Supplementary Information

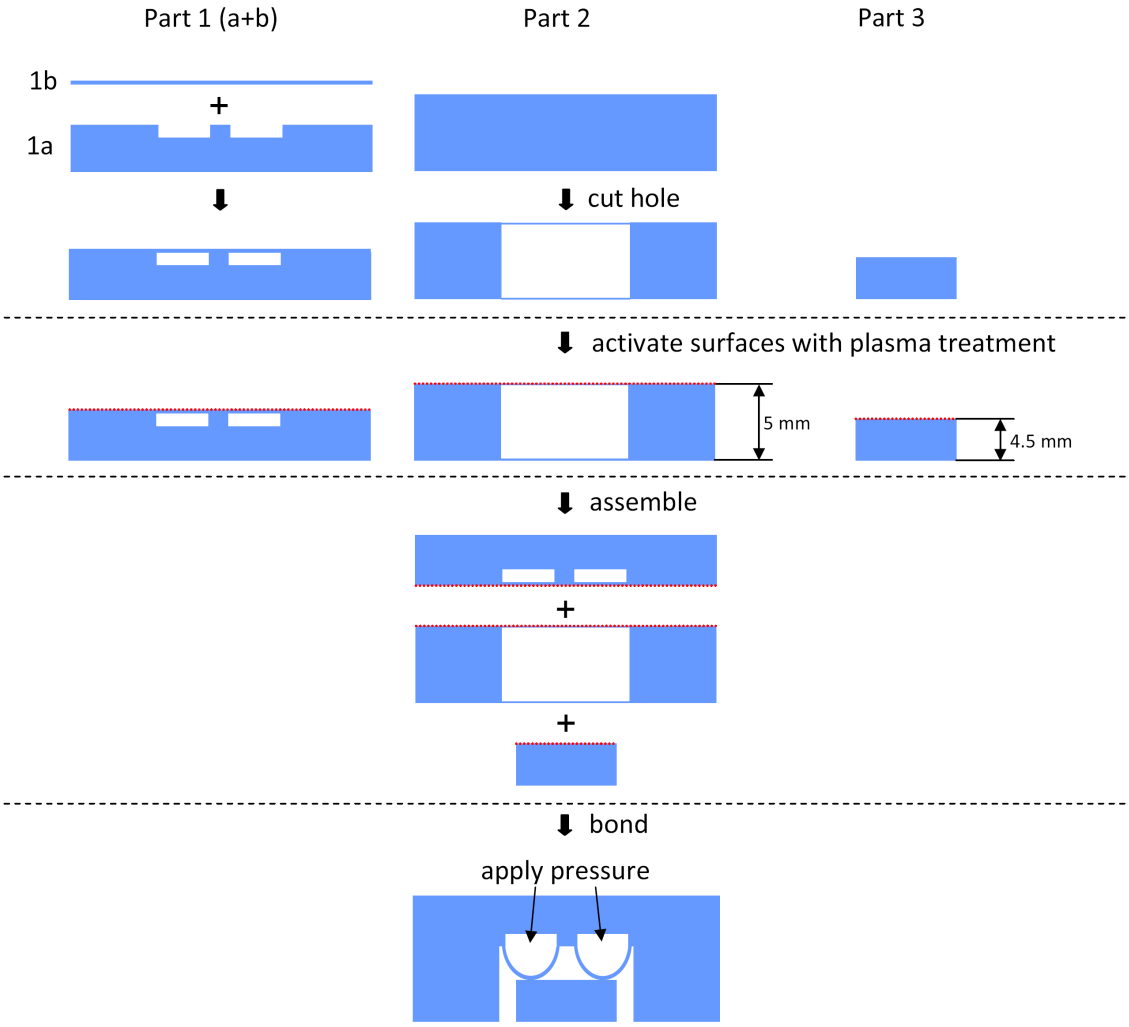

Figure S1: Schematic outline of the manufacturing procedure of the biochip

The chip was manufactured of four separate parts: part 1a, 1b, 2 and 3. Part 1 contains the actuator channel system and was manufactured by covalently bonding a thin PDMS membrane (part 1b) to a ~5 mm thick PDMS piece with 200 µm deep channels (part 1a). Part 2 is the outer frame with a central square hole with 12 mm edge length. Part 3 is an octagonal platform carrying the cells in operation. Note that part 3 has a smaller height (4.5 mm) than part 2 (5 mm). For assembling, the appropriate surfaces were plasma activated and then pressed together resulting in covalent bonding. During assembling, the actuator channels were pressurized to ensure that only the middle part of the membrane covering the actuator channels attached to part 3

