Precision mass measurements in solution reveal properties of single cells and bioparticles

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Abstract

Precise characterization of biological materials ranging from single cells (~1-20 microns) to extracellular vesicles (20-200 nm) is of fundamental interest because of their biological and translational value. Here we discuss the value of precision mass measurements in solution for informing various physical and biological parameters, such as mass accumulation rate, longitudinal cell growth or cell density. We introduce how the limits of the single-particle mass measurements can be pushed down to nano-scale dimensions enabling the resolution of extracellular vesicles and viruses in solution. We believe with future advancements on the precision and throughput of this approach, the capability of analyzing biologically relevant particles in solution will have broad biological and translational impact.

Introduction

Biologically relevant particles span more than four orders of magnitude in size, from a couple nanometers for proteins to tens of microns for single cells. It is challenging to precisely characterize the physical properties of this wide range of particles in solution. Flow cytometry performs well for fluorescently labeled cells, but important biophysical properties like mass, density, growth rate or deformability cannot be measured directly. Furthermore, flow cytometry techniques are limited for particles below one hundred nanometers [1]. Light scattering approaches like dynamic light scattering (DLS) can precisely measure average particle size of a rather homogenous population with nanometer precision, but cannot resolve heterogeneity within and across mixed populations. Approaches such as resistive pulse sensing [2] or impedance-based measurements can provide single particle information resolving heterogeneity, but they are not precise enough to resolve nanoparticles below ~70 nm and require high salinity medium, which hinders practical applications for biological analytes such as protein aggregates. Another single-particle approach, nanoparticle tracking analysis (NTA) is a powerful technique to characterize nanoparticles in solution [3]. By using optical microscopy to track the trajectory of particles within the frame of view, the Stokes- Einstein relation can be used to obtain the hydrodynamic radius assuming the temperature and viscosity of the fluid are known. The size resolution of NTA reported in the literature varies between 50 to 90 nm. In addition, NTA requires a concentration of 10^8 particles per mL, which is sufficient for most *in vitro* models but not for rare particles in clinical samples. Therefore, high-precision techniques to interrogate various fundamental properties of a wide range of biological particles in their native environments are required.

Suspended Microchannel Resonators (SMRs)

Micro- and nanomechanical resonators operating under vacuum conditions enable extremely precise mass measurements, enabling the resolution of individual analytes down to a single molecule. However, the performance of these devices dramatically degrades when operated in solution due to viscous loss. The suspended microchannel resonator (SMR) was developed to overcome this limitation [4]. The SMR is a mechanical resonator comprised of a microfluidic channel running through the length of a cantilever (Figure 1). The cantilever is kept oscillating at its resonant frequency by a feedback loop. As particles flow through the integrated channel, the resonant frequency of the cantilever is transiently modulated by the particle's buoyant mass. Monitoring the instantaneous oscillation frequency enables precise mass measurements in solution (Figure 2).

The limit of mass precision is dictated by the inherent mass sensitivity of the device and the frequency stability of its oscillation. The mass sensitivity of an SMR is determined by the ratio of its mass to the resonant frequency.

Here, we will discuss the recent advancements of the

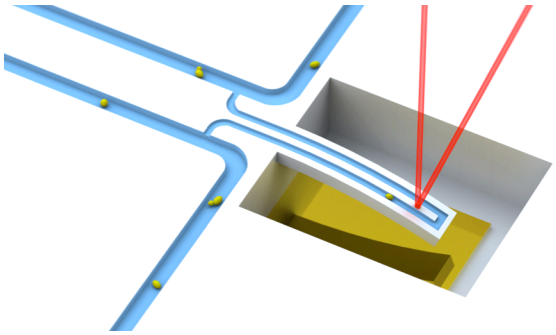

Figure 1: *A cartoon of a suspended microchannel resonator (SMR). A suspended cantilever with an integrated fluidic channel is oscillated at its resonant frequency in a vacuum cavity. The cantilever is actuated by electrostatic forces (or by a piezo-shaker integrated underneath the chip). The vibration of the cantilever is detected by an optical lever (or by a piezoresistor doped at the cantilever base). The fluidic channel buried in the cantilever is flanked by two larger by-pass channels to fetch and discard the analytes.*

SMR technology that enabled various precision measurements leading to quantification of single cell growth and density, and resolving nanoparticles down to 10 nm with single attogram precision. Finally, we will present a new technology for simultaneously monitoring multiple resonances that enables an array of SMRs to be oscillated simultaneously and independently. We will discuss how the same technology can potentially determine the distribution of mass within nanoparticles.

