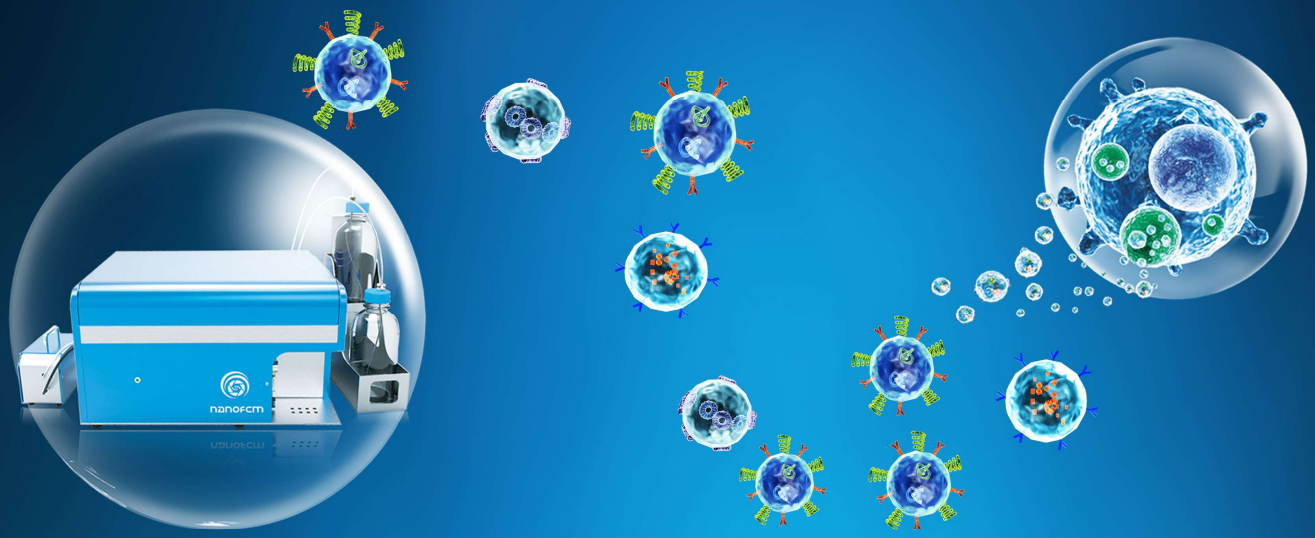
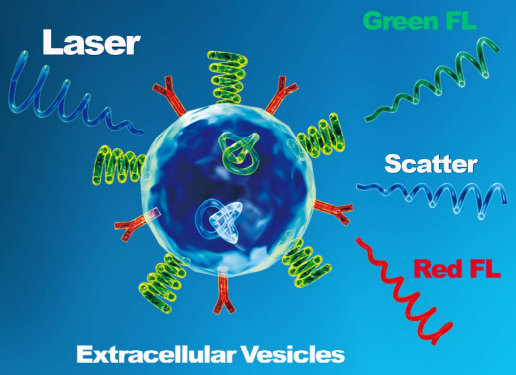
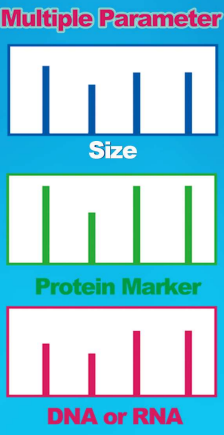
NanoFCM

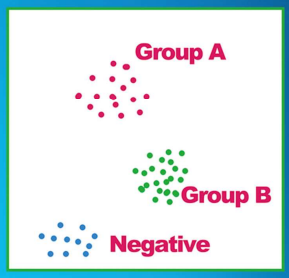
Deciphering EVs

Sizing, Concentration & Phenotyping









HIGH-PERFORMANCE

Ultra-Sensitive and High Resolution for both scatter and fluorescence.

STOP SWARM DETECTION

The state of the probe volume and raw data are displayed and monitored in real time.

