Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary Information for:

Mechanics of Migrating Platelets Investigated with Scanning Ion Conductance Microscopy

Jan Seifert*, Johannes Rheinlaender*, Hendrik von Eysmondt, and Tilman E. Schäffer

Institute of Applied Physics, University of Tübingen

Auf der Morgenstelle 10, 72076 Tübingen, Germany.

* These authors contributed equally

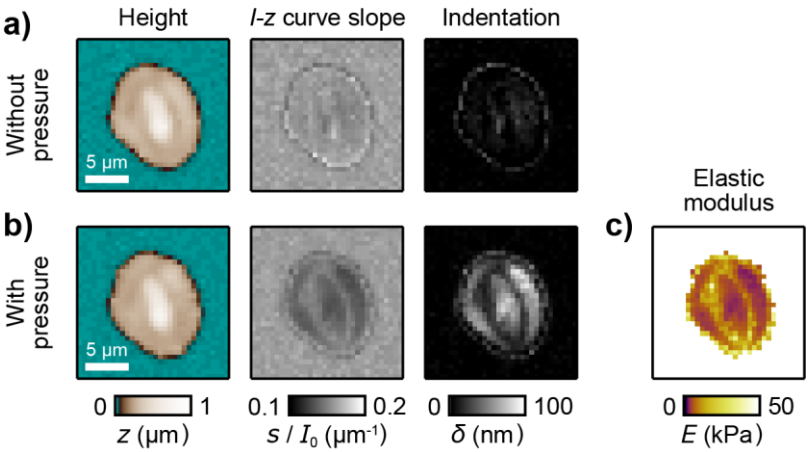
Correspondence to:

E-Mail: johannes.rheinlaender@uni-tuebingen.de & tilman.schaeffer@uni-tuebingen.de

