

**Supporting information for: “Using single nanoparticle tracking obtained by
nanophotonic force microscopy to simultaneously characterize nanoparticle size
distribution and nanoparticle-surface interactions”**

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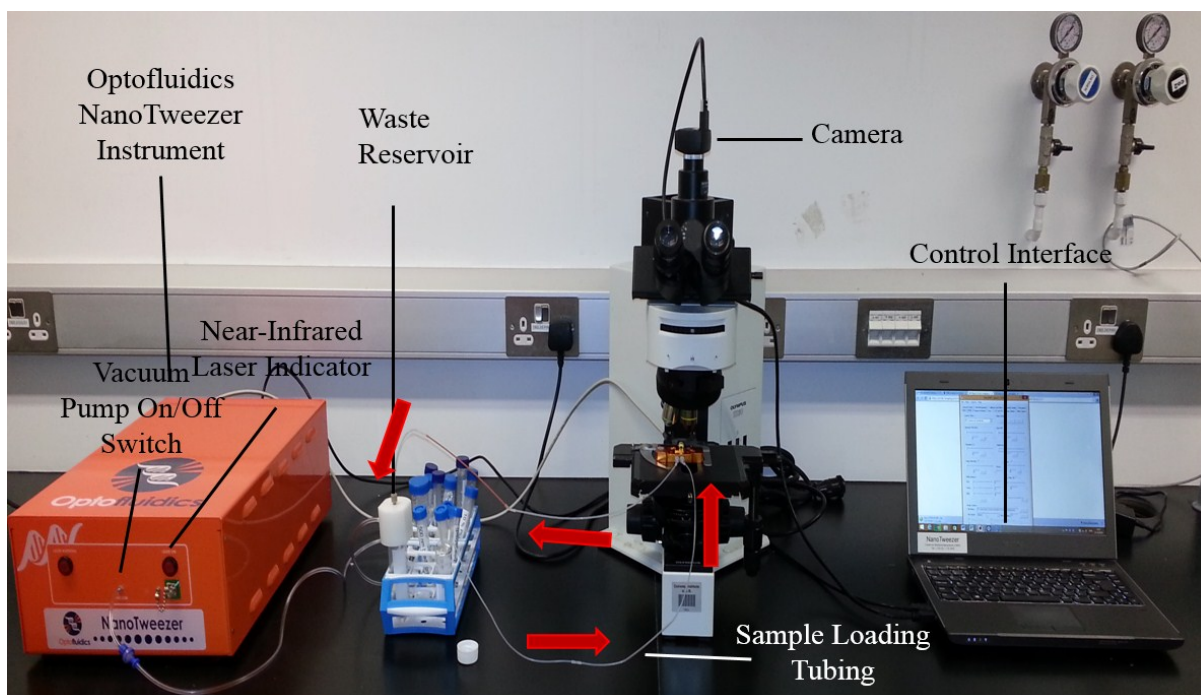


Figure S1. Picture of the NFM instrument setup. The red lines show the flow of liquid from the sample to the waste chamber.

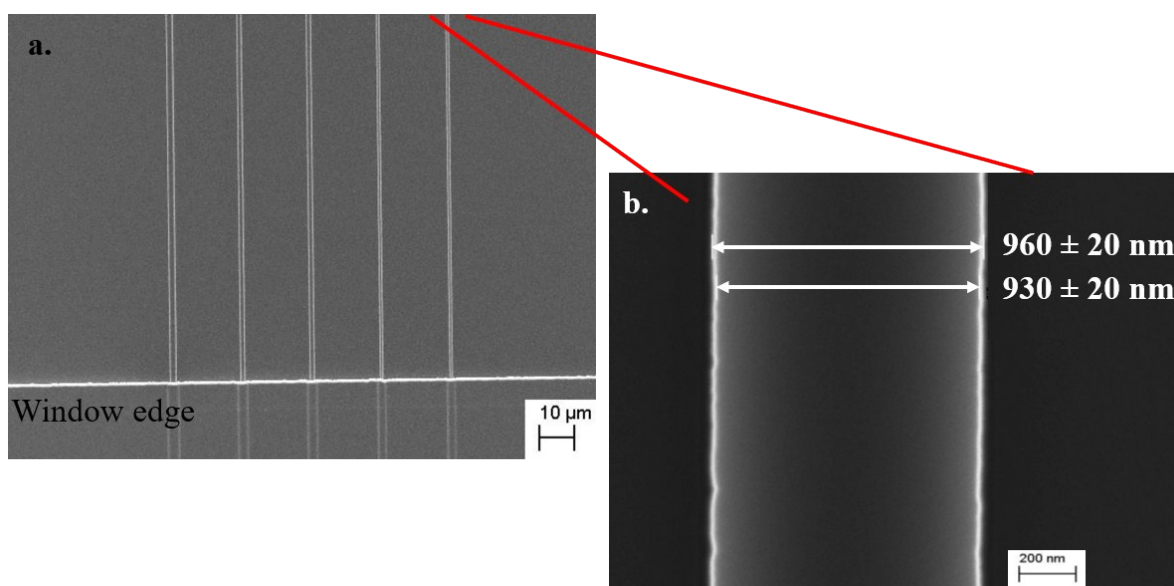


Figure S2. Anatomy of a standard measurement window. (a) Shows a view of a large section of the measurement window while (b) is an SEM close-up of the edge of the experimental window and a single WG, the same size as the ones used in the study.