LABEL FREE

Direct size and concentration measurement without manipulation

PHENOTYPING

Based on single -molecule fluorescence detection, Phenotype quantify direct from marker labeling intensity.

SIZE DISTRIBUTION

High-resolution size distribution comparable to TEM

PARTICLE CONCENTRATION

Count the number of whole population and sub population with 100% efficiency, e.g. the ratio of positive

MULTIPARAMETER

A range of markers within a sample can be Measured and quantified in simultaneous

FLEXIBLE

Flexible Antibodies& Dyes ; no photobleaching; low sample requirements; sample can be recovered

福 流

nanofcm

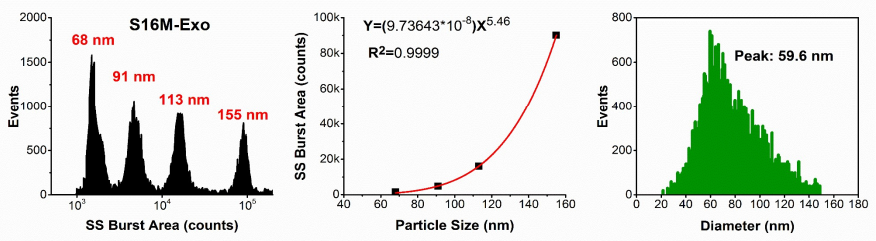
Nano-flow Cytometry

nanoFCM Flow NanoAnalyzer

福流 nanoFCM

Nano-Flow Cytometry: Next-Generation Platform for Comprehensive EV Analysis

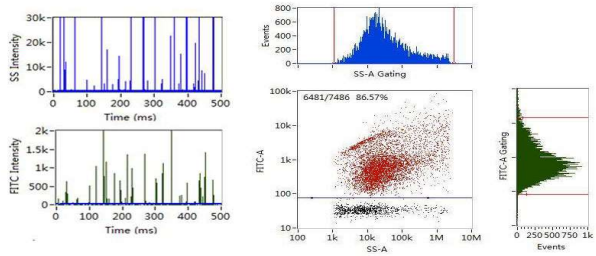
High-Resolution Size Distribution Analysis



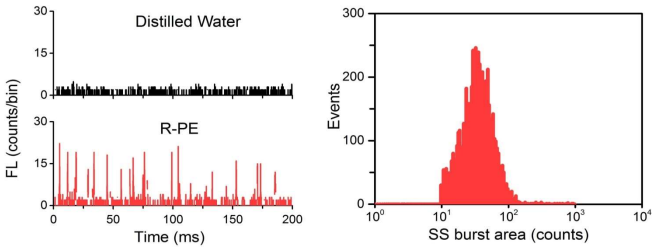
Employing **S16M-Exo** (NanoFCM) as size standards, a calibration curve will be constructed between the particle size and side scatter intensity, the SS intensity of each EV particle could be converted to size. The size distribution of EV matches well with that acquired from cryo-EM.

