Electronic Supplementary Material (ESI) for Lab on a Chip.

Electronic Supplementary Information

Control experiments

Table ESI 1: Overview of the experiments included in this study – EV capture on the antibody-conjugated substrates and negative control experiments.

	EDC/NHS activation	**Antibody**	**EVs**
Sample *EVs to be analyzed*	+	+	+
Control i	-	+	+
Control ii	+	-	+
Control iii	+	+	-

ESI 1. Raman Spectroscopy of Stainless steel

In order to assess the suitability of functionalized stainless steel as a substrate for Raman spectroscopy, an area of 40 µm x 40 µm was scanned with a laser power of 35mW and an illumination time of 250 ms with a step size of 1 µm. The spectrum of clean stainless steel is comparable to standard CaF_2 and commercial stainless steel (µRIM™, BioTools) used as substrate for Raman spectroscopy. A quartz spectrum is also shown for comparison.

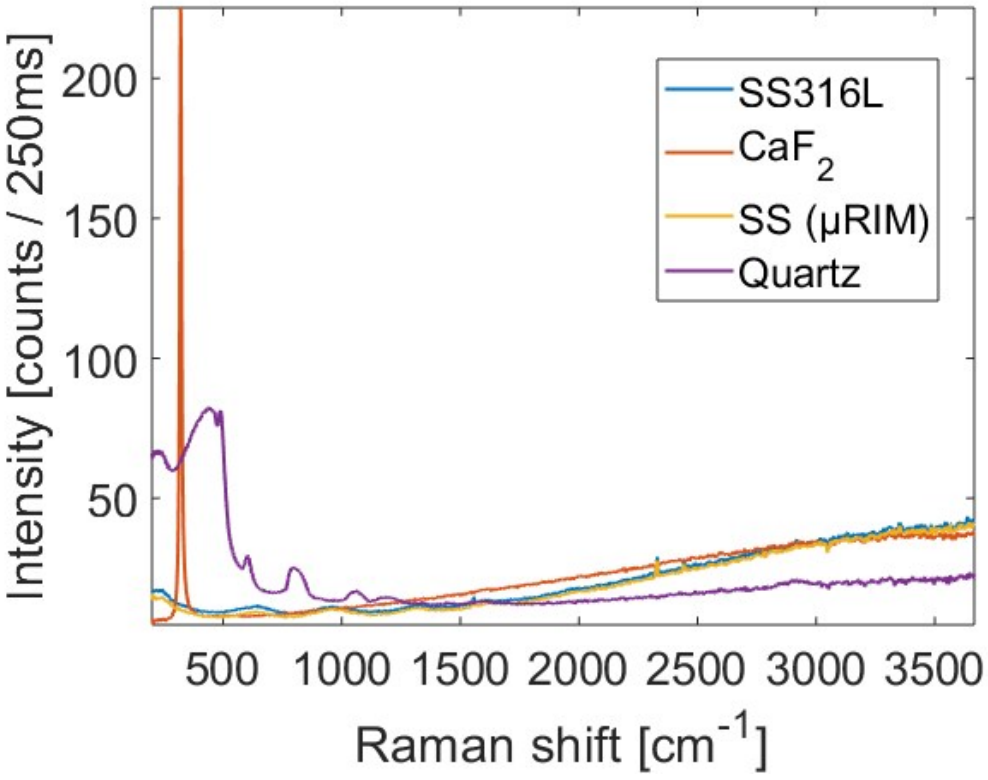

Figure ESI 1. Mean Raman spectra of Stainless steel (SS316L), CaF_2, Stainless steel (µRIM™) and Quartz.

