Combined High-Speed Atomic Force and Optical Microscopy Shows That Viscoelastic Properties of Melanoma Cancer Cells Change during the Cell Cycle

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Current high-speed atomic force microscopy (HS-AFM) setups reach imaging speeds of several images per second but often have limited options for imaging live cells because of a small scan range, a lack of environmental control, or a missing combination with optical phase-contrast or fluorescence microscopy. A HS-AFM setup is therefore developed with a large scan range optimized for imaging live cells. The setup is equipped with temperature and CO₂ control and is mounted on an inverted optical microscope providing high-quality phase-contrast and fluorescence microscopy. To demonstrate the capabilities of the setup, fast force mapping on live human platelets is performed. Further, HS-AFM images and optical phase-contrast and actin fluorescence images of live cancer cells are simultaneously recorded, and two state-of-the-art AFM modes for imaging viscoelastic sample properties, force clamp force mapping and resonance compensating chirp mode, are compared. The setup is then applied to the investigation of viscoelastic material properties of cells in different cell cycle states. Using a melanoma cell line with a fluorescent cell cycle sensor, it is found that during the cell cycle not only cell volume and morphology, but also viscoelastic material properties significantly change, with increasing stiffness and decreasing fluidity from the G1 through the G1/S to the S/G2/M phases.

1. Introduction

Measuring mechanical properties of live cells is important to gain a better understanding of cell physiology or diseases,[1–3] and atomic force microscopy (AFM)[4] has become a key technique for imaging the mechanics of cells.[5–12] For example, using conventional AFM it was shown that healthy and cancerous cells differ in their elastic[13] and viscoelastic[14] properties and that cell stiffness,[15] tension,[16] and viscoelastic properties[17] change during cell division. However, imaging the mechanics of live cells at high speed with AFM is still challenging, even more than 20 years after the invention of small cantilevers[18–20] and high-speed AFM.[21–26] This is because current high-speed AFM setups usually have a limited scan range, a lack of simultaneous optical microscopy capabilities, a lack of environmental control (e.g., temperature or CO₂ level), a small sample holder excluding the application of conventional cell culture dishes, or are missing the means for mechanical measurements. Consequently, current high-speed AFM setups can usually image parts of mammalian cells only.[27–30]

Here, we present a high-speed, large-range AFM setup that is combined with optical phase-contrast and fluorescence microscopy. The setup can be operated with small cantilevers and allows to image live cells in standard cell culture dishes with temperature and CO₂ level control. Fast force mapping to obtain the Young’s modulus of live human platelets is demonstrated. Taking advantage of the capability of fluorescence microscopy, we applied the setup to two model systems for cell mechanics: First, we simultaneously recorded high-speed AFM images and optical phase-contrast and actin fluorescence images of live cells, allowing us to compare two state-of-the-art imaging modes for imaging viscoelastic material properties, force clamp force mapping (FCFM) and resonance compensating chirp mode (RCCM), and to directly relate the material properties to the actin cytoskeleton structure obtained from fluorescence microscopy. Second, using a melanoma cancer cell line with a fluorescent cell cycle sensor, we measured cell volume, morphology, and viscoelastic material properties of live cells as a function of their cell cycle state. We show that cell volume, area, and stiffness increase and fluidity decreases during the cell cycle. We thereby demonstrate that the combination of high-speed AFM with a large scan range in combination with optical microscopy can provide new insight into cell mechanics in physiologically or pathologically relevant situations.
2. Results and Discussion

2.1. High-Speed Atomic Force Microscopy (HS-AFM) Setup with a Large Scan Range Combined with Optical Microscopy

Commercially available AFM setups for live cell investigation of the latest generation are routinely combined with optical microscopy and are established in laboratories all around the world, but lack a fast z-scanner and the possibility to use small cantilevers, which is necessary for high-speed measurements. Recently developed high-speed AFMs, like the FastScan (Bruker Corporation) or Cypher (Oxford Instruments plc) are compatible with small cantilevers, but have small xy scan ranges (on the order of 30 × 30 µm²), which are too small for imaging whole eukaryotic cells, and they do not provide combined optical phase-contrast or fluorescence microscopy.

