Supporting Material for

High resolution correlative microscopy: Bridging the gap between Single Molecule Localization Microscopy and Atomic Force Microscopy

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1. MEF cell sample preparation

The mouse embryonic fibroblast (MEF) cells are kindly provided by Dr. Luca Scorrano, and the mEos2-paxillin-22 vector is kindly provided by Dr. Michael Davidson. The MEF cells are grown in DMEM supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin, 1% non-essential amino acids and 1% glutamine, at 37 °C with 5% CO_2. A 25 mm diameter round cover slip (# 1.5, Electron Microscopy Sciences) is prepared by first treating it with an oxygen plasma for 5 min and then incubating it with PBS containing 50 µg/ml fibronectin (bovine plasma fibronectin, Invitrogen) for 30 min at 37 °C. To remove the excess of fibronectin, the cover slip is washed 1× with PBS. The cells are transfected by electroporation (Neon Transfection System, Invitrogen). The electroporation is performed on ~500,000 cells using 2 µg of DNA, using 1 pulse of 1350 V with a width of 35 ms. The transfected cells are seeded on the cover slip and grown in DMEM supplemented with 10% fetal bovine serum, 1% non-essential amino acids and 1% glutamine, at 37 °C with 5% CO_2. At least 24 h after transfection, the cells are washed 1× with PHEM (60 mM Pipes, 25 mM HEPES, 10 mM EGTA, and 2 mM $MgCl_2$ at pH 6.9) and fixed by incubating them in PHEM with 4% paraformaldehyde at 37°C for 30 min. After removing the fixative, the cells are again washed 3× with PHEM and the cover slip is glued into a custom made holder that is positioned on the microscope stage.

2. Imaging procedure

AFM images were then recorded using the Dimension Icon head (Bruker) in peak force tapping mode. Lever A of a Hydra-All-G cantilever (AppNano) with a nominal spring constant of 0.292 N/m was used. Images were recorded at a line rate of 0.5 Hz and a pixel resolution of 512x256.

3. Overlay between AFM and STORM images

Both AFM and STORM imaging can involve various forms of aberrations. This is especially pronounced in the case the optical aberrations in STORM imaging. Correlative imaging therefore should account for these. Additionally, since the images are not necessarily of exactly the same size and of the same region (our STORM images typically cover a larger field of view than the AFM image), accurate overlay also involves the matching of structures in the image.

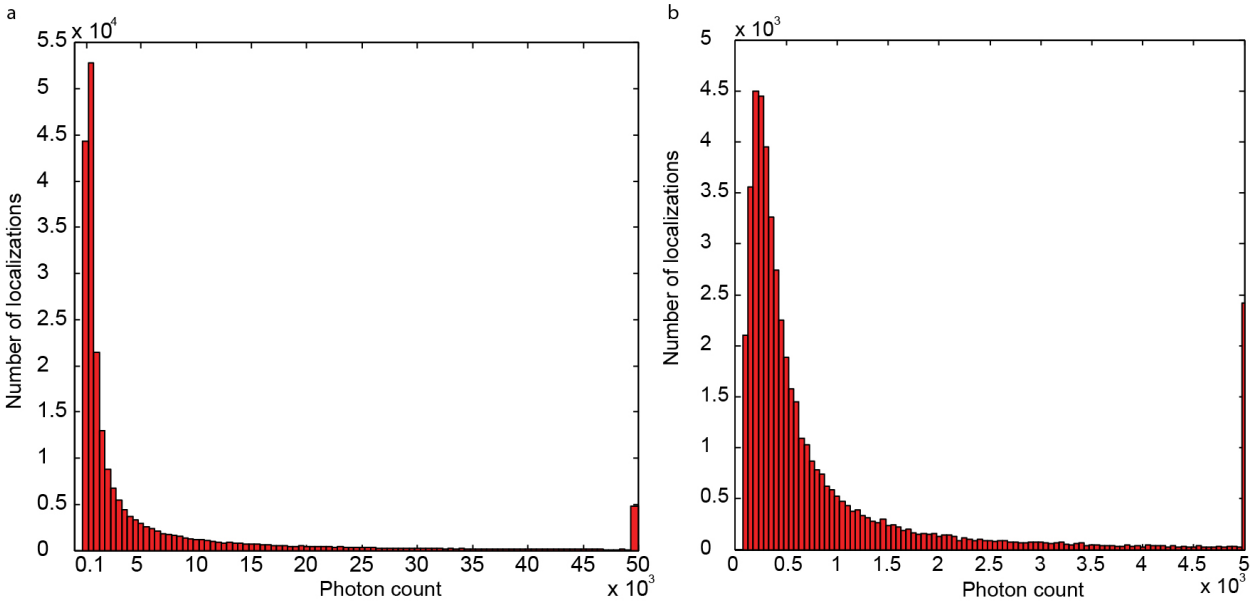
We approximate the net effect of these as an affine transformation of STORM localizations. We then estimate the transformation parameters from data to obtain the more accurate overlay.

