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Lab-on-a-Chip for the isolation of circulating Adipose Stem Cells

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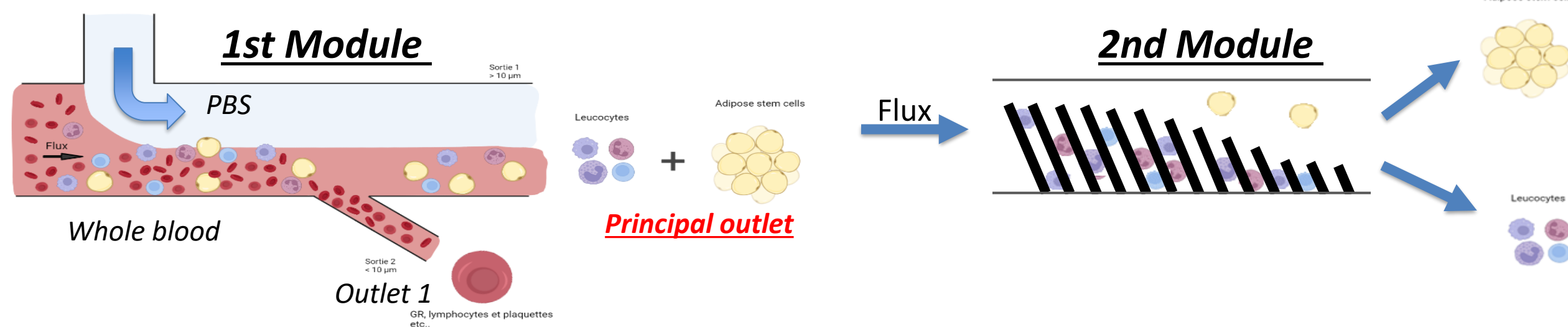
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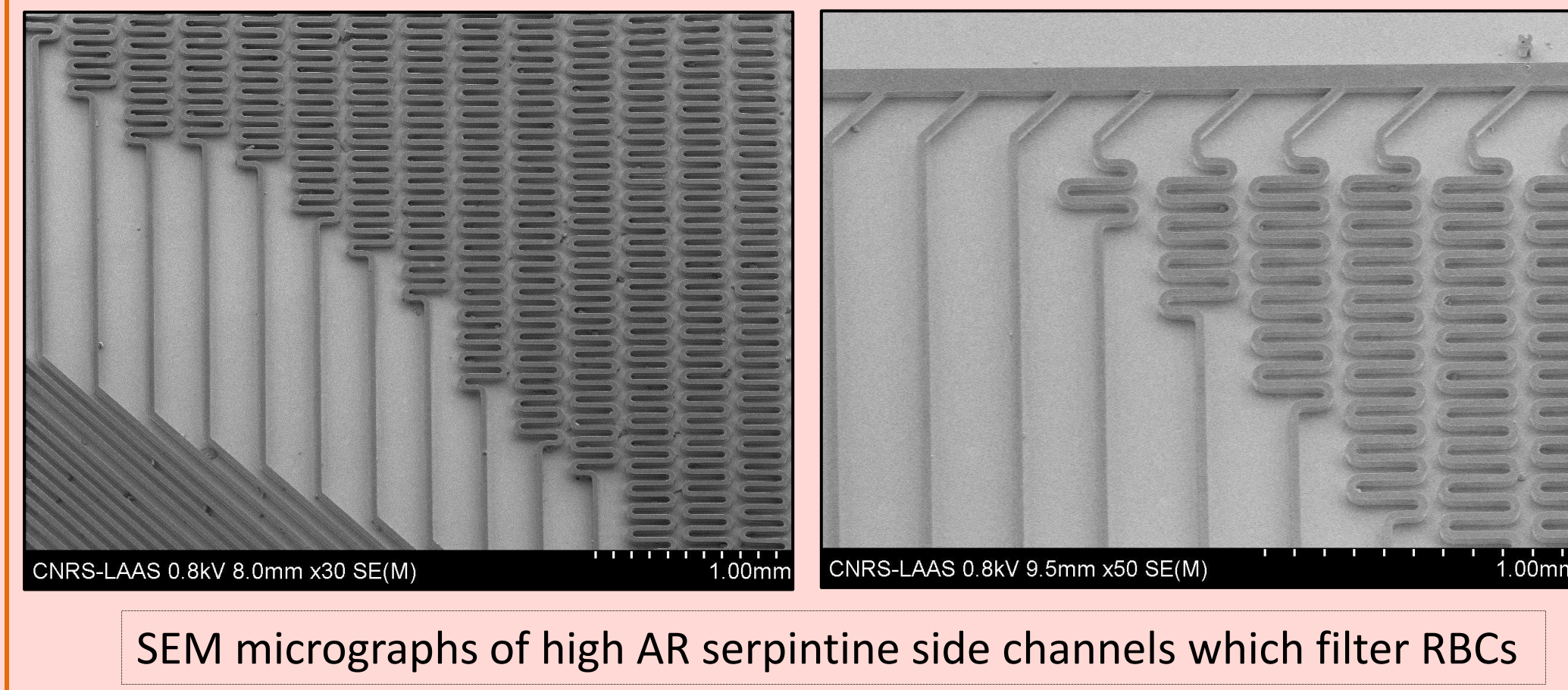
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CONTEXT

Context: Adipose stem cells (ASC) are relatively abundant multipotent stem cells dispersed in fat tissue in the body. They could be considered as diabetes biomarkers since studies showed that they are released by subcutaneous adipose tissue during type 2 diabetes development and are supposed to circulate in blood. However detecting circulating ASCs is rather difficult since they do not express specific unique membrane markers compared to other hematopoietic cells, which renders their isolation from whole blood a hard task. In this work, we aimed to separate ASC from whole peripheral blood, using a setup combining two complementary depletion microfluidic modules. The first one is a **hydrodynamic filtration module** that sorts particles according to their diameter ($R_c = 10 \mu\text{m}$) and which therefore will eliminate small cells as red blood cells and platelets. The second one is a **"cell colling" module** that deplete remaining leucocytes according to their surface antigens, leading to a sample that ideally contains only ASC.

