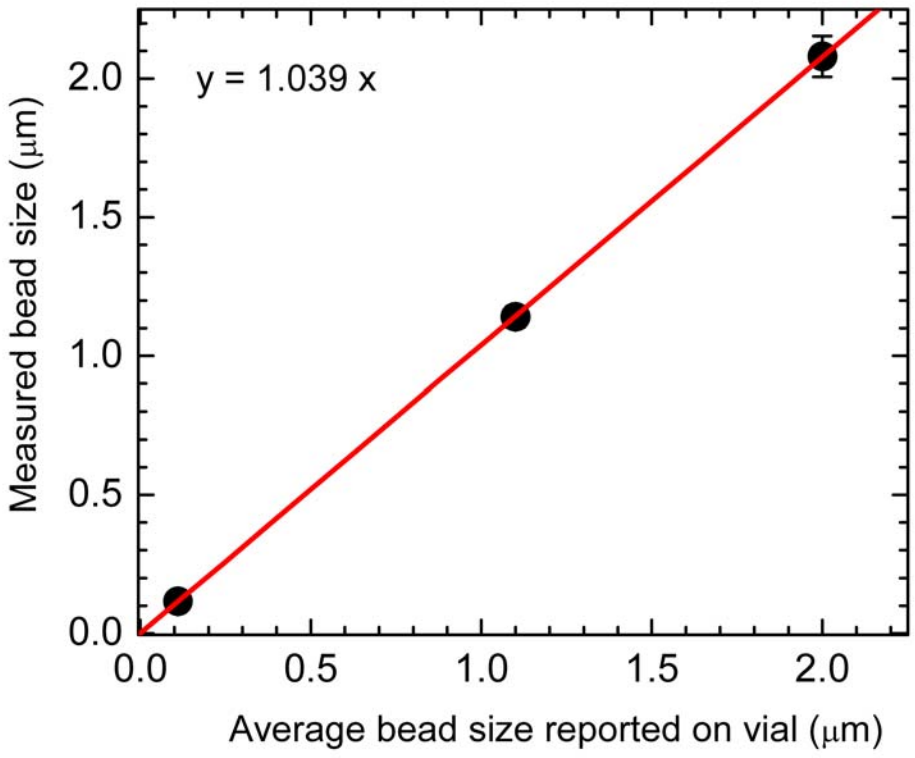
Figure 5. Display of the intensity and normalized ratio along the surface of the microvillus. The size of the box is 1 um. The smaller structures in the surface are about 200 nm in size. (A) Painting according to intensity in channel 1 (colors scale 0-256KHz). (B) Painting according to normalized ratio (color scale -1 to +1). The bottom of the image corresponds to the microvillus base.

Figure 6. RICS analysis for Cerulean-NaPi2c. (A) RICS autocorrelation function. (B) Fit (lower surface) and residues (upper surface) according to a model for 2D diffusion. The recovered diffusion coefficient for Cerulean NaPi2c is 0.02$\mu m^2/s$.

