Microcantilevers track single-cell mass

Rashid Bashir

An array of microfluidic cantilevers measures the mass of single cells in a population over time and detects drug-induced changes in cell growth.

For millennia, determining the mass of objects by weighing them has been an everyday practice that informs all manner of decisions. In modern chemistry and biology, mass spectrometers are used to ionize the contents of complex samples and sort the ions based on their mass to charge ratio in the gas phase, providing a mass spectrum of the ions in the sample. Similarly, gel electrophoresis separates molecules as they move through a sieving matrix, determined largely by their mass when the charge to mass ratio of all molecules is constant. However, direct measurement of the mass of sub-microgram objects, especially in fluid, is still a technological grand challenge. Microscale cantilever sensors operated in resonance mode may address this need (R.B. and colleagues[1]). In two new studies[2,3] published in *Nature Biotechnology,* the Manalis group at MIT presents important advances in this technology that position it for real-world applications. The approach could introduce mass in the biological sciences as a sensitive measure of cell growth and, more specifically, of protein or DNA synthesis.

Micro- and nanoscale cantilevers are mechanical sensors that utilize either static bending or dynamic vibrations as a means to perform biochemical sensing. Biomolecular interactions at the surface of a cantilever can result in changes in surface energy that lead to static bending of thin cantilever beams. The amount of bending can correlate to the number of molecules captured on the cantilever. Similarly, measurement of the resonant frequency of vibration of the cantilever can be used to determine the mass of the cantilever and also of micro- and nanosized objects attached to it. Because the resonant frequency of a cantilever is a function of its mass, the smaller the mass of the cantilever, the more sensitive the cantilever is to minute changes in added mass.

In a vacuum, where viscous damping is minimal, the quality factor, defined as the ratio of the amplitude of resonance to the bandwidth of the resonance, is very high, and detection of attograms has been been demonstrated. However, in fluid, viscous damping dominates, decreasing the sensitivity and increasing the minimal detectable mass to picogram resolution (R.B. and colleagues[4]). How can we then increase the measurement sensitivity—the ability to detect even small masses—in fluid using this mechanical sensing method?

This conundrum was addressed over nine years ago by a pioneering approach from the Manalis group[5]. They fabricated a U-shaped, vibrating, hollow cantilever on a chip—no bigger than the diameter of a human hair—and flowed fluid containing micro and nano-sized particles through the tube. Placement in a vacuum made the quality factor of the vibration very high, providing a very sensitive method for measuring the mass of the tube and of the particles suspended in the fluid. They applied the device to measure the mass of nanoparticles, viruses, bacteria, and cells suspended in fluid[6].

In their new studies[2,3], the group describes advances in the fabrication and application of microfluidic mass sensors. Cermak *et al.*[2] construct arrays of hollow cantilevers and measure buoyant mass accumulation of growing cells. By adding delay elements in between the cantilevers, they can track the growth rate of individual cells for up to a few minutes. The array measures over 60 cells per hour, with a mass resolution of 0.2 pg per hour for mammalian cells and 0.02 pg per hour for bacteria. Previous microfluidic mass sensors could only obtain mass accumulation rates for whole cell populations or measure

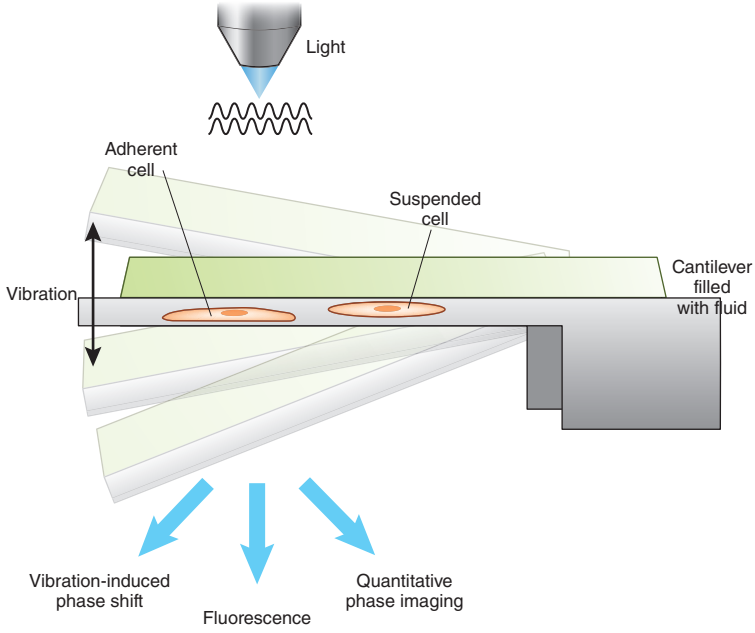

Figure 1 Schematic of the hollow microcantilever sensor and possible combinations with optical microscopy approaches.

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the mass accumulation rates of single cells one at a time using one sensor by flowing the cell back and forth[5]. The latter approach had very low throughput, whereas the current innovative array format is capable of measuring many single cells in a population repeatedly over time in a high-throughput manner.

