Coated microspheres as enhanced probes for optical trapping

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ABSTRACT

In an optical trap, micron-sized dielectric particles in aqueous solutions can be held by a tightly focused laser beam. The optical force on the particle is composed of an attractive gradient force and a destabilizing scattering force. To optimize the trapping potential, we reduced the scattering force by using coated microspheres. The shell of the particle was designed such that it acts as an anti-reflection coating. We made and characterized such particles and found that in comparison with the uniform microspheres of the same diameter a more than two-fold stiffening of the trap. Compared to larger spheres, we achieved an increase in trap stiffness of up to 10-fold. These results quantitatively agree with our calculations based on the generalized Lorenz-Mie theory. By improving the trapping potential higher overall forces can be achieved with the same laser power, or vice versa the same force can be reached by using less laser power. A higher maximal force increases the range of possible experiments, and a reduced laser intensity leads to less photo-toxic interactions or laser heating relevant for biological applications.

Keywords: Optical tweezers, core-shell particles, anti-reflective coating, generalized Lorenz-Mie theory, back focal plane detection, calibration

1. INTRODUCTION

Optical tweezers are a sensitive position and force transducer widely employed in biophysics, colloid research, micro-rheology, and physics.[1–7] For many experiments, trapped microspheres—so-called beads—are the object of interest or are used as handles for the measurements. In a high-numerical aperture focus, the beads can be stably trapped if for the axial direction the gradient force in the direction of highest light intensity is larger than the scattering force that pushes the particle away from the focus in the direction of light propagation. In a geometric optics picture, to first order, the scattering force is proportional to the reflectivity which scales with the square Δn^2 of the refractive index difference of the particle with respect to the surrounding medium. Due to Snell's law, the gradient force is to a first order approximation proportional to Δn. For a Rayleigh scatterer, the same scaling is true.[1] Thus, eventually for high-refractive materials the scattering force dominates. This limits optical trapping.

Based on arguments from geometrical optics, we hypothesized that using anti-reflection-coated microspheres would reduce scattering and lead to stronger trapping. We designed, fabricated, and characterized polystyrene (PS) microspheres coated with silica (SiO_x) to test whether the trap stiffness of optical tweezers can be improved. An overview of the results is presented elsewhere.[8] Here, we focus on the trap stiffness for coated and uncoated microspheres, the linear range of detection and force, and the optimal coating parameters based on the Mie theory. We observed for the coated microspheres that in addition to an increased trap stiffness, the linear range of both the force gradient and the back focal plane positional detection was increased compared to uniform polystyrene

