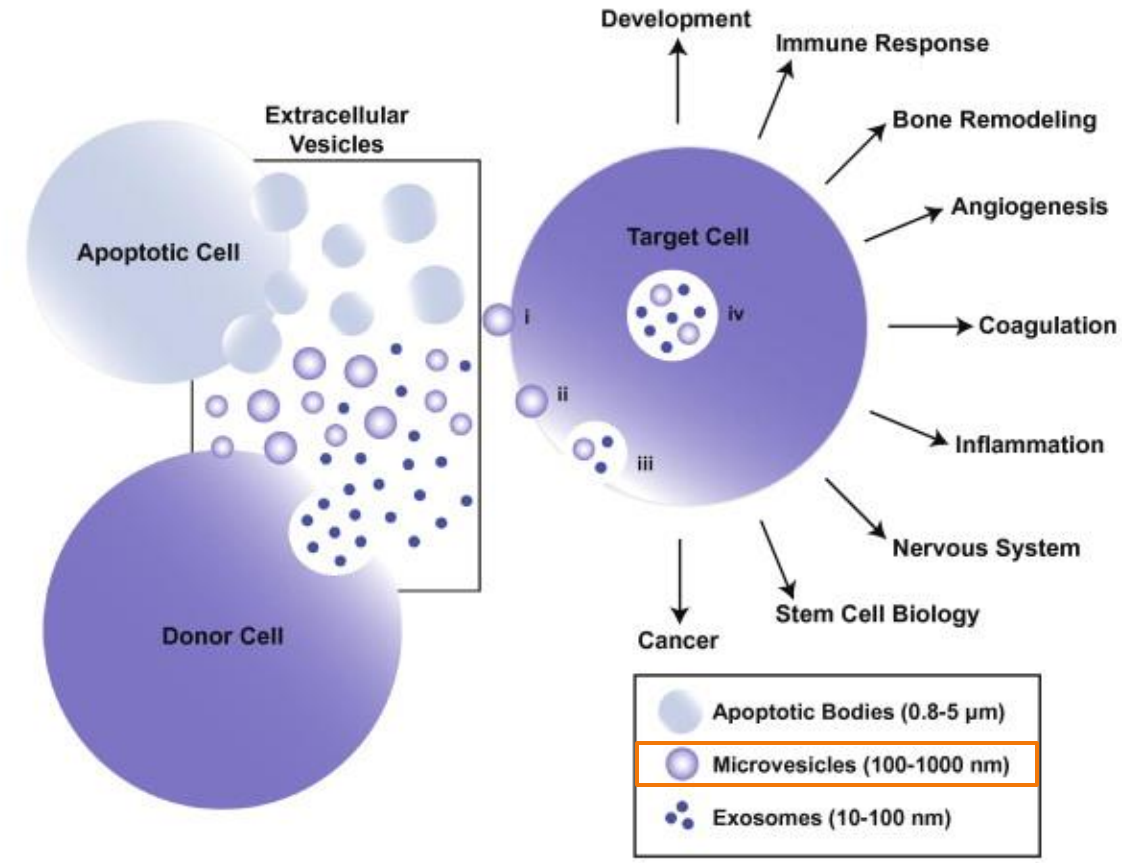
“Applicazioni dell’imaging flow cytometry in ricerca biomedica e clinica"

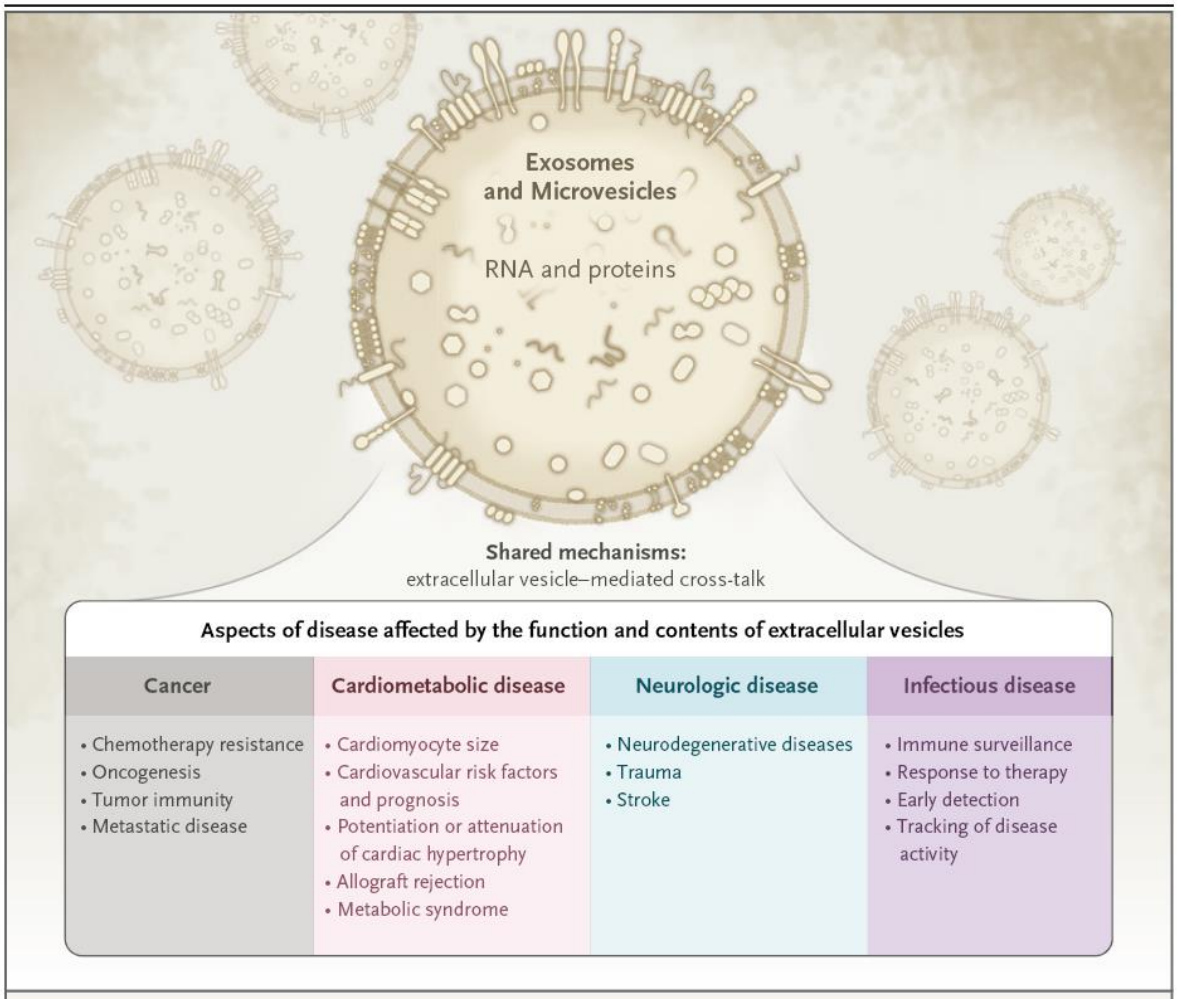
Paola Lanuti
Università “G. d’Annunzio”, Chieti-Pescara

Vescicole Extracellulari (VE)



Morhayim J et al., Arch Biochem Biophys, 2014

Ruolo delle Vescicole Extracellulari

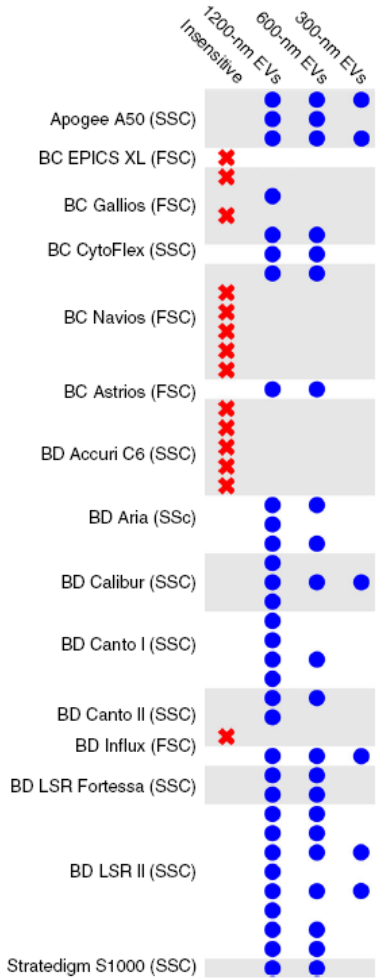


Aspects of disease affected by the function and contents of extracellular vesicles

Cancer	Cardiometabolic disease	Neurologic disease	Infectious disease
• Chemotherapy resistance • Oncogenesis • Tumor immunity • Metastatic disease	• Cardiomyocyte size • Cardiovascular risk factors and prognosis • Potentiation or attenuation of cardiac hypertrophy • Allograft rejection • Metabolic syndrome	• Neurodegenerative diseases • Trauma • Stroke	• Immune surveillance • Response to therapy • Early detection • Tracking of disease activity

