Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary Information for

Time-resolved MIET measurements of blood platelet spreading and adhesion

Anna Zelená,[a] Sebastian Isbaner,[b] Daja Ruhlandt,[b] Anna Chizhik,[b] Chiara Cassini,[a] Andrey S. Klymchenko,[c] Jörg Enderlein,[b] Alexey Chizhik[b] and Sarah Köster*[a,d,e]

[a] *Institute for X-Ray Physics, University of Göttingen, Friedrich-Hund-Platz 1, 37077 Göttingen, Germany.*
[b] *Third Institute of Physics – Biophysics, University of Göttingen, Friedrich-Hund-Platz 1, 37077 Göttingen, Germany.*
[c] *Laboratoire de Biophotonique et Pathologies, UMR 7021 CNRS, Université de Strasbourg, Faculté de Pharmacie, 74, Route du Rhin, Illkirch 67401 Cedex, France.*
[d] *German Center for Cardiovascular Research (DZHK), partner site Göttingen, Germany.*
[e] *Cluster of Excellence "Multiscale Bioimaging: from Molecular Machines to Networks of Excitable Cells" (MBExC), University of Goettingen, Germany.*

*sarah.koester@phys.uni-goettingen.de

Supplementary figures

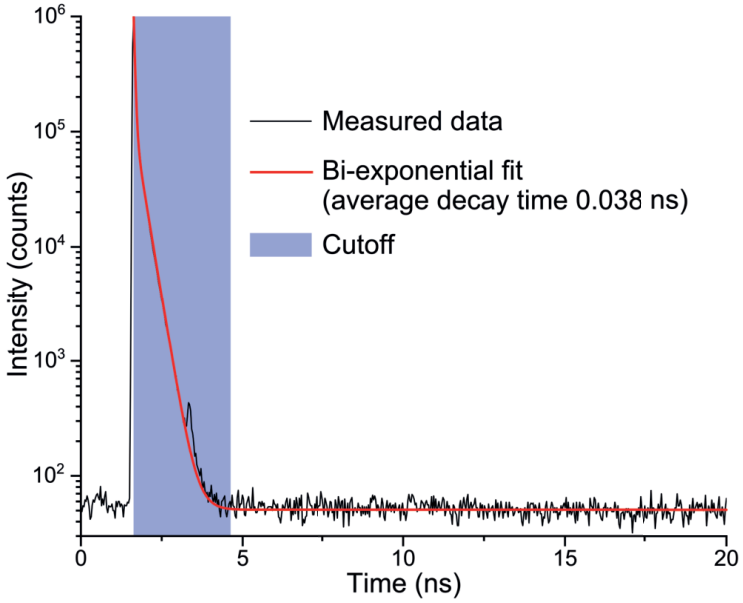

Fig. S1 The instrument response function (IRF) of the microscope that was used for static MIET measurements. The curve was measured until reaching 10^6 counts at the maximum. The additional small peak at $\sim$ 3 ns corresponds to a reflection of the excitation light from one of optical elements. Its intensity is, however, only 0.04% of the main maximum and does not lead to any change in the measured fluorescence decay curves.

