Fluidity as a mechanical property of the fully suspended cell

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OVERVIEW

Mechanical characteristics of single cells are used to identify and possibly leverage interesting differences among cells or cell populations. Fluidity (i.e., phase lag normalized to the extremes of an elastic solid or a viscous liquid) can be extracted from various rheological measurements of cells, including oscillatory phase lag [1]. This nondimensional property may serve as a useful and robust parameter for distinguishing cell populations, and also for understanding the physical origins of deformability in soft matter. Here, for single eukaryotic cells deformed via optical stretching, we examine the dependence of fluidity on chemical and environmental influences.

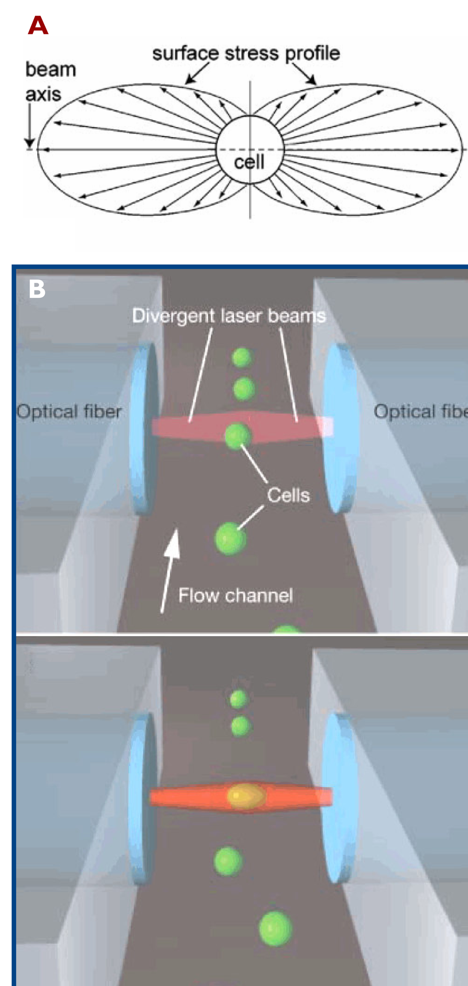

Figure 1. Optical stretching of single cells. **(A)** Impinging photons create an outward surface stress on a cell membrane as they move from a medium of lower to higher refractive index; **(B)** suspended cells are positioned between two optical fibers and stretched one by one to build a data set of population mechanics [2].

Why deform *suspended* cells?

1. Cells in suspension can be processed with higher throughput (similar to flow cytometry).
2. Suspension avoids stress concentrations, adhesion site formation, and stress fibers—factors that can complicate interpretation in other cell mechanics techniques.
3. Stem cells are often re-implanted in the suspended state, and metastatic cancerous cells are migratory; deformability likely affects their movement through tissue.

APPROACH

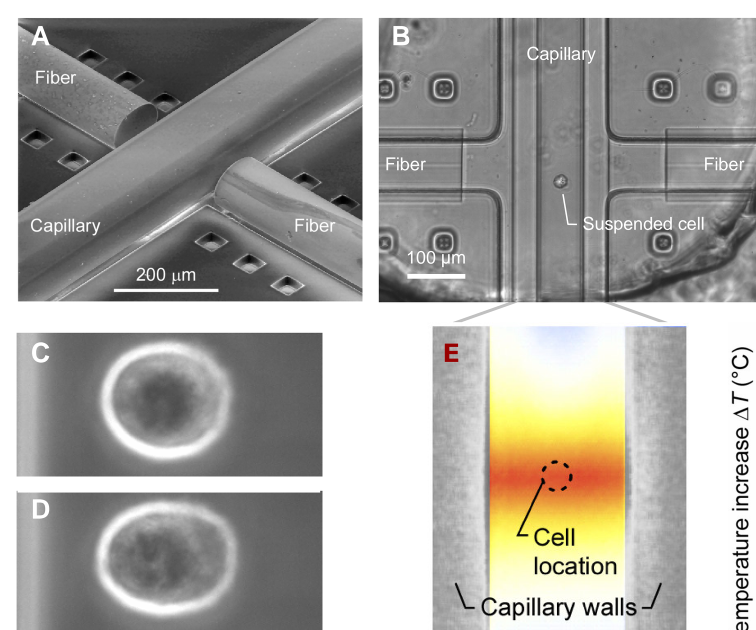

Figure 2. **(A)** Scanning electron micrograph and **(B)** phase contrast photograph of opposing optical fibers positioned to face a hollow glass capillary filled with cell suspension (here, CH27 lymphoma cells) during operation [3]. **(C, D)** Single cell before and during irradiation. The cell response is characterized by its deformation along the laser axis as a function of time [2]. We select a timescale around 1 s; at much higher frequencies, water viscosity dominates, while at much lower frequencies, mechanical sorting is less practical. **(E)** Infrared laser impingement also unavoidably heats the cell; such heating can be characterized by a temperature-sensitive fluorescent dye and usefully employed to explore cell mechanics as a function of temperature (Fig. 7). **(F)** We derive and confirm a ln(t) form for the laser-induced temperature increase in a cell [1].