Shah R et al., The New England Journal of Medicine, 2018

Vescicole Extracellulari & Citometria a Flusso

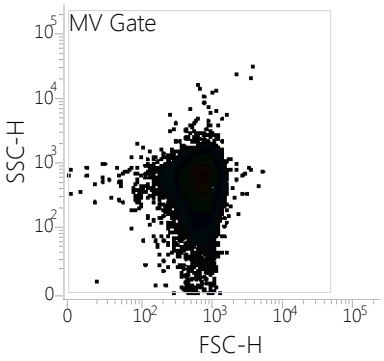


Van der Pol E. et al., J. Thromb. Haemost., 2018

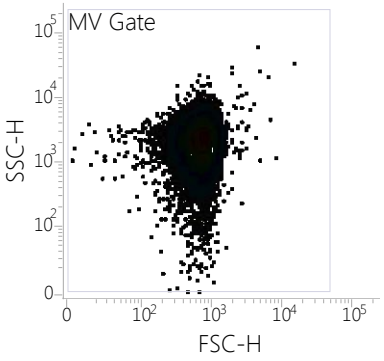
Liposomi Fluorescenti

Analizzatore 1

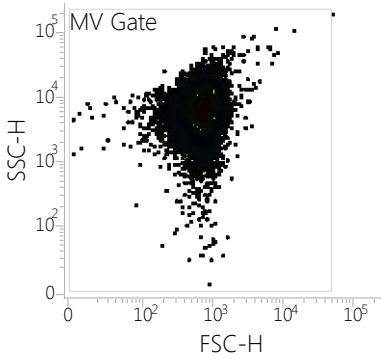
50 nm



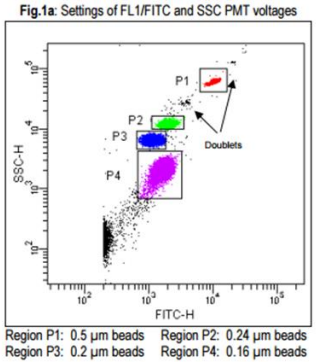
100 nm



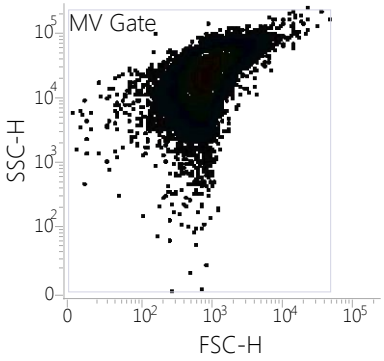
200 nm



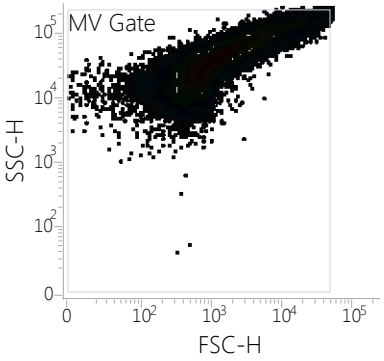
Megamix-Plus SSC



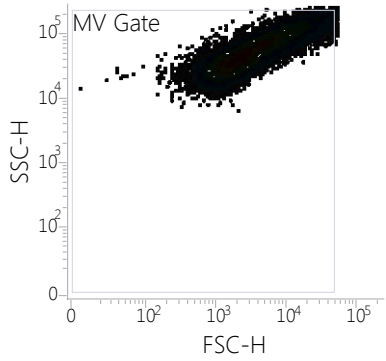
400 nm



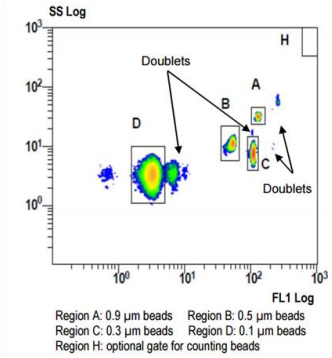
600 nm



1 µm



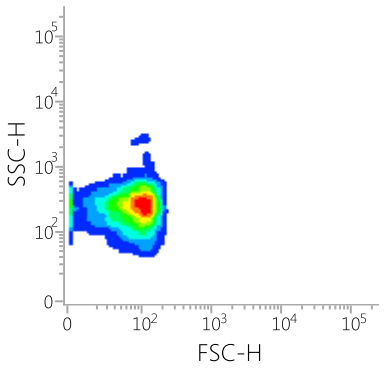
Megamix-Plus FSC



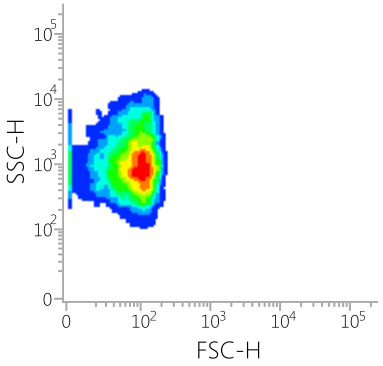
Liposomi Fluorescenti

Analizzatore 2

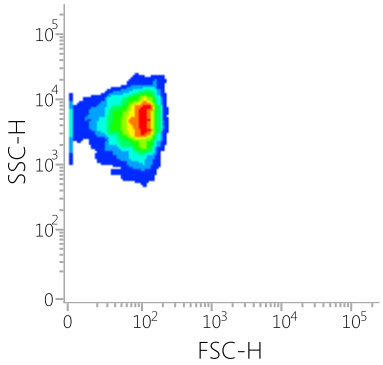
50 nm



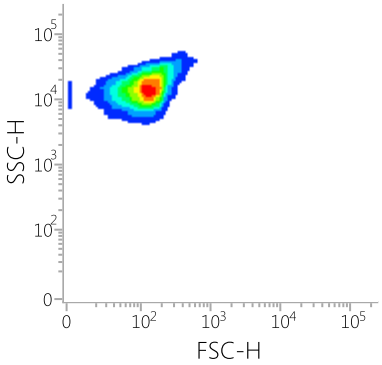
100 nm



200 nm



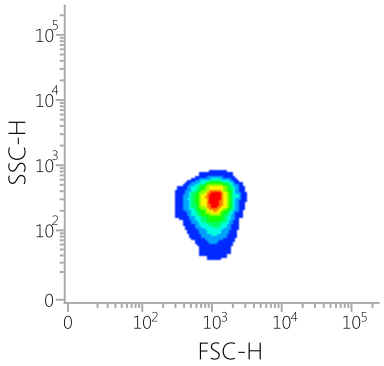
400 nm



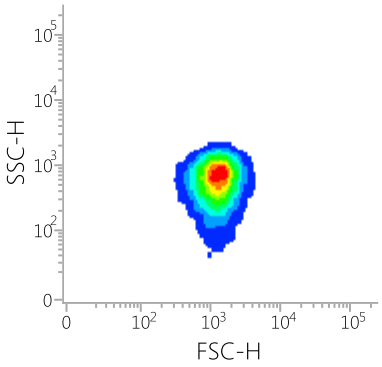
Liposomi Fluorescenti

Cell Sorter

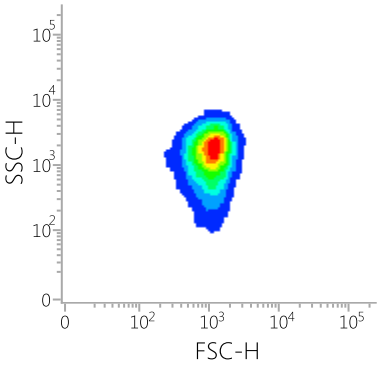
50 nm



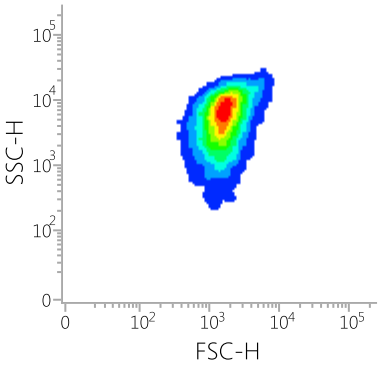
100 nm



200 nm



400 nm



Procedure di arricchimento delle Vescicole Extracellulari

Journal of Extracellular Vesicles

co-action PUBLISHING

SHORT COMMUNICATION

High-speed centrifugation induces aggregation of extracellular vesicles

Romain Linares[1], Sisareuth Tan[1], Céline Gounou[1], Nicolas Arraud[1] and Alain R. Brisson[1,2]*

[1]Molecular Imaging and NanoBioTechnology, University of Bordeaux, Pessac, France; [2]Institut Universitaire de France, Paris, France

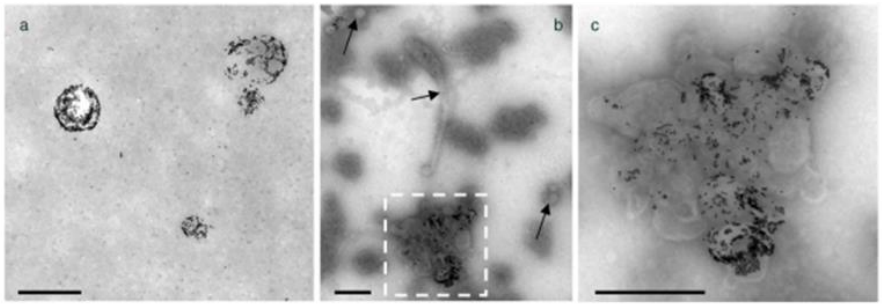
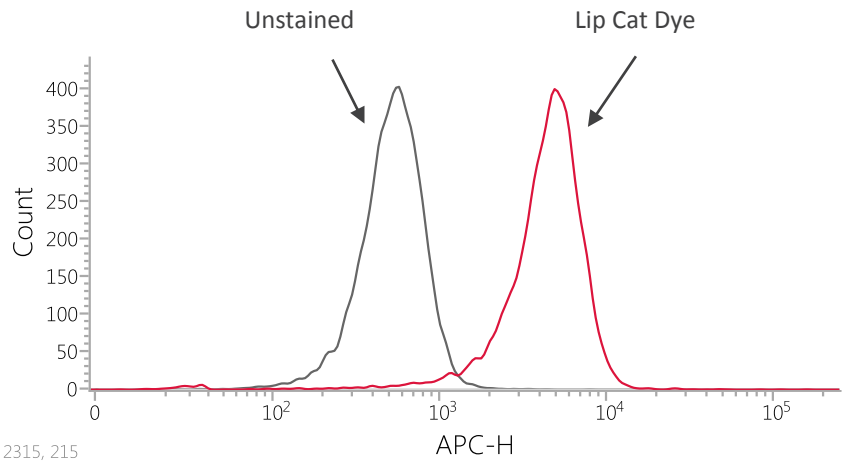


Fig. 3. Representative images of EVs from (a) PFP and (b, c) 100k-PFP sedimented onto electron microscopy grids after Anx5-gold labelling. (a) Isolated Anx5-positive EVs are observed, with no EV aggregates. (b) An EV aggregate, about 800 nm in overall size, is observed, together with isolated EVs (arrows). (c) High magnification view of the dashed box from b; the EV aggregate contains Anx5-positive and Anx5-negative EVs. Scale bars: 500 nm.

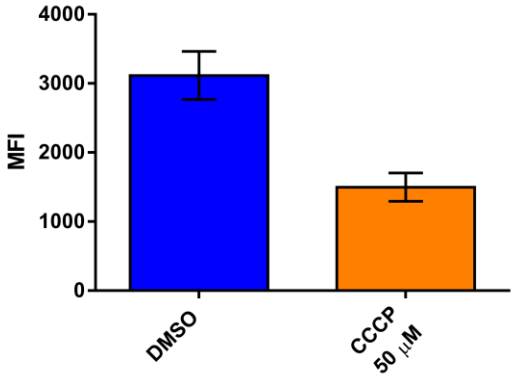
Vescicole Extracellulari & Sonde Fluorescenti

Dyes for MV staining
PKH26
TMRE
JC-1
$DiOC_{18}(3)$
$DiIC_{12}(3)$
CSFE/CFDA-SE/VPD
Amine Reactive Dyes
Lipophilic cationic dye

Liposomes



PB-EVs



Metodo

2 Tubi (Sodio Citrato)

- Sangue periferico
- Staining: 195 µl PBS 1X + Reagents* + 5 µl of Peripheral Blood (prelevati dal 2° tubo)
- Incubazione: RT, 45 minuti

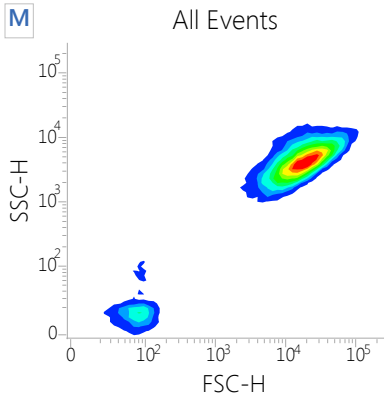
Frank A.W. et al., Circ. Res., 2017

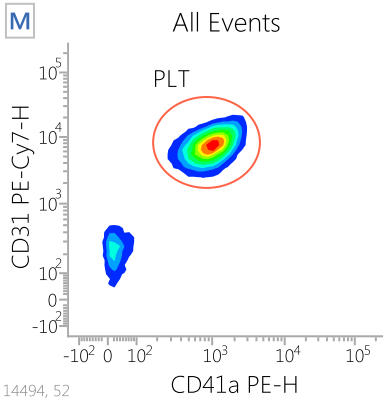
*Reagenti:
- *Lipophilic Cationic Dye*
- Falloidina
- Mix di Anticorpi (CD45/CD31/CD41a)

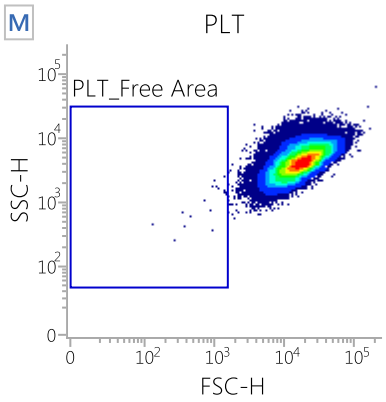
Ottimizzare la diluizione del campione e il flow rate dello strumento!

