Biophysical Journal

Supporting Material

Feeling for Filaments: Quantification of the Cortical Actin Web in Live Vascular Endothelium

Cornelius Kronlage,[1] Marco Schäfer-Herte,[2] Daniel Böning,[3] Hans Oberleithner,[1] and Johannes Fels[1,2,*]

[1]Institute of Physiology II, [2]Institute of Cell Dynamics and Imaging, and [3]Institute of Medical Physics and Biophysics, University of Münster, Münster, Germany

Figure legends:

Supplementary figure 1: Contact-mode scan of an endothelial cell‘s apical cytoskeleton – Difference of Gaussians filtering applied to height data in comparison with error images. (a) raw height data; (b) height data after processing with the same routine as used for force-mapping mode acquired images; (c) and (d) respective trace and retrace error images. It can be appreciated that the resolution in the filtered contact-mode image is much lower when compared to the error mode images or filtered force-mapping mode images (fig. 4-6). Scale bars are 1 µm.

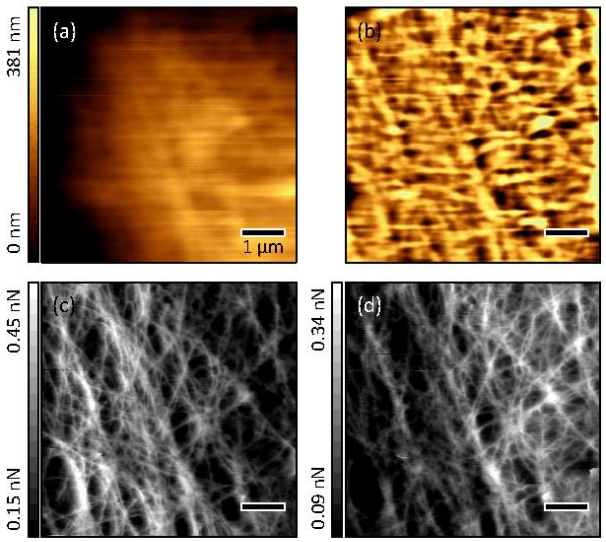
Supplementary figure 2: Intracellular calcium concentration as measured by Fluo4 fluorescence during mechanical indentation of the cell by force-mapping mode AFM. As indicated, after a short control phase AFM scanning is started and at the end of the experiment Ionomycin is added to a concentration of 2.5 µM in order to obtain positive control values. The black trace represents the scanned cell, the red trace corresponds to a non-affected control cell. AFM scanning does not induce any apparent increase in the intracellular calcium concentration, whereas Ionomycin expectedly elicits a strong response. The steady decay of fluorescence intensity over the course of the control phase and AFM scanning period is likely to be caused by photobleaching.

Supplementary figure 3: Movement-related distortion apparent in force-mapping mode imaging AFM. (a) processed force-mapping mode scan; (b) and (c) Lifeact-mKate2 confocal views of the same area acquired much faster at different points of time. In (a) the slow scan direction is indicated by a white arrow, blue arrows signify the lines acquired at the same time as (b) and (c) respectively. The dashed blue line approximates to a large fibre structure in (a) and is overlayed in the same position in (b) and (c). When comparing AFM and fluorescence images a distortion of the AFM image due to the rightward motion of the actin structures over time can be appreciated. Scale bars are 1 µm.

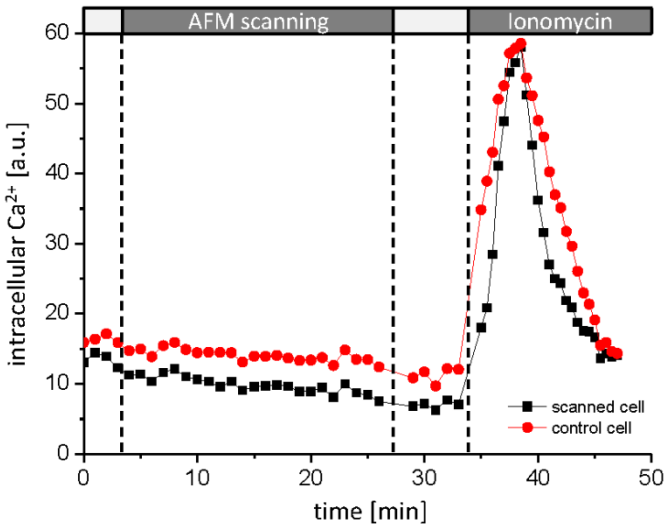
Supplementary figure 4: Estimation of an upper boundary of imaging resolution by inspection of the smallest apparent features, for contact-mode (a)-(c) and force-mapping mode imaging (d)-(f) respectively. (a) and (d) representative images with enlarged views in (b) and (e) as indicated; cross-section profiles of the dotted lines are shown in (c) and (f). The smallest distances between seperate distinguishable features in the range of 100-150 nm, thus the imaging resolution of AFM in this application can be assumed to be at least equal to this figure. Scale bars are 1 µm for (a) and (d), 500 nm for (b) and (e).

