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Kishan Dholakia
Gabriel C. Spalding
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The CID Number appears on each page of the manuscript. The complete citation is used on the first page, and an abbreviated version on subsequent pages.

Contents

- vii *Authors*
- ix *Conference Committee*
- xi *Nano-bio-optomechanics: nanoperture tweezers probe single nanoparticles, proteins, and their interactions (Plenary Paper) [9544-501]*

OUTREACH AND EDUCATION: INTERNATIONAL YEAR OF LIGHT

- 9548 08 **Tutorial: opto-mechanical cooling by the back-action of cavity photons [9548-8]**

TOWARD (OR IN) THE QUANTUM LIMIT OF OPTO-MECHANICS

- 9548 0B **Dispersive light-matter interaction in programmable optical tweezers [9548-11]**

OPTICAL MANIPULATION OF MATTER THROUGH GASEOUS MEDIA

- 9548 0C **Extinction cross section measurements for a single optically trapped particle (Invited Paper) [9548-13]**
- 9548 0D **Quadruple Bessel beam trap for single aerosol droplet studies [9548-14]**
- 9548 0E **Forces and dynamics for optically levitated polystyrene particles in air using electrostatic modulation [9548-15]**
- 9548 0F **Optical trapping of gold aerosols [9548-16]**

TRAPPING AT EXTREMES (I.E., "GONZO" TRAPPING)

- 9548 0H **Toward steering a jet of particles into an x-ray beam with optically induced forces [9548-18]**

FOUNDATIONS OF THE ELECTROMAGNETIC THEORY OF FORCE AND MOMENTUM

- 9548 0J **Continuum electrodynamics and the Abraham-Minkowski momentum controversy [9548-21]**
- 9548 0K **Energy and linear and angular momenta in simple electromagnetic systems [9548-22]**

- 9548 OL **Physics of electromagnetic and material stresses in optical manipulation** [9548-23]
- 9548 OM **Discriminatory effects in the optical binding of chiral nanoparticles** [9548-24]
- 9548 ON **1D problems of radiation pressure on elastic solids** [9548-25]
- 9548 OO **Optodynamic description of optical manipulation** [9548-26]

LASER-INDUCED SHOCKWAVES AND CAVITATION

- 9548 OP **A model for traumatic brain injury using laser induced shockwaves** [9548-27]
- 9548 OQ **Periodic cavitation in an optical tweezer** [9548-28]
- 9548 OR **Interesting physics and applications using microbubbles in thermo-optic tweezers** [9548-29]

USING THE PHOTONIC TOOLBOX TO STUDY CELLS AND THEIR ORGANELLES I

- 9548 OT **Integrated 3D macro-trapping and light-sheet imaging system** [9548-31]
- 9548 OU **Single-cell diffraction tomography with optofluidic rotation about a tilted axis** [9548-32]

PHOTONIC DEVICES FOR OPTICALLY INDUCED FORCES

- 9548 10 **Photonic force microscopy of surface electromagnetic waves in a one-dimensional photonic crystal** [9548-38]
- 9548 12 **Submicron particle manipulation using slotted tapered optical fibers** [9548-40]

OPTICAL LAB-ON-A-CHIP

- 9548 13 **Optical manipulation of single microparticle for microfluidic flow rate sensing** [9548-41]
- 9548 14 **Optically induced transportation of dielectric particles with fiber optic Bessel beam** [9548-42]
- 9548 15 **Ultrathin optical fibers for particle trapping and manipulation** [9548-43]

STATISTICAL MECHANICS OF SMALL SYSTEMS

- 9548 18 **Avalanches and plasticity for colloids in a time dependent optical trap** [9548-47]