Cermak *et al.*[2] use the device to study single lymphocytic cells, mouse and human T cells, primary human leukemia cells, yeast, *Escherichia coli* and *Enterococcus fecalis*. Interestingly, they detect differences in mass accumulation rate after a few hours of *in vitro* drug exposure—an effect that requires multiple cell divisions over days in standard cell culture apparatus or 10–20 h of exposure in a mouse model. Such rapid assessment of susceptibility to antibiotics and antimicrobial peptides could be very important for the emerging field of functional chemosensitivity testing at the single-cell level.

The second study, by Stevens *et al.*[3], further investigates the challenge of chemosensitivity testing. It demonstrates that the drug sensitivity of single glioblastoma cells and B cells can be predicted by weighing each cell repeatedly over a 15-min period in the resonant microchannel sensor in both the presence and absence of cancer therapeutics. The results show that knowledge of minute changes in mass, which cannot be acquired through standard optical measurements of cell growth and division, can provide rapid assessment of the effect of chemicals or drugs on single mammalian or bacterial cells and may be very important for identifying (and in the future separating) chemosensitive cells from a heterogeneous cell population.

It should be noted that quantitative phase imaging[7], and spatial light interference microscopy (SLIM) (R.B. and colleagues[8]) can also measure the 'dry mass' of cells in fluid—that is, the mass of any entity with a dielectric constant different than water. A quantitative comparison of different approaches for mass sensing showed that the suspended microresonator technology is still the most sensitive method to detect the buoyant mass of living cells in fluid at 0.05% of cell buoyant mass, which corresponds to ~25 fg for typical lymphocytes of 50–100 pg of buoyant mass (R.B. and colleagues[9]).

One can imagine many opportunities to enhance the power of this elegant approach (**Fig. 1**). If the materials used for the hollow channel were optically transparent, cell-cycle reporters or other fluorescence markers could be introduced and directly correlated to mass accumulation measurements. Also, although the technique has been demonstrated to date with cells suspended in solution, the majority of cells of interest in the body are adherent as they grow and divide. Dielectrophoretic, magnetic, or optical traps could be used to position cells at specific locations in the cantilever to measure adherent cells with very high accuracy.

Another key goal of biophysical cell analysis is the ability to measure a cell's mechanical properties and elastic modulus, as a decrease in mechanical stiffness has been correlated to the metastatic potential of cancer cells. While the hollow microcantilever sensors have been used to determine the mechanical stiffness of cells[10], integration of optical and mechanical approaches might provide for new ways to probe cellular biophysical properties. In a recent report from our laboratory, optical measurement of the oscillation in a cell's height on a vertically vibrating reflective substrate, as measured by a laser doppler vibrometer, revealed apparent changes in the phase of the optical signal through the cell[11].This apparent optical phase shift and the cell height oscillation were affected by the cell's mechanical properties. As this example begins to suggest, the ability to combine mass and mechanical measurements with simultaneous optical measurements could lead to unprecedented insights into the biophysical properties of cells.

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.

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Defining cell type with chromatin profiling

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Enhancer cytometry outperforms gene expression profiling in identifying hematopoietic cell subtypes.

Most genes are expressed in many or all cell types, and relatively few are transcribed in a truly cell-type-specific manner. Although gene expression analysis can identify different cell types, it may not be able to distinguish closely related cell subtypes that have largely overlapping patterns of transcription. Writing in *Nature Genetics*, Corces *et al.*[1] have now shown that chromatin profiling is a better way to identify similar cell subtypes among primary human blood cells (**Fig. 1a**). Applying the method to investigate leukemia, they find that cancer samples contain both healthy cell chromatin profiles and cancer-specific features.

Chromatin at promoters and enhancers of actively transcribed genes is open and hyperaccessible. Genome-wide studies over the past decade have shown that the patterns of chromatin accessibility and histone modifications in the genome are highly cell-type-specific, paving the way for using chromatin state information to identify cell types[2,3]. However, until very recently, a major limitation has been the large numbers of cells (typically, millions) required for the analysis, limiting it to cell lines and primary cells that can be isolated in large quantities. The latest methodological advances have made it possible to profile the chromatin state using as few as 5,000 cells[4,5] and, at a more limited coverage, even single cells[6,7].

In previous work by some of the authors of the present study, highly accessible regions were profiled by a method called ATAC-seq, in which the Tn5 transposase inserts oligonucleotide primers into highly accessible chromatin regions in cell nuclei[4]. The primers are then used to PCR amplify a library of regulatory elements that can be sequenced to create a profile of accessible regions with regulatory potential (**Fig. 1b–d**).

In the new work, Corces *et al.*[1] introduced Fast-ATAC, an optimized version of ATAC-seq for rapid analysis of small numbers of blood cells. Using this method, they found that, like gene expression profiles, chromatin accessibility at gene promoters has limited power to distinguish different cell types. Strikingly, however, chromatin accessibility of distal regions, including enhancers, which can be hundreds of kilobases or megabases away from their target

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