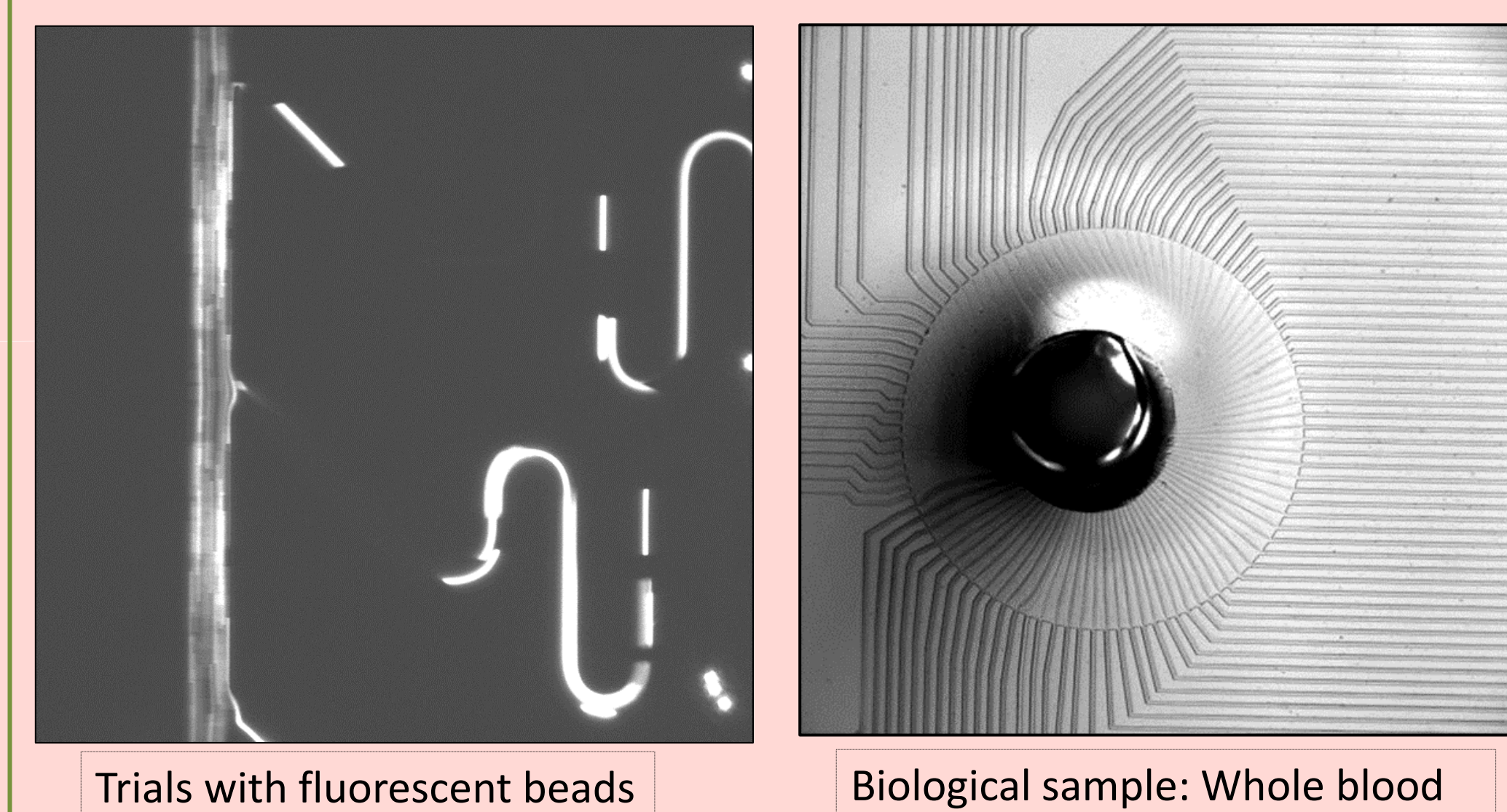


CHARACTERIZATION



SEM micrographs of high AR serpentine side channels which filter RBCs

