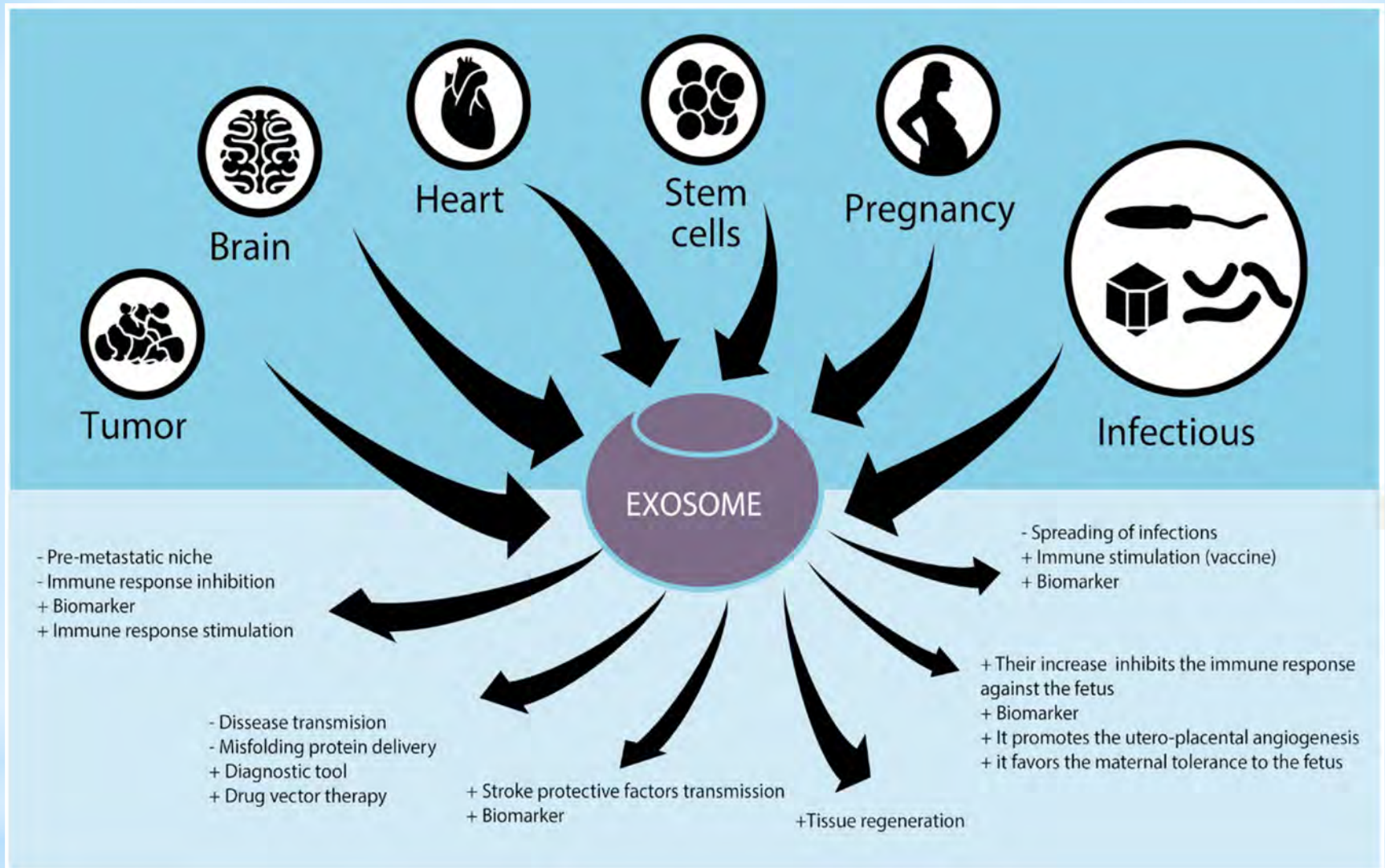


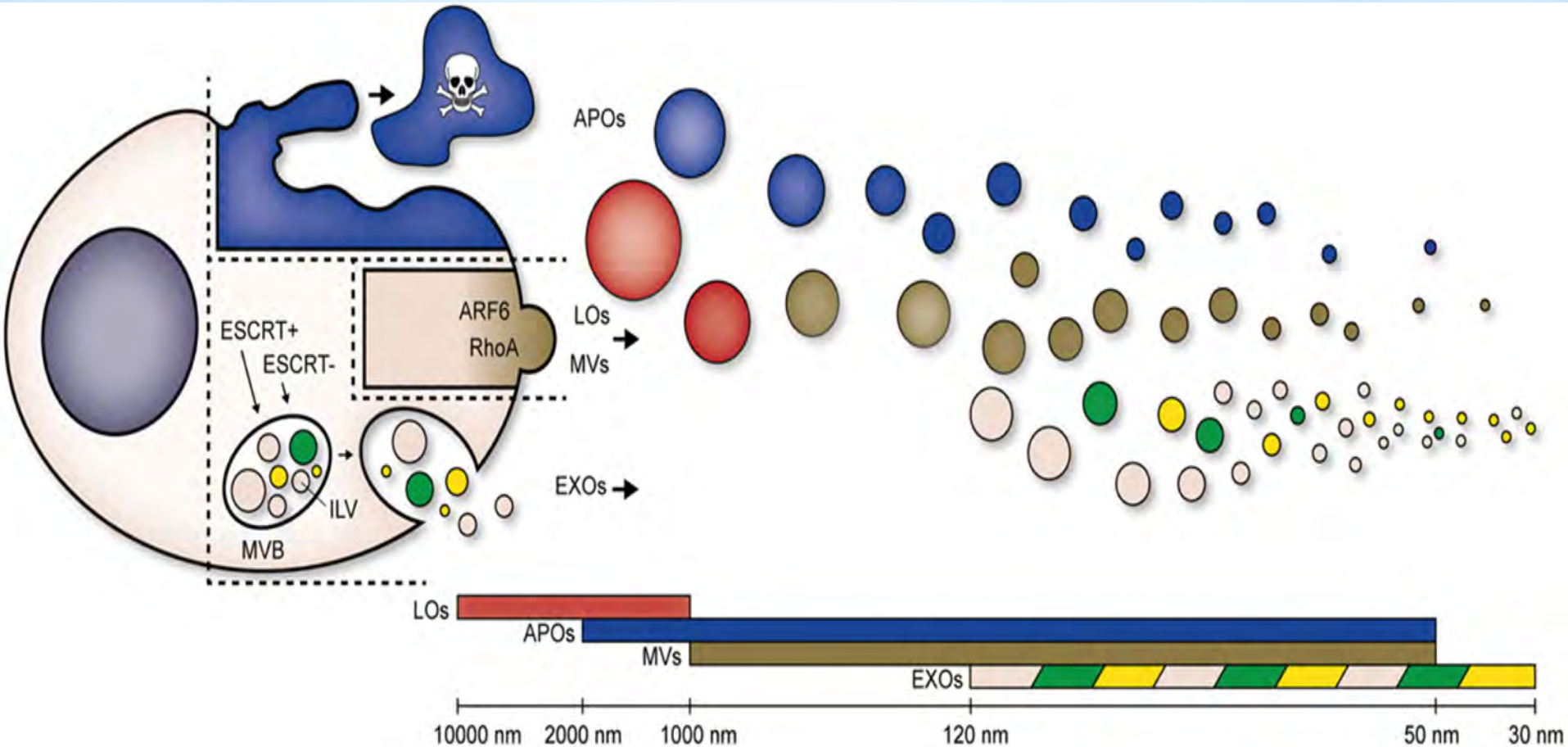
INTEGRATED NANOPARTICLE ISOLATION AND DETECTION SYSTEM FOR COMPLETE ON-CHIP ANALYSIS OF EXOSOMES

Marcella Chiari and Marina Cretich
Institute of Chemistry of Molecular Recognition
C.N.R. Italy



<http://journal.frontiersin.org/article/10.3389/fimmu.2015.00203/full>

Extracellular Vesicles (EV): an heterogeneous population



Modified from Willms et al. Frontiers Immunol

Hurdles in the clinical utilization of exosomes

- Lack of methods to isolate a pure exosome population
- Difficulty in accurately measuring the quantity and purity of exosomes.
- Exosomes have diameters in the range from 30-100 nanometers, i.e., which is too small to be accurately sized by conventional methods such as optical microscopy and flow cytometry (FC) without labels.



Integrated nanoparticle isolation and
detection system for complete on-
chip analysis of exosomes

■ The Trustees of Boston University (BU)

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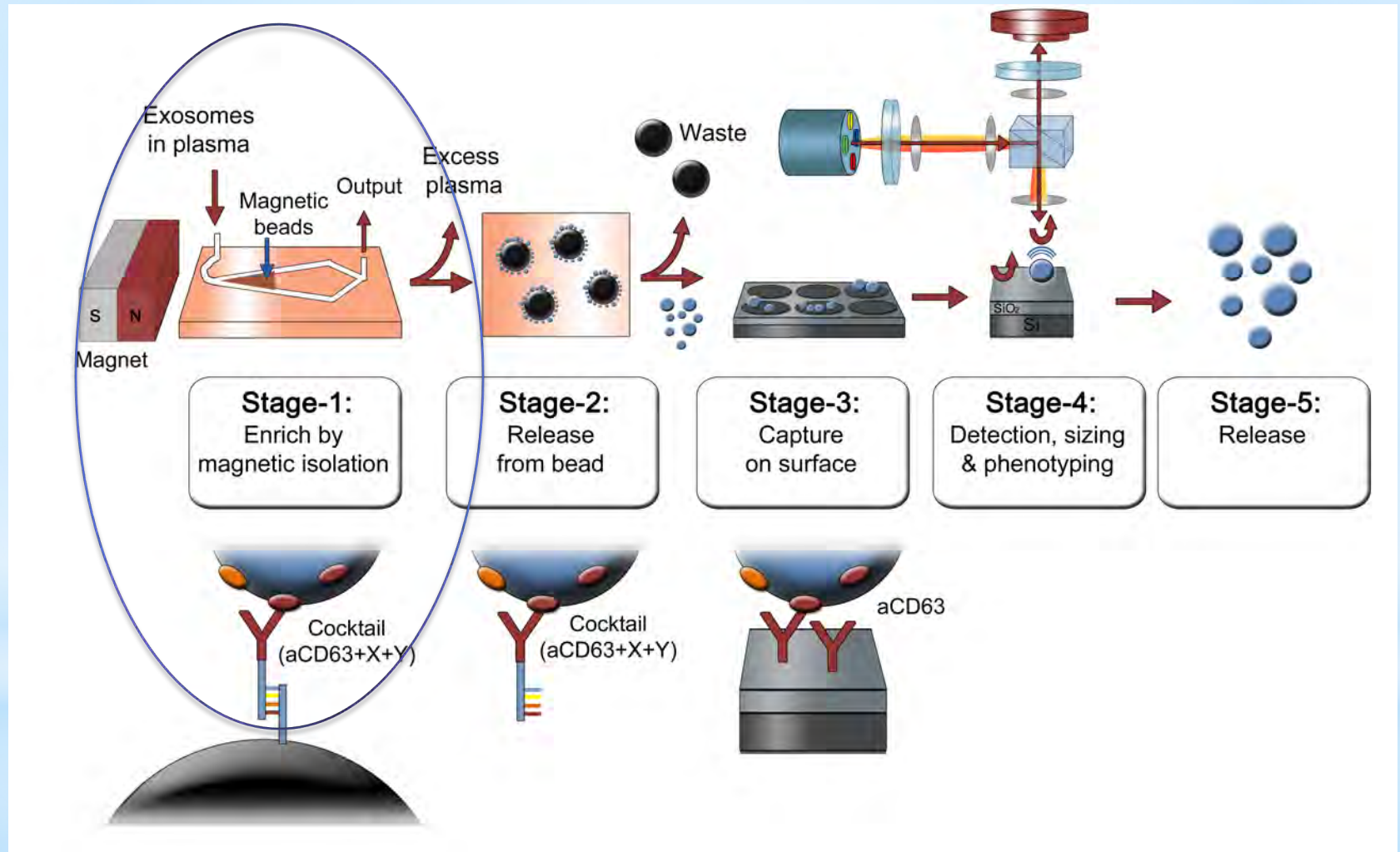