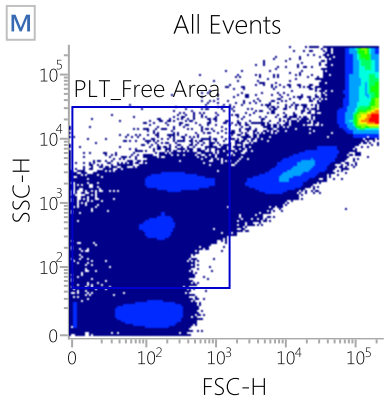
Sangue Periferico

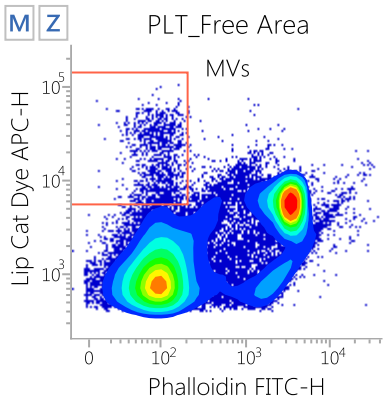
Dovizio M. et al., Mol. Pharm., 2013

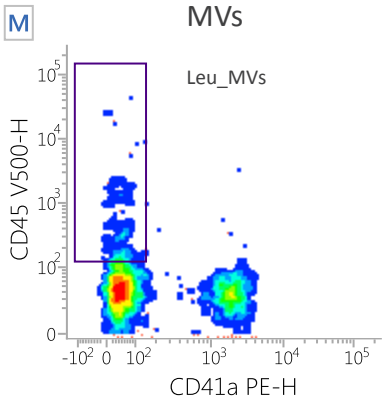


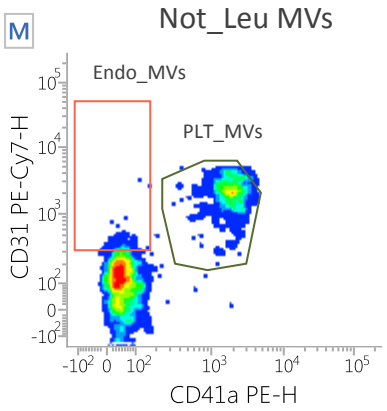




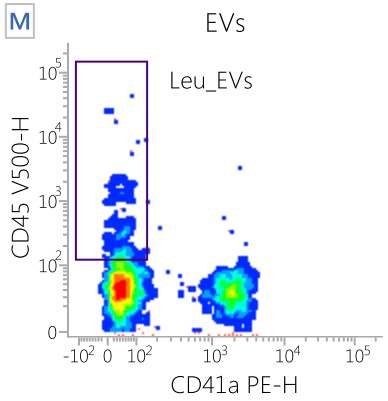


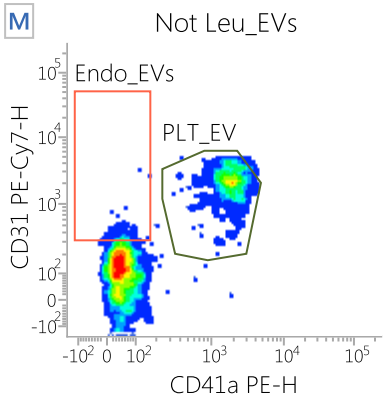




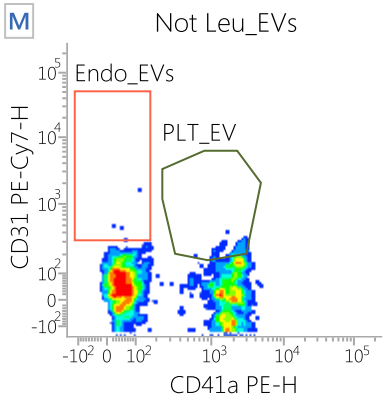


Sangue Periferico - FMO

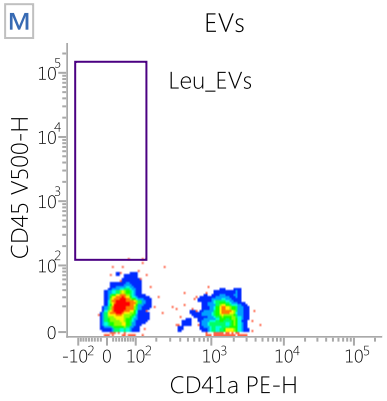




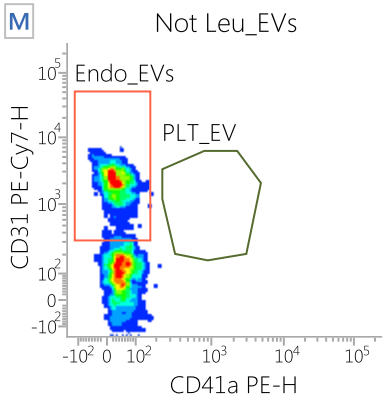
CD31 FMO



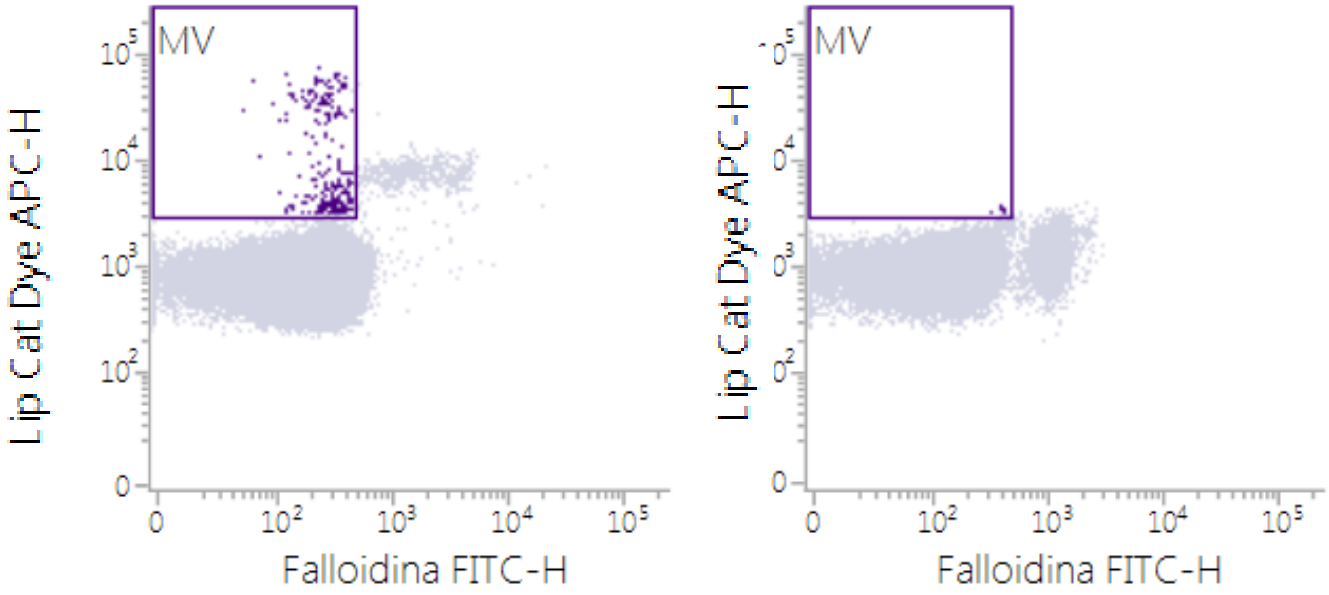
CD45 FMO



CD41a FMO

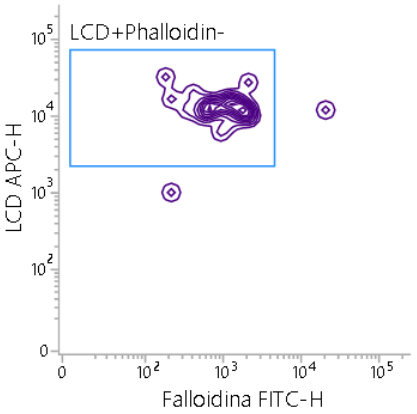


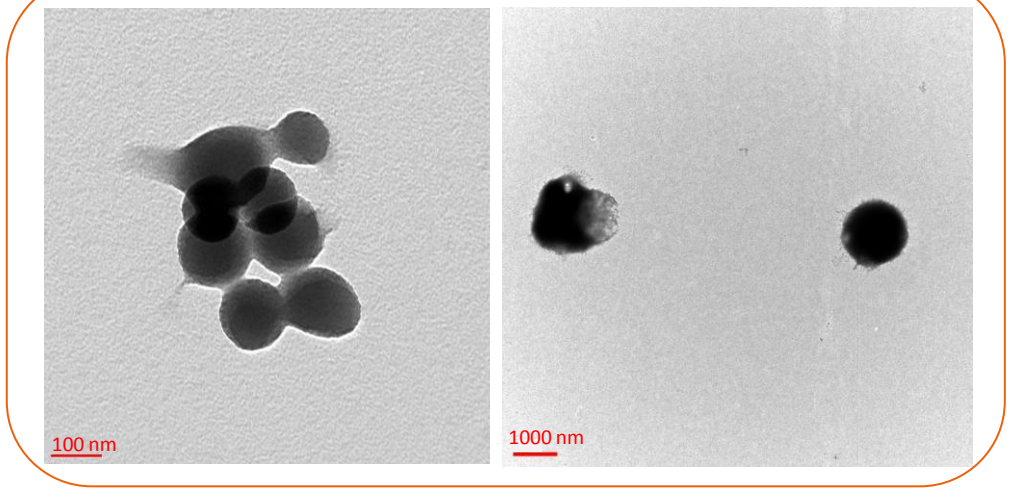
1 % Triton X-100



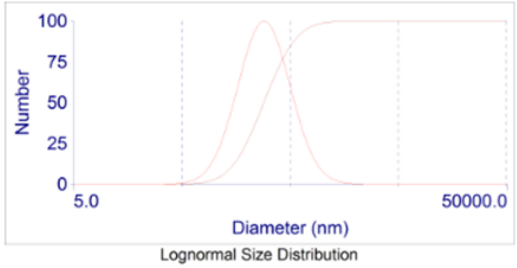
Dimensione delle Vescicole Extracellulari

91.30 %





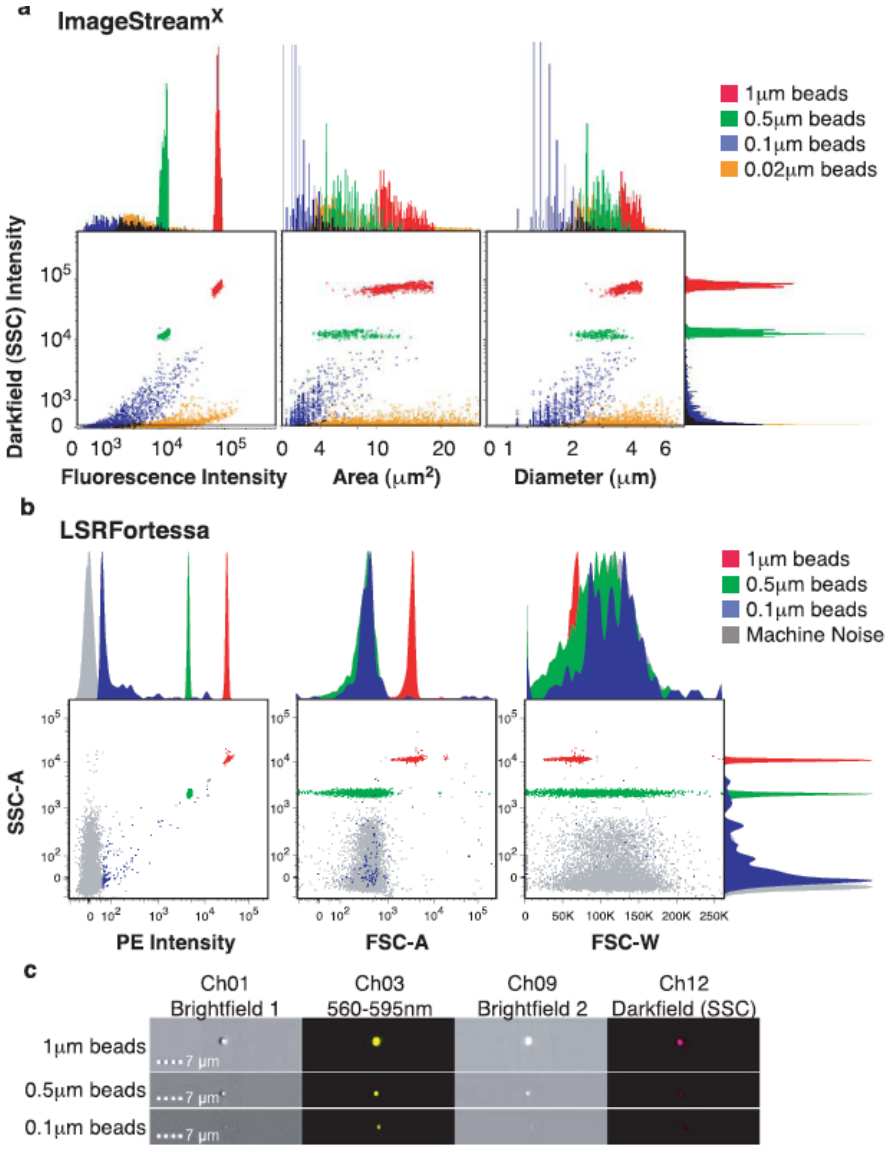
Elapsed Time	00:05:00
Median Diam.	287.1 nm
Mean Diam.	334.1 nm
Polydispersity	0.374
GSD	1.757



