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Multilevel (3D) microfluidic technology for an innovative magnetic cell separation and counting platform

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Introduction

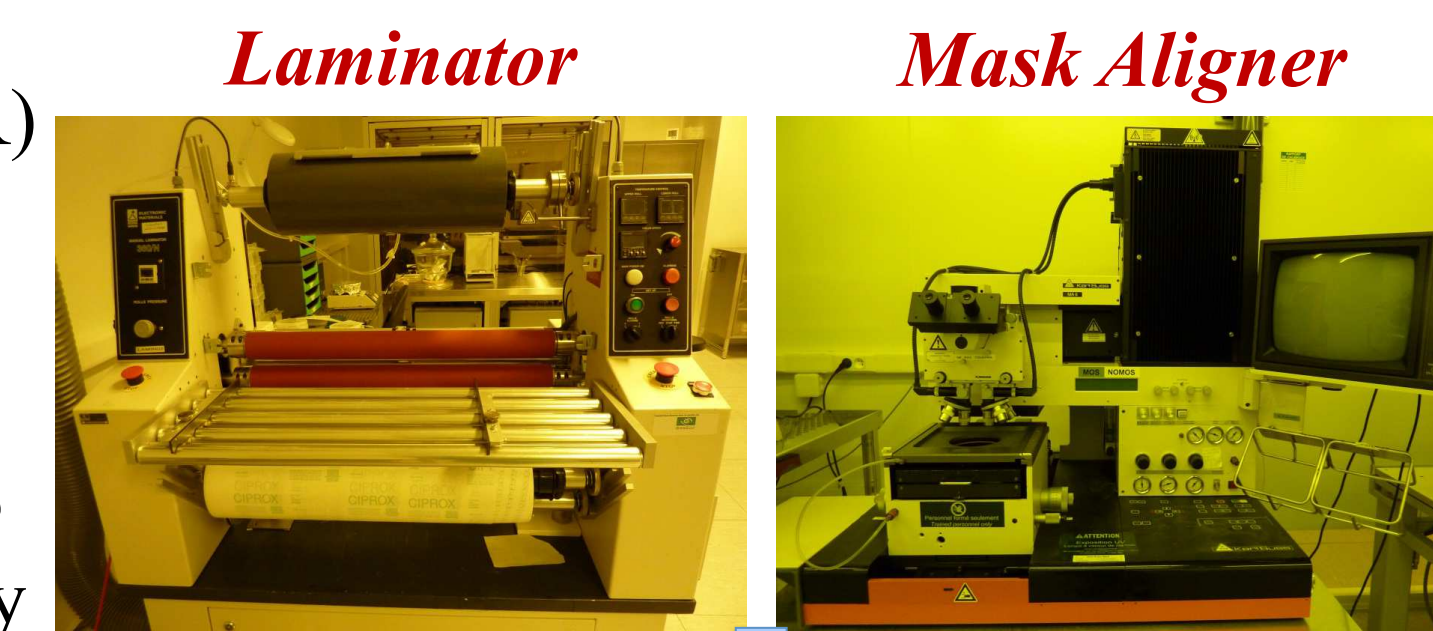
Currently, the technique for the quantitative detection of cells is flow cytometry. This technique has the advantage of being sensitive and reliable but is expensive, time consuming and not suited to both routine screening and point-of-care diagnostics. Miniaturized cell separation devices offer many advantages such as the use of small volumes, portability and low cost.

We propose a new concept of device which, by combining 3D fluid engineering and localized magnetic actuation, enables the full integration of cell tagging, magnetic separation and cell counting in a single device. The labs on chip are manufactured by laminating commercially available photosensitive dry film that fits microfluidic requirements and gives the possibility to build easily 3D microfluidic systems.