Weighing single cells in solution

The SMR technology opened up the possibility to weigh single cells, e.g. bacteria, yeast or mammalian cells, with femtogram precision in solution. A typical mammalian cell weighs on the order of 100 picograms, whereas *E. coli* cells weigh on the order of 100 femtograms in media. Godin *et al.* [5] exploited the precision mass measurement capability of SMRs to determine the growth rates of single cells as they grow in culture by weighing individual cells multiple times in the same sensor (Figure 3). The authors determined that

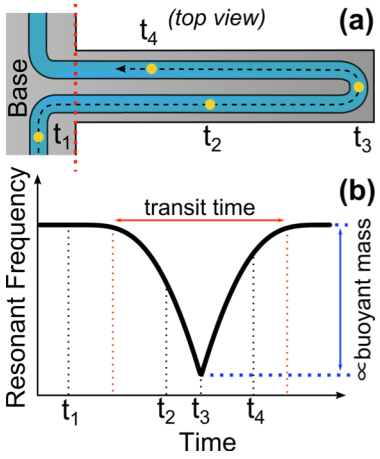

Figure 2: SMR Operation. ***(a)*** *Top view of a SMR with a particle indicated at four different positions in the integrated channel as it flows through the device.* ***(b)*** *Corresponding resonant frequency waveform caused by the flowing particle at the four positions. The signal amplitude is proportional to the buoyant mass of the particle.*

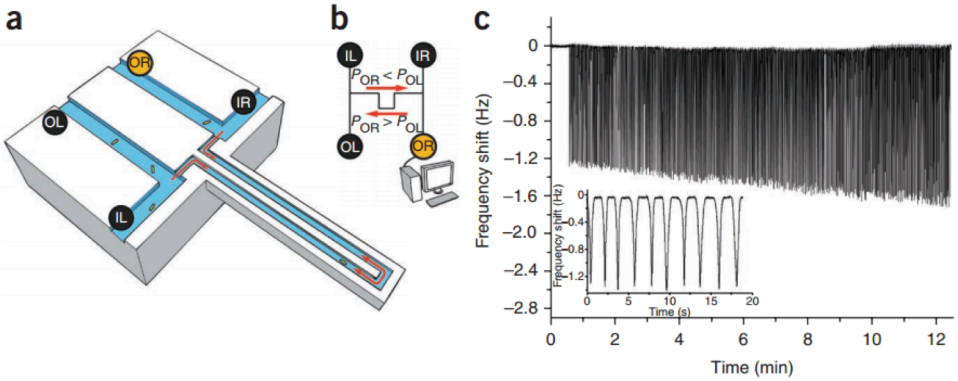

Figure 3: Dynamic trapping of single cells, from Godin, Delgado et al (2010). ***(a)*** *Illustration of the suspended microchannel resonator (SMR) trapping a single cell.* ***(b)*** *Schematic of fluidics: sample is injected in parallel through the left and right inlets (IL and IR) and collected at the left and right outlets (OL and OR).* ***(c)*** *Raw data showing 400 measurements of one B. subtilis cell's buoyant mass. The frequency shift increase with time indicates cellular growth. Inset shows a zoomed in version of a part of the data.*