The 2D affine transformation can be described as $x' = Ax + By + T_x$ and $y' = Cx + Dy + T_y$, where x' and y' are the coordinates obtained by the transformation of the original x and y coordinates (STORM localizations); A, B, C and D are lumped parameters that can cause scale, shear and rotation operations; and T_x and T_y are translation parameters. We estimate these parameters by the optimization of the overlap between the structures in the STORM and AFM images. The cost function for optimization is computed based on the total number of the affine-transformed STORM localizations with a signal in the corresponding pixel of the AFM image. The optimization and parameter estimation are then done by means of generic algorithm. An example of the estimation process is shown in **Supporting Fig. 4** which shows the overlays before and after the estimation.

For the overlay figures (**Fig. 4g** and **Supporting Fig. 3c**), since the probability map rendering of the dSTORM localizations involved the shifting of the origin estimated by the overlay algorithm to accommodate the localization error distributions, an additional manual translation operation was performed.

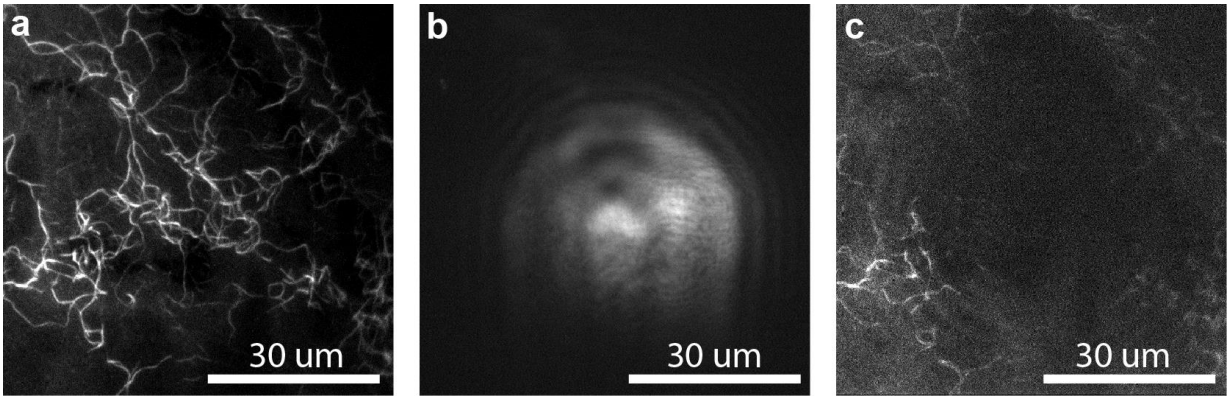
4. Supporting Figures

4.1 Photon count histogram of alexa647 and ATTO488



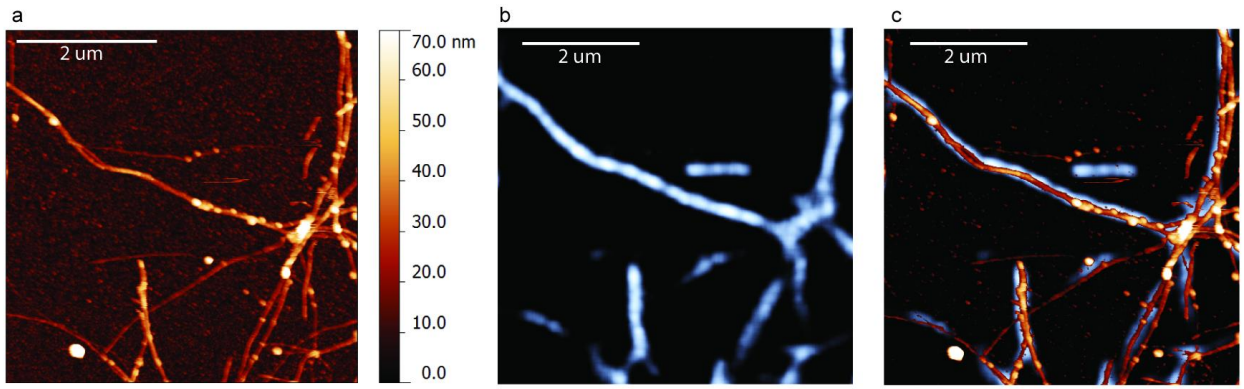
Supporting Fig. 1 Photon count histograms of STORM images. **(a)** corresponding to **Fig 2e** (with alexa647) and **(b) Fig. 3** (ATTO488).

4.2 Bleaching effect of AFM laser on alexa647



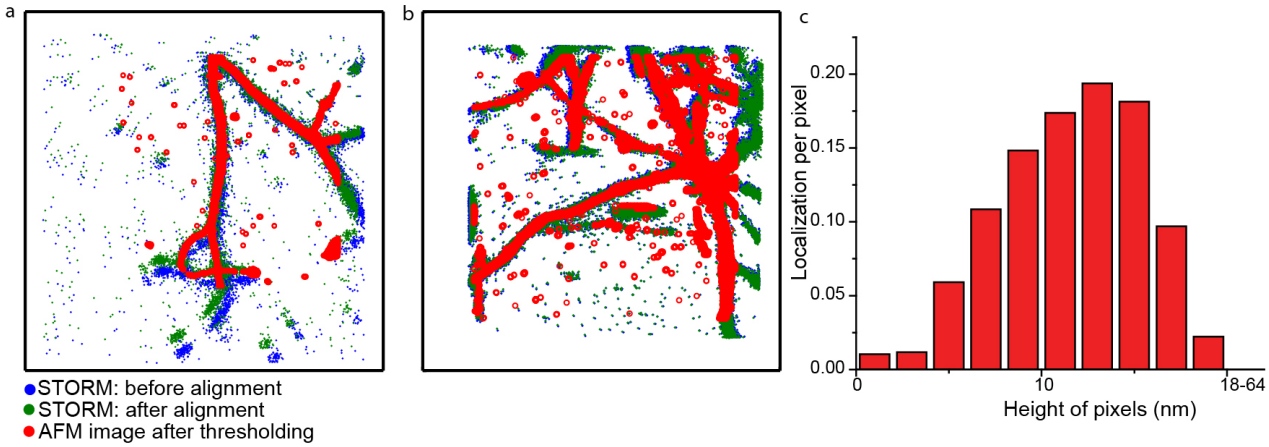
Supporting Fig. 2. TIRF images of F-actin labelled with phalloidin-alexa647 exposed to the laser of the AFM **(a-c)** and effect of dSTORM on F-actin structure. **(a)** TIRF image taken of a fresh sample of F-actin labelled with phalloidin-alexa647 deposited on glass coverslip. **(b)** The AFM tip was then brought close to the sample surface by false engaging without scanning and kept at this position for 30 minutes. **(c)** The fluorescence in the area where the cantilever was positioned was substantially bleached after 30 minutes of exposure to the laser of the AFM, making a subsequent dSTORM image impossible. Therefore for correlated AFM/dSTORM atto488 was used.

