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Biosensing with nanoaperture optical tweezers

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ABSTRACT

Nanoaperture optical tweezers extend the range of optical tweezers to dielectric particles below 50 nm in size. This allows for optical trapping of proteins, DNA fragments and other biomolecules, as well as small viruses. With this label-free, tether-free approach proteins have been trapped, sized and their conformational changes observed in real-time. The molecular weight of proteins in the nanoaperture trap was determined from their Brownian motion statistics. This is useful for analysis of heterogeneous solutions: since this is a single molecule technique, it can be operated in "dirty" solutions with minimal sample preparation. The acoustic modes of proteins, DNA fragments and other trapped nanoparticles can be measured using a nanoaperture optical tweezer with two lasers creating a GHz to THz beat frequency. Interactions between proteins and DNA, small molecules (i.e., binding) and other proteins have also been demonstrated. This single molecule technique allows for measuring the dissociation constants of small molecules binding to proteins, both at equilibrium and at the single molecule level. For DNA fragments in the trap, it has been shown that the protein p53 can suppress unzipping and mutant p53 is ineffective to do so. This is promising for the discovery of drugs that effectively restore the function to p53. Integration of nanoapertures on the ends of fibers allows for translocation of the trapped object and may function as an optical "nanopipette" for microwell single molecule protein sampling. There is also potential to combine nanoapertures with nanopore translocation studies as well as fluorescence correlation microscopy studies and several researchers are already pursuing these areas of research.

1. Introduction

Optical tweezers allow for the manipulation of particles by changes in momentum from light scattering and absorption. For example, a particle that approaches the focus of a laser beam will scatter light away from the focus, which causes a change in the momentum of the photons away from the focus of the beam. The balancing force acting on the particle will push it towards the focus. The first works to use gradient force optical tweezers recognized that smaller particles of less than 100 nm were difficult to trap [1]. The main reason for this is that the polarizability of the particle scales as the third power of the particle size, from Rayleigh scattering. Therefore, a particle that is 10 nm particle will have 1000 times lower force acting on it than a 100 nm one [2].

For this reason, conventional gradient force optical tweezers have been very successful in manipulating particles in the size range from microns to 100 nm. These particles include bacteria and large viruses [3]. For smaller objects, tethers are often used [4]. For tether-free and label-free studying of small viruses, DNA, proteins, protein complexes and other non-biological nanoparticles, it is of interest to be able to trap particles smaller than 100 nm, down to the single digit nanometer range.

Researchers from the near-field optics and plasmonics communities have considered the use of high local fields for trapping, with the goal of reaching smaller particles or using lower powers to create a trapping landscape. Several works proposed using evanescent fields [5,6] or sharp tips [7] to achieve trapping and manipulation of particles. Optical antennas, or gold pads on a dielectric substrate, were able to trap micrometer sized particles and bacteria [8–10]. These works were still limited to rather large dielectric particles exceeding 100 nm in size. For metal nanoparticles, smaller particles could be trapped with plasmonic antennas, down to 10 nm [11]; however, metal nanoparticles below 20 nm had been trapped previously with conventional gradient force tweezers [12]. The approach of using nanostructures to increase the focus intensity in subwavelength dimensions allowed for stiffer traps and an efficient parallel trapping landscape, but to achieve trapping of smaller particles still required prohibitively large field intensities (albeit focused to a much smaller volume).

In 2009, a nanoaperture in a metal film was used to trap polystyrene particles down to 50 nm in size with low laser powers [13]. Previous works considered the trapping of particles with

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nanoapertures in metal films, but these works still used large (>200 nm) particles [14,15]. A key component of this nanoaperture optical tweezer was that the particle itself played an important role in the trapping by modifying the transmission through the aperture in a way that significantly exceeded the conventional zeroth-order perturbation theory. The forces calculated when including the particle in the simulated trapping landscape far exceed the forces given when the particle was omitted. This was referred to as self-induced back-action: the particle played an important role in its own trapping. A simplistic way of understanding this is to consider that subwavelength apertures in metal films have a fourth power diameter transmission dependence [16], but adding a particle makes the aperture effectively bigger by dielectric loading. Therefore, the nanoaperture leverages the strong fourth power scaling of Bethe's aperture theory to overcome the third power polarizability of Rayleigh scattering. While circular apertures allowed for trapping 50 nm particles with relatively low powers, researchers have investigated rectangular [17], coaxial [18,19], double-nanohole [20–22], and bowtie [23,24] apertures for trapping objects down to the single digit nanometer range.