MICRO-SCALE STUDIES OF HYDRODYNAMICS, OPTO-FLUIDICS, AND BINDING

- 9548 1A **Optically controlled hydrodynamic micro-manipulation (Invited Paper)** [9548-49]
- 9548 1B **Hydrodynamic synchronisation of optically driven rotors** [9548-50]
- 9548 1D **Boundary element method for optical force calibration in microfluidic dual-beam optical trap** [9548-52]
- 9548 1E **Rotational behavior of oblate golden nanoparticles in circularly polarized dual beam optical trap** [9548-53]
- 9548 1F **Optical spatial solitons in bidisperse fluorescent nanocolloids** [9548-54]

OPTICALLY DRIVEN MICROHEOLOGY AND MECHANICAL PROPERTIES

- 9548 1M **Artifacts from photobleaching and dye-dissociation in fluorescence correlation spectroscopy** [9548-61]
- 9548 1N **Calibration of femtosecond optical tweezers as a sensitive thermometer** [9548-62]

USING THE PHOTONIC TOOLBOX TO STUDY CELLS AND THEIR ORGANELLES II

- 9548 1O **3D living neural networks** [9548-63]

NEAR-FIELD MICROMANIPULATION, PLASMONIC, AND NANOPARTICLE TRAPPING

- 9548 1T **Characterization of periodic plasmonic nanoring devices for nanomanipulation** [9548-69]

CONSIDERING SHAPED PARTICLES IN OPTICAL TRAPS

- 9548 1Z **Studies on shape anisotropy in red blood cells** [9548-74]

POSTER SESSION

- 9548 20 **Low frequency dynamical stabilization in optical tweezers** [9548-76]
- 9548 21 **Laser trapping and assembling of nanoparticles at solution surface studied by reflection micro-spectroscopy** [9548-77]
- 9548 22 **Magnon-like properties of 1D spin-3/2 chains** [9548-79]
- 9548 23 **Correlated fluctuations of optically trapped particles** [9548-80]

- 9548 25 **A study of red blood cell deformability in diabetic retinopathy using optical tweezers**
[9548-82]
- 9548 27 **Remarkable nonlinear optical effect in plasmon-assisted radiation force** [9548-84]
- 9548 28 **Three-dimensional trapping with a focused Bessel beam** [9548-85]

Authors

Numbers in the index correspond to the last two digits of the six-digit citation identifier (CID) article numbering system used in Proceedings of SPIE. The first four digits reflect the volume number. Base 36 numbering is employed for the last two digits and indicates the order of articles within the volume. Numbers start with 00, 01, 02, 03, 04, 05, 06, 07, 08, 09, 0A, 0B...0Z, followed by 10-1Z, 20-2Z, etc.