4.3 Additional correlated AFM/dSTORM on F-actin



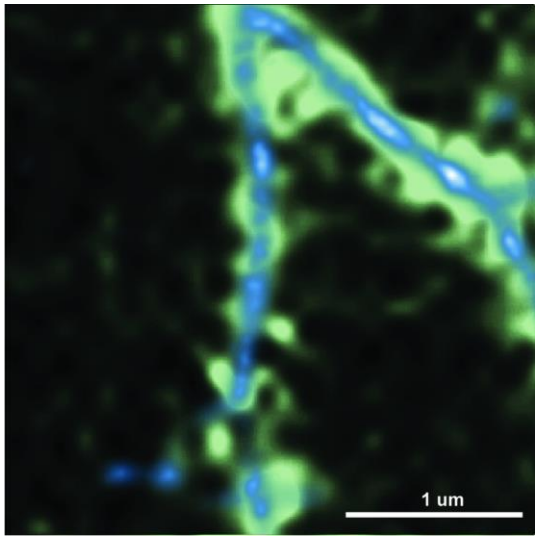
Supporting Fig. 3. F-actin labelled with phalloidin-atto488 imaged by AFM and dSTORM. **(a)** AFM height image **(b)** dSTORM image of the same area **(c)** Overlay of a and b. Details to the overlay is shown in **Supporting Fig. 4b.**

4.4 Alignment process for correlated AFM/STORM



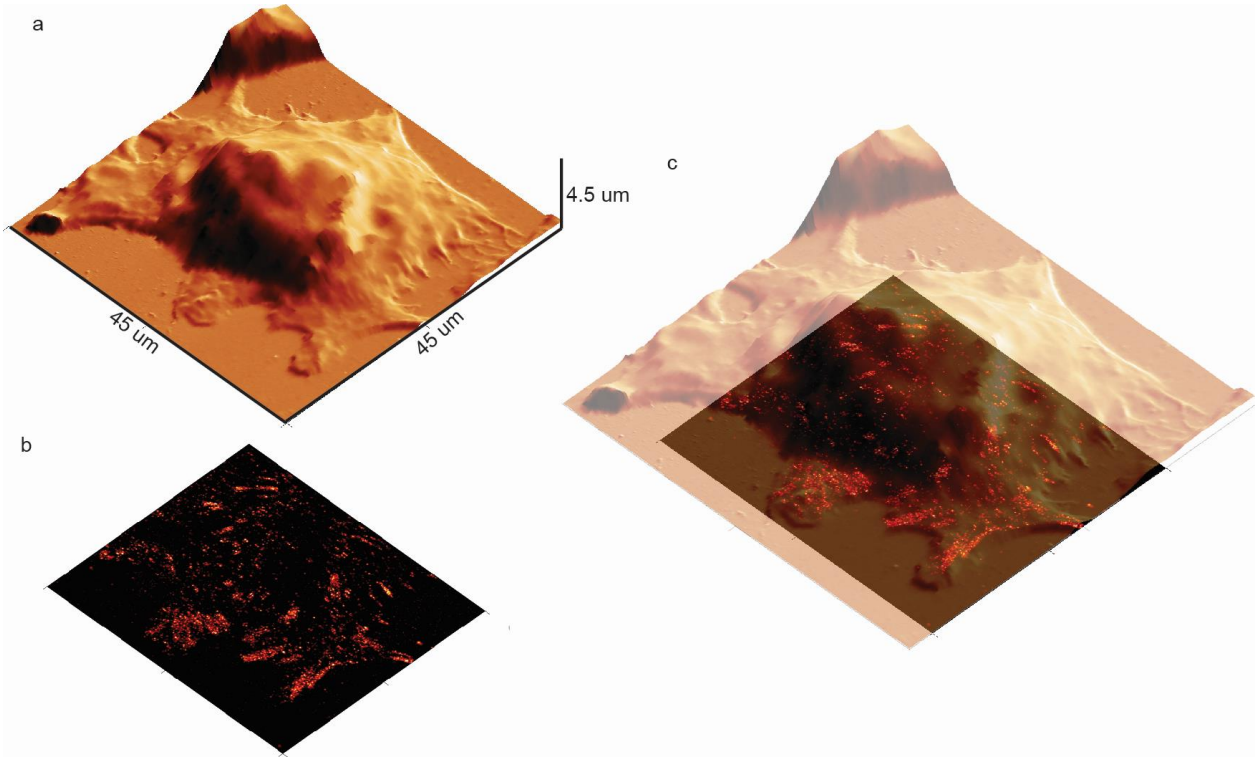
Supporting Fig. 4. Alignment process for STORM images of **Fig 3k (a)** and **Supporting Fig 3 (b)** The localizations obtained from STORM imaging (blue) is transformed by a 2D affine transform to obtain the coordinates (green) that are better aligned with the structure in the AFM image (red). The parameters of the transform are estimated by the procedure mentioned in the Supporting material text. **(c)** Histogram showing the distribution of localizations per area of a particular height as measured by AFM. By correlating dSTORM data and AFM data as shown in **Fig. 4g**, a height value was assigned to pixels of the dSTORM image. The sum of localizations occurring within a bin (bin size 2 nm) was divided by the total number of pixels falling into that particular bin size. Most localizations relative to the number of pixels appeared at heights between 8 nm - 14 nm, which corresponds to the measured physical height of F-actin, while very few localizations were detected on the level of the substrate (0 - 2 nm). This histogram is a quantitative representation of the correlation of dSTORM and AFM data.