ESI 2. Navigation markers

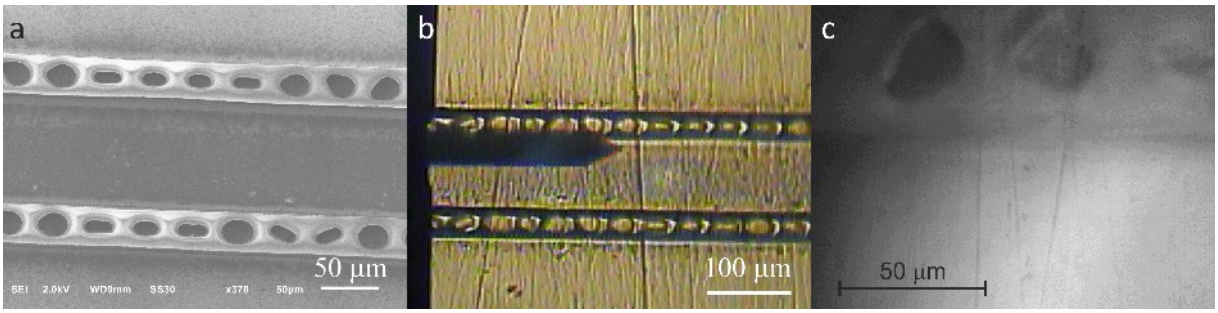

Figure ESI 2: Navigation markers patterned on the stainless steel substrates next to the sample region, using cyanoacrylate glue. a) SEM image of the sample region flanked by the navigation markers; navigation markers as seen through the sample stage optics of the AFM (b) and the Raman spectroscopy imaging setup (c). Knowing the position of a particular object of interest relative to a (unique) combination of navigation features allows easily retracing it in all instruments.

ESI 3. EV isolation protocol from prostate cancer cell lines (LNCaP)

The LNCaP Prostate cancer cell line purchased at the American Type Culture Collection (ATCC) was used to produce prostate tumor-derived EVs. LNCaP cells were cultured at 37 °C and 5% CO_2 in RPMI-1640 with L-glutamine medium (Lonza, cat.# BE12-702F) supplemented with 10% v/v fetal bovine serum, 10 units/mL penicillin, and 10 μg/mL streptomycin. The initial cell density was 10,000 cells/cm^2 as recommended by the ATCC. Medium was refreshed every second day. When cells reached 80−90% confluence, they were washed three times with PBS and FBS-free RPMI medium supplemented with 1 unit/mL penicillin and 1 μg/mL streptomycin was added to the cells. After 48 h of cell culture, the cell supernatant was collected and centrifuged at 1000g for 30 min. The pellet containing dead or apoptotic cells and the largest EVs was discarded. The supernatant was pooled, and aliquots of 50 μL were frozen in liquid nitrogen and stored at −80 °C. The size distribution of the harvested EVs was assessed with nanoparticle tracking analysis (NTA) (See ESI 4).