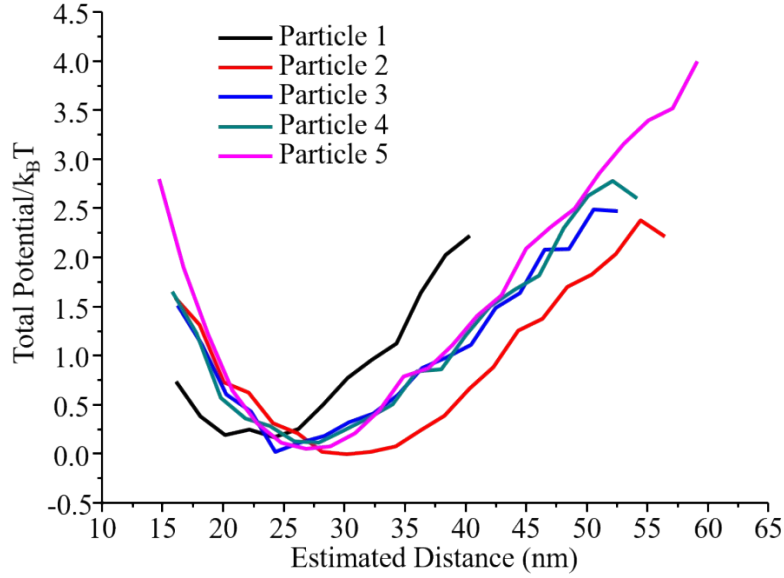


Figure S3. Example of the variability of the total potential energy and the distance from the WG surface for five representative individual particles. Sample analyzed is 200 nm silica at 1.4×10^{-3} M.

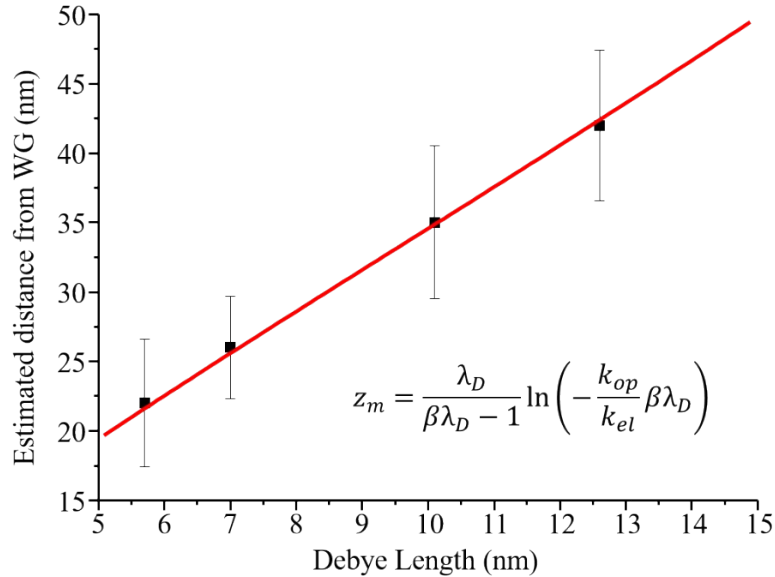


Figure S4. Estimated distance to the WG as a function to the Debye length. The continuous line is a fit to the theoretically predicted expression shown in the figure (see equation S2 and the methods section in the SI for more details). Error bars correspond to the standard deviations of the average estimated distance to the WG.