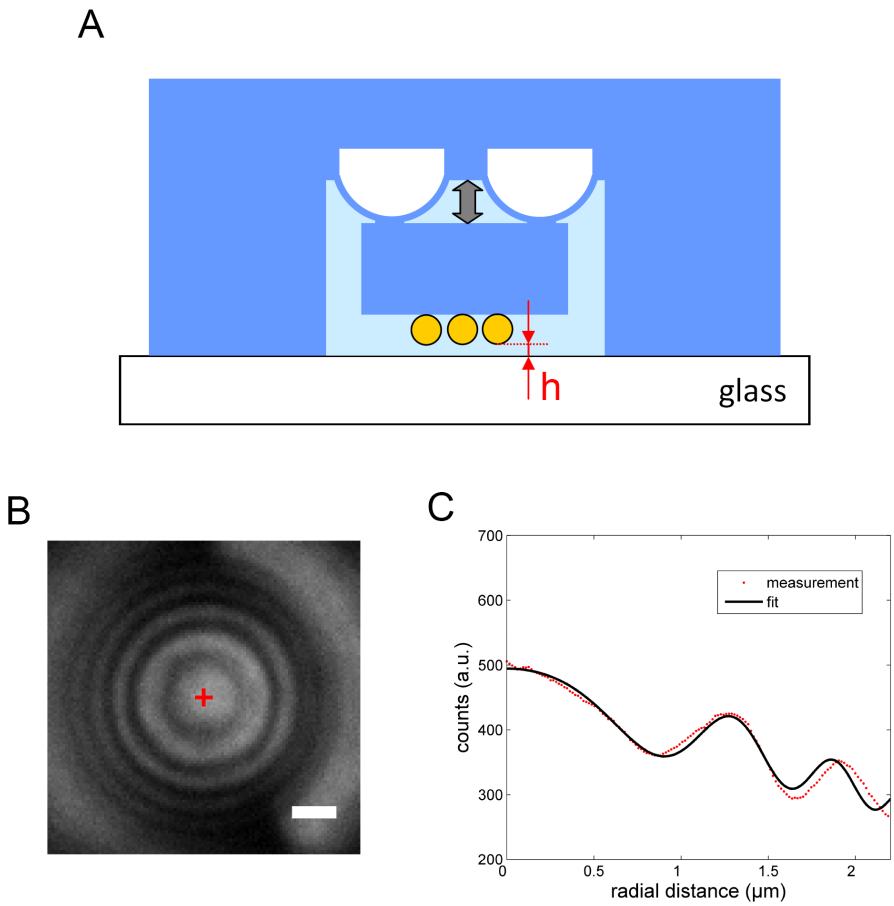

Figure S2: Reflection interference contrast (RIC) microscopy

(A) Scheme of the setup for determining the pressure - distance behaviour of the biochip by RIC microscopy. Note the beads (shown in orange) attached to the cell culture surface of the biochip. (B) RIC image of a bead at a height of 121 nm above the glass slide. The automatically determined centre of the RIC-rings is marked by a red cross. The scale bar corresponds to 1 µm. (C) The figure shows the radial intensity distribution of the RIC image from Fig. S2B (red dots) and the fitted intensity distribution according to equation (1) (black line).

