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A generic Microfluidic Approach for Deciphering Nanoscale biovesicles properties

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INTRODUCTION

Extracellular vesicles (EVs) are subcellular species present in all biological fluids which are increasingly considered as pathologies indicators. Therefore identifying and characterizing nanoscale vesicles in biofluids could lead to unprecedented diagnosis/prognosis assays for a wide set of pathologies. Owe to their complexity in size, origin, membrane markers, there is currently no ideal technology available yet to quantify and characterize cell-derived microvesicles, and fully relate their structure and functions which have high potential in clinical diagnostics and early treatment of life threatening diseases such as cancer, cardio vascular diseases....

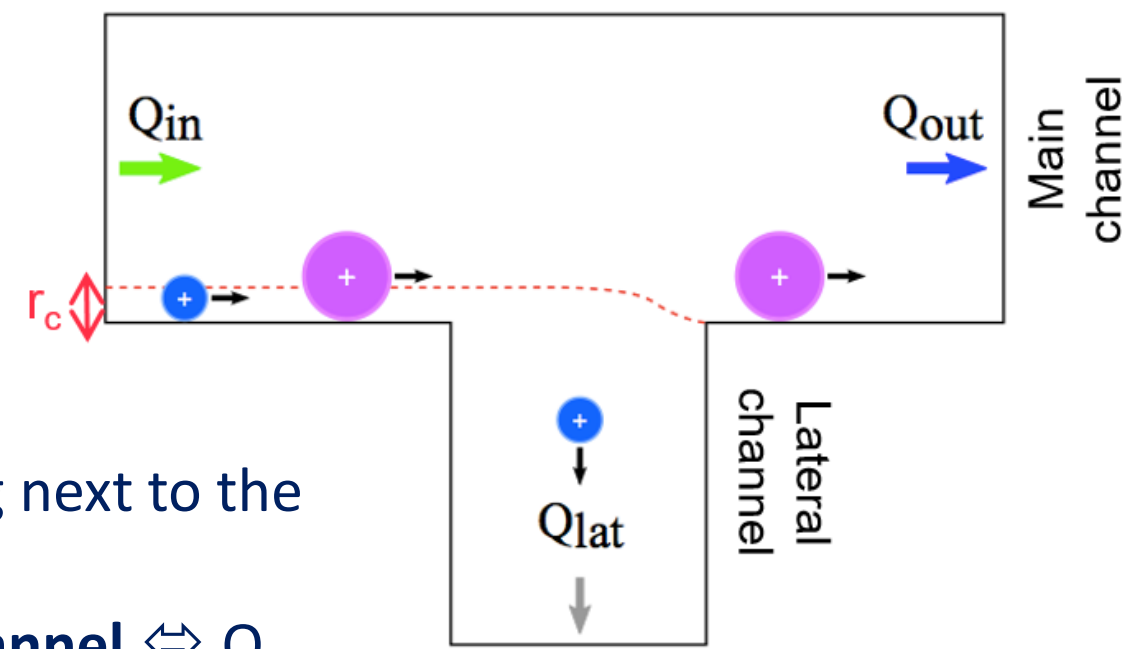
In that goal, we propose to couple a **microfluidic device (lab-on chip)** enabling to **separate particles** from a complex sample to a second device allowing the **capture of particles on specific spots** and their analysis. These methods allow obtaining important information about nanoscale vesicles in terms of size, membrane markers expression, and structure-function properties.

METHOD

1- Separation Principle: Hydrodynamic filtration

Exclusion of large particles from the streamlines located close to a wall

Critical sorting r_c = portion of fluid flowing through the side channel



After a bifurcation, a particle flowing next to the sidewall:

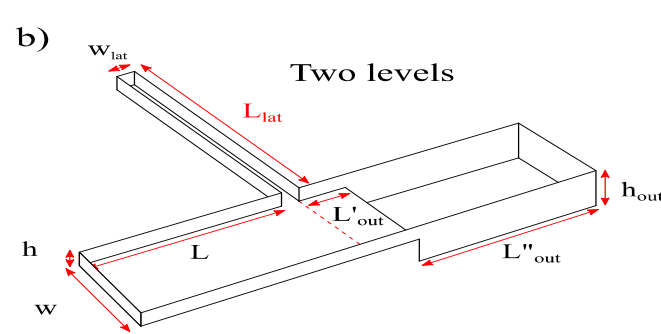
- radius $< r_c$ → sucked in lateral channel $\Leftrightarrow Q_{lat}$
- radius $> r_c$ → stay in the main channel $\Leftrightarrow Q_{out}$

Advantages and drawbacks:

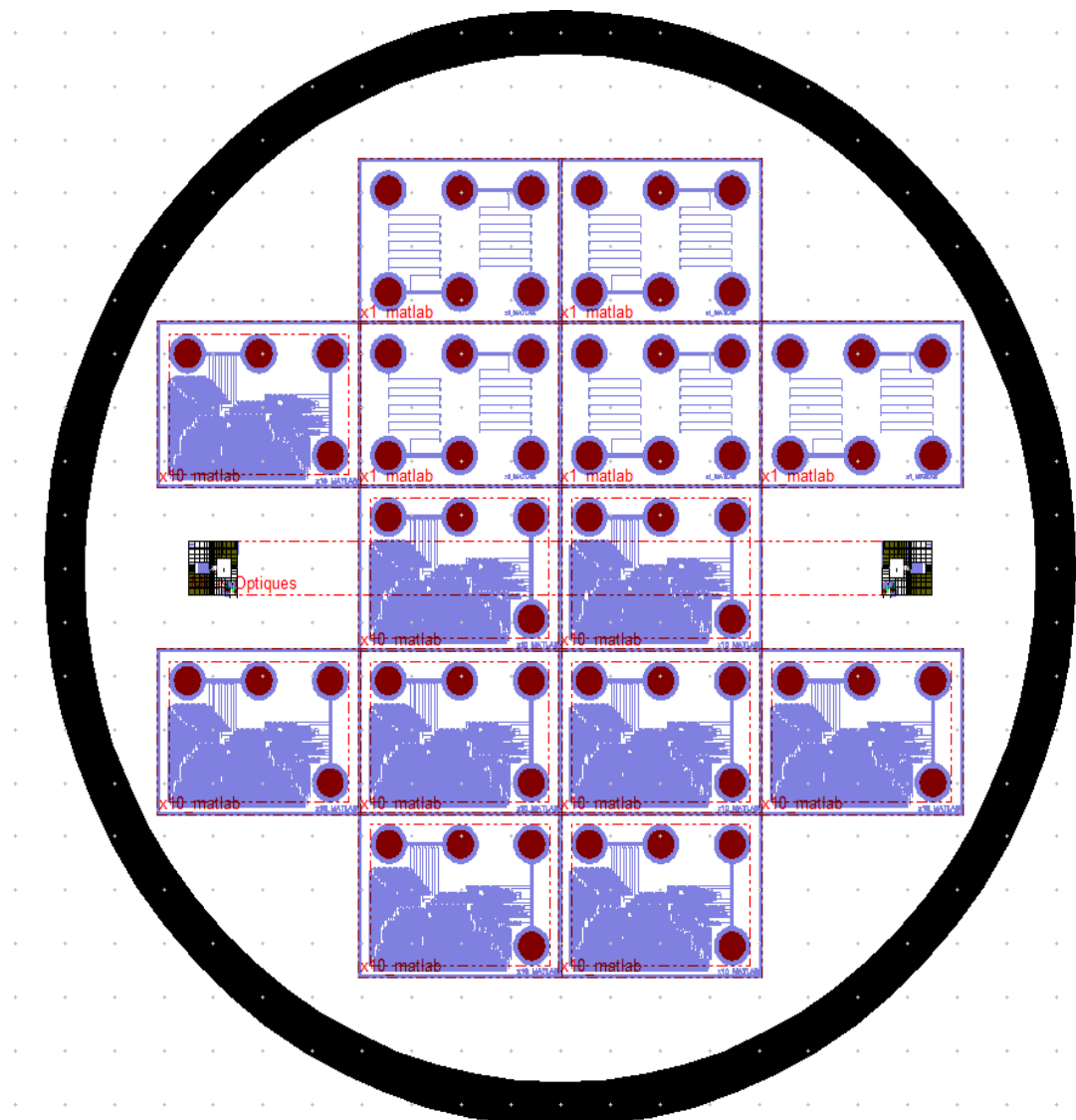
- ✓ Passive method \Leftrightarrow compatible with various applications
- ✓ Robustness \Leftrightarrow less prone to clogging than filter-based geometries
- ✓ Q_{lat} is weak \Leftrightarrow numerous channels necessary to obtain bigger volume

Proof of concept (Fouet et al LOC 2016):

2.5D configuration



Exclusion of 100 nm
Fraction of fluid extracted too low ($\approx 10^{-3}$)



Design channels lateral 1 and 10 channels (4" wafer)

2- Device Design:

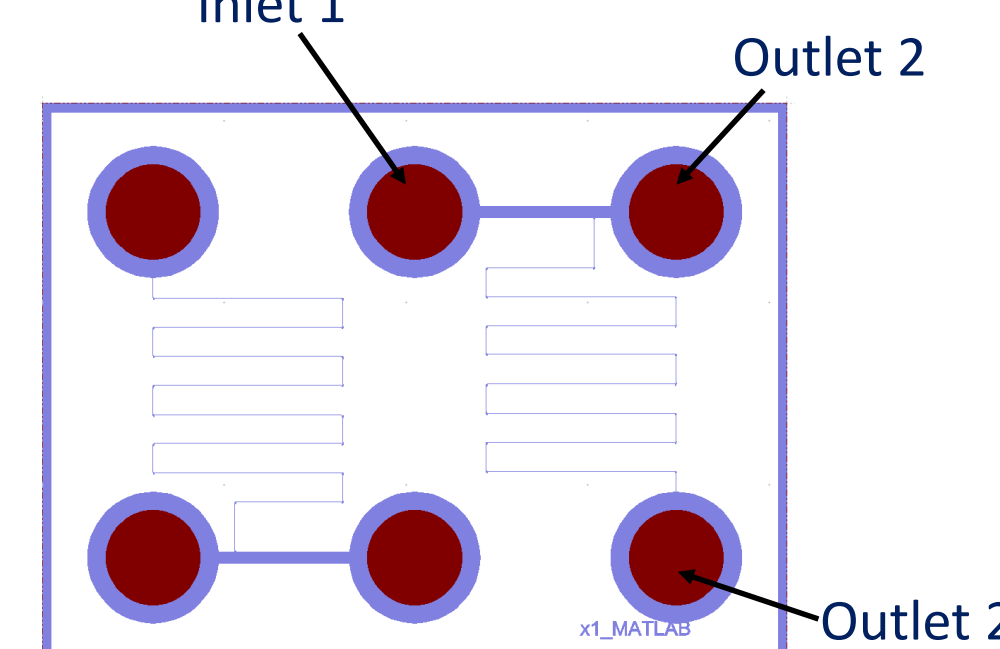
r_c and microchannel geometry (width, height) defined:

$r_c = 350\text{nm}$	Main channel		Lateral channel
	Input	Output	
w (μm)	300	300	10
h (μm)	100	100	100

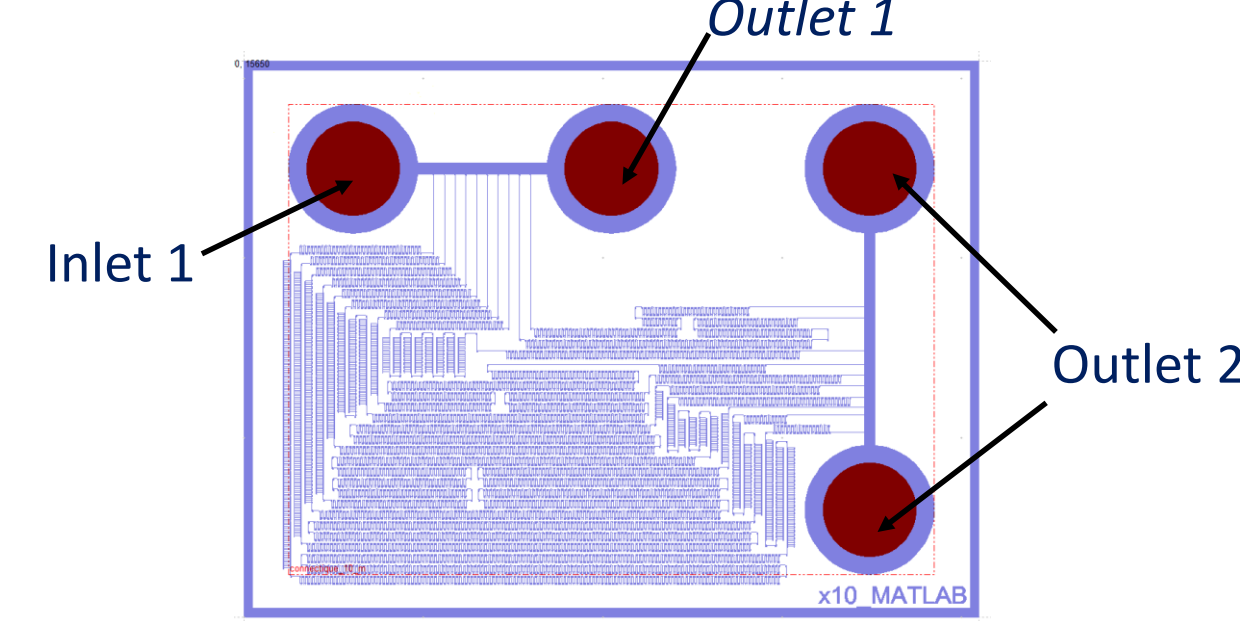
➤ Calculate L_{lat} with Matlab program to 1, 10 lateral channels:

1 channel	$L_{lat} = 4.72\text{ cm}$
10 channels	$4.72 < L_{lat} < 30.20\text{ cm}$