Agrawal, Rupesh, 25	Ishihara, H., 27
Aguilar-Ayala, Yareni, 1F, 28	Jákl, Petr, 1E
Alba-Arroyo, José E., 0Q	Jauffred, Liselotte, 0F
Andrews, David L., 0M	Jones, Philip H., 20, 23, 25
Arzola, Alejandro V., 1E, 1F, 28	Joo, Boram, 14
Awel, Salah, 0H	Kafle, Rudra P., 1M
Banerjee, Ayan, 0R, 1Z	Kemp, Brandon A., 0L
Baranoğlu, Besim, 1D	Kirian, Richard A., 0H
Basta, Tamara, 1O	Kjærgaard, Niels, 0B
Bera, Sudipta, 1Z	Küpper, Jochen, 0H
Berns, M. W., 0P	LeBrun, Thomas W., 0E
Bhardwaj, B. S., 1T	Lee, Sungrae, 14
Bhatnagar, Rhythm, 25	Liebeskind, Molly R., 1M
Biyikli, Necmi, 1D	Linke, Heiner, 0F
Bourg, Julia T., 1M	Linnenberger, Anna, 1O
Bowman, Richard, 0H	Liu, Qunfeng, 13
Box, Stuart, 1B	Lyubin, Evgeny V., 10
Bradshaw, David S., 0M	Maimaiti, Aili, 15
Brzobohatý, Oto, 1E	Mansuripur, Masud, 08, 0K
Carmona-Sosa, Viridiana, 0Q	Mason, Bernard J., 0C
Çetin, Barbaros, 1D	Masuhara, Hiroshi, 21
Chan, Chii J., 0U	McDermott, D., 18
Chapman, Henry N., 0H	McLeod, Robert R., 1O
Chvátal, Lukáš, 1E	Meiners, Jens-Christian, 1M
Cotterell, Michael I., 0C	Mondal, Argha, 1Z
Crenshaw, Michael E., 0J	Mondal, Dipankar, 1N
Cubero, David, 20	Možina, Janez, 0N, 0O
Daly, M., 12	Müller, Paul, 0U
David, Grégory, 0D	Nic Chormaic, Síle, 12, 15, 1T
Deb, Amita B., 0B	Nobuhiko, N., 27
Debono, Luke J., 1A, 1B	Oddershede, Lene B., 0F
Dholakia, Kishan, 0T	Oh, Kyunghwan, 14
Eckerskorn, Niko, 0H	Olson Reichhardt, C. J., 18
Fedyanin, Andrey A., 10	Orr-Ewing, Andrew J., 0C
Ferrier, David E. K., 0T	Padgett, Miles J., 0H, 1A
Forbes, Kayn A., 0M	Park, Haesung, 0E
Ghosh, Subhrokoli, 0R	Pavesio, Carlos, 25
Gomez, V., 0P	Peng, Gangding, 13
Gong, Yuan, 13	Phillips, David B., 1A, 1B
Gordon, Reuven, xi	Piksarv, Peeter, 0T
Goswami, Debabrata, 1N	Požar, Tomaz, 0N, 0O
Guck, Jochen, 0U	Prakash, P., 1T
Gunn-Moore, Frank J., 0T	Preece, D., 0P
Han, Xiang, 23	Preston, Thomas C., 0C
Hanna, Simon, 1B	Quinto-Su, Pedro A., 0Q
Hernández-Cordero, Juan, 1F	Rao, Yunjiang, 13
Hernández-Sarria, J. J., 22	Reichhardt, C., 18
Horvath, Milena S. J., 0B	Reid, Jonathan P., 0C
Hoshina, M., 27	Richards, Christopher J., 20, 23, 25

Rode, Andrei V., 0H
 Rodríguez, K., 22
 Roy, Basudev, 0R, 1Z
 Roy, Soumyajit, 0R
 Salazar-Romero, M. Yadira, 1F
 Sawyer, Bianca J., 0B
 Schloss, J. R., 1T
 Schmitt, Regina K., 0F
 Schürmann, Mirjam, 0U
 Selfridge, A., 0P
 Sergides, M., 1T
 Serhathoğlu, Murat, 1D
 Sheppard, Cheyenne J., 0L
 Shi, L. Z., 0P
 Shilkin, Daniil A., 10
 Signorell, Ruth, 0D
 Šiler, Martin, 1E
 Simpson, Stephen H., 1A, 1B, 1E
 Siwiak-Jaszek, Stefan, 23
 Smart, Thomas J., 20, 23, 25
 So, Byunghwy, 14
 Soboleva, Irina V., 10
 Solmaz, Mehmet E., 1D
 Stowell, Michael H. B., 1O
 Suigiyama, Teruki, 21
 Taheri, S. Mohammad-Reza, 0F
 Truong, Viet Giang, 12, 15, 1T
 Volke-Sepúlveda, K., 1F, 28
 Wang, Shun-Fa, 21
 White, Elizabeth, 1M
 Wiedorn, Max, 0H
 Wu, Yu, 13
 Yang, Zhengyi, 0T
 Yuyama, Ken-ichi, 21
 Zemánek, Pavel, 1E
 Zhang, Chenlin, 13