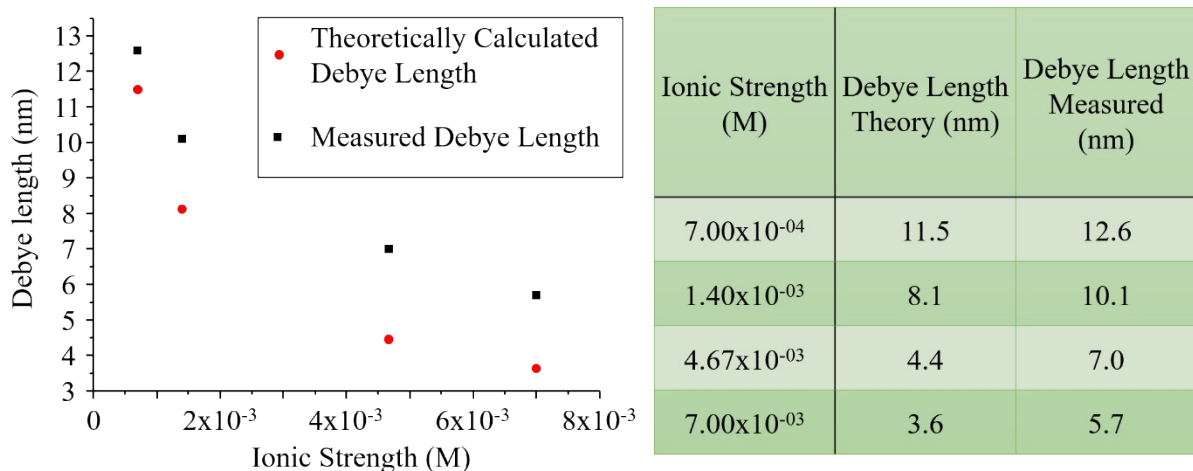


Figure S5. Debye lengths measured by NFM (Eq. 10 in main text) compared to theoretical values.

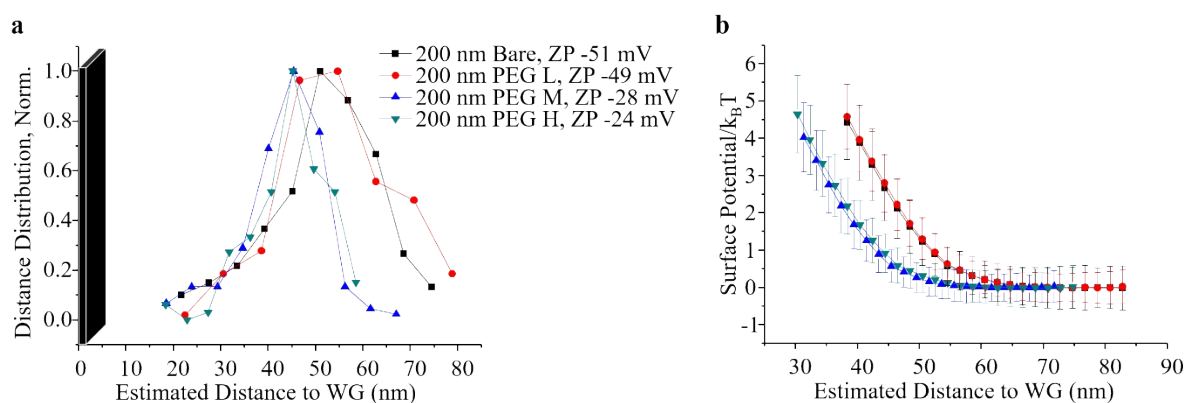


Figure S6. (a) Distance distribution of a particle dispersion with varying PEG surface concentration (PEG H: 7×10^{-2} PEG/nm²; PEG M: 4×10^{-2} PEG/nm²; PEG L: 3×10^{-3} PEG/nm²) and (b) surface potentials of the same particles. All observations were made at a solution ionic strength of 1.4×10^{-3} M and pH 7. Error bars correspond to the standard deviations of the average value.

