Supplemental Material

Spatial Correlation of Cell Stiffness and Traction Forces in Cancer Cells Measured with Combined SICM and TFM

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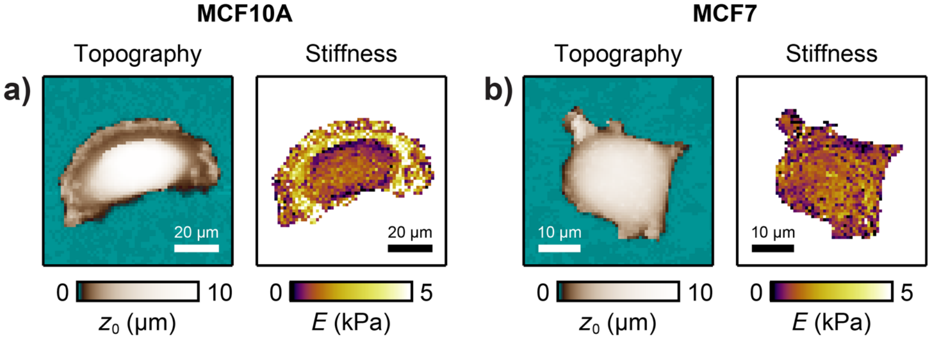
Cell Morphology and Stiffness on Rigid Substrates



Supplementary Figure S-1 | Cell morphology and stiffness on rigid substrates. (a) SICM topography image (left) and stiffness map (right) of a normal MCF10A and **(b)** of a cancerous MCF7 human breast epithelial cell on rigid cell culture dishes. On rigid substrates, the cells are more spread, but show a similar morphology compared to cells on elastic TFM substrates (see Figure 4).

Theoretical Modell and Effect of Finite Cell Thickness

The apparent cell stiffness E_{app} was measured as described previously.[1] Briefly, a constant pressure p_0 was applied to the upper end of the capillary and *IZ*-curves were recorded. The sample stiffness in terms of the apparent Young's modulus E_{app} was then obtained from the slope s of the *IZ*-curve between 98 and 99% relative ion current as

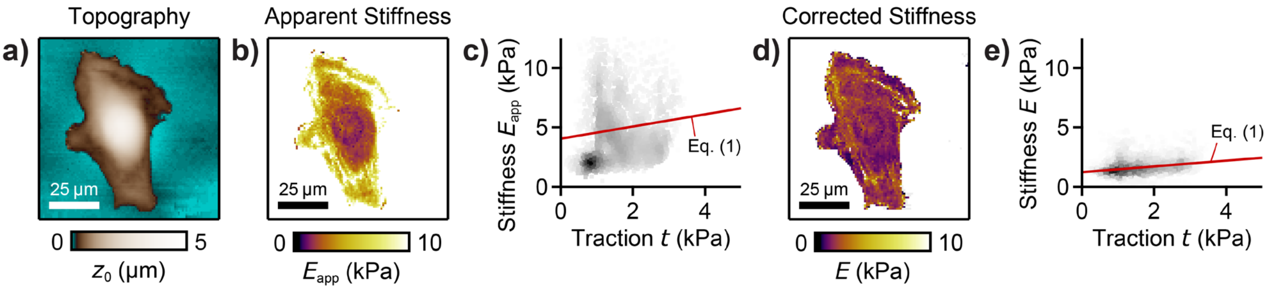
$$E_{\mathrm{app}} = p_0 A \left(\frac{s_\infty}{s} - 1\right)^{-1}, \tag{S1}$$

where s_∞ is the slope measured on the substrate and $A = 0.26$ is a pipette-dependent geometrical parameter.

As demonstrated for the cell shown in Figure 2b, flat extensions of the cells sometimes appear comparably stiff (also see, e.g., Supplementary Figure S-2a and b). The presence of large traction forces in the extensions might induce an artificial correlation between apparent stiffness and traction force density (Supplementary Figure S-2c, $\rho = 0.12 \pm 0.02$). To avoid this possible artifact, the effect of the finite cell thickness was corrected by[2]

$$E = E_{\mathrm{app}} \cdot \left\{\exp\left(a\frac{r_{\mathrm{i}}}{h}\right) + b\frac{r_{\mathrm{i}}}{h}\left[1 - \exp\left(-c\frac{r_{\mathrm{i}}}{h}\right)\right]\right\}^{-1} \tag{S2}$$

with cell thickness h (here equivalent to the cell height z_0) and pipette inner opening radius r_{i} and using $a = 1.462$, $b = 3.30$, and $c = 0.66$, assuming a Poisson's ratio $\nu = 0.499$ and the cell sticking to an infinitely stiff underlying substrate. The corrected cell stiffness (Supplementary Figure S-2d) is generally lower than the apparent cell stiffness (Supplementary Figure S-2b), for example by a factor of 1.7 for a thickness of $1\ \mu\mathrm{m}$, but still shows a similar subcellular distribution with a soft cell body and stiffer extensions. The correlation between cell stiffness and traction force density is even stronger for the corrected cell stiffness (Supplementary Figure S-1e, $\rho = 0.29 \pm 0.02$). Both the apparent and the corrected cell stiffness (median stiffness here $4.9\ \mathrm{kPa}$ and $1.6\ \mathrm{kPa}$, respectively) are significantly softer than the substrate ($E \cong 15\ \mathrm{kPa}$), indicating that the assumption of an infinitely stiff underlying substrate was valid.



Supplementary Figure S-2 | Effect of cell thickness on measured cell stiffness. (a) SICM topography image, **(b)** map of apparent stiffness, and **(c)** apparent local cell stiffness as a function of local traction force density for the cell shown in Figure 2b and 3a. **(d)** Map of corrected stiffness calculated using Equation (S2) (with $r_{\mathrm{i}} = 300\ \mathrm{nm}$) and **(e)** corrected local cell stiffness as a function of local traction force density. The red lines are fits of Equation (1). The scatter plot grayscale level indicates point density.

References

1. J. Rheinlaender and T. E. Schäffer, *Soft Matter*, 2013, **9**, 3230-3236.
2. J. Rheinlaender and T. E. Schäffer, *Appl. Phys. Lett.*, 2020, **117**, 113701.