Electronic Supplementary Material (ESI) for Nanoscale.

Supplemental Material

Mapping the creep compliance of living cells with scanning ion conductance microscopy reveals a subcellular correlation between stiffness and fluidity

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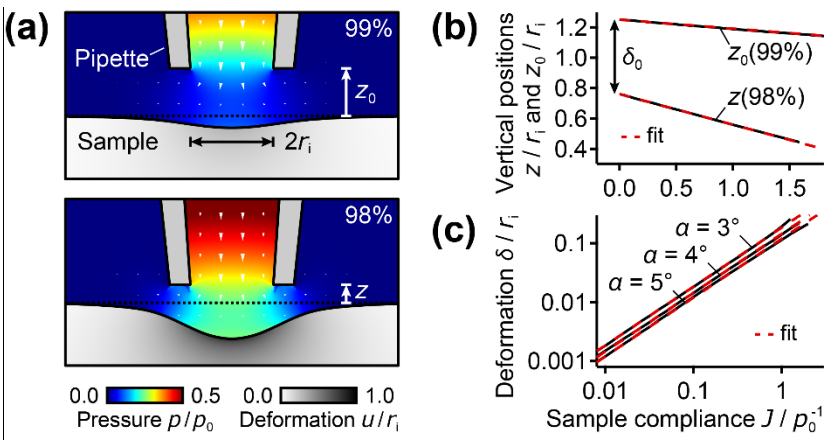
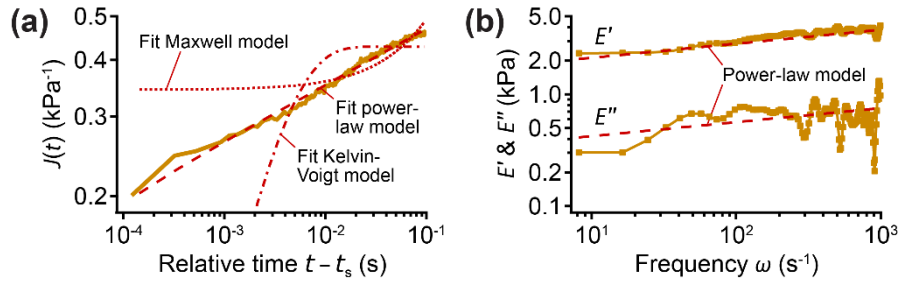
Numerical Model



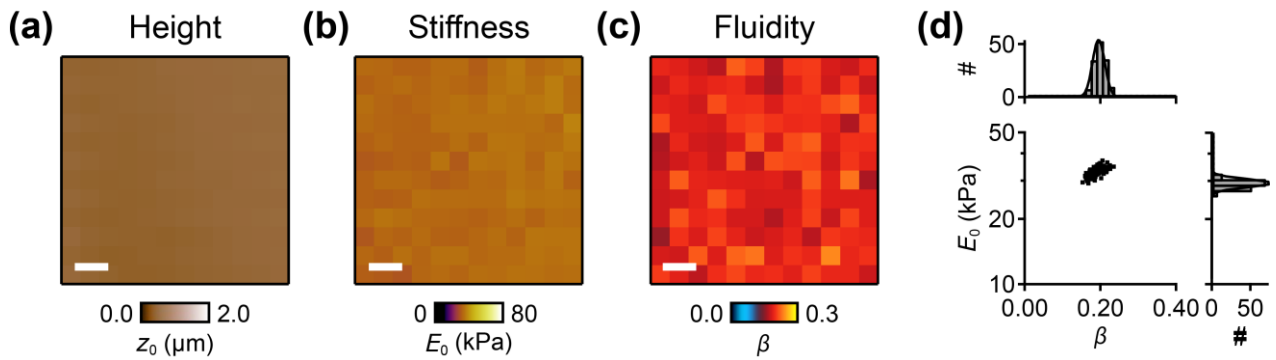
Suppl. Fig. S-1 | Numerical model for quantification. (a) Finite element simulations for fluid flow and deformation of an elastic sample calculated for vertical pipette positions corresponding to 99% ion current (top panel, $z_0 = 1.6r_i$) and to 98% ion current (bottom panel, $z = 0.4r_i$). **(b)** Vertical pipette position at 99% ion current, z_0, and at 98% ion current, z, as a function of sample compliance J. δ_0 denotes the difference between the two positions at zero sample compliance ($J = 0$). **(c)** Relative sample deformation, defined as $\delta = z_0 - z - \delta_0$, as a function of J for different values of inner half cone angle α. The parameters for the shown FEM simulations are $J = 1.5\ p_0^{-1}$ (panel a), $\alpha = 4°$ (panels a and b), and ratio of outer to inner opening radius $r_o/r_i = 1.5$. The dashed red traces denote linear fits (b) and fits of Equation (5) (c).