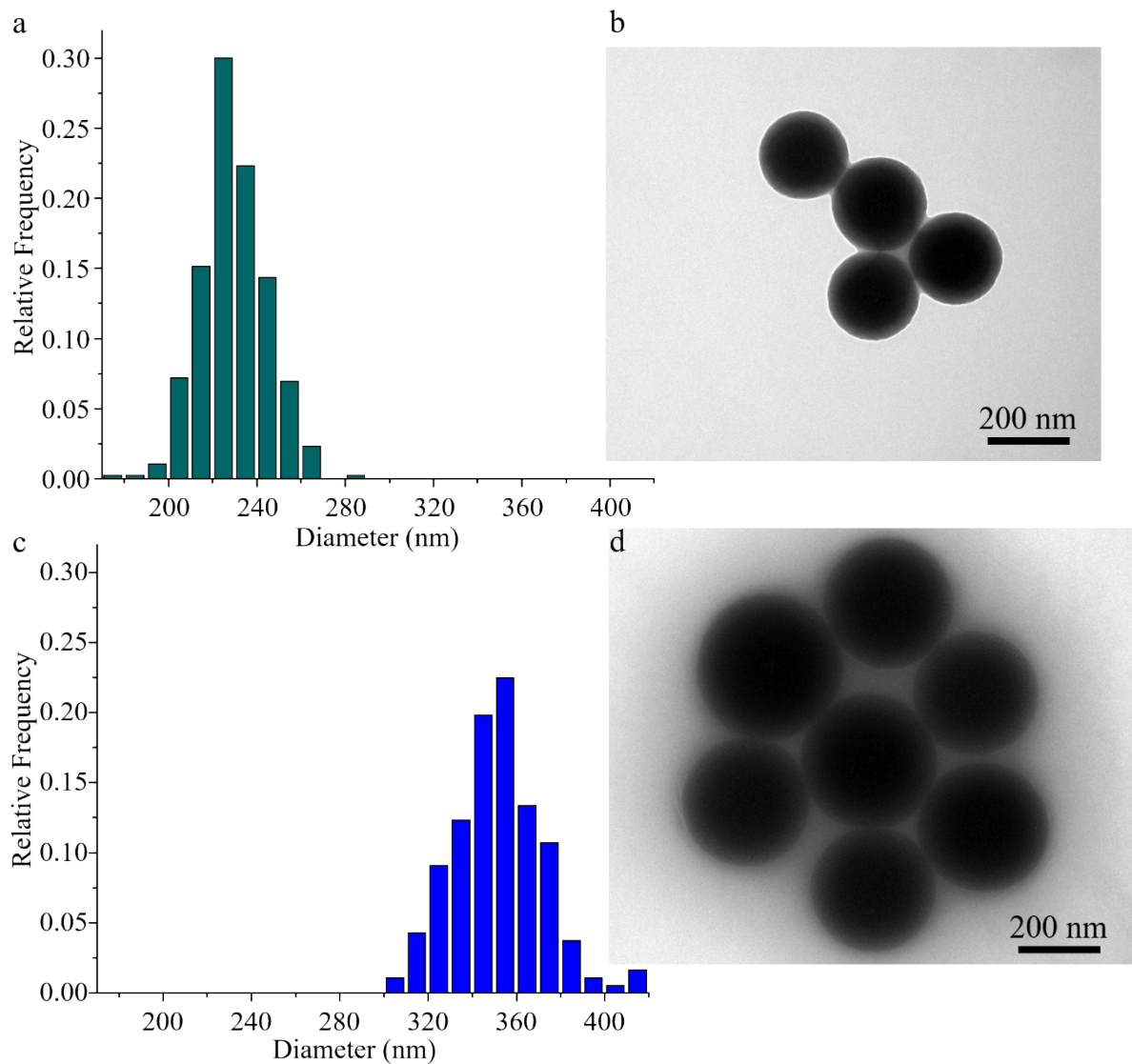


Figure S7. TEM analysis of bare SiO₂ particles used in this study. (a) Histogram and (b) representative TEM image of 200 nm SiO₂ particles. (c) Histogram and (d) representative TEM image of 300 nm SiO₂ particles.

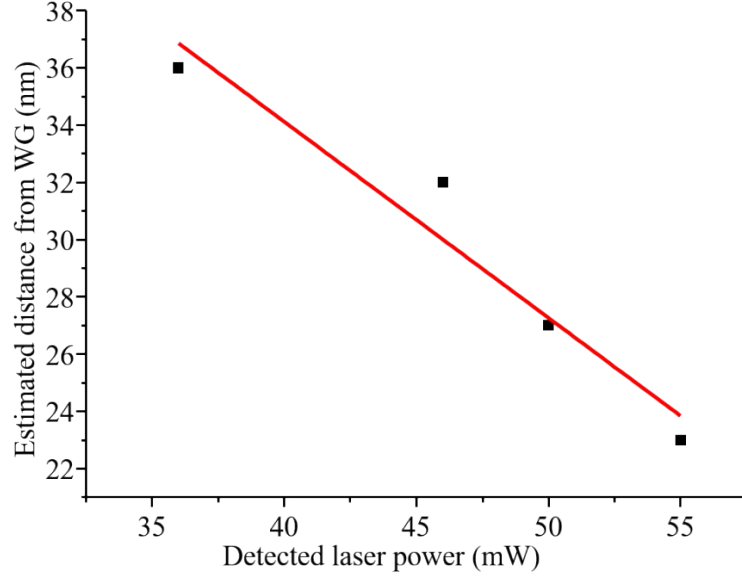


Figure S8. Changes in average particle distance with increase of detected laser power. The continuous line is a linear fit to the data.

Dependence of the position of the minimum of the total potential energy on the Debye length

As mentioned in the method section in the main text, the total potential energy for the interaction of a NP with the surface of the WG can be approximated by the sum of two main contributions: the optical potential generated by the evanescent field (φ_{op}) and the electrostatic repulsion from the electric double layer (φ_s). The complete expression for these two contributions are given in Eq. 5 and Eq. 9 in the main text. Taking the derivative of the total potential energy with respect to z , we have:

$$\frac{d\varphi(z)}{dz} = -\frac{k_{el}}{\lambda_D} e^{-\frac{z}{\lambda_D}} - k_{op}\beta e^{-\beta z}, \quad (S1)$$

with k_{el} as defined in the main text and $k_{op} = 2\pi\alpha l_0/c$. Expression S1 is zero at the position of the minimum (z_m) and solving for z_m we obtain:

$$z_m = \frac{\lambda_D}{\beta\lambda_D - 1} \ln\left(-\frac{k_{op}}{k_{el}}\beta\lambda_D\right), \quad (\text{S2})$$

which gives the dependence of z_m with the Debye length. Notice that we have neglected the dependence of k_{el} on λ_D . This is justified as k_{el} depends as the tanh of the Stern potentials.

