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# A LOW-COST AND EASY TO USE SORTING DEVICE FOR THE SEPARATION OF EXTRACELLULAR VESICLES FROM COMPLEX FLUIDS

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## CONTEXT

Identifying and characterizing **Extracellular vesicles** (EVs) in biofluids could lead to unprecedented diagnosis/prognosis assays for a wide set of pathologies.

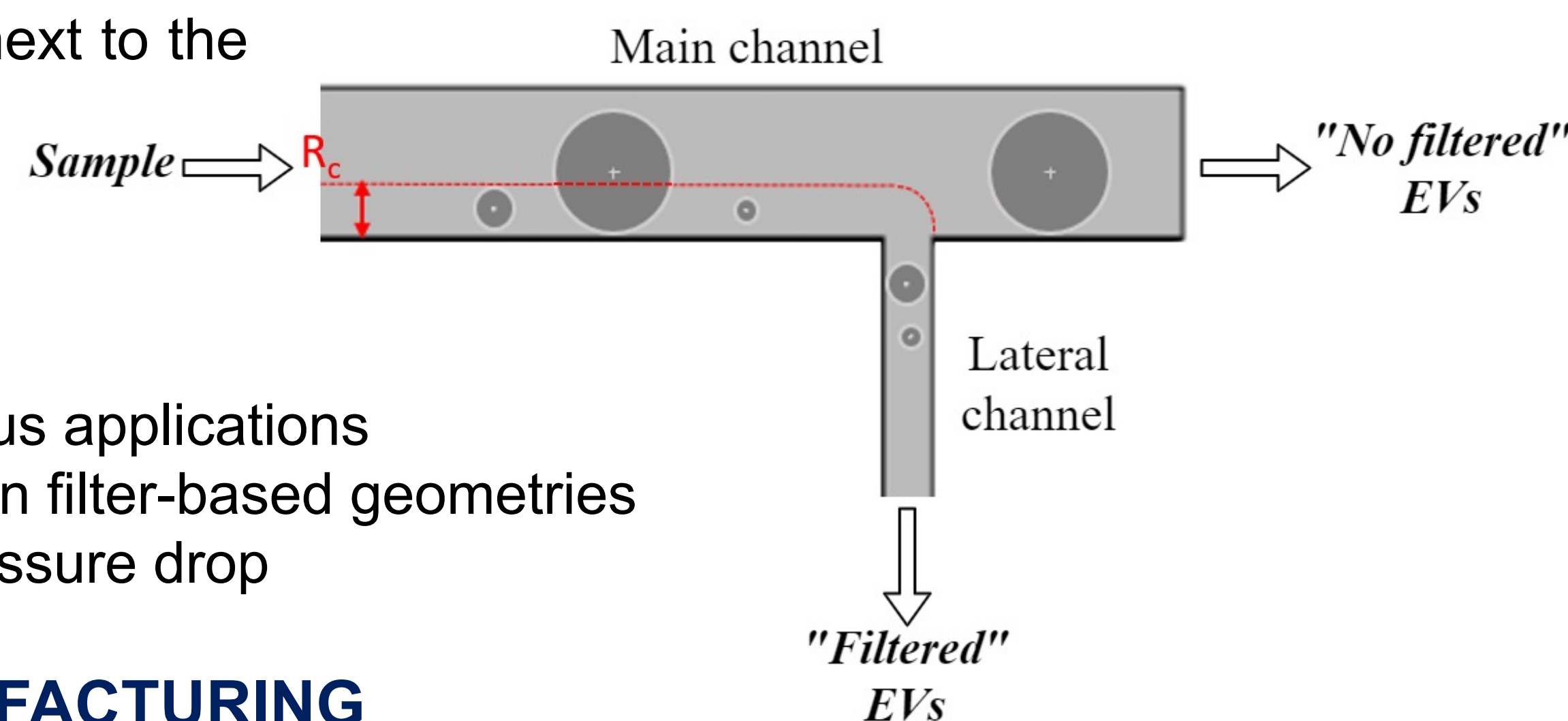
Owing to their complexity in size, origin, membrane markers, there is currently no ideal technology available yet to sort, to quantify and characterize cell-derived microvesicles, and fully relate their structure and functions which have high potential in clinical diagnostics and early treatment of life threatening diseases such as cancer, cardio vascular diseases....

