

## *Supplementary Information*

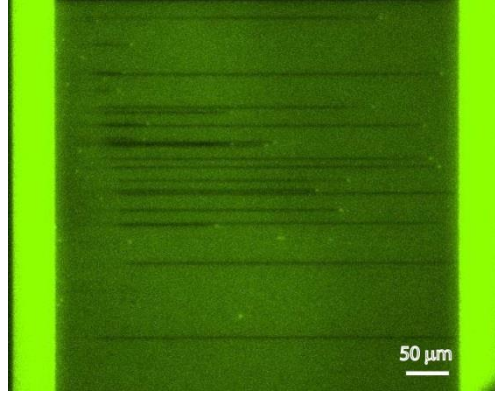
### A nano flow cytometer for single lipid vesicle analysis

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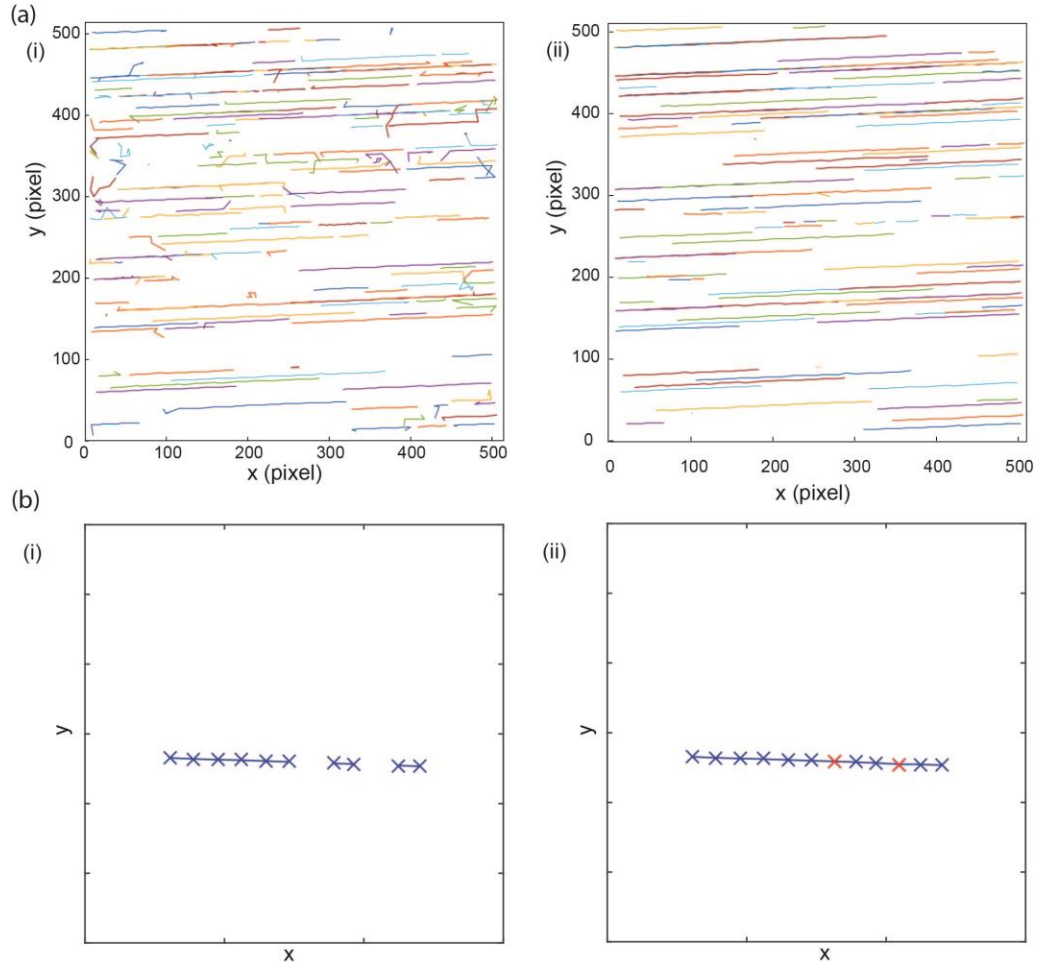
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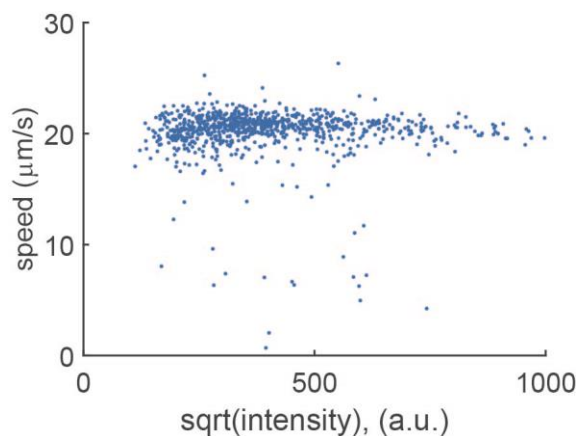


**Figure S1.** Device passivation with vesicles containing 1% NBD-PC lipids. Once the bilayer is formed, it appears smooth. In this example, some of the channels were clogged by non-ruptured vesicles.