Synthesis

Bare silica synthesis

Before use a jacketed three necked flask was rinsed with methanol and left to dry. Then it was placed on a magnetic stirred and connected to a water bath with a build in pump (temperature is set to 40°C). The flask was supplied with a thermometer (neck 1), a stopper (neck 2) and another stopper (neck 3) and a 25 mm egg shaped rare earth magnetic stirrer. Typically neck 2 was used for addition of compounds during the reaction.

A Syrris Atlas asp - 030 syringe pump was used to add all the reagents to the flask. The pump and tubing were rinsed with methanol. 85 mL of methanol were dosed from a bottle to the flask (intake though port A and discharge though port B where the tube was put though neck 2).

After the dosing was complete the liquid was removed by pumping though with air in a waste bottle for two syringe fulls (5 mL). In the meantime 35 mL of a 1 to 1 (V/V) methanol to ammonia (36%) solution was prepared. The syringe was equilibrated with the methanol/ammonia solution for two syringe volumes (5 mL) and then 25 mL of the solution was dosed into the flask in the way described above.

The pump and tubing were again dried by running air though them and rinsed first with methanol for two cycles and then with water for another two. 7 mL of water was then dosed into the flask after which the system was closed and left to equilibrate for 10 minutes. At this time the temperature was measured to be 40°C.

Port C was rinsed using methanol followed by air for two syringe cycles of both. The pump was equilibrated with TEOS by flushing for one syringe volume. 3.5 mL of TEOS were added to the synthesis mixture. Following this neck 2 was closed and the mixture was left to

react for an hour followed by a maturation for a further hour. The dispersion was diluted with the methanol ammonia solution made as described above by a factor of three and TEOS was pipetted into the unwashed dispersion at a rate of 1 mL/30 minutes until the particle size was measured to be 200 nm (characterized by DLS, number mean and DCS, relative weight). Some of the 200 nm particles were taken diluted again and regrown to 300 nm in the same way. It is important to keep the particle concentration relatively low to minimize aggregation.

The dispersion was spun at 4000 (3220 rcf) rpm for 20 minutes, the supernatant was replaced by MiliQ water. Particles were washed a total of four times. The final particle concentration was measured to be concentration of 65 mg/mL

Characterization of bare, HSA covered and PEGylated silica particles

ζ potential by Zetasizer ZS

After the size measurement, particles were transferred to a zeta potential cuvette (DTS1070) and measured three times using manual measurements with eleven runs each for a total of thirty three runs. The zeta potential presented is an average of the final values. All measurements used the Smoluchowski model. The conductivity obtained from the Zetasizer instrument was always above 0.2 mS/cm. pH of all dispersant solutions was between 7 and 7.4.

Surface of the WG zeta potential measurement

The surface zeta potential was measured following the protocol provided by the company¹. Briefly, the Si₃N₄ surface, provided in the appropriate dimensions by Optofluidics Inc. was glued to a holder and left to settle. The holder was inserted in a ZEN1020 cell. Before the measurement was carried out the zero distance of the surface was found¹⁻². The surface zeta potential was measured using 200 nm bare silica particles at a concentration of 100 μg/mL

dispersed in a solution with an ionic strength of 1.4×10^{-3} , 2.8×10^{-3} and 1.4×10^{-2} M. The measured zeta potential was -77 mV (n=2) for 1.4×10^{-3} , -71 mV (n=2) for 2.8×10^{-3} and -67 mV (n=1) for 1.4×10^{-2} . The pH of all solutions was around 7.2. These results are similar to the ones described in ³.

PEG density analysis

Particle dissolution studies in DLS (data not shown)

Core silica particles were placed in a plastic cuvette ($l = 1$ cm) at a concentration of 10 mg/mL and NaOH was added so that the final concentration was 50 mM, 100 mM and 200 mM. The cuvette was sealed well with parafilm and placed in the instrument where a size measurement was taken every 5 minutes for twelve hours (temperature was set to 37°C) with a fixed attenuator of 11. The drop in count rate over time was observed. In this way the standard particle dissolution procedure was established. Particles were dispersed in 200 mM NaOH in a 2 mL Safe Lock® Eppendorf and left 16 hrs at 37°C . Particle dissolution is then confirmed using a standard DLS measurement.

Nuclear Magnetic Resonance

In all cases a 5 mm thin wall, 8 inch, 500 MHz NMR tube was used (Wilma Lab Glass). Oxford instruments 400MHz was used for all measurements. Samples were measured with a minimum of 16 scans and a relaxation time of 25 s. No changes in the angle or temperature were made to the default protocol (25°C with no equilibration time and 45° detection angle).

Silicate PEG5000 OMe calibration curves

5 kDa methylated PEG silicate at a concentration of 5.7 mg/mL (1.1 mM), 2.8 mg/mL (5.7×10^{-1} mM), 1.4 mg/mL (2.8×10^{-1} mM), 0.7 mg/mL (1.4×10^{-1} mM), 0.35 mg/mL (7×10^{-2} mM) was dissolved a 1 mL solution of 200 mM NaOD with 1mM DMF as an internal

standard. The solution was left overnight at 37°C to match dissolution conditions and then measured. Relaxation time in the measurement was 25 seconds and each measurement consisted of 32 scans. Two calibration curves were averaged. The mean PEG peak (3.6 ppm) was used to determine concentrations for the calibration curve.