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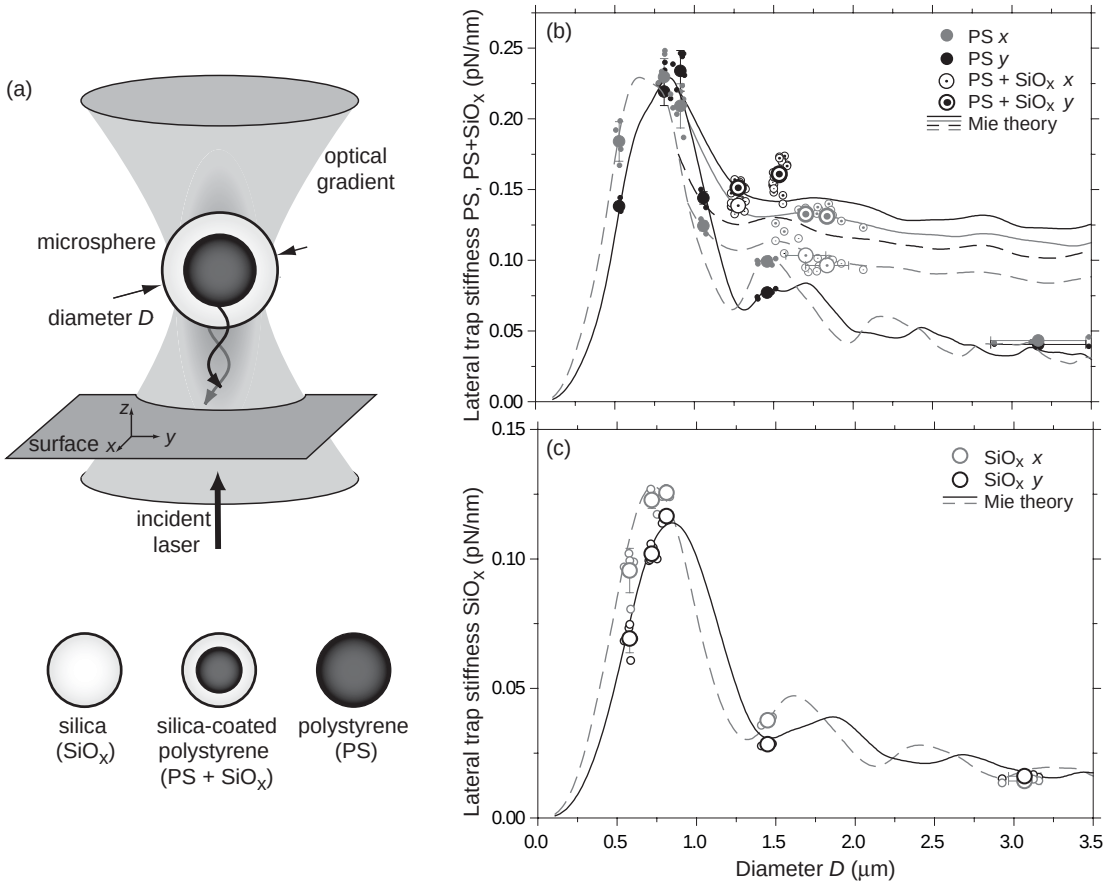

Figure 1. (a) Setup geometry: A coated microsphere (bead) with diameter D is trapped in a tightly focussed laser beam near a surface in an aqueous medium. The axial direction (propagation direction of the laser) is referred to as z. The laser was linearly polarized in the y-direction. (b,c) Lateral trap stiffness as a function of bead diameter for (b) PS, SiO_x-coated PS, and (c) SiO_x. The darker symbols and the solid lines are the y-direction. The coated beads had two different cores: for $D \leq 1.5\,\mu$m the core diameter was 913 nm (black symbols) and for $D > 1.5\,\mu$m the core size was 960 nm (gray symbols). The small symbols are the measured values for individual beads. The large symbols are averages ($N \approx 6$) for each bead type plotted with standard deviation. The lines are the Mie theory results.

spheres. Within the linear detection range, we achieved at least a 40% higher trap force compared to any uniform polystyrene sphere. Moreover, we performed calculations of the trapping potential varying coating parameters, bead size, and materials. We found quantitative agreement of the size dependence for coated and uncoated beads. Thus, we can use the theory to design core-shell particles to obtain the optimal trapping properties for an experiment, for example, microspheres having a high-refractive index core that cannot be trapped unless coated.

2. RESULTS

2.1 Characterization

We coated PS microspheres ($n_{\mathrm{PS}} = 1.57$) with SiO_x ($n_{\mathrm{SiO_x}} = 1.45$) using the method of Graf *et al.*[9] (see Bormuth and Jannasch *et al.*[8]). The refractive index of SiO_x nearly corresponds to the geometric mean of the indices from PS and water ($n_{\mathrm{H_2O}} = 1.326$). All refractive indices are with respect to a wavelength of 1064 nm. We used bright field, differential interference contrast, and transmission electron microscopy to verify the smooth and homogeneous coating and the monodispersity of the overall size of the microspheres.[8]

2.2 Trap Stiffness

We measured the trap stiffness with which SiO_x, PS, and SiO_x-coated PS microspheres with a total diameter D ranging from $0.5\,\mu$m to $3\,\mu$m were held in an optical trap created with a linearly polarized (y-direction), $\lambda = 1064\,$nm laser. All measurements were done in aqueous solutions close to a surface [Fig. 1(a)]. To calibrate position and force, we used our recently developed technique[10,11] which is based on a drag force method using a small sinusoidal stage movement combined with power spectral analysis. This allowed us—in the absence of assumptions—to measure the diameter of the bead, its drag coefficient, the distance from the surface, and as a function of this microsphere-surface distance, the displacement sensitivity (the volt-to-meter conversion factor of the photodiode) and the trap stiffness for all spatial directions. Foremost, we determined the lateral trap stiffness [Fig. 1(b,c)] at the surface for the three different bead types.