Lognormal Size Distribution

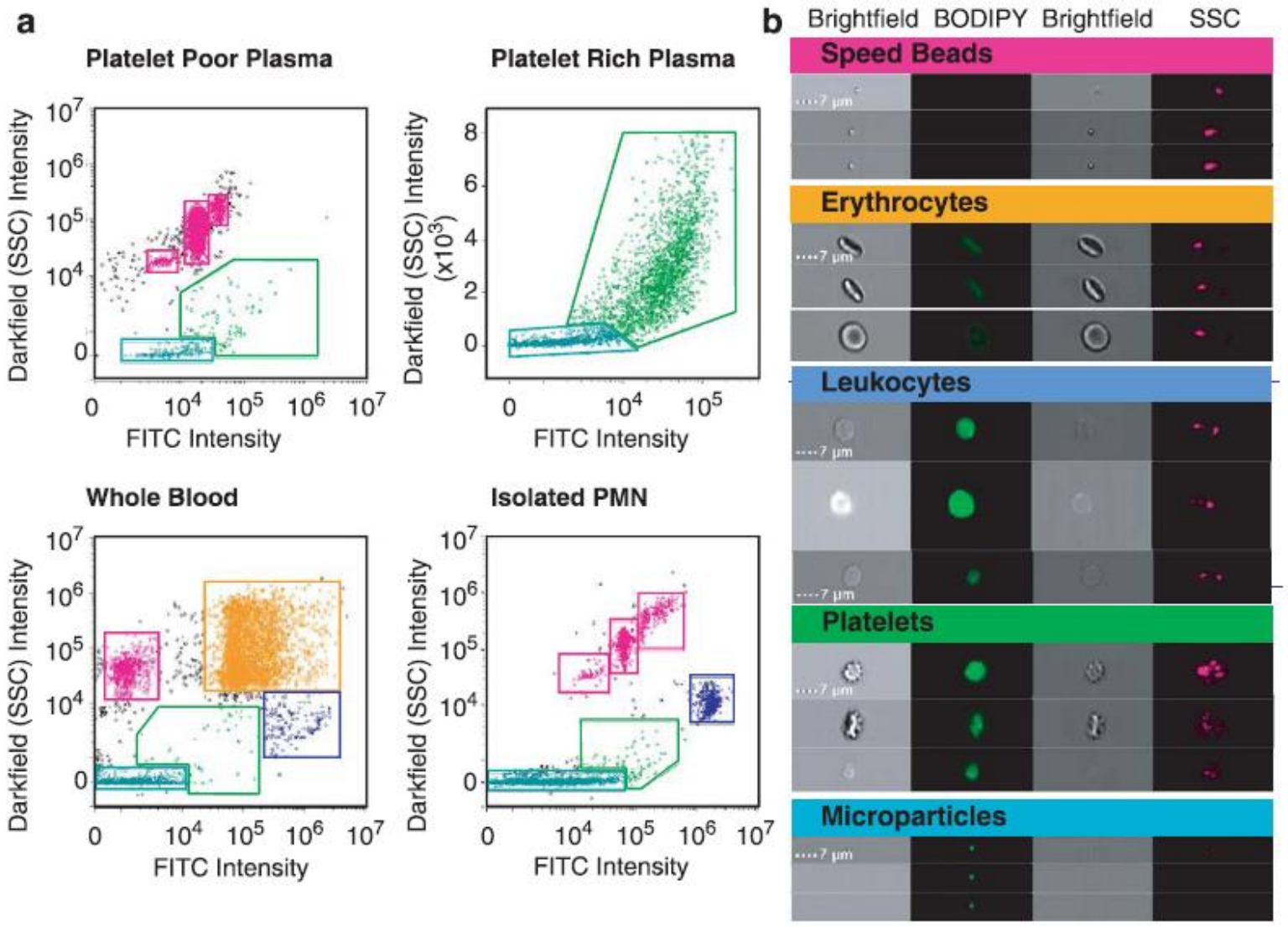
r(nm)	G(r)	C(r)	d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)
116.0	26	5	249.7	97	40	416.1	80	75
141.7	44	10	267.8	99	45	456.4	70	80
162.2	58	15	287.1	100	50	507.9	58	85
180.5	70	20	307.7	99	55	581.6	44	90
198.0	80	25	330.0	97	60	710.3	26	95
215.1	87	30	354.9	93	65			
232.2	93	35	383.1	87	70			

Vescicole Extracellulari & ImageStream



Haedland SE et al., Sci. Rep., 2014

Vescicole Extracellulari & ImageStream



Haedland SE et al., Sci. Rep., 2014

Vescicole Extracellulari in campioni eterogenei

CD31 positive lymphocytes

CD31/CD42a positive platelets (white arrow → platelet derived EV)

Platelet-derived EVs

RBC

RBC
(white arrow → platelet derived EV)

EV Aggregates

Erdbrügger U et al., Cytometry A, 2014

Vescicole Extracellulari & ImageStream

~ *0,1 µm*

0,1 – 0,5 µm

~ *0,5 µm*

	% of MV < 0.16 µm	% of MV 0.16-0.5 µm	% of MV > 0.5 µm
Sample 1	50	40	10
Sample 2	60	30	10
Sample 3	59,2	26,8	12

Ingrandimento 60 X → dimensione pixel = 0.3 µm^2

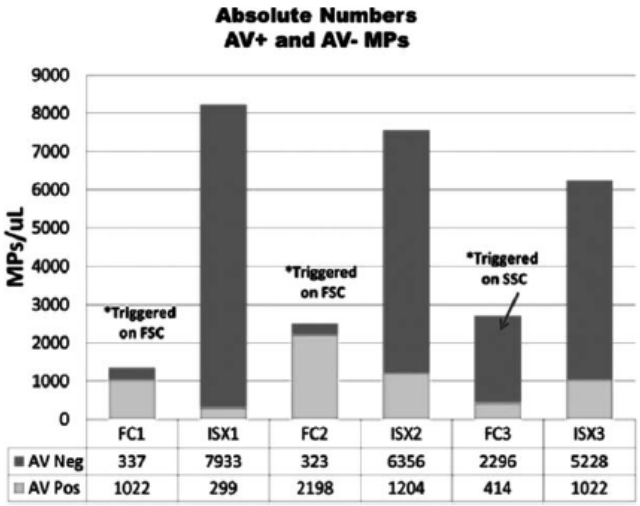
Vescicole Extracellulari & ImageStream

- Laser → Potenza massima;
- Ingrandimento → 60 X;
- Fluorocromi brillanti.

Lannigan J et al., Methods, 2017

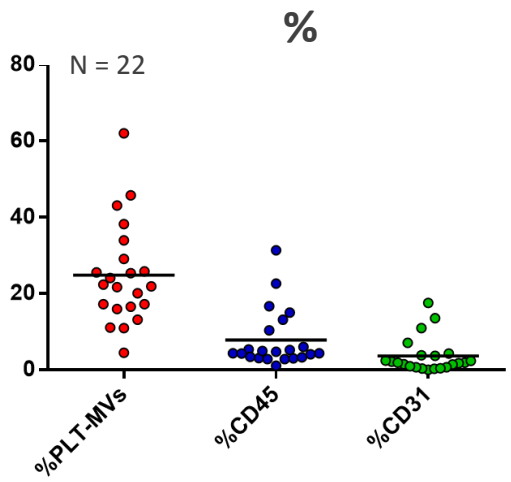
Table 1. Comparison of MP detection between ISX and FCM Fortessa™ and FCM FACSCalibur™ (number of MPs per µl) at high (maximal laser power) and low (same laser power as FACSCalibur™) laser powers

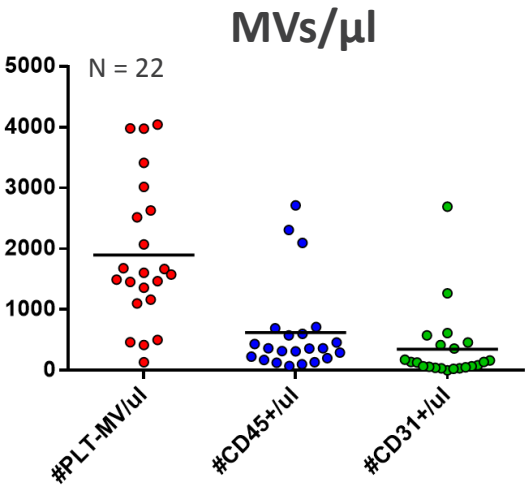
	FCM CALIBUR	FCM FORTESSA		ISX	
TYPE OF MP/µl	LOW LASER[A]	LOW LASER	HIGH LASER	LOW LASER	HIGH LASER
Total MP	290	197	227	2035	4954
AV pos Mps	140	135	129	1523	4549
AV neg Mps	148	66	98	510	401
AV+CD41+	114	91	92	1633	4238
AV+CD45+	0	0	0	0	0



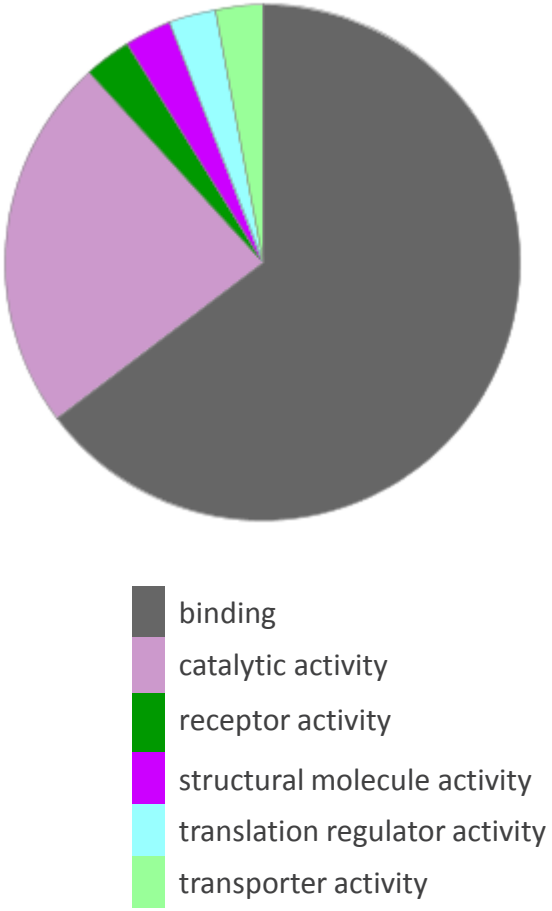
Erdbrügger U et al., Cytometry A, 2014

Vescicole extracellulari nel sangue periferico



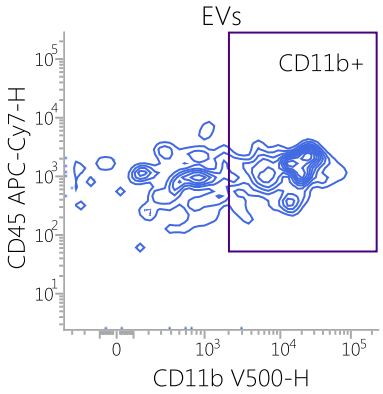


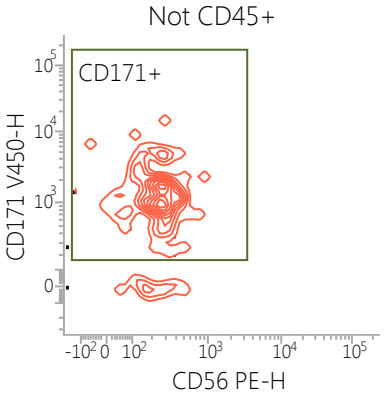
Proteomics of MVs from peripheral blood of healthy donors