■ Institut Curie

■ Fluigent SA

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INDEX: Integrated nanoparticle isolation and detection system for complete on-chip analysis of exosomes

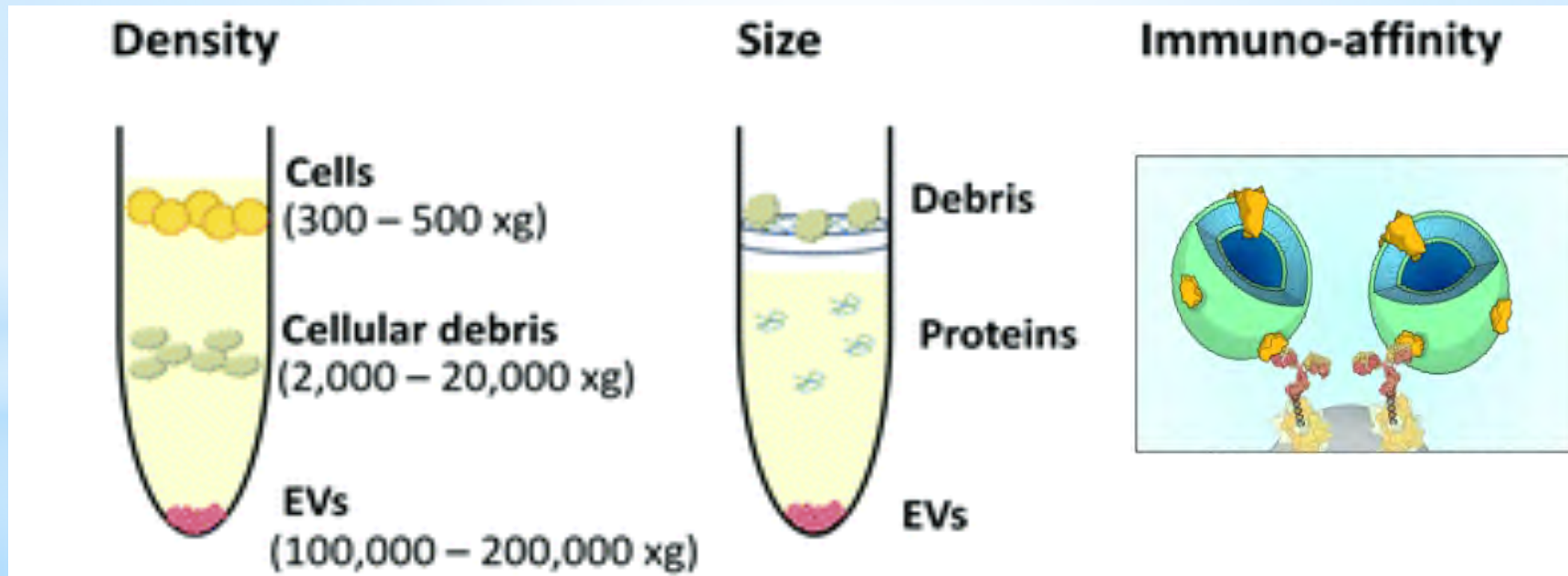


EV separation and enrichment methods

Ultracentrifugation,
sucrose gradient
techniques, and
precipitation reagents

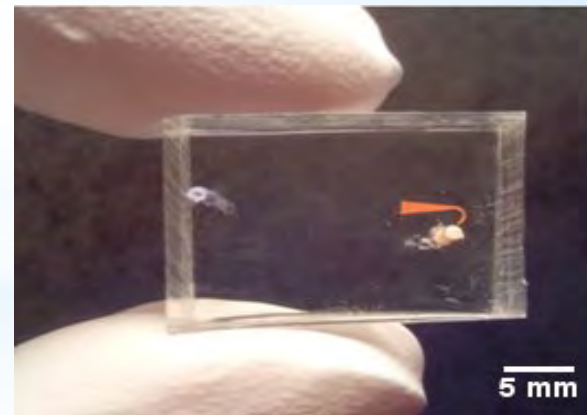
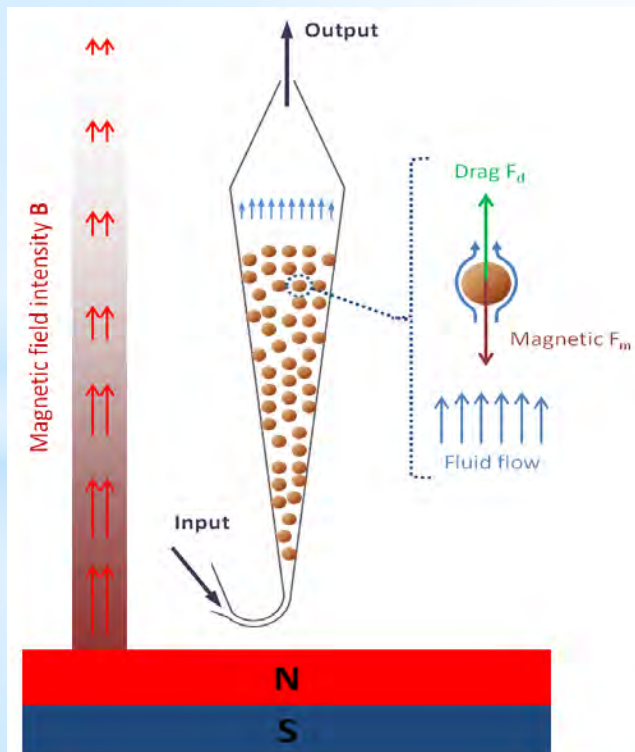
Filtration through
membranes filters
SEC: size exclusion
chromatography

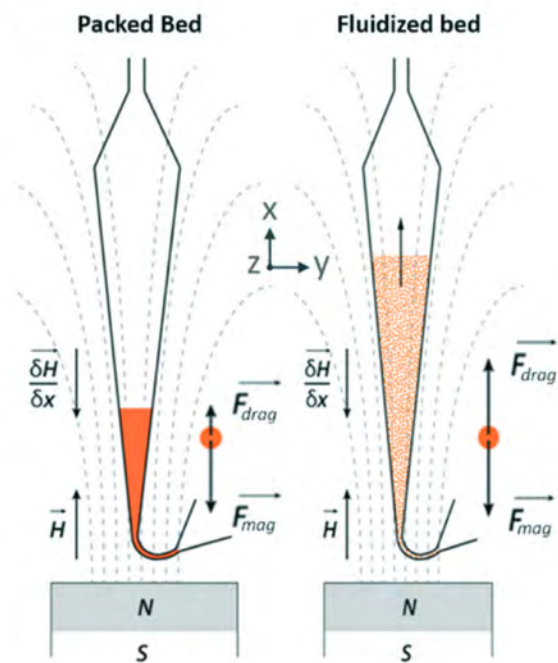
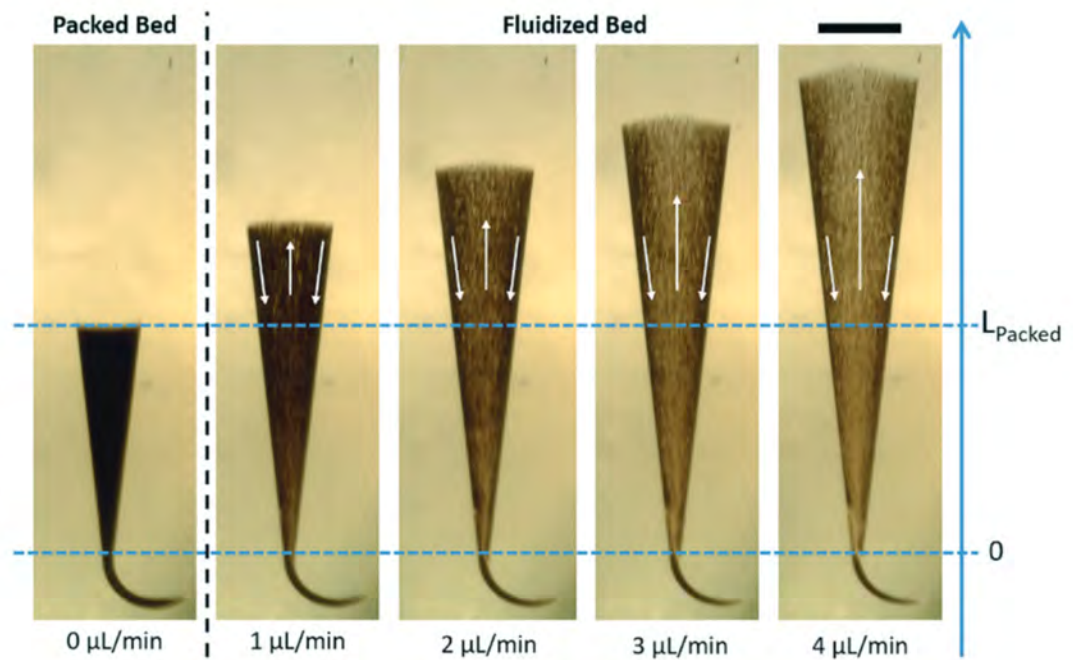
Affinity-based
separation