## SEPARATION PRINCIPLE: HYDRODYNAMIC FILTRATION

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

At the channel crossing, a particle flowing next to the sidewall:

- radius <  $R_c$  → flow in **lateral channel**
- radius >  $R_c$  → stay in the **main channel**



### Advantages and drawbacks:

- ✓ Passive method ⇔ compatible with various applications
- ✓ Robustness ⇔ less prone to clogging than filter-based geometries
- ✓ Reliability ⇔ independent of flow and pressure drop

## LAB ON CHIP: DESIGN AND MANUFACTURING

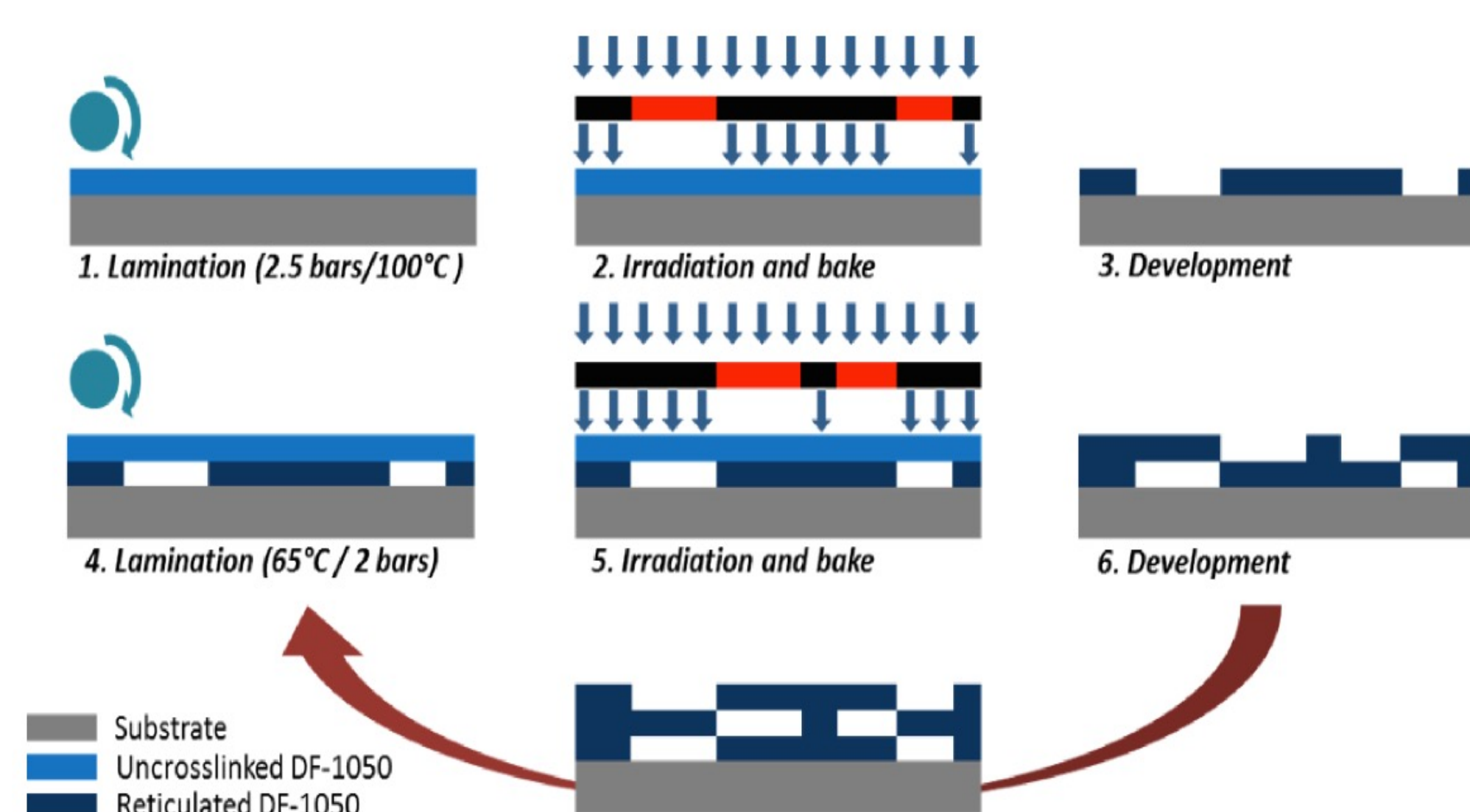
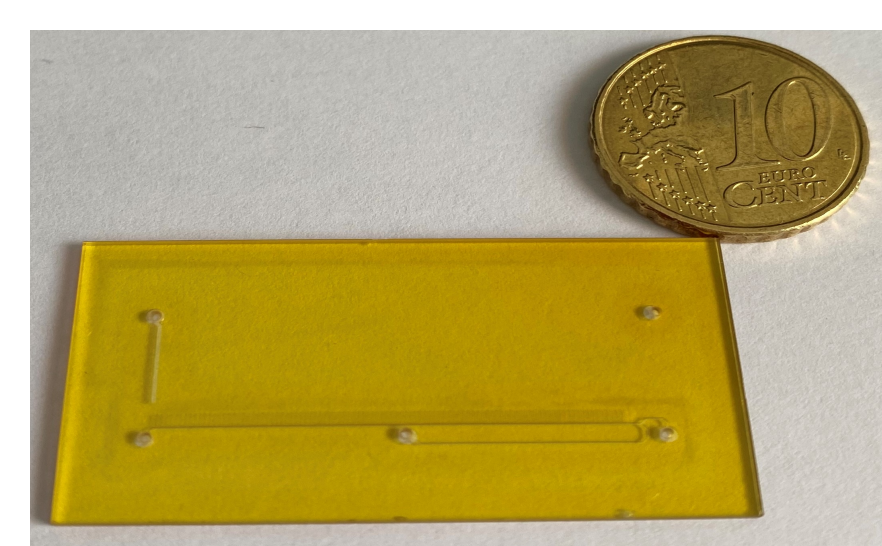
Choosing the appropriate dimensions of the channels, we designed and fabricated chips enabling the separation of vesicles at tunable cut-off (150 - 900 nm).