To overcome these limitations, we developed a high-speed AFM for small cantilevers that provides a large scan range, the possibility of imaging samples in standard cell culture dishes, environmental control, and combination with optical phase-contrast and fluorescence microscopy. The setup ([Figure 1a,b]) contains four main units: A laser unit i), a z-scanner unit ii), an xy-scanner unit iii), and a base construction iv). The laser unit consists of a superluminescent laser diode, a collimator, a beam expander, a mirror glued on a tip/tilt platform for xy-adjustment of the laser beam, and a lens to focus the laser beam on the cantilever ([Figure 1b, i]). The superluminescent diode (SLD-380-MP-T056-PD, Superlum Diodes Ltd., Carrigtwohill, Ireland) provides a spot size (diameter) of 3.7 µm (minor axis) and 9.6 µm (major axis) in air, and 4.9 and 12.8 µm, respectively, in water. The z-scanner unit is attached to the laser unit and consists of an aluminum support that the z-piezo is glued to, a mirror to redirect the reflected laser beam from the cantilever to the detector, and a detector with a four-segment photodiode ([Figure 1b, ii]). The z-scanner (SA050510, 5 × 5 × 10 mm³, PiezoDrive, Callaghan, Australia) has a range of 10 µm, which is large enough to image most cells, and has a high resonant frequency around 17 kHz ([Figure S1a, Supporting Information]) enabling fast force curve rates ([Figure S1b, Supporting Information]). The z-piezo is driven by a low-noise voltage amplifier (PX200, PiezoDrive) (390 kHz signal bandwidth, 35 V µs⁻¹ slew rate) in the unipolar 0–150 V output configuration. The z-position sensor is a strain gauge (1-LY11-3/350, Hottinger Baldwin Messtechnik GmbH, Darmstadt, Germany) with a strain gauge amplifier (DMS03, Piezomechanik, München, Germany) modified for a maximum bandwidth of 200 kHz. The xy-scanner unit consists of a commercial xy-scanner (NPXY100-126 with LC402 controller, nPoint, Middleton, USA), a sample holder, and an xy coarse adjustment ([Figure 1b, iii]). The xy-scanner provides a scan range of 100 × 100 µm² (closed loop) or 160 × 160 µm² (open loop). To characterize the performance of the system for high-speed imaging, we imaged two calibration gratings with an image rate as high as 78 images per s in contact mode ([Figure S2, Supporting Information]).

As the setup aims to measure mechanical properties of live cells in point-spectroscopy-like imaging modes, where the force curve rate is typically limited to 200–300 Hz even with small cantilevers, a commercial xy-scanner with decent scan speed is sufficient here. For maintaining cells at 37 °C, a custom-made sample heater was developed, consisting of a sample holder with a 20 × 20 mm² indium tin oxide (ITO) coated glass for optical access, which can be heated directly by resistive heating using a home-build heater controller with a PT100 sensor for feedback control (temperature stability 0.1 °C over a period of 12 h). The base construction ([Figure 1b, iv]) consists of a large plate that is mounted on the optical microscope (not shown, for more details see the Experimental Section), a mounting for the dovetail slide and a micrometer screw for coarse adjustment of the z-position using the dovetail slide. The screw is turned by a stepper motor, enabling 80 nm steps for a well-controlled approach of the cantilever to the sample, which is especially useful when using small cantilevers. An optional incubator system (for details see the Experimental Section) allows for long-term investigations of live cells over more than 8 h ([Figure S3, Supporting Information]).

Figure 1. High-speed AFM setup for combined optical microscopy and AFM cell imaging. a) Schematic of the setup showing the different components in front view. b) 3D view showing the four main units of the setup: i) laser unit including the focusing lens, ii) z-scanner/detector unit, iii) xy-scanner unit including the sample heater, and iv) base construction on which all four units are mounted to. The positions of the four units on the base construction are marked with black dashed contours. The base construction is mounted on top of an inverted optical microscope (not shown) for optical imaging.
2.2. Fast Force Mapping on Live Cells