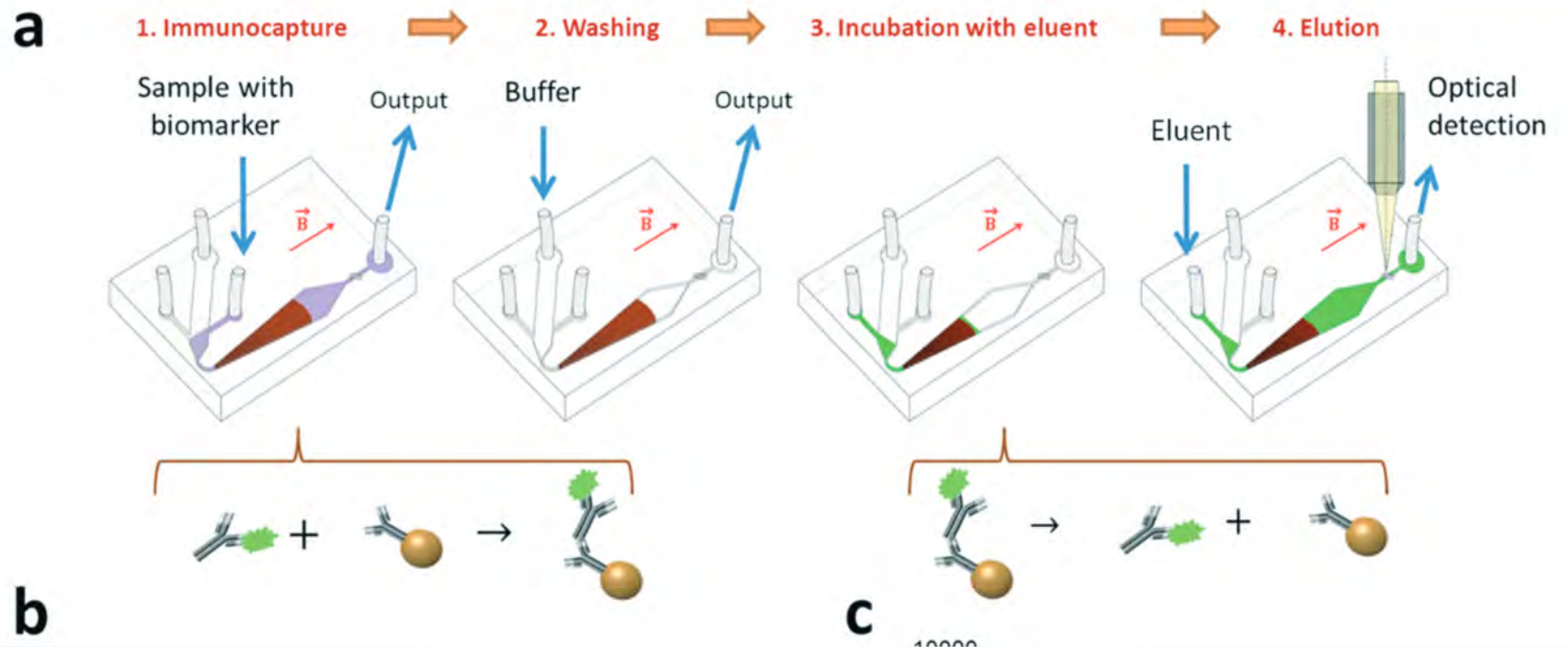




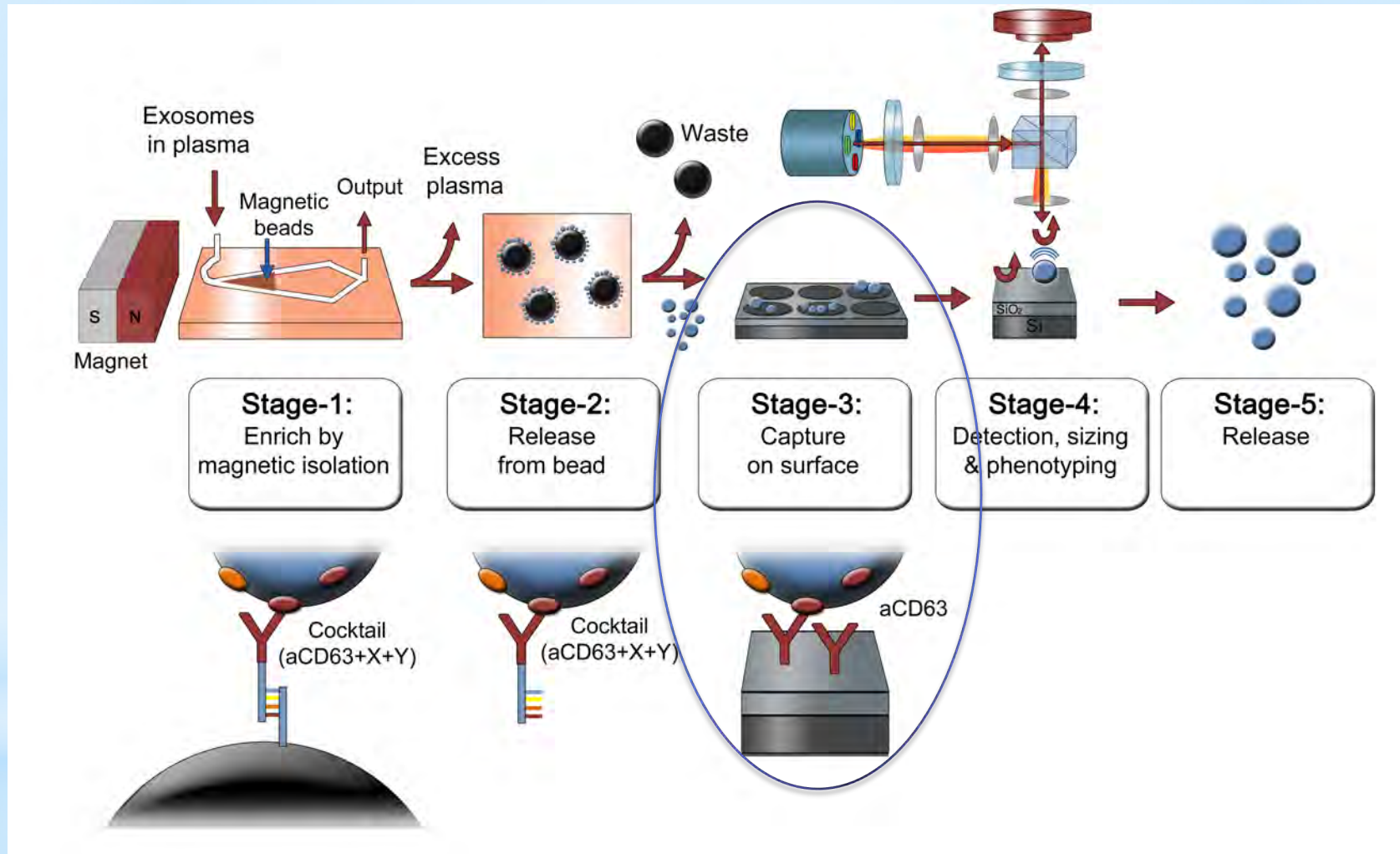
Micro-fluidized bed based on the equilibrium between two forces: a drag force created by the fluid that flows inside the microchamber and a magnetic force that maintains the particles in the microchamber under the hydrodynamic flow.



a**b**



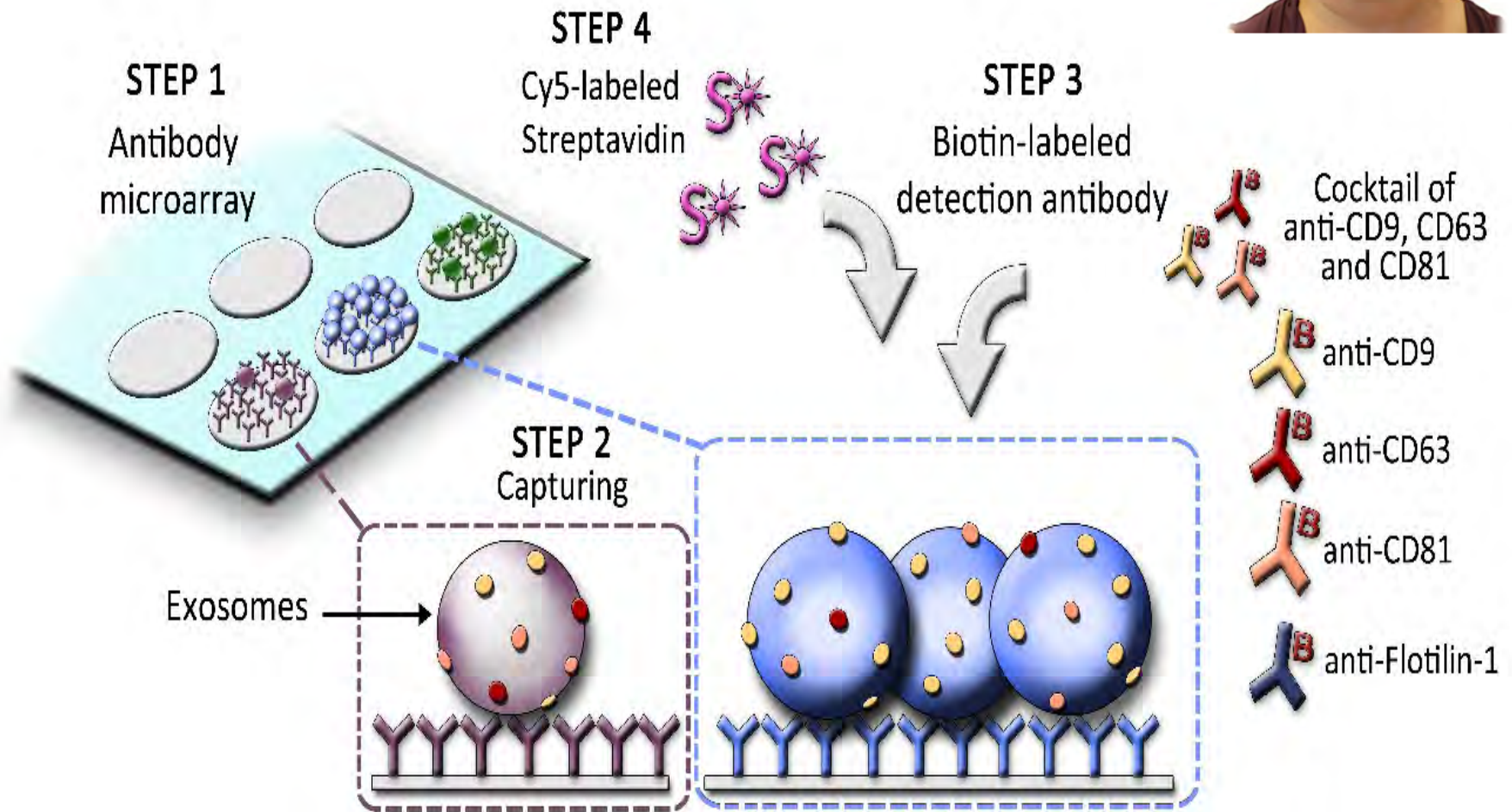
INDEX: Integrated nanoparticle isolation and detection system for complete on-chip analysis of exosomes



