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High Trap Stiffness Microcylinders for Nanophotonic Trapping

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Abstract

Nanophotonic waveguides have enabled on-chip optical trap arrays for high-throughput manipulation and measurements. However, realization of the full potential of these devices requires trapping enhancement for applications that need large trapping force. Here, we demonstrate a solution via fabrication of high refractive index cylindrical trapping particles. Using two different fabrication processes, a cleaving method and a novel lift-off method, we produced cylindrical silicon nitride (Si_3N_4) particles and characterized their trapping properties using the recently developed nanophotonic standing-wave array trap (nSWAT) platform. Relative to conventionally used polystyrene microspheres, the fabricated Si_3N_4 microcylinders attain an approximately 3- to 6-fold trap stiffness enhancement. Furthermore, both fabrication processes permit tunable microcylinder geometry and the lift-off method also results in ultrasmooth surface termination of the ends of the microcylinders. These combined features make the Si_3N_4 microcylinders uniquely suited for a broad range of high-throughput, high-force, nanophotonic waveguide-based optical trapping applications.

Graphical Abstract

Keywords

nanophotonics; microparticle; optical trapping; lab-on-a-chip; microcylinder; nanofabrication; trap stiffness; high refractive index

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Author Contribution Note

All authors contributed to the writing of the manuscript. M.D.W, R.P.B., and F.Y. planned the experiments and analyzed the data. R.P.B. and W.C. fabricated the microcylinders. R.P.B and F.Y. performed the trap stiffness experiments. R.P.B and F.Y. wrote and analyzed the simulation programs. F.Y. fabricated the nSWAT devices used in this work.

Conflict of Interest Note

The authors declare that there is no financial or other conflict of interest related to this work.

1. Introduction

Over the past three decades, optical trapping has proven to be a powerful tool in the physical and biological sciences. Trapped particles, which serve as handles for biological molecules, can be manipulated with piconewton (pN) forces and nanometer (nm) precision^{1, 2}, making optical trapping especially valuable for biophysical, chemical, biochemical, and cellular studies. Recent advancements in a broad range of nanophotonic structures hold promise to enable optical trapping experiments at high-throughput on-chip^{3–12}. These nanophotonic devices are compact and portable, and can be fully integrated with microelectronic and microfluidic components^{13,14}.

While nanophotonic trapping potentially offers tremendous benefits over free space traps^{14, 15}, challenges remain in creating stiff traps for various manipulation applications. Thus far, significant effort has been devoted to the optimization of the coupling of a free-space laser to a given device¹⁶ and to the minimization of waveguide propagation losses^{17, 18}. Several strategies have also been developed to enhance laser power at the trapping region and trapping efficiency. Slotted waveguides afford significant power enhancement but require trapping particles to have a size smaller than the slot¹⁹. Photonic crystal waveguides also provide substantial local power enhancement, but are limited in their flexibility of particle position manipulation^{5, 6, 20}.

For biological applications, power enhancement is not a viable option when the waveguide materials cannot sustain high powers due to non-linear power absorption²¹ or must be operated at sufficiently low power to minimize laser heating of the aqueous solution⁴. Thus while greater power in a device may be advantageous, a more desirable solution is power usage efficiency in applications involving biomolecules. This need requires a method that permits further nanophotonic trap stiffness enhancement.

In this work, we take an alternative and complementary approach to trap stiffness optimization by creating high refractive index microcylindrical trapping particles. Trap enhancement from higher refractive index particles has not been explored in nanophotonic traps though it has been demonstrated in free space optical traps and laser fibers^{22–24}. The new particles developed in this work are nanofabricated out of silicon nitride (Si₃N₄) thin films, which offer a higher index of refraction ($n = 2.0$ at wavelength $\lambda = 1064$ nm) over conventional polystyrene ($n = 1.57$) or silica ($n = 1.45$) microspheres. In addition, as compared to spherical particles, their cylindrical shape allows for more overlap of a particle with the evanescent trapping field of a nanophotonic waveguide. These characteristics are congruent with stiffer traps. We characterized their trap stiffness using a nanophotonic standing-wave array trap (nSWAT) device capable of precise manipulation of a trap array^{7, 17, 25} and found that the microcylinders provide significantly higher trap stiffness than conventionally used polystyrene microspheres. We anticipate that this approach for trapping enhancement should also be applicable to many other nanophotonic trapping functionalities in a broad range of microfluidic devices.