To demonstrate the capabilities of the setup, we investigated two adjacent live cells in fast force mapping mode and compared the topography image, showing the “true” (zero-force) height calculated from the contact point of the force curve, and the Young’s modulus map with optical images showing phase-contrast and fluorescently labeled β-actin (Figure 2a). The phase-contrast image shows the shape of the cells (Figure 2a, i). The fluorescence image shows β-actin all over the cells with stress fibers and with a slightly darker area at the site of the nucleus of the left cell (Figure 2a, ii). The topography image shows the two cells with a height of about 4 µm each with the highest regions around the nucleus (Figure 2a, iii). Softer areas around the nuclei and areas with pronounced fibers are visible in the Young’s modulus map (Figure 2a, iv). Some areas with high Young’s modulus (Figure 2a, iv, green arrows) correspond to areas with visible fibers in the fluorescence image (Figure 2a, ii, green arrows), while areas with lower Young’s modulus correspond to areas with less β-actin (Figure 2a, ii and iv, orange arrows). Two areas that show nearly no intensity in the fluorescence image and a considerably smaller Young’s modulus (Figure 2a, ii and iv, red arrows) are presumably blebs.[10,31] However, not all of the fibers are visible in the Young’s modulus map as they may be located at the bottom of the cell and thus are not detectable in this AFM measurement that mainly probes the upper cell cortex.[12,32] The geometric mean of the Young’s modulus (Figure 2a, iv) is 6.5 kPa (Figure S4, Supporting Information), which is similar to the Young’s modulus of other cell types measured with sharp AFM cantilevers.[33]

Next, we imaged a live human platelet[34] in fast force mapping mode and recorded topography images and Young’s modulus maps as a function of time (Figure 2b). The platelet was imaged for a total of 6 min (26 s per frame); a movie of the sequence can be found in the Supporting Information (Movie S1, Supporting Information). Areas with a high dynamic behavior showing extending and retracting of the platelet lamel-lipodium are indicated by arrows.

2.3. Comparison of FCFM and RCCM Viscoelastic Imaging Modes

To demonstrate that the setup can measure viscoelastic properties of live cells and to correlate these properties with the

![Figure 2](https://www.advancedsciencenews.com)
structural components of the actin cytoskeleton, we performed a combined FCFM and RCCM measurement on a live U2OS cell that had pronounced fibers along its long axis (Figure 3a).

In the FCFM measurement, the indentation increases over time (Figure 3b), showing the viscoelastic creep of the cell.[13] In the RCCM measurement (Figure 3c), the storage $G'$ and loss $G''$ modulus as well as the loss tangent $G''/G'$ continuously increase with frequency, with an intersection of $G'$ and $G''$ around 4 kHz. The FCFM and RCCM measurements are well fit by power-law models (dashed lines in Figure 3b,c, see the Experimental Section for details), providing the modulus scaling parameter $G_0$ as a measure for the viscoelastic stiffness, the fluidity $\beta$, and (in the case of RCCM) the power-law Newtonian viscous damping coefficient $\mu$. The maps of the modulus scaling parameter $G_0$ from FCFM (Figure 3d) and RCCM (Figure 3f) are visually very similar. The modulus scaling parameter on the fibers ($G_0 \approx 10 \text{kPa}$) is about 10-fold higher compared to the surrounding regions ($=1 \text{kPa}$). The maps of the fluidity $\beta$ from FCFM (Figure 3e) and RCCM (Figure 3g) are also visually similar; the fluidity $\beta$ on the fibers ($\beta = 0.1$) is smaller compared to the surrounding regions ($\beta = 0.2–0.3$). Quantitatively, both the modulus scaling parameter $G_0$ and the fluidity $\beta$ from FCFM and RCCM mostly match, but deviate for low $G_0$ and high $\beta$ values (see Figure S5b,c, Supporting Information for details). The modulus scaling parameter maps do not show any correlation with the topography image (Figure S5a, Supporting Information), proving that it represents the undisturbed cell surface. The map of the power-law Newtonian viscous damping coefficient $\mu$ from RCCM (Figure S5d, Supporting Information) shows higher values on the fibers ($\mu = 2 \text{Pa s}$) compared to the surrounding regions ($\mu = 1 \text{Pa s}$). This observation is in line with the common assumption that the higher frequency domain characterized by $\mu$ is associated with the viscous damping of the cytoskeleton filaments.[36,37]