➤ Chip designs



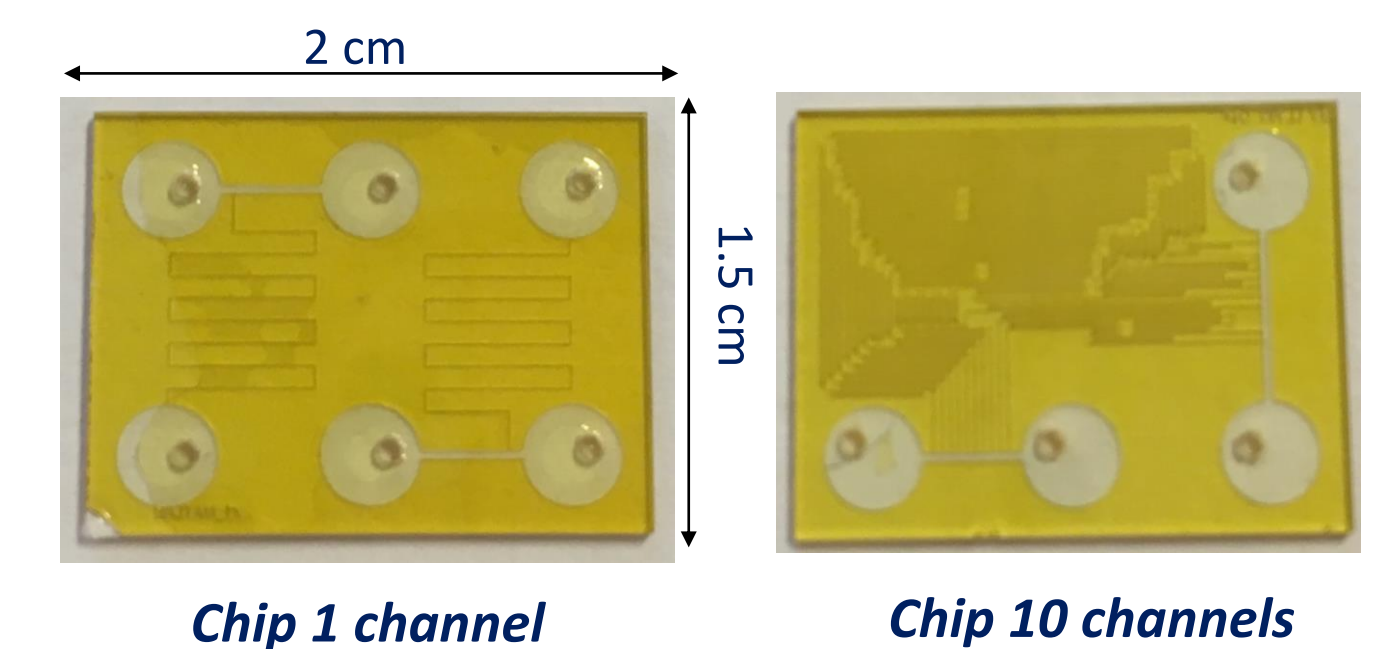
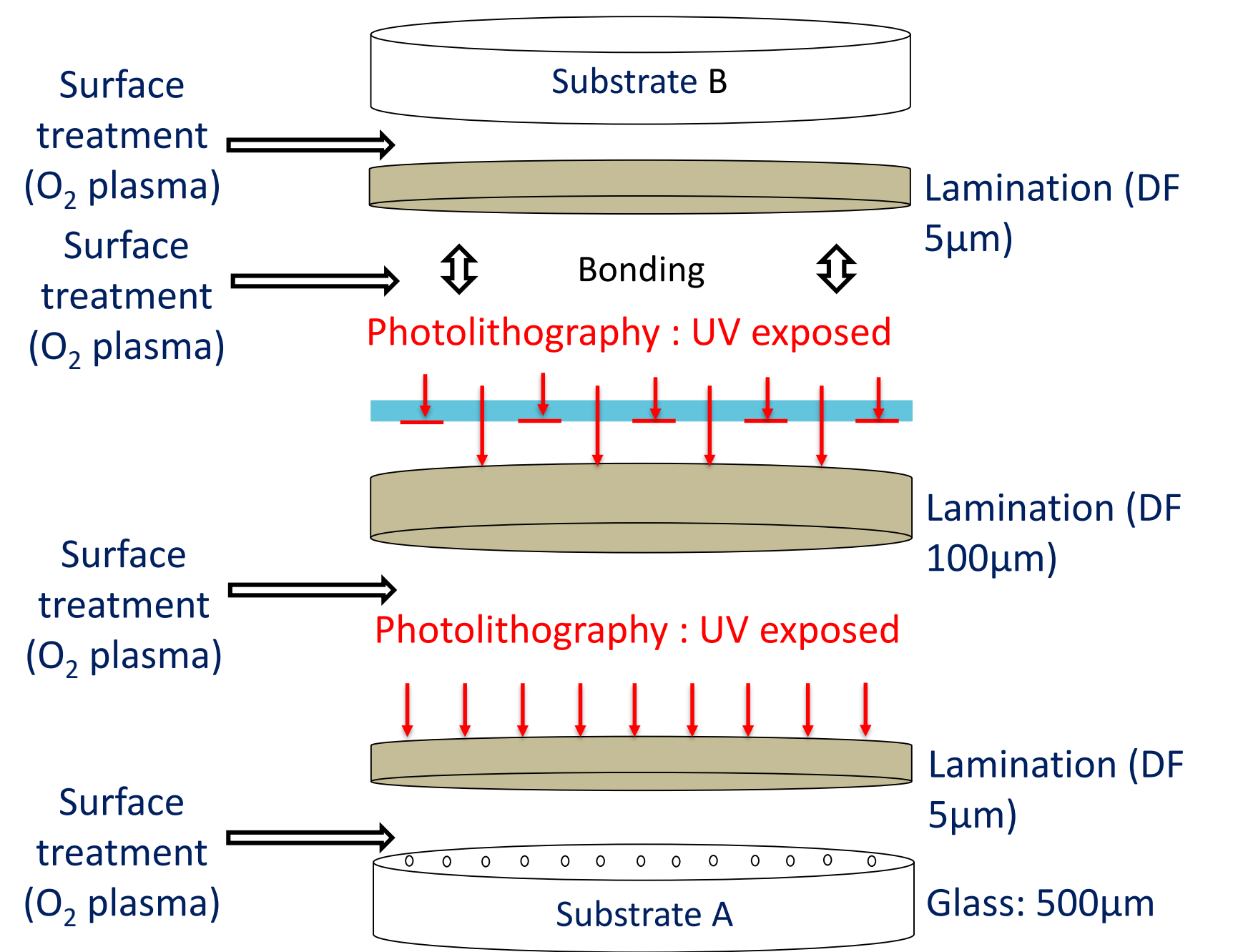
Design of chip with 1 lateral channel