EXPERIMENTATION

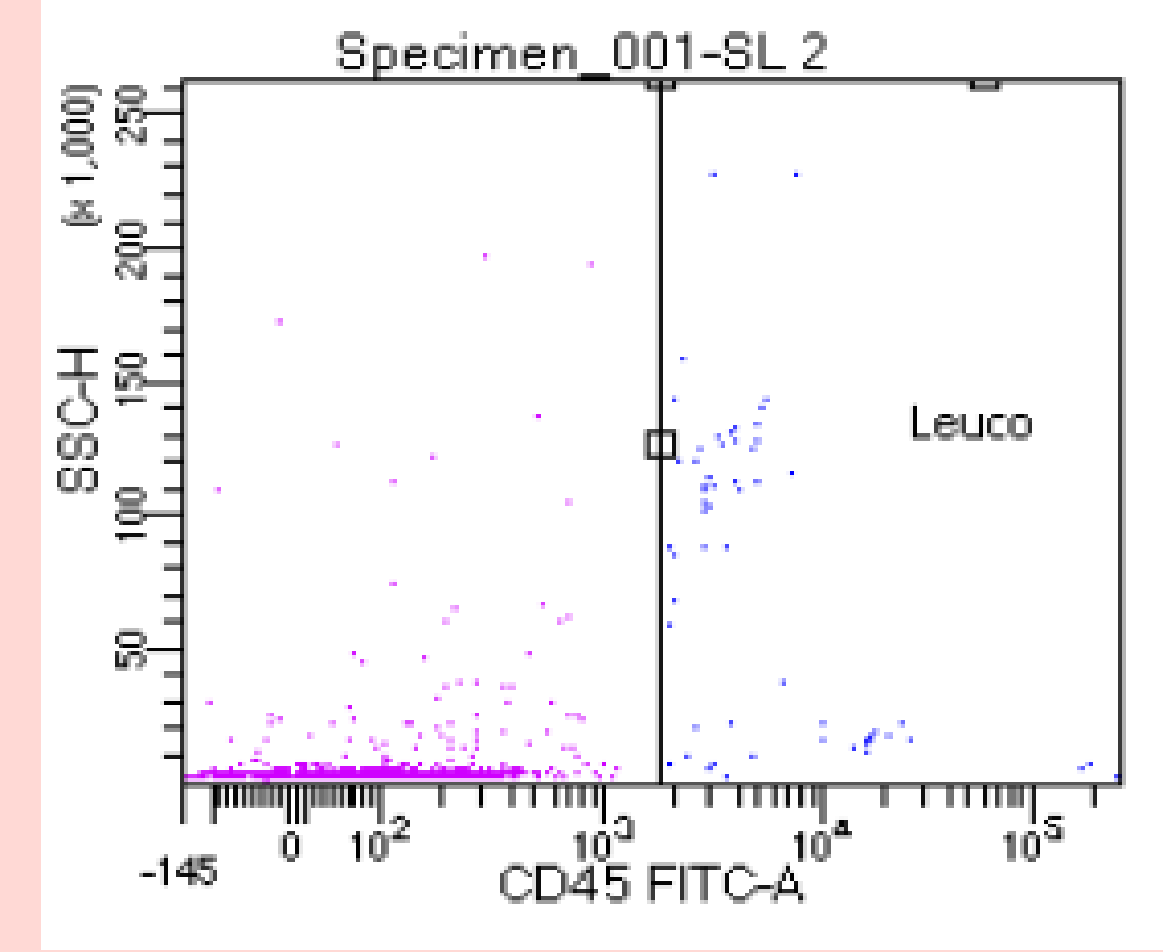


Trials with fluorescent beads

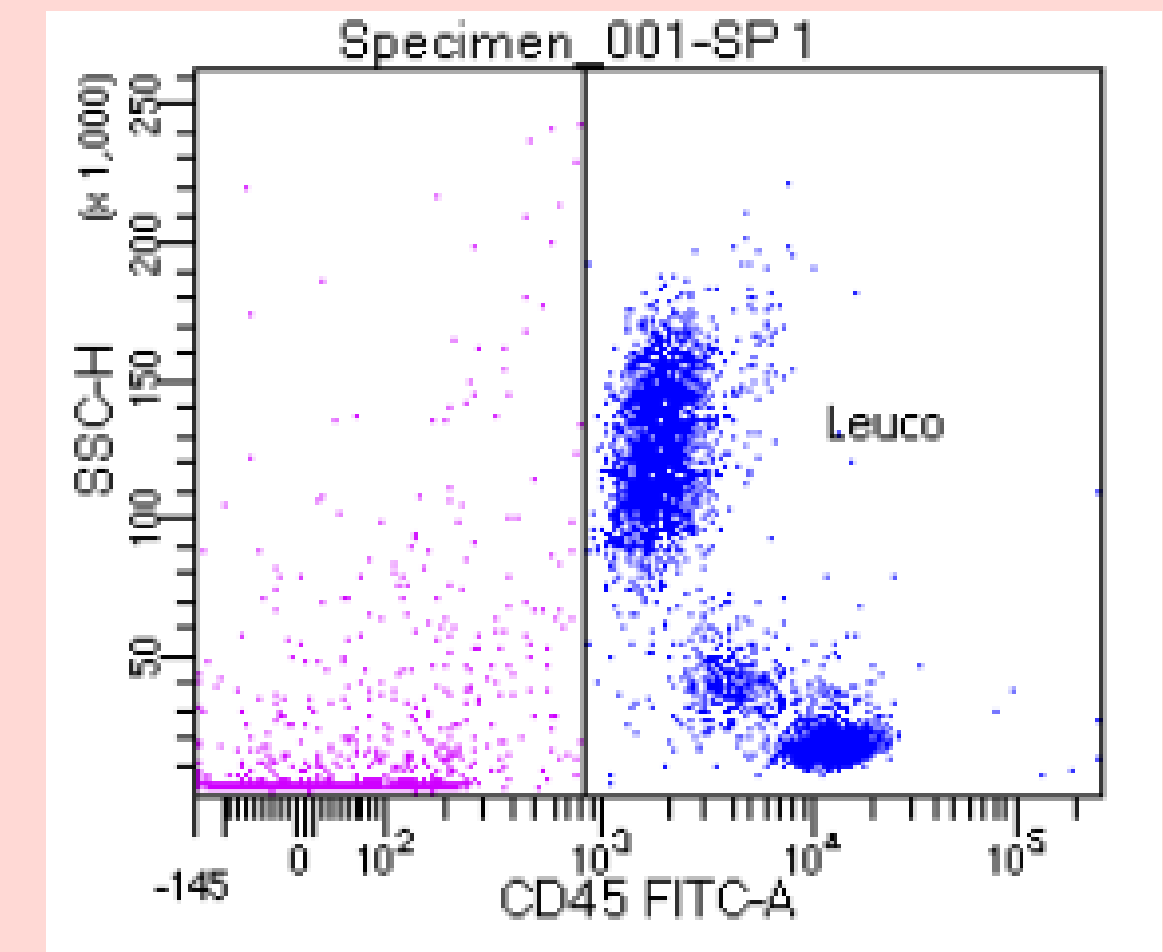