Supplementary figure 5: Number of detected cytoskeleton network ‘holes‘ in force-mapping mode imaging – plot of absolute values for individual control experiments and thus individual cells. It is apparent that the absolute values for this AFM based measurement differ considerably (from experiment to experiment or from cell to cell respectively).

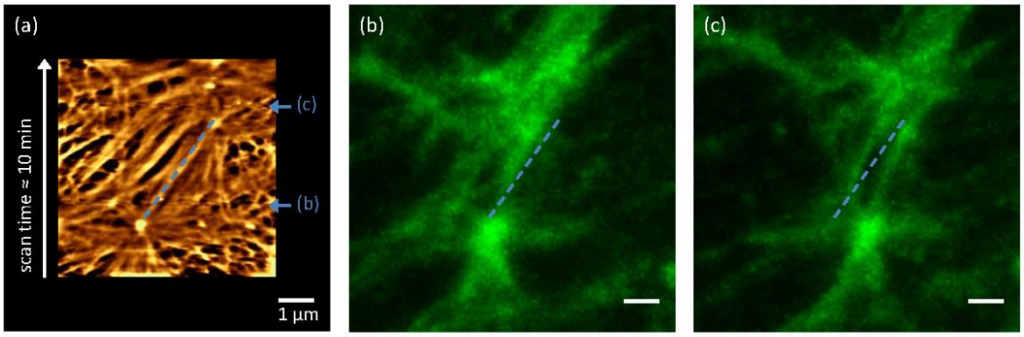
Figures:



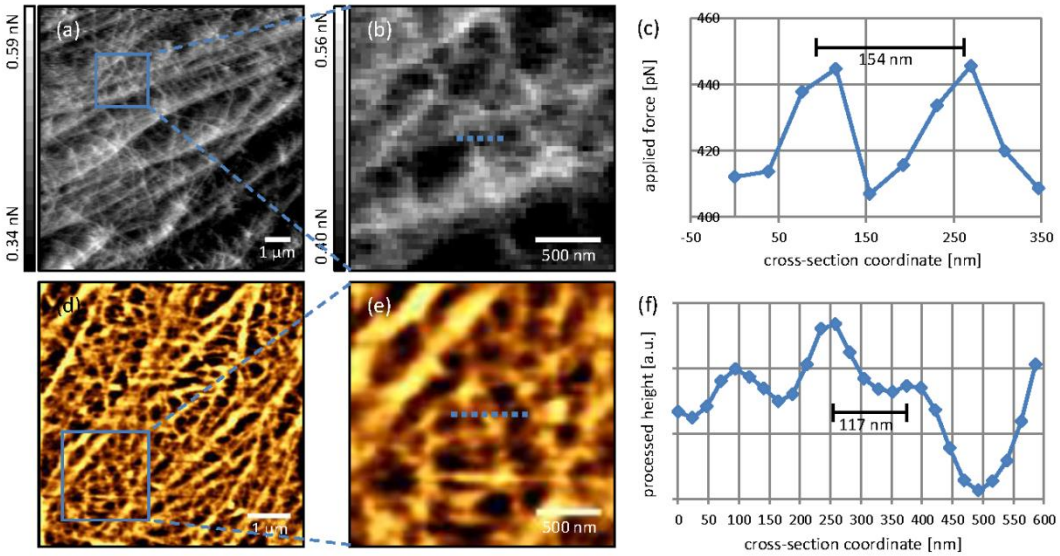
supplementrary figure 1



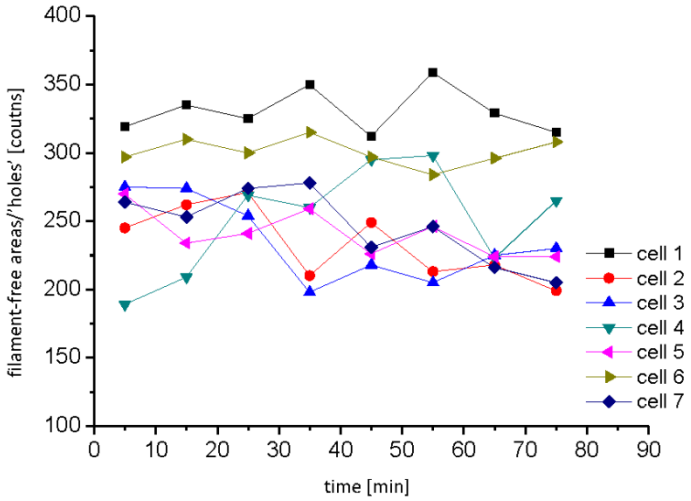
supplementrary figure 2



supplementrary figure 3



supplementrary figure 4



supplementrary figure 5