MEASURING SINGLE-CELL DENSITY

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ABSTRACT

Using a microfluidic mass sensor, we have measured the density of single cells. We find that cell density is the most tightly regulated aspect of cell size: the cell-to-cell variation in density is almost 1000 times smaller than the mass or volume variation. As a result, we can measure changes in cell density that are undetectable in cell mass or volume. We demonstrate this with four examples: distinguishing malaria-infected erythrocytes from healthy cells, discriminating transfused erythrocytes from an individual’s own cells, identifying irreversibly-sickled cells from a patient with sickle-cell anemia, and identifying leukemia cells in the early stages of drug-induced apoptosis.

KEYWORDS: Suspended Microchannel Resonator, Cell Density, Cell Mass, Cell Volume

The density of a cell—its mass to volume ratio—changes during important processes like cell growth, differentiation, apoptosis, and the onset of disease. But while tools like density gradient centrifugation have been used to separate cells by their density and estimate the average density of a population of cells, there has been no way to measure the density of large numbers of individual cells with meaningful accuracy.

According to legend, around 250 BC Archimedes of Syracuse measured the density (and purity) of a gold crown by weighing it twice, in two fluids of different densities. From these two measurements of buoyant mass, Archimedes could calculate the absolute mass, volume, and density of the crown (Figure 1). We have implemented Archimedes’ technique in a microfluidic device to measure the density of single cells. The “scale” we use to weigh single cells is the Suspended Microchannel Resonator (SMR) [1]. The SMR consists of a silicon beam containing an embedded microfluidic channel (Figure 2); the cantilever vibrates at a frequency proportional to the mass of the cantilever. When a cell passes through the cantilever, the resonance frequency changes momentarily by an amount proportional to the buoyant mass of the cell. The SMR has previously been used to measure the average density of a population of cells [2,3], but those approaches cannot quantify the density of single cells or the distribution of densities in a population.

To measure single-cell density in the SMR, we load the device with two fluids of different densities, the first containing the cells of interest in any media or buffer (the red fluid in Figure 2), and the second identical to the first fluid but more dense (the blue fluid in Figure 2). The SMR is initially filled with red fluid, and the resonance frequency of the cantilever is used to calculate the density of the red fluid (Figure 2, step 1). When a cell passes through the cantilever, the height of the peak in the resonance frequency of the cantilever is used to calculate the buoyant mass of the cell in red fluid (Figure 2, step 2). The cell then enters the blue fluid, where fast flow quickly dilutes the red fluid surrounding the cell. The direction of flow is reversed, and the density of the blue fluid is measured (Figure 2, step 3). Finally, the cell’s buoyant mass in blue fluid is measured (Figure 2, step 4). From these four measurements of fluid density and cell buoyant mass, the absolute mass, volume, and density of the cell are calculated. The process takes ~5 s per cell and can measure ~500 cells per hour.

By measuring a polystyrene bead standard, we find an upper estimate of the resolution of our method: 3 pg mass, 3 fL volume, and 0.001 g/mL density (Figure 3). In a plot of erythrocyte mass vs. density, we can clearly discriminate less-dense Plasmodium falciparum malaria-infected cells from healthy cells (Figure 4). We can also distinguish an individual’s own erythrocytes from those received via transfusion several days prior to analysis, and identify irreversibly-sickled erythrocytes in a patient with sickle-cell disease (Figure 5). Finally, we find that mouse lymphocytic leukemia cells treated with staurosporine (a kinase inhibitor) undergo an increase in density that is statistically more significant than the already well-known volume decrease accompanying apoptosis (Figure 6). In each of these examples, we discriminate cells by density that would be impossible to distinguish by mass or volume alone.

This simultaneous measurement of cell mass, volume, and density provides what is arguably the most complete metric of cell size currently available, and we anticipate that our method will find uses in a variety of clinical and analytical applications.

Figure 1: By plotting the buoyant mass of a cell vs. the density of the fluid in which the cell was weighed, the absolute mass, volume, and density of the cell can be calculated.

Figure 2: Using the Suspended Microchannel Resonator (SMR, left) to measure the buoyant mass of a cell in two fluids of different densities (red and blue, below). Measurement starts with the mass-sensitive cantilever filled with the red fluid (step 1). The density of the red fluid is determined from the baseline resonance frequency of the SMR. When a cell (yellow) passes through the SMR, the resulting peak in resonance frequency has a height that is proportional to the buoyant mass of the cell in the red fluid (step 2). The cell then enters a channel containing blue fluid, and the higher flow rate quickly dilutes and replaces the remaining red fluid around the cell. The direction of fluid flow is reversed, and the density of the blue fluid is determined from the new baseline frequency of the SMR (step 3). Now surrounded by blue fluid, the cell passes through the SMR a second time, and the buoyant mass of the cell in blue fluid is recorded (step 4). From these four measurements of fluid density and cell buoyant mass, we calculate the mass, volume, and density of the cell.

Figure 3: Measuring the volume, mass, and density of 5.0 micron polystyrene beads (n = 1,069). The coefficient of variation (CV) of bead density is 35 times smaller than the mass or volume variation. In the plot of bead mass vs. density (right), the error bars estimate our resolution: 3 pg mass, 3 fL volume, and 0.001 g/mL density (mean ± 1 standard deviation).
Figure 4: Previous work by others has shown that erythrocytes infected with *P. falciparum* malaria are less dense than healthy cells. Using our technique, we can identify single malaria-infected erythrocytes in a culture by their reduced density (right) and confirm that these cells are absent from an all-healthy culture (left).

Figure 5: (Left) Single-cell mass vs. density for 502 erythrocytes from a patient with suspected thalassemia trait (red), compared to an equal number of cells from a healthy individual (black). Patients with thalassemia trait have a decreased average erythrocyte volume; we observe this along with a decreased erythrocyte mass and density. The small fraction of red points overlapping the black points correspond to normal erythrocytes the thalassemia patient received via transfusion 4 days prior to collection. In spite of being in another individual’s body for several days, these transfused erythrocytes are still identifiable by their mass and density. (Right) A similar plot with 502 erythrocytes from a patient with sickle cell disease (red) compared to an equal number of cells from a healthy individual (black). The widened distribution of cell densities is consistent with other studies, with the region of more-dense cells likely representing irreversibly-sickled erythrocytes.

Figure 6: Single-cell volume, mass, and density measurements from a culture of L1210 mouse lymphocytic leukemia cells, measured before (black points) and after (red points) adding staurosporine to the culture. Staurosporine is a general kinase inhibitor that induces apoptosis in the cells. Staurosporine treatment is expected to cause an apoptotic volume decrease in the cells, and we observe this post-treatment decrease in the volume and mass time courses. But because the intrinsic range of cell volumes pre-treatment is so large (400–1200 fL), single cells cannot be identified as apoptotic on the basis of volume or mass alone. However, we also measure a pronounced increase in cell density after treatment. Since the pre-treatment range of cell density is much smaller (1.072–1.086 g/mL), the density change accompanying drug treatment is much more statistically significant and individual cells can be identified as apoptotic by their density.