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Weighing of Biomolecules, Single Cells, and Single Nanoparticles in Fluid

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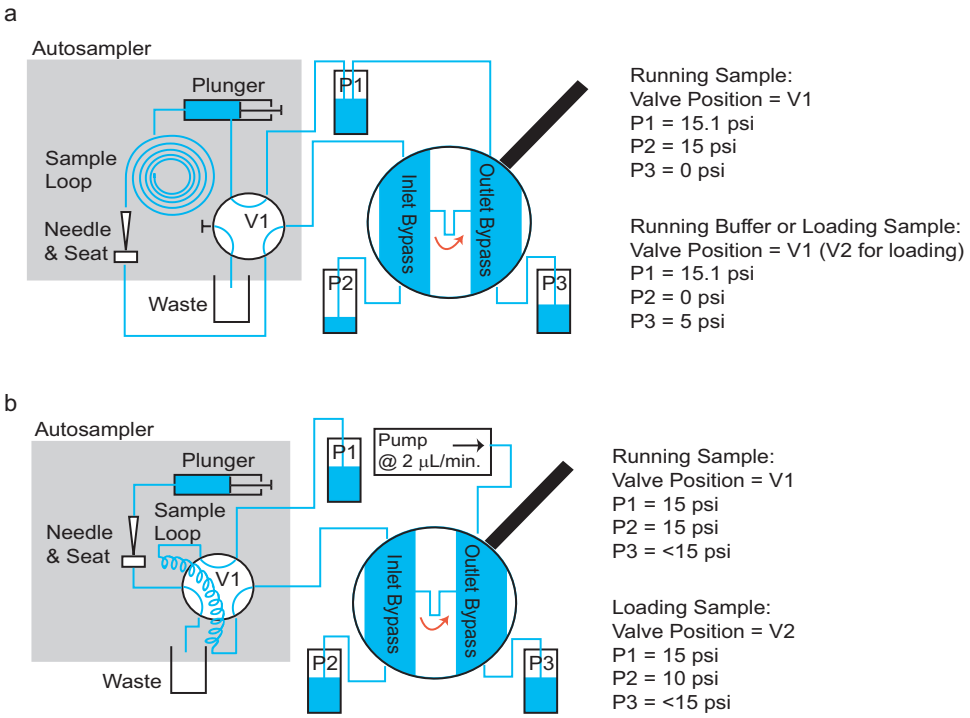
Supplementary Methods

Suspended microchannel resonator devices: Suspended microchannel resonators were fabricated on six inch silicon wafers at Innovative Micro Technology (Santa Barbara, CA). Analogous to the process described by Corman et al.,[1] wafer bonding of silicon to silicon and silicon to pyrex was used to create free-standing vacuum packaged silicon microchannels. An important difference between our devices and previously described micromachined fluid density sensors is a substantially smaller cross section, resulting in improved sensitivity towards total mass at the expense of sensitivity for bulk fluid density. Electrostatic excitation electrodes are placed on the inside of the vacuum cavity to minimize charge buildup, which could result in sensor drift. Devices are vacuum sealed at sub-millitorr pressure, and an on-chip getter provided by SAES (Italy) ensures stability of the low pressure micro-environment over extended time periods. Bypass channels for fluid delivery were etched 30 μm deep into pyrex wafers (Corning 7740), which were ultrasonically drilled (Bullen Ultrasonics Inc.) and anodically bonded to the silicon wafer. At this etch depth, good pattern transfer and minimal undercut was achieved with a chrome-gold mask for the wet etching of glass in 49% hydrofluoric acid. Fluidic interconnects to the chip are made by a Teflon manifold and perfluoroelastomer o-rings as described previously,[2] with the difference that the fluid path in the new devices only contacts silicon and pyrex, which are inert to most chemicals.



Supplementary Figure S1 | Fluidic setups for surface-binding (a) and flow-through (b) mass measurements.