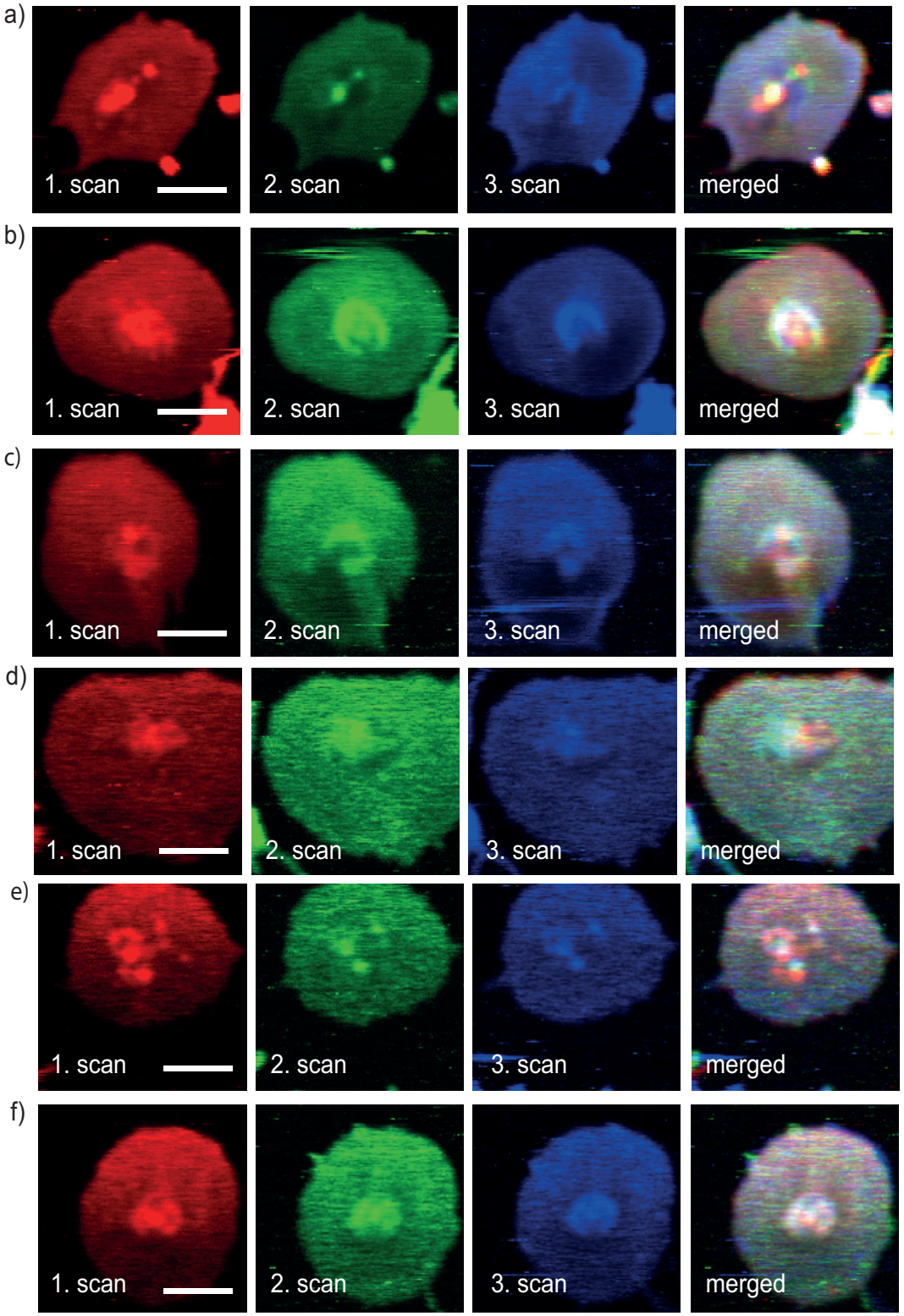

Fig. S2 Triple scan for each platelet studied in static conditions. Each subfigure (a-f) represents a different platelet. Left to right: Individual scans of each blood platelet as well as a merged image from these three individual scans. The data show that the platelet area remains unchanged from the time point of the first scan on. Thus, we add the data of all three scans before further analysis. The total acquisition time of the three scans varies from 5.1 to 10.2 min depending on the size of the scanned area. Scale bars correspond to 3 μm.