Dissolution ¹H NMR

An aliquot of NaOD (5M in D₂O) was added to a known concentration of particles, typically between 3 and 10 mg/mL, so that the final base concentration is 0.2 M. The dissolution procedure was as described above i.e. incubation for ~16 hrs at 37°C

The dissolved particles were put in a clean NMR tube and measured in a 400 MHz NMR (16 scans and 25 seconds relaxation time).

NMR spectra Processing

MestReNova 8.0 software was used for peak fitting to determine the integrated area and FWHM. All NMR spectra were processed in the following fashion: the obtained spectra was chemical shift referenced using the internal standard (DMF) and standard solvent peak, an exponential apodization of 0.3 Hz was then applied, the phase of the spectra was corrected manually (if required). Spectra were baselined using Polynomial Fit in all cases. Peak picking and integration and peak fitting were done in manual mode using the MNova software and the areas were compared to the calibration line described above.

Calculations of PEG density

The following assumptions were made in all calculations used in the work: all particles are perfect spheres of the same size, the density of all particles is the same and equal to 2 g/cm³. DCS was assumed to give an accurate description of “true size” for bare particles and is used for further calculation. To obtain the concentration of the PEG molecules per surface area

unit we need to calculate the total surface ($S_{A_{total}}$) area. As a first step we calculate the surface area of a single nanoparticle ($S_{A_{NP}}$):

$$S_{A_{NP}} = 4\pi R^2, \quad (S3)$$

where R is the radius in nm (taken from DCS analysis). The number of nanoparticles can be calculated using the concentration of silica particles, the volume of a sphere and the material density as:

$$N_{NP} = \frac{3m10^{18}}{4\pi d r^3}, \quad (S4)$$

where m is the mass of the silica sample (measured through vacuum drying of aliquots of known volume) and d is silica density (taken as 2.0 g/cm³).

Combining Eqs. 3 and 4 we obtain for the total surface area:

$$Total\ SA = \frac{3m10^{18}}{4\pi d R^3} \cdot 4\pi R^2 = \frac{3m10^{18}}{d R}, \quad (S5)$$

We can obtain the number of PEG molecules directly from our NMR results. When we combine Eq. 5 with the NMR results we obtain for the PEG concentration on the surface:

$$\frac{PEG}{nm^2} = \frac{N_{PEG}}{Total\ SA} = \frac{1}{3} \frac{d R N_{PEG}}{m} 10^{-18}, \quad (S6)$$

where $\frac{PEG}{nm^2}$ is the concentration of PEG on the particle surface, N_{PEG} is the concentration of PEG in the dissolved particle solution measured by NMR

NFM Measurement

Optofluidics instrument

The Optofluidics NanoTweezer instrumental setup consists of an instrument which contains a laser (635 mW, 1064 nm), a pneumatic pump (can regulate from 0 to 70 mBar of pressure) for fluid flow and additional electronic and optics. The instrument is linked to a microchip mounted on a microscope stage and the microscope was further equipped with a camera (figure S1).

Each Optofluidics NanoTweezer chip contains five silicon nitride (Si_3N_4) waveguides (WG) two 1, two 1.5 and one 2 μm wide which is situated in a 200 μm x 200 μm experimental chamber. The rest of the chips is covered by SiO_2 (~ 8 μm thick outside the WG chamber and several nm in the chamber) (figure S2). 1064 nm laser light (TE mode polarized) is supplied by the instrument laser, coupled to the waveguides by the pre-aligned optical fibers, and guided to the waveguide outputs where optical power is measured with a photodiode. A sample is introduced by inserting an aspirator into the solution of interest. The sample is drawn through the system with vacuum pressure and ultimately collected in a waste reservoir. Vacuum pressure is regulated in the range of 0 to 70 mBar, and can be increased to ~ 300 mBar for rapid sample loading and washing. Precise flow rate control in the range of 0 - 7 $\mu\text{L}/\text{min}$ is achieved by using an in-line flow rate sensor and a PID feedback control loop. This movement enables steady-state imaging of particles as they travel along the waveguide and pass through the imaging region of interest. The intense scattering generated by particles enables high signal to noise imaging at low (100 μs) exposure time and high frame rates 2555

fps. Images recorded by the camera are analyzed with a custom software package that performs automated particle tracking.

Each WG is coupled to optical fibers at both its ends, as the laser light exits the WG the optical power is measured with a photodiode in order to monitor the optical properties of the chip. The sample is loaded onto the chip using the pneumatic pump at increased pressure (~300 mBar).