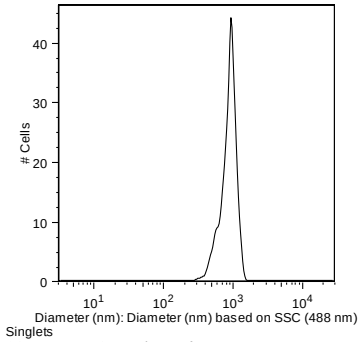


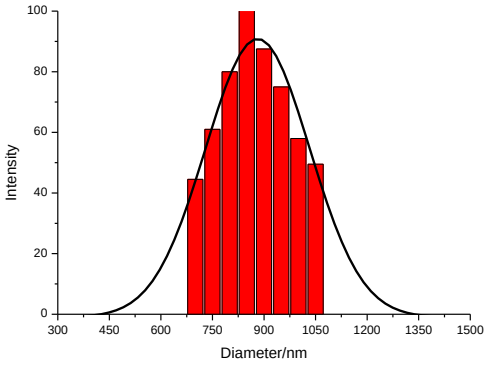
Liquor *versus* Lacrime

CSF

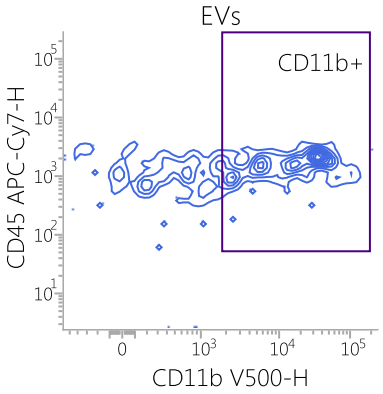


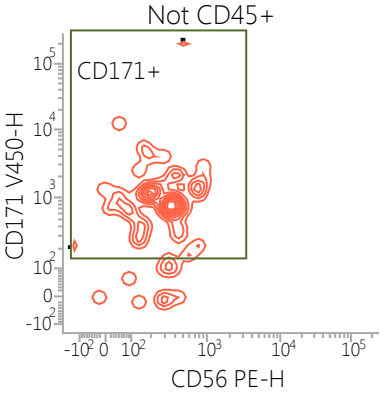


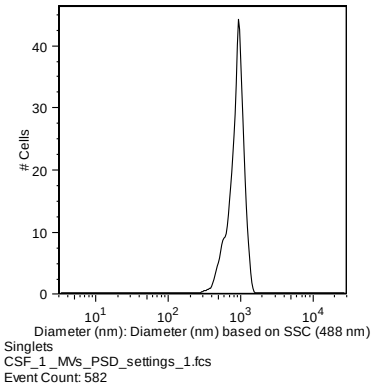




Tears







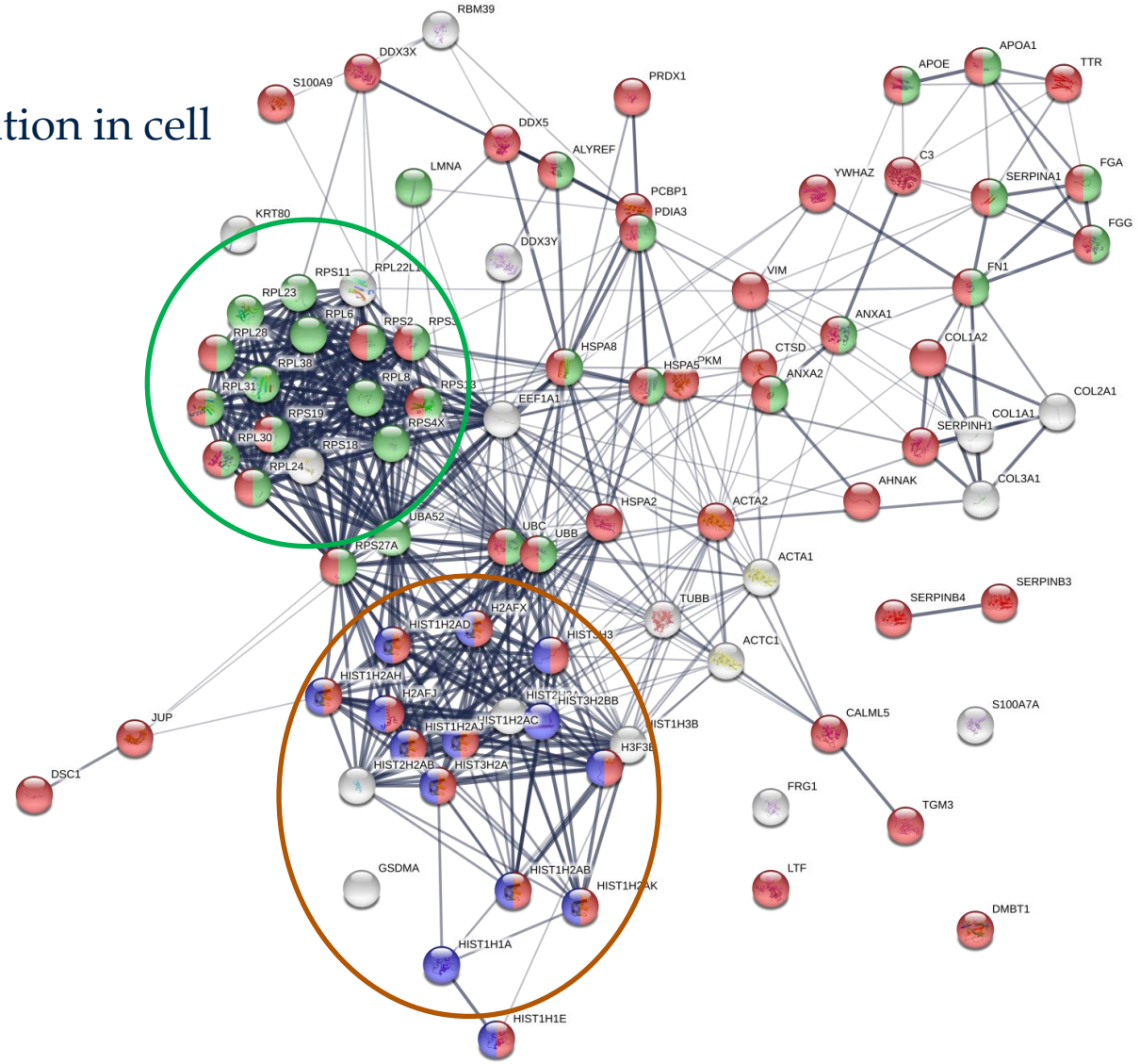
Proteomica delle Vescicole separate da Luquor

Extracellular exosomes

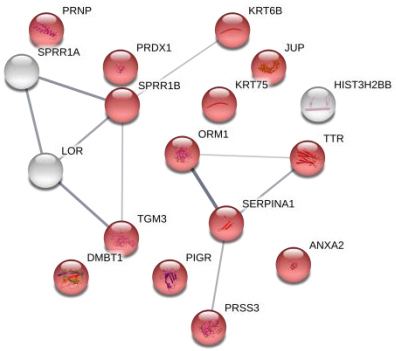
Establishment of localization in cell

Nucleosome

Sclerosi Multipla



Volontari Sani



Proteomica delle Vescicole separate da Luquor

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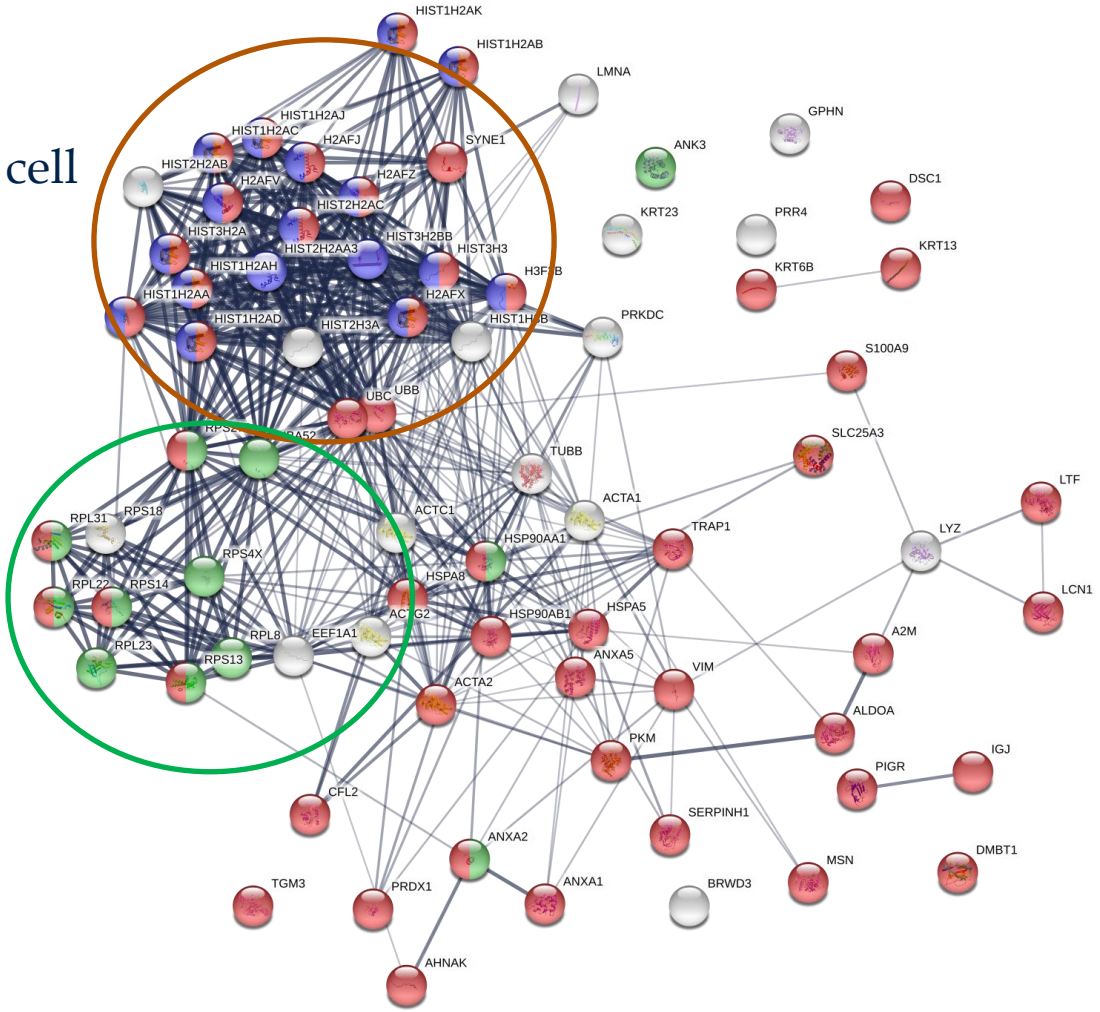
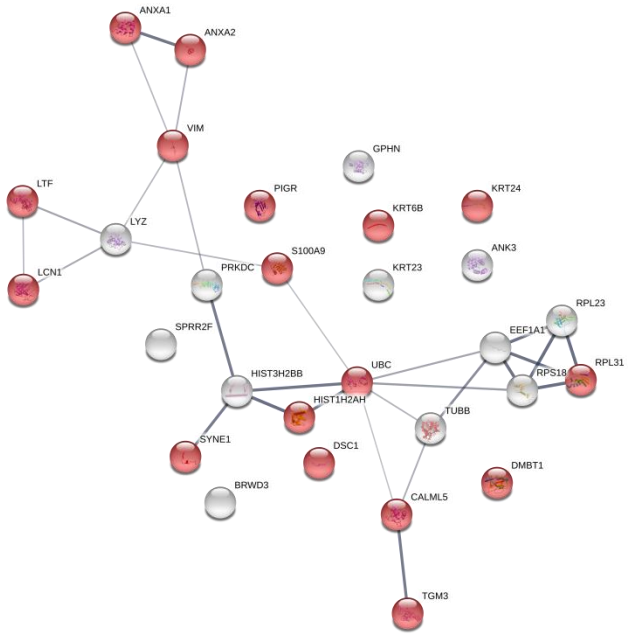
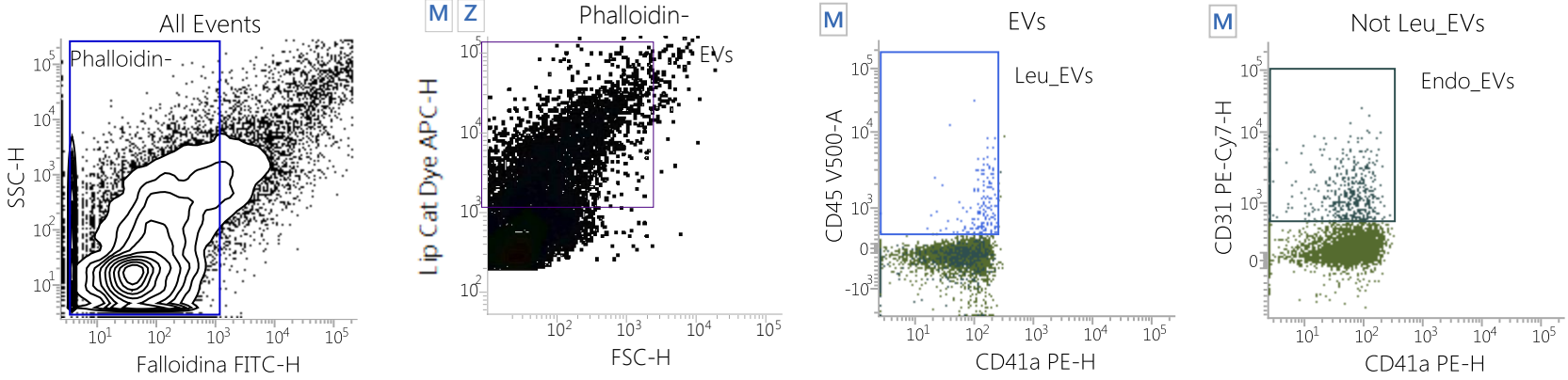