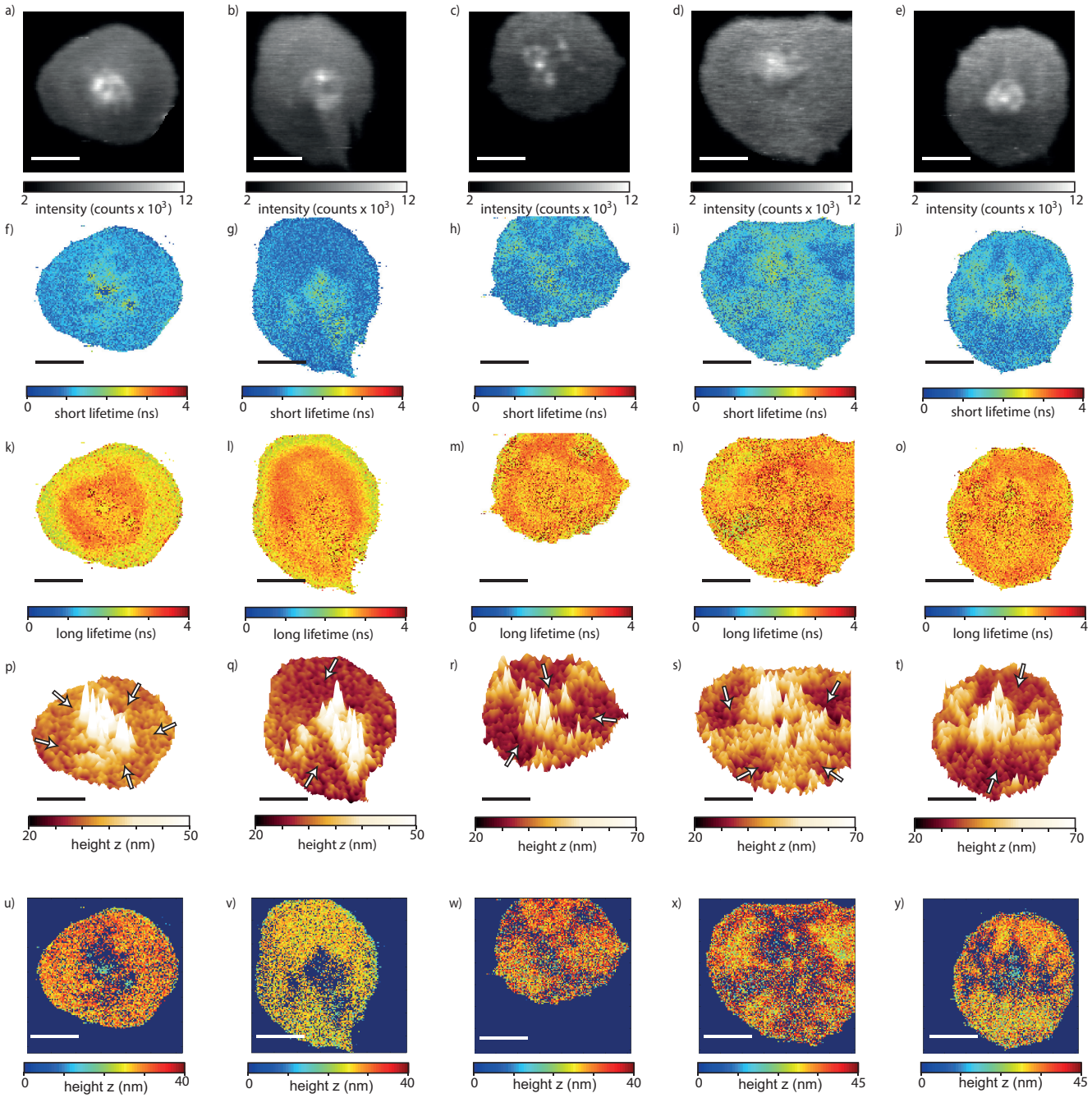

Fig. S3 Additional examples of spread platelets. (a-e) Fluorescence intensity images. (f-j) Maps of the short life time components. (k-o) Maps of the long life-time components. (p-t) 3D reconstruction of the basal membrane; note the areas with smaller membrane-to-substrate distance (marked by the white arrows) in each platelet; for platelets (p-r) the color scale bar range is reduced compared to (s-t). The difference is due to a different noise level of the reconstruction. For clarity, the color scaling is chosen such that central bright areas show values above the color bar range. (u-y) Maps of membrane-to-substrate distance below the second tercile threshold. This representation highlights the localization of the areas with a smaller distance to the surface. The choice of the threshold is detailed in Fig. S4. Scale bars correspond to 3 μm.