Waveguides were imaged with an upright transmission light microscopy (Olympus BX60, Japan). The system was firstly run with deionized water for 5 min, and then the waveguides were evaluated with a dry 20 X objective lens (0.40 NA) for any presence of debris. The inlet tube was placed into a desired sample and purged at a pressure rate of ~300 mBar until the chip was entirely filled. A lower laser power was firstly applied (50 mW) to make sure there was no bubble on the WG. Before a movie was taken the power was set to what was required and flow to 0.1 $\mu\text{L}/\text{min}$. Trapping objects on a waveguide were focused with an Olympus LUCPLFLN40X objective lens (0.6 NA) and images were captured for 20 seconds using a Basler acA2000-165uc camera. For each particle type, between 15 and 25 movies were acquired depending on sample behavior. In between measurement of different particle types, sufficient amount of deionized water was run through the waveguides to avoid cross-contamination. In some rare cases of tough contamination ethanol was flowed through the system.

Trajectory selection

It was found that a further trajectory selection procedure is required after the movie was analysed. The same movie analysis conditions were used through this study which in many cases led to “artefact trajectories” which needed to be removed manually. We plotted and considered the intensity over time, place of the trajectory on the WG, the diffusion along the

X and Y axis and the total potential for each particle (figure S9). Using this information we can exclude many of the artefacts observed.

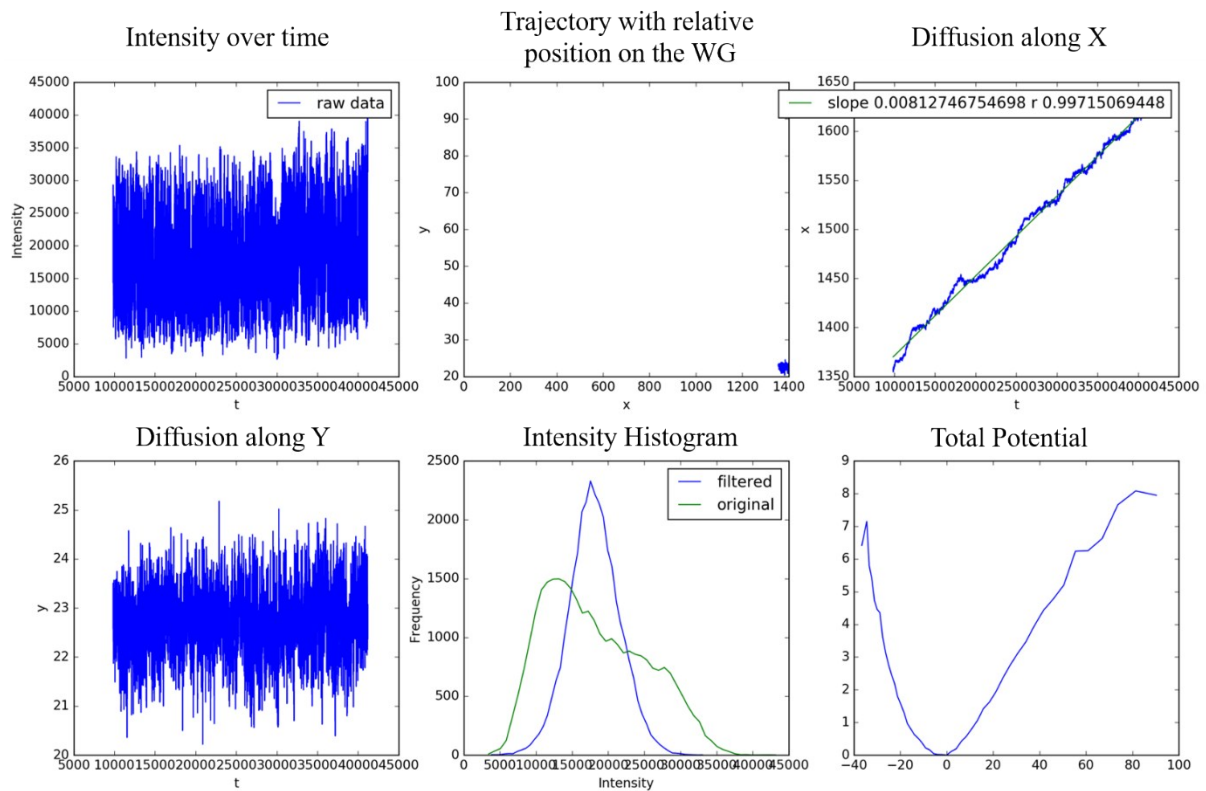


Figure S9. Example information sheet acquired for each particle trajectory. It includes the change of particle intensity over time, its movement in the X and Y axis, the relative position on the WG, the particle intensity histogram and total potential.

Examples of trajectories which were considered for analysis are presented in Figure S10a and S10b. Others which were excluded manually include tracks at which the particle is there only for a small fraction of the total frames (Figure S10c and S10d). This type of artefact is characterized by a lack of mobility on either axis and an intensity several times lower than a typical particle trajectory. The particle may interact with features of the WG surface or get stuck for parts of its trajectory this leads to abnormal behavior (figure S10e and f). Such trajectories typically have a very distinguishable total potential. In some cases the Airy disk

of the particle may be mistaken for another particle. Similarly to background, these trajectories are defined with a very low intensity and move together with another trajectory.