Volontari Sani

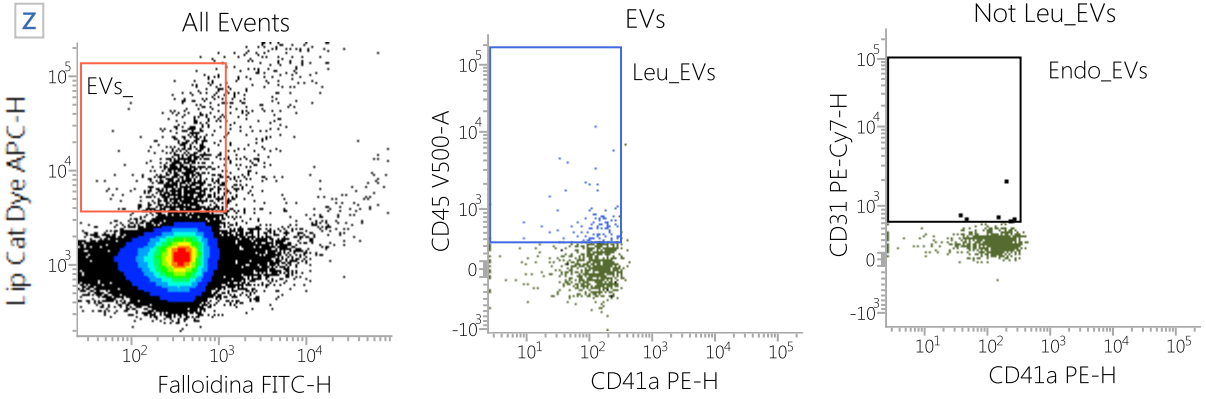


Saliva & Urine

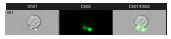
Saliva

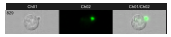


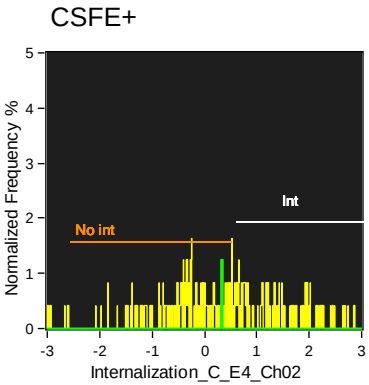
Urine



Funzionalità e ImageStream

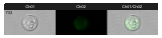


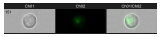




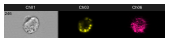
Internalization_C_E4_Ch02

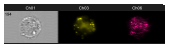
Population	Count	%Gated
CSFE+ & Singlets & Focus	242	100
Int & CSFE+ & Singlets & Focus	109	45
No int & CSFE+ & Singlets & Focus	117	48.3

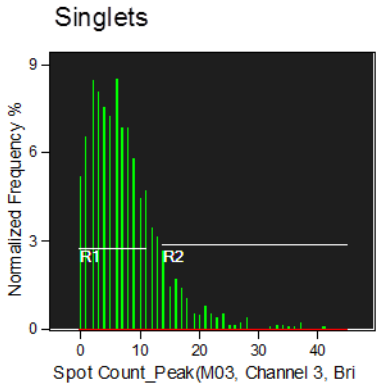




Funzionalità e ImageStream







Spot Count_Peak(M03, Channel 3, Bri

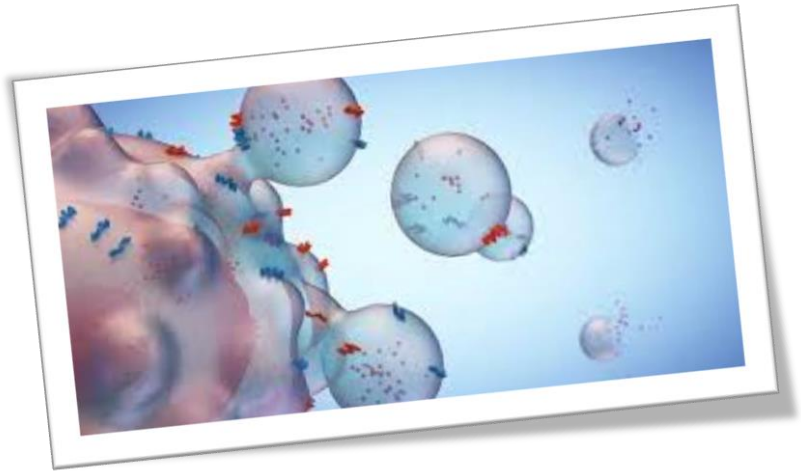
Population	Count	%Gated
Singlets & Best Focus	1423	100
R1 & Singlets & Best Focus	1141	80.2
R2 & Singlets & Best Focus	188	13.2

Beer

A rich source of yeast extracellular vesicles

Take home Messages

- Lo studio delle vescicole extracellulari mediante *Imaging Flow Cytometry* consente di risolvere l'annoso problema legato alla necessità di misurarne le **dimensioni**;
- La possibilità di analizzare l'internalizzazione delle vescicole nelle cellule target apre prospettive interessanti nello studio della **funzionalità** delle vescicole extracellulari;
- L'impiego dell' *Imaging Flow Cytometry* nell ricerca biomedica e clinica rappresenta un **valore aggiunto**.



Prof. Sebastiano Miscia
Prof. Marco Marchisio
Dott.ssa Laura Pierdomenico
Dott.ssa Giuseppina Bologna
Dott.ssa Eva Ercolino
Dott. Pasquale Simeone
Dott. Domenico Bosco

Prof.ssa Antonella Fontana
Dott.ssa Romina Zappacosta

Dott.ssa Rossella Grande
Dott. Christian Celia

Prof. Piero Del Boccio
Dott.ssa Daminana Pieragostino
Dott.ssa Ilaria Cicalini

UNIVERSITY OF AMSTERDAM

Dott. Edwin van der Pol

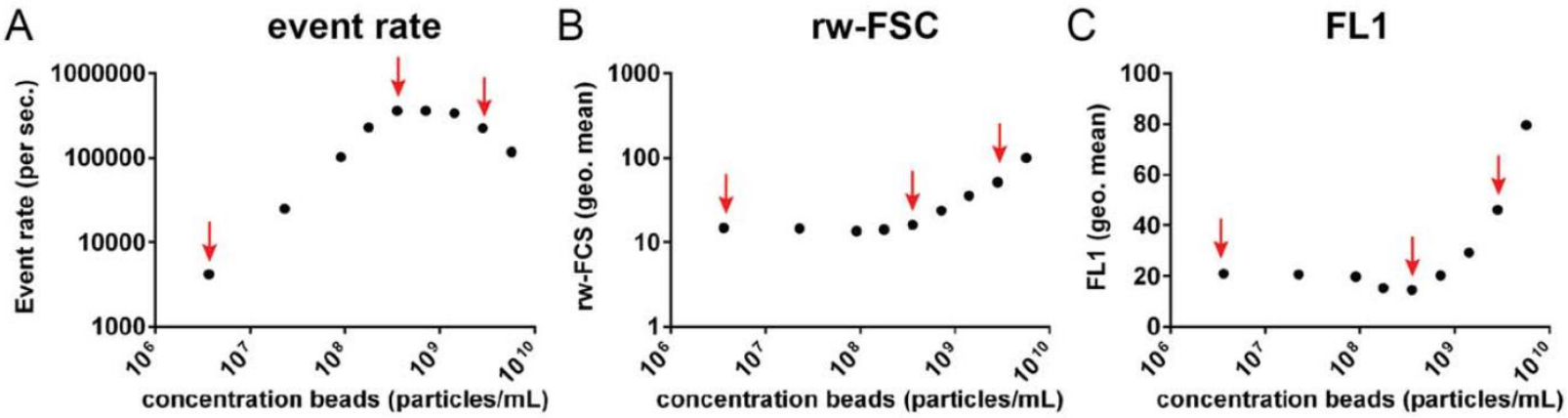
Swarm Effect!

Journal of Thrombosis and Haemostasis, **10**: 919–930 DOI: 10.1111/j.1538-7836.2012.04683.x

IN FOCUS

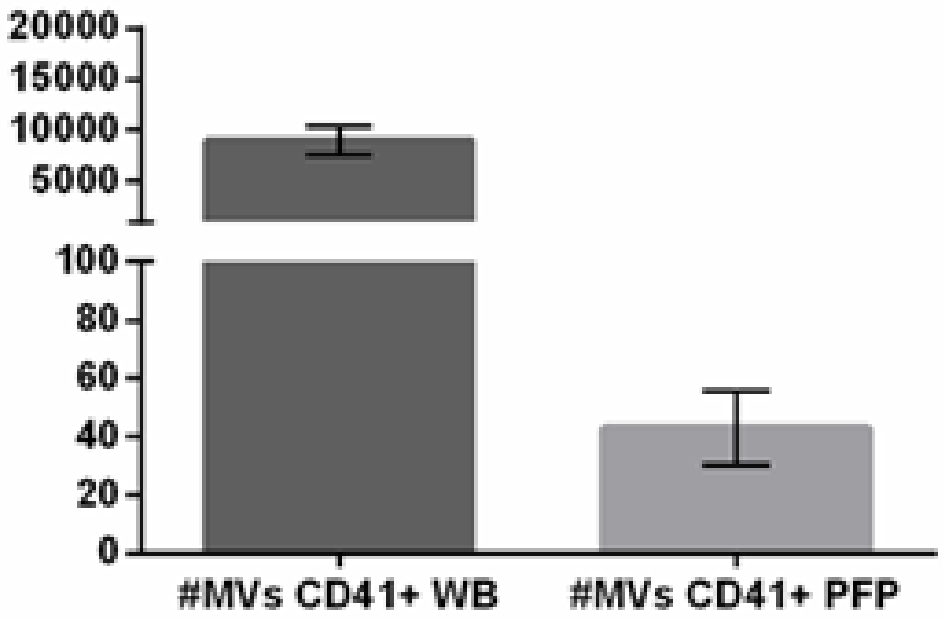
Single vs. swarm detection of microparticles and exosomes by flow cytometry