Frequency-domain optical stretching deforms single cells in the linear viscoelastic regime, with well-defined fluidity:

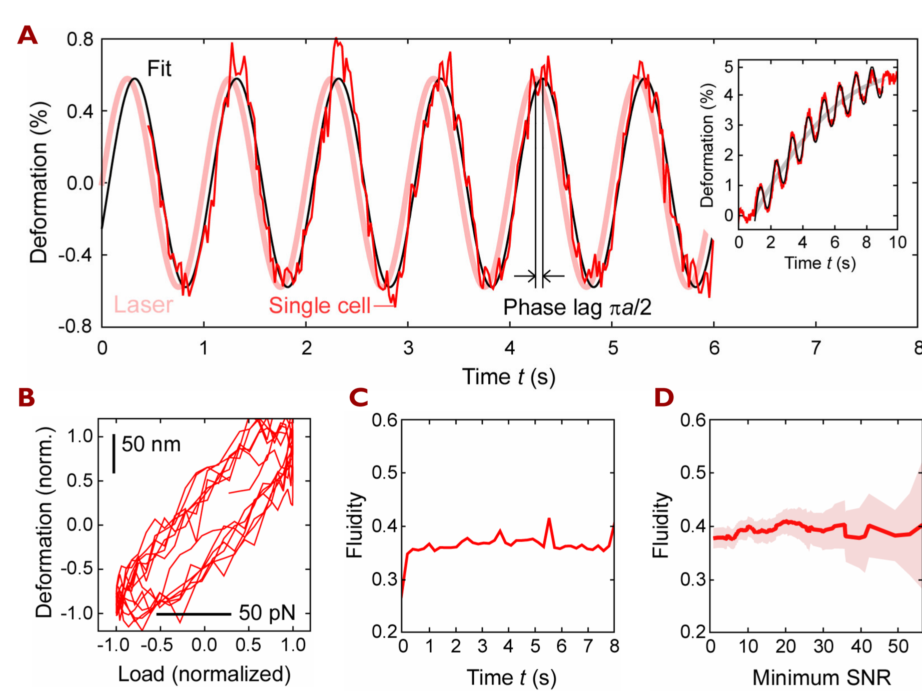

Figure 3. **(A)** Oscillatory deformation of a single cell in response to sinusoidal photonic pressure with frequency 1 Hz. (Inset, total deformation before subtracting background creep.) The viscoelastic phase lag φ of the cell in radians is also a measure of cell fluidity as $a = 2\varphi/\pi$. **(B)** Symmetric and elliptical Lissajous figure indicates linear viscoelasticity with an estimated noise floor of 10 nm. **(C)** At 10 Hz, brief 0.2-s-wide analysis windows provide consistent fluidity estimates across 8 s of oscillatory stretching. Fluidity can thus be acquired from subsecond sampling of the cell. **(D)** Population average of fluidity (with 95% confidence intervals) is independent of signal-to-noise (SNR) ratio; even the noisiest deformation signals contribute useful information to population estimates.

Why focus on fluidity?

Fluidity, as a measure of viscoelastic damping, complements cell stiffness and provides an additional parameter for characterization, sorting, and biophysical study of the cell. Because the cell cannot be easily divided into elastic and viscous components (Fig. 4), a structural damping model ("power-law rheology") based on fluidity is the *simplest* way to represent cell deformation at our selected timescale. Consider, for example, how a single measurement of fluidity (via phase lag) usefully predicts the time response of creep compliance and the frequency response of stiffness (Figs. 4, 5, and 6). Furthermore, fluidity is nondimensional and requires no calibration before comparison across tools.

