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Injection moulded pinched flow fractionation device for cell separation

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In general cancer cells are bigger than most other cells found in blood. This size difference can be exploited to separate and enrich cancer cells from blood for further investigation. One microfluidic method which utilizes size separation is called pinched flow fractionation (PFF). It is continuous and no external fields need to be applied, making it very simple and ideal for processing large samples. So far it has been used to separate polymer beads of different sizes [1][2], and it has been suggested to use it for separation of red blood cells (RBC) from other blood constituents [3], but this has not been achieved yet. Other methods have been used for continuous separation or enrichment of different cells, such as hydrophoretic separation [4] and lateral displacement [5]. While these methods have a high throughput, the enrichment of cells has not been high enough for separation of cancer cells from blood taken from a cancer patient, since the amount of cancer cells compared to other cells is very small. There is a need for a device with a high throughput and enrichment, which is inexpensive and disposable. Some of these challenges were addressed when we designed an injection moulded PFF chip to use for separation of cancer cells from whole blood.

The PFF chip design was injection moulded in a polymer called TOPAS. The chips are equipped with 12 luer fittings connecting the micro channels (figure 1B). The luer fittings can both contain small amounts of fluid samples and be used to attach the chips to a pressure control system. A piece of 500 μm thick TOPAS foil was used to seal each chip. The chips and foil were treated with UV light to get rid of potential contamination. There are two similar PFF devices on each chip; one is shown in figure 1A. The shown device has three outlets, one for cells with a size below a critical radius r_c , and one for cells with a size above r_c . The devices are designed so that it is possible to tune r_c making the chips applicable to many different samples. The last outlet works as a drain for the buffer fluid, thus avoiding dilution of the sample. Since the chips are injection moulded, they are both cheap and disposable.

The chips were used to separate cancer cells from a cell line mixed with diluted whole blood. FACS buffer mixed with BSA was used as buffer to prevent cells from sticking to the channel walls. Pressures in the mbar range were applied at the two inlets and the cancer cells were separated into Outlet 2, while the RBC went into Outlet 1. Images were taken at the luer outlets to be able to count the cells. The separation was successful with a very high enrichment of cancer cells. Approx. 99.3 % of the RBC went to Outlet 1, while the rest got through to Outlet 2 together with all of the cancer cells. The size of the cancer cells is assumed to be close to the size of some of the larger white blood cells (WBC), which poses a challenge for the separation. The chips will be tested for separation of cancer cells and WBC, and we are expecting to have results from these experiments at the time of the conference.

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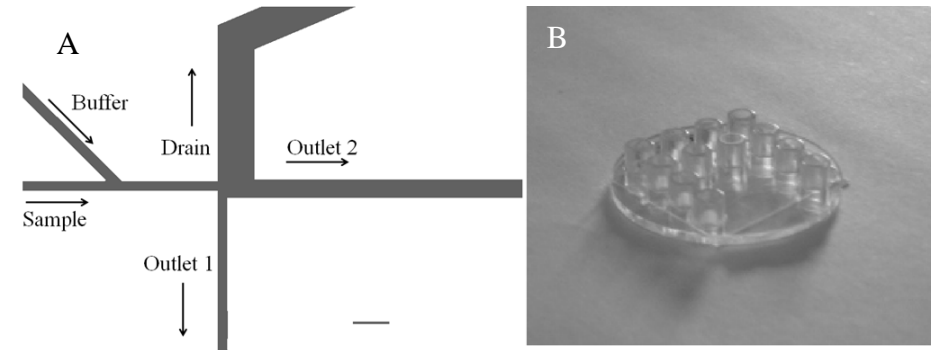
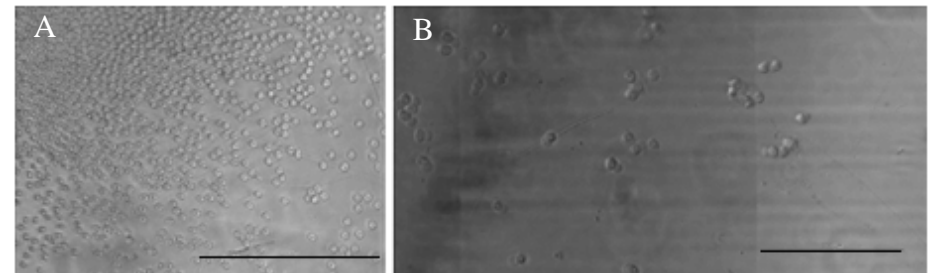


Figure 1. A) Top view of the PFF design. The sample goes through one inlet and the cells are pinched against the sidewall because of flow from the buffer inlet. Cells will follow different streamlines depending on their size, and can be separated into different outlets. There are a total of three outlets, one for small cells (Outlet 1), one for big cells (Outlet 2) and one for the buffer fluid (Drain). The scale bar in the lower right corner is 200 μm . B) An injection moulded chip with 12 luer fittings for the pressure control system and an attached TOPAS foil lid at the bottom.



	RBC	Cancer cells
Count Outlet 1	5000	0
Count Outlet 2	15	180

Figure 2. Optical images of red blood cells (RBC) in Outlet 1 for small cells (A) and cancer cells in Outlet 2 for big cells (B). The scale bars are 200 μm .

The table shows the counted number of RBC and cancer cells in each outlet after the separation.