E. VAN DER POL,*† M. J. C. VAN GEMERT,† A. STURK,* R. NIEUWLAND* and T. G. VAN LEEUWEN†‡
*Laboratory of Experimental Clinical Chemistry and †Biomedical Engineering and Physics, Academic Medical Center, University of Amsterdam, Amsterdam; and ‡Biomedical Photonic Imaging, University of Twente, Enschede, the Netherlands



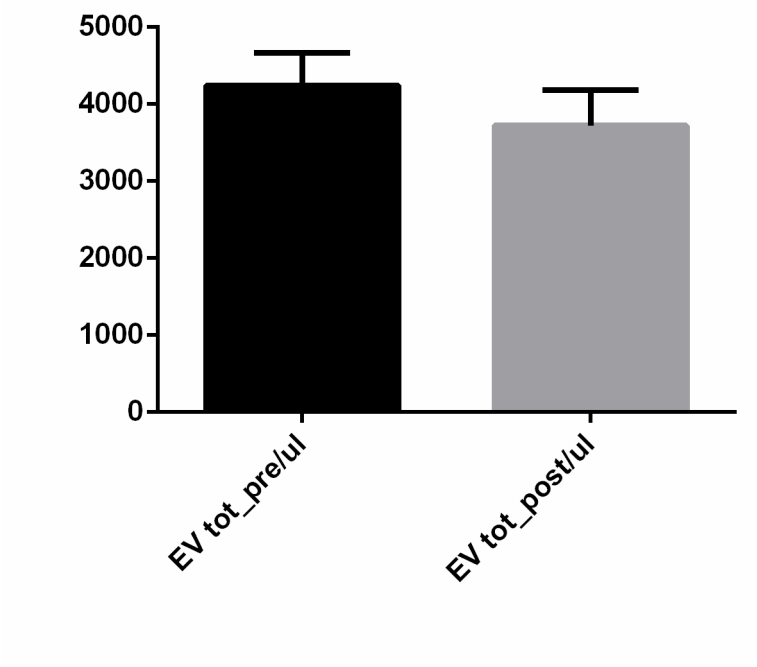
Optimization of sample dilution and flow rate!

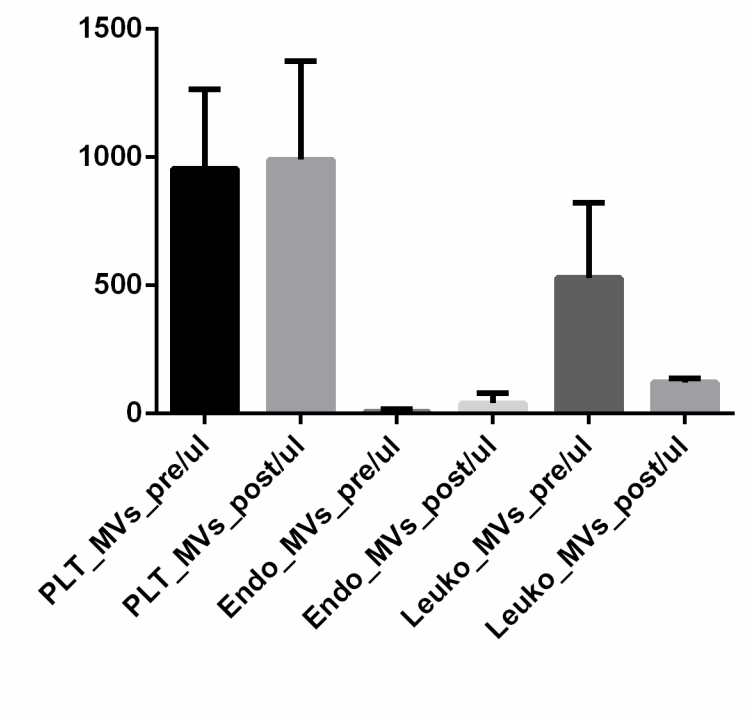
Whole peripheral blood *versus* PFP



(2 x 2500g for 15 min)

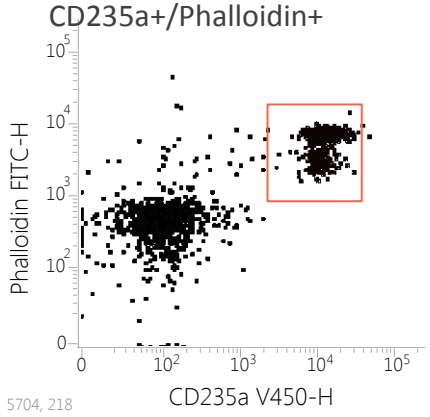
Frank A.W. et al., Circ. Res., 2017

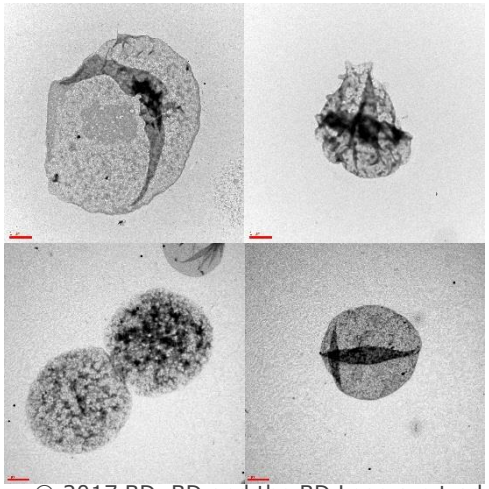

5000
4000
3000
2000
1000
0
EV tot_pre/ul
EV tot_post/ul


1500
1000
500
0
PLT_MVs_pre/ul
PLT_MVs_post/ul
Endo_MVs_pre/ul
Endo_MVs_post/ul
Leuko_MVs_pre/ul
Leuko_MVs_post/ul

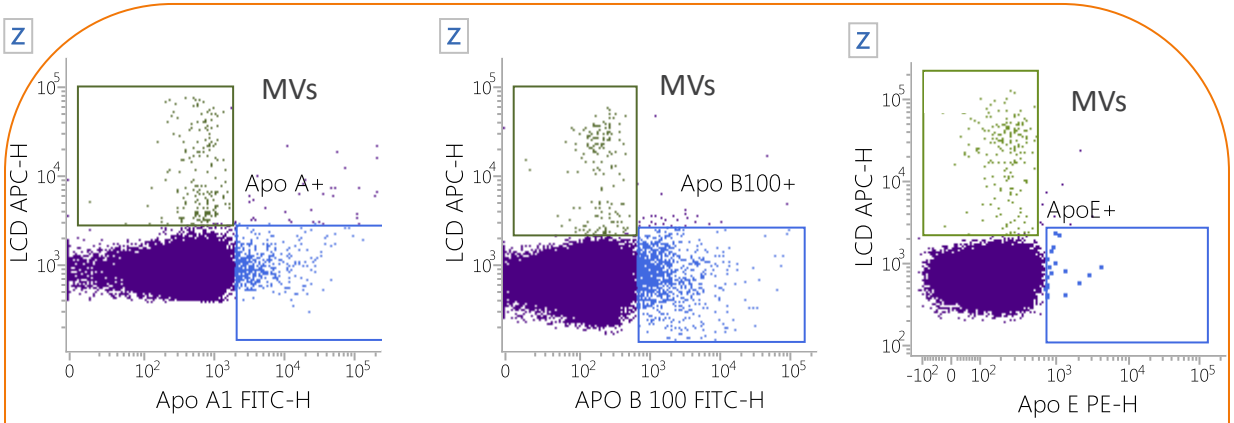
MV contaminants (Peripheral Blood)

RBC Ghosts

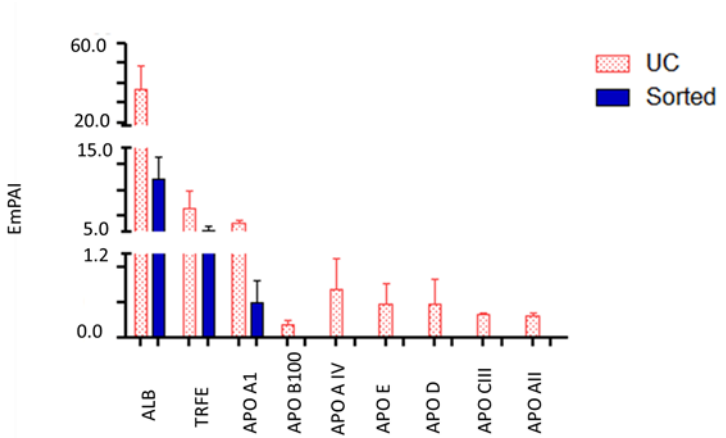




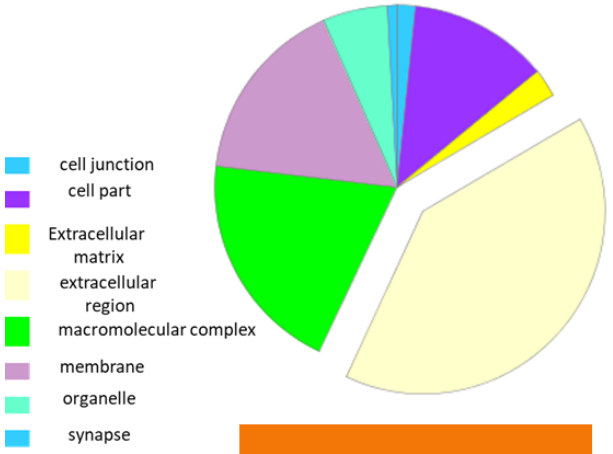
Apolipoproteins



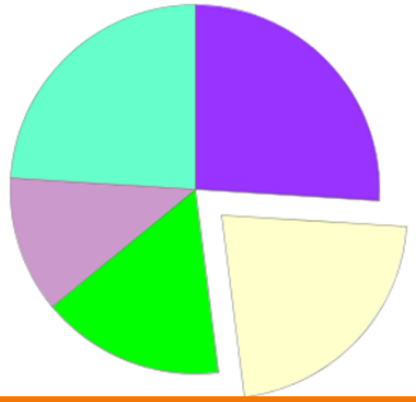
Mass spectrometry analyses of MV proteins



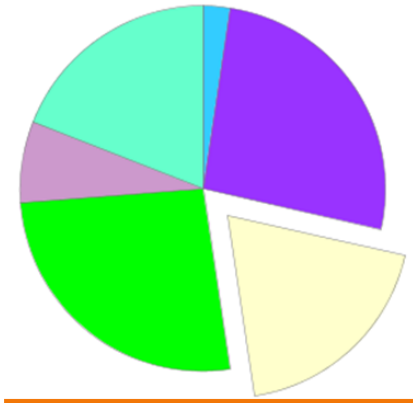
MV contaminants (CSF)