Benefitting from the combination with optical microscopy, we selected regions on and off the stress fibers in the actin fluorescence image (Figure S5e, Supporting Information) and averaged $G_0$, $\beta$, and $\mu$ over these regions (Figure S5f–h, Supporting Information). First, we found that $G_0$ and $\mu$ are significantly higher and $\beta$ is significantly lower on the fibers compared to off the fibers, for both FCFM and RCCM. Second, FCFM and RCCM give identical results on the fibers with $G_0$ around 10 kPa and $\beta$ around 0.1 (Figure S5f–h, Supporting Information, green bars), consistent with a previous study using AFM stress relaxation and micro rheology.[38] In areas off the fibers (Figure S5f–h, Supporting Information, blue bars), where $G_0$ is lower and $\beta$ is higher than on the fibers, FCFM and RCCM deviate. The reason for this deviation might be inaccuracies in the characterization of the force history in both creep compliance and stress relaxation experiments, which may cause an overestimation of the power law exponent $\beta$ in FCFM at high values of $\beta$.[38] As $G_0$ is inversely correlated to $\beta$,[39] this would result in an underestimation of $G_0$ in FCFM at low values.

2.4. Imaging the Mechanics of Melanoma Cells in Different Cell Cycles

Next, we studied the volume, calculated from the “true” (zero-force) height, morphology, and viscoelastic parameters of live cells as a function of their cell cycle using WM938 cells, a human melanoma cell line, which was stably transfected to express the fluorescence ubiquitination cell cycle indicator (FUCCI).[40] With FUCCI staining, the nuclei are colorless shortly after cytokinesis, show red (RFP) during the G1 phase, and green (GFP)
from the start of the S to the G2 and M phase. At the beginning of the S phase, both RFP and GFP are present, resulting in an orange color. As WM938 cells are very sensitive to their environmental conditions, the use of an incubator for environment control is essential here. We identified the phase of the cell cycle using fluorescence images (Figure 4a–d, first column) and investigated the WM938 cells with FCFM (Figure 4a–d, second to fourth column). The cells were identified as either early G1 = colorless (Figure 4a), late G1 = red (Figure 4b), G1/S = orange (Figure 4c), and S/G2/M = green (Figure 4d).

From the topography images, it can be seen that the cell morphology changes from a well-spread to a more rounded shape (Figure 4a–d, second column). As expected, the mean cell volume significantly increases during the cell cycle (Figure 5a) from early G1 (gray, 3755 µm³)/late G1 (red, 4055 µm³, p = 0.66) to G1/S (orange, 5214 µm³, p = 1.71 × 10⁻⁶) and approximately doubles for S/G2/M (green, 6233 µm³, p = 1.62 × 10⁻¹⁰) compared to early G1. Also, the mean cell area significantly increases during the cell cycle (Figure 5b) from early G1 (gray, 1551 µm²)/late G1 (red, 1682 µm², p = 0.70) to G1/S (orange, 2078 µm², p = 1.85 × 10⁻⁴) to S/G2/M (green, 2152 µm², p = 1.69 × 10⁻³).

Interestingly, also the viscoelastic properties of the cells change during cell cycle. From the maps of modulus scaling parameter and fluidity (Figure 4a–d, third and fourth column) a slight increase in modulus scaling parameter and decrease in fluidity can be seen. The mean modulus scaling parameter significantly increases during the cell cycle (Figure 5c) from early G1 (gray, 201 Pa)/late G1 (red, 194 Pa) to G1/S (orange, 229 Pa) to S/G2/M (green, 249 Pa, p = 2.30 × 10⁻⁵). The mean fluidity significantly decreases (Figure 5d) from early G1 (gray, 0.214)/late G1 (0.221) to G1/S (0.204) to S/G2/M (green, 0.186, p = 2.23 × 10⁻² and 1.95 × 10⁻³). A similar difference between the different cell cycle phases in terms of viscosity is observed.
deformability was recently measured qualitatively for non-adherent HL60 cells with real-time deformability cytometry.\[31\] The mean modulus scaling parameter $\bar{G}$ and the mean fluidity $\bar{f}$ of many individual cells are highly correlated (correlation coefficient $R = -0.80$, $p = 6.37 \times 10^{-22}$, t-test) and are distributed along a line in a semi-log plot (Figure 5e), commonly denoted as “master curve” in the literature.\[42\] The distribution along a master curve for a population of cells was reported for other cell lines previously\[35,39,43\] and is here now also shown for WM938 cells. Moreover, we found that the location of cells on the master curve depends on the cell cycle phase: cells in the G1, G1/S, and S/G2/M phases are located predominantly on the lower right, in the center, and on the upper left of the scatter plot, respectively. This finding is even more apparent when viewing the cells’ mean values (Figure 5e, inset). The interpretation of this behavior is still unclear, but as cell stiffness and fluidity are also correlated with traction forces,\[44\] this behavior may be explained by increased actomyosin contraction and pressure generation,\[45\] increased traction forces,\[46\] or stiffening of the actin cortex\[47\] during cell cycles and division.

