



HAL
open science

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

Lyne Pillemont, Daniel Guneyusu, Wilfrid Boireau, Celine Elie-Caille, Anne
Marie Gué

► **To cite this version:**

Lyne Pillemont, Daniel Guneyusu, Wilfrid Boireau, Celine Elie-Caille, Anne Marie Gué. A LOW-COST AND EASY TO USE SORTING DEVICE FOR THE SEPARATION OF EXTRACELLULAR VESICLES FROM COMPLEX FLUIDS. MicroTAS 2021, Oct 2021, Palm Spring, United States. , MicroTAS 2021, 2021, The 25th International Conference on Miniaturized Systems for Chemistry and Life Sciences. hal-03514467

HAL Id: hal-03514467

<https://laas.hal.science/hal-03514467v1>

Submitted on 6 Jan 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Lyne PILLEMONT¹, Daniel GUNEYSU², Wilfrid BOIREAU², Céline ELIE-CAILLE², Anne-Marie GUE¹

¹LAAS-CNRS, CNRS, Université de Toulouse, Toulouse, France

²FEMTO-ST, Université Bourgogne Franche-Comté, CNRS, Besançon, France



CONTEXT

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

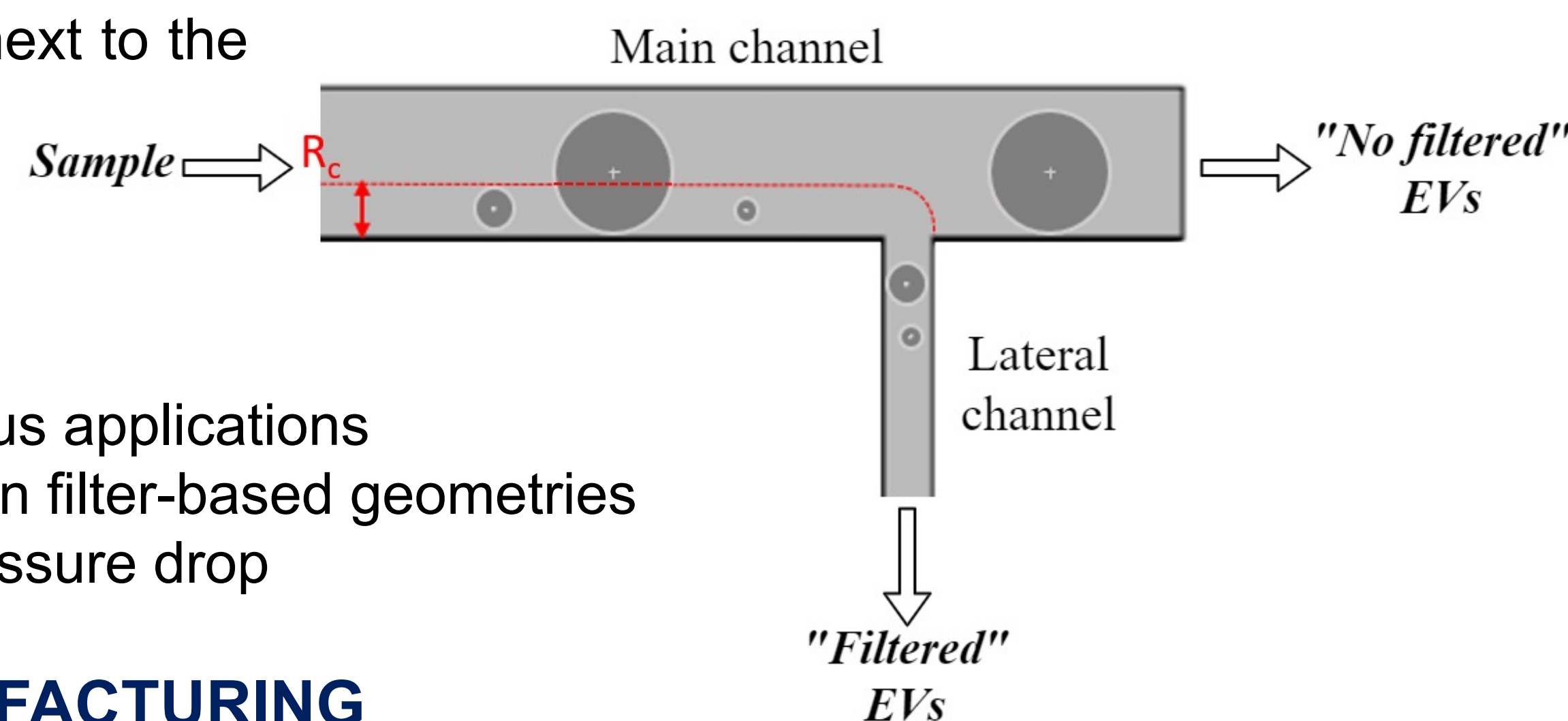
Owe to their complexity in size, origin, membrane markers, there is currently no ideal technology available yet to sort, to quantify and characterize cell-derived microvesicles, and fully relate their structure and functions which have high potential in clinical diagnostics and early treatment of life 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