w (μm)	h (μm)	L (cm)	Flow rate (μL/s)
10	10	0.12 to 7.59	0.035

### Fabrication process: successive lamination and photolithography

Dry Film: manufacturable process by laminating dry photosensible films

- Low cost
- Compatible with micro-nanotechnologies
- Biocompatible



### Samples preparations

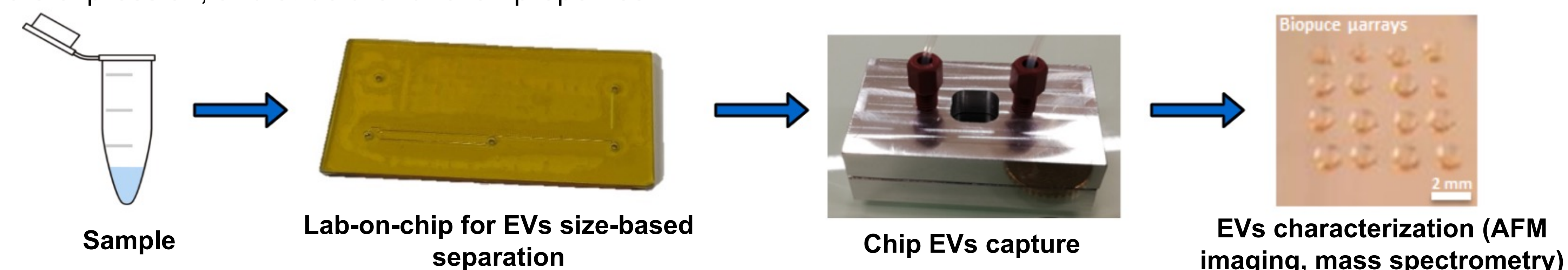
- Development of NP 140 / NP 480 / NP 920 calibrators: Biofunctionalization by proteins(OVA, CAS)
- Approx. 3500 CAS / NP 140 and 17000 OVA / NP 480
- Selective trapping verification by anti-CAS and anti-OVA