2. Experimental Methods

For our previous nSWAT measurements^{7, 17, 25}, we employed spherical trapping particles which are commercially available and widely used for optical trapping. However, microcylinders of desired dimensions are not commercially available and must be fabricated. We thus developed methods to fabricate Si_3N_4 microcylinders using two approaches: a cleaving method or a lift-off method. Here, we refer to the resulting microcylinders as “cleaved Si_3N_4 microcylinders” or “lift-off Si_3N_4 microcylinders” respectively. Both methods are based on high-throughput and cost-efficient deep ultraviolet (DUV) lithography to pattern at the sub-micron scale and are detailed below.

2.1 Cleaved Si_3N_4 Microcylinders

Microcylinders were cleaved from top-down fabricated micropillars composed of a Si_3N_4 core with a 50 nm thick hafnium dioxide (HfO_2) inner shell and a ~5 nm silicon dioxide (SiO_2) outer shell (Figure 1a, Figure S1). This approach is inspired by our previously established protocol to fabricate quartz microcylinders^{26–32} and we adapted our protocol for Si_3N_4 microcylinders.

To begin the fabrication process, a 4-inch standard silicon wafer was coated with 930 nm of low stress Plasma Enhanced Chemical Vapor Deposition (PECVD) Si_3N_4 . We used DS-K101–312 for the underlayer antireflective coating (ARC) on the wafer, as this ARC is readily removed by standard 726 MIF developer. The negative-tone DUV photoresist UVN 2300–5 was spun on the ARC-coated wafer at 1300 rpm with a 70 s 110 °C bake, for a final thickness of approximately 800 nm. The photoresist was then patterned in a 4x lithographic stepper (ASML 300C DUV Stepper) with 248 nm light, exposing a pattern of microcylinders spaced in a hexagonal lattice pattern, with center to center distance of 1 μm between a cylinder and its 6 closest neighboring cylinders (5.1 billion cylinders total). The patterned Si_3N_4 was then etched by high power (3000 W) $\text{CH}_2\text{F}_2/\text{He}$ plasma chemistry¹⁷, with etch rates of approximately 200 nm/min for Si_3N_4 and 80 nm/min for the photoresist.

After etching, we used isotropic, fluorine-based, Buffered Oxide Etch (BOE) wet etching of Si_3N_4 , and SiO_2 and HfO_2 layer growth by Atomic Layer Deposition (ALD) to attain target cylinder dimensions guided by our simulations (see below). This allowed fabrication of smaller diameter cylinders (~350 nm) than possible with the DUV photolithography equipment used, but is not generally necessary if the photolithographic patterning tool achieves target dimensions at the start. Specifically, we coated a 50-nm layer of ALD HfO_2 ($n = 2.0$) on the Si_3N_4 core after the BOE etch to reach our target dimensions, with core and post-ALD dimensions verified by scanning electron microscope (SEM). ALD HfO_2 was chosen over ALD Si_3N_4 because oxide ALD processes have substantially shorter deposition times, and these two materials have nearly identical refractive indices. We then added a final 3–5 nm ALD SiO_2 shell for potential chemical functionalization of the cylinders. The microcylinders were mechanically cleaved off with a razor blade and suspended in 10 mM Tris-HCl pH 8.0 for measurements. See Figure S1 for a schematic of microparticle material layers.

2.2 Lift-off Si₃N₄ Microcylinders

Lift-off Si₃N₄ microcylinders were composed entirely of Si₃N₄, fabricated top-down, and were recovered by dissolving an Al₂O₃ sacrificial layer (Figure 1b, Figure S1). To our knowledge, Al₂O₃ has not previously been used as a sacrificial layer for microparticle generation. In addition, this novel lift-off method results in a minimally toxic and biocompatible product compared to other microparticle lift-off methods used in optical trap applications which involve dissolving an entire gallium arsenide wafer to lift-off the microparticles³³.