Biological sample: Whole blood

RESULTS

Flow Cytometry analysis

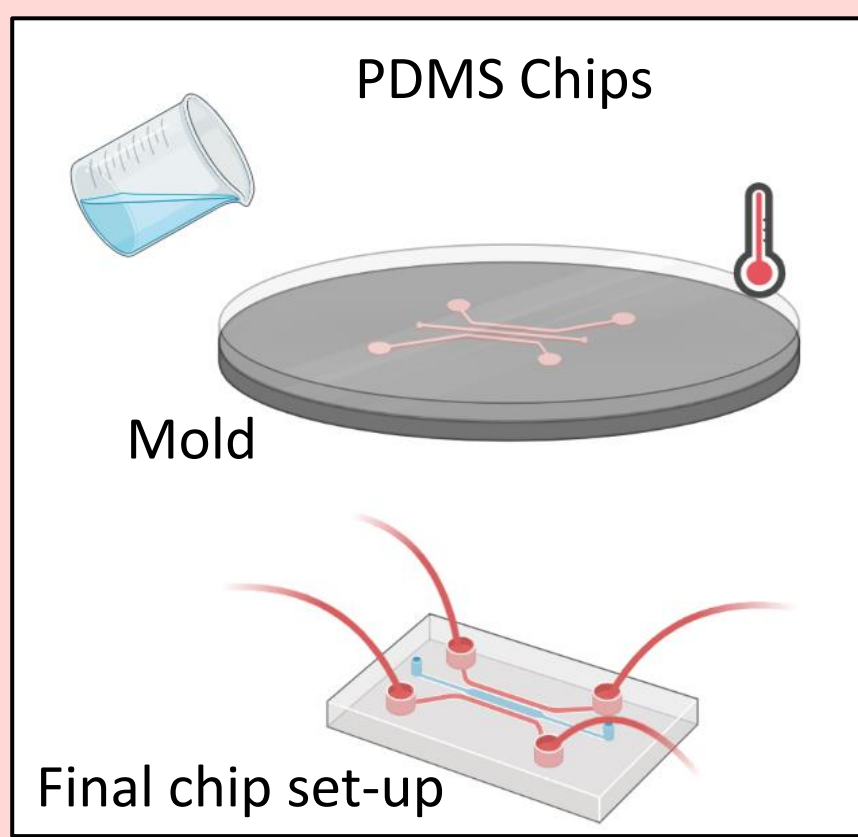
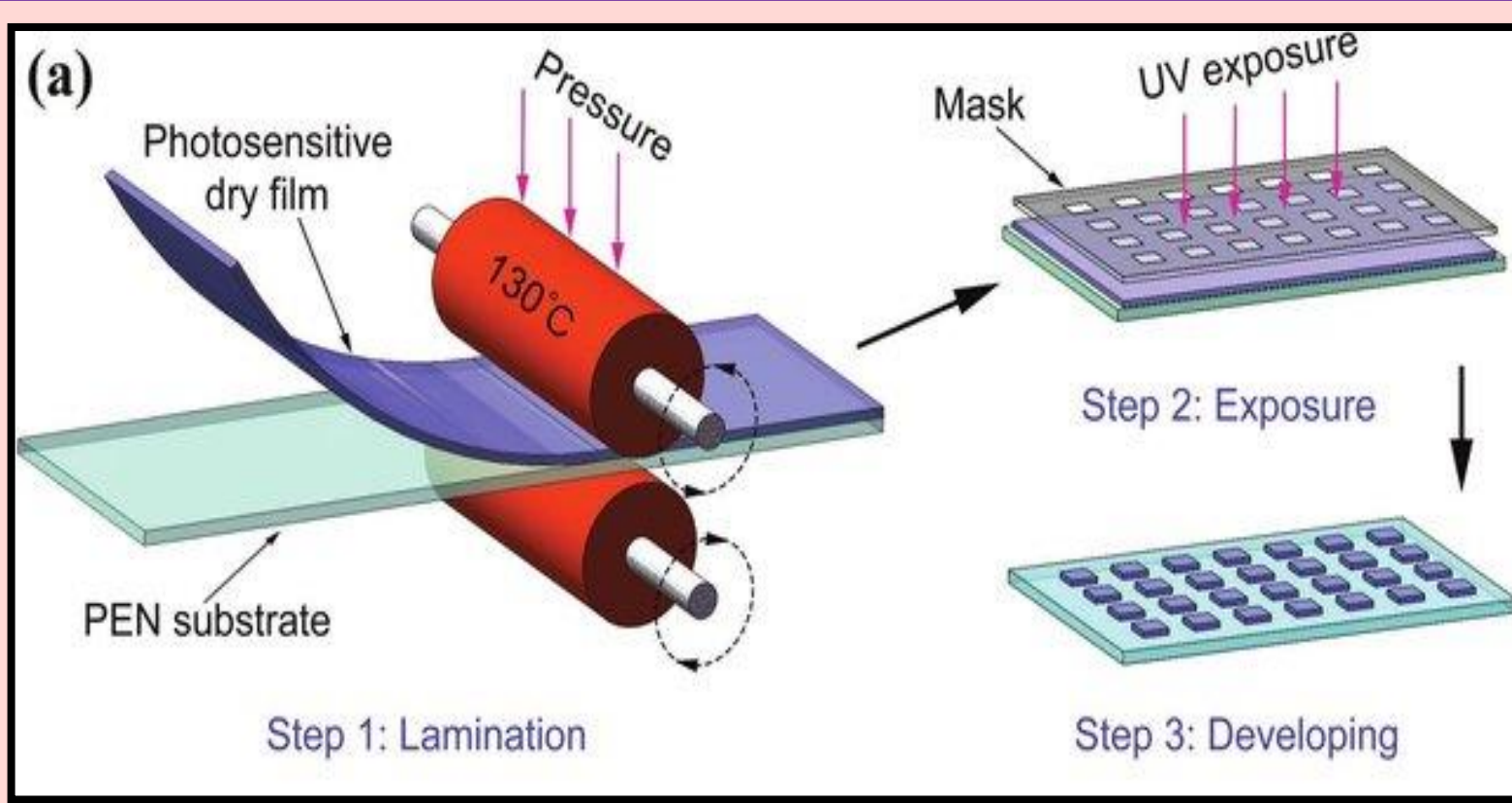