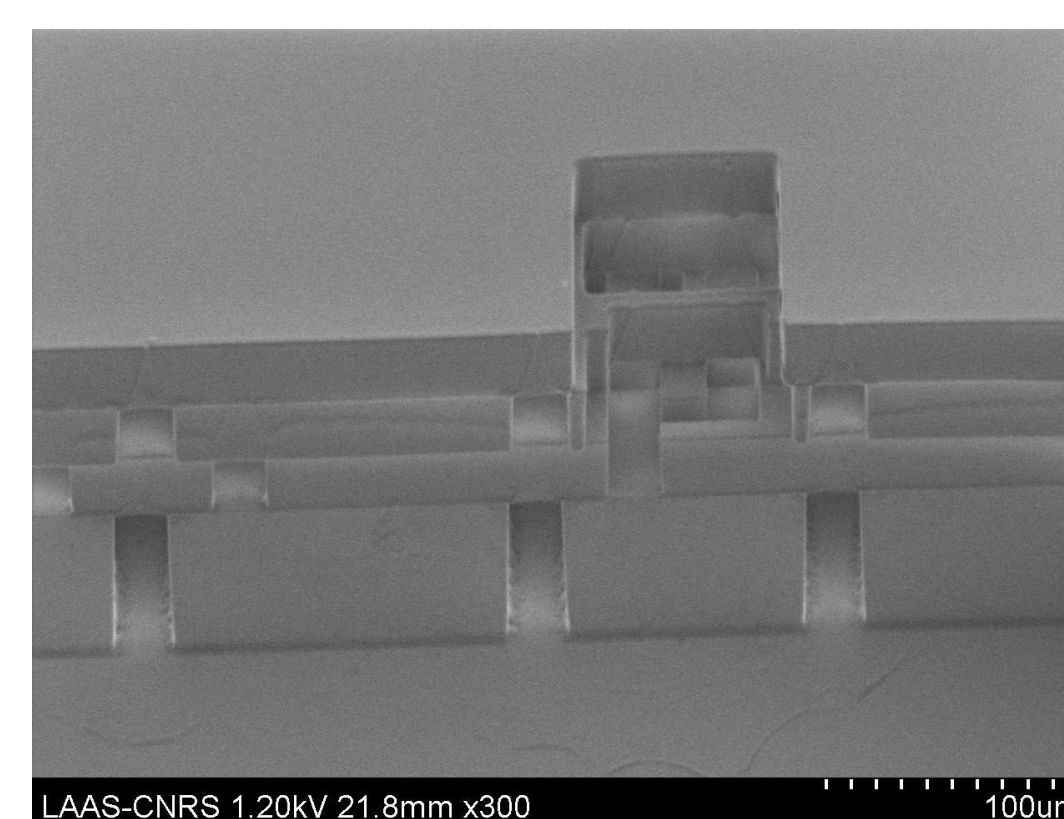
We show we can tag efficiently THP1 monocytes and subsequently sort them through magnetic trapping on integrated micro-coils. The separation efficiency is studied at different flow rates. Cell counting capacity, evaluated by using non-faradic impedance spectroscopy revealed that the cell trapping is selective, depending on the specific antibody grafted and quantitative with the range of detection being 1000 to 30000 infected cells. This range of detection is consistent with the targeted application.