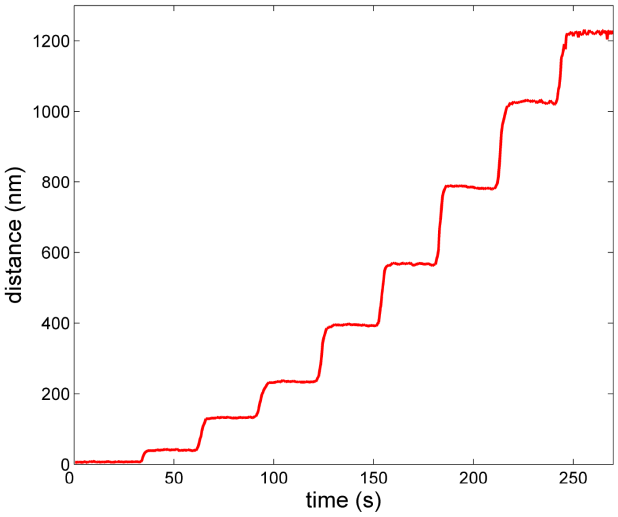

Figure S3: Total pressure - distance curve

The distance between beads attached to the cell culture surface of the chip and a glass slide was determined by RIC microscopy as a function of the pressure applied to the actuator channel system. The applied pressure was controlled via a water column with adjustable height and was determined from the height readings. Before measurement, the pressure was increased till the bead touched the glass surface. This pressure was defined as 0 mbar. For recording of the pressure - distance curve, the pressure was decreased by 0.5 mbar every 30 s; the distance was determined in every 0.5 s.