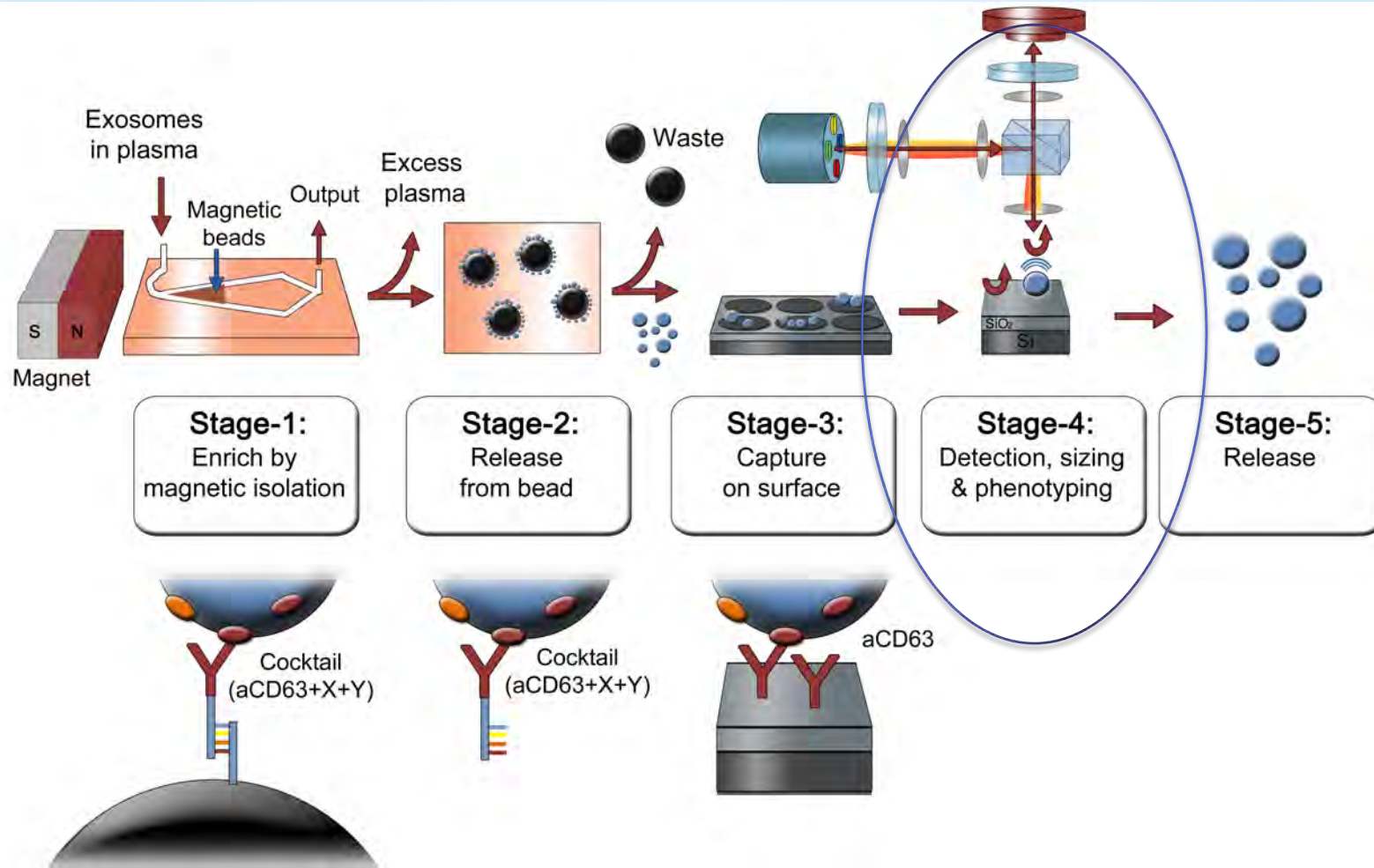
EV microarrays



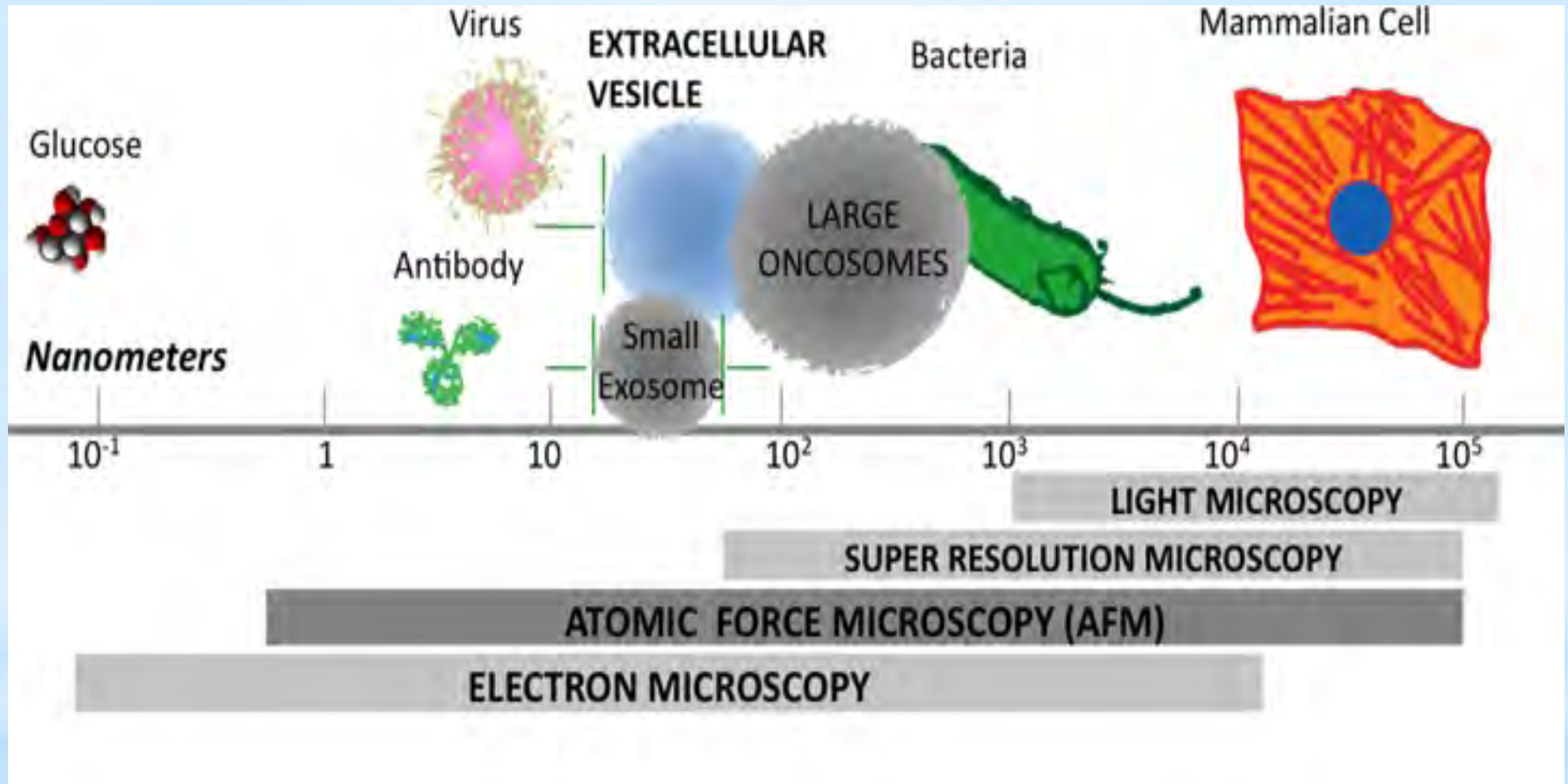
Region Nordjylland (AALBORG University)



INDEX: Integrated nanoparticle isolation and detection system for complete on-chip analysis of exosomes

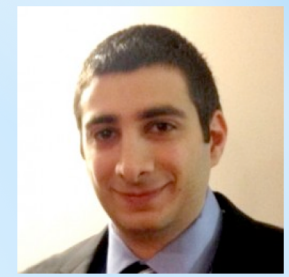


Sensing/detecting EVs: a challenging task





Prof. Selim Unlu
Boston University



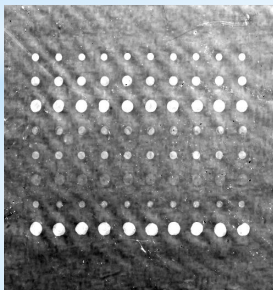
Dr. George Daabaoul
Nanoview, Boston

Interferometric detection platforms



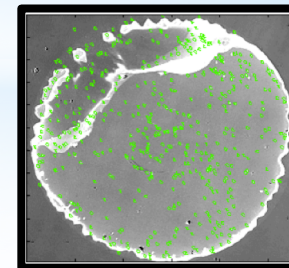
IRIS

Interferometric Reflectance
Imaging Sensor

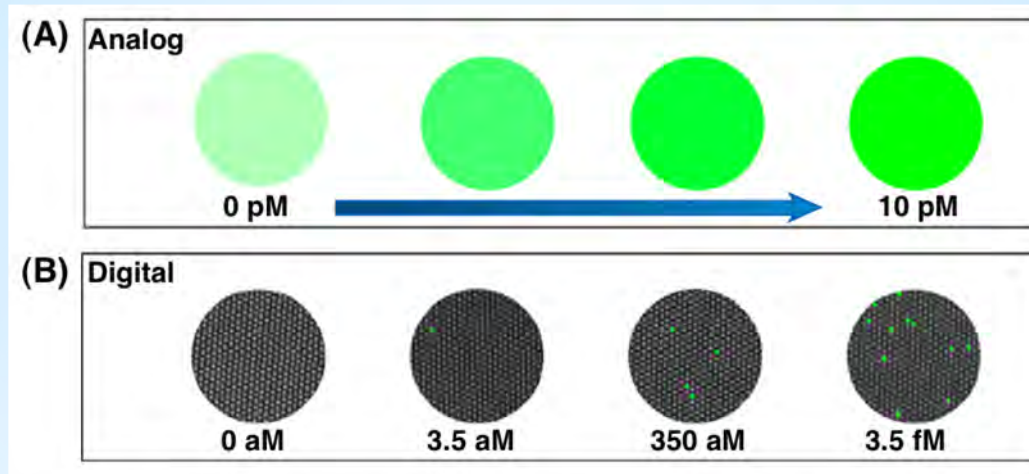


SP-IRIS

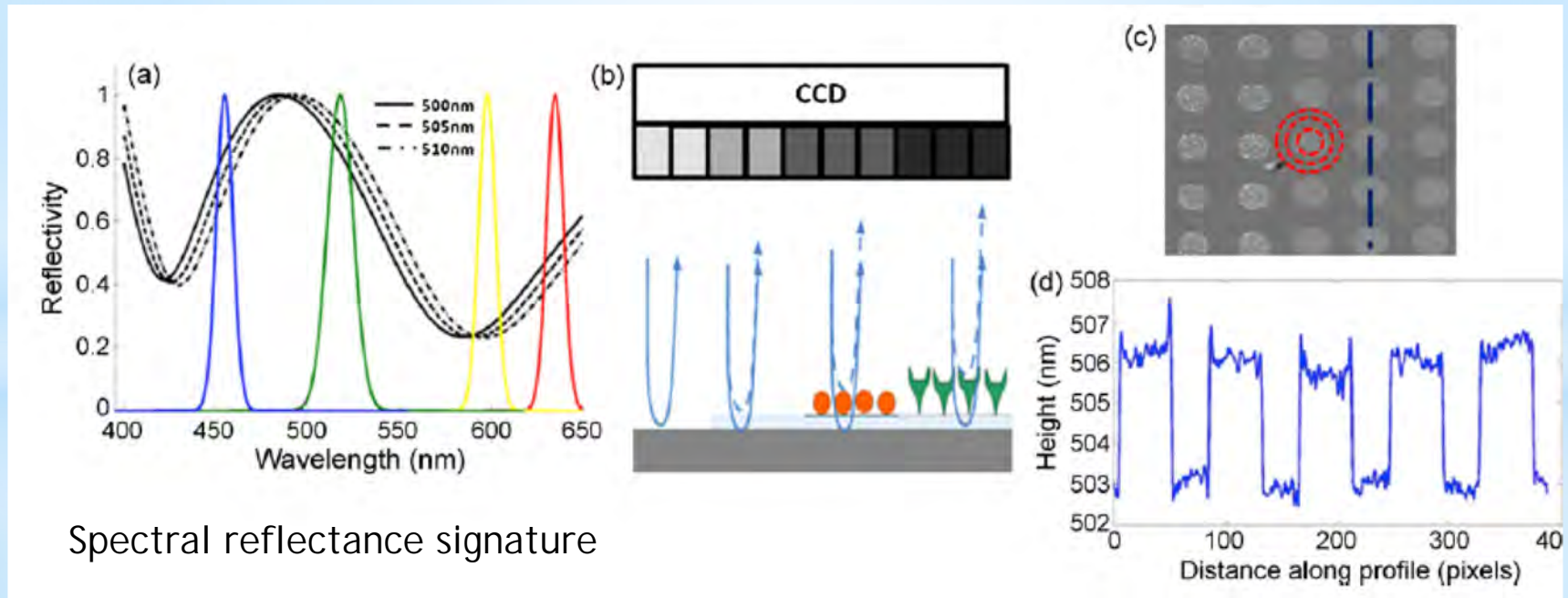
Single Particle
Interferometric Reflectance
Imaging Sensor