The modulus scaling parameter and the fluidity are also correlated on a subcellular level on the same master curve as from the population of cells (Figure S6a–d, Supporting Information), in line with recent findings obtained with scanning ion conductance microscopy.\[48\] Despite the changes in volume and area, the height skewness\[49\] does not change during the cell cycle (Figure S6e, Supporting Information).

### 3. Conclusion

In summary, we presented a home-built, high-speed AFM for small cantilevers with a large scan range, optical access, and environmental control. This allowed us to map the mechanical properties of whole cells with combined phase-contrast and fluorescence microscopy and to identify similar fiber structures in Young's modulus and actin fluorescence images. The combination of FCFM and RCCM allowed us to measure viscoelastic properties of live cells in both the time and frequency domain up to 5 kHz and to correlate them to actin stress fibers, showing that the viscoelastic modulus scaling parameter, fluidity, and Newtonian damping coefficient strongly differ on and off stress fibers. Finally, we investigated the dependency of morphology and viscoelastic mechanical properties of WM938 cells on the cell cycle phase and found significant differences in volume, area, and – most interestingly – also in their viscoelastic modulus scaling parameter and fluidity. We showed for the first time that viscoelastic properties of live cells are clustered at different locations along the master curve depending on the cell cycle phase. In particular, cells in the S/G2/M phase are stiffer and less fluid-like on average than cells in the G1 phase.

### 4. Experimental Section

#### Optical Setup

The system was mounted on a commercial optical microscope (Eclipse Ti-S, Nikon Corporation, Tokyo, Japan) with a changeable objective (20×, NA 0.45, Ph1, Nikon), a fluorescence module using an epi-fluorescence illuminator (CHGFI Intensilight, Nikon), and a low-noise CMOS camera (DS-Qi2, Nikon).

#### Cell Culture: U2OS cells expressing GFP-labeled actin (BioCat GmbH, Heidelberg, Germany) were cultured in Dulbecco’s modified Eagle’s medium (DMEM, Biochrome GmbH) supplemented with 10% (v/v) fetal bovine serum (Biochrome GmbH, Berlin, Germany), 2 × 10^{-5} M l-alanyl-l-glutamine (Biochrome GmbH), 1% non-essential amino acids (Biochrome GmbH), and 100 U mL^{-1} penicillin–streptomycin (Biochrome GmbH) at 37 °C and 5% (v/v) CO₂. Cells were detached for splitting using 0.05% trypsin/EDTA solution and centrifuged at 265 g for 3 min. Cells were seeded in polystyrene cell culture dishes (CELLSTAR 627160, Greiner Bio-One, International GmbH, Kremsmünster, Austria; Figure 3; Figures S3 and S5, Supporting Information) or in CELLview cell culture dishes (627860, Greiner Bio-One; Figure 1) and imaged in DMEM with supplements (see above) at 37 °C and 5% CO₂ (Figure S3, Supporting Information). In CO₂-independent live cell imaging solution (A14291DJ, Life Technologies Corporation, Eugene, USA; Figure 3), or in L-15 medium (Biochrome GmbH; Figure 3 and Figure S5, Supporting Information) at 37 °C.

#### WM938 melanoma tumor skin cells expressing Fucci were cultured in MCDB153 (Biochrome GmbH), supplemented with 20% (v/v) L-15 medium (Biochrome GmbH), 2% (v/v) heat inactivated fetal bovine serum (Biochrome GmbH), 1% non-essential amino acids (Biochrome GmbH), and 1.68 mmol CaCl₂. They were kept at 36 °C with 5% (v/v) CO₂. Cells were detached for splitting using 0.25% trypsin/EDTA solution and centrifuged at 265 g for 5 min. The WM938 cells duplicated slower than other cells like MEF or U2OS cells and should not be seeded too thin. Cells were seeded on polystyrene cell culture dishes (CELLSTAR 627160, Greiner Bio-One) and imaged in MCDB153 with supplements (see above) at 36 °C and 5% CO₂ (Figures 4 and 5).

