A microfabricated platform for chromosome separation and analysis

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Abstract. More and more diseases find their cause in malfunctioning genes. There is therefore still need for rapid, low-cost and direct methods to accurately perform genetic analysis. Currently the process takes a long time to complete and is very expensive. We are proposing a system that will be able to isolate white blood cells from blood, lyse them in order to extract the chromosomes and then perform chromosome sorting on chip. As the physical properties of the chromosomes, such as size and dielectric properties, are needed for designing the chip, we have measured them using an AFM microscope.

1. Introduction
The identification and function of the genes in the human genome is an area where lot of research is being carried out. Of particular interest are malfunctioning genes that more and more seem to be the causes of various serious diseases, whose origin was previously unknown. Genes are particular DNA strands that can be transcribed and subsequently translated into proteins. In a malfunctioning gene, some of the DNA bases are deleted, added or exchanged with other ones. This can happen during cell division, when the chromosomes in the nucleus are copied and condense into compact structures that then separate into two different cells. During this process whole parts of a certain chromosome can be cut off and attached to a different chromosome. This particular mutation is called a translocation and is responsible for the well-known Down’s syndrome.

For translocated chromosomes it is important to find the exact location of the breakpoint. A number of steps have to be carried out before this can occur. First, the white blood cells (WBCs) have to be isolated from a patient’s blood and cultured, so that enough material can be available for further processing. This is currently done by blood centrifugation and incubation for a few days. Then the division of the WBCs has to be stopped in the so-called metaphase, a point in the cell-cycle when the chromosomes are condensed but before they separate into two cells. Then the cells are lysed and a drop of material is placed on a microscope slide along with a fixative chemical in order for the chromosomes to stick on the substrate. A karyotype is made of the chromosomes and a trained lab technician can then identify translocated chromosomes from the banding patterns that are formed. When a pair of translocated chromosomes is found, the samples are sent to a dedicated laboratory where chromosome sorting by flow cytometry [1] is carried out. This process relies on fluorescence signals and can sort two chromosomes at a time. When the sorted chromosomes are returned to the
lab, lab technicians use FISH (Fluorescence In Situ Hybridization) and sequencing in order to find the exact breakpoint.

This entire process usually takes more than a few months to complete, involves at least two different locations and highly trained personnel and is rather expensive. Therefore any system that can reduce the cost and time for each analysis will be of great interest both for genetic scientists and for medical doctors. In this paper we will describe such a system, which will be able to complete chromosome isolation and sorting in a small chip within a few minutes. We take advantage of the physical properties of cells and chromosomes for the purpose, therefore an investigation of these will also be presented.

2. Materials and methods
In order to study the physical properties of the chromosomes such as size and dielectric constant, an Atomic Force Microscope (CP-II SPM system from Veeco) working in tapping mode was used. For the dielectric constant measurements a conducting cantilever with a platinum coating from Budgetsensors (model BS-ElectriMulti75) [2] was used. The principle of the measurement method has been described in [3-5]. The chromosome suspension was prepared from human lymphocytes by the method described in [6]. This ensures a stable stock of chromosomes in hexylene glycol buffer from which the chromosomes can be resuspended in fixative (methanol and acetic acid, 3:1) and deposited on a substrate. Further treatment with pepsin ensured that a minimum of cell debris was present on the surface of the chromosomes.

For the isolation of WBCs from blood a method called pinched flow fractionation, first described in [7] has been used. In short the method takes advantage of the fact that small particles exiting a small channel into a large chamber will follow the streamlines passing through their center of mass. Separation can be achieved by making sure that particles of different sizes follow different streamlines that end up in different outlets. A device for the sorting of WBCs of a particular dimension corresponding to human lymphocytes from other WBCs and from smaller blood constituents such as platelets and red blood cells (RBCs) has been designed, simulated (using Comsol Multiphysics [8]) and fabricated. The fabrication process is very simple: A mould of the channel is made in SU8 2075 obtained from Microchem [9] and PDMS (polydimethylsiloxane) obtained from Dow Corning [10] is poured on the mould and cured at 65 °C for 3 hours. The PDMS cast is then removed from the SU8 mould and bonded to a pyrex wafer using a plasma asher treatment with an oxygen flow of 100 ml/min for 30 s with a power of 100 W. Inlet and outlet holes are then made on the PDMS part of the bonded chip.

After the WBCs are isolated they need to be trapped and lysed in order to extract the chromosomes. A structure inspired from [11] has been designed and fabricated for the purpose. Design considerations and results from our simulations will be presented in section 3.