4.5 Overlay of dSTORM and TIRF



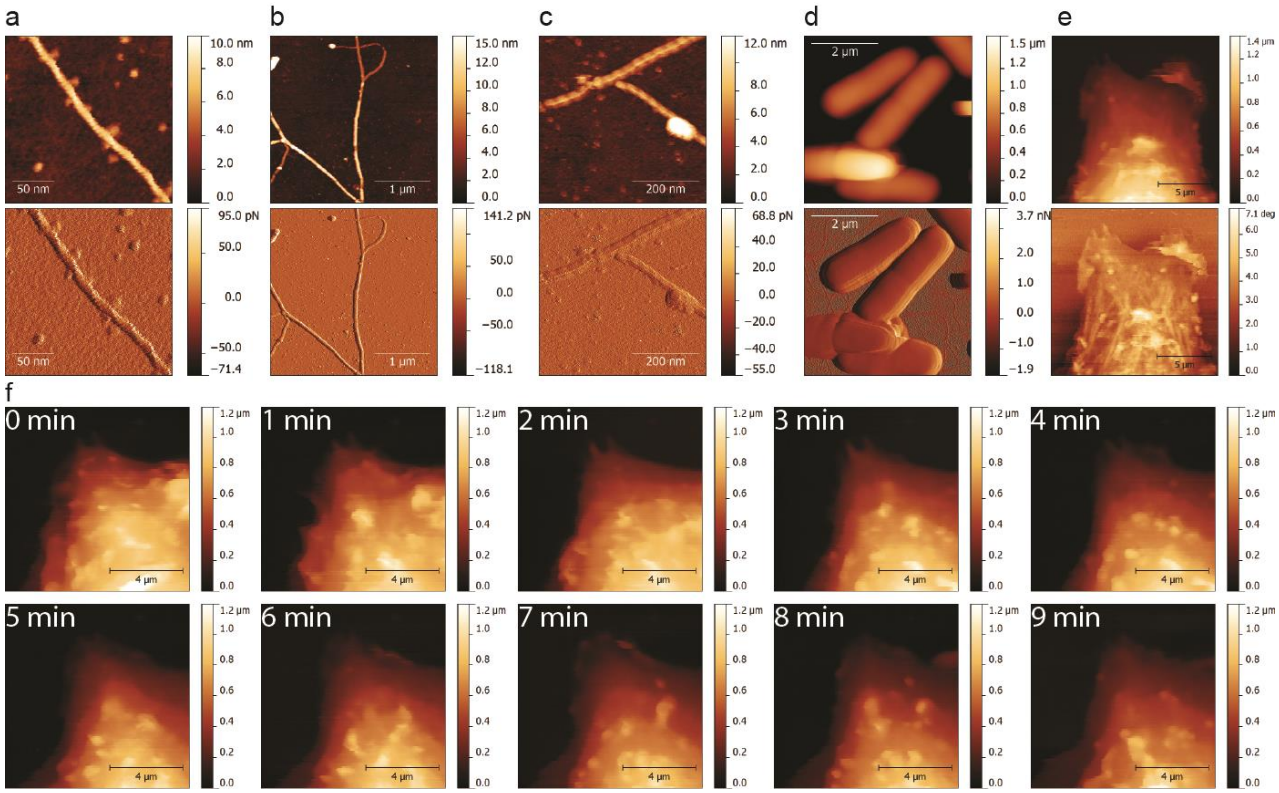
Supporting Fig. 5. Overlay of dSTORM (blue) image and total internal reflection fluorescence (TIRF) image (green) of the same area as shown in **Fig. 4g**. The TIRF image shown here was used for the analysis in **Fig. 4e & f**.