Lateral outlet: Negligible number of Leucocytes left



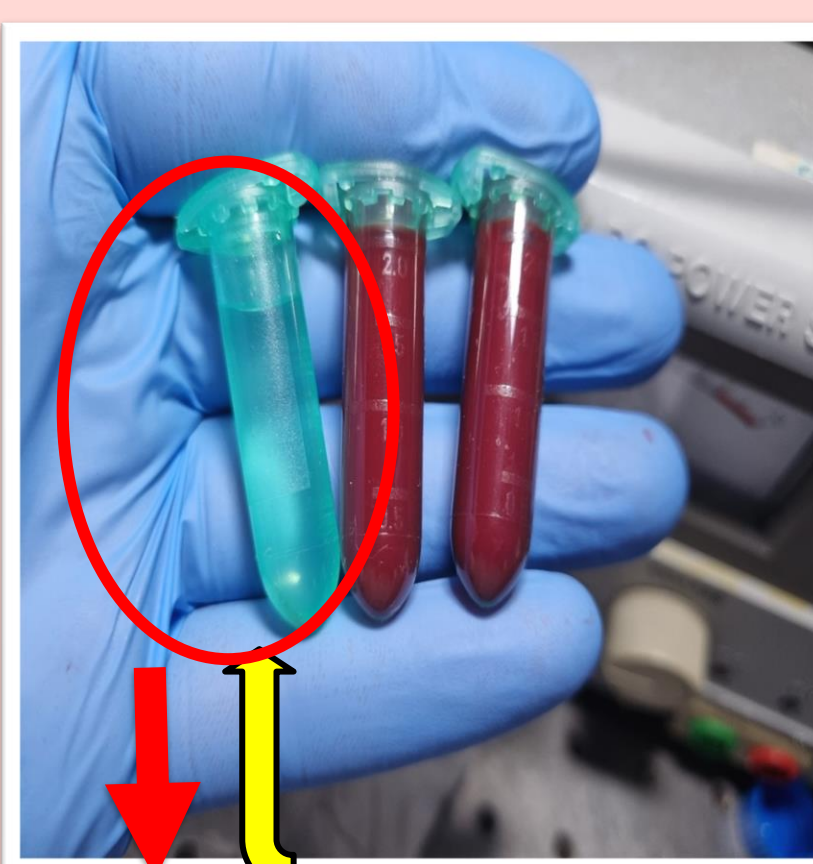
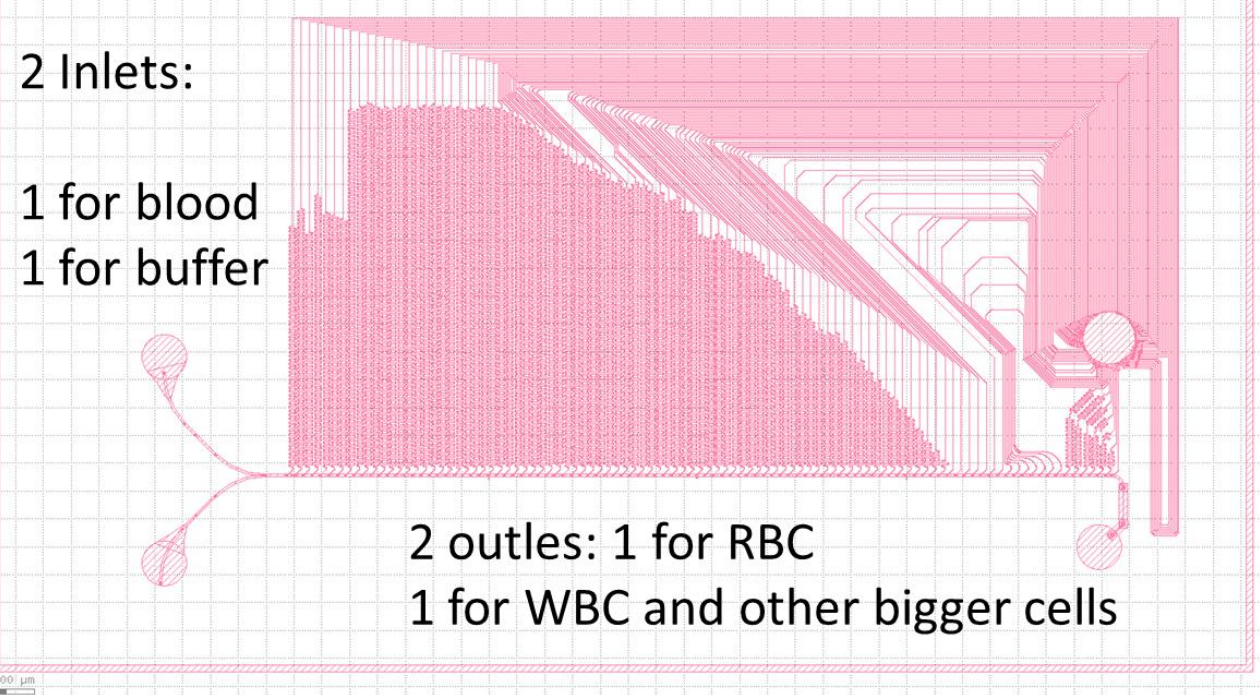
Principal outlet: majority Leucocyte population

