Development of a Microfluidic Device for Blood Cells Extraction in Liquid Biopsy

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Abstract. This project aims to produce a microfluidic device capable of separating 6 µm and 20 µm diameters particles by inertial sorting. This Lab-on-Chip (LoC) was designed with a trapezoidal cross-section for better fluid control and effective particle manipulation at the microscopic level, as demonstrated by COMSOL simulations. The device was manufactured on a substrate of Polymethyl Methacrylate (PMMA) by femtosecond laser technology and then assembled using an innovative geometry-preserving Isopropyl alcohol-based procedure. The LoC was test with spherical plastic microparticles of two diameters (6 µm and 20 µm) suspended in distilled water. The separation efficiencies were (98.2 ± 1.6) % for 20 µm diameter particles and (70.0 ± 1.8) % for 6 µm diameter particles in good agreement with the simulation results. Finally, after a microfluidic channels' acetone vapors treatment, the device demonstrated a good ability to separate biological particles (Red Blood Cells) at different concentrations (20%, 30%, 40%, 50%) in a PBS buffer.

1 Introduction

Lab-on-Chips (LoCs) are miniature devices that can integrate into a single chip different laboratory analysis [1]. They use active (external force assisted) and passive sorting processes to work. In particular, passive methods rely upon the inherent characteristics of the cells, such as shape, size or density to separate cells. The simplicity of process led to choose the inertial sorting technique for the development of this LoC device [1]. Polymeric matherials, such as Polymethil Methacrylate (PMMA) have become very common for this type of applications (due to their cost-effectiveness, excellent mechanical strenght, biocompatibility, recyclability and ease of processing). In particular, they are suitable for processing with fs-laser technology, which is a technique that allows the substrate processing in a fast, repeatable way and with micrometric precision. Here, an innovative process based on fs-laser fabrication and an Isopropyl alcohol-based technique assembling process was exploited to create a LoC device able to separate 20 µm and 6 µm diameter particles by inertial cell sorting. The separation efficiency of the device was simulated using COMSOL and its characterization was conducted using plastic spherical microparticles in distilled water and blood samples at different Red Blood Cells (RBCs) concentrations.

2 Materials and methods

This LOC device was fabricated on a PMMA substrate (Vistacryl CQ; Vista Optics, Ltd., UK). The plates were 30 mm side squares of 5 mm thickness. Thus, the used fabrication setup (Figure 1) is based on a 1030 nm ultrafast laser system (Pharos Light Conversion) set with a pulse energy of 13.5 µJ and a repetition frequency of 200 kHz. In particular, two kinds of microfluidic channels were created: an acetone vapours treated one and an untreated one. After this, two kinds of LoCs were assembled following the Isopropyl-alcohol based method [2]. Thus, the devices were connectorized using flat tip needles (SNP-D 091-060) and Loctite 3430 A&B resinous glue. Finally, the laboratory characterization was conducted using an OBI MK3+ Elveflow system and plastic microparticles of 6 µm diameter (micromod, product code: 01-02-204 S05118) and 20 µm diameter (Polysciences, Inc. 15714) suspended in distilled water. For blood tests, samples were obtained from anonymous donors. They were centrifuged and RBCs samples at different concentrations in PBS were prepared. A flow of 2 mL/min was used as suggested by the simulations for the designed geometry. The separation efficiencies were calculated using a hemocytometer (HL – 8100204).

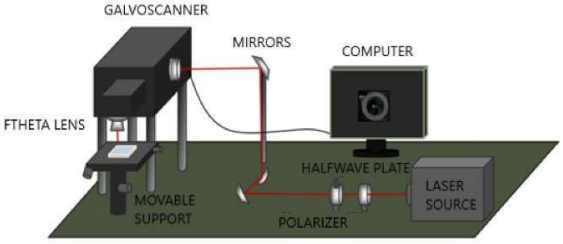

Fig. 1. Ultrafast laser fabrication process experimental setup.

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2.1 Design of the microfluidic device

Figure 2 shows the device design. It is an Archimedean 5-loop spiral with an inlet and two symmetrical 350 µm large outlets. The chosen cross-sectional channel geometry was a trapezoid of the shown dimensions. The outlet area was characterized by an expander 1.2 mm long and with initial and final section sides of 0.4 mm and 0.7 mm respectively.

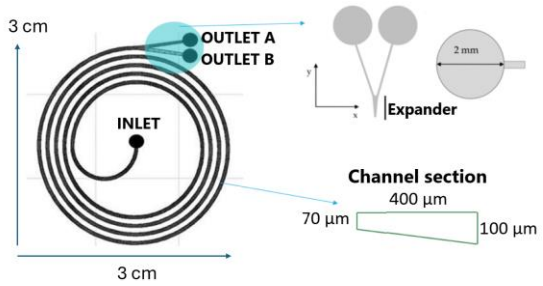

Fig. 2. LoC geometry design scheme.

3 Results

First, the designed geometry separation efficiency was simulated using COMSOL. This, allowed an optimization of the device introducing an asymmetry in the outlets as shown in figure 3.

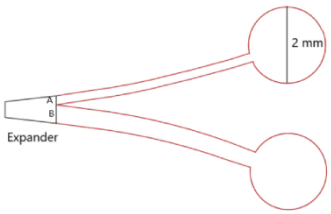

Fig. 3. Modified outlet area scheme; channel A width 230 µm; channel B width 570 µm.

The simulations lead to a separation efficiency of 100% for the 20 µm diameter partciles and 66.7 % for the 6 µm diameter particles. Then, a study on the laser ablation of PMMA was conducted in order to obtain the designed geometry. First, the device was studied using spherical microparticles in distilled water and varying the flow rate. The flow rate of 2 mL/min was chosen consistently based on the highest separation efficiencies observed at this value, which represents the maximum achievable flow rate with the current setup. The separation efficiencies for both the untreated and acetone vapors treated device are reported in table 1.

Table 1. Separation efficiencies for the two kinds of devices (flow = 2 mL/min)

Particle diameter	Untreated Device Efficiency (%)	Acetone Treated Device Efficiency (%)
6 µm	70 ± 1.8	74.8 ± 1.7
20 µm	98.2 ± 1.6	94.4 ± 1.2

The device treated with acetone vapor exhibited superior separation efficiency for 6 µm particles (mimicking the behavior of RBCs). Consequently, it was further evaluated using samples of RBCs at varying concentrations in PBS solution (refer to Table 2).

Table 2. Sorting efficiencies at varying red blood cells concentrations for the acetone vapors treated device.

RBCs concentration	Efficiency (%)
20 %	59.1 ± 1.8
30 %	57.2 ± 1.5
40 %	54.1 ± 1.6
50 %	50.6 ± 1.2

Conclusions

The ultrafast laser fabrication and assembly procedures met expectations for process speed and micrometric precision. Subsequent simulations optimized the device geometry, and tests with synthetic microparticles yielded promising results. The tests conducted with RBCs showed good sorting efficiencies, up to 60 %. Further optimization of this LOC device can be pursued by testing higher flow values and exploring the use of multiple devices in a stack.

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