Novel Optical Trapping Particles for Biological Experiments

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Abstract

Optical trapping is a powerful technique used to investigate the mechanical properties of the molecular motors that govern cellular processes. In order to examine such mechanisms, trappable "handles" must be developed that can be used for attachment to biological samples. This project involves the design and fabrication of cylindrical trapping particles to be used in measuring forces and torques exerted on deoxyribonucleic acid (DNA), in addition to optimizing existing fabrication protocols. In previous work, the entire top surface of a cylinder was chemically functionalized for binding to DNA. During the first stage of this project, this protocol was repeated with slight modifications in order to generate cylinders which dramatically reduced the unwanted precessing of particles in an optical trap, thereby minimizing measurement noise. The second stage principally dealt with fabricating smaller cylinders (600 nm height, 300 nm diameter) to allow for more accurate and precise measurements. Progress in this area has proved challenging, likely due to having approached the optical limits of the nanofabrication tools being used. It may ultimately prove more beneficial to design and fabricate a new type of trapping particle.

Introduction

There have been a myriad of uses for optical traps in the field of single molecule biophysics, with special emphasis on systems involving DNA. Briefly, an optical trap can be described as an instrument that uses collimated light, normally provided by a single mode laser, which is brought into a tight focus by high numerical aperture (NA) objective lenses to trap dielectric particles. The principal forces involved in an optical trap are the scattering force (a result of the momentum of photons) and the gradient force, which is the force that actually does the trapping. The scattering force "is proportional to the light intensity and acts in the direction of the propagation of light" while the gradient force is "proportional to the spatial gradient in light intensity and acts in the direction of that gradient" [1]. The diameter of the particles is on the order of the wavelength light that is being used.

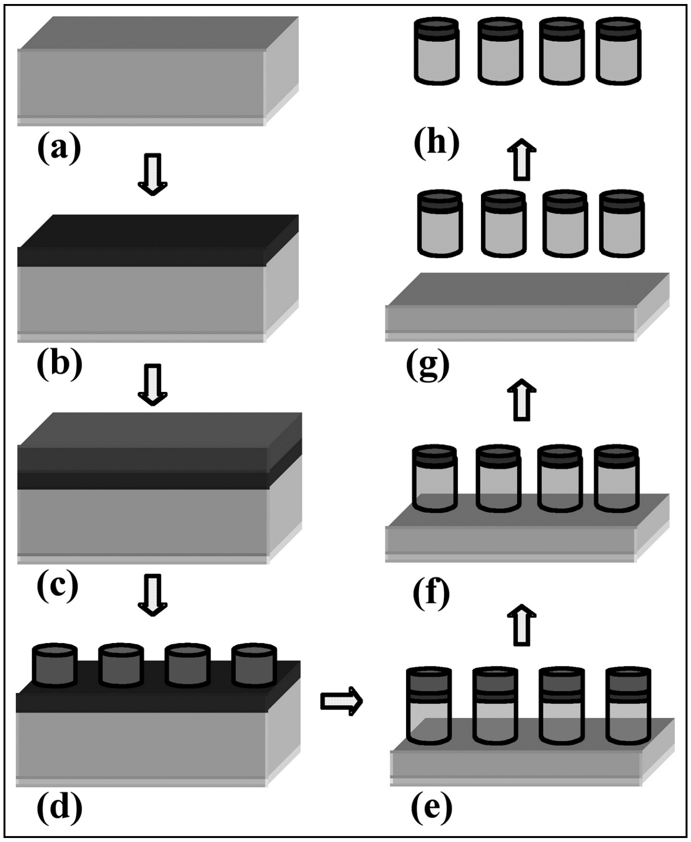

Figure 1: Optimized protocol.

In this project we used crystalline quartz, which is birefringent, as our substrate for fabricating cylindrical trapping particles in order to make measurements of torque and force on DNA in an angular optical trap. In addition to being a well understood fabrication material, quartz was used since "angular trapping occurs in particles made from materials such as quartz, in which the extraordinary axis of the crystal is more easily polarized [due to the birefringent nature of quartz] than the ordinary axes" [2]. When such a particle is positioned in an electric field, a polarization is induced on the particle. Any misalignment between the electric field and the polarization of the particle results in a torque [3]. By monitoring the ellipticity introduced in the trapping beam by this particle, the torque can be determined with great accuracy (typically ~ a few kT).

Fabrication

The primary purpose of this project was to master and improve the existing cylindrical nanoparticle fabrication protocols [4]. Figure 1 outlines the optimized fabrication protocol. Part (a) depicts the initial step in our protocol; here a thin anti-reflective coating (ARC) has been applied (this coating was used to prevent unwanted ring like structures from appearing on the wafer from the reflective chucks used in the exposure process). In (b) the top surface has reacted with 3-aminopropyltriethoxysilane (APTES); this is the functionalized area to which we want to attach a biological molecule. Approximately 660 nm of OIR 620-7i has been spun onto the wafer in part (c).

A 10x stepper is used to expose the pattern in (d). Section (e) depicts the first anisotropic dry etch (CHF_3/O_2 used as etching gas). Etching times were selected such that particles of height ~ 1 micron were generated.

In the next step, part (f), the resist is removed by sonicating the cylinders/wafer in an acetone solution. The resist was successfully separated from the cylinder, leaving behind the APTES layer for attachment to streptavidin protein. The cylinders, in section (g), have been cleaved using a microtome blade. Finally, part (h) depicts the end product; from here a sample chamber will be prepared and the localized APTES on the top of the cylinders has to be reacted with streptavidin in order for use in an optical trap.

Summary

A protocol for the production of anisotropic quartz cylinders for use in novel optical trapping experiments was successfully performed with improvements made over the published protocol. Further optimization of the fabrication protocol would involve reducing the overall size of the cylinder. Initial attempts proved challenging due to the optical limits of the nanofabrication tools being reached.

In addition, it may be possible to further refine and improve the trapping handles by employing new particle geometries or perhaps considering alternate fabrication substrates.

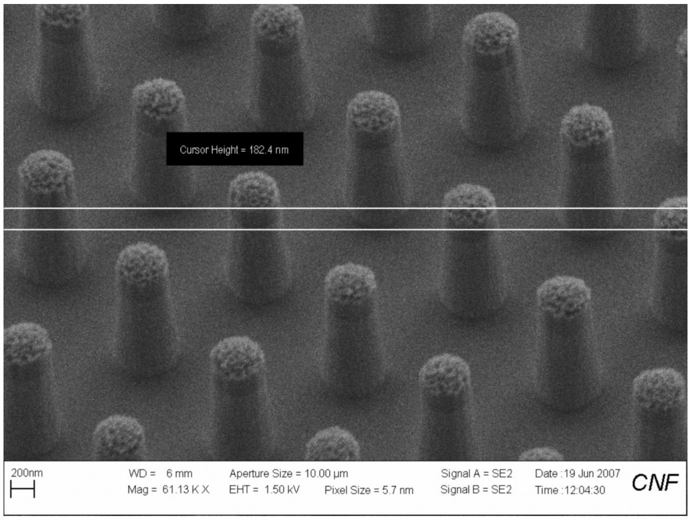

Figure 2, top: Quartz cylinders with resist on top after anisotropic dry etch is performed.

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[3] Ibid.

[4] Deufel, C. et al, "Nanofabricated quartz cylinders for angular optical trapping: torque detection during DNA supercoiling," Nature Methods Vol. 4, 223 (2007).