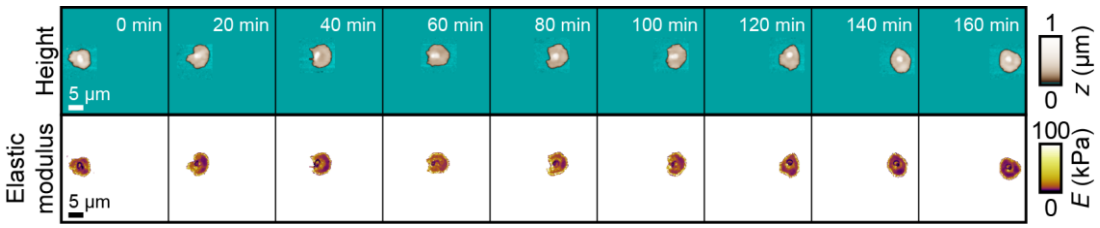
Telephone: +49 7071 29 76030

Fax: +49 7071 29 5093

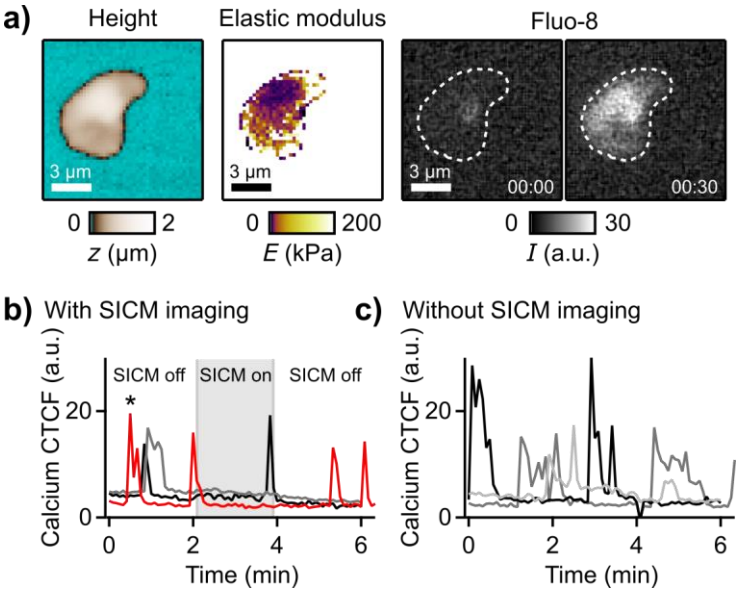
Supplementary Figures



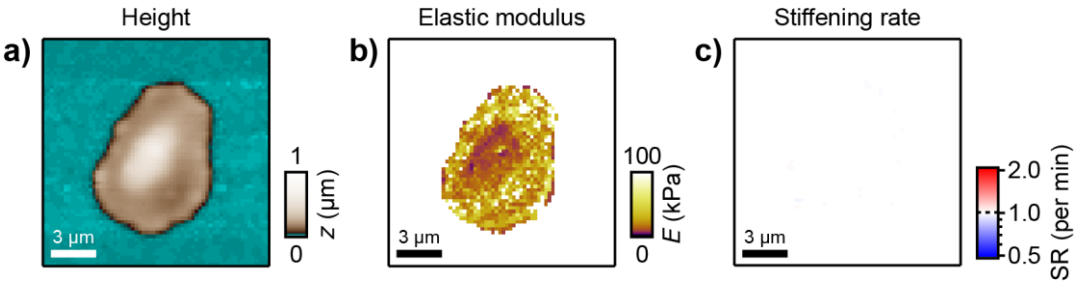
Supplementary Figure S1 | SICM stiffness mapping. Topography image, slope of the *I-z* curves between 99% and 98% of the saturation current, and indentation depth of a living platelet **(a)** without pressure and **(b)** with 10 kPa pressure applied to the nanopipette. **(c)** Elastic modulus map for the measurement shown in (b). Image resolution in a) – c): 32×32 pixels (546.9 nm pixel size).



Supplementary Figure S2 | Topography and elastic modulus of a migrating platelet. Sequence of topography images and elastic modulus maps of the migrating platelet shown in Fig. 3. Pixels outside the scan range were set to a height of $z = 0$. Image resolution: 64×64 pixels (187.5 nm pixel size).



Supplementary Figure S3 | Calcium signaling in platelets during SICM imaging. **(a)** Topography image, elastic modulus map, and calcium signal of a non-migrating platelet. Platelets were incubated with 4 µM calcium dye (Fluo-8 AM, ab142773, abcam) for 30 min at 37 °C prior to activation and spreading. A fluorescence image was recorded every 5 s with an acquisition time of 50 ms using a monochrome digital camera (DS-Qi2, Nikon) and a 60x objective. Time is displayed as mm:ss. **(b)** Calcium signal (corrected total cell fluorescence, CTCF) of platelets before (0 – 2 min), during (2 – 4 min), and after (4 – 6 min) SICM imaging and of **(c)** control platelets without SICM imaging. The red line shows the calcium signal of the platelet shown in a). Platelets sporadically showed calcium oscillations (one marked with an asterisk). SICM imaging did not remarkably alter the calcium signal. Image resolution: 48×48 pixels (250 nm pixel size).



Supplementary Figure S4 | Non-migrating platelets usually show a homogenous subcellular stiffness distribution. (a) Topography image, **(b)** elastic modulus map, and **(c)** stiffening rate map of a non-migrating platelet. The stiffening rate of the whole platelet is close to 1 (white when using the same color scale as in Fig. 3c). Image resolution: 64×64 pixels (234.4 nm pixel size).

Supplementary Tables

Supplementary Table 1 | Pipette puller parameters. Parameters used to pull pipettes with an inner opening diameter of 200 nm or 80 nm. Pipettes were pulled from borosilicate glass capillaries (1B100F-4, World Precision Instruments Germany GmbH, 1.0 mm outer diameter, 0.58 mm inner diameter) using a CO_2 laser-based pipette puller (P-2000, Sutter Instruments).

Pipette diameter 200 nm					
LINE	HEAT	FIL	VEL	DEL	PUL
1	350	4	30	200	0
2	350	4	30	200	0
3	350	4	26	140	118

Pipette diameter 80 nm					
LINE	HEAT	FIL	VEL	DEL	PUL
1	350	4	30	200	0
2	350	4	30	200	0
3	370	4	36	140	210

Supplementary Videos

Supplementary Video 1: SICM image sequence of the migrating platelet shown in Fig. 1. Image resolution: 112×112 pixels (125 nm pixel size).

Supplementary Video 2: SICM image sequence of the migrating platelet shown in Fig. 2a and b. Image resolution: 60×60 pixels (200 nm pixel size).

Supplementary Video 3: SICM image sequence of the non-migrating platelet shown in Fig. 2c. Image resolution: 112×112 pixels (125 nm pixel size).

Supplementary Video 4: SICM topography and elastic modulus image sequences of the migrating platelet shown in Fig. 3. Image resolution: 64×64 pixels (187.5 nm pixel size).

Supplementary Video 5: SICM topography and elastic modulus image sequences of the migrating platelet shown in Fig. 4 during the addition of 2 µM cytochalasin D. Image resolution: 64×64 pixels (218.75 nm pixel size).