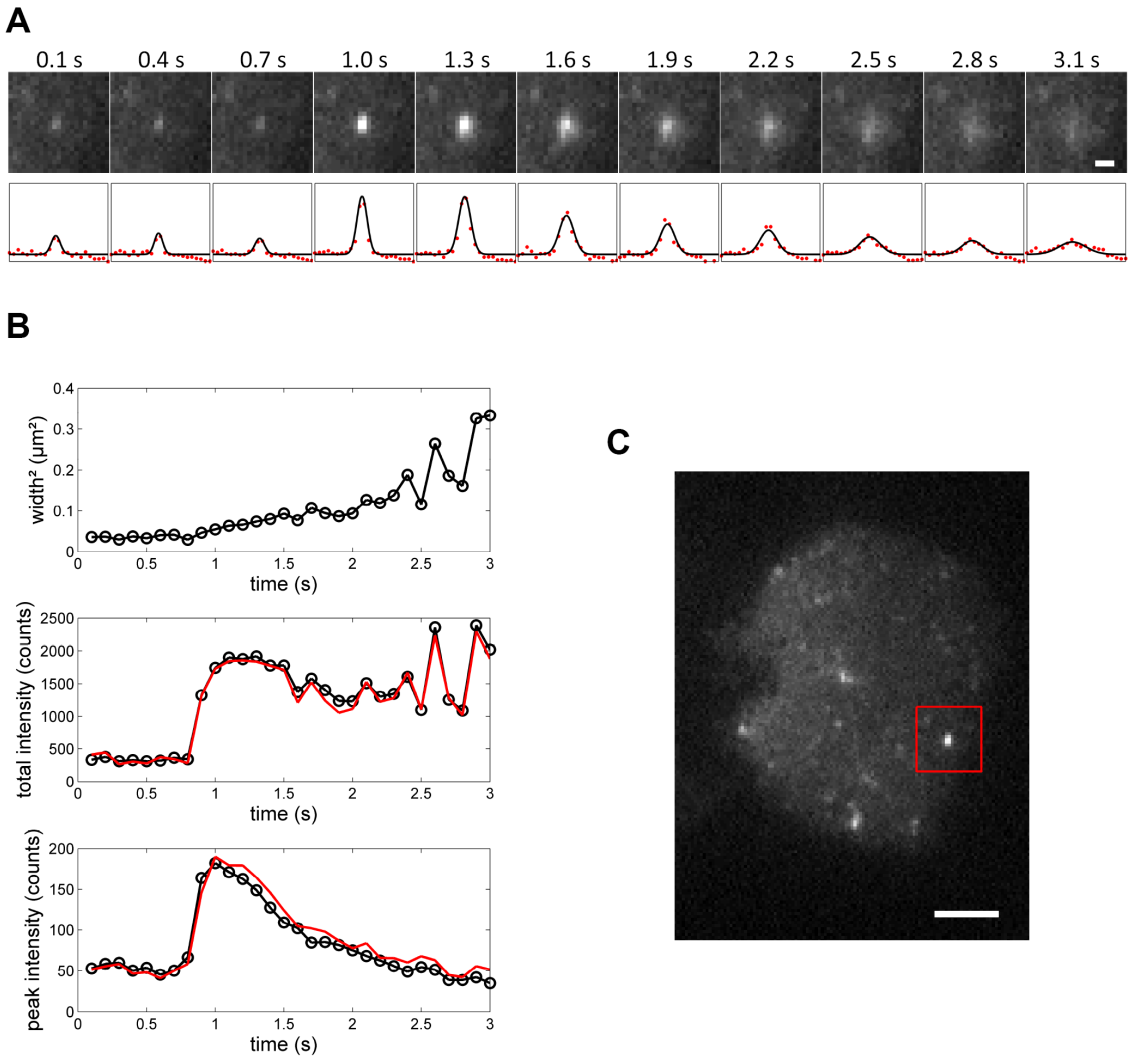

Figure S4: An example for a GPI-GFP vesicle fusion event at the apical membrane

(A) Image sequence of a GPI-GFP vesicle fusion event recorded by TIRF microscopy at the apical membrane. Beneath each image an intensity profile along a horizontal cross-section through the centre of the intensity peak representing the vesicle is displayed (red dots). An equivalent cross section through a two-dimensional Gaussian fitted to the intensity peak is also shown (black line). The scale bar corresponds to 1 µm. (B) Time dependence of the (width)², total intensity and peak intensity of a two-dimensional symmetrical Gaussian fitted to the initially diffraction limited and then spreading fluorescence signal of the vesicle in Fig. S4A (black curves). As a control, the total intensity was alternatively determined by measuring the cumulated intensity in a 11 × 11 pixel region centred at the fusion site. Also the peak intensity was alternatively determined by measuring the maximum intensity in the same 11 × 11 pixel region. The resulting values are displayed as red lines in the corresponding diagrams. The time course of the parameters shows the characteristic time dependence as expected from a vesicle fusion event: After fusion onset (~0.8 s), the volume of the Gaussian representing the total intensity suddenly increases, the amplitude of the Gaussian representing the peak intensity first increases and then decays exponentially, while the (width)² increases. (C) Apical TIRF image of the whole cell from which the fusion event was recorded. The image was taken after release of the Golgi block and removal of pre-existing plasma membrane signal by photo-bleaching of the evanescent wave region. The red square outlines the region where the apical fusion event shown in Fig. S4A was recorded. The scale bars correspond to 5 µm.