4.6 Correlative AFM/PALM image of MEF cell



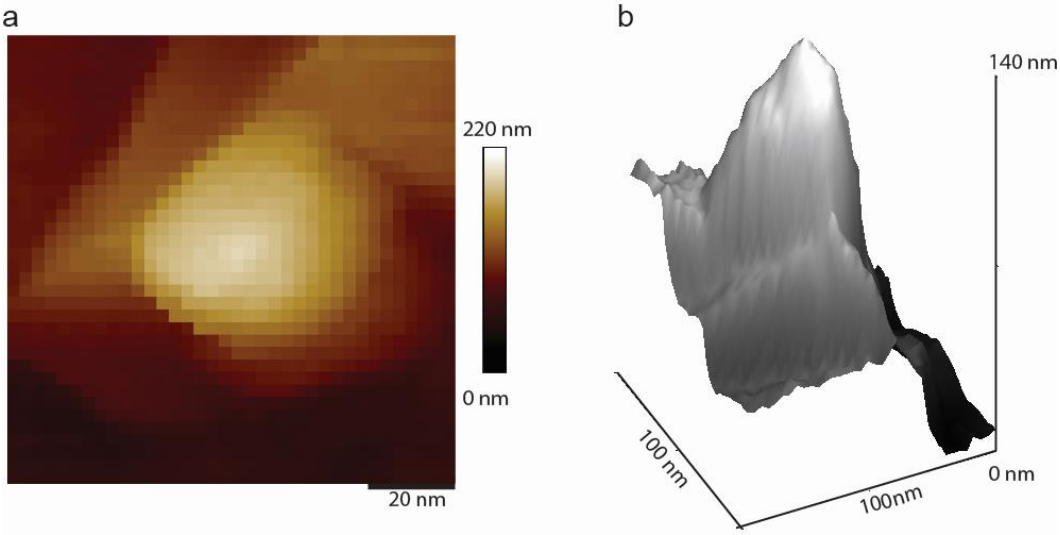
Supporting Fig. 6. Correlative AFM/PALM image of a fixed mammalian cell (mouse embryonic fibroblast (MEF) cell) expressing the fusion protein paxillin-mEOS2. **(a)** 3D render of AFM image with error channel as mask. **(b)** PALM image. **(c)** Correlated AFM and PALM image. Details describing the sample preparation are discussed in the text of the Supporting material.

