Electronic Supplementary Material (ESI) for Nanoscale.

SUPPLEMENTARY INFORMATION

High-speed scanning ion conductance microscopy for sub-second topography imaging of live cells

Stefan Simeonov and Tilman E. Schäffer*

Institute of Applied Physics, University of Tübingen,

Auf der Morgenstelle 10, 72076 Tübingen, Germany

*E-mail: tilman.schaeffer@uni-tuebingen.de

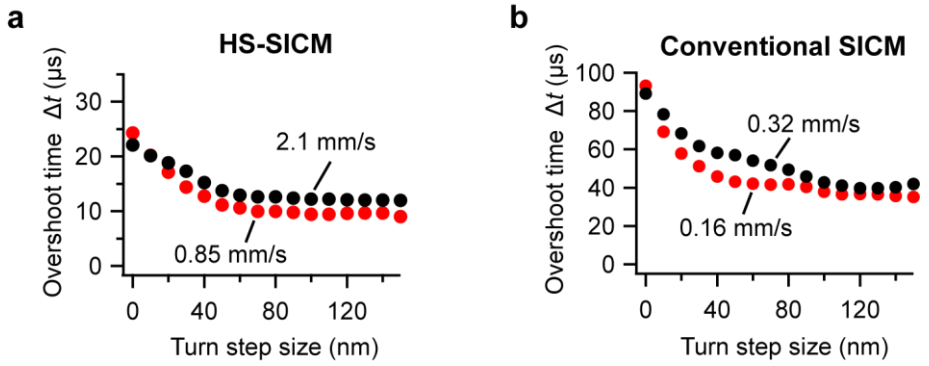

Figure S1: Evaluation of the overshoot time from approach curves (example curves shown in Fig. S2) recorded with **(a)** the HS- and **(b)** the conventional SICM setup in hopping mode with turn step for different turn step sizes and for two different approach speeds (see red and black curves; approach speed indicated in the graph). Increasing the turn step size beyond ≈ 70 nm (HS-SICM) or ≈ 100 nm (conventional SICM) does not further decrease the overshoot time.