EVs Phenotyping at Single Particle Level

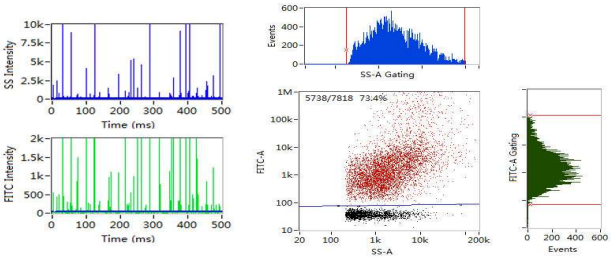
CD63-GFP



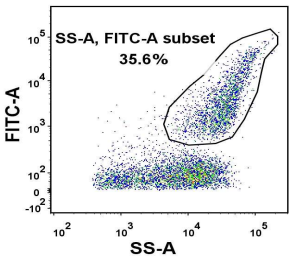
Single Molecule Detection



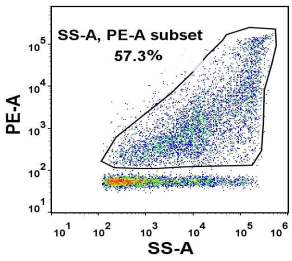
CD9-Alexa Fluor 488



RNA

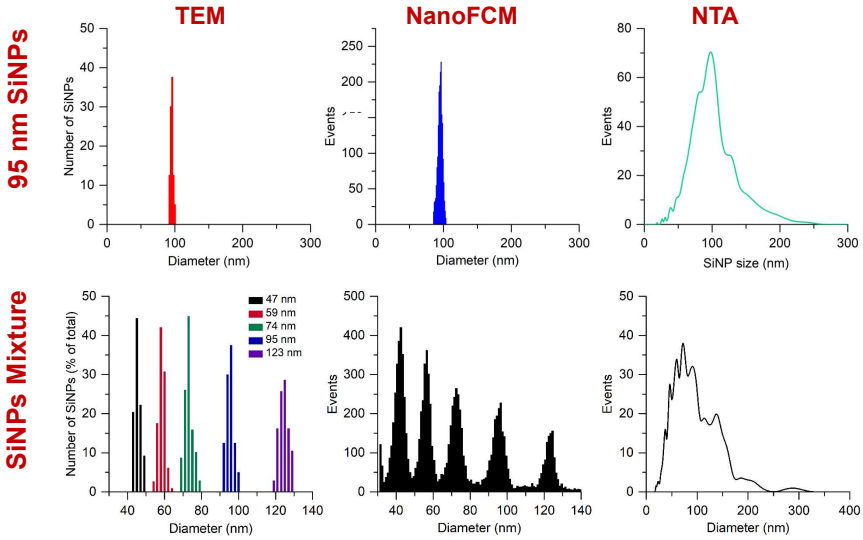


Lipid



With single molecule detection sensitivity in fluorescence channel, nano-flow cytometry allows the phenotyping of EVs at single particle level, including surface proteins, nucleic acids and lipids. The positive ratio could be evaluated at real time.

Comparison with First-Generation Techniques

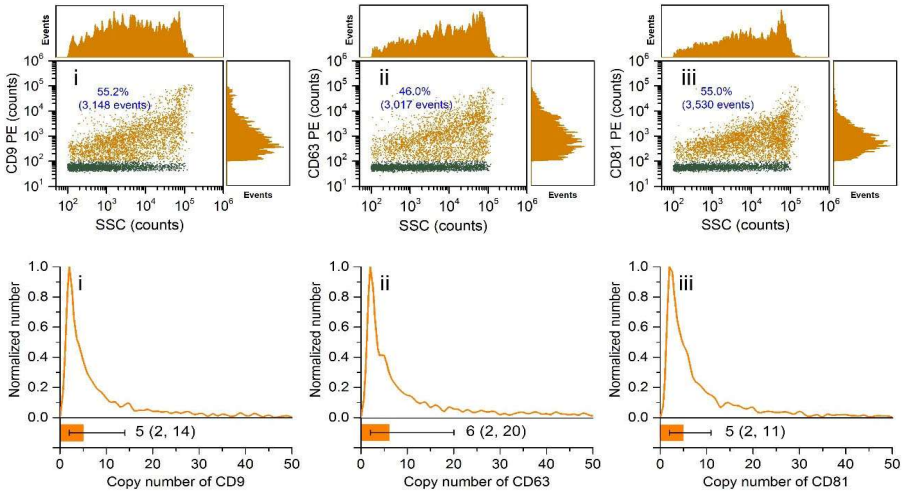


For silica NPs of 95 nm, the size distribution measured by NanoFCM is consistent with TEM, that is, sharp histogram centered at 95 nm is achieved. In contrast, a broad size distribution (20-250 nm) containing massive false signals is observed by NTA, originating from the lack of both sensitivity and resolution.