A 4-inch Si wafer was first coated with 60 nm of Al₂O₃ using plasma-based ALD before being coated with approximately 385 nm of low stress PECVD Si₃N₄. The wafer was then subjected to the same photolithography protocol for patterning and etching as detailed above for the cleaved microcylinders, except that the diameter shrinking of the pillars post-etching was not needed due to the larger ~500 nm target diameter being attainable via the DUV exposure. To perform the microcylinder lift-off, the alumina sacrificial layer was dissolved by placing the wafer in 726 MIF developer and sonicating at 60°C, for one hour and then heating for an additional 4 hours without sonication. The sample quality was then inspected by SEM and finally the microcylinder solution was placed in centrifuge tubes and centrifuged for 5 minutes at 8000 RCF and 20°C. The supernatant was decanted and the microcylinder pellet, easily visible by the naked eye, was washed, spun and resuspended twice with DI water before final suspension in 10 mM Tris-HCl pH 8.0.

Both methods produce 1 ml stock solutions of approximately 10 pM (close to 100% yield) at ~\$500 (excluding labor) per 4" wafer using the Cornell NanoScale Science & Technology Facility. We anticipate significant cost reduction if these cylinders were to be mass-produced commercially. In our biophysics laboratory, multiple users can use one such stock solution for 10–12 months of experiments²⁶.

We found the lift-off method to be especially advantageous over cleaving for fabricating shorter cylinders (<500 nm height) that would otherwise be difficult to cleave, producing cylinders with a smoother end termination on *both* the top and bottom surfaces, compared to the cleaved protocol where only the microcylinder top is smooth.

2.3 Polystyrene Microspheres

The carboxylated polystyrene microspheres were purchased from Polysciences Inc. (product #21753). The manufacturer measured the microspheres as 380 ± 10 nm diameter (CV 3%) and highly spherical geometry. These specifications were verified by SEM.

2.4 Numerical Force Simulations

To quantitatively assess trap stiffness enhancement with the use of Si₃N₄ microcylinders, we first performed 3D full-field electromagnetic simulations of the microcylinders using the COMSOL Multiphysics finite element method software. These simulations for microcylinders used the same waveguide parameters as those previously published for polystyrene microspheres^{17, 25}.

2.5 Trap Stiffness Measurements

To experimentally determine the trap enhancement factor provided by the microcylinders, we monitored the Brownian fluctuations of a microcylinder trapped along the waveguide of an

nSWAT device and then analyzed these motion trajectories to determine trap stiffness using both power spectrum and variance analysis methods, including corrections for blurring and aliasing introduced by the camera^{17, 34–36}.

Positions of microcylinders were tracked by a cross-correlation-based method³⁷. The power spectrum of tracked microcylinder positions were resampled by box-car windowing in the log frequency space, and then fitted by a modified Lorentzian function with weighting factors proportional to the sample mean^{35–38}. The modified Lorentzian function takes into account the camera blurring effect due to finite integration time, and the aliasing effect due to finite camera frame rate, and a white noise term^{35,36}.

$$P(f) = \sum_{n=-\infty}^{+\infty} \frac{\frac{k_B T}{2\pi^2 \beta}}{f_c^2 + (f + n f_s)^2} \times \left(\frac{\sin(\pi(f + n f_s)W)}{\pi(f + n f_s)W} \right)^2 + \frac{\epsilon^2}{f_s}$$

where $k_B T$ is the thermal energy, f_c is the corner frequency, f_s is the sampling frequency, W is the camera integration time, β is the drag coefficient, and ϵ^2 is the white noise term. Fitting this modified Lorentzian function (truncating at $n = 4$) yields f_c and β , and trap stiffness $k_{\text{psd}} = 2\pi f_c \beta$ can be calculated. The variance analysis of the microcylinder traces was also modified for camera blurring and aliasing effects and white noise taken into account³⁵:

$$\text{Var} = \frac{k_B T}{k_{\text{var}}} \left(\frac{1}{\pi W f_c} - \frac{1}{2\pi^2 W^2 f_c^2} \left(1 - e^{-2\pi W f_c} \right) \right) + \epsilon^2,$$

yielding the trap stiffness k_{var} . The above mentioned power spectrum analysis and variance analysis correspond to two methods of trap stiffness determination. When they are properly implemented and executed, k_{psd} and k_{var} are expected to show consistent values^{35,36}.