Design of chip with 10 lateral channels

3-Microfabrication: Lab on chip in clean room

Fabrication of multilevel microfluidic devices by combining a lithography technique and lamination technology



Chip 1 channel

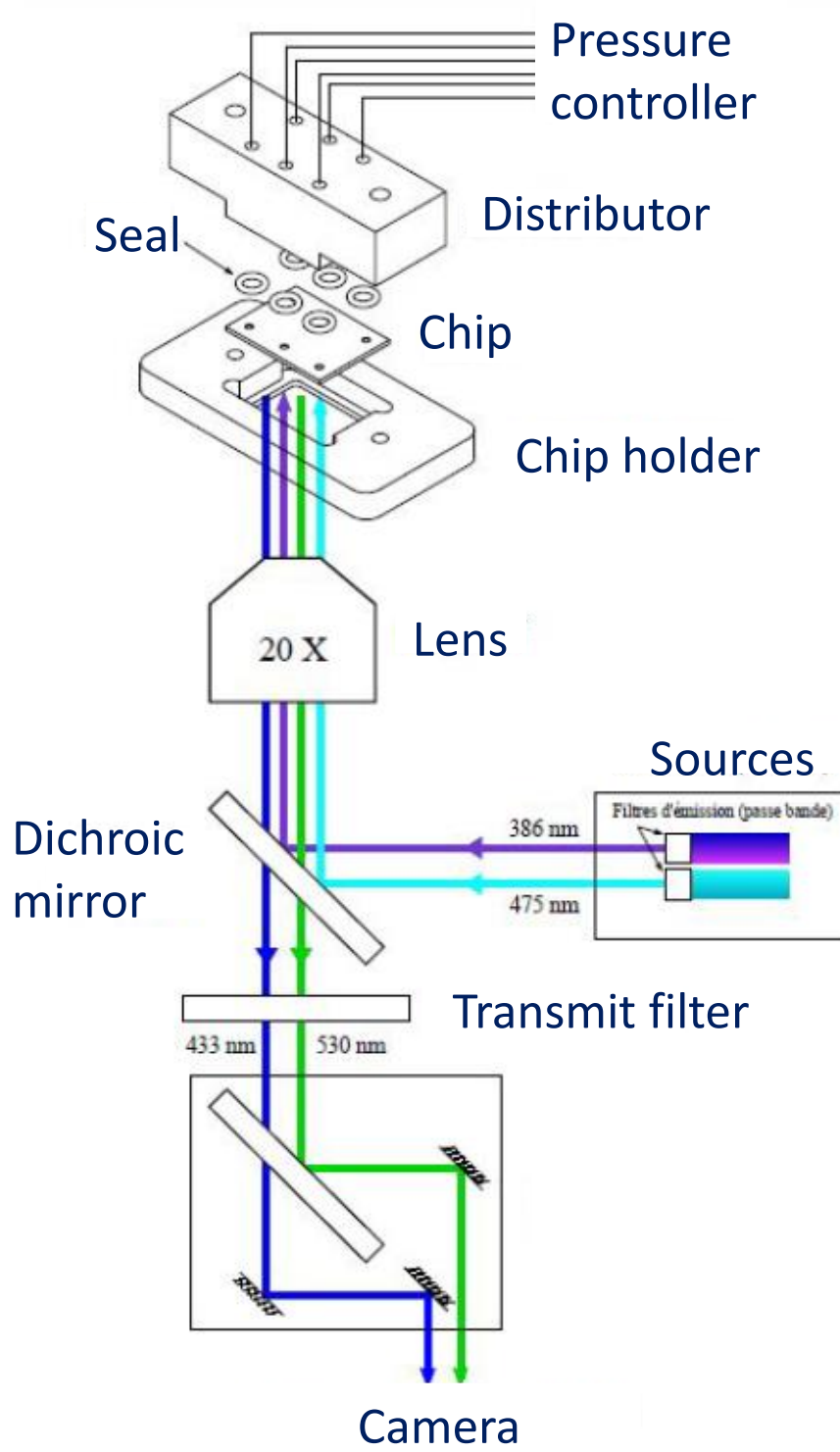
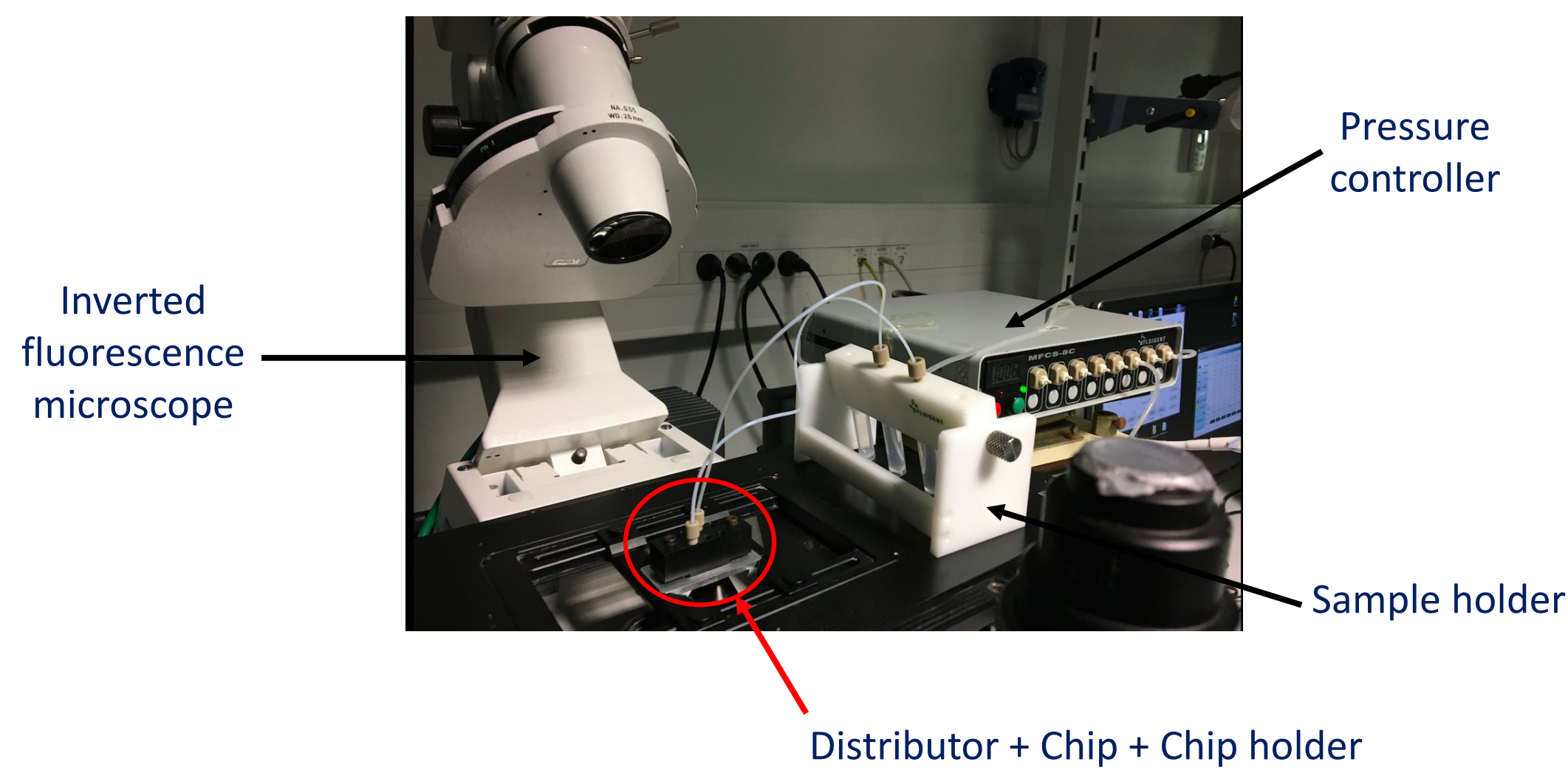
Chip 10 channels