3D Microfabrication

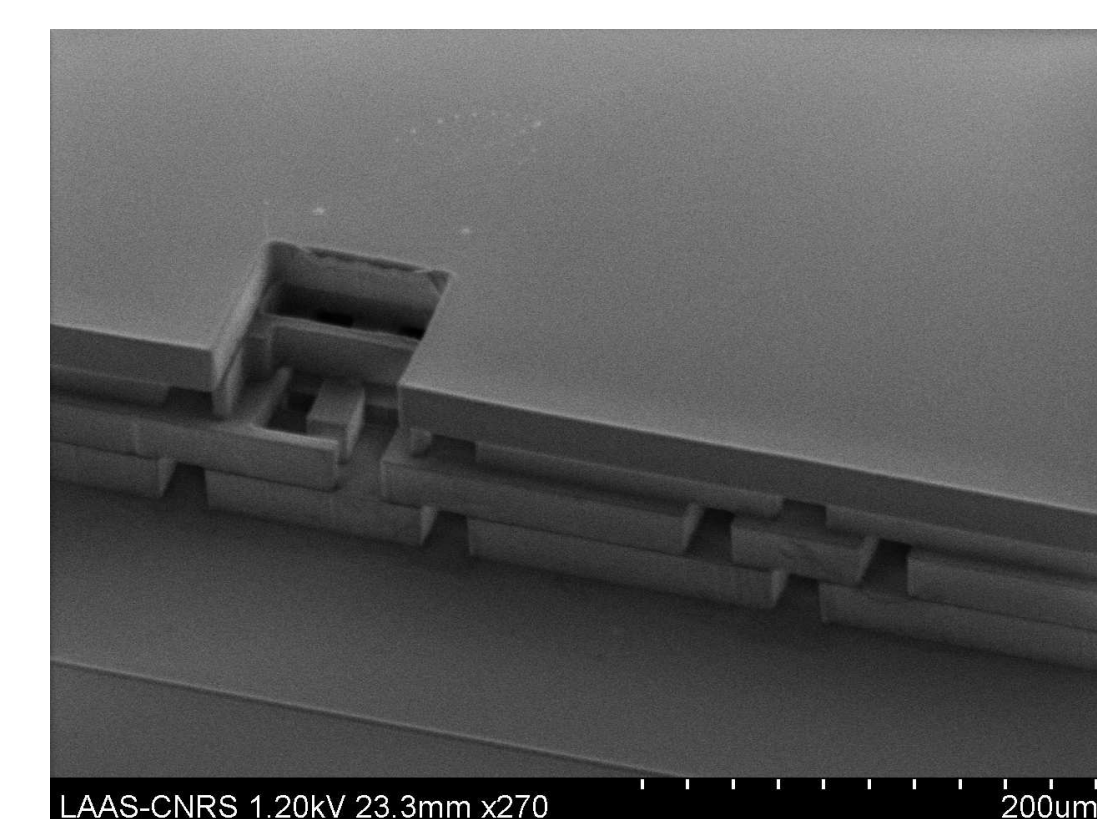
- Lamination of a dry film photoresist (DFR) DF-1000 series¹ onto a substrate.
- Structuration of this DFR by photolithography.
- Lamination of a second layer of DFR onto the first structured one and structuration by photolithography.
- Repeating these steps allows to create a 3D microfluidic devices.



Advantages: fast deposition of thick layer and high accuracy alignment.



SEM pictures of four stacked layers obtained with the combination of multiple lamination and photolithography steps.