ESI 4. NTA measurements of LNCaP EVs

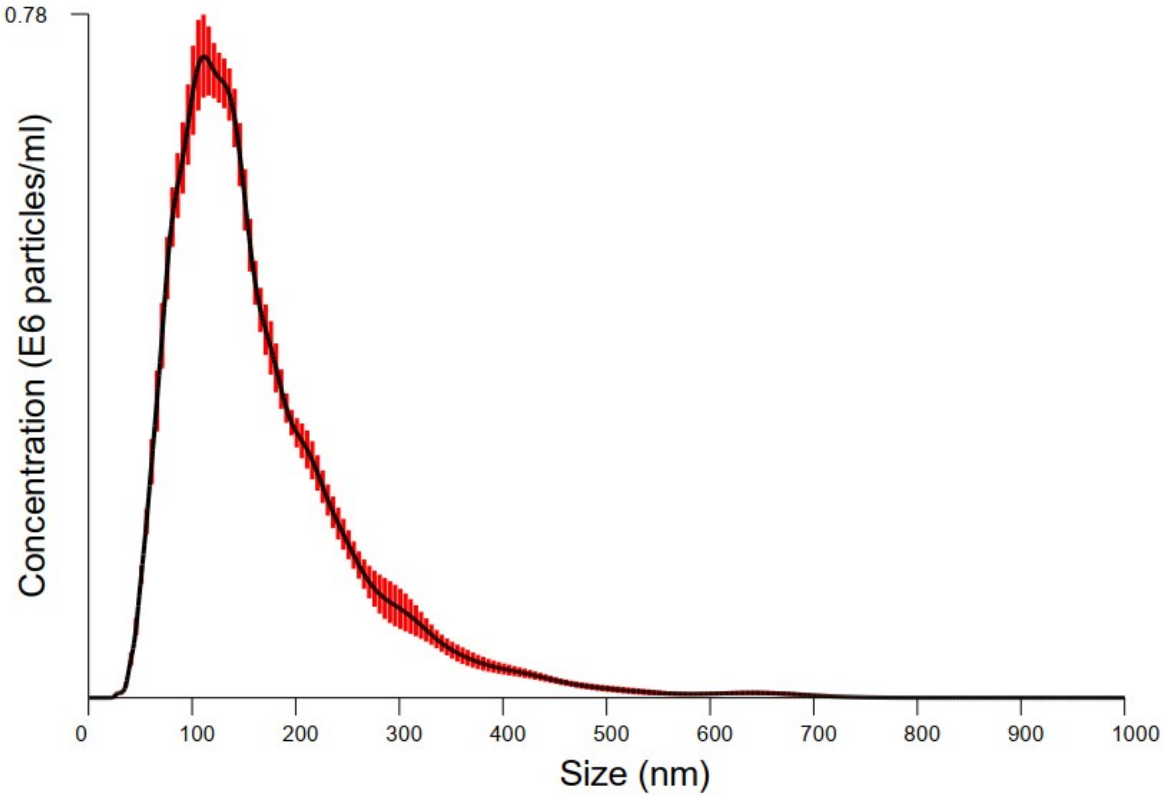

Figure ESI 4: NTA average concentration over 10 samples. Measured using a NanoSight 500 dark field microscope (Nanosight, Amesbury, UK). 5607 particles were measured with an average diameter of 167 ± 91 nm (diameter ± s.d.), corresponding to a total concentration (before 10x dilution) of $1.06 \times 10^{9} ml^{-1}$. The raw data from this measurement was used to obtain a histogram compatible with the AFM data from Figure 8.

ESI 5. IRRAS measurements of CDPA as monolayer on stainless steel substrates, and as a powder

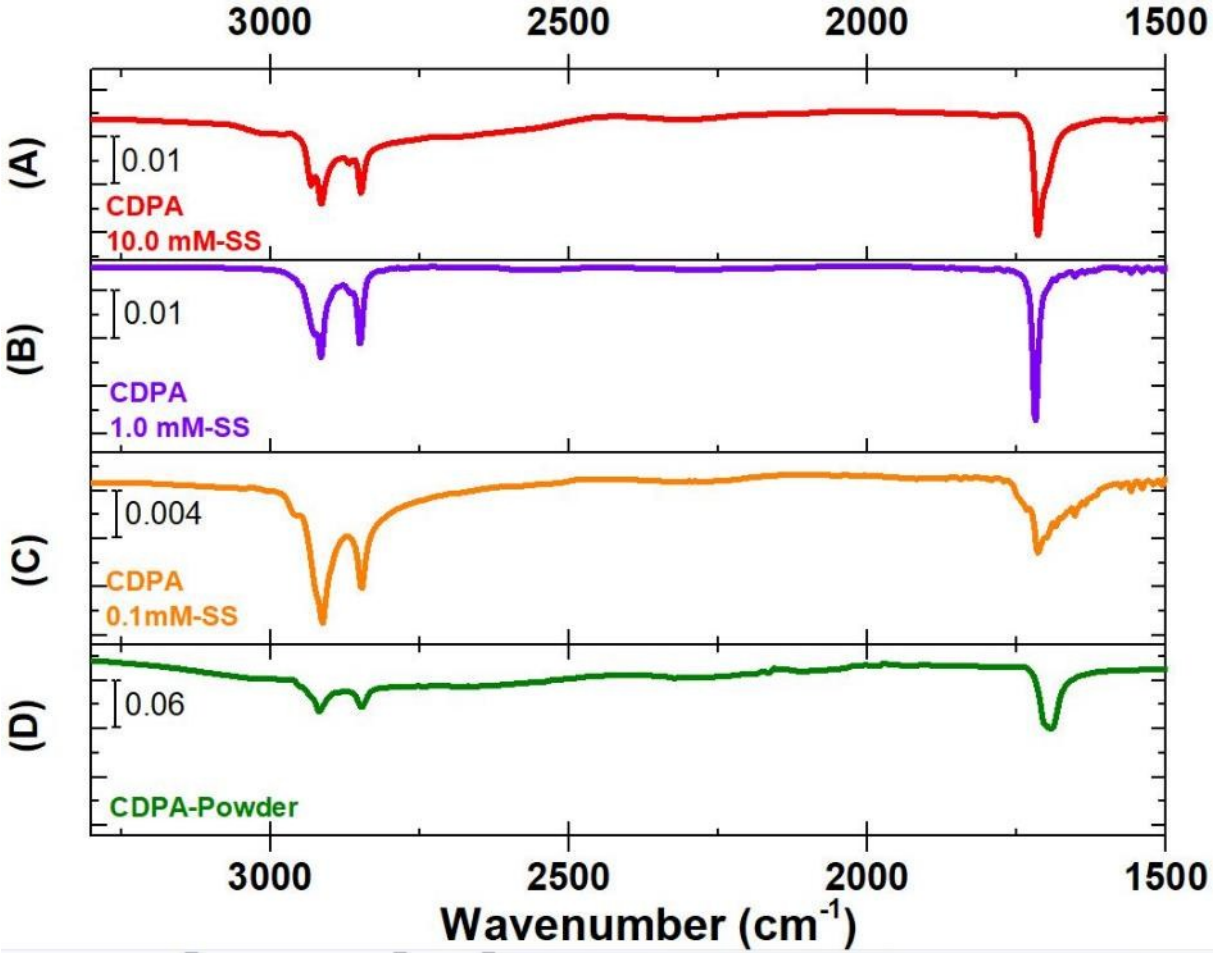

Figure ESI 5: IRRAS reflection spectra for CDPA monolayers prepared using a CDPA concentration of (A) 0.1 mM, (B) 1 mM, and (C) 10 mM, and using CDPA powder.