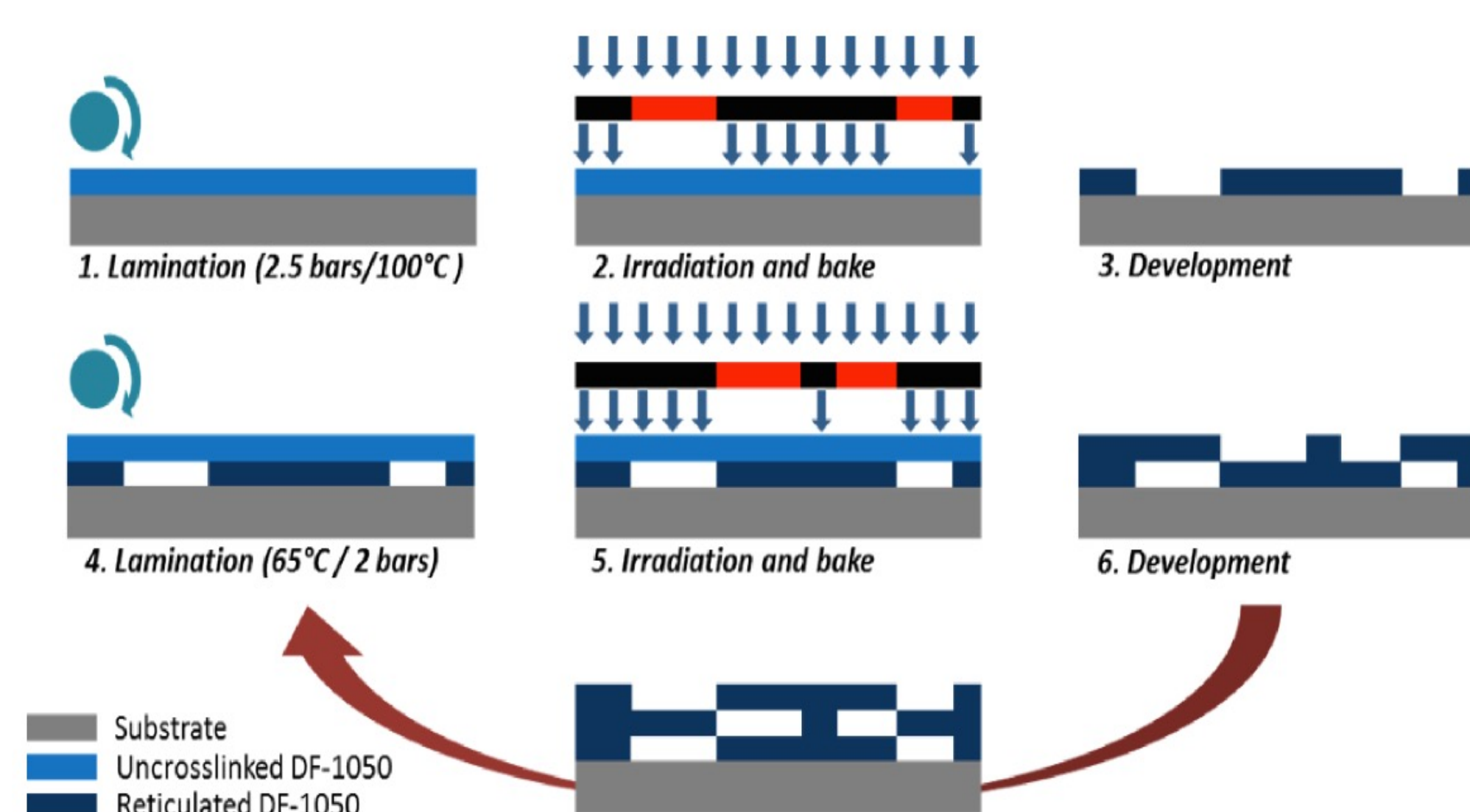
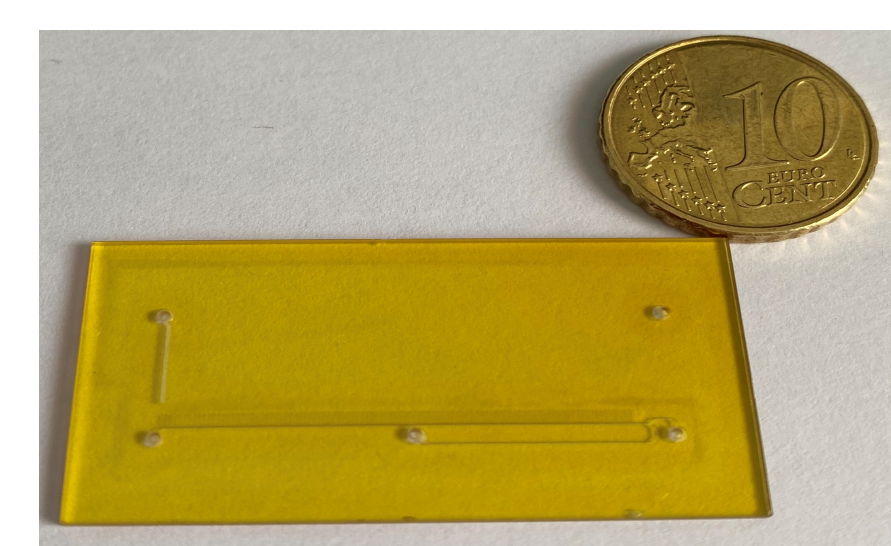
Choosing the appropriate dimensions of the channels, we designed and fabricated chips enabling the separation of vesicles at tunable cut-off (150 - 900 nm).