3. Results

3.1 Microcylinder Characterization

We characterized the microcylinders' dimensions using SEM. The cleaved Si_3N_4 microcylinders ($N = 45$) were 742 ± 75 nm (mean $\pm$ SD) in height, 303 ± 27 nm in top diameter, and 353 ± 21 nm in bottom diameter (Figure 2a). The lift-off Si_3N_4 microcylinders ($N = 76$) were 386 ± 20 nm in height and 517 ± 35 nm in diameter (Figure 2b). These variations correspond to a coefficient of variation of approximately 10% in height and 6–9% in diameter for the cleaved microcylinders, and approximately 5% in height and 7% in

diameter for the lift-off microcylinders. We attribute the reduced height variation of the lift-off microcylinders to the more controlled removal of microcylinders from the wafer.

We further investigated the orientations of these microcylinders on an nSWAT device¹⁷ that operates with a bio-friendly 1064 nm laser for minimal sample heating. In such an nSWAT device, the nanophotonic standing-wave platform results from a standing wave that is generated by the interference of two counter-propagating laser beams in a Si₃N₄ nanophotonic waveguide, forming an array of stable three-dimensional optical traps at the antinodes of the evanescent field. By tuning the phase difference between the two counter-propagating laser beams using a microheater^{7, 39}, the optical trap locations can be precisely repositioned and manipulated.

We found that when trapped on an nSWAT device, a cleaved microcylinder was always oriented with its cylinder axis along the waveguide's top surface (Figure 2c), whereas a lift-off microcylinder was always oriented with its cylinder axis perpendicular to the waveguide's top surface (Figure 2d). In each case, the microcylinder was orientated in such a way that allowed for optimal volume overlap between the microcylinder and the evanescent wave.

3.2 Numerical Force Simulations

Trapping forces along three directions (Figure S2) were calculated across one spatial period of a standing wave trap of an nSWAT device, which is approximately 355 nm¹⁷. For each type of microcylinder, we performed these simulations at or close to the mean values of the fabricated cylinder dimensions (Figure 3a). As a comparison, we also performed similar simulations using 380 nm polystyrene microspheres, which are commercially available and have a mean size close to the optimal diameter for trapping for this type of microsphere^{17, 25} (Figure S3). In our simulations, all microparticles were assumed not to rotate, which is a standard treatment in the nanophotonic trapping field¹⁷. For a symmetric and optically isotropic microcylinder, rotation about its cylindrical axis will not alter the trapping force. However, due to unavoidable fabrication imperfections, a microcylinder may have some slight shape asymmetry. This may give the microcylinder a slight preference for rotational orientation.

In this work, we are primarily concerned with forces along the waveguide (*z*) because this is the direction along which trapped particles can be translocated along the waveguide by the modulation of the microheater. Figure 3b shows how the trapping force along *z* varies with displacement from their equilibrium positions for cleaved Si₃N₄ microcylinders, lift-off Si₃N₄ microcylinders, and 380-nm polystyrene microspheres. For all three types of particles, the trapping force reaches a maximum when the center of the particle is approximately 90 nm from the trap center. However, the maximum force of a microcylinder, of either type, is approximately 6 times that of the 380-nm polystyrene microspheres, representing significant trapping force enhancement.

Figure S4 shows that both types of fabricated microcylinders have dimensions close to those that which would support high trapping forces. Although longer cleaved Si₃N₄ microcylinders or wider lift-off Si₃N₄ microcylinders may allow for somewhat higher

trapping forces, increased sizes of these microcylinders will reduce measurement throughput when they are used to form an array on the nSWAT.

In addition, we found that both types of microcylinders also afford similar force enhancement relative to the microsphere along the other two directions (x and y) (Figure S2).

Finally, further simulations demonstrated that the force enhancement is due to significant contributions from both increasing the refractive index of the microparticle material, and switching from spherical to cylindrical geometry (Figure S3 & S5).