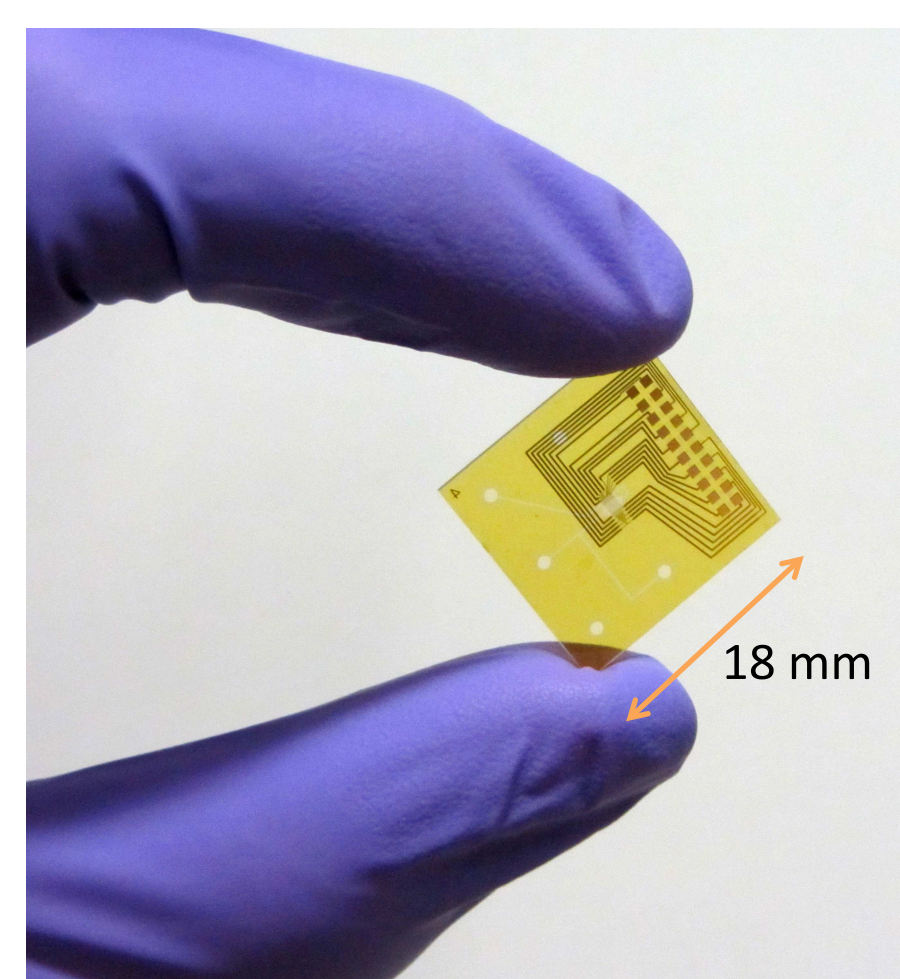


¹Manufactured by Engineered Materials Systems

Microfluidic Device

Three dimensional microfluidic structures can be created with dry film lamination techniques. Mixing applications have been investigated, especially with designs dedicated for cells and beads tagging reactions.

High integration could be achieved with other functions like magnetophoresis and specific impedance electrical detection: the device is then placed in a chip holder and connected to fluidic controllers and electronics.



Microfluidic chip with 10 coils embedded

An interesting application is the diagnosis of infectious diseases based on monocytes subpopulations. An immunotagging is performed: magnetic beads are attached to monocytes.

A serie of microcoils then separate them from the sample, and the different subpopulations are detected with an immunosensor based on electrical impedance measurements.