MICROFABRICATION

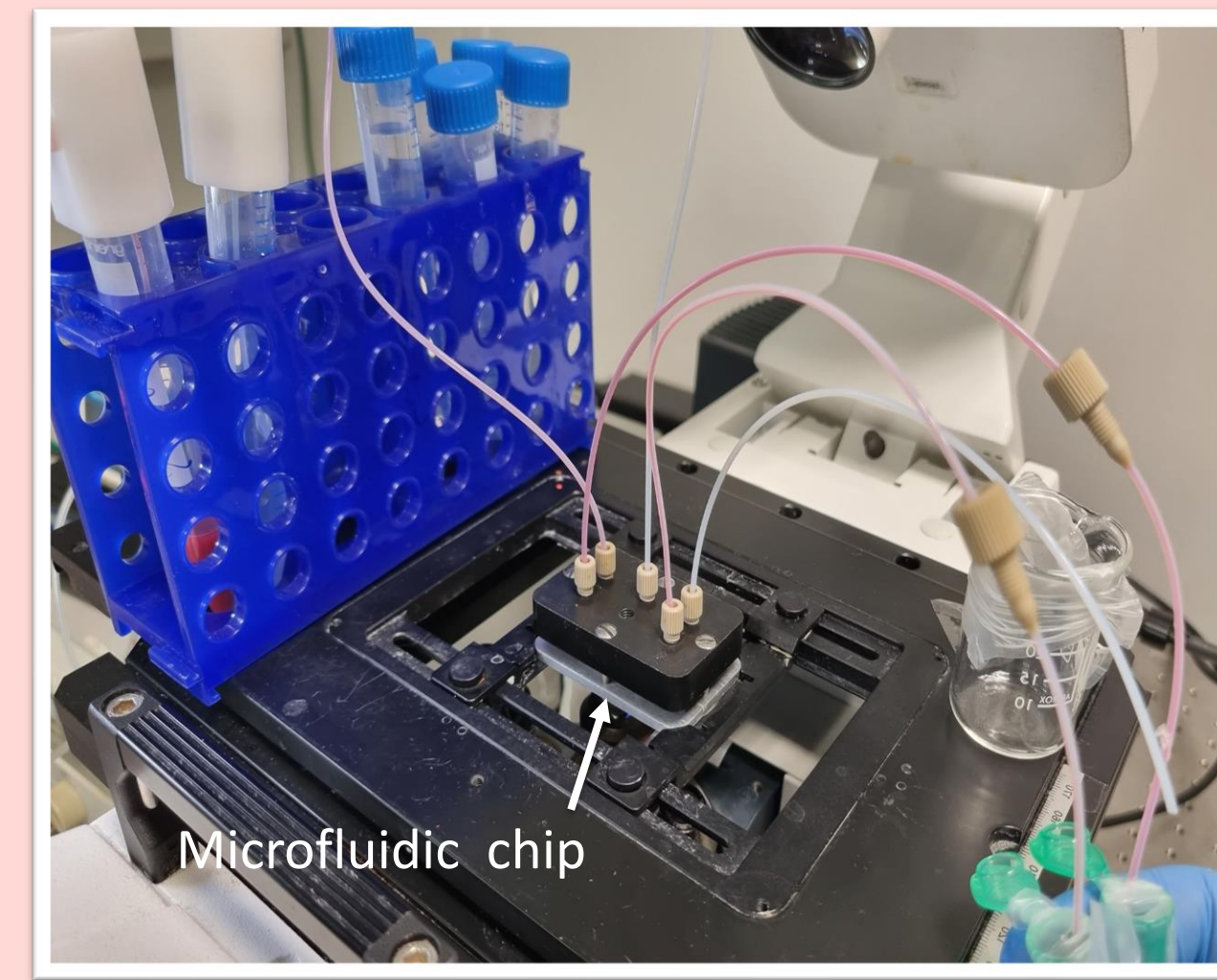


Chip design:

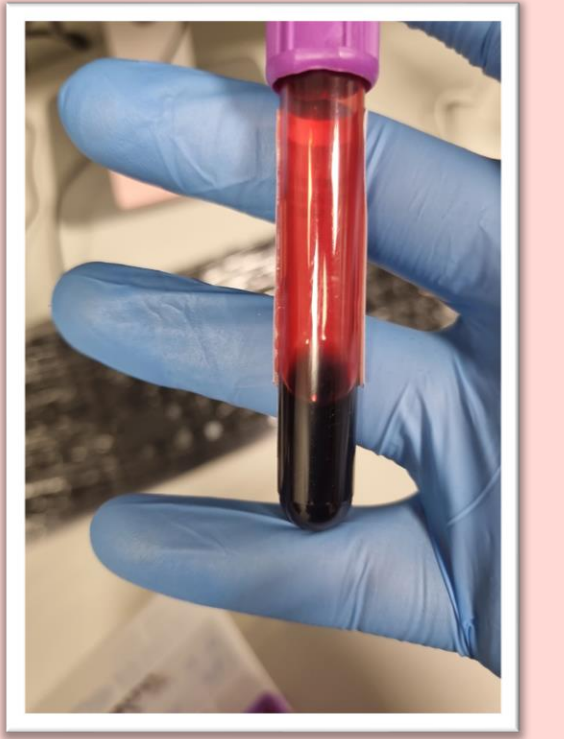
- 100 side channels to filter RBCs
- 2 inlets: 1 for blood and 1 for buffer
- 2 outlets: 1 for RBCs and 1 for cells of interest



