ISOLATION, DIGITAL DETECTION AND CHARACTERIZATION OF BIOLOGICAL NANOPARTICLES FOR EXOSOME-BASED DIAGNOSTICS

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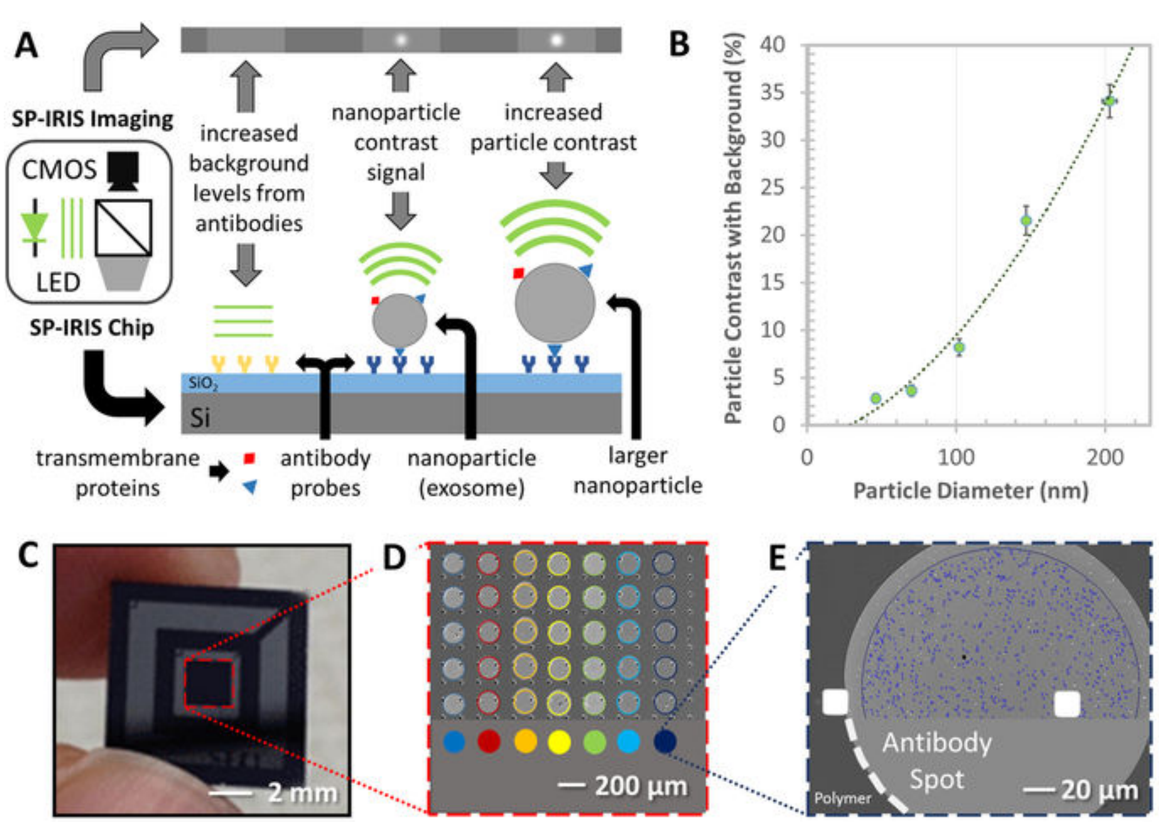
Introduction

Cells produce and release membrane vesicles in cell cultures and different bodily fluids. A particular type of such vesicles, named exosomes, are 30-100 nm in diameter.

Exosomes are known to mediate communication between cells, playing an essential role in inflammation, cellular homeostasis, survival, transport and regeneration; accordingly, minimally-invasive diagnosis of a number of diseases can be achieved through detection of these vesicles.
In particular, exosomes are considered valuable for liquid biopsies in cancer diagnosis since they carry molecular and proteomic cargo from their tumour cell of origin. In human cerebrospinal fluid (hCSF), exosomes are rich reservoirs of biomarkers for neurological disorders and there is increasing evidence that deregulation of vesicle secretion play a pathological role in Alzheimer's disease.