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

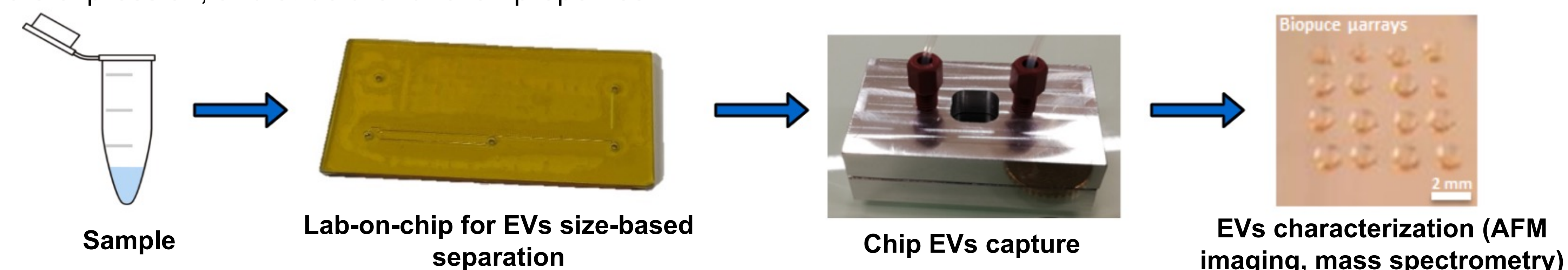
- Development of NP 140 / NP 480 / NP 920 calibrators: Biofunctionalization by proteins(OVA, CAS)
- Approx. 3500 CAS / NP 140 and 17000 OVA / NP 480
- Selective trapping verification by anti-CAS and anti-OVA