For both SiO_x and PS beads, there was a pronounced and sharp maximum in trap stiffness for beads with a diameter corresponding roughly to the trapping laser wavelength in the medium ($\approx 0.8\,\mu$m).[12] The exact position of the maximum depended on the lateral direction. For larger diameters, the lateral trap stiffness approached quickly the geometric optics limit and for very small diameters it converged to the Rayleigh theory.[8] Based on preliminary calculations (see Theory and Discussion), we did not expect to find improvements in trap stiffness for PS-SiO_x core-shell particles with a core diameter of $\lesssim 0.8\,\mu$m. Therefore, we used core sizes of around 900 nm. The coated beads with a total diameter of $1.3\,\mu$m to $1.8\,\mu$m resulted in a more than two-fold stiffer trap compared to uniform PS and SiO_x beads of the same size (see also Theory). Relative to the peak trap stiffness of PS, the coated beads were held with a $\approx 0.7\times$ smaller trap stiffness. In contrast, compared to larger uniform microspheres the coated microspheres produced an up to 10-fold stiffer trap. The trap stiffness differed for the two lateral directions which we attributed to the linear polarization of the laser.[8] All measurements agreed quantitatively with Mie scattering calculations (see Theory).

2.3 Linearity

We measured the detector response and found an increased linear range for the $1.5\,\mu$m-diameter coated particles compared to the uniform beads. In order to measure the response, we scanned fixed beads through the laser by moving the stage laterally. The resulting detector signal as a function of stage position is plotted in Fig. 2(a,c,d). The slope of this signal at the origin corresponds to the inverse of the displacement sensitivity at the surface. Compared to the $1.5\,\mu$m-diameter uncoated beads, the detector response for the coated particles showed that the gradient in the center was not only the highest but also remained nearly constant over a more than 2-fold larger region ($\pm 500\,$nm with $\leq 10\%$ deviation) [Fig. 2(a)]. In particular, the curve did not show points of inflection at the extent of the lateral laser focus ($\approx \pm 0.4\,\mu$m). Compared to the smaller $0.9\,\mu$m-diameter uncoated beads, the linear range was also at least 2-fold larger.

In order to test whether the reduced scattering force for the coated beads also improved the overall potential, we calculated the restoring force as a function of the displacement from the trap center [Fig. 2(b)]. The curves resembled in shape those of Fig. 2(a). Therefore, based on the theory, also the force gradient of the coated particles had a larger linear range compared to the uniform beads. To confirm this experimentally, we used drag force measurements and the force induced melting plateau of DNA at $\approx 60\,$pN as a force standard.[8] In addition to the $1.5\,\mu$m-diameter beads, we measured the detector response and calculated the restoring force for two additional bead sizes [$\approx 0.6\,\mu$m and $\approx 3\,\mu$m, Fig. 2(c,d)]. For both PS and SiO_x the linear range increased with the diameter. For the $3.2\,\mu$m-diameter PS beads, the linear range was $\pm 650\,$nm with $\leq 10\%$ deviation. Interestingly, the calculated force profile could be overlayed onto the detector response curve for all bead types. This implies that a good estimate for the linear force range can be obtained form the detector response curve. Furthermore, this means, that the detector response is directly proportional to the force, irrespective of any nonlinearity. The reason for this is that nonlinearities in the displacement sensitivity are canceled out by the nonlinearities in the trap stiffness.