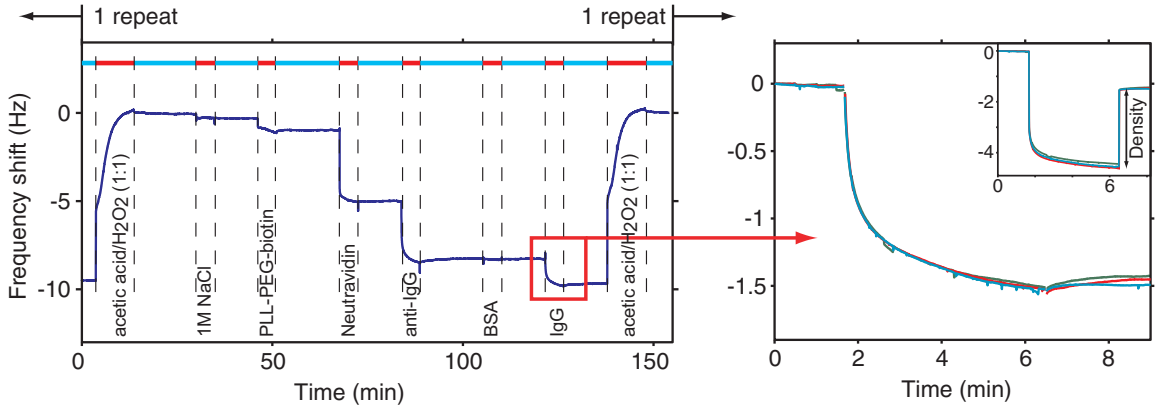
Experimental setup: Fluids are supplied to the SMR using an autosampler in conjunction with fluid vials maintained at different pressures to control the direction of flow; for details, see Figure S1. Surface binding experiments were performed using an Agilent 1100 capillary autosampler, while particle and bacteria flow-through experiments were performed using a Hitachi AS-4000. The primary difference between the two systems is that the flow-through experiments require a more precise control of the pressure differential between the inlet and outlet bypasses. During sample data acquisition for these experiments, the pressure of the outlet bypass (P3 in Figure S1) is maintained at just below the 15 psi of the inlet bypass, so that the transit time of particles traveling through the resonant portion of the SMR is long enough to be accurately measured. Pressures are controlled with precision manual regulators (Omega) and by a computer-addressable regulator (ProportionAir Inc.). The temperature of the sensor mount is controlled to 20 ºC or 25ºC (depending on the experiment) by a ThermoNESLAB RTE7 water circulator.

Data acquisition and analysis: Resonance frequency in the biomolecular detection experiments was measured using a digital frequency counter (Agilent 53131A) with a 300 ms gate time and a data rate of 2 Hz. The times at which the flow in the cantilever was switched were also recorded with the same time resolution. Instantaneous changes in resonance frequency caused by the density difference between samples and the running buffer could therefore be discerned from slower binding signals. The magnitude of this constant offset for each sample was derived from the step height at the end of the injection and then subtracted from the data during the injection interval. (c.f. inset in Figure S2)

Particle mass measurements require frequency to be measured at a rate of several hundred Hertz. To this end, the resonator signal (~200 kHz) is mixed down with a reference oscillator whose frequency is ~1 kHz below the mechanical resonance frequency of the SMR. The down-converted signal is then rectified and measured with the time-frequency counter of a National Instruments PCI-MIO-16 multifunctional DAQ card. With this scheme, the data rate is dictated by the frequency of the mixed-down signal. Noise is reduced off-line by processing the data with a Savitzky-Golay filter, which preserves the shape and magnitude of transient frequency changes induced by particles passing through the resonator.

Biomolecular detection experiments: Prior to surface binding experiments, the fluidic system up to and including the cantilever is cleaned with an equivolume mixture of acetic acid and 30% hydrogen peroxide by injecting the solution as if it were a sample, followed by a 15 minute rinse with running buffer (PBS pH 7.4, 0.01% sodium azide). The surface is then functionalized for analyte binding by successive injections of biotinylated poly(L-lysine)-grafted poly(ethylene glycol) (1 mg/mL),[3] Neutravidin (Pierce, #31000; 0.5 mg/mL), and biotinylated anti-goat IgG antibodies (Abcam, ab6740; 0.5 mg/mL) in running buffer. Between injections, the system is rinsed with running buffer for 5 minutes. In addition, all proteins (including subsequent samples) were supplemented with 0.1% BSA (Sigma, A3059) and 0.01% Tween 20 (Sigma, P1379). Prior to analyte injection, the acetic acid/hydrogen peroxide cleaning solution, followed