EXPERIMENTAL SET-UP

Experimental set-up

Observations are done :

- Using an inverted fluorescence microscope (ZEISS)
- Images are recorded with a sCMOS camera (ZYLA)
- ImageJ allows to process data



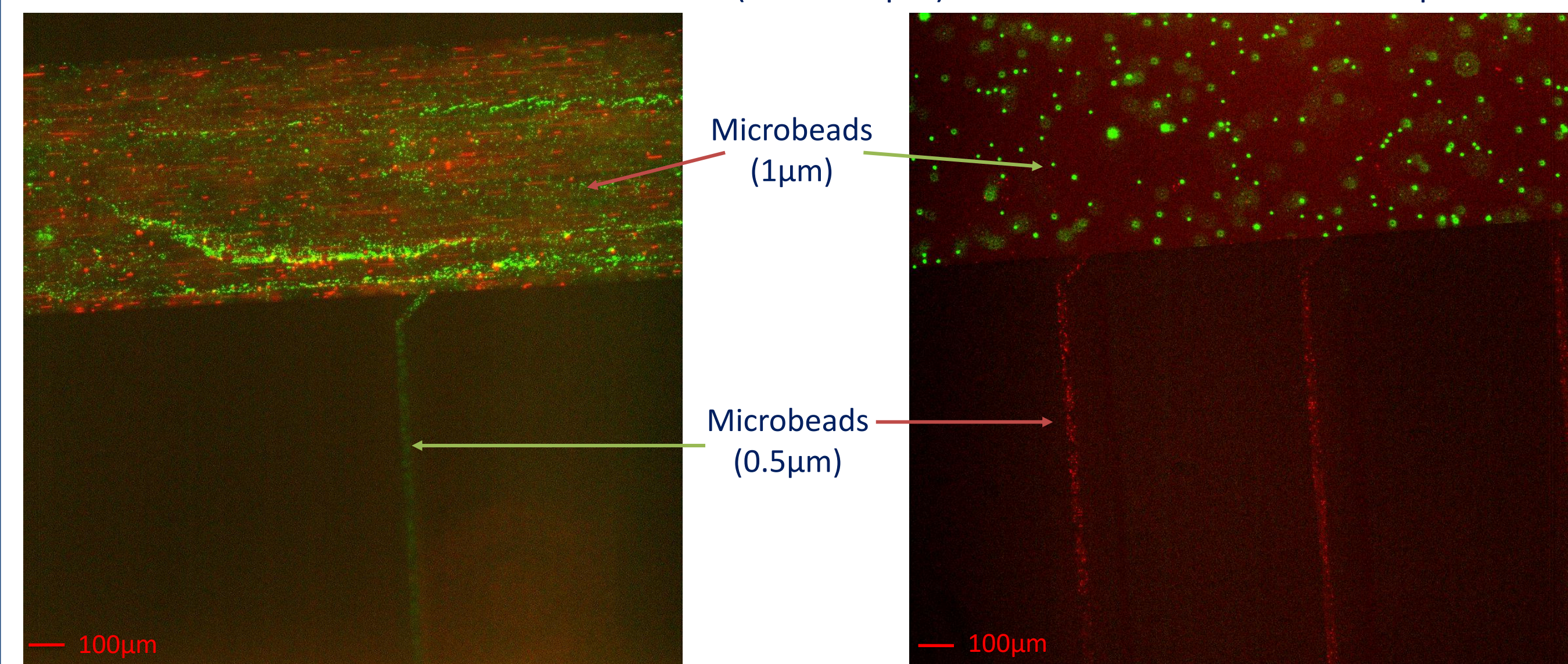
2-Microfluidic experiment

Proof-of-concept: Filtration of synthetic solution mimicking biological sample
 $R_c = 350\text{nm}$

Fluorescent calibrated polystyrene microbeads are used as fluorescent tracers:

Fluorescent microbeads	Diameters (μm)	Concentration
microbeads A	0.2	$7.5 \cdot 10^{11}$ microbeads/ $3\mu\text{L}$
microbeads B	0.5	$48 \cdot 10^9$ microbeads/ $3\mu\text{L}$
microbeads C	1	$6 \cdot 10^9$ microbeads/ $3\mu\text{L}$