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

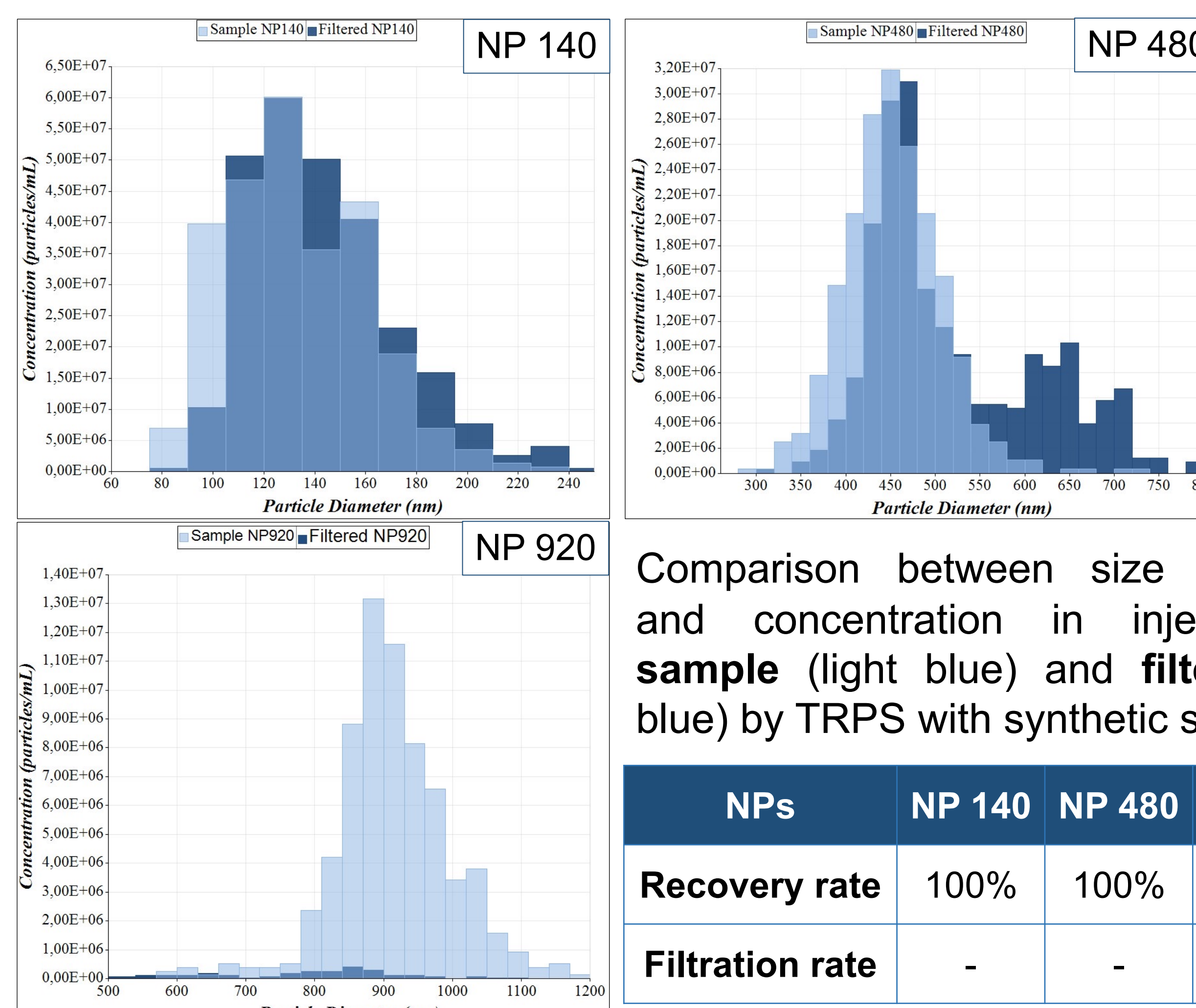
Synthetic solution	NPs in PBS-Tween 20 (0.1%)	[NPs] = 1.10 ⁸ part/mL
Blood sample	Plasma diluted x 10	[NPs] in PBS-Tween20: 2.10 ⁶ to 1.10 ⁸ part/mL
	Blood diluted X 10 and x 100	

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.