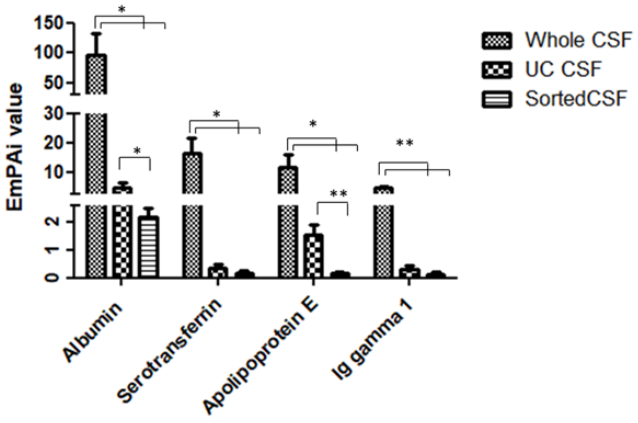
Whole CSF



UltraCentifugation



FACS Sorting

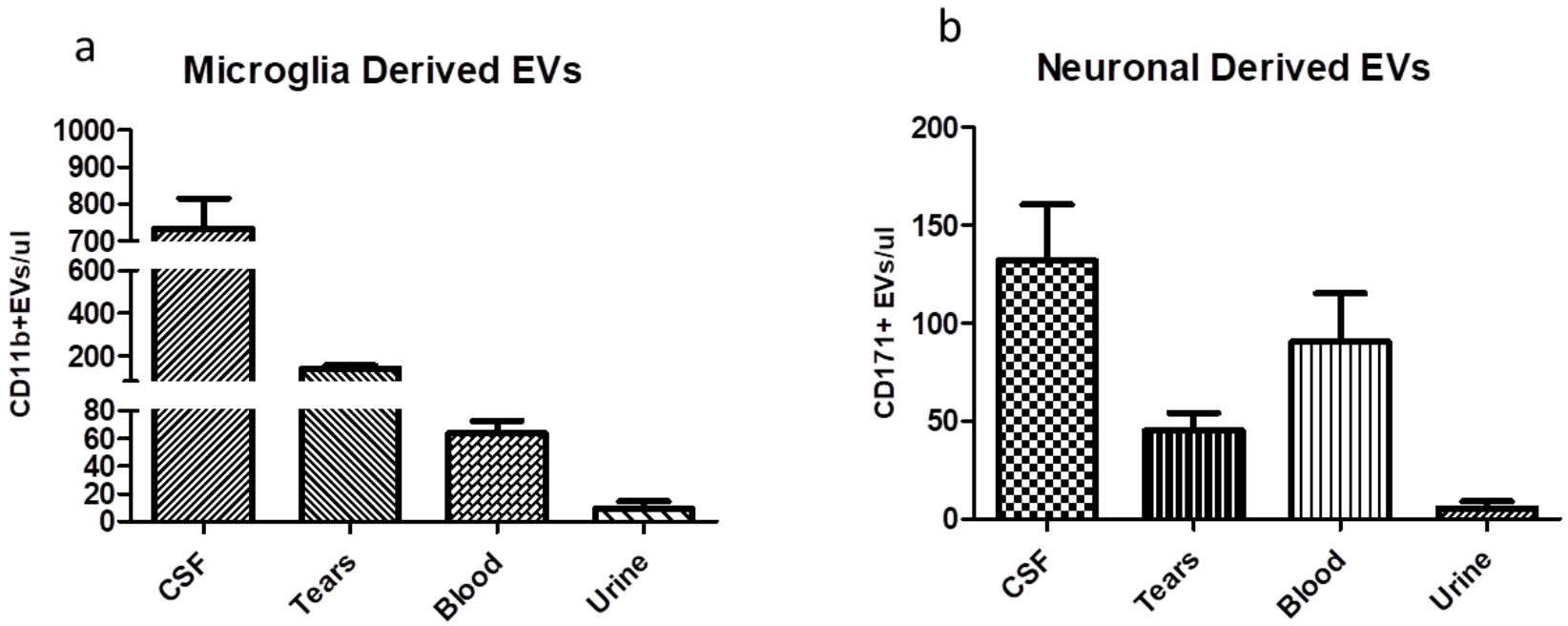


1X10^6 MVs

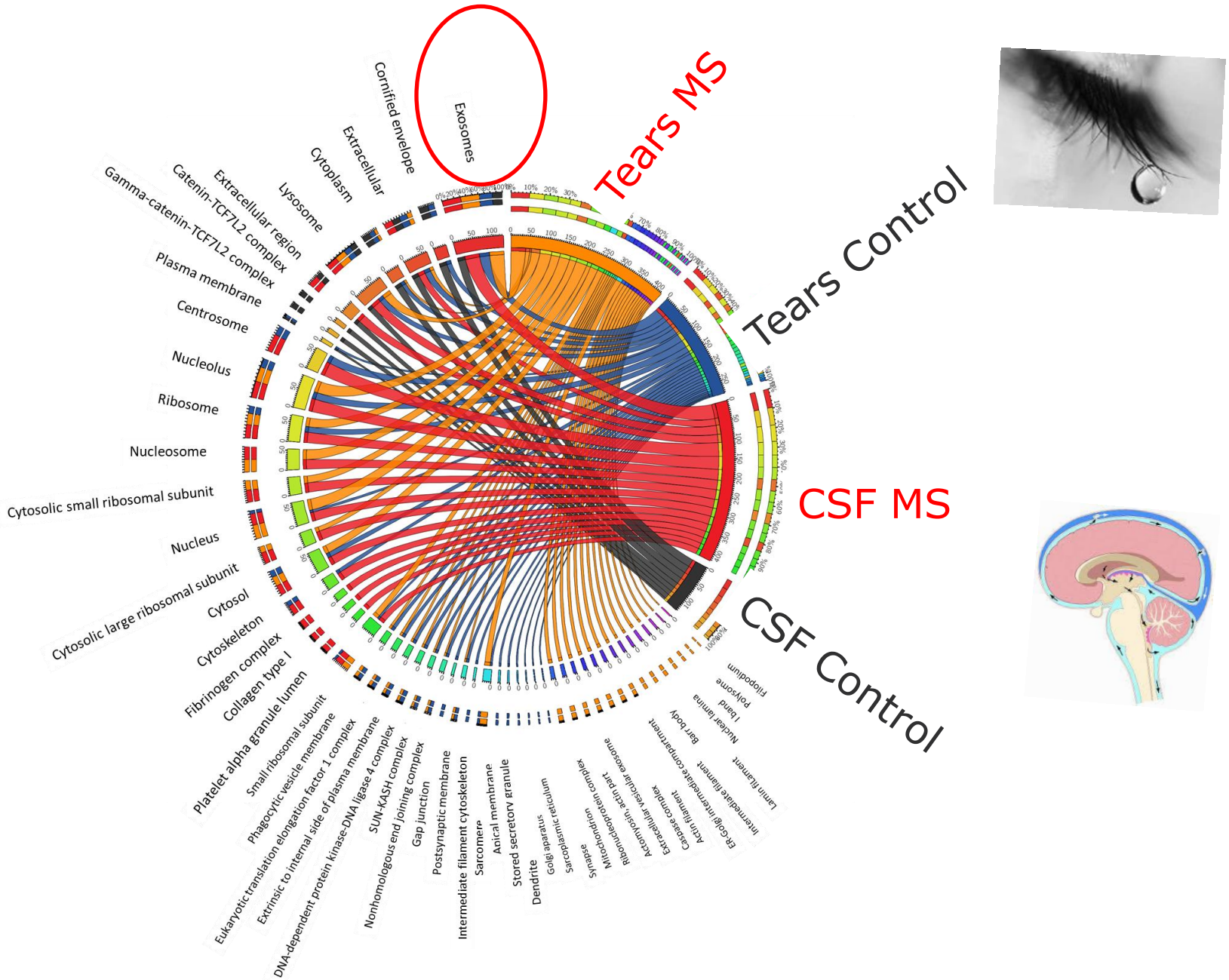
↓

More than100 CLEANED PROTEINS

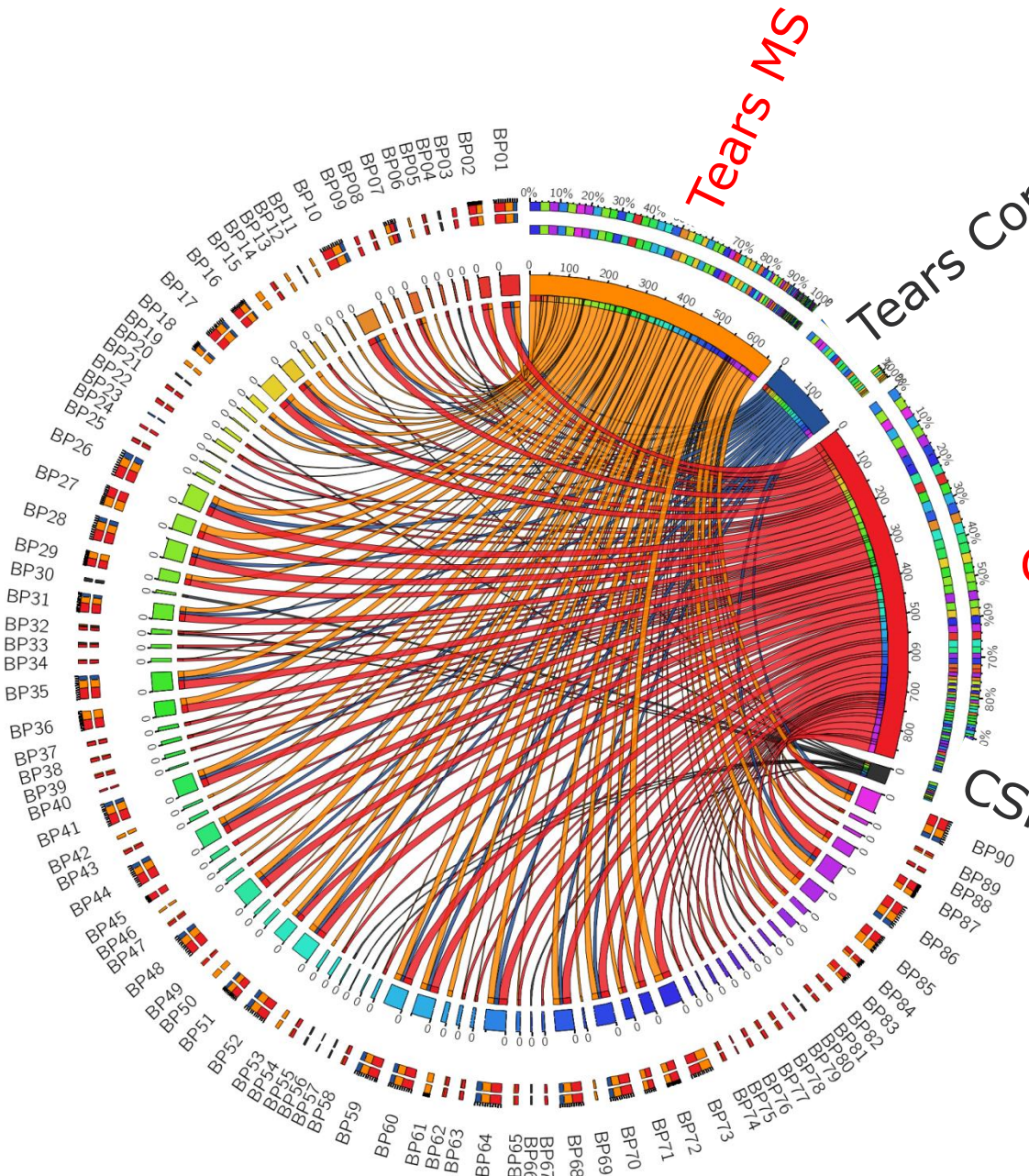
Microglial and Neuronal MVs from different biofluids

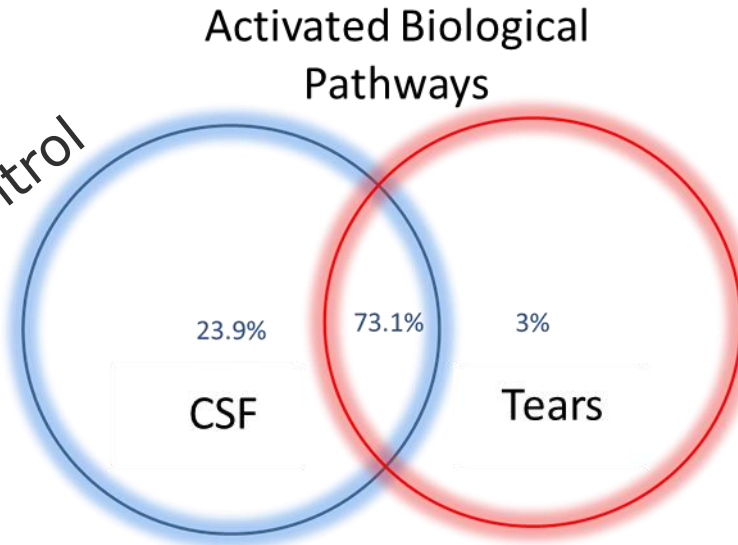


Cellular component classification



Biological pathways classification





- **Immune response**
- **Apoptosis**
- **Angiogenesis**