3. THEORY

Since the size of particles that are typically used for optical trapping falls in the range where Rayleigh scattering and geometric optics are not applicable, computational approaches are necessary to quantitatively calculate the trapping efficiency. Most pertinent for optical tweezers is the theory based on Mie's now 100 year old

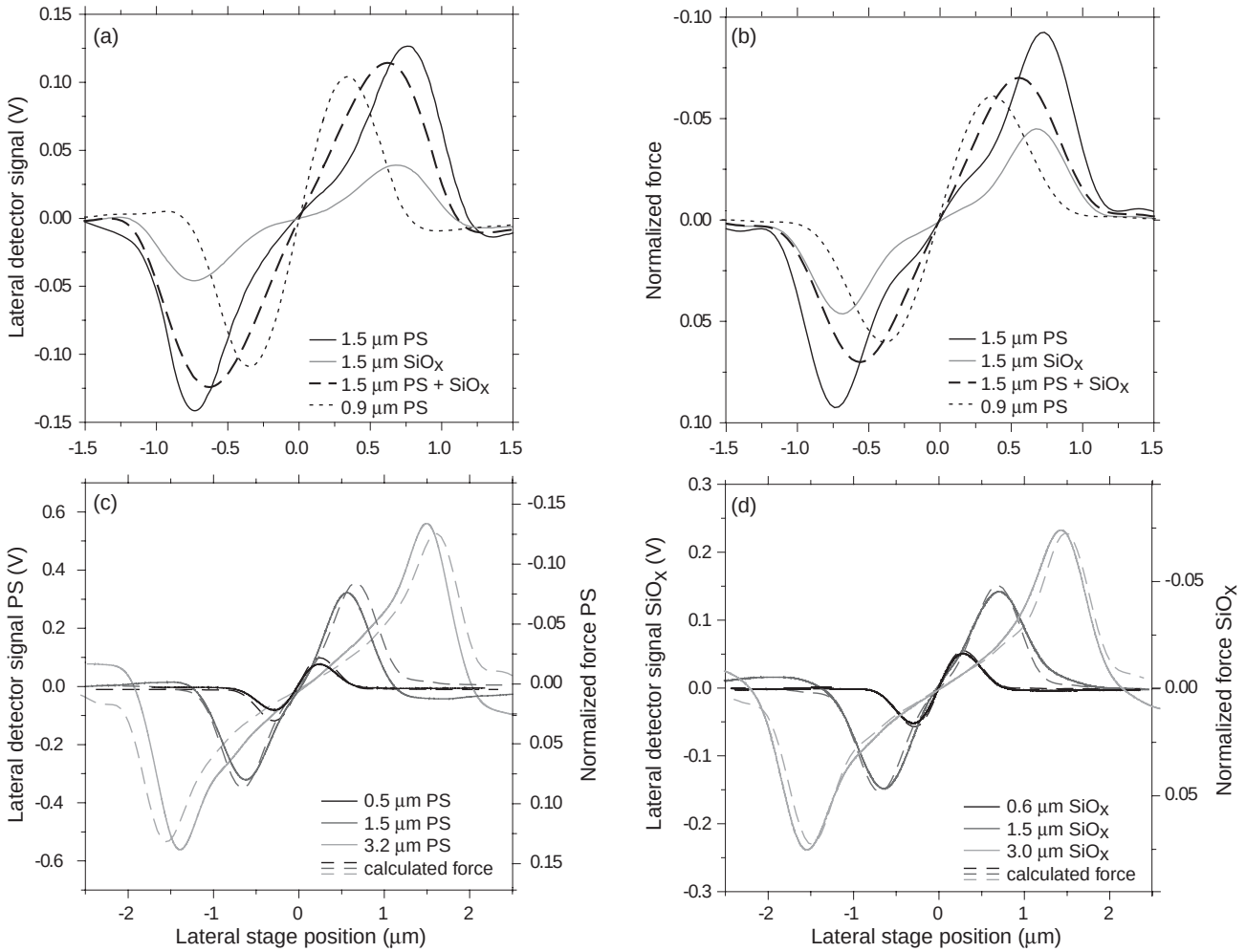

Figure 2. (a) Lateral detector signal measured as a function of lateral stage position for surface-immobilized beads. (b) Normalized restoring force calculated as a function of the displacement from the trap center. (c,d) Lateral detector signal measured as a function of lateral stage position for (c) PS and (d) SiO_x surface-immobilized beads. The dashed curves are the calculated normalized restoring force scaled to the detector response curve. The scaling factor depended only on the material, but not on the bead size.