ESI 6. Influence of flow dynamics on capturing efficiency

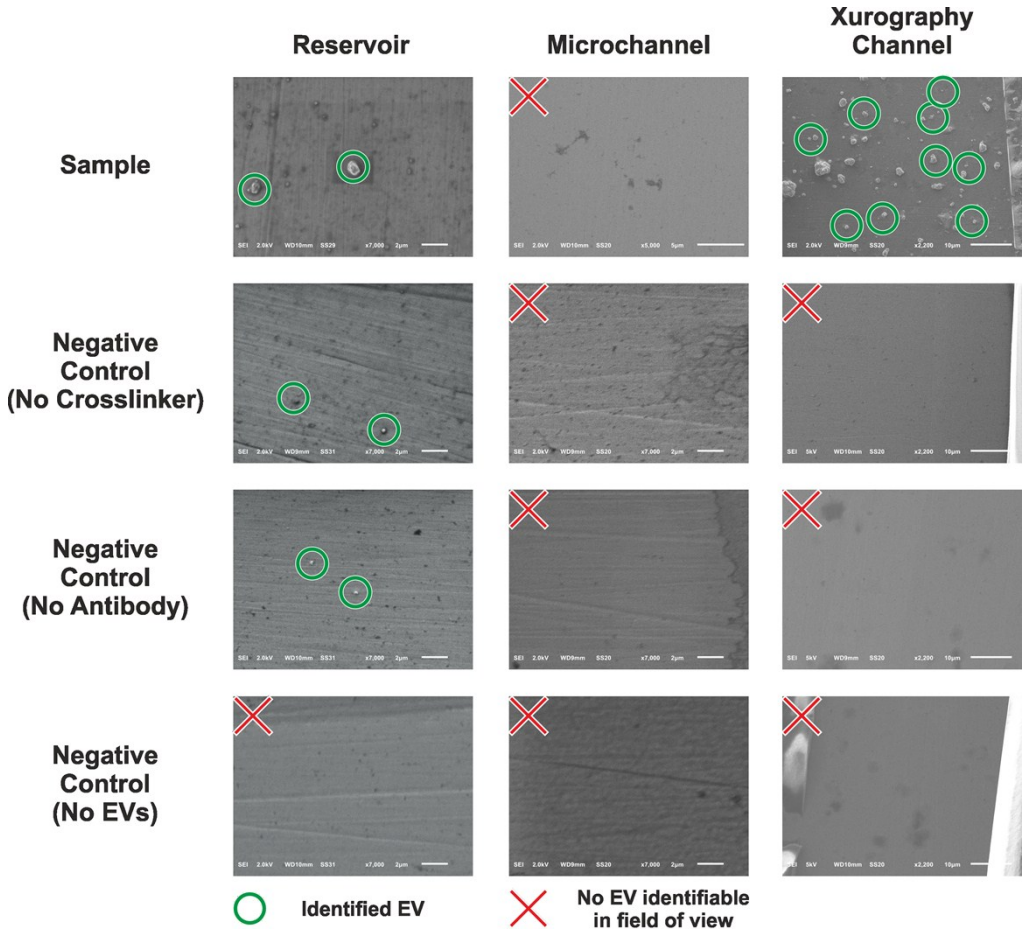

Figure ESI 6: SEM images; comparison of contrast between specific and non-specifically bound species in various fluidic systems. In a reservoir (left column, scale bar: 2 μm), where EVs are incubated and dehydration agents are exchanged under semi-static conditions, there is an apparent lack of a force to remove physisorbed EVs as can be seen from the negative controls. Using a microchannel of 20-μm height (middle column, scale bars: 5 μm (top) and 2 μm) entails imposing shear forces so high that specifically bound species are also removed when introducing dehydration agents. An intermediate option, use of exceptionally high (∽200μm) microchannels produced by xurography (right column, scale bars: 10 μm) yielded expected results, where EVs were only identified in the positive control.