The ultrahigh sensitivity and resolution of NanoFCM is perfectly illustrated by the measurements of silica NPs with mixed sizes (47, 59, 74, 95, and 123 nm). NanoFCM offers comparable results as TEM, far beyond the best result obtained by NTA. NanoFCM enables users to accomplish complicate analysis for heterogeneous samples without losing details.

Copy Number of Classic Markers

ACS Nano, 2018, 12, 671-680



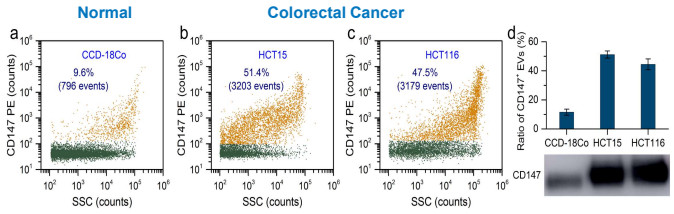
Immunostained individual EVs can be readily detected by NanoFCM, the bivariate dot-plot of PE orange fluorescence *versus* SSC shows two clearly resolved populations.

The number of MAbs bound on each individual EV can be derived by calibrating the PE fluorescence intensity against the median fluorescence of single PE-conjugated MAbs. The median copy numbers for CD9, CD63, and CD81 expressed on each individual EV are 5, 6, and 5, respectively.

Early Diagnosis of Colorectal Cancer

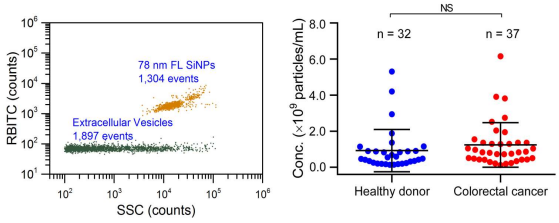
ACS Nano, 2018, 12, 671-680

CD147+ EVs in Colorectal Cancer Cell Lines



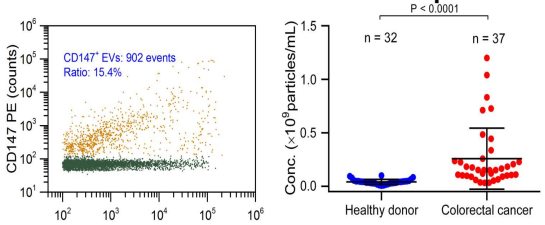
CD147 expression is analyzed quantitatively at single EV level by NanoFCM. Moreover, NanoFCM allows correlating the protein abundance with vesicle size at the single-particle level, CD147-positive EVs exhibit a range of sizes depending on their cell origin, e.g., small size for HCT 15 and large size for HCT 116 cells.

EVs Concentration in Clinical Blood Samples



Total EV Concentration	n	Lowest	Highest	Ratio	Mean ± s.d.
Healthy donor	32	1.3 × 10⁹/mL	5.3 × 10⁹/mL	41	(0.9 ± 1.2) × 10⁹/mL
Patients	37	1.2 × 10⁹/mL	6.2 × 10⁹/mL	52	(1.2 ± 1.2) × 10⁹/mL

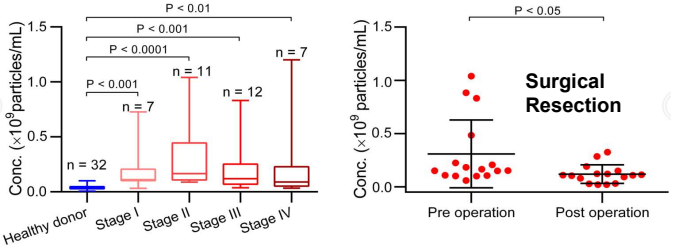
CD147+ EVs in Clinical Blood Samples



CD147+ Concentration	n	Lowest	Highest	Ratio	Mean ± s.d.
Healthy donar	32	0.6 × 10⁷/mL	1.0 × 10⁸/mL	17	(4.1 ± 2.3) × 10⁷/mL
Patients	37	3.2 × 10⁷/mL	12 × 10⁸/mL	38	(2.9 ± 2.9) × 10⁸/mL
Ratio	/	/	/	/	7.1

EV concentration in plasma of both cancer patients and healthy donors varies a lot for different individuals, there is no significant difference in the mean concentration of EVs between cancer patients and healthy controls, while the concentration of CD147 positive EVs of cancer patients is significantly higher than that of the healthy donors.