Creep Compliance and Complex Modulus of Living Cells Follow a Power-Law Model



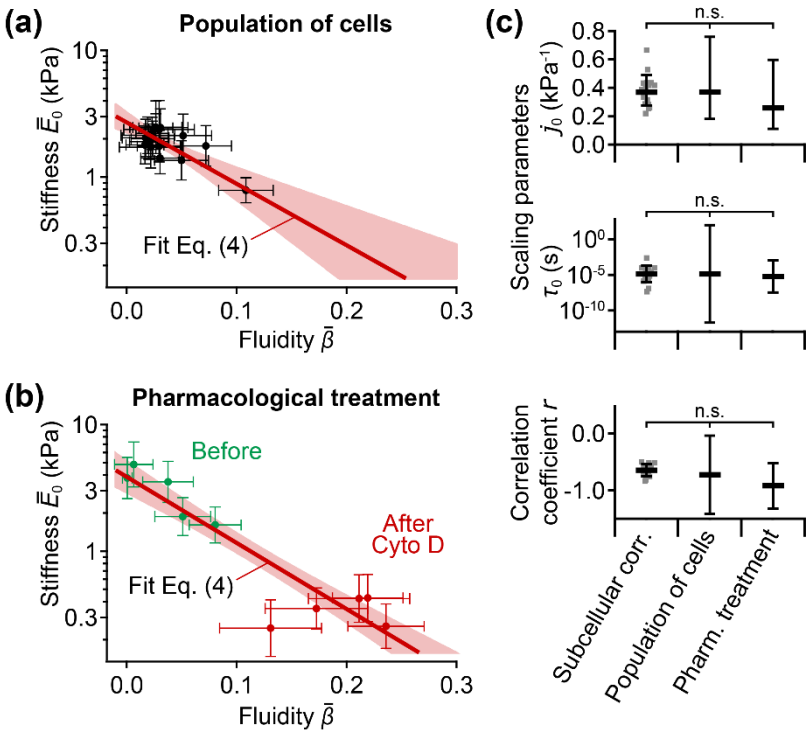
Suppl. Fig. S-2 | Creep compliance and complex modulus of a living cell and power-law model. (a) Creep compliance $J(t)$ with a time relative to t_s (start of creep measurement) recorded on a living cell (same data as Figure 1c, bottom panel) shown on a log-log scale with fits of power law (Equation (3), red dashed trace), Maxwell $[J(t) = E^{-1}(1 + t/\tau)$, gives $E = 2.9$ kPa and $\tau = 0.23$ s, red dotted trace], and Kelvin-Voigt $[J(t) = E^{-1}(1 - e^{-t/\tau})$, gives $E = 2.3$ kPa and $\tau = 3.5$ ms, red dashed-dotted trace] models. Here, E, η, and $\tau = \eta/E$ denote modulus of elasticity, viscosity, and time constant, respectively. Interpreting the time constant in terms of a poroelastic material model[1] gives poroelastic diffusion constants of typically $D_p = L^2/\tau \approx 10\ \mu m^2 s^{-1}$ (using $L \approx r_i$ as characteristic length scale), consistent with AFM experiments.[1] **(b)** Complex modulus $E^*(\omega) = E'(\omega) + i\,E''(\omega)$ calculated by the modified Fourier transform[2] of the creep compliance data (solid traces) and power-law model (red dashed-dotted trace, prediction from the fit in the time domain data).

Verification on a Silicone Polymer Sample



Suppl. Fig. S-3 | Verification on a silicone polymer sample. Map of **(a)** sample height, **(b)** stiffness, and **(c)** fluidity recorded on a CY52-276 polymer with the nominal mixing ratio of 1: 1 (part A to part B). **(d)** Scatter plot of stiffness E_0 vs. fluidity β and histograms of stiffness E_0 (right) and fluidity β (top) with an indication of log-normal and normal distributions (black curves), respectively. As expected, the polymer is homogenous in stiffness and fluidity, within a narrow range of $\bar{E}_0 = 28 \pm 1$ kPa and $\bar{\beta} = 0.25 \pm 0.03$ (average ± standard deviation). No strong correlation between E_0 and β was observed. For a mixing ratio of 6: 5 (part A to part B) we measured averages $\bar{E}_0 = 10$ kPa and $\bar{\beta} = 0.4$, which is in very good agreement with AFM data on the same polymer with a similar mixing ratio.[3] The applied pressure was $p_0 = 150$ kPa. Scale bars: 5 µm (a-c).