by 0.1% BSA in PBS are injected with P2 turned off (see Figure S1) in order to clean and passivate the fluidic system up to, but not including the cantilever. This cleaning step is important to ensure that the analyte is not depleted by binding in the fluid delivery system or inside the microfluidic bypass channels. The antigen and control samples are then injected as above. Goat anti-Mouse IgG (Abcam, ab6708) and Human IgG (Sigma, I4506) were diluted from the storage solution to 1 mg/mL in PBS, buffer exchanged to the running buffer plus 0.01% Tween 20 (PBST), and then serially diluted to low concentrations in PBST + 1 mg/mL BSA. The presence and concentration of Human IgG in the control sample was verified prior to serial dilution by measuring the UV absorbance at 280 nm.



Supplementary Figure S2 | Surface regeneration and experimental repeatability for surface-based biomolecular detection experiments.

Device regeneration and repeatability: Prior to all experiments, devices were cleaned with either a mixture of hydrogen peroxide and either sulfuric acid (1:3) or acetic acid (1:1). For surface binding experiments, the less aggressive solution of acetic acid/H_2O_2 was found to be effective at resetting the surface to a state that allowed repeatable binding experiments. Figure S2 shows the second out of three fully automated consecutive goat IgG binding experiments. At the beginning of each sequence, the surface was cleaned, followed by a test injection of one molar sodium chloride solution. Antibodies were then immobilized as described in the paper, followed by injections of 1 mg/mL BSA alone or 1 mg/mL BSA plus 20 μg/mL goat IgG in PBS + 0.01% Tween 20. The right plot in Figure S2 shows the binding signals of all three IgG injections

superimposed. The same data is also plotted in the inset without subtraction of the bulk density signal. The entire sequence was run overnight, and the baseline at the end of the experiment (six hours total duration) returned to within 450 mHz of the starting frequency after acetic acid/hydrogen peroxide clean.

Particle mass measurements: The microfluidic channel surface does not need to be functionalized for the flow-through experiments. Prior to an experiment, piranha solution (3:1 Sulfuric Acid:30% Hydrogen peroxide) was flowed through the device to ensure clean microfluidic channels free of particles from a previous experiment and to eliminate the possibility of bacterial growth and contamination. Measurement buffer was then flowed through the device for at least one hour to ensure a complete rinse. All colloidal and bacterial samples were injected at concentrations between $1 \times 10^7 - 5 \times 10^8$ ml^{-1}. The 1.51 μm polystyrene beads dispersed in water were NIST size standard (Bangs Laboratories NT16N) and were also used to calibrate the device for mass. The 100 nm Au particles (Corpuscular Inc. 790122-200) were also dispersed in water. *E. coli* (CGSC # 4401) and *B. subtilis* (ATCC #29056) were grown by incubation in Luria-Bertani (Miller) broth (Sigma L2542) at 37ºC for 20 hours and then thrice pelleted by centrifugation, rinsed and resuspended in PBS.

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Supplementary Discussion

Comparison with other methods: When comparing the suspended microchannel resonator (SMR) to other mass sensors, there are two important metrics: **i)** the minimum detectable mass per area which is useful for comparing relative concentration sensitivities for situations where there is unlimited target, and **ii)** the minimum detectable mass which is useful for assessing performance for weighing particles or for measuring concentration when the amount of target is limited. To our knowledge, the SMR is the only device that can weigh particles in a flow-through mode while all other approaches require surface attachment.

Supplementary Table S1 | Mass resolution comparison of SMR to other mass-based sensors

	Mass per area [ng/cm^2]	Total mass [fg]
Suspended microchannel resonator (SMR)	0.01	1
Quartz crystal microbalance (QCM)[1]	1	10^6
Surface plasmon resonance (SPR)[2]	0.05	10^3
Resonant microcantilever[3]	1000	10^6
MEMS acoustic resonator[4]	10	10^3

When comparing the SMR to sensors that do not provide a direct measure of mass (e.g. ELISA and microcantilever stress sensor), one must compare the concentration resolution for a particular assay. This metric depends both on the inherent mass sensitivity of the detector and the properties of the reagents that are used to capture the target. Such properties include the binding affinity constant, surface density of capture molecules, and their overall activity.

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