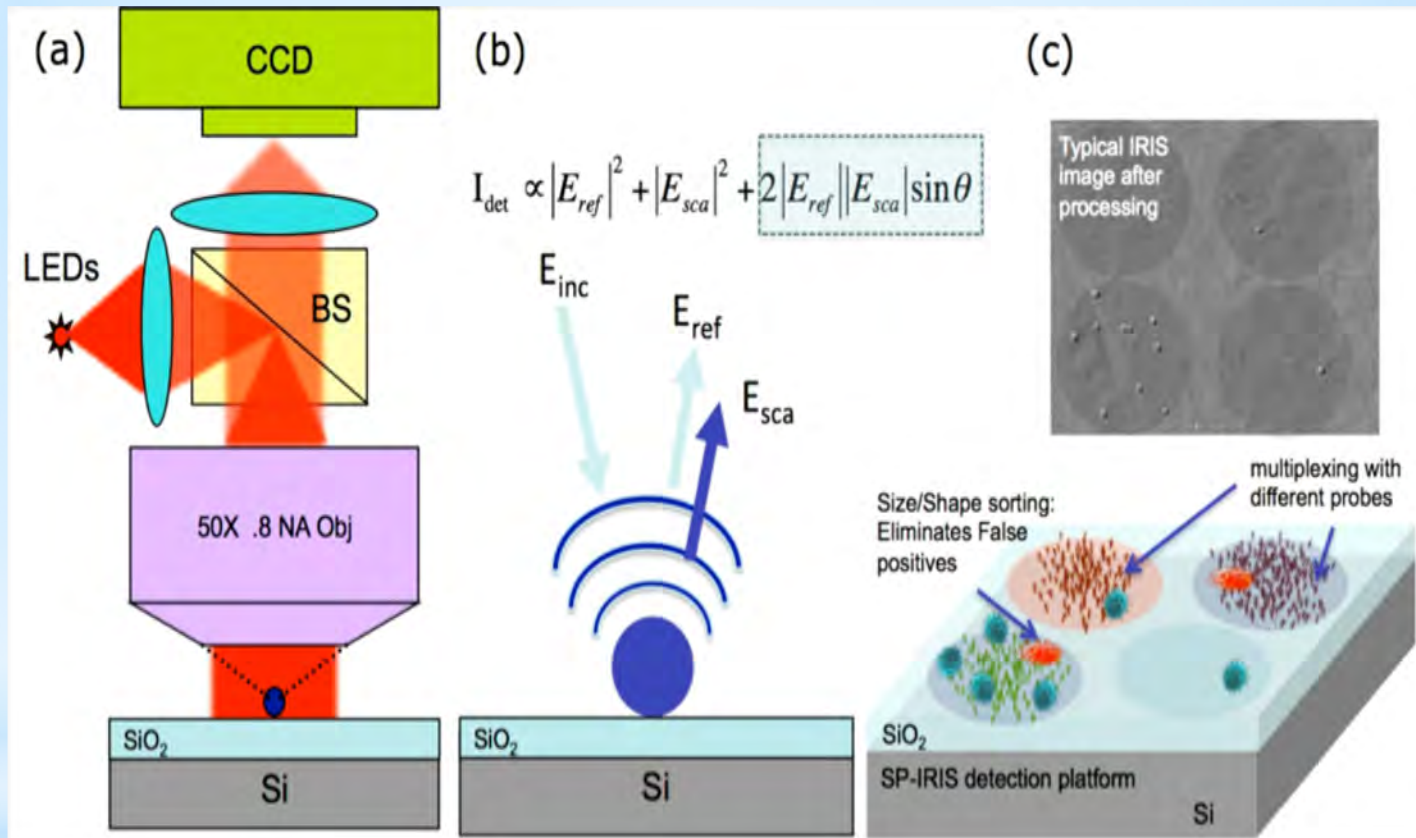
* Digital detection



Label-free detection by LED based Interferometric Reflectance Imaging Sensor (IRIS)



Single particle-IRIS

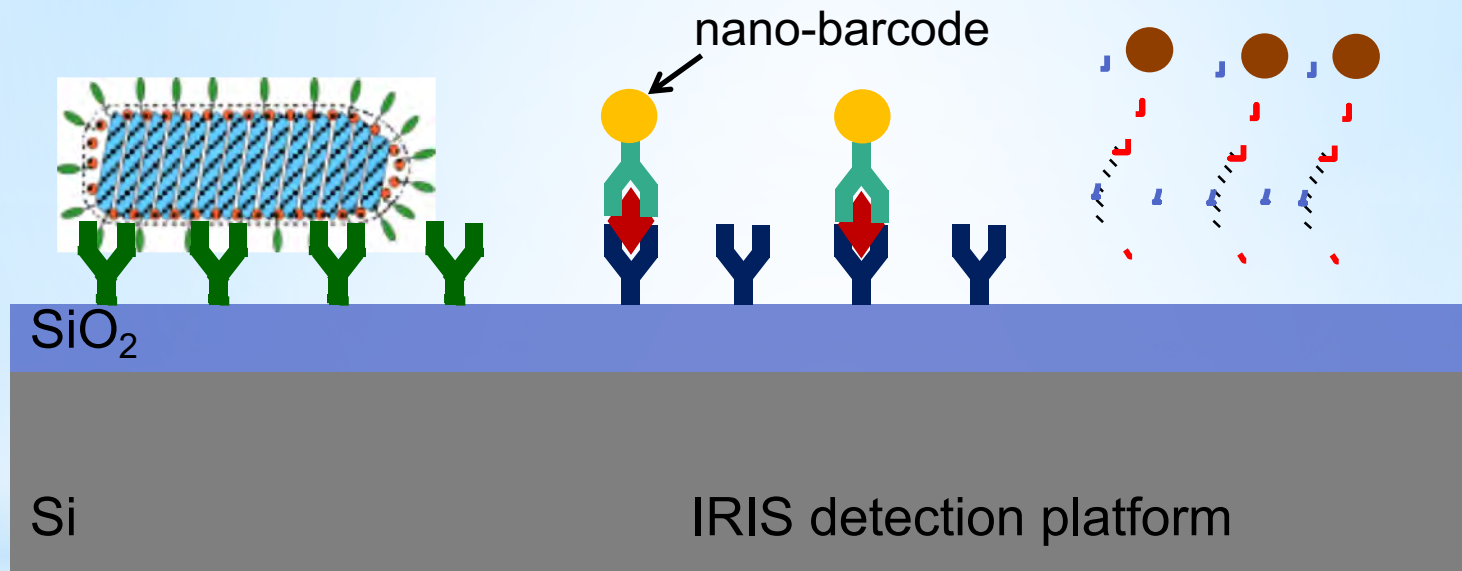


A visible LED provides illumination and bright field reflection image is captured on a CCD camera. The key to improved visibility of nanoparticles on the SP-IRIS system is mixing of the scattered light with reference field reflected from the Si surface.

Single Particle - IRIS

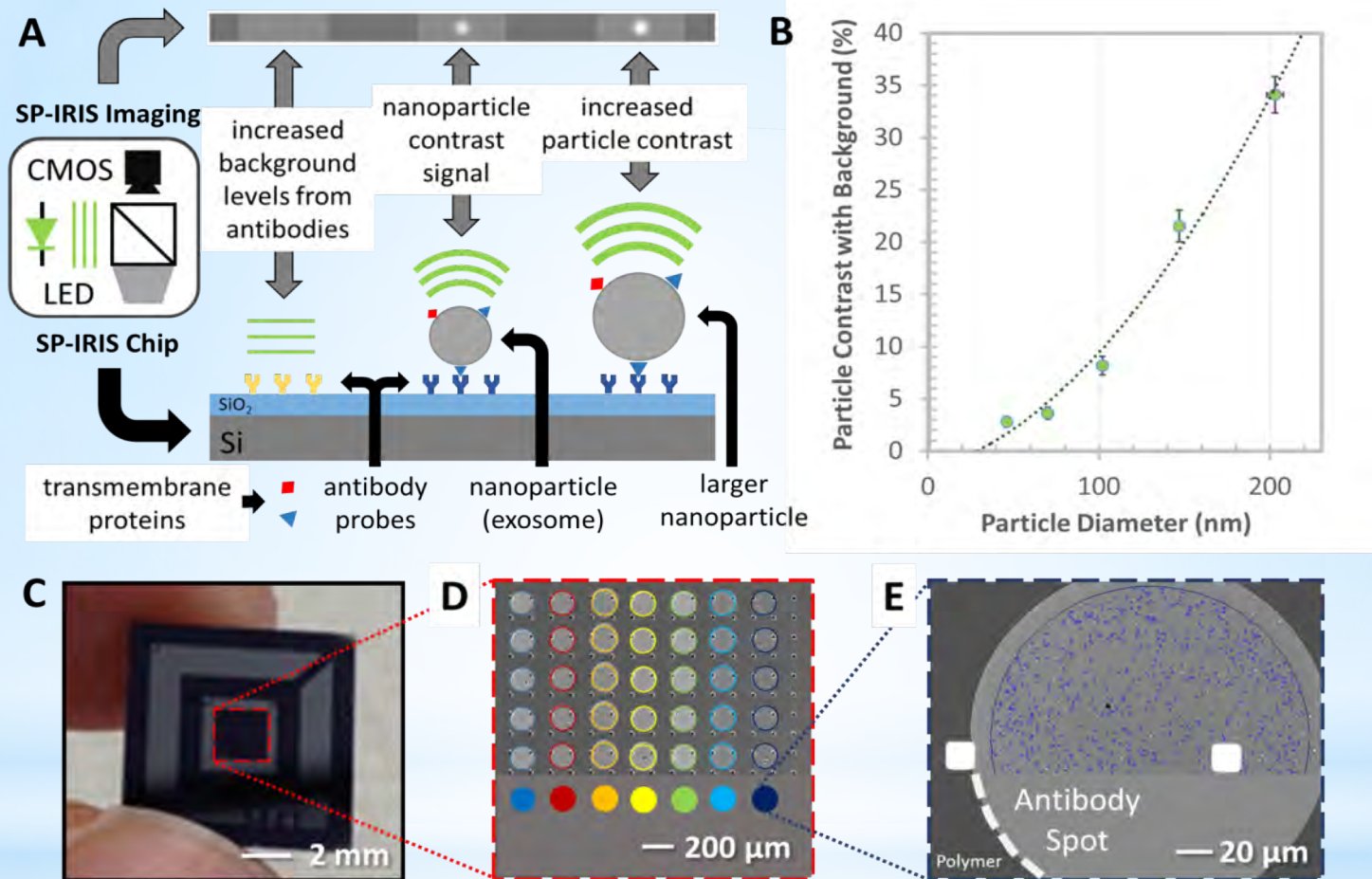
Single Virus Detection
(label –free)