Despite exosomes hold the great promise of revolutionizing the standard of clinical care, their detection and molecular profiling is still technically challenging due to their small dimension and low refractive index.
In this work we present a method based on Single Particle Interferometric Reflectance Imaging Sensor (SP-IRIS) that allows multiplexed phenotyping and digital counting of various populations of individual exosomes (>50 nm) captured on a protein microarray-based solid phase chip. We demonstrate these concepts using purified exosomes from cell culture and directly from hCSF.
Our interferometric imaging method could capture, from a very small hCSF volume (20 uL), nanoparticles that have a size compatible with exosomes, using antibodies directed against tetraspanins. With this unprecedented capability, we foresee revolutionary implications in the clinical field with improvements in diagnosis and stratification of patients affected by different disorders.

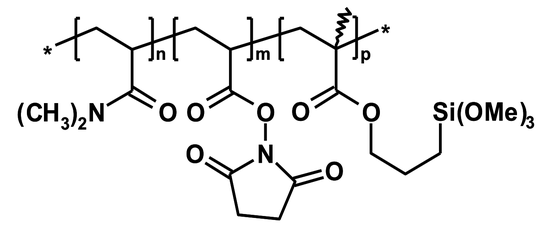
The detection platform



(A) SP-IRIS detection principle, monochromatic LED light illuminates the sensor surface and the interferometricly enhanced nanoparticle scattering signature is captured on a CMOS camera.
(B) Demonstrates SP-IRIS signal for polystyrene nanoparticles with a diameter from 50–200 nm which can be used to infer size of captured EVs.
(C) Image of the SP-IRIS chip.
(D) Low-magnification interferometric image showing microarray of immobilized capture probes.
(E) SP-IRIS image of a capture probe. NVDX analysis software recognize capture spot and detects nanoparticles captured.

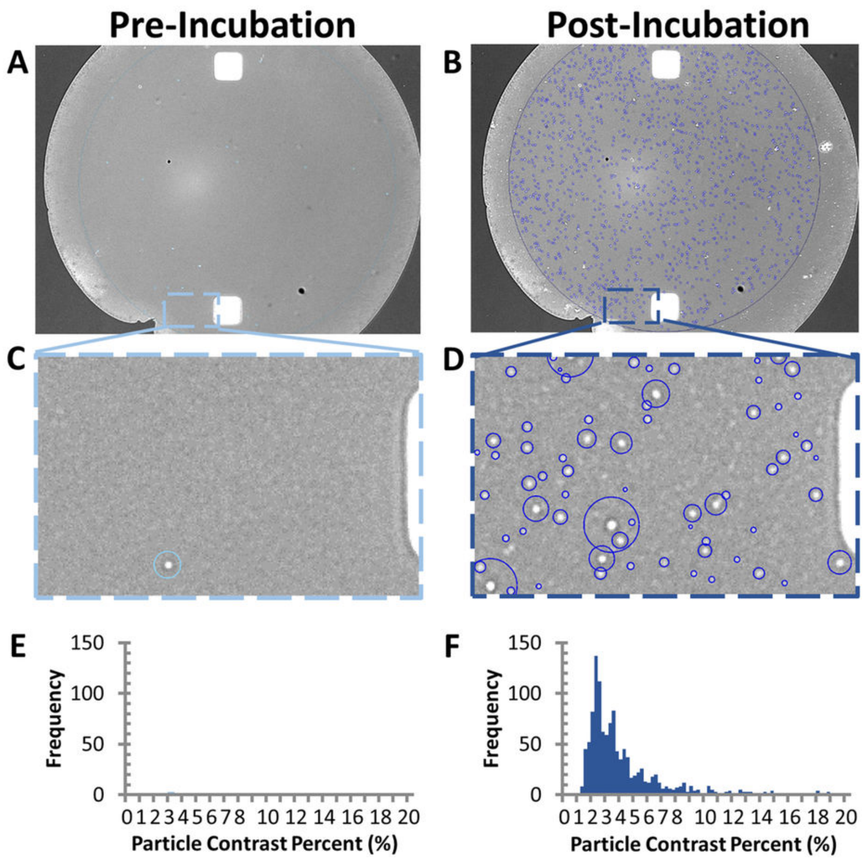
Silicon coating

Silicon CHIPS are functionalized by adsorption of copoly(DMA-NAS-MAPS) , a ter-copolymer based on N,N-dimethylacrylamide (DMA), N-acryloyloxysuccinimide (NAS) and 3-(trimethoxysilyl)propyl-methacrylate (MAPS)