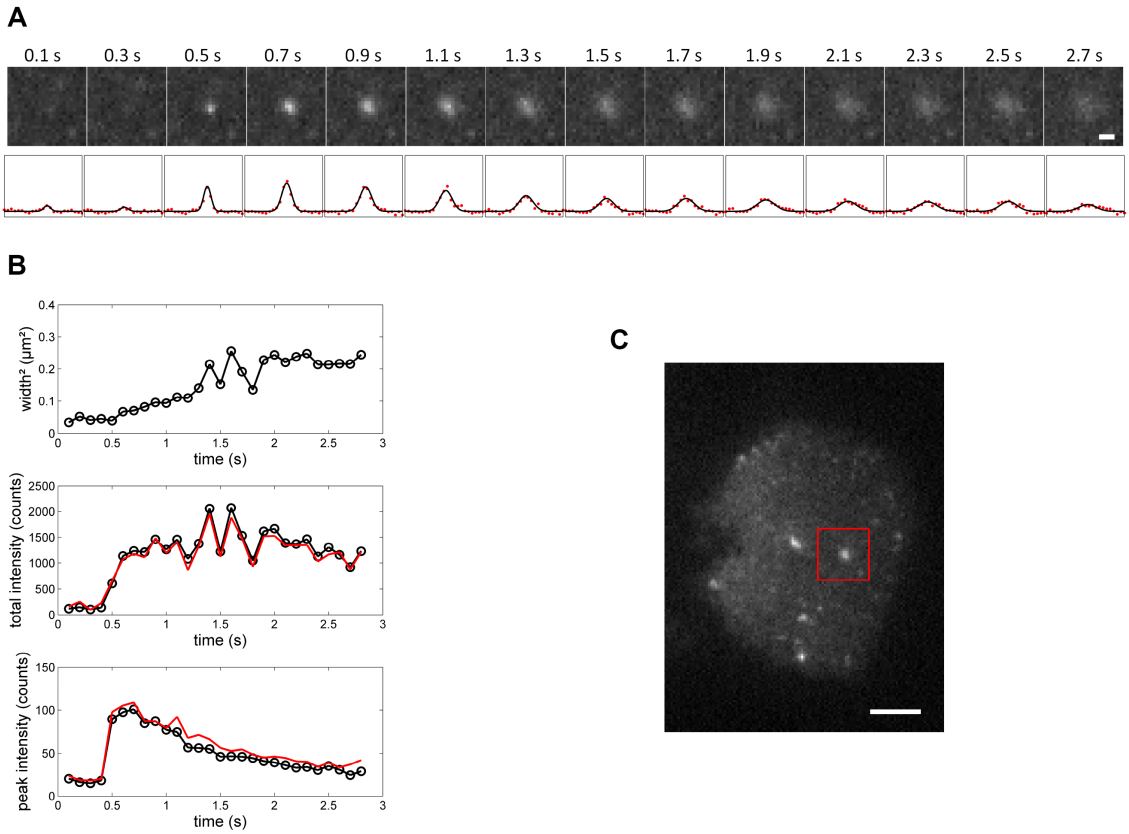

Figure S5: An example for a GPI-GFP vesicle fusion event at the apical membrane

(A) Image sequence of a GPI-GFP vesicle fusion event recorded by TIRF microscopy at the apical membrane. Beneath each image an intensity profile along a horizontal cross-section through the centre of the intensity peak representing the vesicle is displayed (red dots). An equivalent cross section through a two-dimensional Gaussian fitted to the intensity peak is also shown (black line). The scale bar corresponds to 1 µm. (B) Time dependence of the (width)², total intensity and peak intensity of a two-dimensional symmetrical Gaussian fitted to the initially diffraction limited and then spreading fluorescence signal of the vesicle in Fig. S5A (black curves). As a control, the total intensity was alternatively determined by measuring the cumulated intensity in a 11 × 11 pixel region centred at the fusion site. Also the peak intensity was alternatively determined by measuring the maximum intensity in the same 11 × 11 pixel region. The resulting values are displayed as red lines in the corresponding diagrams. The time course of the parameters shows the characteristic time dependence as expected from a vesicle fusion event: After fusion onset (~0.4 s), the volume of the Gaussian representing the total intensity suddenly increases, the amplitude of the Gaussian representing the peak intensity first increases and then decays exponentially, while the (width)² increases. (C) Apical TIRF image of the whole cell from which the fusion event was recorded. The image was taken after release of the Golgi block and removal of pre-existing plasma membrane signal by photo-bleaching of the evanescent wave region. The red square outlines the region where the apical fusion event shown in Fig. S5A was recorded. The scale bars correspond to 5 µm.

Electronic Supplementary Material (ESI) for Lab on a Chip

Supplementary movie 1: Movie of the apical vesicle fusion event shown in Fig. 5

The movie is playing at 10 fps (real time).

Supplementary movie 2: Movie of the apical vesicle fusion event shown in Fig. S4

The movie is playing at 10 fps (real time).

Supplementary movie 3: Movie of the apical vesicle fusion event shown in Fig. S5

The movie is playing at 10 fps (real time).