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Session Chairs

- 1 OAM Transfer: Celebrating 20 Years
Halina Rubinsztein-Dunlop, The University of Queensland (Australia)
- 2 Outreach and Education: International Year of Light
Gabriel C. Spalding, Illinois Wesleyan University (United States)
- 3 Toward (Or In) the Quantum Limit of Opto-Mechanics
Masud Mansuripur, College of Optical Sciences, The University of Arizona (United States)

- 4 Optical Manipulation of Matter Through Gaseous Media
Kishan Dholakia, University of St. Andrews (United Kingdom)
- 5 Trapping at Extremes (i.e., "Gonzo" Trapping)
David B. Phillips, University of Glasgow (United Kingdom)
- 6 Foundations of the Electromagnetic Theory of Force and Momentum
Simon Hanna, University of Bristol (United Kingdom)
- 7 Laser-Induced Shockwaves and Cavitation
Tomaz Pozar, University of Ljubljana (Slovenia)
- 8 Using the Photonic Toolbox to Study Cells and Their Organelles I
Anna S. Bezryadina, San Francisco State University (United States)
- 9 Enhanced Sensitivity, Resolution, and Range of Force Actuators
Anna M. Linnenberger, Meadowlark Optics, Inc. (United States)
- 10 Photonic Devices for Optically Induced Forces
Jeremy N. Munday, University of Maryland, College Park
(United States)
- 11 Optical Lab-on-a-Chip
Ayan Banerjee, Indian Institute of Science Education and Research
Kolkata (India)
- 12 Statistical Mechanics of Small Systems
Daniel H. Ou-Yang, Lehigh University (United States)
- 13 Micro-Scale Studies of Hydrodynamics, Opto-Fluidics, and Binding
Giovanni S. Volpe, Bilkent University (Turkey)
- 14 Optical Force Studies of Active Micro-Swimmers
Samira Aghayee, University of Maryland, College Park (United States)
- 15 Optically Driven Microheology and Mechanical Properties
Daniel H. Ou-Yang, Lehigh University (United States)
- 16 Using the Photonic Toolbox to Study Cells and their Organelles II
Michael W. Berns, University of California, San Diego (United States)
- 17 Near-Field Micromanipulation, Plasmonic, and Nanoparticle
Trapping
Gabriel C. Spalding, Illinois Wesleyan University (United States)
- 18 Considering Shaped Particles in Optical Traps
Halina Rubinsztein-Dunlop, The University of Queensland (Australia)

Nano-Bio-Optomechanics: Nanoaperture Tweezers Probe Single Nanoparticles, Proteins, and their Interactions

Reuven Gordon

Department of Electrical and Computer Engineering, University of Victoria,
Victoria, BC, Canada, V8P5C2

ABSTRACT

Nanoparticles in the single digit nanometer range can be easily isolated and studied with low optical powers using nanoaperture tweezers. We have studied individual proteins and their interactions with small molecules, DNA and antibodies. Recently, using the fluctuations of the trapped object, we have pioneered a new way to “listen” to the vibrations of nanoparticles in the 100 GHz – 1 THz range; the approach is called extraordinary acoustic Raman (EAR). EAR gives unprecedented low frequency spectra of individual proteins in solution, allowing for identification and analysis, as well as probing their role in biological functions. We have also used EAR to study the elastic properties, shape and size of various individual nanoparticles.

Keywords: Optical Tweezers, Optomechanics, Nanoparticles, Biosensors, Plasmonics

1. INTRODUCTION

Nanoparticles in the size range of 1-50 nm are important building blocks for technology and biology. DNA, proteins, protein complexes, antibodies and viruses are biological nanoparticles in this size range. Quantum dots, nanocarbon and colloidal nanoparticles are among the technologically relevant nanoparticles similarly sized. Our aim is to develop new tools to understand such nanoparticles at the single particle level.

We are looking at approaches to *hear* the acoustic vibrations of nanoparticles. What does a protein *sound* like and how does this relate to its functioning? What happens when the protein is a mutant form? Does it sound “sick”? If so, can we use this as a diagnostic or as an assay to discover drugs?

