

As it can be seen, the results are very similar to the real ones calculated with ImageJ. A $R^2 = 0.977$ is a very close value to 1, which indicates a very good correlation between calculated and real value.
4.5 Second application - "Left-and-Right algorithm"
Although the previous results were already very close to the real ones, with all the cycles running during the analysis, the duration of the measurement was still above one minute. Therefore, the next task was to optimize the algorithm to perform the calculation faster.

Instead of counting all the target pixels, and then filling them in, this new approach takes into consideration only the height of the red blood cell layer. Then, it compares with the height of the readout chamber, avoiding then the performance of new cycles, to target the plasma area. With this, the time performance is reduced.

Afterwards, the image is ran again one time, in order to find where the previously found red pixels are. While doing this, it starts collecting the X-value of the pixel more on the right, and the X-value of the pixel more on the left. That is, concerning the pixel more on the right, it starts by saving the X-value of the pixel more on the right of the first line read. The, while running the different lines, if there is a red-pixel more on the right when compared with the saved one, it replaces the former value with this one. It does the same with the left pixel, and keeps replacing the values on the next lines, if the values are more on the right or more on the left.

Furthermore, each time that pixel coordinate value is modified, also the Y-value of that pixel is saved, as it will be needed later on, for both right and left pixels (Figure 13).

![Figure 13. Calculation of the height of the red blood cell high, after knowing the distance between the two pixels, and the vertical distance between them](image)

The height in pixels of the chamber is known, and uniform, as it is always fixed the distance and angle of the phone towards the cartridge. By doing the ration between the height in pixels of the read area found by algorithm, and knowing beforehand the height in pixels of the total chamber, it is possible to know the ratio between the two heights, hence the percentage of red-blood cells present in the sample. Finally, with these both calculated distances, it is possible to know the height of the layer, using the Pythagoras theorem.

4.6 Algorithm improvement - "Area" algorithm
After all the experiments, and having such good results, the program was integrated in the system on the device. There was already on the program, attached to the device, parameters that allowed the user to have control over the final position of the cartridge. Having the phone already on a restraint position, a good angle, in front of the camera, was chosen, for the photo taken.

Knowing that the final position would always be the same, image analysis became easier to define. Concerning the image analysis, instead of having to run the whole image, it would only have to run a square around the readout chamber.

![Figure 14. Chosen area to run the algorithms, after knowing the final position of the cartridge](image)

4.7 Results
For the experiment mentioned before, for each picture taken, two algorithms were tested, in order to realize which one would have a better performance.

The first algorithm tested was the "left-and-right" algorithm (App 1), whereas the second tested was the improvement of the first algorithm, concerning pixels areas (App 2).

The results of the cartridges readouts, from the two Apps were as follows:

![Figure 15. Comparison between the two described algorithms, when presented the same images, taken from different blood samples with different concentrations](image)

Through analysis of the linear approach to each app, there are clear differences. On App1, the $R^2 \approx 0.8$ whereas in App2 the $R^2 \approx 0.98$ which is a considerable difference.
4.7.1 Error in blood loading
It is very important when doing measurements in microfluidics, to have precise volumes. When considering a biological sample, reagents or beads, volume control is crucial in order to perform assays reliably and reproducibly. Since it is important to have precisely metered sample volumes to perform subsequent analysis, the program for hematocrit measurement was expanded to assure that sufficient sample was loaded into the cartridge. To this end, the algorithm checks whether there is blood present in the waste chamber, since the absence of blood would indicate that insufficient sample was loaded. If there is no blood in the waste chamber, the app notifies the user that not enough samples was loaded and the test is aborted.

Figure 16. Error in the app, when the loading sample is not enough to fill the readout chamber

4.7.2 Results from error in blood loading
The volume required to fill the readout chamber is approximately 12 $\mu$L, and the loading chambers can handle up to 20 $\mu$L, on the previous showed designs.

Although in the other experiments the loading chambers were filled or nearly filled, in this experiment the objective is not to fill at all the loading chamber, but to load less than the volume of the separation chamber.

