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 μL), 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**

![Detection Platform Diagram](image)

**Silicon coating**

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

![Silicon Coating Diagram](image)

**Results**

(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 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 fibroblasts cells and EV depleted supernatant, captured with antibodies against CD81, CD63, CD9 and IgG negative control and detected by SP-IRIS.

**Pre-incubation**

![Pre-incubation Image](image)

**Post-incubation**

![Post-incubation Image](image)

**Results Table**

<table>
<thead>
<tr>
<th>Condition</th>
<th>CD81</th>
<th>CD63</th>
<th>CD9</th>
<th>Control IgG</th>
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<tbody>
<tr>
<td>1.5 HEK</td>
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<tr>
<td>1:10 HEK</td>
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<td>Supernatant</td>
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**References**


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**Horizon 2020**