Acoustic modes of proteins have been studied in detail using various theoretical approaches; of particular note, molecular dynamics simulations [1]. While these theoretical approaches are valuable to better understand protein function and their interactions (for example, for applications in drug discovery), there are limited experimental approaches to validate such models. Raman (and its relative, Brillouin scattering) has been used to probe the acoustic vibrations of nanoparticles. For example, C60 (Buckminsterfullerene) has Raman active vibrations at 273 cm^{-1} and 497 cm^{-1} . For larger nanoparticles, the typical vibration frequencies start below 100 GHz (around 3 cm^{-1}). This is a challenge for Raman due to the Rayleigh line overwhelming the weak Raman signal, and also for micro-Brillouin due to limited scattering for nanoparticles below 200 nm. Ideally, we would like a method to probe individual nanoparticle vibrations in the range from 10 GHz to 1 THz. For proteins in particular, the vibrational density of states is relatively sparse in this range, and so it is promising for analysis. This can be compared with typical vibration frequencies founding in conventional optomechanics of micron-scale structures; which rarely reaches to GHz frequencies due to the larger size (although there are fairly recent examples at 5 GHz [2]).

Single nanoparticle/molecule studies have been developed for just over a decade. Of note, single molecule fluorescence approaches developed by W. E. Moerner and co-workers were recognized with the 2014 Nobel Prize in Chemistry [3]. There are many reasons to attempt single nanoparticle studies, including:

- removing heterogeneity from a population,
- seeing richer statistics (non-averaged behavior),
- studying dynamic processes without the need for synchronization,
- studying nanoparticles over a wide range of concentrations, and
- sensing vary rare particles/events.

Even with highly innovative and impactful developments using single molecule fluorescence approaches, there are still drawbacks to existing methods. For example, a tour de force 2012 study of protein folding using Förster resonance energy transfer at the single protein level illustrates the challenges [4]:

1. Adding fluorescent markers alters the native protein, so there is always the question about whether the marker is influencing the actual biophysical process.
2. A tether is usually used to immobilize the protein for study (otherwise it would diffuse away).
3. Fluorescence can be a relatively slow process, so photon counting is required to resolve faster dynamics and usually the molecule has to be pumped quite “hard” to ensure enough signal is achieved. Even so, glycerol needs to be used to slow down the protein folding [4].
4. The fluorophores bleach, so observation time is limited.
5. The signal observed is open to interpretation since it is coming from an exogeneous marker, not the protein itself.

Despite all these challenges, single molecule fluorescence approaches have advanced significantly our understanding of biological processes. Even so, it is highly desirable to develop methods that are not subject to these limitations. In particular, we would like a single nanoparticle detection platform that has the following features:

- No labels/markers/fluorophores
- No tethers
- Fast response
- Extended observation times
- Direct measurement of nanoparticle (not an exogeneous signal open to interpretation)
- Fairly agnostic to concentration (can isolate single particles in dilute or dense mixtures)

Since 2009, we have been developing laser tweezers using apertures in metal films to study nanoparticles down to 50 nm [5]. In 2011, we had a breakthrough in this approach to allow trapping of much smaller particles by using the double nanohole (DNH) aperture shape, with two sharp cusps where the holes overlap, allowing for trapping of 12 nm dielectric nanoparticles [6], and then single proteins [7]. Even in the first single protein studies, we observed protein conformational changes [7], which showed the potential of this work to observe directly dynamics in individual proteins without labels or tethers at timescales that are limited by the detector response (typically into the GHz). Recently, however, we have developed this approach further to modulate resonantly the nanoparticles at their vibration frequency and thereby probe their dynamics at ~100 GHz [8]. As mentioned previously, this is exactly the regime where nanoparticles in the 1 to 50 nm size range start to show vibrational resonances, and so it is of interest for identifying them and understanding their behavior.