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RESULTS

Single-cell deformation follows a structural-damping, or fractional-derivative, constitutive law:

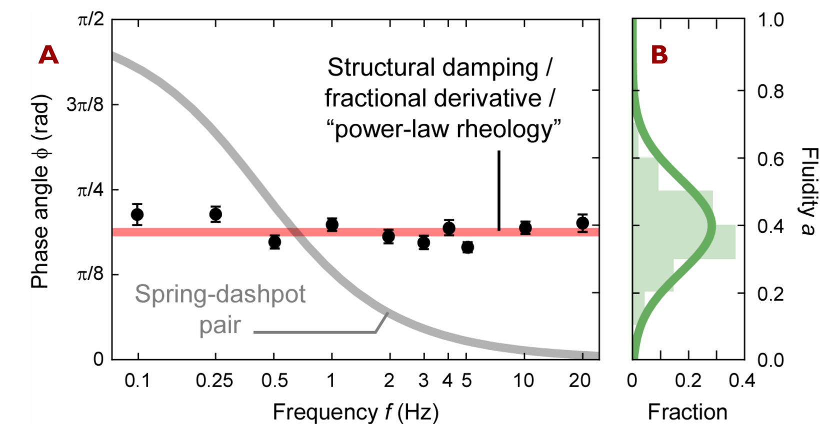


Figure 4. **(A)** Across >2 decades around 1 s, fluidity a is frequency independent and compatible with the complex-modulus constitutive law $G(\omega) = g_0(i\omega)^a$ where $a = 0.38 \pm 0.01$. (This model is also known as "fractional-derivative," "constant-phase," or "power-law" rheology.) Lumped-component models of one or several spring-dashpot pairs are incompatible with these measurements. **(B)** Fluidity across cells is Gaussian-distributed with a standard deviation of approximately 0.1.

The same constitutive law and fluidity values are obtained via measurements of creep compliance vs. time and complex modulus vs. frequency:

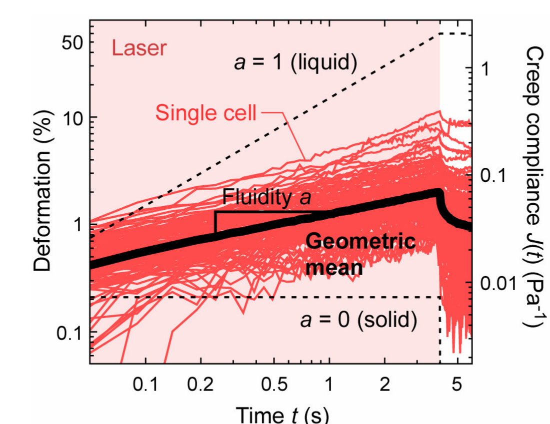


Figure 5. Time-dependent creep compliance $J(t)$ (acquired at 0.9 W/fiber unit step for 4 s) for single cells. Thick black line shows geometric mean, well fit during stretching by $J(t) = j_0 t^a$ where $a = 0.34 \pm 0.02$. Dotted black lines contrast behavior of perfectly elastic ($a = 0$) and viscous ($a = 1$) materials.

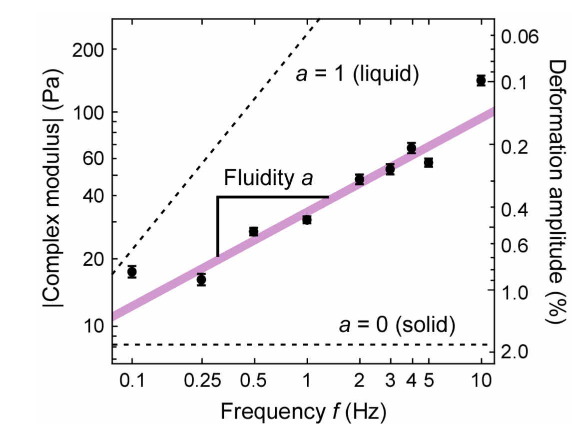


Figure 6. Frequency-dependent stiffness $|G(\omega)|$ (acquired at 1 W/fiber mean, 0.5 W/fiber sinusoidal amplitude) is well fit by $|G(\omega)| = g_0\omega^a$ where $a = 0.41 \pm 0.02$. Dotted black lines contrast behavior of perfectly elastic ($a = 0$) and viscous ($a = 1$) materials.

These conclusions hold across nonadherent, immortalized CH27 lymphoma cells; adherent, immortalized 3T3 fibroblasts; and adherent, primary human mesenchymal stem cells [1].

What is the problem with using springs and dashpots to model cell mechanics?

Each spring-and-dashpot pair implies a characteristic time constant (Fig. 4) where the system transitions from solid toward liquid character or vice versa. We find no such transitions near the 1 s timescale.

Importantly, good-looking spring-dashpot fits to creep in the *time* domain are revealed to be inconsistent in the *frequency* domain—the time constants derived with these fits are artifactual and misleading. The models shown in red above are, in contrast, consistent across domains.