classic paper.[13] This exact theory was recently implemented using the $\boldsymbol{T}$-matrix method in an optical tweezers computational toolbox[14] written in MATLAB® which we extended to include coated spheres.[8] Recently, the authors of the toolbox also extended it to include coated spheres.[15] Their calculations are in agreement with our calculations and experiments. Apart from our work,[8] several studies demonstrated quantitative agreement between theory and experiment for uniform microspheres, however, with limited ranges of bead diameters.[12,16,17]

Compared to these studies, we have quantitative agreement for different materials and bead sizes covering both the Rayleigh and geometric optics limits. The lines in Figs. 1 are the best fit of the Mie theory to all trap stiffness measurements of SiO_x and PS. The best fit was obtained with respect to the following parameters: the refractive indices of the particles ($n_{SiO_x} = 1.45$, $n_{PS} = 1.57$), the effective numerical aperture ($NA_{eff} = 1.25$), the amount of overfilling described by a truncation angle ($\theta = 71°$ corresponding to $a/\omega = 0.9$ filling of the objective back aperture having a radius a with respect to the Gaussian beam waist ω consistent with our setup), and the laser power (e.g. in Fig. 1(b,c) the calculated power in the focus was 62 mW). No other adjustable parameters entered. The effective numerical aperture is lower than the NA = 1.3 specification for the trapping objective.