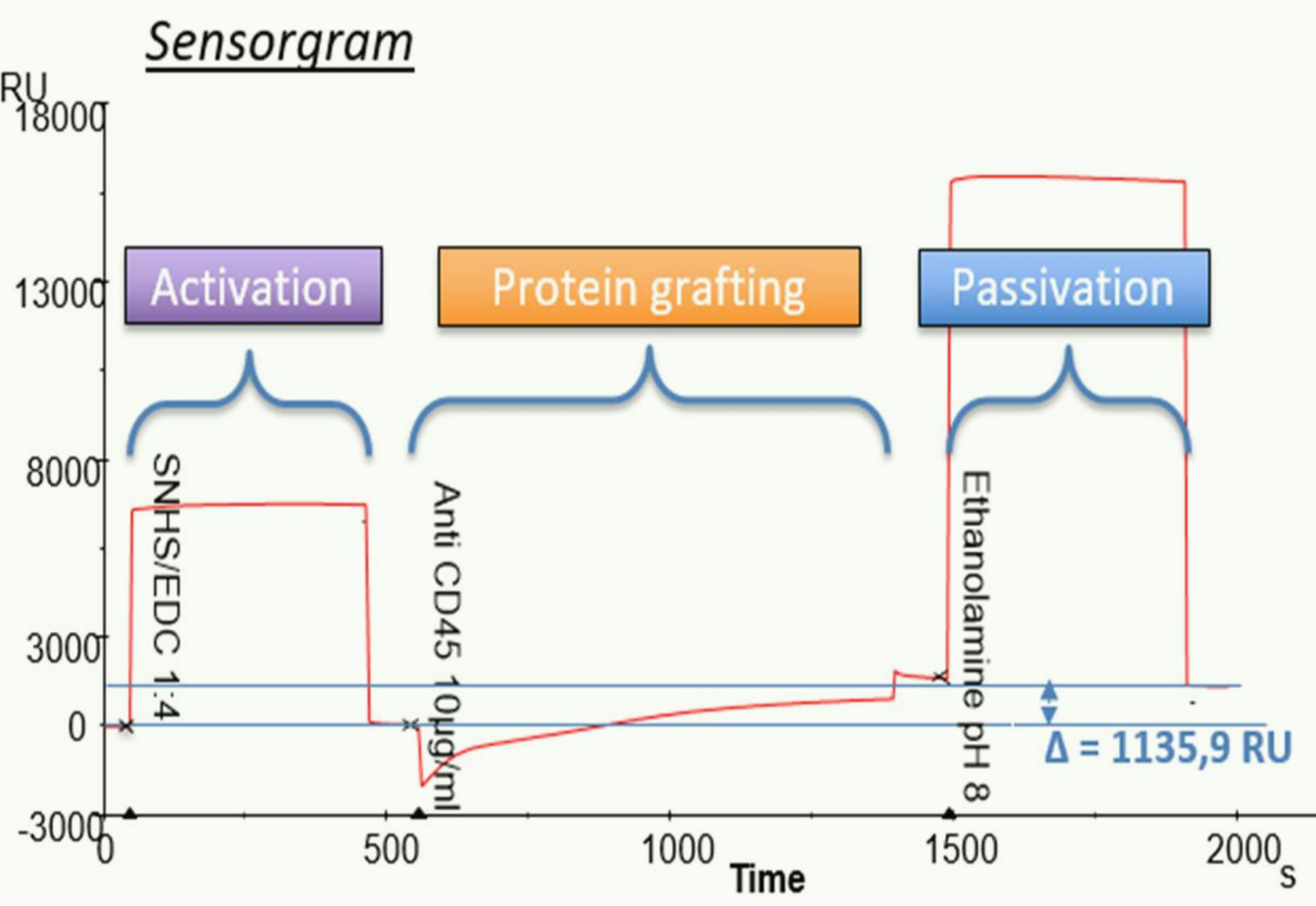
Cells of interest



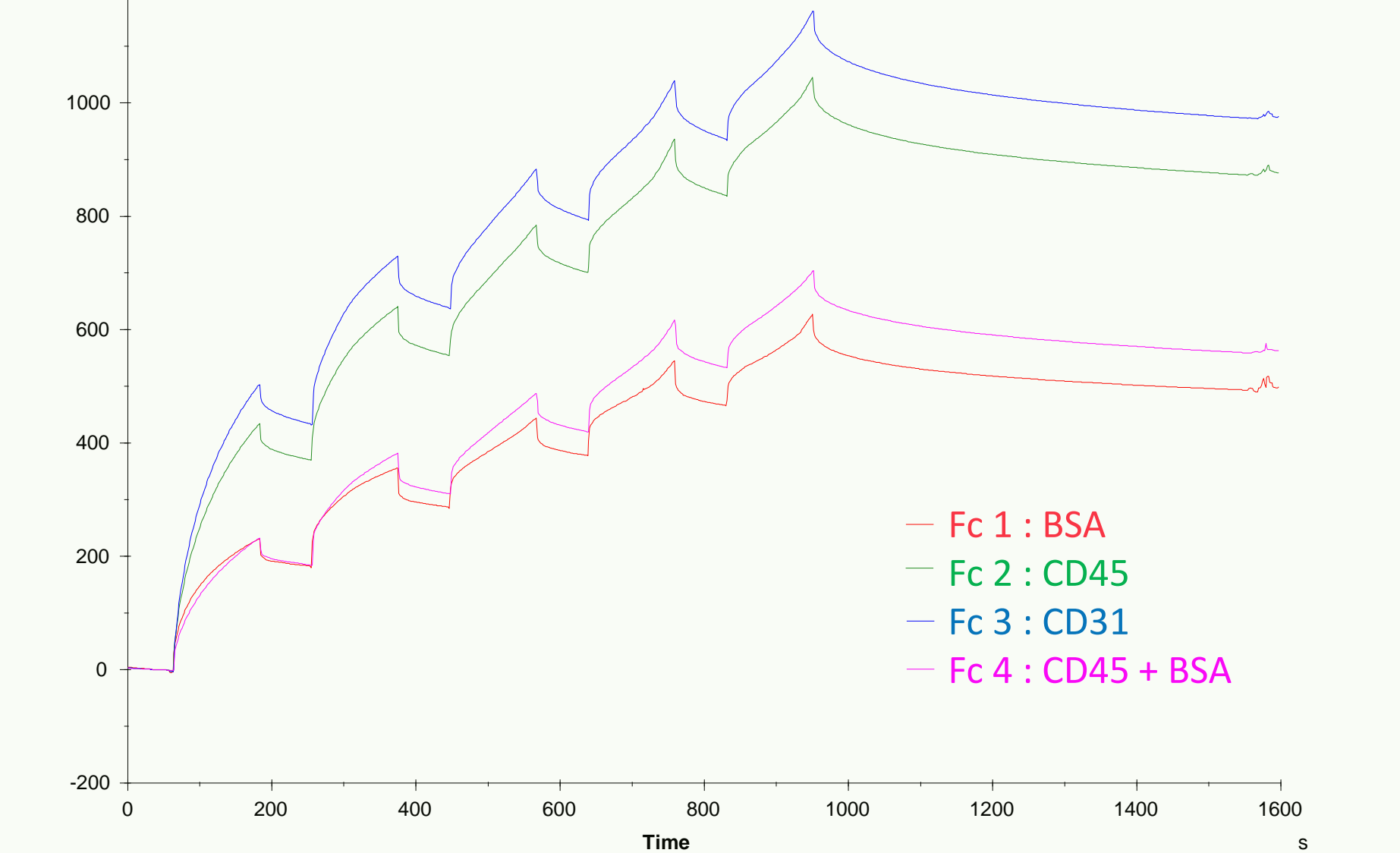
Microfluidic chip



Of the surface functionalization (Ab on SAM)

