BIOPHYSICS

# Rapid mass changes measured in cells

**An ultrasensitive balance has been developed to weigh single or multiple cells, at high time and mass resolution — revealing fast and subtle mass fluctuations during the cell cycle and viral infection.** SEE LETTER P.500

DAVID ALSTEENS & YVES F. DUFRÊNE

A long-standing challenge in cell biology is to measure the physical properties of living cells as they grow or interact with viruses and drugs. Such knowledge is crucial to our understanding of fundamental processes, such as those involved in cellular shape changes, differentiation and disease. More specifically, how cells regulate their volume and mass during the cell cycle is an important but poorly understood problem. On page 500, Martínez-Martín *et al.*[1] describe a balance that addresses these challenges by enabling tiny changes of cell mass to be measured in just milliseconds.

Instruments known as flow cytometers and Coulter devices have conventionally been used to analyse the volume and size of cells suspended in fluids. Flow cytometers typically take measurements by analysing how cells scatter a laser beam, whereas Coulter devices detect changes in the electrical conductivity of fluids as cells pass through them. But although these methods are useful for studying cell populations, they are not suited to probing single cells at high precision, or to tracking variations in properties over time.

In the past few years, there has been progress in the development of mechanical resonators for measuring the masses of single cells[2]. Nanomechanical resonators are devices that incorporate a tiny vibrating cantilever. If a cell is attached to the cantilever, or passed through a fluid-filled micrometre-scale channel within it, then the frequency of the vibration shifts in a way that depends on the mass and position of the cell[3].

Microchannel resonators have been used to weigh single, suspended cancer cells repeatedly over time in the presence of cancer therapeutics[4]. This method accurately determined the drug sensitivity and resistance of glioblastoma and primary leukaemia cells. Arrays of microchannel sensors have been used to precisely and rapidly measure the growth rates of many suspended individual cells simultaneously[5]. The mass and growth rates of single colorectal-carcinoma cells attached to nanomechanical resonators have also been determined[6]. But although these assays are powerful, they cannot analyse individual adherent cells (those that attach to surfaces in biological settings) in physiologically relevant conditions at the mass and time resolution needed to track fast cellular dynamics.

Martínez-Martín *et al.* now report an optically excited microresonator that can weigh single or multiple attached cells in physiological conditions, at millisecond time resolution and picogram mass sensitivity (1 pg is $10^{-12}$ g). When a single cell is attached at the end of the device's cantilever, the vibrational frequency of the cantilever shifts, as in previously reported devices. But the authors also irradiate the cantilever near its fixed end with a low-power blue laser (Fig. 1), the use of which is compatible with living mammalian cells. The intensity of the laser is varied to generate extremely small cantilever oscillations. An infrared laser is simultaneously focused on the free end of the cantilever, to read out the amplitude and phase signal of the cantilever movement. The mass of the cell is deduced from the vibrational frequency measured before and after cell attachment, and by taking into account the precise location of the added cell mass on the cantilever.

Using the balance, Martínez-Martín and colleagues showed that the mass of living mammalian cells fluctuates by a few per cent over timescales of seconds throughout the cell cycle. Cells that had been fixed — chemically preserved in a way that stops biochemical processes from occurring — did not show mass fluctuations, indicating that these are a feature intrinsic to living cells. The authors linked the fluctuations to basic cellular processes such as ATP synthesis and glycolysis (both of which are involved in cellular energy production), and to water transport across the cell membrane.

The researchers went on to show that cells infected by vaccinia viruses stop growing, but continue to fluctuate in mass until cell death. The authors also developed a method for transmitting viruses from one cell to another by making mechanical contact between cells, thus providing new avenues for studying the mechanisms of virus transfer.

Collectively, Martínez-Martín and co-workers' experiments suggest that fast and subtle mass fluctuations are widespread in living cells and occur throughout the cell cycle. This work represents a major step forward in the development of ultrasensitive tools for monitoring the mass of living cells, and might find applications in many fields, including cell physiology, drug discovery and studies of stem-cell differentiation.