3. Results
The first step towards a total analysis system for chromosomes (C-TAS) is to make sure that the biological matter does not stick to the surfaces of the microfluidic channels. For this purpose all surfaces have been covered with one or more layers of PEG (Polyethylene glycol) molecules. This makes the surfaces more hydrophobic, as can be seen in figure 1, where the increased hydrophobicity is visualised by a droplet of water on a pure pyrex and on a PEG surface.

![Water drop on glass](image1.png) ![Water drop on PEG](image2.png)

Figure 1. On a pure pyrex surface the water droplet perfectly wets the surface. When the surface is functionalised with PEG it becomes more hydrophobic.
The second obstacle towards C-TAS is the isolation of the WBCs. For this purpose the structure shown in figure 2 was designed, simulated and fabricated in PDMS. The system consists of two inlet channels, one for the blood sample containing the cells and one for a pure buffer solution, a pinched segment, where the particle solution and the buffer come into contact and the particles can be pressed towards the upper wall, and a separation chamber with several outlet channels. Through Comsol simulations on this structure and considering that we would like to separate WBCs (average diameter of 10 μm) from RBCs (average minimum dimension of 3 μm) it can be shown that for given dimensions of the pinched segment the most critical parameters for the separation are the inlet flow rate ratio as well as the relative hydraulic resistance of the channels. It was calculated that a flow rate ratio of 0.1 between the particle carrying flow (channel a) and the buffer carrying flow (channel b) is necessary for a 40 μm wide pinched segment, as well as a hydraulic resistance which is 7 times larger for the first outlet (top left outlet E) than for the remaining five outlets.

![Figure 2. The chip design for the isolation of WBCs. A is the inlet for the blood, B is the inlet for the buffer, E are the long outlets (the hydraulic resistance from the separation chamber c to E is 7 times larger than the hydraulic resistance from c to the short outlets D) and d are the outlet channels. For the separation, outlet E is used for the first channel and outlets D are used for the remaining channels.](image)

After the WBCs have been collected in the second from the top outlet D (s. figure 2) they need to be isolated and lysed. The isolation is necessary due to the inevitable changes in chromosome sizes between cells occurring when stopping the cell cycle at different points in time in the metaphase. Figure 3a shows the structure designed and simulated for the purpose. The cells entrance and exit is indicated by the two arrows. There are two different structures, a meander and a cell trap. At each intersection the cell has to choose whether to move into the trap or continue through the meander. The structure is designed in a way so that the cell will always choose to move through the trap and as it is bigger than the trap it will stop there and block access for the remaining cells. The next incoming cell will therefore pass through the first meander structure and go down the second trap instead and so on. It can be shown that the system will function best when more than 4 traps are included in the structure. Once the cells are captured, a voltage applied through electrodes placed on each side of the trap will provoke lysis, releasing the chromosomes from the cell.

The chromosomes will then be taken to the sorting part of the chip. Electrical forces (electrophoresis and/or dielectrophoresis) will be used for sorting the chromosomes. In order to find the optimal parameters an estimate of the chromosome dielectric constant, conductivity and dimensions is needed. The chromosomes were therefore imaged by AFM and the permittivity measurements were performed on several different chromosomes with cantilevers working at four different vibration frequencies. The results are shown in figure 3b and indicate that the permittivity is a constant independent on the chromosome height and the frequency of vibration, at least in the kHz range. The length of the chromosomes was in the range of 1 to 10 μm.
4. Discussion and Conclusion

We have shown experimental and theoretical results relating to the fabrication of a total analysis system for chromosome translocations. By surface functionalisation with PEG, sticking of the biological matter on the channels is avoided. The sorting of the WBCs from blood can be achieved without centrifugation by a method called pinched flow fractionation. We have shown that effective separation is possible for a 40 μm wide pinched segment when the flow rate ratio is adjusted between the two inlets. A system where each outlet channel has two possible outlets has been designed and this makes it possible to tune the separation radius further.

After separation the cells are trapped in a special structure where they are lysed by help of electric fields generated by electrodes placed on each side of the traps. The extracted chromosomes are then led to the sorting chip. As electrical forces are going to be used for the sorting, it is necessary to measure the chromosomes dielectric constant and conductivity as well as their dimensions in order to be able to predict the correct parameters for the separation. The dielectric constant was measured for chromosomes of different heights and with cantilevers vibrating at different frequencies. It was shown that the dielectric constant is independent of the chromosome height as well as the frequency of the cantilever vibration (which corresponds to the frequency of the electric field for a dielectrophoresis experiment) in the range of ca. 60 – 300 kHz.

Future work will involve the experimental confirmation of our theoretical results as well as measurements of the conductivity and charge of the chromosomes.

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