#### Platelet Isolation: All procedures were approved by the institutional ethics committee (237/2018BO2) and comply with the declaration of Helsinki. Informed consent was obtained from all participants. Human platelets were isolated as described previously.\[34\] Briefly, human platelets were isolated from freshly drawn blood of healthy volunteers mixed with acid citrate dextrose to avoid coagulation. First, platelet-rich plasma was gained from whole blood by centrifugation at 250 g for 20 min. Tyrode-HEPES buffer with a pH-value of 6.5 was added at a ratio of 1:1. Remaining red blood cells were removed with a second centrifugation step if required (100 × g for 20 min). The platelet-containing supernatant was transferred into a new tube and centrifuged at 900 × g for 10 min to receive a platelet pellet. Finally, washed platelets were gained by careful resuspension of the platelet pellet in Tyrode-HEPES buffer, pH 7.4. For AFM measurements, platelets were added to a “TC”-treated (“tissue culture”) polystyrene culture dish (CELLSTAR 627160, Greiner Bio-One), containing Tyrode-HEPES buffer, pH 7.4, and allowed to adhere and spread at room temperature.

#### AFM Cantilevers: A HS-AFM cantilever (SD-USC-SIN-0.5, NanoWorld AG, Neuchâtel, Switzerland) with a 14 μm length, 4 μm width, 100 nm thickness, a nominal spring constant of 0.2 N m⁻¹, a frequency of the first flexural vibration mode of around 500 kHz in air and 220 kHz in water, and an electron-beam-deposited conical tip with a length of 2.5 μm, a tilt compensation of 8°, and a half opening angle of 9° was used for the live cell investigation with high spatial resolution in Figures 2b, 3, and Figure S5, Supporting Information. However, sharp tips are known to possibly penetrate the cell membrane or damage live cells.\[50–52\] It was observed that the response of a cell to the cantilever highly depends on the cell type. While more sensitive cells like the WM983C used in Figure 4 tended to show a reaction such as a retraction of filopodia and lamellipodia after a few frames, other cells like fibroblasts could often be investigated for hours even with sharp tips. Alternatively, sphere tip cantilevers (SD-Sphere-CONT-M, NanoWorld AG) with a nominal spring...
constant of 0.2 N m\(^{-1}\), a resonant frequency of 13 kHz, and a tip diameter of 2 \(\mu\)m were used to reduce possible damage to cells (Figures 2a, 4, 5, and Figures S3 and S6, Supporting Information). As the indentation into the cells was typically a few 100 nm, the contact radius and therefore the lateral resolution was typically between 0.5 and 1 \(\mu\)m and was therefore not a limiting factor at the chosen pixel resolution. The contact mode measurement (Figure S2, Supporting Information) was performed using a PPP-NCH cantilever (NanoWorld AG) with a pyramidal tip and a nominal spring constant of 19.5 N m\(^{-1}\). The spring constants of the cantilevers were calibrated using the thermal noise method.[35,36]

### Viscoelastic Measurement Modes: A combination of force clamp force mapping (FCFM)[35] and resonance compensating chirp mode (RCCM)[35] allowed to investigate the viscoelastic properties in both the time and frequency domain at the same position of the sample right after each other. At each pixel, the cantilever was approached to the sample just as in force mapping mode with a constant speed until a predefined trigger force \(F_{\text{Clamp}}\) was reached and the force was then kept constant for the clamp duration \(\Delta t_{\text{Clamp}}\) (0.2 s for Figure 3, 0.1 s for Figure 4) using a force feedback loop. The force increase during the approach was fitted by

\[
F(t) = F_{\text{Clamp}} \left( \frac{t-t_c}{\Delta t_{\text{Clamp}}} \right)^a
\]