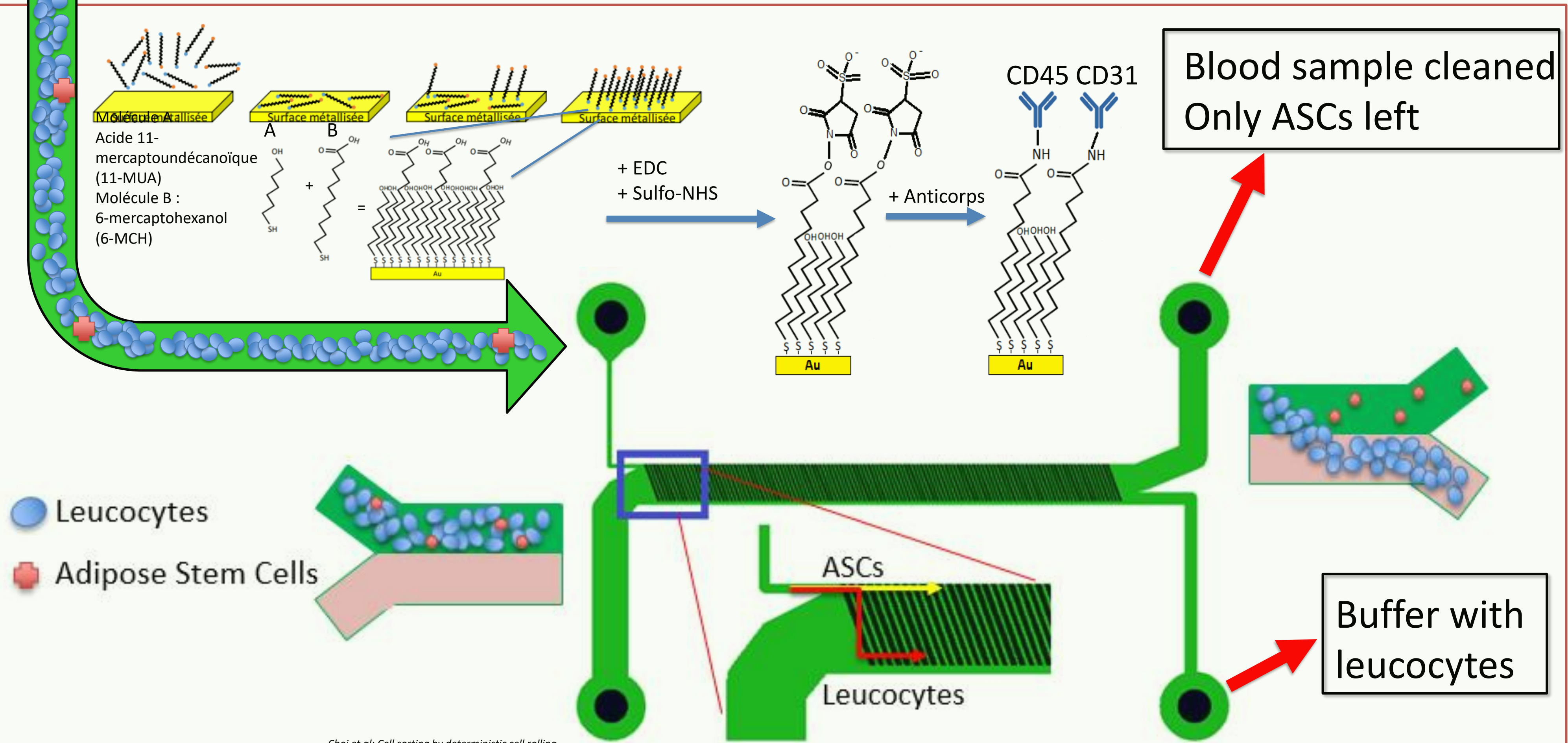


Of the interaction between Ab functionalized and THP-1 cells



→ We are able to produce reproducible results on the grafting of antibodies.
→ We still have trouble with non-specific adhesion of cells on the surface and trying to find the best way to passivate it.

AN IDEA OF THE MODULE'S CONCEPT INSPIRED FROM THE LITERATURE

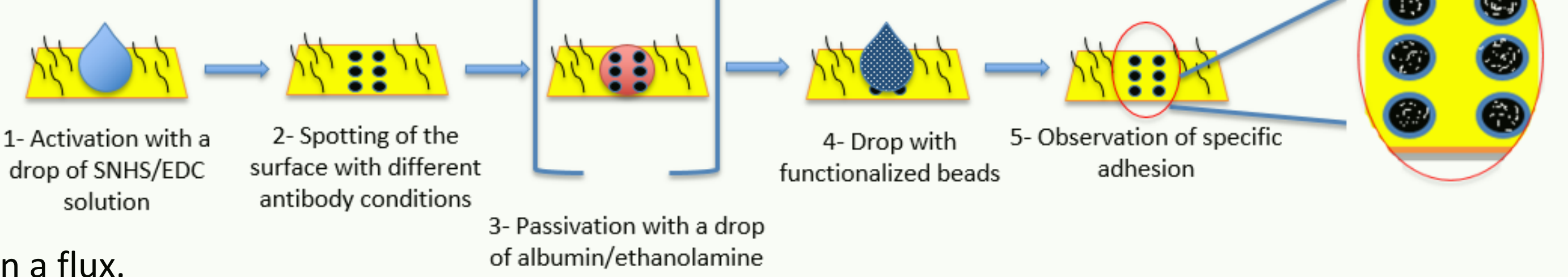


Inspired from the literature on CTC isolation: Choi et al., Cell sorting by deterministic cell rolling; Edington et al., Tailoring the Trajectory of Cell Rolling with Cytostatic Surfaces

RESULTS AND PERSPECTIVES

- Optimal conditions allowing reproducible results :
 - pH = 5
 - Injection's flow rate = 5µL/min
 - Activation done by SNHS/EDC
- Contact time = 14 minutes
- Antibodies concentration = 10µg/mL

- Proof of a specific adhesion on functionalized surface.



- Proof of rolling concept in a flux.

CONCLUSION AND PERSPECTIVES

- The first hydrodynamic filtration module is already able to eliminate almost all RBC and platelets (99 %) from whole blood samples. Optimizations may include increasing filtration rate without compromising the purity of the filtrate (depleting as much RBC as possible).
- The second module allowing the depletion of remaining leucocytes by cell rolling is still in progress but we are already able to graft relevant antibodies on a gold surfaces, in order to deplete remaining leucocytes and collect circulating ASCs at the end of the process.

Using this Lab-on-a-chip configuration, we should be able to directly isolate circulating ASC from whole blood without any prior treatment.

REMERCIEMENTS :

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