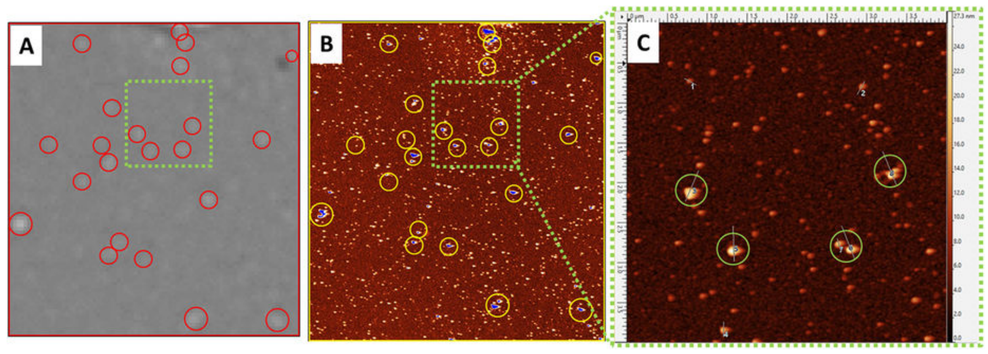


Coating procedure is easy and robust: chips are immersed in a polymer solution (1% w/v in 0.9 M ammonium sulfate) for 30 minutes. The chips are then rinsed with water, dried under nitrogen and cured under vacuum at 80°C

Results

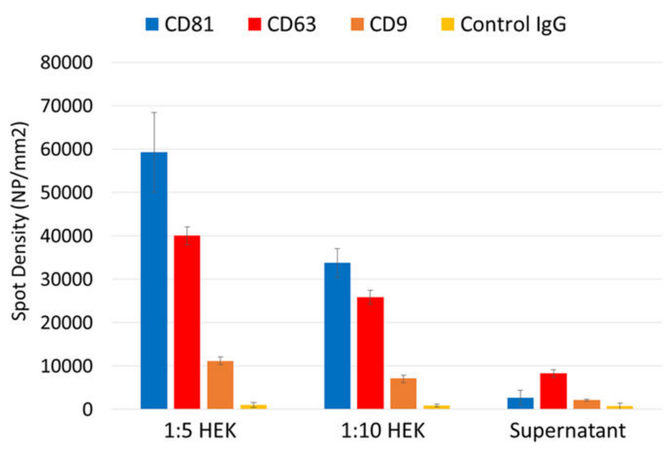


(A,B) Anti-CD81 capture probe image acquired before and after incubation with purified HEK293 cells derived exosomes.
(C,D) Zoom-box of particles detected pre- and post-incubation.
(E–F) Particle contrast histogram pre- and post-incubation.



Exosomes purified from HEK cell line, captured with anti- CD81 antibody on silicon chip and detected by SP-IRIS.

(A) and AFM (B,C). (A) SP-IRIS Image of the anti-CD81 spot incubated with a suspension of exosomes (6.75E + 10 exosomes/mL), the red circles highlight the countable nanoparticles. (B) AFM image of the same spot area: the blue dots identify particles larger than 15 nm; the yellow circles show in image (B) perfectly match the particles detected by SP-IRIS in image (A). (C) Zoom in area of the anti-CD81 spot shown in the green frame highlights the particles larger than 15 nm in height detected by AFM and SP-IRIS. Particles smaller than 15–10 nm are detectable only with AFM as they are below SP-IRIS detection limit



Exosomes, isolated from HEK fibroblast cells and EV depleted supernatant, captured with antibodies against CD81, CD63, CD9 and IgG negative control and detected by SP-IRIS.

REF: M. Chiari, G. Daaboul, P. Gagni, L. Benussi, P.Bettotti, M. Ciani, M. Cretich, D. Freedman, R. Ghidoni, A. Yalcin Ozkumur, C.Piotto, D. Prosperi, B.Santini and M. Selim Ünlü. Digital detection of exosomes by interferometric imaging Scientific Reports (2016) 6: 3746

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