2. DOUBLE NANO HOLE OPTICAL TWEEZERS

Even the first works on gradient-force optical tweezers recognized the size-dependent limitation of Rayleigh scattering, requiring extremely large intensities to hold onto non-resonant particles below 100 nm [9]. The third power scaling of the polarizability for Rayleigh scatterers means that 1000 times more intensity is required to have the same force on a 10 nm particle as on a 100 nm particle.

Our trick to overcome this strong size-scaling limitation was to use an aperture in a metal film. Like nanoparticles, nanoapertures also have strong size scaling dependence: their transmission depends on the ratio between the aperture size and the wavelength to the fourth power according to Bethe’s theory. Adding a dielectric material into the aperture effectively reduces the wavelength or makes the aperture *appear* larger. Therefore, there is a strong change in the transmission due to the strong scaling from Bethe’s theory, even though the nanoparticle is a Rayleigh scatterer. We used this nanoaperture to observe optical trapping; when a particle enters the aperture, there is a jump in the optical

transmission [5]. The particle is kept in the aperture by the resulting optical forces, as was verified by a maintained higher level of transmission. The transmission dropped if the particle escaped. Also, multiple particle trapping events could be distinguished from single particle trapping events (all showing the same step height), but a distinct second step in the transmission, as shown in Figure 1. The trapping of multiple particles is rare for typical experimental configurations used, which we believe is due to steric hindrance, repulsion of like-charged particles, and the improbability of such coincidences (at lower concentrations).

Figure 1. Trapping of two identical nanoparticles in sequence showing two steps in the transmission of almost the same size. (Single nanoparticle trapping events show a single step equal to the height of the first trapping event).

It should be noted that others had considered the use of apertures in optical tweezer systems prior to our work, both theoretically [10] and in experiment [11], yet we are unaware of any previous works that exploited this effect to hold onto nanoparticles smaller than ~ 100 nm.

While a cylindrical aperture was able to trap nanoparticles down to ~ 50 nm, going to smaller sizes required shaped apertures. In particular, we used the double nanohole (DNH), which we studied since 2004 [12-14] because it creates a local hot-spot of around 10 nm in size in the electric field that interacts more strongly with the nanoparticle of interest. With the DNH, we were able to trap 12 nm silica spheres [6], single proteins [7], and 20 bases of single stranded DNA [15], among other things. More recently, researchers have started to use bowtie nanoapertures [16]; these share the same features of the DNH structure that enable trapping down to the size of single proteins – in particular, sharp cusps with a narrow gap where two holes overlap.

3. PROTEINS, DNA AND THEIR INTERACTIONS

The ability to trap single proteins has allowed for studying protein-antibody binding [17], protein-DNA interactions [15], protein sizing [18], protein-protein interactions [18], protein small-molecule interactions (both in the strong binding [19] and weak binding limits [20]). In typical studies, the aperture transmission is used to monitor light scattering changes by the protein or DNA particles, thereby giving direct information about their structure (via their diffractive properties).

The ability to directly monitor proteins and their interactions is of obvious interest for drug discovery applications. Not only is it possible to see directly if a small molecule is binding to a protein (through changes in the fluctuations of the optical transmission through the aperture – representing thermal motion of the particle) [19], but it is also possible to monitor on-off binding kinetics to determine the binding affinity [20]. In this way, potential small molecule drug candidates can be probed for their interactions with proteins. Because this approach operates at the single molecule level, it has the potential to conserve valuable protein sample.

One of the interesting systems we studied with this approach is the so-called tumor suppressor protein, p53 [15]. This protein is reported to be mutated in the majority of known cancers. It is well known that p53 binds to DNA; however, the mutant form can also bind with almost the same affinity. To probe the function of p53, we first trapped a hair-pin of single-stranded DNA (ssDNA) that binds with p53. This hairpin is unzipped by electrostriction in the DNH trap: the unzipped ssDNA is longer and has a higher polarizability; therefore, its dipole interaction with the local electric field is greater. In the presence of p53, the unzipping time of the hairpin is increased by orders of magnitude. Interestingly, a point mutation to p53, which still binds strongly to the hairpin, does not increase the unzipping time. Therefore, our

experiment has shown that p53 acts to suppress the unzipping of DNA and that it cannot perform this task with a common mutation. (We also quantified the energy barrier to unzipping that is created by wild-type p53 binding using Arrhenius scaling). This approach may be used as an assay to screen for drugs that could correct the function of p53, and possibly play an important role in suppressing the development cancers.