Inlet 2: Buffer

Separation stage
Vertical magnetic sorting

Tagged Monocyte

Magnetic beads

Mixing stage

3D microfluidics – baker's transformation

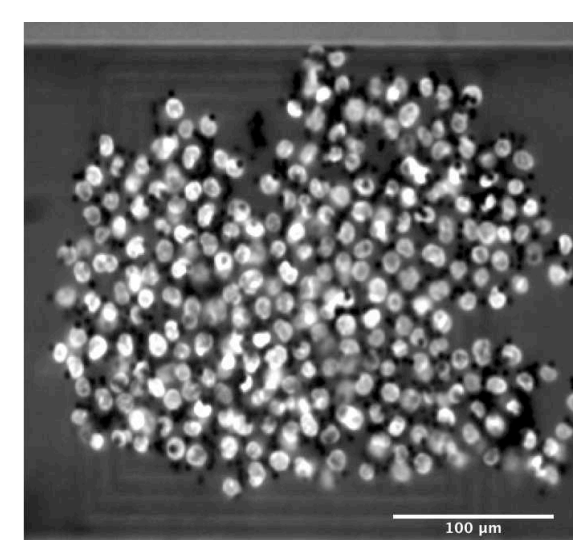
Inlet 1: Sample

Detection stage

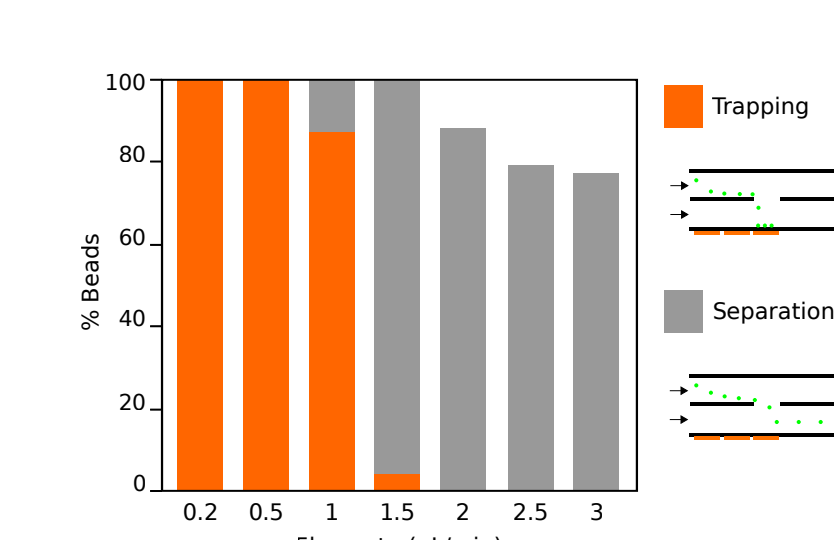
Electrical impedance measurements

Magnetic Separation and Trapping

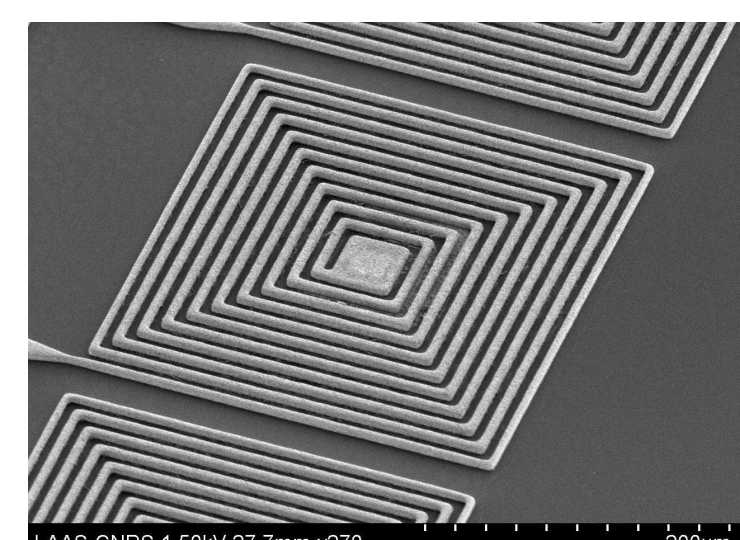
The use of super paramagnetic microbeads that can be functionalized with specific biomolecules (e.g. antibodies) enables micro manipulation, separation and trapping of cells. A magnetic field is created with microcoils, which can be also coupled to magnets to amplify the force (hybrid systems).