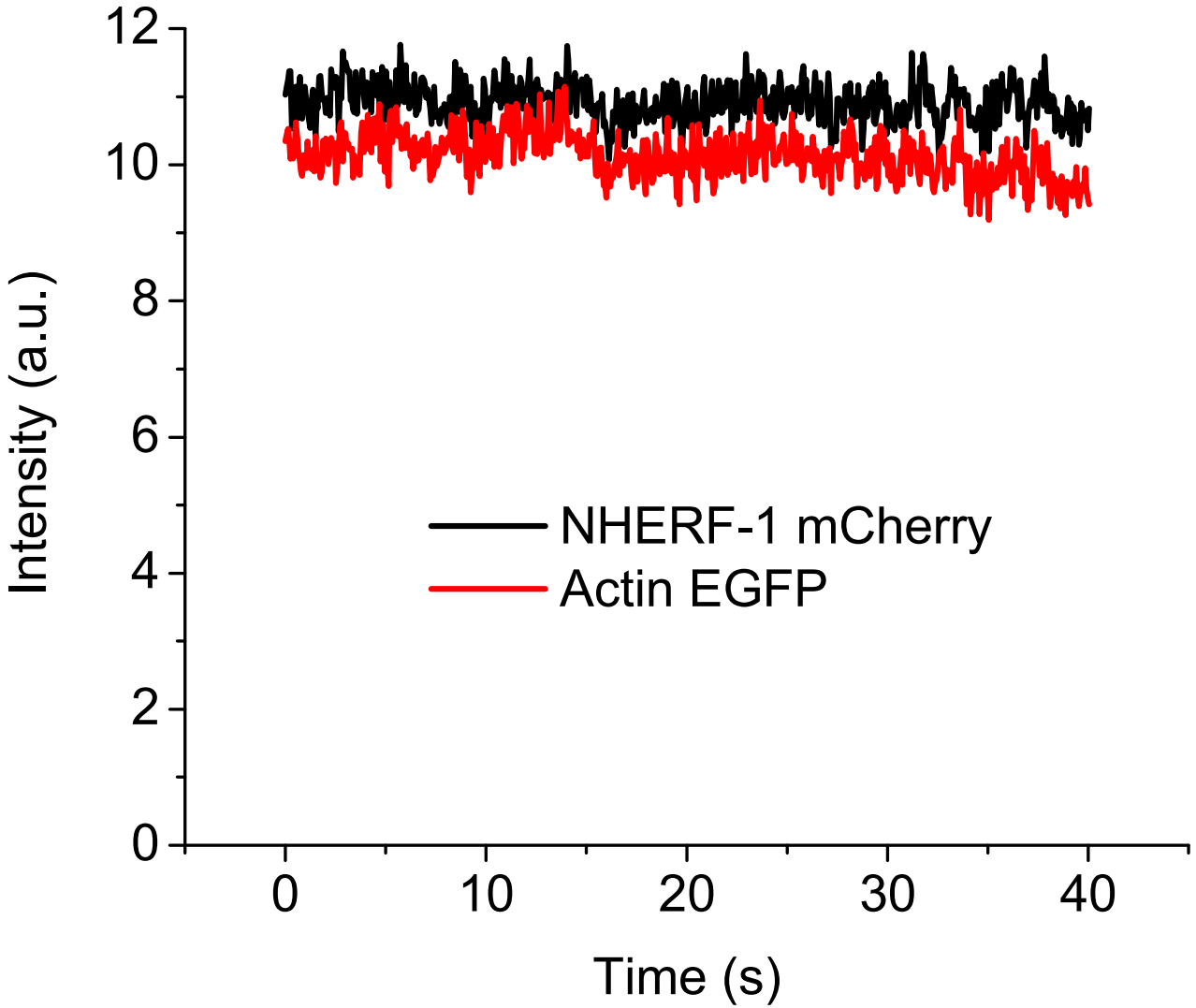
Figure 7. (A) 3D raster scan image of a protrusion of MB231 cell growing in a 3D collagen matrix. (B) MT image of a small portion of the protrusion indicate in (A). The diameter of the protrusion changes along the filopodium. The fluorescence is not uniform on the cell surface but clusters at specific direction where contacts are made with the collagen matrix.

Figure 1S. Calibration of the MT method using fluorescent beads. In the x-axis the nominal diameter of the bead according to the manufacturer is shown. In the y axis is the value of the diameter given by the MT method. Seven beads were measured for each size. The errors are within the size of the dot in the figure except for the largest beads where the standard deviation of the seven measurements is shown.

Figure 2S. Typical fluorescence intensity trace during single measurements at a constant height along the microvilli. During the 40 second duration of these experiments, no appreciable change of the fluorescence was recorded.



Supplemental Figure S1



Supplemental Figure S2