NPs	Diametre	Proteins	Immunocapture
NP 140	140 nm	Casein	anti-CAS
NP 480	480 nm	Ovalbumin	anti-OVA
NP 920	920 nm	-	-

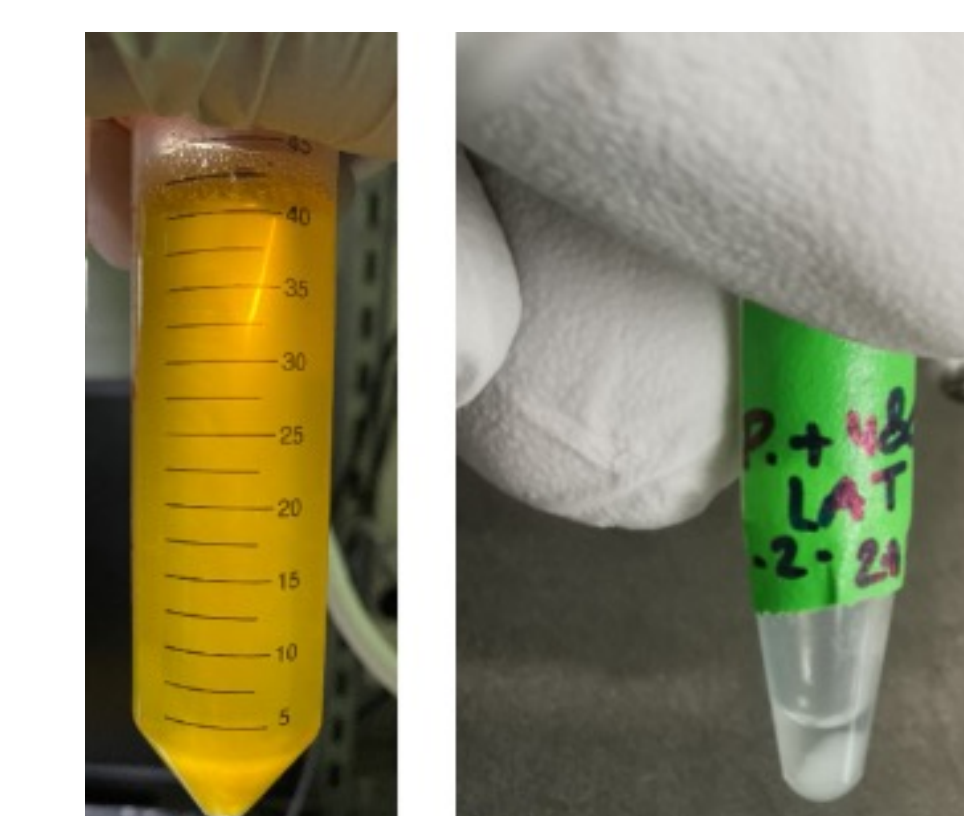
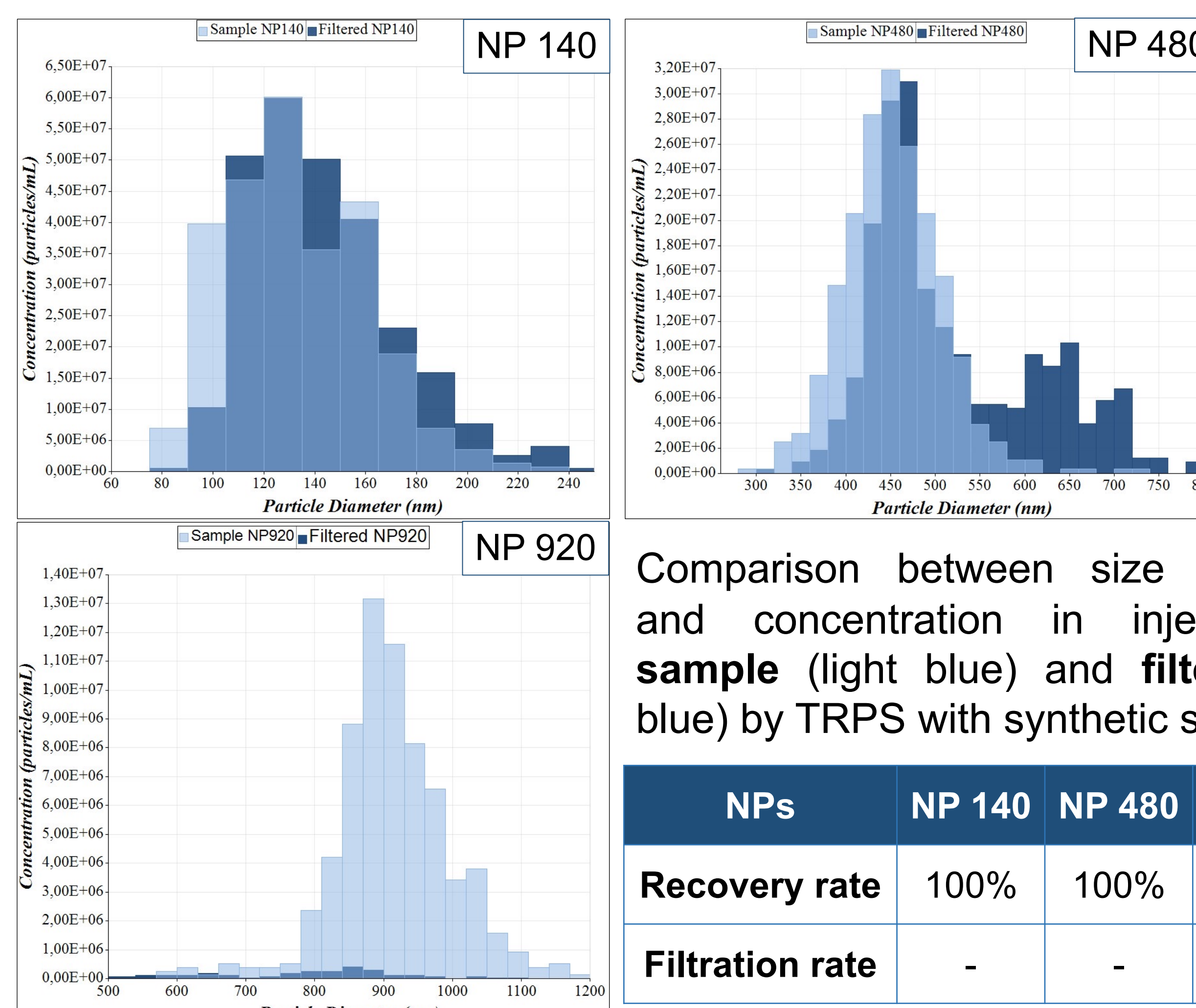
Synthetic solution	NPs in PBS-Tween 20 (0.1%)	[NPs] = 1.10 <sup>8</sup> part/mL
Blood sample	Plasma diluted x 10	[NPs] in PBS-Tween20: 2.10 <sup>6</sup> to 1.10 <sup>8</sup> part/mL
	Blood diluted X 10 and x 100	