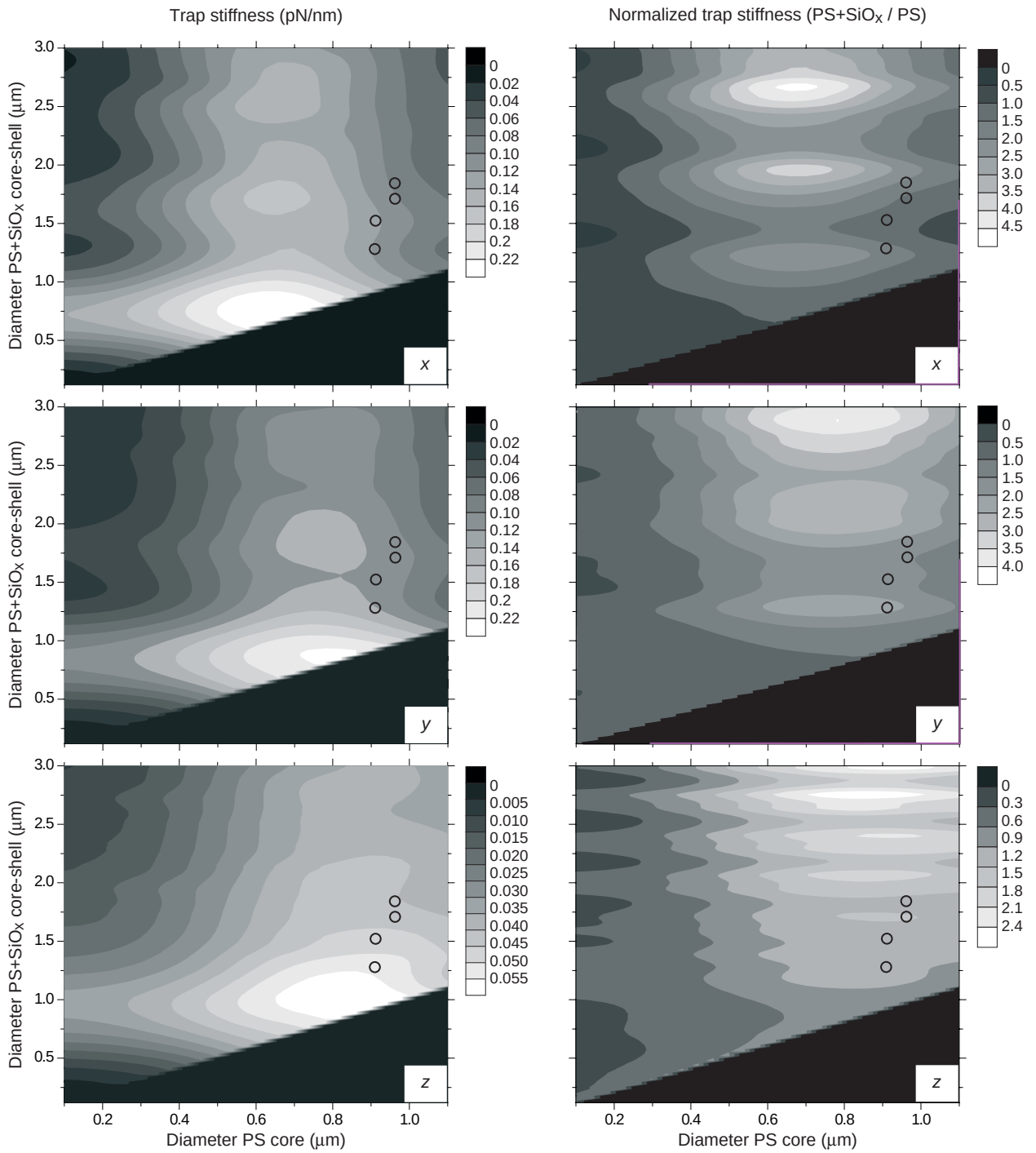

Figure 3. Calculated contour plots of trap stiffness for a range of core and total microsphere sizes. In the left column, the lateral (x,y) and axial (z) trap stiffness (in pN/nm) is plotted. In the right column, an improvement/quality factor is plotted. Here, the values of the left-hand side were normalized by the trap stiffness of a uniform PS microsphere having the same overall size as a coated one. The black region in the lower right-hand corners corresponds to undefined parameter space. The total diameter was varied with 20 nm and the core with 40 nm increments. Contours were interpolated. The parameters that entered the calculation are given in the Theory section.

We think that the reduction is mostly caused by a smaller transmission of the objective at the outer edges for the incoming beam.[18] The magnitude of the reduction is similar to the reported one.[18] Other factors that contribute to a lower effective NA are the non-ideal Gaussian laser beam and deficiencies in the theory such as a phenomenological description of the effect of overfilling and a lack of treatment of spherical aberrations. These factors may also explain discrepancies between individual data points and the theory. In addition, beads from individual batches may have different refractive indices or be slightly elliptical in shape.

The relative root-mean-squared deviation of the data from the theory was 10%. This quantitative agreement implies that a force calibration is unnecessary if our achieved accuracy is sufficient and/or calibration procedures are difficult, for example, when working inside cells. Furthermore, it lends trust to the calculations that the theory and computations can be used to design optimal-coated microspheres.

To find optimal coating parameters, we calculated the trap stiffness for a range of PS core sizes having varying SiO_x-coating thicknesses (Fig. 3). Using the above parameters for the theory, we calculated both the lateral and axial trap stiffness (left column) and normalized these values by the trap stiffness of a uniform PS microsphere with the same overall size (right column). With a PS core size of $0.8\pm0.1\,\mu$m, the trap stiffness of the coated microspheres decreases from the pure PS maximum trap stiffness to a level of $\approx$70% of the latter. A constant level of 70% is maintained for increasing coat thicknesses.[8] This is remarkable since the trap stiffness of uniform microspheres decreases proportional to D^{-1}. Thus, for increasing coat thickness, the trap stiffness of coated microspheres compared to uniform PS microspheres increases on average proportional to D (right column). Modulations of this behavior, i.e. maxima in trap stiffness or the improvement factor are due to Mie resonances. The black circles in Fig. 3 mark the coated microspheres that we made.

4. DISCUSSION

Anti-Reflection. We set out to reduce the amount of backscattering on microspheres trapped in optical tweezers. For the coated beads, the overall amount of backscattered light is reduced compared to the PS core.[8] The lowest amount of backscattering was achieved with the $1.3\,\mu$m-diameter, coated beads. These beads have a silica layer thickness that corresponds to the optimal coating for a planar anti-reflection coating.[8] The contour plots (Fig. 3) showed that these have also the best overall performance of the different coated beads that we produced. Based on our preliminary calculations and intention to optimize trapping, in particular, in the axial direction, we chose core sizes of around 900 nm. The exact calculation showed that the beads that we synthesized are not optimal. Based on the theory, a slightly smaller core size would have yielded slightly better overall performance.