Single Molecule Detection of Antigen
proteins and DNA/RNA

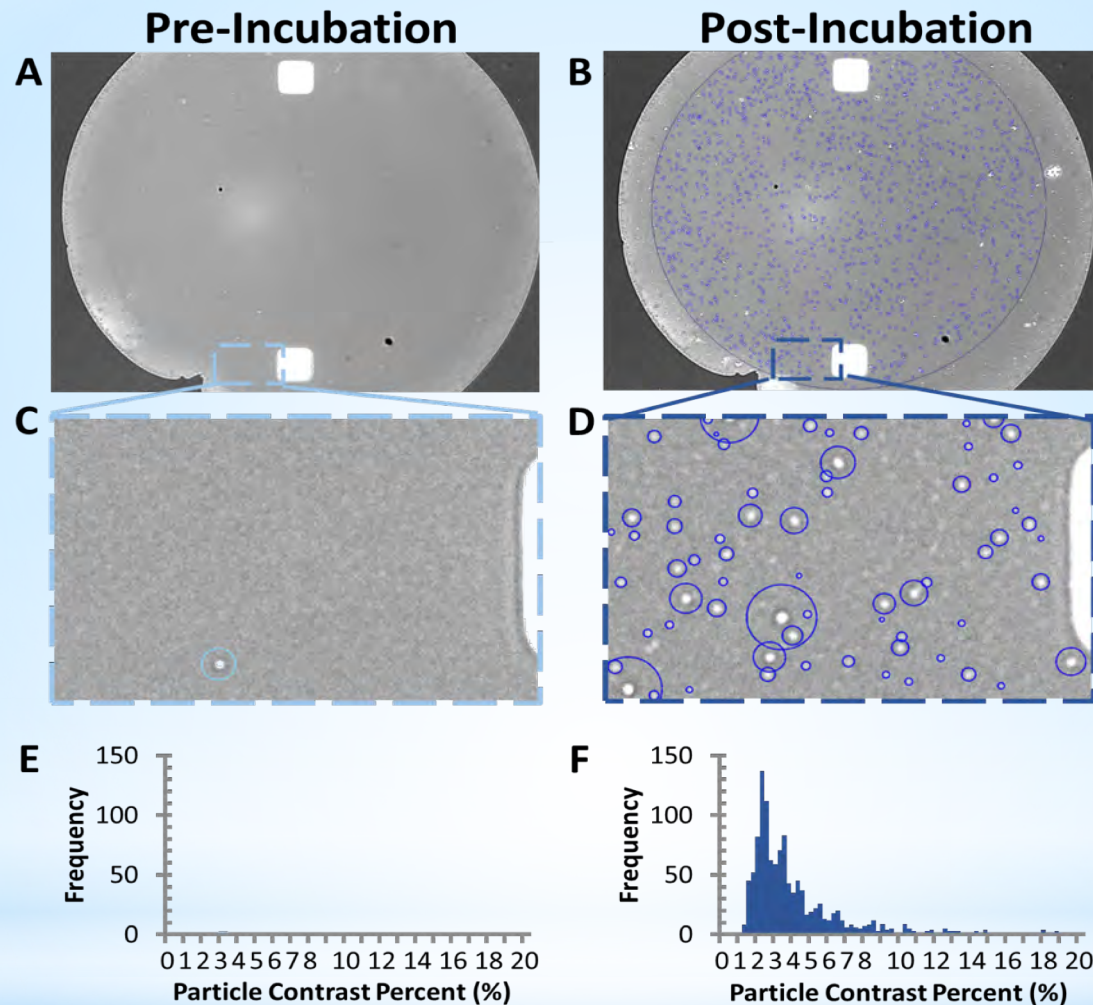


- Label Free direct sensing of individual viruses
- Digital Detection: Single molecule level detection of Nucleic Acids and Proteins
- ULTIMATE BIODETECTION PLATFORM?

Single Particle Interferometric Reflectance Imaging Sensor SP-IRIS

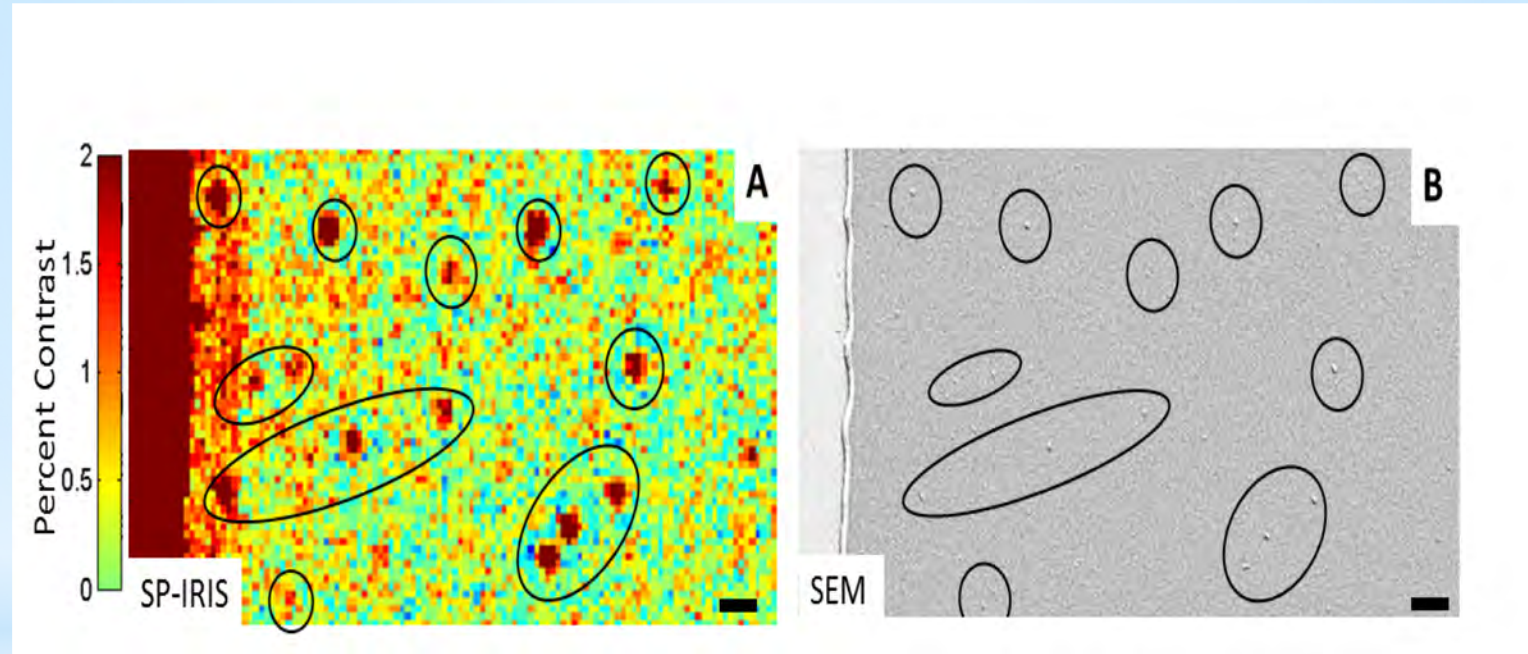


SP-IRIS detection principle, monochromatic LED light illuminates the sensor surface and the interferometrically enhanced nanoparticle scattering signature is captured on a CMOS camera.



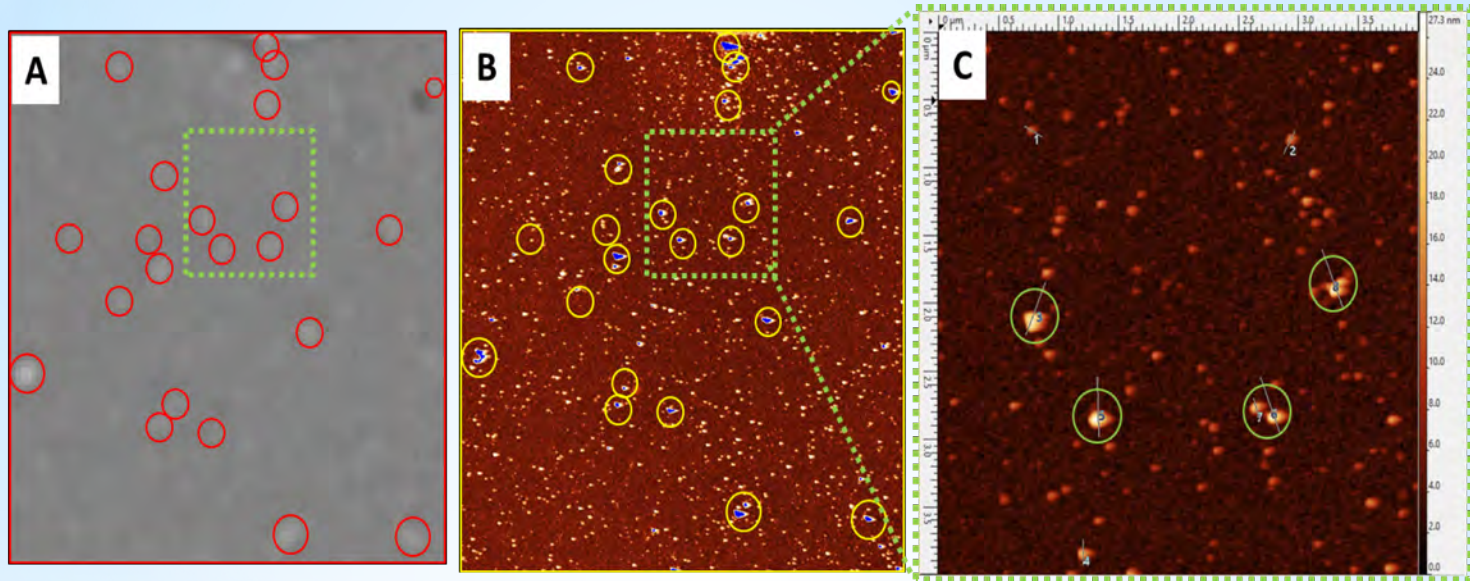
Exosome capture, digital counting, and relative sizing. A-B) Anti-CD81 capture probe image acquired before and after incubation with purified Human Embryonic Kidney 293 (HEK293) cells derived exosomes. C-D) zoom-box of particles detected pre- and post-incubation. E-F) particle contrast histogram pre- and post-incubation

Nanoparticle capture validation with scanning electron microscope (SEM)