Correlation for Cell Population and for Pharmacological Treatment

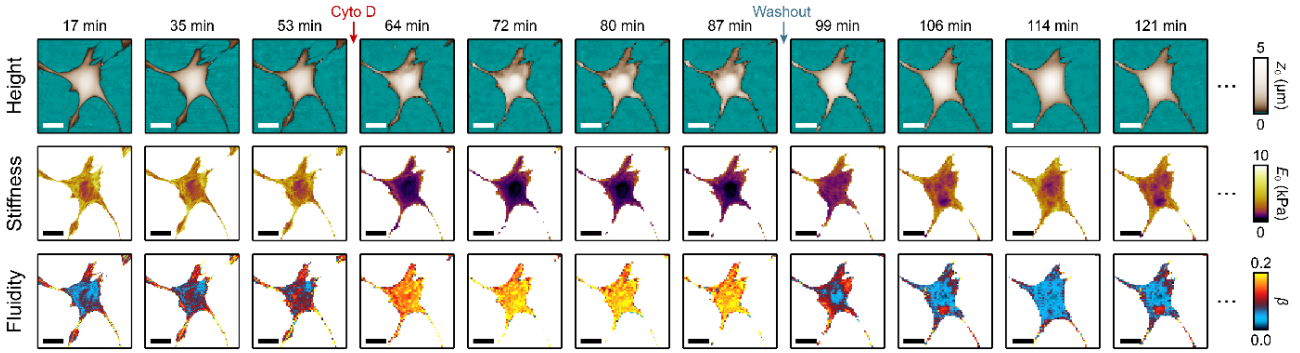


Suppl. Fig. S-4 | Correlation between average stiffness and fluidity for the population of cells and for cells during pharmacological treatment. (a) Average stiffness $\bar{E}_0$ vs. average fluidity $\bar{\beta}$ for the population of cells ($N = 17$ cells) with fit of Equation (4) (red line). **(b)** Average stiffness $\bar{E}_0$ vs. average fluidity $\bar{\beta}$ for cells ($N = 5$ cells) before and 30 min after pharmacological treatment with 2 µM cytochalasin D with fit of Equation (4). **(c)** Average scaling parameters j_0 and τ_0 and average correlation coefficient r obtained from subcellular correlations (see *e.g.* Fig. 3), from the population of cells (see panel a), and from pharmacological treatment (see panel b). Plots show average values (markers) and data of individual cells (dots); error bars indicate estimated standard deviation. The light red areas represent standard error of the fit (a, b).

Table S1 | Average scaling parameters j_0 and τ_0 and average correlation coefficient r obtained from subcellular correlations (see *e.g.* Fig. 3), from the population of cells (see Suppl. Fig. S-4a), and from pharmacological treatment (see Suppl. Fig. S-4a), provided as average ∗ (scaling parameters) or ± (correlation coefficient) standard error.

	Scaling parameters		Correlation coefficient	Number of cells
	j_0 (kPa^{-1})	τ_0 (µs)	r	N
Subcellular correlation	$0.368 * 1.1$	$13.2 * 1.9$	-0.65 ± 0.03	17
Population of cells	$0.371 * 1.2$	$14.3 * 60$	-0.72 ± 0.18	17
Pharmacological treatment	$0.258 * 1.3$	$6.01 * 6.4$	-0.91 ± 0.14	5

Stiffness and Fluidity of a Living Cell during Cytoskeleton Disruption and Recovery



Suppl. Fig. S-5 | Stiffness and fluidity of a living cell during cytoskeleton disruption and recovery. Whole sequence of topography images (top row) and maps of stiffness E_0 (middle row) and fluidity β (bottom row) of the living fibroblast cell from Figure 4 during addition and washout of 2 µM cytochalasin D. Scale bars: 20 µm. See also Supplementary Video S1 for an animation of this sequence.

References

1 E. Moeendarbary, L. Valon, M. Fritzsche, A. R. Harris, D. A. Moulding, A. J. Thrasher, E. Stride, L. Mahadevan and G. T. Charras, *Nat. Mater.*, 2013, **12**, 253-261.

2 R. M. L. Evans, M. Tassieri, D. Auhl and T. A. Waigh, *Phys. Rev. E*, 2009, **80**, 012501.

3 R. Takahashi and T. Okajima, *Appl. Phys. Lett.*, 2015, **107**, 173702.