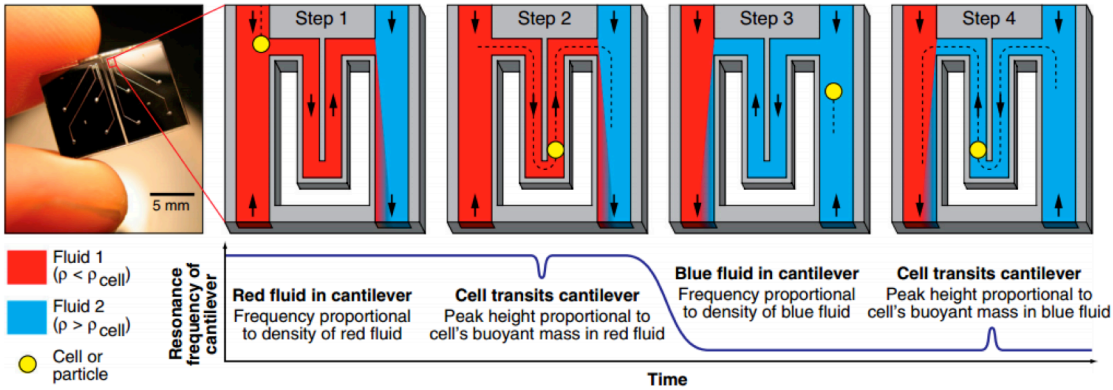

Figure 4: Using the SMR (Left) to measure the buoyant mass of a cell in two fluids of different densities, from Grover et al (2011). *Measurement starts with the cantilever filled with any buffer or media less dense than the cell (red, step 1). The density of the red fluid is determined from the baseline resonance frequency of the cantilever. When a cell passes through the cantilever (step 2), the buoyant mass of the cell in the red fluid is calculated from the height of the peak in the resonance frequency. The direction of the fluid flow is then reversed, and the resonance frequency of the cantilever drops as the cantilever fills with a fluid more dense than the cell (blue, step 3). The buoyant mass of the cell in the blue fluid is measured as the cell transits the cantilever a second time (step 4). From these four measurements of fluid density and cell buoyant mass, the absolute mass, volume, and density of the cell are calculated.*

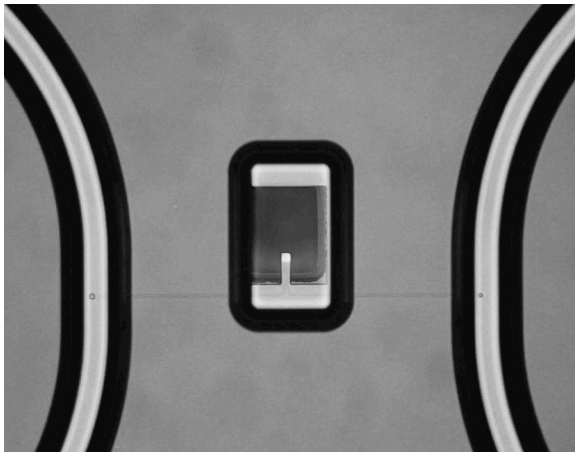

***Figure 5: A micrograph of the fabricated SNR**. The cantilever, which is seen in the vacuum cavity, is 27 microns in length. Integrated channel running the length of the cantilever is 400 nm by 1 micron in cross section. Larger microfluidic channels at the sides are the by-pass channels for delivering and discarding the sample.*

heavier cells grow faster than lighter cells. Later, Son *et al.* [6] developed a microfluidic protocol to trap a single cell and monitor cell cycle progression over multiple generations (over 100 h). This technique provided insight on how growth rate is regulated during the cell cycle.

Longitudinal measurements of single cell mass enable fundamental studies on cell biology. In addition to mass, cells change their mass-to-volume ratio during important processes such as cell cycle progression, apoptosis or differentiation. Just like Archimedes did for the golden crown, Grover *et al.* measured single cell density by using buoyant mass measurements in two different fluids with different densities (Figure 4) [7]. Using the extreme density measurement precision of 1 mg/mL, they found that intrinsic cell-to-cell variation in density is nearly 100-fold smaller than the mass or volume variation. Therefore measuring cell density at high precision can indicate cellular processes that would be otherwise undetectable by mass or volume measurements.