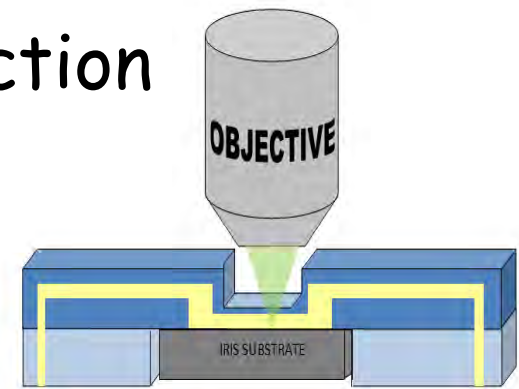
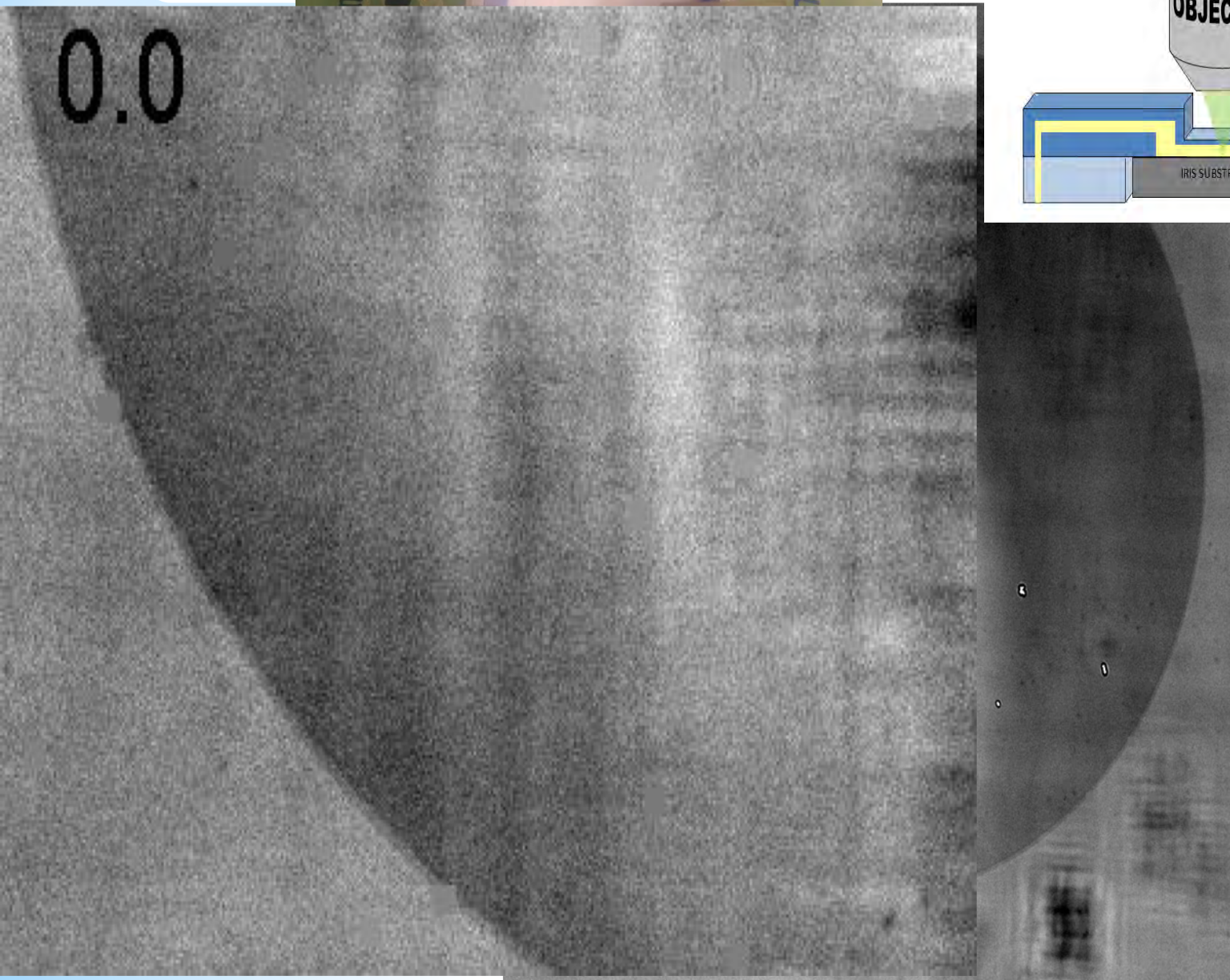
A) SP-IRIS image of exosomes being captured by anti-CD81 antibody. B) Exosomes visualized by SEM of the same field-of-view for comparison. Scale bar is 1 micron.

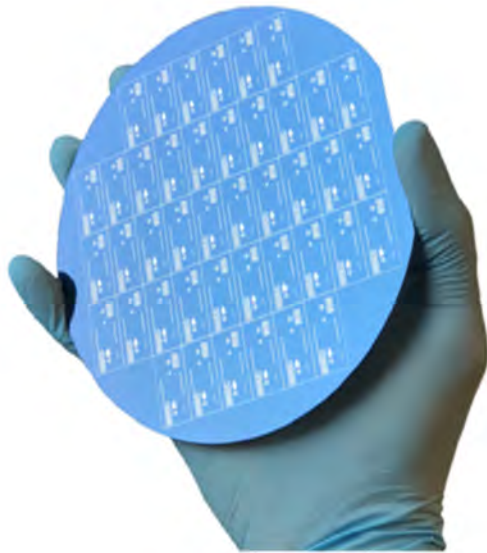
Nanoparticle capture validation with AFM



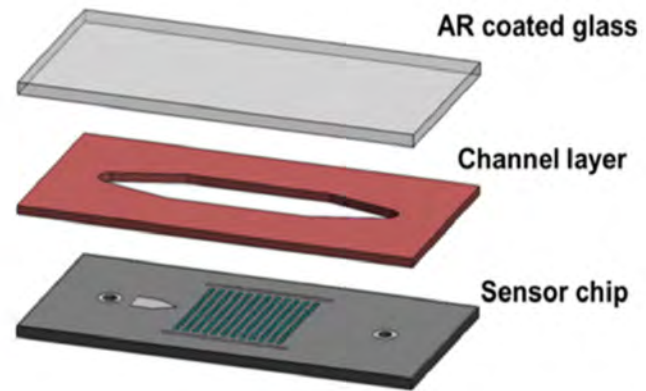
Exosomes purified from HEK cell line, captured with anti- CD81 antibody on silicon chip and detected by SP-IRIS (A) and AFM (B, C).

Real-Time in-liquid Virus Detection





(A)

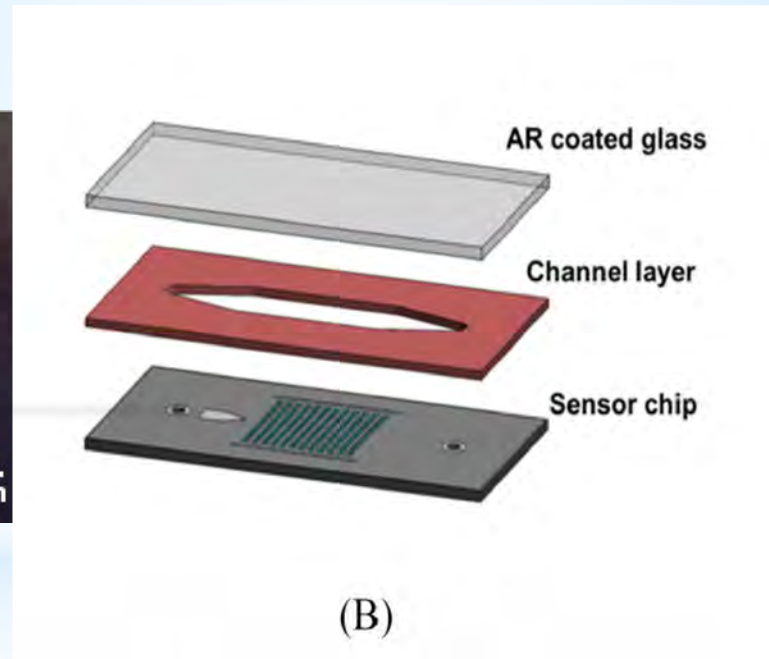
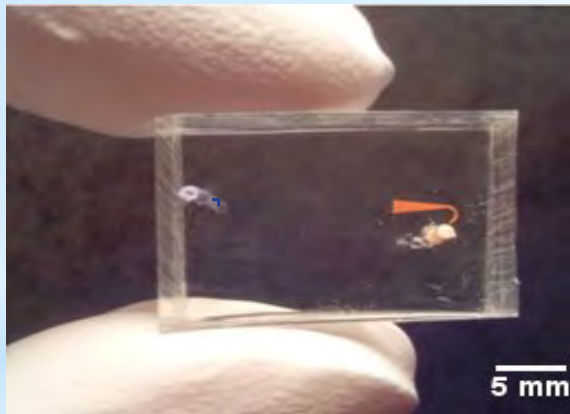


(B)

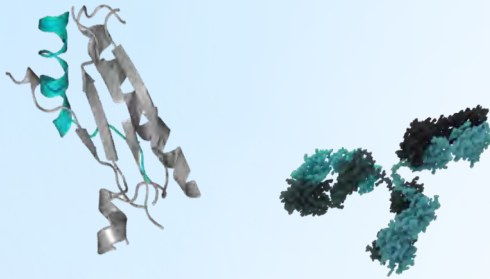
Integration



Marine Verhusel
Fluigent (Paris)



The key to silicon based multimodal analysis: Surface functionalization



- Functional groups for immobilization of molecules of interest
- The surface is inherently inert and resists non-specific adsorption
- Binding strong enough to retain the protein on the surface, but also sufficiently non intrusive to have minimal effect on its 3D structure
- Retention of optical properties

Transparent
Spacer (SiO_2)

High index absorbing
substrate (Si)

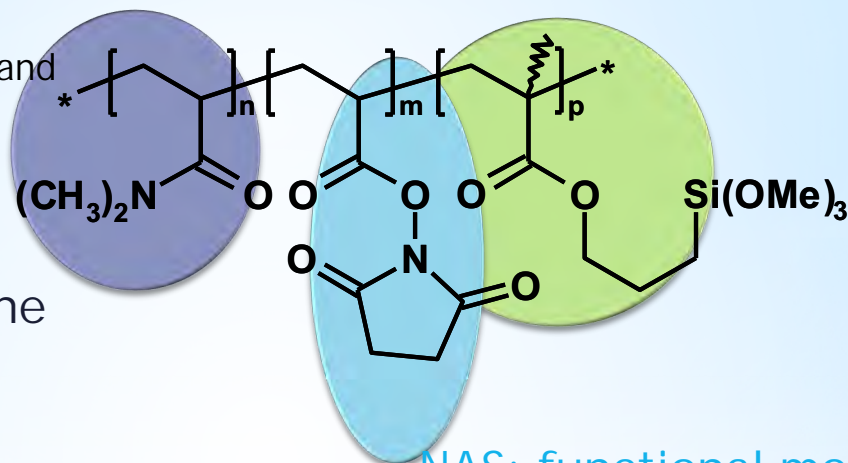
Surface functionalization: polymeric coatings

Copoly(DMA-NAS-MAPS)

a ter-copolymer based on N,N-dimethylacrylamide (DMA), N-acryloyloxysuccinimide (NAS) and 3-(trimethoxysilyl)propyl-methacrylate (MAPS)

DMA: polymer backbone
self-adsorb onto SiO_2
surfaces

MAPS: silanating
monomer stabilize
the coating



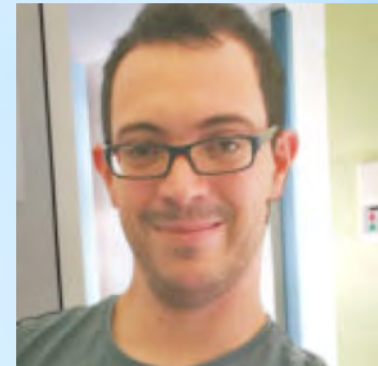
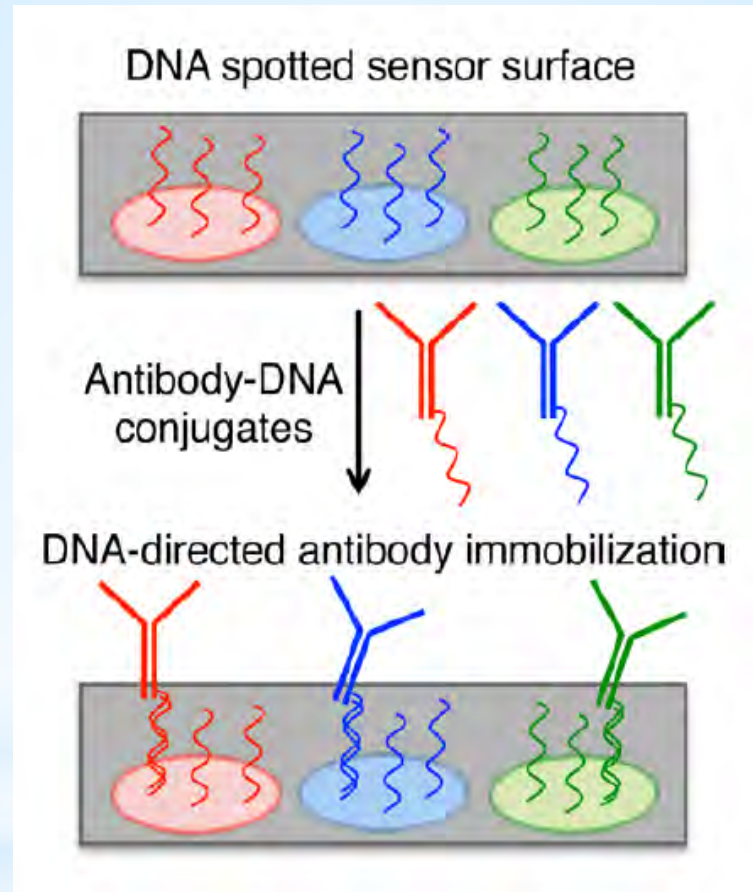
NAS: functional monomer
reacts with
amines/nucleophiles