4.7 2D AFM images of 3D representations shown in main figures



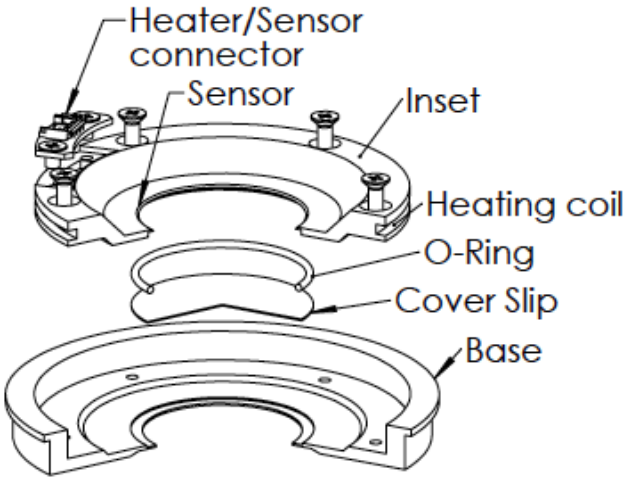
Supporting Fig. 7. 2D AFM height images of 3D representations shown in main figures. **(a)** Corresponds to AFM image shown in **Fig. 2a**. **(b)** Corresponds to AFM image shown in **Fig. 3i & 4g**. **(c)** Corresponds to AFM image shown in **Fig. 3j**. **(d)** Corresponds to AFM image shown in **Fig. 4j**. **(a-d)** Top height image, bottom peak force error image. **(e)** Corresponds to AFM image shown in **Fig. 5a**. Top height image, bottom phase image. **(f)** Corresponds to AFM time-resolved images shown in **Fig. 5b**.

4.8 Reconstruction of tip used for AFM for Fig. 3 & 4



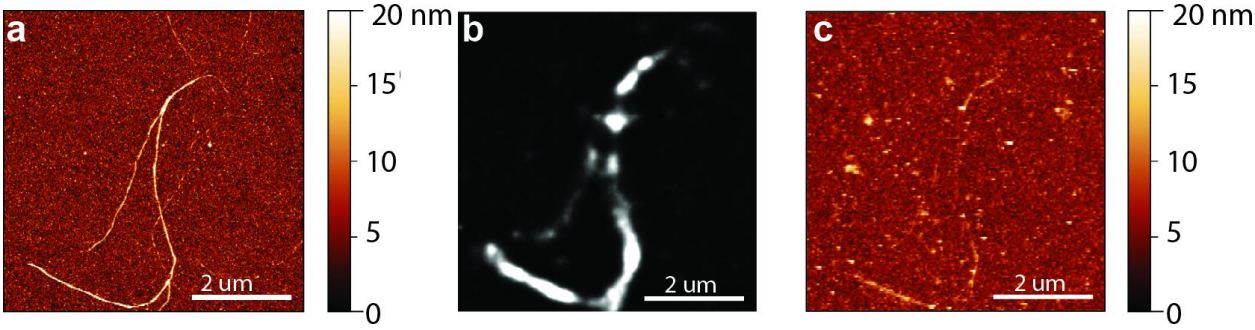
Supporting Fig. 8. Tip reconstruction as obtained by Nanoscope Analysis software of the AFM tip used for imaging F-actin filaments shown in **Fig. 3 & 4**. The tip shape was reconstructed from an image of a tip characterization sample. **(a)** 2D height image. **(b)** 3D representation of tip reconstruction.