A crucial challenge in the next few years will be to make the technology more user friendly, so that it becomes accessible to a broad range of life-sciences researchers. Parallelization of the method will also be required to investigate multiple cells simultaneously, and to enable high-throughput measurements for basic research and for drug-screening applications.

There is a need for innovative tools to

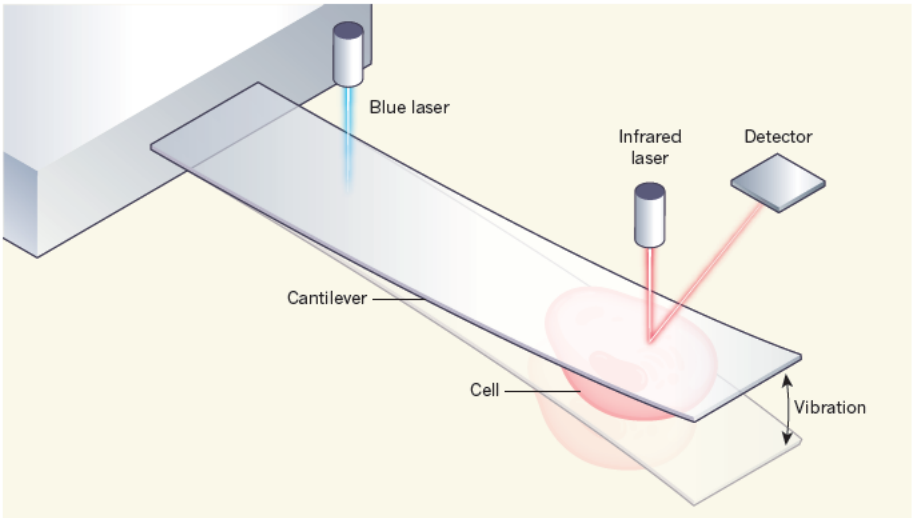


**Figure 1 | An ultrasensitive balance to weigh living cells.** Martínez-Martín *et al.*[1] report a balance based on a micrometre-scale cantilever. The cantilever is vibrated by irradiating it with a blue laser beam, and the vibrations are tracked by detecting an infrared laser beam reflected from the cantilever's free end. The frequency of the vibration depends on the mass and location of a cell attached to the cantilever. By monitoring the vibrations, changes in cell mass can be monitored with millisecond time resolution and picogram mass resolution (1 pg is $10^{-12}$ g).

rapidly identify and fight microbial pathogens, because many of these have become resistant to antibiotics. Fluid-filled cantilevers have previously been used[3] to weigh single living bacterial cells with sub-femtogram mass resolution (1 fg is $10^{-15}$ g), suggesting that this method could be used for detecting very low concentrations of pathogens. Moreover, cantilever technologies have been used to study the interaction of bacterial cell walls with the clinically valuable antibiotics vancomycin and oritavancin[7], providing insights into how these drugs work. Cantilevers have also allowed the sensitivity and resistance of bacteria to antibiotics to be measured in minutes, which is much faster than conventional culture methods[8]. Martínez-Martín and colleagues' cell balance therefore holds great promise for studying the mechanisms of action of antibiotics, and for identifying the most efficient antimicrobial therapies. ■

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*In Retrospect*

# Twenty years of drying droplets

**When a particle-laden droplet evaporates on a solid surface, the particles form a ring-like deposit. The explanation for this phenomenon, provided in 1997, has led to advances in many areas of science and engineering.**

RONALD G. LARSON

Why does a drying droplet, such as a spilt drop of coffee, leave a circular ring as its residue, rather than a uniform spot? Twenty years ago this month, Deegan *et al.*[1] published a paper in *Nature* that explained this commonly observed phenomenon, which is known as the coffee-ring effect. Their results, and the broad interest in and applications of the effect, have spawned thousands of publications, and advances including potential improvements to ink-jet printing[2], methods of patterning surfaces[3] and techniques for disease detection[4].