Early Diagnosis and Treatment Monitoring



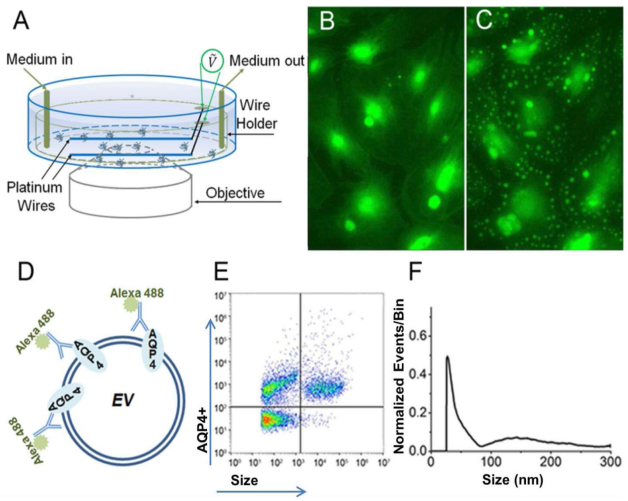
NanoFCM is able to identify the elevated level of CD147 positive EVs for patients at all the cancer stages, even stage I. Moreover, this strategy can be used to track the level of CD147 expression after surgical resection.

Programmable Modulation for Extracellular Vesicles

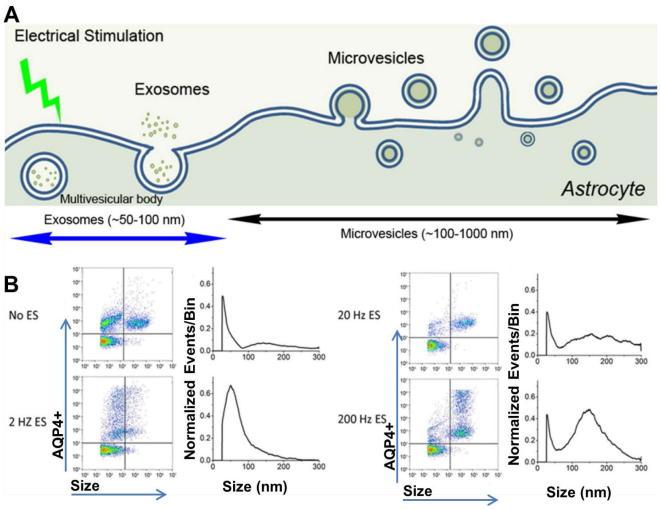
Quantitation of Astrocytes EVs by their Size and Surface Proteins

DOI: *http://dx.doi.org/10.1101/566448.*

Characterization of EVs in Astrocytes



Distributions of EVs after Electrical Stimulation



The sensitivity and high-resolution of NanoFCM in both side scatter and fluorescent channels make it possible to monitor the effect of electrical stimulation on single EV level. The astrocyte-specific water channel, aquaporin-4 (AQP4) is employed as an indicator.

In control condition without electrical stimulation, three populations are differentiated, with one AQP4 negative and two AQP4 positive, which was further interpreted as exosomes (smaller) and microvesicles (larger) according to their size.

The size distribution of AQP4-positive EVs are differentially affected by the frequency of electrical stimulation. After stimulation at 2 Hz, a near uniform distribution of EVs with peak size at ~70 nm is identified, the population of exosomes express more AQP4 than control condition. Stimulation at 20 Hz produces fewer EVs of both sizes, particularly exosomes. While electrical stimulation at 200 Hz has the opposite effect of that of 2 Hz. Instead of yielding exosomes, microvesicles with peak at ~170 nm dominates, and this population is enriched in AQP4 expression.