(1)

with the free variable \(a\) describing the shape of the force increase and with the clamp force \(F_{\text{Clamp}}\), time at contact \(t_c\), and duration of the force increase \(\Delta t_{\text{Clamp}}\). The topography image was calculated from the “true” (zero force) height at the time at contact \(t_c\). For FCFM, the cell was modeled as a power-law material with a shear creep compliance

\[
J(t) = \frac{1}{G_0} \left( \frac{t}{t_c} \right)^\beta
\]

(2)

In contrast to RCCM (see below), for FCFM the cell with a single power-law model was modeled, as the contribution of the viscous damping term is relevant only in the higher frequency range above several kilohertz,[37] which corresponds to the time range below 1 ms; the contribution of the viscous damping term is therefore neglected here. Assuming a constant Poisson’s ratio \(\nu\), the indentation becomes

\[
\delta(t) = \left[ \int_0^t J(t-t') \frac{dF}{dt} dt' \right]^{1/2}
\]

\[
= \left[ CF_{\text{Clamp}} \frac{1}{G_0} \left( \frac{t-t_c}{\Delta t_{\text{Clamp}}} \right)^\beta \left( \frac{\Delta t_{\text{Clamp}}}{t-t_c} \right)^a \beta (a+1) \right]^{1/2}
\]

(3)

with the modulus scaling parameter \(G_0\) and the fluidity \(\beta\) (also referred to as the power-law exponent) as free parameters and a time scale factor \(t_0\) (arbitrarily set to 1 s), the incomplete beta function \(B\), and a tip-geometry dependent variable \(C = \pi(1-\nu)/(4 \tan \alpha)\) with the half opening angle of the cone shaped tip \(\alpha\) and the Poisson’s ratio \(\nu = 0.5\) for live cells here. For details of the calculation and derivation see Hecht et al.[35]

Immediately after the force clamping, the z-scanner was oscillated with an exponential frequency chirp using RCCM (amplitude 20 nm, frequency 1 Hz–6.5 kHz, duration 0.2 s).[35] The complex shear modulus was calculated using

\[
G'(\omega) = \frac{k \pi(1-\nu)}{B_0 \tan \alpha} \frac{\delta'(\omega)}{\delta(\omega)}
\]

(4)

where \(G'(\omega)\) and \(G''(\omega)\) are storage and loss modulus, respectively, \(k\) the cantilever spring constant, \(\delta'(\omega)\) the indentation after the force clamping, \(\delta(\omega)\) the Fourier-transformed deflection, and \(\delta'(\omega)\) the Fourier-transformed indentation.[36] The complex shear modulus was fitted by a power-law model[36,37]

\[
G'(\omega) = G_0 \left[ \beta \left( 1 + i \eta(\beta) \right) \right]^{\beta} \left( \frac{\omega}{\omega_0} \right)^\beta + i\omega G''(\omega)
\]

(5)

where \(G_0\) is the modulus scaling parameter, \(\beta\) the fluidity, \(\mu\) the Newtonian viscous damping coefficient, \(\omega_0\) a frequency scale factor set to \(\omega_0 = 1\ \text{rad} \mbox{s}^{-1}\) (allows a direct comparison with the time domain data with \(t_0 = 1\ \text{s}\)), \(\eta(\beta) = \Gamma(1-\beta) \cos(\pi\beta/2)\) where \(\Gamma\) denotes the gamma function, and \(\eta(\beta) = \tan(\pi\beta/2)\) the hysterescivity.

### Statistical Analysis: Data processing and statistical tests were performed in Igor Pro (WaveMetrics Inc., Portland, OR). AFM raw data was analyzed as described above. Topography images were flattened using first-order line fitting. To avoid an influence of the underlying stiff substrate on the modulus scaling parameter, pixels with height values below 0.5 \(\mu\)m were excluded from the analysis. As the modulus scaling parameter values were found to be log-normally distributed,[35,36] the geometric mean and the geometric standard error were calculated and statistical tests were performed on logaritimized values. Data is shown as (geometric) mean ± standard error and the sample size is given in the respective figure captions. Statistical significance was tested using the two-sided t-test or Tukey’s test as indicated and the data were considered as statistically significant for \(p < 0.05\).

### Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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### Conflict of Interest

The authors declare no conflict of interest.

### Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

### Keywords

fast force mapping, FUCCI, high-speed atomic force microscope, platelets, power-law rheology, U2OS cells, WM938 cells

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