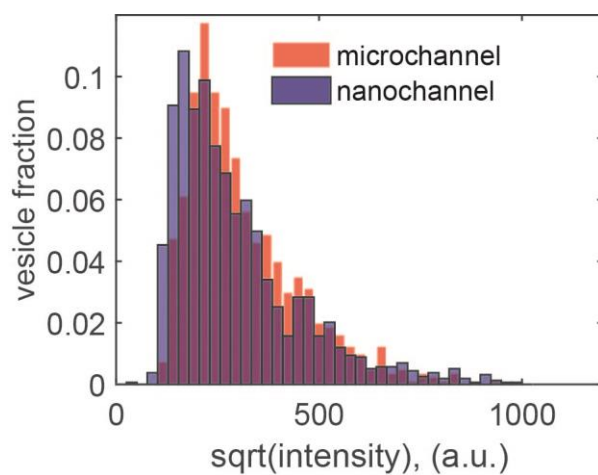


**Figure S2.** Single particle tracking improvements. (a) Tracking before (i) and after imposing movement constraints. For (ii) the maximum angle tolerated was set to  $15^\circ$ . (b) Tracks before (i) and after imposing movement constraints.

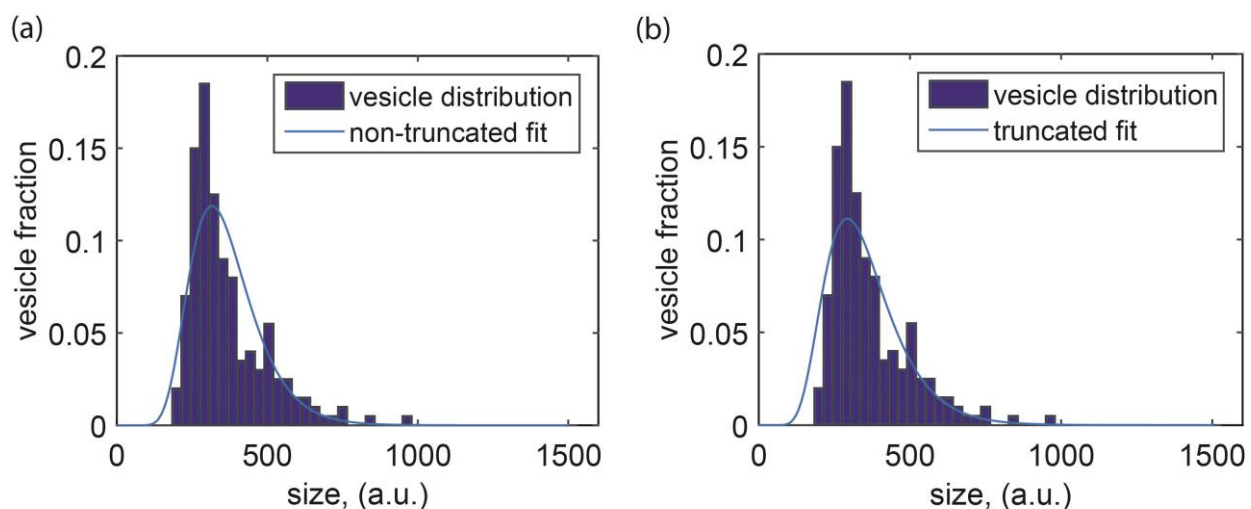
and after (ii) applying the extrapolating algorithm. Blue crosses: detected events in individual frames. Red crosses: extrapolated events.



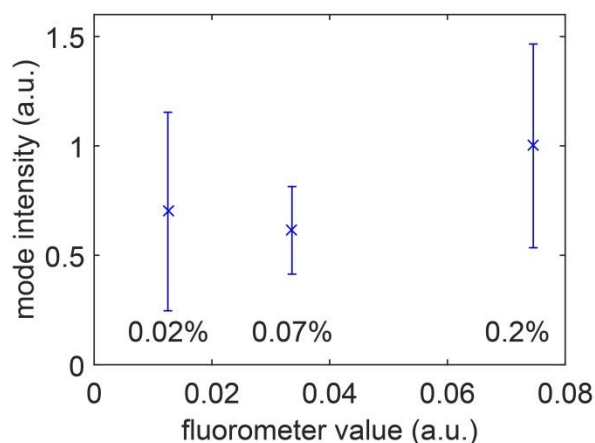
**Figure S3.** Vesicle speed versus the square root of the intensity which is proportional to the vesicle size. No influence of the particle size on the flow speed is observed.



**Figure S4.** Vesicle detection in the micro and nanochannels. No significant difference in particle concentration or size distributions were observed. The measured concentration was 1.43 pM in the microchannel and 1.57 pM in the nanochannel.



**Figure S5.** Effect of fit truncation. A non-truncated log-normal fit (a) is compared to a truncated one (b). To improve the accuracy of the fit, a truncated fit with a cutoff at the vesicle with the lowest intensity was used. This truncated fit neglects the non-detectable fraction in the experimental data below the cutoff and thereby allows for an improved determination of the mode value.



**Figure S6.** Influence of the fluorophore content on the vesicle intensity. Vesicle mode intensity values as determined with the nanofluidic-based assay using 50 ms exposure times vs. average fluorescence intensity as measured by fluorometry. The average and standard deviation for at least 3 independent measurements is reported. The corresponding mol% of ATTO 550 lipids in the vesicles is indicated in the graph.

**Movie M1.** Sample movie showing individual vesicles containing 0.6 mol% ATTO 550 imaged in the nanofluidic device using an epi-fluorescence microscope. 25 mbar were applied to the channel. Lipid concentration: 50  $\mu\text{g/ml}$ , pressure: 50 mbar. The movie is in real time.