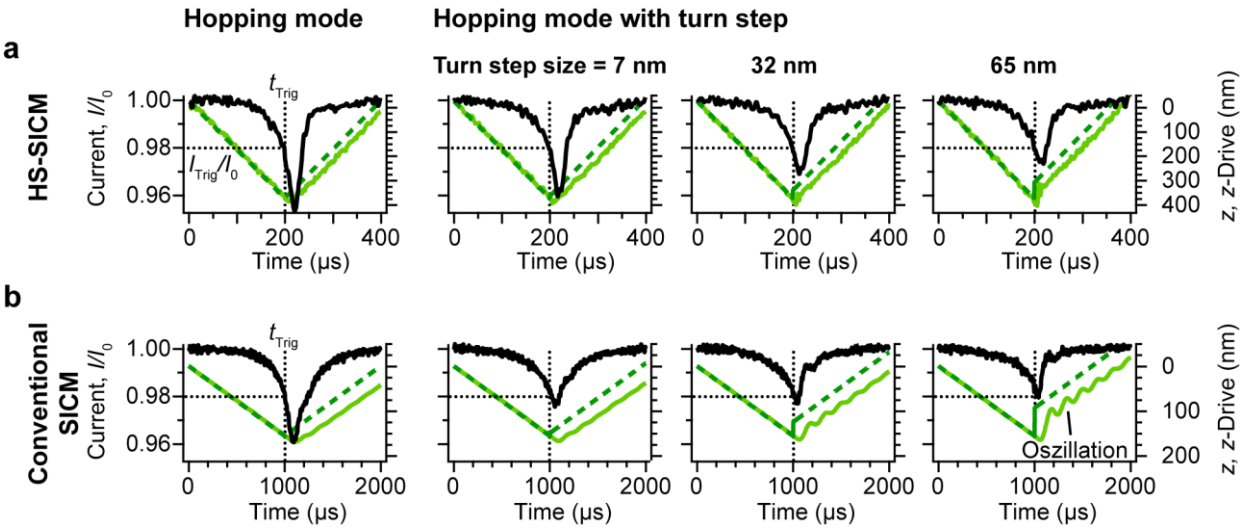

Figure S2: Approach curves (black trace: ion current, green solid trace: *z*-sensor, green dashed trace: *z*-drive) recorded with **(a)** the HS- and **(b)** the conventional SICM setup, in regular hopping mode (left column, turn step size = 0 nm) and in hopping mode with turn step using different turn step sizes (right 3 columns). For the conventional SICM setup, piezo oscillations are observed when using hopping mode with a turn step size larger than 32 nm, likely owing to the large inertial mass of the *z*-scanner. No oscillations are visible for the HS-SICM. Approach velocity was 0.32 mm/s (a) and 0.16 mm/s (b).

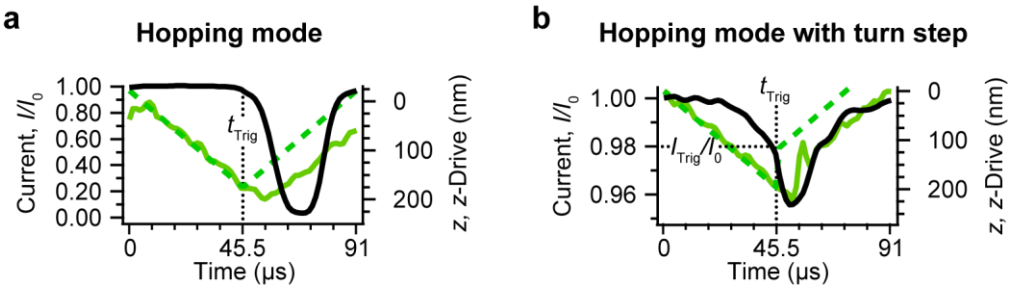

Figure S3: Fast approach curves using an approach velocity of v_{Appr} = 4.8 mm/s (corresponding approach rate: 11.0 kHz) and a current trigger level of I_{Trig} / I_0 = 0.98 (black trace: ion current, green solid trace: *z*-sensor, green dashed trace: *z*-drive) recorded with the HS-SICM **(a)** in regular hopping mode and **(b)** in hopping mode with turn step using a turn step size of 65 nm. In regular hopping mode, the ion current decreases to zero, clearly indicating mechanical contact between the nanopipette and the sample. In hopping mode with turn step, the relative ion current decreases to just 0.955, indicating a non-contact measurement.