Knowing that the volume to be loaded had to be less than 12 $\mu$L, 10 experiments were conducted, and of the 10, all of them did not have the chambers filled, and in all of them this was detected in the App 2, resulting in an error message.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Loading volume</th>
<th>App result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>Insufficient volume</td>
</tr>
<tr>
<td>2</td>
<td>1.5</td>
<td>Insufficient volume</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>Insufficient volume</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>Insufficient volume</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>Insufficient volume</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>Insufficient volume</td>
</tr>
<tr>
<td>7</td>
<td>4.5</td>
<td>Insufficient volume</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>Insufficient volume</td>
</tr>
<tr>
<td>9</td>
<td>6.5</td>
<td>Insufficient volume</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>Insufficient volume</td>
</tr>
</tbody>
</table>

Table 4. App results, knowing that the loading volume would not fill the readout chamber

These results also enhance the precision of the app, adding another important feature to it, the measurement of initial volume loaded into the microfluidic system. Through this development, it is possible to stop running the readouts of an application quicker, giving the user time and perception to change the cartridge and do another loading and measurement. With this, it is avoided time consumption, reagents waste, as well as all the costs involved in operating all the components of the system.

5. Optical analysis of bead resuspension
For a commercial product, beads are stored in the disc in dry form, and are resuspended in buffer when the test is performed. As discussed in the previous chapter, during this project a software for image analysis has been developed to measure hematocrit of a blood sample as well as performing a check that sufficient sample was loaded. The next step is to integrate a function into the software to monitor whether dried reagents are resuspended properly.

Having the same structure and interface as the hematocrit count (HCT) application, this new bead analyser program takes two pictures of the assay chamber, one before running the cartridge, and another after finalising the specific protocol of the assay. Subsequently both images are analysed to determine whether the dried beads resuspended correctly.

Figure 17. Readout chamber for analysing if the beads were well dissolved

5.1 Results
The new application takes a picture before the protocol, where the beads are untouched, and takes another picture in the end, when the beads should be dissolved in plasma. Then, after the comparison, it is possible to know how if the assay worked.

Figure 18. Bead optical analysis, where the beads did not dissolve

Figure 19. Beads optical analysis, where the beads dissolved

With the previous coding, RGB comparison was possible to be made, and through that analyses, it was possible to reach some results.
<table>
<thead>
<tr>
<th>Experiment</th>
<th>Beads, after protocol</th>
<th>App result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Resuspended</td>
<td>Beads Dissolved</td>
</tr>
<tr>
<td>2</td>
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</tr>
<tr>
<td>10</td>
<td>Resuspended</td>
<td>Beads Dissolved</td>
</tr>
</tbody>
</table>

Table 5. Results from the beads analysis, where the beads did resuspend, after spinning

These results allowed the user to already have some certainty in beads resuspension. The experiment made concerned 10 samples of beads that were successfully resuspended in plasma. Although it was only 10 experiments made, the percentage of right results (100 %) can already state that the algorithm is trustworthy and can be usable for future analysis, as well as integration in the full program.

6. Conclusions

This project had a very important and unique purpose. Step by step, from the design, to the manufacturing of the most suitable cartridge, with the right bonding and coating materials, until the setting of the readout platform where the developed algorithms would run, all of this development was done with the clear objective of having a final product to be used in the future.

Beginning with the tape characterization and the choosing of the most suitable one, it was possible to have different results from them. That brought some new enlightening about the tapes at disposal, as this characterization can also be useful in the future, for all the different experiments and assays to be executed.

Concerning the applications, it turned out to be a very effective way of measuring the hematocrit. It was developed a way of quickly and easily calculate the hematocrit of an human blood sample. An alternative way of calculating this measurement. When compared to the traditional way, in this option any user is able to run it.

The nanobeads also had very promising results, that can lead to new experiments in the future.

It is very gratifying to acknowledge that my work will be part of the official android application from the company. It shows how much my work was taken into consideration, and how much my work was trusted.

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References