Weighing nanoparticles in solution

The mass precision of the SMR is better than 0.1% for weighing mammalian cells. However, the buoyant mass of an HIV virus or an extracellular vesicle (e.g. exosome) is below 100 attograms. Therefore, we developed an extremely precise resonant mass sensor by fabricating smaller (lighter) cantilevers with higher resonant frequencies (Figure 5). The suspended nanochannel resonators (SNRs) are 1000-fold lighter and have up to 10-fold higher resonant frequencies (1-4MHz) compared to a SMR. SNRs can weigh nanoparticles in solution with a single attogram precision [8]. To quantify the potential for further improvement in the precision, we calculated the ultimate limit of frequency stability imposed by intrinsic thermomechanical fluctuations

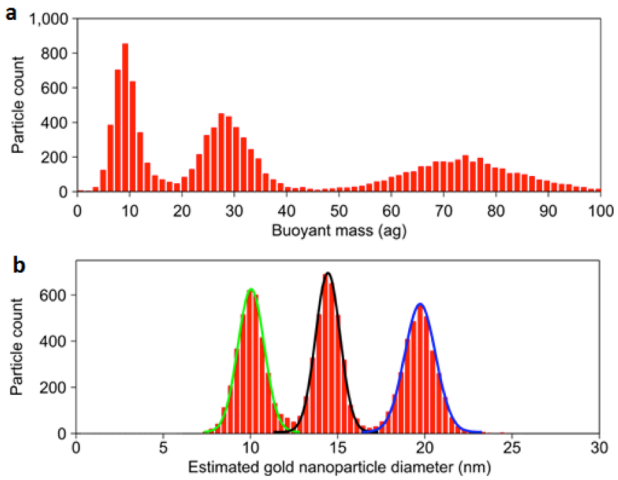

***Figure 6, from Olcum, Cermak et al. (2014): (a)** Buoyant mass histogram of 10,600 particles detected during an experiment. **(b)**, Diameter histogram, calculated assuming particles are spheres of density 19.3 g/cm^3. Colored lines show fits to Gaussians.*

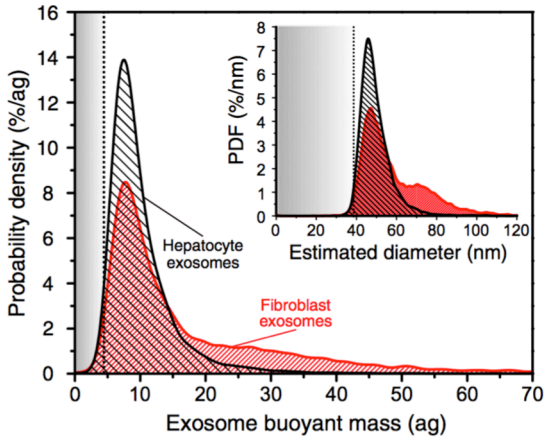

***Figure 7, from Olcum, Cermak et al. (2014):** Buoyant mass distributions of fibroblast-derived (red) and hepatocyte-derived (black) exosomal vesicles. Some 7,100 fibroblast exosomes and 9,600 hepatocyte exosomes are weighed using an SNR in 65-min and 76-min experiments, respectively. The limit of detection is depicted with a vertical dotted line close to 5 ag. (Inset) Estimation of exosome diameter by assuming a spherical shape and a constant, uniform exosome density of 1.16 g/mL throughout the populations. The vertical dashed line indicates the corresponding limit of mass detection as 39 nm.*

of the resonator. We determined that the frequency stability of the SNRs at the operation bandwidth (1 kHz) is only 1.8- to 3-fold above the thermomechanical noise limits. We demonstrated the use of SNRs by measuring the buoyant mass distribution of a mixture of gold nanoparticles (10 nm, 15 nm and 20 nm) in water (Figure 6). Furthermore, we compared the mass distributions of exosomes produced by different cell types and showed that their distributions show differences in terms of their heterogeneity (Figure 7).