In this work, I will focus the manipulation, sensing and spectroscopy capability of nanoaperture optical tweezers for biological nanoparticles. The types of biological particles that are probed are less than 50 nm in size, including proteins, DNA, and viruses, as well as their interactions (including with antibodies and small molecules). This work will focus specifically on optical tweezers using lasers, although there is clear potential for related works using apertures with electrostatic [25] and dielectrophoretic [26] forces. Many other non-biological objects have been trapped and probed with nanoaperture optical tweezers including dielectric spheres [20], lanthanide upconverter nanoparticles [27], quantum dots [28,29,24], magnetic nanoparticles [30] and metal nanoparticles [31]; a detailed discussion of these studies will not be given.

2. Protein and DNA analysis

The detection and monitoring of proteins is useful for many health applications, including basic biophysical investigation, drug discovery, and pathology. Single molecule methods are certainly not required for most of these applications; however, there are considerable advantages in using single molecule methods including the ability to observe dynamic processes without the need for synchronization, richer statistics, the ability to capture rare events and the ability to study systems at equilibrium (rather than approaching equilibrium as in conventional binding studies) [32,33]. In many cases, single molecule studies are required to interrogate the physical properties of individual biomolecules [4]. Flourescence-based techniques have dominated optical single molecule studies due to sensitive detection at high data rates. Even so, high intensities are required to observe fast processes, such as protein folding, and glycerol is often used to slow things down [34]. Furthermore, fluorescence based methods suffer from bleaching of the dye molecules, the requirement for fluorescent labels that are known to alter the natural interactions of proteins [35], and the requirement for tethers which act as hindrances to molecular reorientation and binding sites. For larger biomolecules, such as 4 kbp DNA, trapping with circular apertures can be sufficient [36]; however, for smaller biomolecules gap sizes of around 5–50 nm are desired [20].

2.1. Protein trapping and conformational changes

In 2012, it was demonstrated that a single protein could be trapped with a double-nanohole in a metal film [37]. Fig. 1 shows a schematic of the experimental setup, scanning electron microscope image of the double nanohole in a gold film and a typical transmission trace showing the dynamics of the protein trapping.

The trapping events were recorded as discrete transmission jumps (> 10%) of the laser power through the double-nanohole. When the laser was blocked briefly and re-opened, the power returned to its original value, showing that the protein had left the aperture. In addition, mPEG-thiol was used to coat the gold surface in an effort to reduce protein sticking. This was found in later works to be not essential. When the protein was trapped, the laser power not only increased, but also showed increased noise amplitude from the Brownian motion of the protein in the trap.

A surprising observation of that initial work was that the protein being studied, boving serum albumin, also showed a conformational change from the normal form (N) to the fast form (F) upon trapping. This was observed by an intermediate step in the laser power transmission when the protein was initially trapped in the N form and then a second step as the protein transitioned to the elongated F form. The elongated F form has a larger polarizability and therefore a deeper optical trapping potential as well as more transmission through the aperture. This transition was confirmed by reducing the pH to where the protein was in the F form

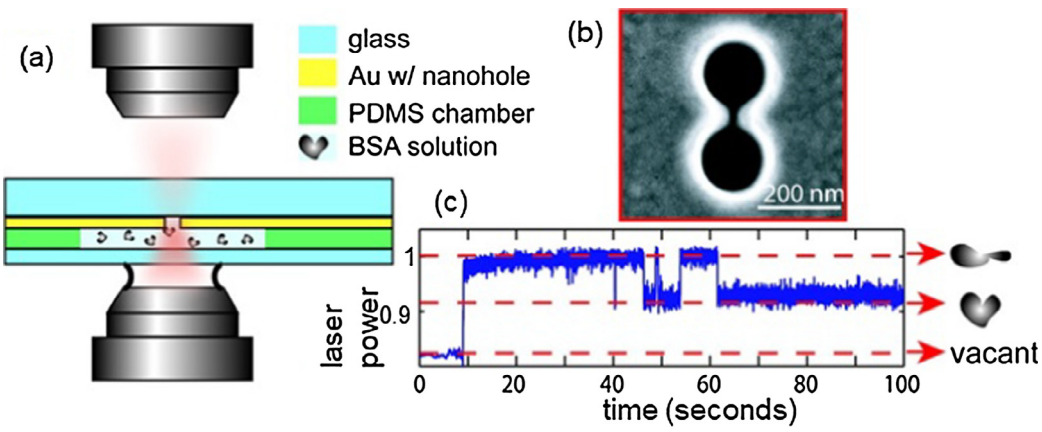

Fig. 1. (a) Schematic of double nanohole in gold film with PDMS/glass microslide chamber and oil immersion inverted microscope objective. The laser is incident from the lower objective and collected with the upper objective to be detected at an avalanche photodiode. (b) Scanning electron microscope image of double nanohole in 100 nm thick gold film as fabricated by focused ion beam milling. (c) Detected laser power on avalanche photodiode showing initial step of trapping event, but also transitions between a higher and lower power level corresponding to the folding and unfolding of the protein being trapped, bovine serum albumin. Adapted from [37] © 2011 American Chemical Society with permission.