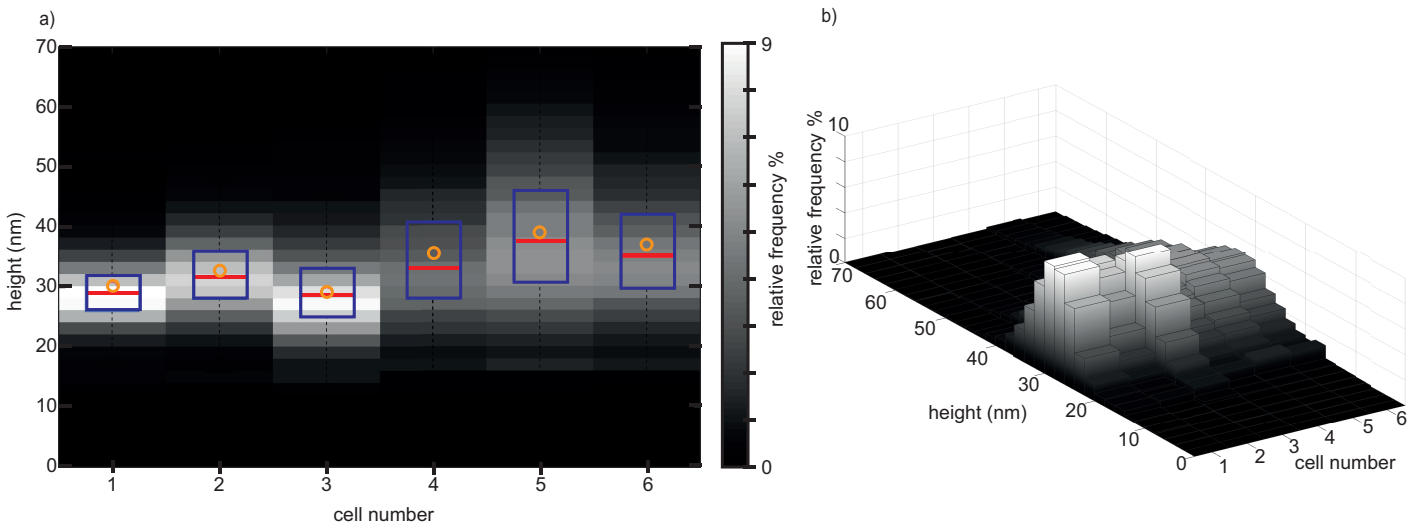

Fig. S4 Histograms of the distance between gold layer and basal membrane for each of the six platelets analyzed here. (a) Top view of all histograms with the relative frequency color coded (see color scale). Each histogram was normalized by the total number of pixels. The overlaid boxplots show the 25-75 % percentile, the orange circle denotes the mean, and the red line the median. Outliers are less than 2% and hidden for clarity. Outliers occur when the separation of the basal and the apical membrane contributions is inaccurate. (b) 3D view of all histograms next to each other. The average height of the basal membrane is 34 ± 4 nm.