Deegan and colleagues' explanation for the coffee-ring effect is surprisingly simple (Fig. 1). When a particle-laden droplet dries on a solid surface, the rate of evaporation is typically highest near the droplet's outer rim, known as the contact line. This line is usually pinned to the surface because of microscopic surface roughness, meaning that it must draw liquid from the droplet's interior to replace what is lost to evaporation. The particles in the droplet are thereby dragged to the contact line, where they form a ring that helps to maintain line pinning.

The authors' intriguing explanation, and their analysis of the associated deposition physics, have led to an outpouring of theoretical and experimental work, much of it extending well beyond the coffee-ring effect. For instance, drying conditions have been discovered that lead to deposits at the droplet centre rather than, or in addition to, the ring at the edge[5]. Conditions have also been found for deposition patterns that resemble the spokes of a wheel[6], snowflakes[7], webs[8], and cones that grow higher than the initial height of the droplet[9].

As observed and explained two years before the authors' study, concentric-ring deposits, rather than single rings, can be produced by stick–slip motion of the contact line[10], whereby the edge of the droplet and the solid surface alternate between sticking to each other and sliding over each other. In other cases, deposition initially occurs at the top of the droplet, along the liquid–air interface. As the droplet dries, the deposit is then brought to rest on the surface, giving rise to other types of distinctive pattern. Finally, when particles make up a large fraction (more than 10%) of the droplet's volume, deposits can resemble structures ranging from volcanoes[11] to arches[12], depending on the drying conditions. It is amazing that such a huge range of patterns can be generated by simply spotting a droplet onto a surface and letting it dry.

Following Deegan and colleagues' work, it was shown that deposition can be steered by more than just evaporation and contact-line pinning. For example, it can be redirected by a type of mass transfer called Marangoni secondary flow[5], which is responsible for producing 'tears of wine' — the ring of liquid that forms near the top of a wine glass, from which droplets constantly form and trickle back into the wine. Depending on the type of material suspended in the droplet, deposition can also be influenced by crystallization dynamics, and the jamming of particles or buckling of

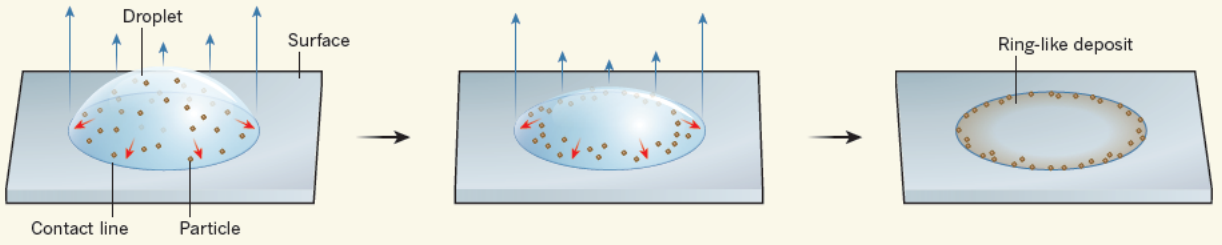


**Figure 1 | The coffee-ring effect.** In 1997, Deegan *et al.*[1] reported an explanation for the coffee-ring effect — the observation that a particle-laden droplet, such as a spilt drop of coffee, leaves a ring-like deposit when it dries on a solid surface, rather than a uniform spot. The rate at which such a droplet evaporates (blue arrows) is typically highest near its outer rim, known as the contact line. This line is usually pinned to the surface as a result of microscopic surface roughness, and so it must draw liquid from the droplet's interior (red arrows) to replace what it loses to evaporation. The particles in the droplet are thereby dragged to the contact line and, when the droplet has fully evaporated, they form a ring-like deposit. The authors' explanation for this effect has led to improvements in, for example, ink-jet printing[2] and the patterning of surfaces[3].