Trapped (magnetically tagged) THP1 monocytes on a coil



Impact of flow rate on beads trapping and separation efficiency



SEM picture of microfabricated spiral coil

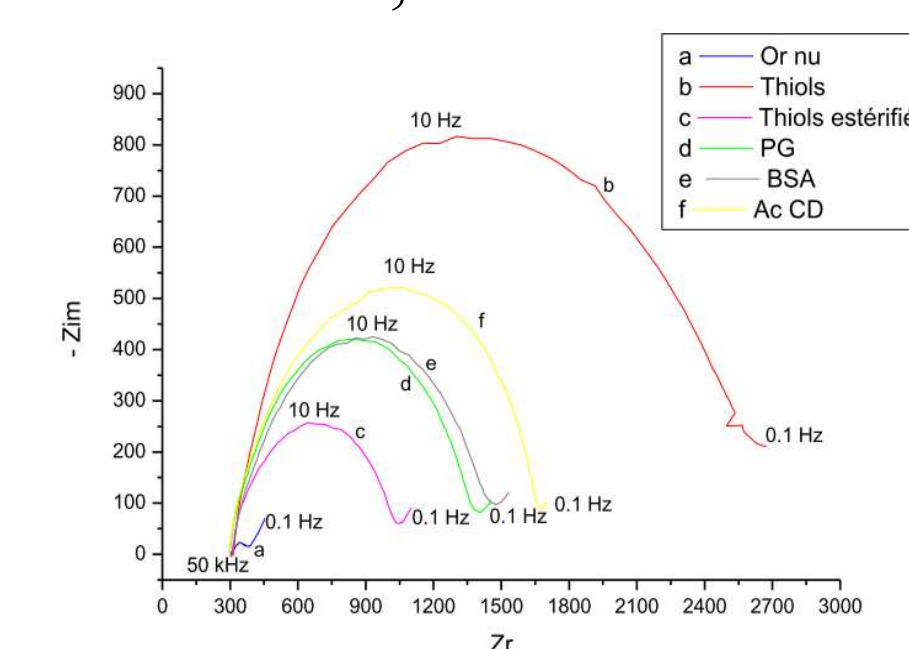
Metal deposition and electroplating are used for the coils fabrication, allowing a high resolution (5 x 5 µm wires) and 3D microfluidic channels integration on the same chip.

We showed tagged monocytes can be separated from a sample using a set of coils for specific parameters (flow rate, electrical intensity...).

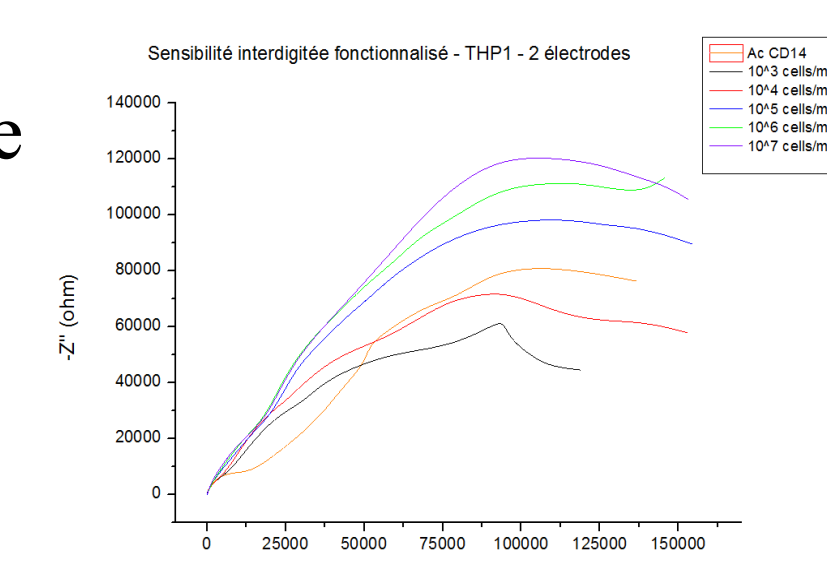
Surface modification and Impedance detection

- Functionalization of gold microelectrodes with antibody specific to the antigen present on the target cell : after activation of the first layer of SAMs, the electrode surface is modified by the addition of the protein G (saturated by adding BSA) before being coated with specific antibody.

- Characterization of electrode modification and cell attachment by impedance spectroscopy.



- Determination of the intrinsic limit of detection (LOD) : minimal detectable number of captured cells on the sensor surface.
- Optimization of the microelectrode design to maximize the signal to noise ratio and hence the LOD.
- Application of a scale cell concentration to determine design sensitivity.



Conclusion

We demonstrated a new concept of devices, which by combining 3D fluid engineering and localized magnetic actuation enables the full integration of a cell tagging, magnetic separation device and cell counting. This approach, whose main drawback is the limitation of flow (and therefore volumes) which can be handled, can be significantly improved by adding external permanent magnets for the generation of high and homogeneous magnetic fields. Such hybrid systems open up the way to more efficient systems but also to the implementation of new kind of functionalities: focusing, shift register, trapping array, manipulation of single cell or chain of clusters.