Trap Stiffness. The measurement and the theory showed that the choice of the correct bead diameter and material could improve the trap stiffness. However, the choice of microsphere is determined by the requirements of the experiment. The highest trap stiffness is not always the only consideration. For instance, to resolve fast dynamics in motor protein studies, beads with a low drag coefficient and, thus, with a small diameter ($<0.8\,\mu$m) are used.[19] Otherwise, large microspheres deliver various advantages and were used in many studies (e.g. 1.4-$4\,\mu$m diameter in[20–26]). For instance, the light intensity at the surface where the protein of interest is adsorbed is reduced. Another advantage is that large microspheres are better visualizable in bright field microscopy in particular if the video image is used for quantitative measurements. Another advantage of larger beads, is less local heating. Since the absorption factor at 1064 nm for water (14–$15\,\mathrm{m}^{-1}$)[27,28] is much larger than the one for polystyrene (4.5–$6\,\mathrm{m}^{-1}$)[28,29] and silica ($0.005\,\mathrm{m}^{-1}$)[28] larger beads cause less heating. If large beads are desired for an experiment it would be very useful to be able to obtain high trapping stiffnesses with less laser power. In this size range, the coated microspheres provide a substantial improvement compared to beads with the same outer diameter; we measured an up to 10-fold increase in trap stiffness compared to $3\,\mu$m-diameter silica beads.

Maximal Forces. Compared to smaller-diameter uniform beads, the coated microspheres can provide an improvement. Even compared to the $0.9\,\mu$m diameter PS beads that had the highest trap stiffness, the highest lateral and axial forces in the linear operating range of the tweezers are still achieved with the coated beads. For a Hookean spring, this maximal lateral force is the displacement times the trap stiffness both of which need to be still within their linear range. With a $>2\times$ larger lateral linear range [Fig. 2(a)] and a $\approx0.7\times$ lower lateral trap stiffness [Fig. 1(b)], the maximal lateral force is $\geq1.4\times$ larger for the coated beads compared to the optimal trap stiffness, $0.9\,\mu$m diameter, PS beads. Even for the large linear range of the $3.2\,\mu$m diameter PS beads,

the maximal force in the linear range is not larger than for the 0.9 μm beads since the trap stiffness decreases proportional to the inverse of the diameter. The trade-off of our coated beads is that they were larger than the 0.9 μm diameter PS beads and therefore had a larger drag in particular near a surface. A larger drag means a lower force sensitivity.

Achieving ultimately even higher trap efficiencies also for smaller beads is only possible for higher refractive index materials. However, there is an upper limit for the refractive index for which uniform microspheres can still be trapped (see Introduction). Coating high-n particles, opens up new possibilities to trap for instance particles with a ZnS[30] or TiO_2 ($n \approx 2.4$) core in a single beam tweezers. For such high-n cores, coated particles with an overall diameter $D \approx \lambda$ can be realized with a higher trapping efficiency than PS. For the parameters of our setup, if we use an optimal coated TiO_2-core microsphere with an outer diameter of $\approx$1 μm, theoretically having 4 W of laser power, we could achieve a trap stiffness of 4.3 pN/nm [$\approx$7.4 pN/(nm W); more than 2-fold larger compared to PS]. Thus, with a displacement of 240 nm, which is still well within the linear detection range for coated particles, a force of more than 1 nN could be realized. Experiments in the nanonewton force range, for instance protein unfolding or intra- and intercellular measurements, with at least a piconewton resolution are therefore feasible.

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AUTHOR CONTRIBUTION

E.S. designed research; V.B., A.J., C.M.v.K., and E.S. performed research; C.M.v.K. and A.v.B. contributed new reagents/analytic tools; A.J., V.B., and E.S. analyzed data; A.v.B. and J.H. provided advice and commented on the manuscript; and A.J. and E.S. wrote the paper.

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