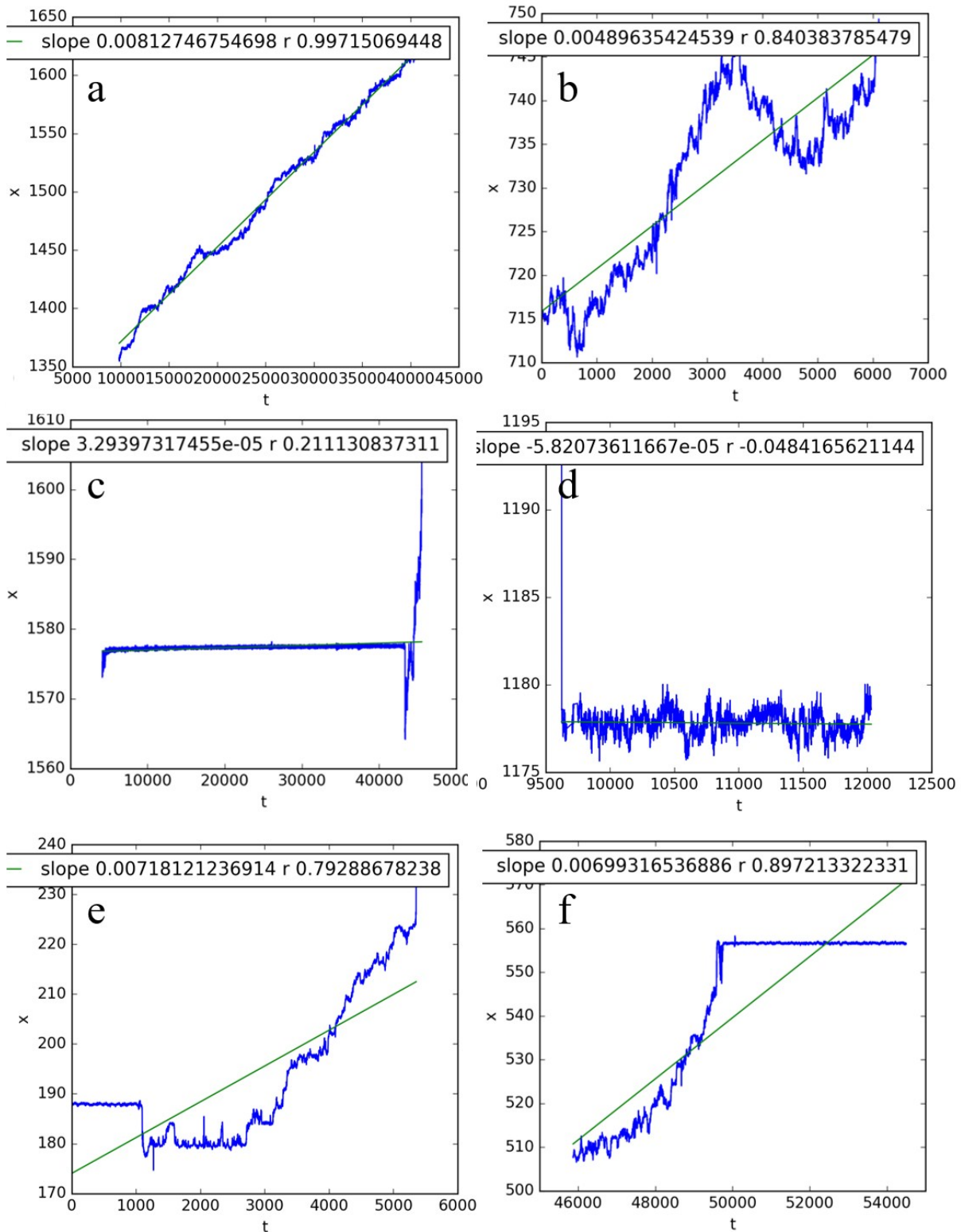


Figure S10. Examples of different trajectories commonly found in the NFM experiment. Those include examples of a (a) perfect trajectory and (b) an acceptable trajectory as well as examples of artefacts such as background (c) with and (d) without a particle appearing in the trajectory.

References

1. Ltd., M. I. Measuring Surface Zeta Potential using the Surface Zeta Potential Cell. <http://www.malvern.com/en/support/resource-center/application-notes/AN120917SurfaceZetaPotentialCell.aspx>.
2. Ltd., M. I., Surface Zeta Potential Cell. 2011.
3. Corbett, J. C.; McNeil-Watson, F.; Jack, R. O.; Howarth, M., Measuring surface zeta potential using phase analysis light scattering in a simple dip cell arrangement. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **2012**, *396*, 169-176.