3.3 Trap Stiffness Measurements

The trap stiffness was measured for cleaved microcylinders, lift-off microcylinders, and 380 nm polystyrene microspheres using the power spectrum (Figure 4a) and variance (Figure 4b) methods. Since trap stiffness of a travelling wave trap is conventionally reported in the units of pN/nm per watt of laser at the trapping center^{37, 40–42}, for ease of comparison, we define an “effective power” for the nSWAT to be the sum of the magnitudes of powers in the counter-propagating waves at the trapping region¹⁷. The effective power in the waveguide at the trapping region was ~ 7 mW. Additionally, based on our COMSOL simulations, this power is expected to increase the local temperature by only ~ 0.007 °C, which should have minimal influence on the measurements.

Figure 4c shows the measured trap stiffness enhancement factors of the two types of microcylinders relative to 380 nm polystyrene spheres, along with the numerically predicted trap stiffness enhancement factors from the COMSOL simulations. Compared with the measured trap stiffness of microspheres, the cleaved microcylinder’s trap stiffness shows a 6-fold enhancement and the lift-off microcylinder’s trap stiffness shows a 3-fold enhancement.

The error bars in the measured stiffness should reflect the sensitivity of trap stiffness to variations in the particle dimensions (Figure S4). It is important to note that although there were some variations in microcylinder trap stiffness, the trap stiffness of each microcylinder was individually calibrated. This *in situ* trap stiffness calibration permits accurate force determination in a force measurement application.

For the both microcylinder types, the measured trap stiffness is in agreement with prediction within measurement and theoretical uncertainties. The measured trap stiffness of the lift-off microcylinders was somewhat smaller than predicted, possibly due to the large contact region, between the flat cylinder bottom and flat waveguide surface, leading to a stronger repulsive electrostatic force that moves the microcylinders away from the waveguide.

4. Discussion

This work demonstrates the promise of using microcylinders of high refractive index ($n = 2.0$) to significantly enhance trap stiffness on nanophotonic waveguides and to our knowledge, represents the highest demonstrated trap stiffness on any biocompatible nanophotonic waveguide device. Comparable trap stiffness in nanophotonics has only been

demonstrated with photonic crystals²⁰ using a silicon waveguide operating with a 1550-nm laser that leads to significant laser absorption and sample heating at high laser powers. Our trap stiffness is also comparable to the highest reported in plasmonic traps⁴³, though plasmonic traps also experience sample heating at higher powers⁴⁴. Notably, in contrast with high refractive index microparticles for free-space traps²³, high refractive index microcylinders for the nSWAT applications do not require any anti-reflective layer particle coating, which when needed is normally difficult to optimize.

While the cleaved microcylinders provide significantly stronger trap stiffness, for biological applications we anticipate that the lift-off microcylinders are better suited at meeting the demands of high-throughput precision measurements because of their smaller dimension along the waveguide. Alternatively, if precision is less critical than achieving high trap stiffness in a particular application, the cleaved longer cylinders could instead be used. If even greater trap stiffness is called for, the lift-off fabrication protocols established here for Si_3N_4 may be readily extended to microparticle materials of even higher refractive indices such as amorphous Ta_2O_5 ⁴⁰, or amorphous or rutile TiO_2 ^{45, 46}. Figure S3 shows simulation of the force enhancement for trapping particles of higher refractive indices.

Our microcylinders are not commercially available and are top-down fabricated on a small scale, thus they incur a higher cost than commercial polystyrene microspheres. A precise cost comparison between our microcylinders and commercial polystyrene microspheres is challenging and produces disparate results because the cost breakdown (including original development costs and benefits from large volume production) for commercial microspheres is unavailable. In addition, an equitable cost comparison would need to quantify the unique experimental benefits conveyed by the microcylinders to the end user. The demonstrated resulting trap stiffness enhancement, which broadens the range of biological experiments with the nSWAT or other nanophotonic trapping devices, is unattainable with polystyrene particles. This stiffness enhancement advantage, coupled with accessible fabrication processes that result in each batch of fabricated microcylinders allowing for thousands of experiments, provides a compelling motivation to utilize these microcylinders even considering their modest production cost.