Observations with microbeads mixture (1 and $0.5\mu\text{m}$) in 1 and 10 lateral channels chips



The critical radius is checked in different chips: the $1\mu\text{m}$ microbeads cannot flow into lateral channels and $0.5\mu\text{m}$ microbeads through in lateral channels

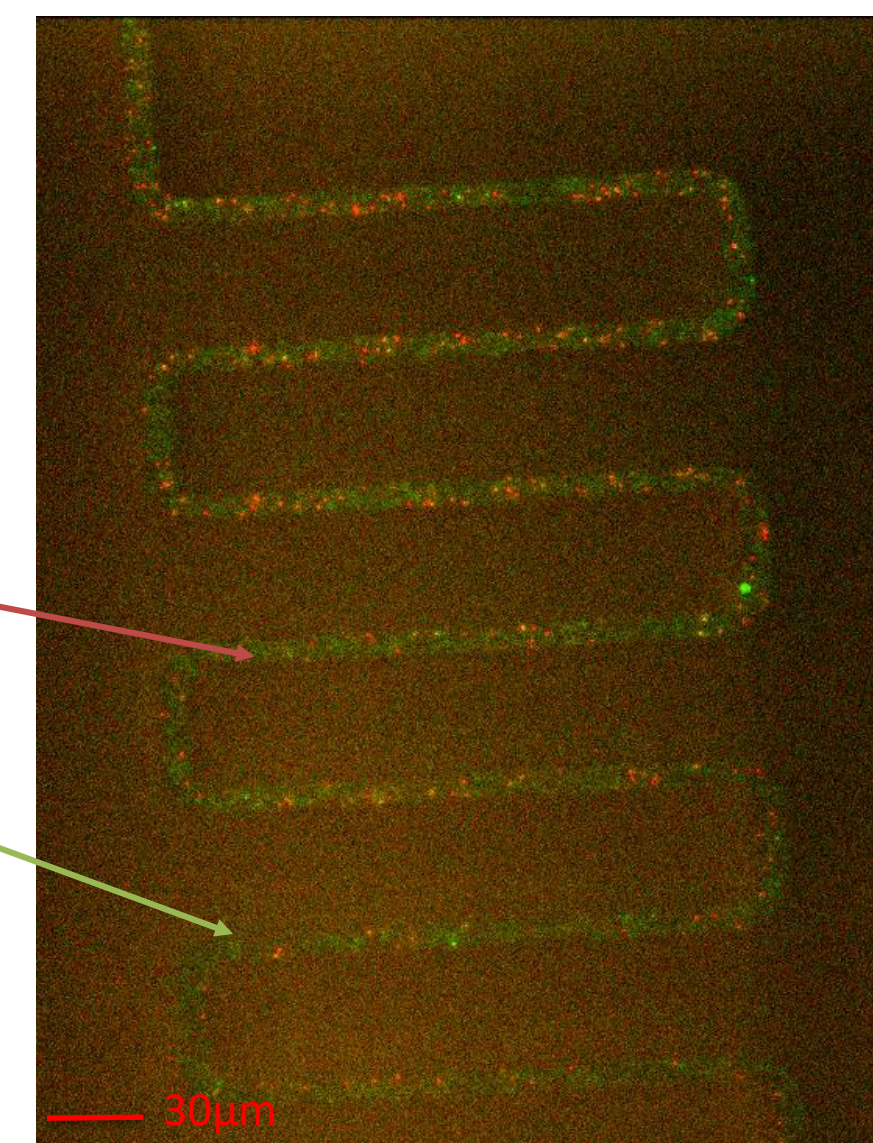
Observations with microbeads mixture (0.2 and $0.5\mu\text{m}$) in 10 lateral channels chip

In 10 lateral channels chips, 0.2 and $0.5\mu\text{m}$ microbeads flow into lateral channels

Filtration of synthetic solution on different chips confirm the correct critical radius $r_c=350\text{nm}$

Channels	Flow rate ($\mu\text{L/s}$)
1	0,000014
10	0,023

Microbeads ($0.5\mu\text{m}$)
Microbeads ($0.2\mu\text{m}$)



CONCLUSION

The purpose of this project is to develop a comprehensive microfluidic method which allows micro and nanoparticles sorting by size (hydrodynamic filtration) and to combine this device with a second device which allows capturing these particles on specific spots.

The proof of concept has been done with synthetic solution using fluorescent calibrated polystyrene microbeads.

PERSPECTIVES :

- Optimization of filtered volumes (with several viscoelastic effects)
- Increase number of lateral channels
- Filtration test with blood sample
- Make new design with critical radius smaller for exosome applications

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REFERENCES

M.Fouet, MA. Mader, S.Iraïn, Z.Yanha, A.Naillon, S.Cargou, AM. Gué and P. Joseph, Lab chip, 2016