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# Preliminary evaluation of a microfluidic device for blood separation and deformation assessment

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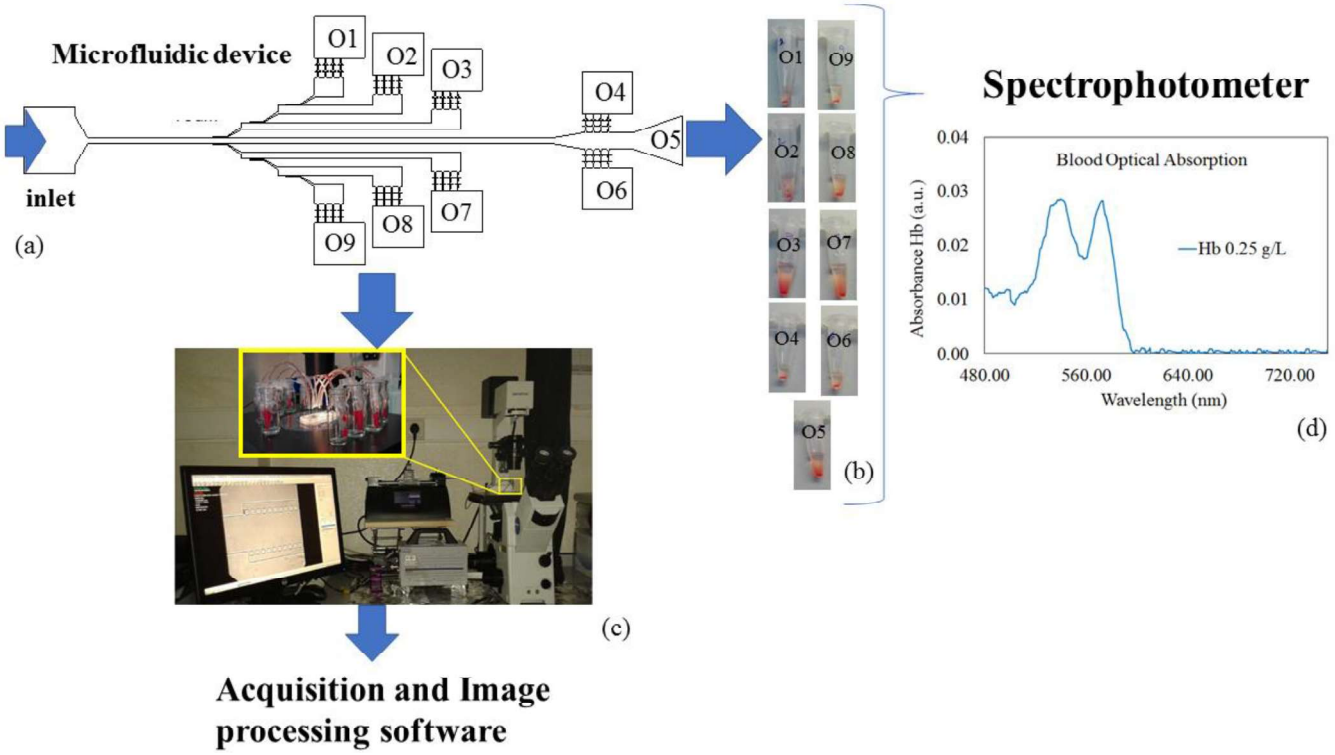


Representation of the conducted experiment: (a) Microfluidic device for red blood cells separation and deformation assessment, which comprises one inlet and 9 outlets (O1 to O9); (b) Eppendorf tubes with the collected samples; (c) data acquisition experimental set-up; (d) the quantification method: optical absorption spectrophotometry.

## Abstract

Microfluidic devices have been widely reported for blood separation experiments [1,2]. Some diseases, such as malaria, influence the Red Blood Cells (RBCs) stiffness and, consequently, their deformability [3,4]. Therefore, the separation of healthy blood cells from infected blood cells (deformable RBCs from rigid RBCs, in the malaria infection case) makes it possible to detect the disease by their ability to deform. Thus, this work intended to evaluate the possibility of using polydimethylsiloxane (PDMS) (Sylgard® 184 Silicone Elastomer, from Dow Corning) microchannels with 3 rows of 10 pillars (with distance between them of 17, 16 and 14 µm, respectively) to perform a preliminary study on the inertial separation of RBCs based on their deformability. In this study, it was used healthy human blood from a volunteer donor, which was collected into a 2.7 mL tube (S-Monovette®, Sarstedt) containing ethylenediaminetetraacetic acid (EDTA). The whole blood was centrifuged at 2500 rpm for 10 min (at 20ºC). Then, the plasma and the buffy coat were removed, and the RBCs were re-suspended and washed once with a

physiological salt solution (PSS) with 0.9% NaCL (B. Braun Medical, Germany). Two working fluids were considered in the experiment: (1) a Dextran 40 (Sigma-Aldrich, USA) solution containing 3% of haematocrit (Hct) was used to represent a healthy (malaria-free) blood sample; (2) a solution with 2% Hct of RBCs chemically modified with 0.025% of glutaraldehyde was studied to mimic the malaria behaviour on the RBCs (by rigidifying them). The high-speed video microscopy system used in the present study consisted of an inverted microscope (IX71, Olympus) combined with a high-speed camera (Fastcam SA3, Photron, USA). The PDMS microchannel was placed and fixed in the microscope. The fluids were injected using a syringe pump (PHD Ultra, Harvard Apparatus, USA) with a 5 mL syringe (Terumo, Japan), keeping a constant flow rate at 50 μL/min. All the collected samples from each microchannel outlet were collected in different Eppendorf tubes, for further quantification. At the same time, the images of the flowing cells at the established flow rate were captured by the high-speed camera at a 2000 frames/s rate and at a shutter speed ratio of 1/75000, which minimized the dragging of the cells at the considered high flow rate. All the experimental assays were performed at room temperature (T= 22 ± 1ºC). To validate and quantify the separation of the cells based on their deformability, the authors propose the use of an optical absorption spectrophotometric setup to compare the optical absorption of the healthy RBCs [5] with the optical absorption of glutaraldehyde chemically modified RBCs. By marking the modified RBCs with a coloured dye, it is possible to obtain different absorption spectra for the samples, accordingly to the rate of healthy/malaria-mimicked RBCs that were collected in the Eppendorf tubes. This preliminary study showed the potential of the proposed microfluidic device, not only to perform the partial separation of RBCs, but also to deform the cells and assess their deformability, by analysing the acquired images.

**Keywords:** RBC Blood separation; RBC deformability; spectrophotometry; microfluidics.

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The novelty of this study is the combination, in the same microdevice, of separation and deformation of healthy and stiffed RBCs. To validate and quantify the separated RBCs will be used an optical absorption spectrophotometric setup.