29



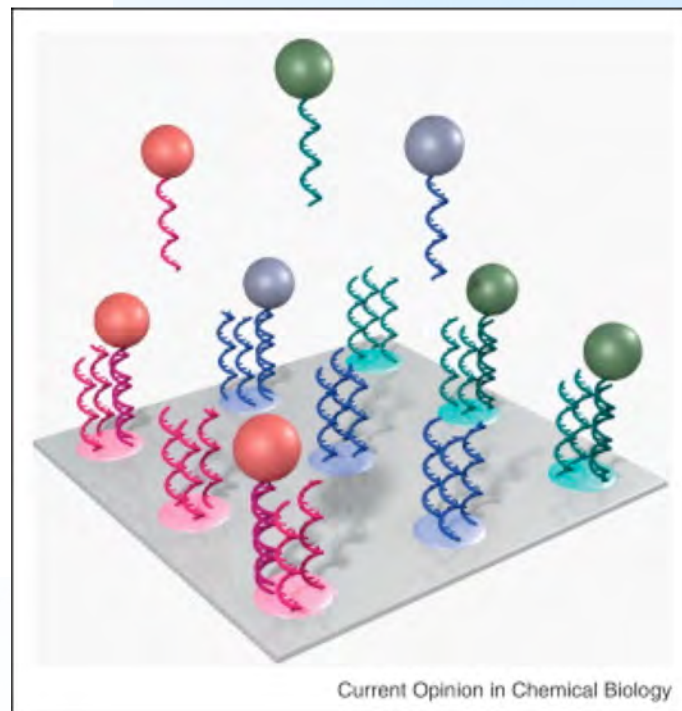
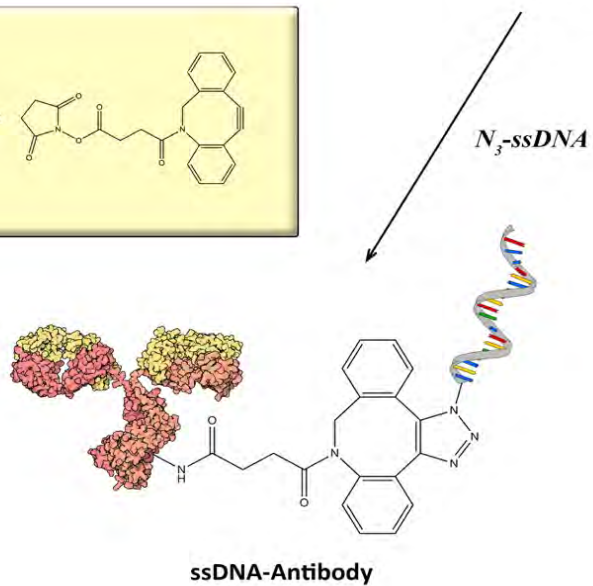
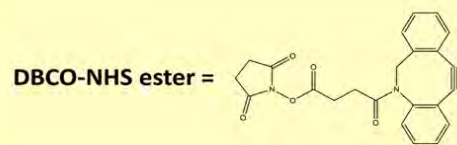
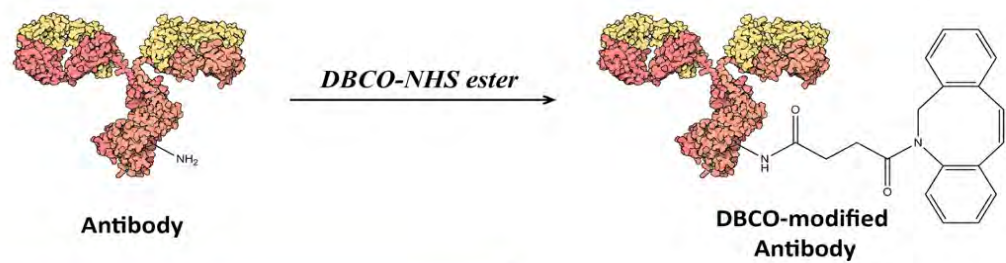
HansaBiomed
Tallin Estonia

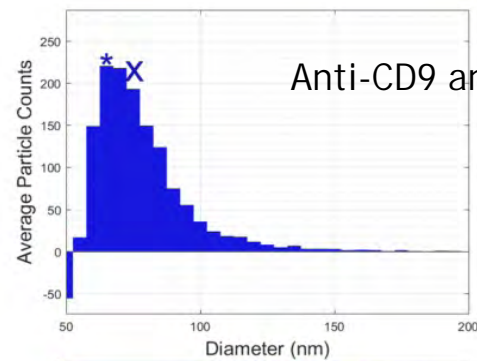
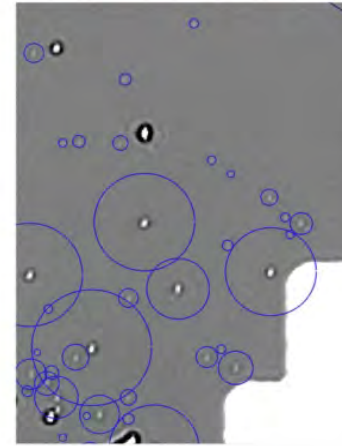
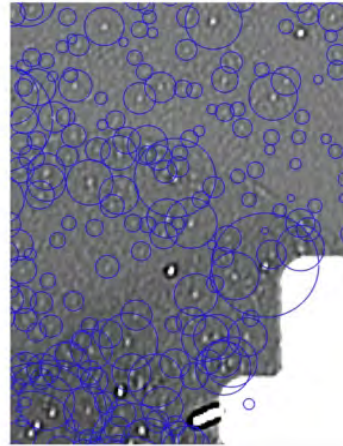
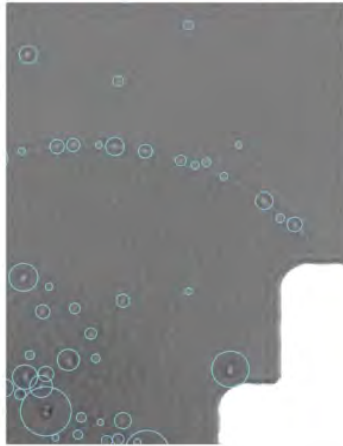
Reversible capture of exosomes



Dario Brambilla
ICRM-CNR

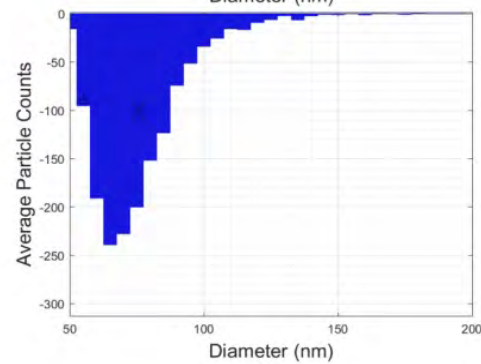
Evs are immunocaptured through **DNA-directed Immobilization** of exosome-specific antibodies (Anti-CD9 and Anti-CD63).



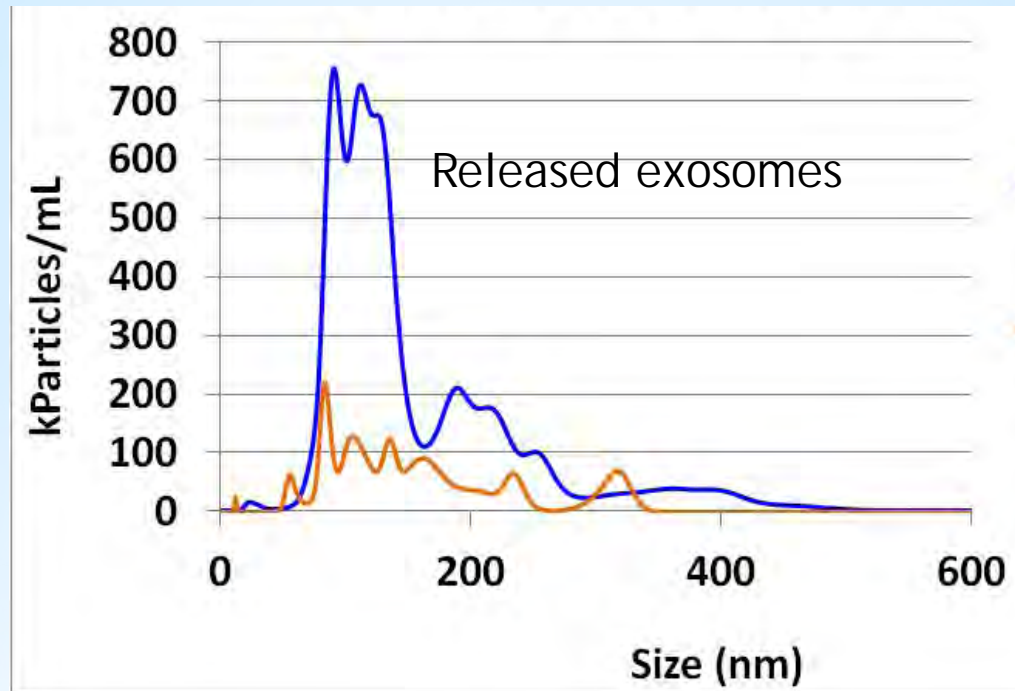


Anti-CD9 antibody.

**Size Distribution
of Captured Particles**



**Size Distribution
of Released Particles**



NTA analysis of a solution of EVs released from magnetic beads through denaturation of the DNA linker (blue trace) and releasing buffer (brown trace)

Conclusions

- ✓ Separation of Evs from serum and plasma
- ✓ DNA directed immobilization of Antitetraspanine antibodies
- ✓ Detachment of intact EVs from the surface
- ✓ Detection of Evs by single particle IRIS
- ✓ Integration of extraction and detection modules into a prototype instrument for Evs sizing and phenotyping

Chemistry and Technology for Bioscience Group
www.ctbio.eu

Marina Cretich

Francesco Damin

Laura Sola

PhD students and post docs

Paola Gagni

Dario Brambilla



Boston University
Selim Unlu

Nanoview
George Doaboul

**Istituto Centro San Giovanni di Dio Fatebenefratelli,
Brescia, Italy**
Roberta Ghidoni, Luisa Benussi

University of Trento, Povo (TN) Italy
Paolo Bettotti



Regione Lombardia

Amanda

Ready