4.9 Glass coverslip heating holder used for live-cell experiment



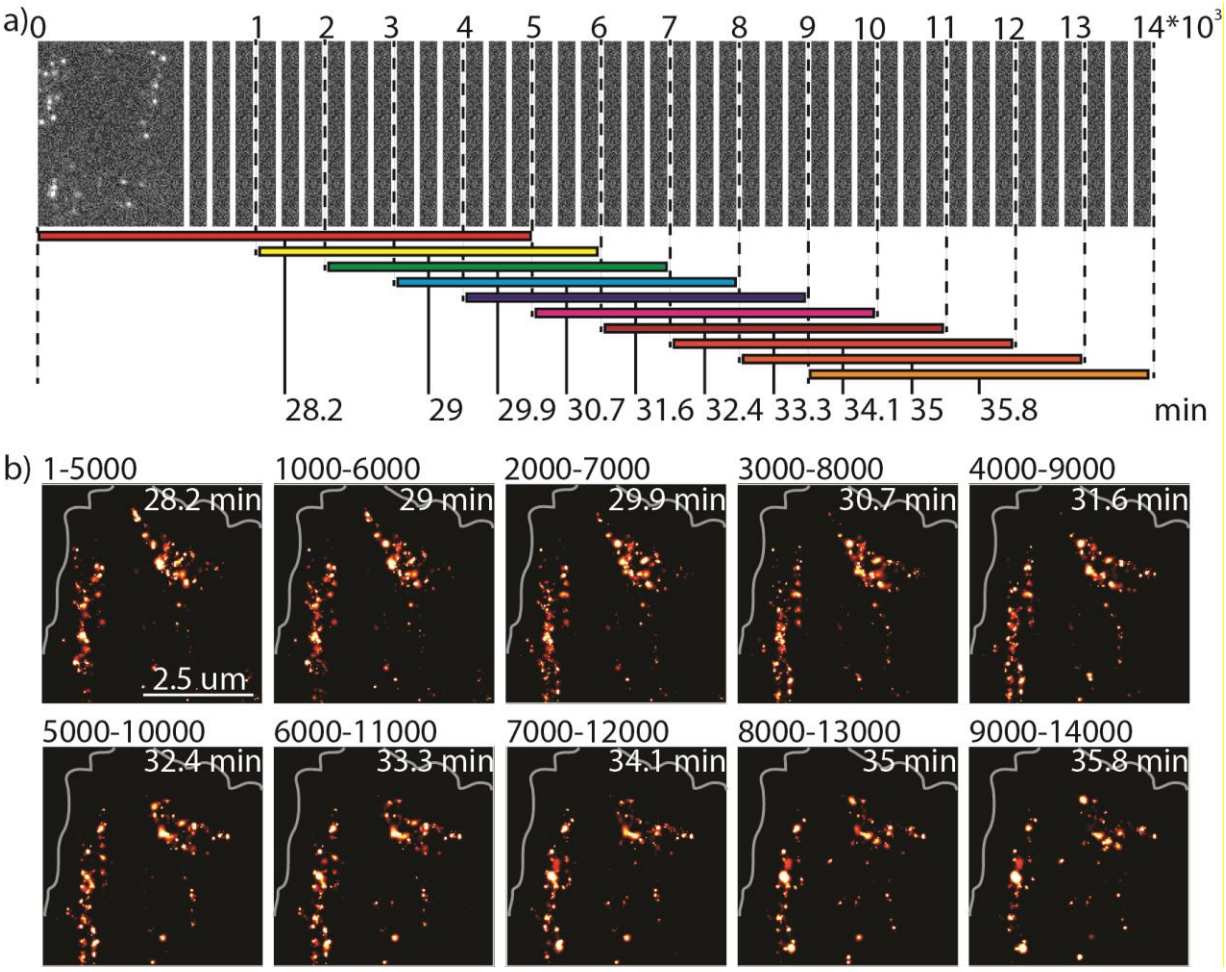
Supporting Fig. 9. Schematic of the home-built glass coverslip holder used to keep the live-cell CHO-K1 sample at 37°C. The temperature was controlled with TC-2-80-15 (Bioscience Tools).

4.10 Effect of dSTORM measurement on structural integrity of actin filament



Supporting Fig. 10. Effect of dSTORM measurement on actin filament interity. **(a)** AFM image of F-actin labelled with phalloidin-atto488 before dSTORM. **(b)** dSTORM image acquired as described in the methods, but no beads were used for dSTORM. Reconstruction was done using the software thunderSTORM. **(c)** After dSTORM image was acquired another AFM was recorded. Parts of actin filaments disintegrated.

4.11 Schematic of PALM image reconstruction and time-sequence



Supporting Fig. 11. Time-resolved PALM images constructed from 5000 frames with a running average approach. **(a)** Schematic showing the time-resolved PALM image reconstruction. Each PALM image is a reconstruction out of 5000 frames. Between each consecutive PALM image there is an offset of 1000 frames. The time reported indicates the time at which the middle frame of the respective PALM frame has been acquired. **(b)** Time-resolved PALM series. On top of each PALM image the number of frames used for its reconstruction is indicated. The outline of the cell is marked in gray. This time-sequence was used for the **Supporting movie 1.**

Supporting movie 1.- live cell PALM time series showing the reorganization of the paxillin-mEos2 clusters in CHO-K1cell transiently expressing a paxillin-mEos2