Fluidity increases with increasing cell temperature:

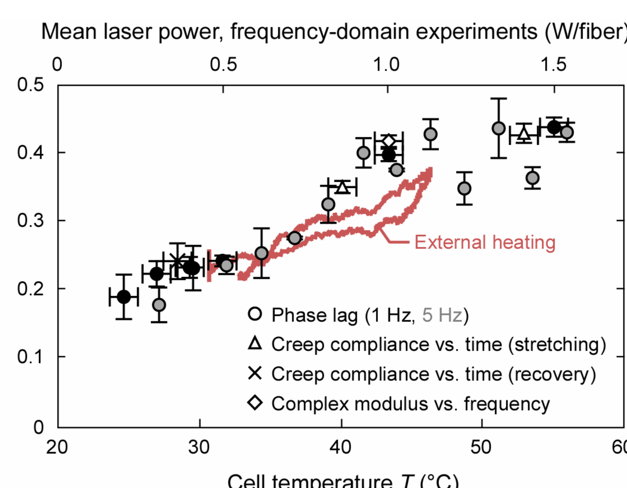
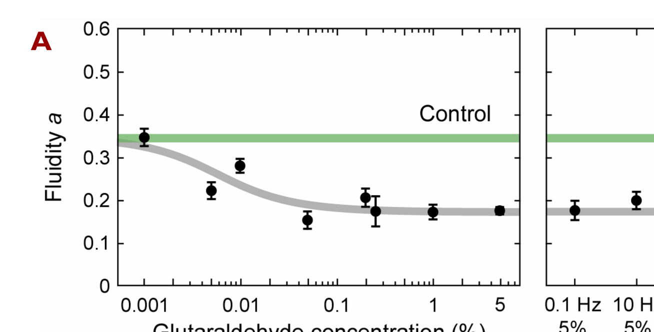


Figure 7. Via four techniques in two rheological domains, fluidity is found to increase with temperature at a rate of 0.01/°C. We generally used the impinging laser to heat the cells (Fig. 2). Our method of estimating fluidity is illustrated in Fig. 4 for phase lag measurements, in Fig. 5 for creep compliance measurements, and in Fig. 6 for complex modulus measurements. To decouple laser-induced stress, we also increased and decreased the temperature of the microscope stage over several hours while measuring fluidity ("external heating", a 100-cell moving average), with equivalent results.

Fluidity is suppressed by fixation, unaltered by ATP depletion:



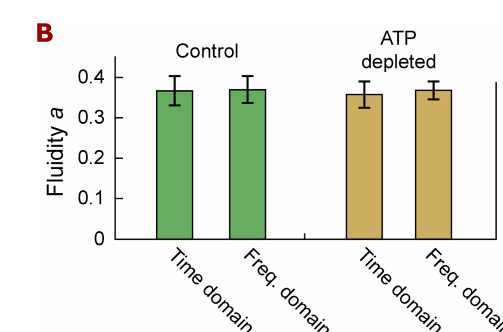


Figure 8. **(A)** Chemical fixation reduces fluidity but does not turn the cell into an elastic solid or even a spring-dashpot-like solid; fluidity remains insensitive to frequency and best described by a constant-phase model even after cells are fixed and killed. **(B)** Another type of cell "death": blocking actomyosin contraction and other metabolic activities leaves the cytoskeleton unresponsive but does not affect fluidity.

What is the physical interpretation of fluidity?

The parameter we term "fluidity" has been interpreted in the literature as an athermal jostling in complex fluids [4], or mathematically as a distribution of material relaxation times τ as $P(\tau) \sim \tau^{-a-1}$ [4]. Its reciprocal has been treated as a frictional resistance to polymer chain rearrangement [5].

Our work shows that the τ^{-a-1} distribution model is sound for cells around a timescale of 1 s, but that deformation mechanisms do have a measurable thermal component. Chemical crosslinking increases cytoskeletal "friction," but ATP-depleted cells in the rigor state are as fluid as control cells with a healthy metabolism.

CONCLUSIONS AND OUTLOOK

The term "fluidity" generalizes a parameter that can be estimated from the phase lag or power-law exponent of cell deformation. We expect that physical sorting approaches will continue to emerge that will test suspended cells at timescales of approximately 1 s or smaller, and that studies of single-cell mechanics will enable theories of soft matter and complex fluids to be tested. Our current focus is to identify chemically modulated *differences* in fluidity between tissue cell subpopulations in contexts relevant to the sorting and delivery of suspended cells.