Nano Flow Cytometry Pipeline

Analysis & Sorting of nano-particules

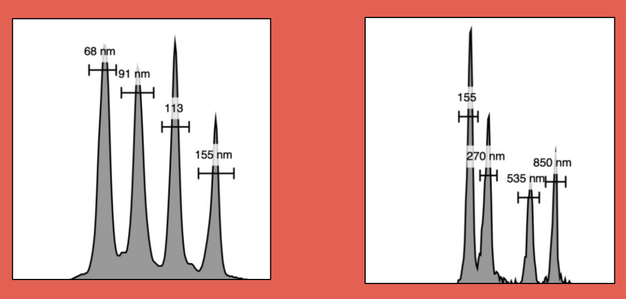
Pierre-Henri Commere[1], Juliana Rizzo[2], Sandrine Schmutz[1], Sebastien Megharba[1], Gerard Pehau-Arrnaudet[4], M. Hasan[1], Nicoletta Casartelli[3], Guilhem Janbon[2], Sophie Novault[1].

1Plateforme de Cytométrie/ CBUTechS, 2Unité de Biologie des ARN des pathogènes Fongiques, 3Unité Virus & Immunity, 5UTechS UBI Institut Pasteur, Paris (France),4 UBI UTechS, Institut Pasteur Paris.

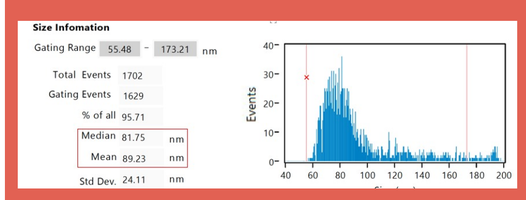
The Cytometry platform of the CB UTechS has collaborated with scientific groups of Virology and Microbiology departments to develop a pipeline that pushes the limits of conventional approaches to allow analysis and sorting of subcellular particles.

With the "*Nano Flow Cytometry pipeline*" that we developed, it is possible to visualize nanoparticles in the 40–850 nm size range. This pipeline takes advantage of a combination of an adapted optical bench and settings of a jet-in-air cell sorter and the new nano analyzer system. The NanoAnalyzer™ (NanoFCM) technology allows detecting and quantifying EVs and viruses in a fast and reliable way without the need of any fluorescent read out. In parallel, with the optimized high-speed sorting (Moflo Astrios™, Beckman Coulter) that combines sample labelling, specific optical parameters, and finely defined laser power settings, we have demonstrated our capacity to detect and sort retroviral particles.

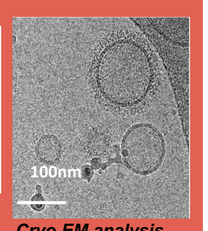
High-Resolution Size Distribution Analysis (40-150 nm 150-850 nm)



S16M-Exo and S17M-MV as size standards



C. Neoformans labelled with ConA-A488



Cryo EM analysis

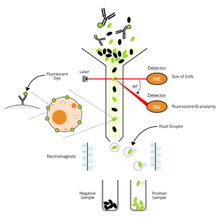
Particules Detection & Quantification

Nano FCM Analyzer (40-850m)

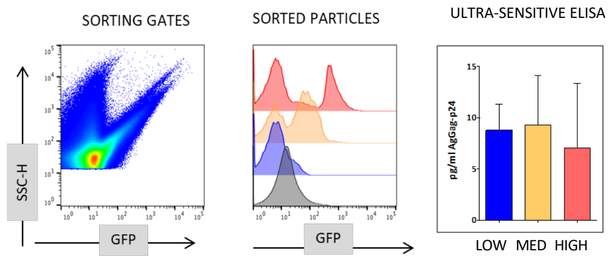
NanoFCM technology with optimized parameters allow the detection and quantification of sub-cellular particles. We have demonstrated the sensitivity of the FlowAnalyzer by detecting silica nanoparticles, the fluorescence sensitivity of which has also been verified. The size and concentration of EV's could be acquired directly from the software. We have compared results obtained with the Nano FCM technology and electron microscopy and they are consistent.

Nano Sorting

Particules Isolation_ Astrios EQ FSC Module



Sorted material contains viral particles : 3 levels of GFP fluorescence intensity are detected. Viral material in each gate was then sorted and re-analyzed to verify that GFP intensities were maintained. We measured the amount of the HIV-1 antigen p24 in the three fraction by the ultra-sensitive ELISA, and confirmed that in each fraction we could find viral particles.



Perspectives:

We applied nano sorting and nano analysis to investigate Virus cell-free particles interaction and we have quantified EVS realized by different micro-organisms in a challenging strategy due to the small size of those sub cellular particules and the lack of specific staining reagents.

The "***NanoFlow Pipeline***" with optimized parameters (laser power and voltage settings combined with an adapted fluidic pathway) allow the detection and quantification of sub-cellular particles. We have demonstrated the sensitivity of the FlowAnalyzer and the "Jet in Air" Sorter by detecting beads and we have compared results obtained with electron microscopy and confocal microscopy.

Broader applications will be supported by the NanoFlow pipeline to any types of viruses, viral vectors, lipid nanoparticules (for the estimation of size, concentration of particles in batch production).

References:

Liu and al " Archaeal extracellular vesicles are produced in an ESCRT-dependent manner and promote gene transfer and nutrient cycling in extreme environments" ISME J. 2021 Oct

Rizzo et al "Cryptococcus extracellular vesicles properties and their use as vaccine platforms" J Extracell Vesicules. 2021 Aug; 10(10): e12129

Staropoli and all "Flow Cytometry Analysis of HIV-1 Env Conformations at the Surface of Infected Cells and Virions: Role of Nef, CD4, and SERINC5)", J Virol. 2020 Feb 28;94(6):e01783-19. doi: 10.1128/JVI.01783-19

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