Future studies in manipulating single biological molecules on nanophotonic devices, such as on the nSWAT, may require a biological molecule of interest to be attached to a sidewall of a microcylinder. This would require surface functionalization of the sidewall only, which could be achieved prior to removal of the photoresist etch mask that blocks the top surface of the microcylinder. Recently, DNA attachment to Si_3N_4 surfaces was found to be as feasible as attachment to SiO_2 surfaces⁴⁶, and thus functionalization of only the sidewalls of the microcylinders is expected to be straightforward. Alternatively, a thin (1–5 nm) layer of ALD of SiO_2 could be coated over the cylinders as was done in the cleaved cylinder protocol, and standard protocols exist to functionalize a SiO_2 substrate²⁶.

5. Conclusions

In this work, we present two methods to fabricate high refractive index microcylinders for nanophotonic trapping and demonstrate that these microcylinders permit significant trap

stiffness enhancement compared with commercially available polystyrene microspheres. We anticipate that the significant enhancement of trap efficiency demonstrated here will enable a broader range of nanophotonic waveguide-based optical trapping applications, including parallelized biomolecular or cellular biophysical experiments under high forces (predicted as tens of pN or higher within demonstrated nSWAT specifications).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1:
Comparison of Si_3N_4 microcylinder fabrication processes between the (a) cleaved method adapted from a previous protocol²⁶ and (b) a novel lift-off method.

Figure 2: Scanning electron microscope (SEM) images of (a) cleaved Si_3N_4 microcylinders and (b) lift-off Si_3N_4 microcylinders. The measured dimensions of these cylinders are shown below each image. The trapped orientations of the microcylinders on an nSWAT device were determined for both (c) cleaved Si_3N_4 microcylinders and (d) lift-off Si_3N_4 microcylinders based on their optical images. On an nSWAT device¹⁷, a 1064-nm laser is coupled into the TM mode of a Si_3N_4 waveguide (250 nm high and 750 nm wide) before passing through a 50/50 splitter that creates counter-propagating waves to form a standing wave within a fluidic pool region. The antinodes of the standing wave form trap centers for an array of traps. The entire trap array may also be translocated via a microheater, which changes the phase of one of the counter propagating waves. Bright field microscopy image scale bars in the inserts of (c) and (d) are 1 μm in length.

Figure 3 Caption:

(a) Simulated distributions of the squared magnitude of the electric field when a cleaved Si_3N_4 microcylinder (top) or lift-off microcylinder (bottom) (white rectangles) is located at its equilibrium position above the waveguide. Each simulated microparticle is located at 10 nm above the waveguide surface¹⁷. (b) Predicted trapping force along the waveguide as a function of displacement of a trapped particle from its equilibrium position, for 1 W of effective power simulated in an nSWAT waveguide.

Figure 4 Caption:

(a) Trap stiffness may be measured by the power spectrum of the trapped particle motion. The example shown here is a measurement from a cleaved microcylinder at ~6 mW of laser power at the trapping region (540 Hz camera sampling rate, ~5–10 seconds per trace). A fit to the power spectrum using a Lorentzian function yields a trap stiffness of 4.16 pN/(nm-W). (b) Trap stiffness may also be measured by the variance of the trapped particle motion. The same trace as shown in (a) was analyzed based on the variance of the particle motion and equipartition theory. This yielded a trap stiffness of 3.94 pN/(nm-W). (c) A comparison of the measured trap stiffness enhancement for the cleaved ($N=11$) and lift-off ($N=6$) microcylinders relative to the measured values for more conventional 380-nm polystyrene microspheres ($N=18$). The viscous drag coefficient was measured to be $22.2 \pm 4.0 \times 10^{-6}$ pN-sec/nm for lift-off microcylinders, and $19.2 \pm 1.9 \times 10^{-6}$ pN-sec/nm for cleaved microcylinders. Error bars are standard deviations. Results from simulations are also shown for comparison with error bars obtained from a distribution of simulated microcylinders that match the size distribution of fabricated microcylinders.