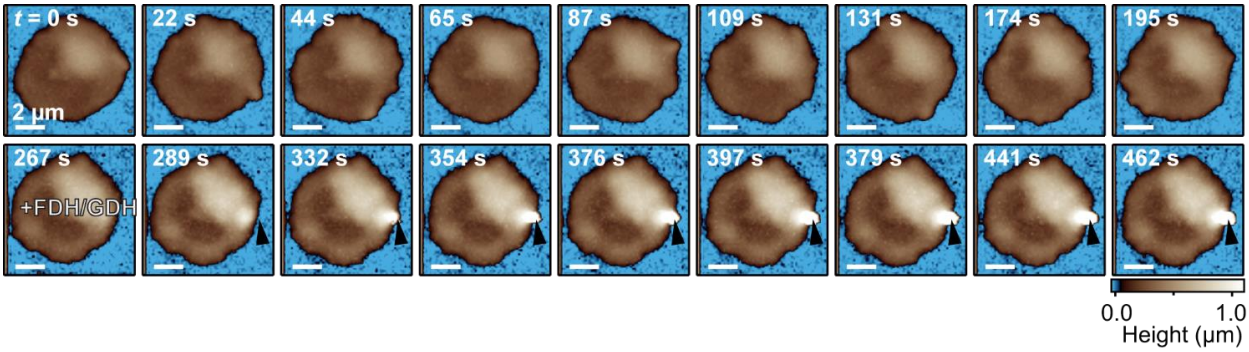

Figure S4: Image sequence of the dynamics of a whole platelet imaged with HS-SICM for 25 min before and 18 min after fixation. Every 5th image of the full image sequence [600 images in 45 min, supplementary movie Mov. 4] is shown. The image at time t = 0 s corresponds to frame #305 from Mov. 4. Adding fixative at t = 217 s, indicated by the tag "+FDH/GDH" in the following frame, stopped the fast dynamics at the platelet periphery. A fixation artifact (back arrow) appeared, which we commonly observed when fixing platelets. Images were recorded with 64 × 64 pixels at an approach rate 0.94 kHz. The approach velocity v_{Appr} was 1.25 mm/s.

Movies

Movie S1: HS-SICM image sequence with 1000 consecutive images of the platelet in Fig. 4, showing rapid morphological changes along the platelet periphery. The image at time $t = 0$ s in Fig. 4b corresponds to frame #242 in the movie. The sequence was recorded at a rate of 0.6 s/frame (corresponds to 1.7 frames per second) (5 × 5 µm, 32 × 32 pixels, approach rate 2 kHz, approach velocity 1.40 mm/s, turn step size 35 nm). The movie is played at 18× time lapse. Scale bar 2 µm.

Movie S2: HS-SICM image sequence with 386 consecutive images of a platelet showing rapid morphological changes along the platelet periphery. The sequence was recorded at a rate of 1.1 s/frame (4 × 4 µm, 32 × 32 pixels, approach rate 0.94 kHz, approach velocity 1.13 mm/s, turn step size 50 nm). The movie is played at 22× time lapse. Scale bar 1 µm

Movie S3: HS-SICM image sequence with 568 consecutive images of a platelet showing rapid morphological changes along the platelet periphery. The sequence was recorded at a rate of 1.1 s/frame (5 × 5 µm, 32 × 32 pixels, approach rate 0.94 kHz, approach velocity 1.13 mm/s, turn step size 50 nm). The movie is played at 22× time lapse. Scale bar 1 µm

Movie S4: HS-SICM image sequence with 600 consecutive images of a whole platelet 25 min before and 18 min after fixation. The fixative was added at $t = 1520$ s (just after frame #349 and 50 s before frame #350), indicated by the tag "+FDH/GDH" (for formaldehyde / glutaraldehyde). The sequence was recorded at a rate of 4.3 s/frame (10 × 10 µm, 64 × 64 pixels, approach rate 0.94 kHz, approach velocity 1.13 mm/s, turn step size 50 nm). The movie is played at 87× time lapse. Scale bar 2 µm

Movie S5: HS-SICM image sequence with 100 consecutive images of a live A6 cell in Fig. 5 showing highly dynamic microvilli at its surface. The image at time $t = 0$ s in Fig. 5a corresponds to frame #0. The sequence was recorded at a rate of 1.4 s/frame (5 × 5 µm, 64 × 64 pixels, approach rate 2.94 kHz, approach velocity 2.68 mm/s, turn step size 50 nm). The movie is played at 28× time lapse. Scale bar 1 µm.

Movie S6: HS-SICM image sequence with 1000 consecutive images of a live U2OS cell in Fig. 6 showing rapid dynamics at the edge and the formation of large membrane protrusions, propagating across the cell in a wave-like motion. The image at time $t = 0$ s in Fig. 6b corresponds to frame #161. The sequence was recorded at a rate of 6 s/frame (10 × 10 µm, 100 × 100 pixels, approach rate 1.67 kHz, approach velocity 1.88 mm/s, turn step size 50 nm). The movie is played at 100× time lapse. Scale bar 2 µm.