In addition to mass, measuring the distribution of mass within a virus in solution could enable distinguishing it in blood serum or could even inform about its efficacy - a challenging prospect using existing biochemical assays. In

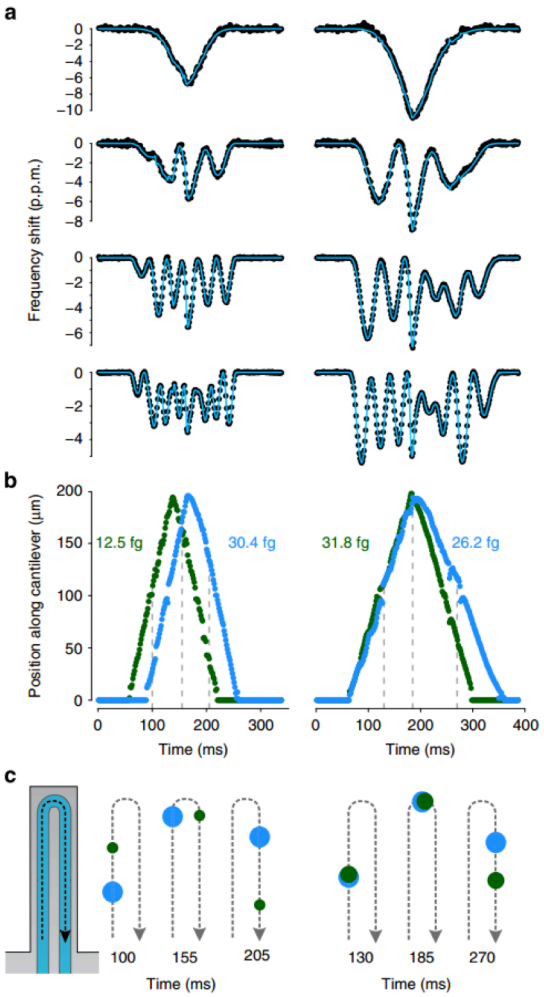

Figure 8 from Olcum, Cermak et al. (2015): *(a) Frequency signals from the first four bending modes (top), with multiple nanoparticles passing through the SNR in close proximity. (b) Inferred positions and masses of nanoparticles in the SNR as a function of time. (c) An illustration of the measured particle positions as they flow through the SNR (not to scale).*

contrast to optical approaches that are limited by wavelength-dependent diffraction, interactions of a particle with the SNR are not diffraction limited. SNRs currently weigh nanoparticles under the assumption that each particle is a point mass. Since each point mass at a specific location on the resonator interacts differently with different eigenmodes, the particle morphology as a superposition of point masses can be reconstructed. Since flowing nanoparticles constantly move, extremely fast measurement techniques are required to precisely analyze them.

Recently, we developed a general platform for independently and simultaneously oscillating multiple modes of mechanical resonators, enabling frequency measurements that can precisely track fast modulation signals [9]. In order to oscillate multiple resonances independently, we dedicated a phase-locked loop (PLL) for each individual resonance. For the highest precision, we required a resonator-PLL closed-loop system for each mode follows the resonant frequency changes as close as possible.

We used this technique to resolve multiple nanoparticles flowing simultaneously through a suspended nanochannel resonator and showed that four resonant modes are sufficient for determining their individual position and mass (Figure 8) with an accuracy near 150 nm and 40 attograms throughout their 150-ms transit. Presented examples demonstrate the ability to resolve multiple masses at close proximity in solution, which suggest the possibility of observing dynamic mass distributions or shapes of single nanoparticles in the future.

The same platform to control multiple resonant frequencies can be extended to simultaneously track multiple resonators instead of multiple modes enabling high throughput measurements with an array of mass sensors. Controlling an array of SMRs or SNRs each operating independently with the highest precision would enhance the sample volume that can be analyzed by these high precision mass sensors.

Conclusions

We believe the precision of the SMR technology brings unique advantages to investigate the properties and variations of biological elements at the single particle level, which creates new paradigms to the way of studying and solving biological and clinical problems.

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