## APPROACH

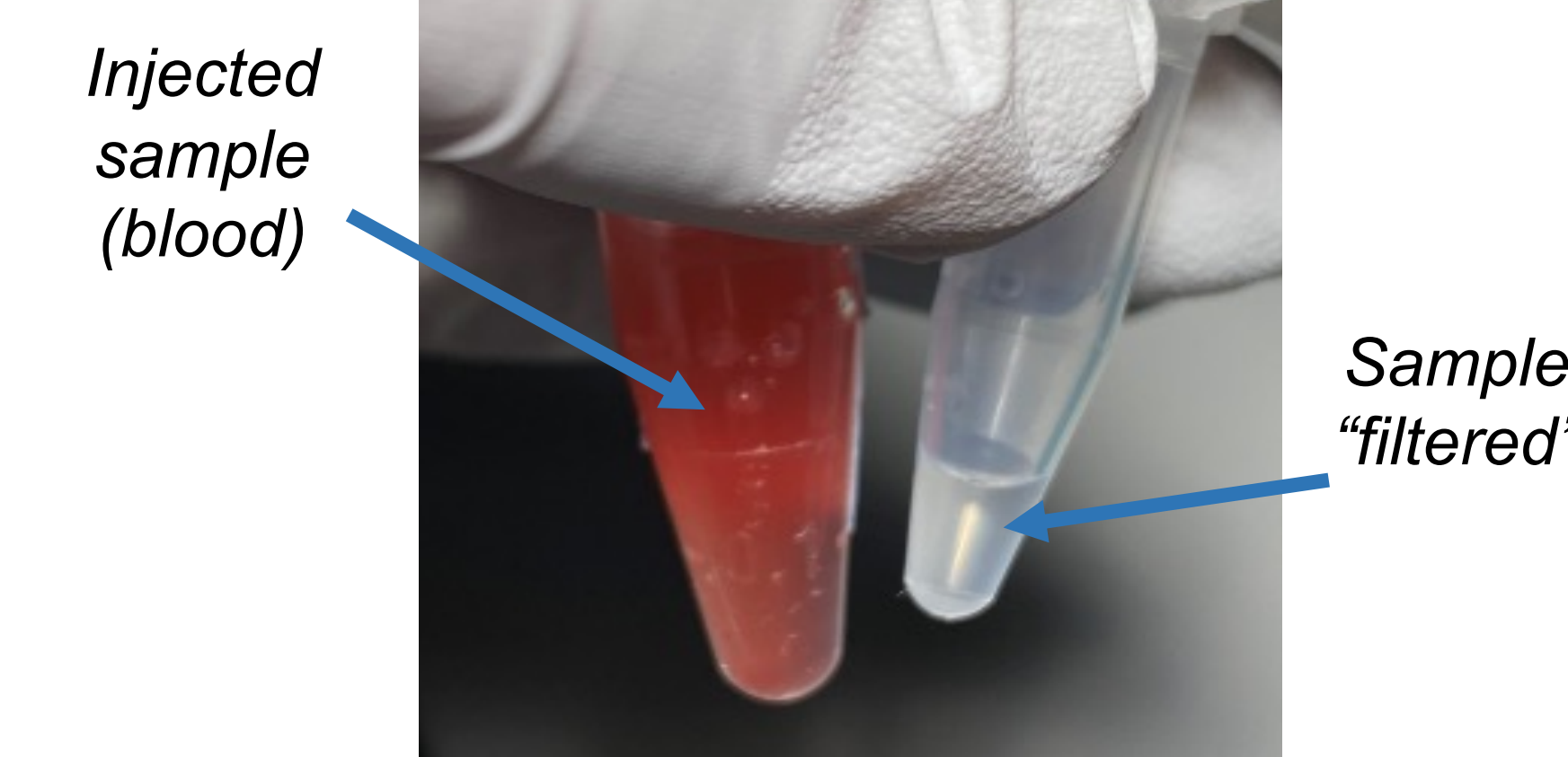
We propose to couple a microfluidic device (LOC) enabling to **separate particles** from a complex sample to a device allowing the **capture of particles on specific spots** and their analysis. These methods allow obtaining important information on a chip about nanoscale vesicles in terms of size, membrane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



## CHARACTERIZATION AND VALIDATION OF LAB ON CHIP



Blood sample: **Malassez cells** characterization → Same filtration rate in biological fluid



## CONCLUSION

- **Proof-on-concept** : NP 140 and 480 were filtered while NP 920 were find in the unfiltered sample.
- Recovery conditions: 35 μL sample filtered in 30 minutes.
- With NPs in PBS-Tween20 and NPs in blood sample, we obtained the same **recovery rate** (100%) and **filtration rate** (96 to 98%).
- We showed high **efficiency separation** with NPs and biological fluids did **not affect** the separation.
- We are also improving the **performances** of the devices (filtration flow rate, filtered volume) and are **coupling** it to an immuno-trapping device in order to provide an **integrated EVs sorting and capture bio-analytical system**.

## REFERENCES

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