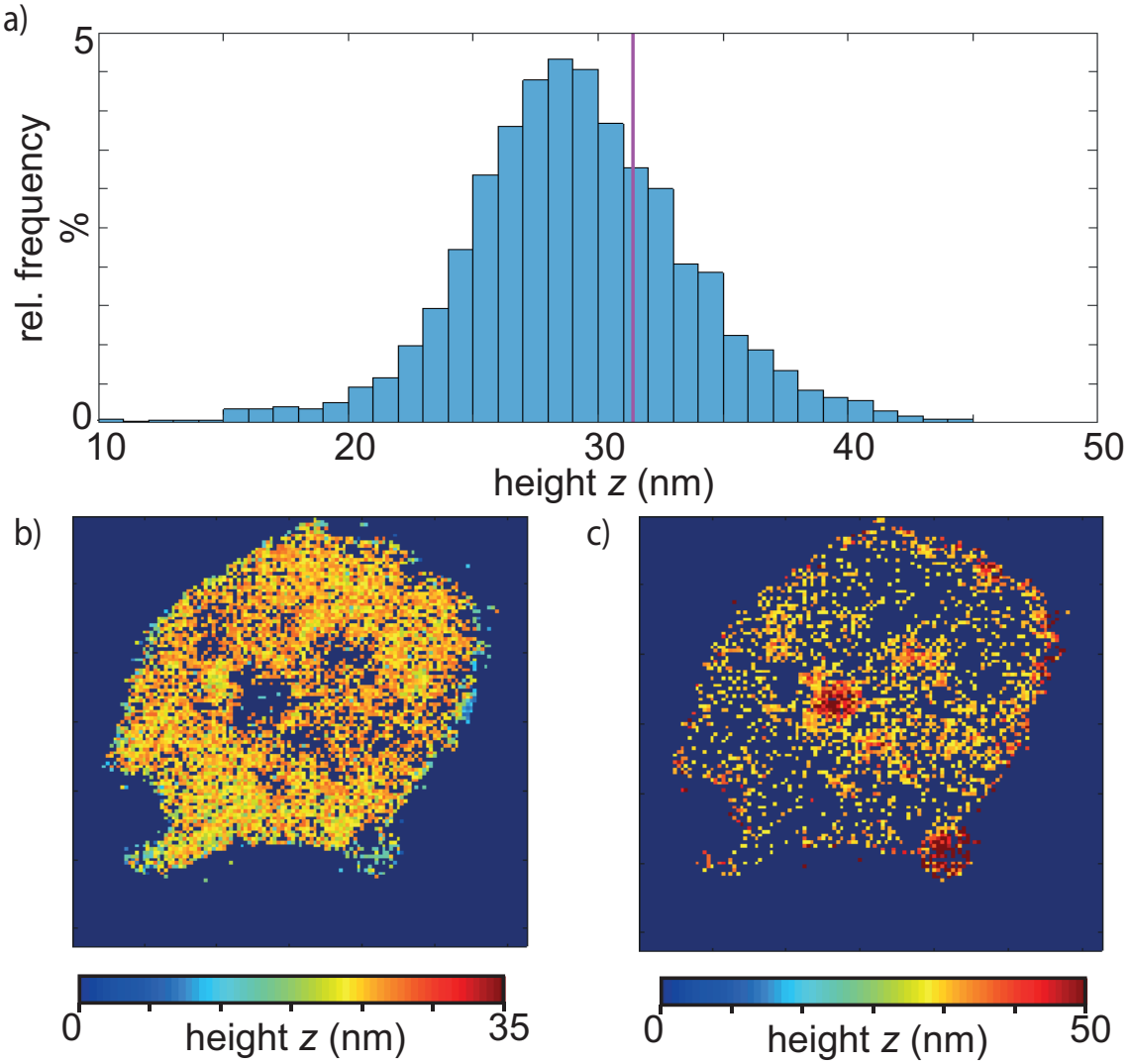

Fig. S5 Analysis of the basal membrane profile. (a) Height histogram for the basal membrane after removal of outliers. Outliers are identified by using the "median absolute deviation" (MAD). For each data point, the MAD computes the absolute deviation from the data's median and defines the median of all deviations. We define an outlier as a value that is more than three times the MAD away from the median.[1] As threshold we set the 2nd tercile, shown as the vertical purple line. (b) Height values below the threshold. (c) Height values above the threshold.

Supplementary movies

Movie S1 Adhesion and spreading of a blood platelet upon stimulation with thrombin. The platelet shows very fast dynamics, especially at the outermost rim of the cell. Data were recorded by rapid FLIM with a scan speed of 3.3 fps (0.3 s per frame). For final display, 15 sequential images each are added to increase the signal-to-noise ratio. The sequence is modified by temporal binning to 4.5 s per frame and shows intensities. Scale bar corresponds to 3 μm.

Movie S2 Adhesion and spreading of a blood platelet upon stimulation with thrombin, segmented into two areas. Each frame shows the averaged height for the outermost rim and the central area without the bright spot for later time points. Frame dimensions are 15 $\times$ 15 μm^2. The differing behavior of the rim and the rest of the cell is clearly visible.

References

[1] C. Leys, C. Ley, O. Klein, P. Bernard and L. Licata, *J. Exp. Soc. Psychol.*, 2013, **49**, 764–766.