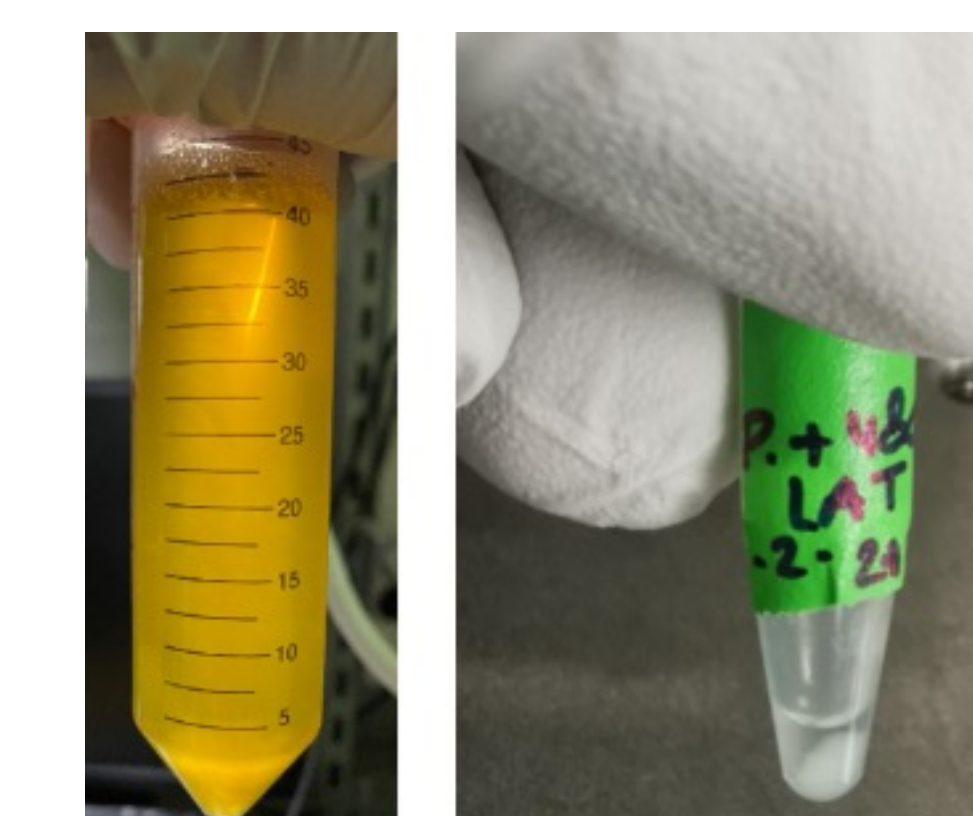


CHARACTERIZATION AND VALIDATION OF LAB ON CHIP

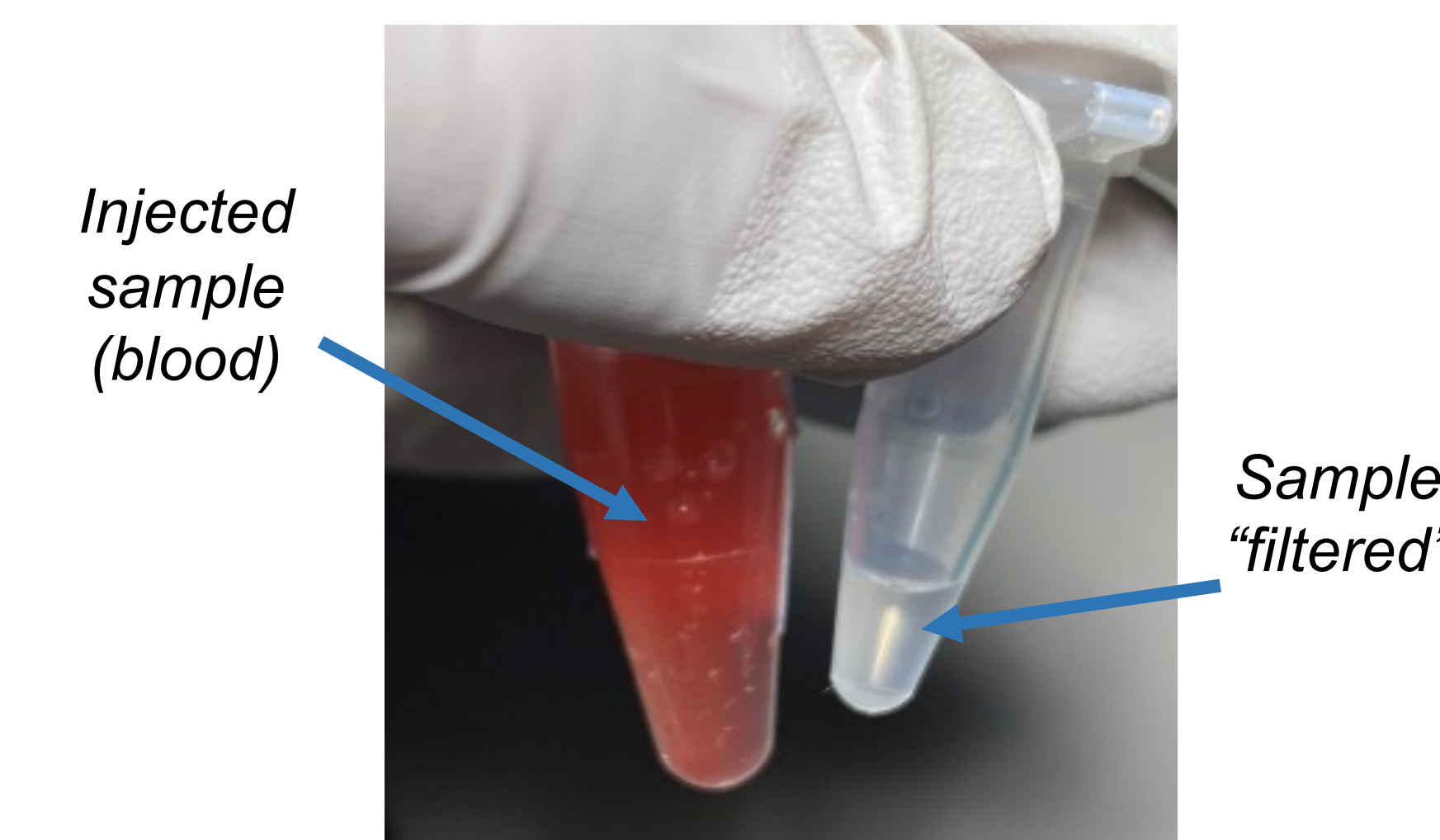


Comparison between size distribution and concentration in injected NPs sample (light blue) and filtered (dark blue) by TRPS with synthetic solutions

NPs	NP 140	NP 480	NP 920
Recovery rate	100%	100%	-
Filtration rate	-	-	96 to 98%



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

- Yamada, M., & Seki, M. (2005). Hydrodynamic filtration for on-chip particle concentration and classification utilizing microfluidics. *Lab on a Chip*, 5(11), 1233-1239.
- Fouet, M., et al. "Filter-less submicron hydrodynamic size sorting." *Lab on a Chip* 16,4 (2016): 720-733.
- Courson, R, et al. "Low-cost multilevel microchannel lab on chip: DF-1000 series dry film photoresist as a promising enabler." *RSC Advances* 4,97 (2014): 54847-54853.

ACKNOWLEDGEMENTS

ANR MADNESS project (ANR-17-CE09-0025)
Toulouse Cancer-Health foundation for funding this work.
This work was supported by the micro and nanotechnology platform of LAAS-CNRS, member of the French network RENATECH.