4. EXTRAORDINARY ACOUSTIC RAMAN

As described above, the DNH optical tweezer pulls on ssDNA in the trap through electrostriction. If we modulate the intensity of the trapping laser beam, then the electrostriction is also modulated and it is possible to drive vibrations in the ssDNA. When this is done, we observed increased amplitude fluctuations of the laser transmission for certain driving frequencies. This increased root mean squared (RMS) amplitude fluctuation is attributed to hitting a resonance of the trapped ssDNA, heating it up, and thereby producing an increase in thermal motion. By scanning the frequency, a spectrum of the vibrational resonances of the ssDNA can be achieved (see Figure 2). We noted changes in the resonance frequency with length of the ssDNA and found good agreement with a linear chain oscillator model [21].

Figure 2. Increased noise amplitude (RMS fluctuations) observed when intensity modulation hits resonance frequency of ssDNA. Top shows increase in noise and bottom shows noise amplitude over a range of modulation frequencies.

We referred to this process as extraordinary acoustic Raman (EAR) because of its single molecule sensitivity and ability to access low wavenumbers with high resolution. Unlike related coherent types of spectroscopy, like the optical Kerr effect, here we measure the random motion of the particle, so we are not as sensitive to energy redistribution that is common in proteins [1]. Figure 3 shows the spectrum taken for a common protein. Other examples can be found elsewhere [8]. Using this approach, we have also measured the vibration spectra of the MS2 bacteriophage (virus).

Figure 3. EAR spectrum of a single protein in the DNH trap.

5. TECHNOLOGICAL DEVELOPMENT OF DNH TWEEZER SYSTEMS

Every nanotechnology faces the challenge of being able to achieve low-cost, high-throughput and reliable fabrication. Furthermore, it would be ideal to integrate the DNH optical tweezer system into a fiber-optic platform to improve scalability. First steps have been taken to solve the nanofabrication challenge by using a process of template stripping [22], where gap sizes down to 7 nm were produced. We have also integrated DNH structures on the ends of cleaved optical fibers and demonstrated trapping with those structures [23]. This is promising for future scalability where it is possible envision having multiple trapping fibers to each address a single well of a microwell plate, and thereby integrate seamlessly with existing biochemistry laboratory protocols. It should be noted that past works have also integrated bowtie nanoapertures on the end of tapered fibers and shown the ability to translate the trapped object [16]; however, care should be taken to avoid damaging the tapered fiber, and the tapering process complicates the fabrication.

6. CONCLUSIONS AND OUTLOOK

The DNH optical tweezer approach allows us to study single proteins and other nanoparticles. Unlike fluorescence based methods, the DNH approach is label-free (uses the intrinsic light scattering properties of the nanoparticle) and does not require tethering to prevent the particle from diffusing away. We have already studied protein sizes, protein-protein interactions, protein-small molecule interactions, protein antibody interactions, protein-DNA interactions with this approach. Adding a second trapping laser of different frequency allows for exciting vibrations in the nanoparticle of interest, which can be used to build the acoustic Raman spectrum of the nanoparticle by sweeping the laser frequency difference in the range of ~100 GHz (extremely high frequency to THz bands). In this way, it is possible to identify individual proteins, as well as study the properties of nanoparticles (such as material anisotropy).

We are developing this method in terms of scalability and ease of nanofabrication to allow for widescale adoption in the areas of drug discovery, diagnosis and basic scientific analysis.

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