Biomedical Optics EXPRESS

Trapping of a single DNA molecule using nanoplasmonic structures for biosensor applications

Jung-Dae Kim and Yong-Gu Lee*

School of Mechatronics, Gwangju Institute of Science and Technology (GIST), 123 Cheomdan-gwagiro (Oryong-dong), Buk-gu, Gwangju, 500-712, South Korea

**lygu@gist.ac.kr*

Abstract: Conventional optical trapping using a tightly focused beam is not suitable for trapping particles that are smaller than the diffraction limit because of the increasing need of the incident laser power that could produce permanent thermal damages. One of the current solutions to this problem is to intensify the local field enhancement by using nanoplasmonic structures without increasing the laser power. Nanoplasmonic tweezers have been used for various small molecules but there is no known report of trapping a single DNA molecule. In this paper, we present the trapping of a single DNA molecule using a nanohole created on a gold substrate. Furthermore, we show that the DNA of different lengths can be differentiated through the measurement of scattering signals leading to possible new DNA sensor applications.

OCIS codes: (350.4855) Optical tweezers or optical manipulation; (240.6680) Surface plasmons; (250.5403) Plasmonics; (140.7010) Laser trapping; (310.6628) Subwavelength structures, nanostructures; (240.3695) Linear and nonlinear light scattering from surfaces.

References and links

1. A. Ashkin, J. M. Dziedzic, J. E. Bjorkholm, and S. Chu, "Observation of a single-beam gradient force optical trap for dielectric particles," Opt. Lett. **11**(5), 288–290 (1986).
2. A. Ashkin, "Forces of a single-beam gradient laser trap on a dielectric sphere in the ray optics regime," Biophys. J. **61**(2), 569–582 (1992).
3. K. Svoboda and S. M. Block, "Optical trapping of metallic Rayleigh particles," Opt. Lett. **19**(13), 930–932 (1994).
4. L. Huang and O. J. F. Martin, "Reversal of the optical force in a plasmonic trap," Opt. Lett. **33**(24), 3001–3003 (2008).
5. M. Nieto-Vesperinas, P. C. Chaumet, and A. Rahmani, "Near-field photonic forces," Philos. Trans. A Math Phys. Eng. Sci. **362**(1817), 719–737 (2004).
6. K. Okamoto and S. Kawata, "Radiation force exerted on subwavelength particles near a nanoaperture," Phys. Rev. Lett. **83**(22), 4534–4537 (1999).
7. R. H. Ritchie, "Plasma losses by fast electrons in thin films," Phys. Rev. **106**(5), 874–881 (1957).
8. W. L. Barnes, A. Dereux, and T. W. Ebbesen, "Surface plasmon subwavelength optics," Nature **424**(6950), 824–830 (2003).
9. F. J. Garcia de Abajo, "Light transmission through a single cylindrical hole in a metallic film," Opt. Express **10**(25), 1475–1484 (2002).
10. F. J. García-Vidal, E. Moreno, J. A. Porto, and L. Martín-Moreno, "Transmission of light through a single rectangular hole," Phys. Rev. Lett. **95**(10), 103901 (2005).
11. T. Ishi, J. Fujikata, K. Makita, T. Baba, and K. Ohashi, "Si nano-photodiode with a surface plasmon antenna," Jpn. J. Appl. Phys. **44**(12), L364–L366 (2005).
12. M. L. Juan, R. Gordon, Y. Pang, F. Eftekhari, and R. Quidant, "Self-induced back-action optical trapping of dielectric nanoparticles," Nat. Phys. **5**(12), 915–919 (2009).
13. Y. Pang and R. Gordon, "Optical trapping of 12 nm dielectric spheres using double-nanoholes in a gold film," Nano Lett. **11**(9), 3763–3767 (2011).
14. A. Zehtabi-Oskuie, H. Jiang, B. R. Cyr, D. W. Rennehan, A. A. Al-Balushi, and R. Gordon, "Double nanohole optical trapping: dynamics and protein-antibody co-trapping," Lab Chip **13**(13), 2563–2568 (2013).
15. Y. Pang and R. Gordon, "Optical trapping of a single protein," Nano Lett. **12**(1), 402–406 (2012).

#207176 - $15.00 USD Received 25 Feb 2014; revised 19 May 2014; accepted 16 Jun 2014; published 3 Jul 2014

16. A. Kotnala, D. DePaoli, and R. Gordon, "Sensing nanoparticles using a double nanohole optical trap," Lab Chip **13**(20), 4142–4146 (2013).
17. T. Shoji, J. Saitoh, N. Kitamura, F. Nagasawa, K. Murakoshi, H. Yamauchi, S. Ito, H. Miyasaka, H. Ishihara, and Y. Tsuboi, "Permanent fixing or reversible trapping and release of DNA micropatterns on a gold nanostructure using continuous-wave or femtosecond-pulsed near-infrared laser light," J. Am. Chem. Soc. **135**(17), 6643–6648 (2013).
18. A. H. J. Yang, S. D. Moore, B. S. Schmidt, M. Klug, M. Lipson, and D. Erickson, "Optical manipulation of nanoparticles and biomolecules in sub-wavelength slot waveguides," Nature **457**(7225), 71–75 (2009).
19. Y.-F. Chen, X. Serey, R. Sarkar, P. Chen, and D. Erickson, "Controlled photonic manipulation of proteins and other nanomaterials," Nano Lett. **12**(3), 1633–1637 (2012).
20. M. L. Juan, M. Righini, and R. Quidant, "Plasmon nano-optical tweezers," Nat. Photonics **5**(6), 349–356 (2011).
21. C. Chen, M. L. Juan, Y. Li, G. Maes, G. Borghs, P. Van Dorpe, and R. Quidant, "Enhanced optical trapping and arrangement of nano-objects in a plasmonic nanocavity," Nano Lett. **12**(1), 125–132 (2012).
22. C. M. Galloway, M. P. Kreuzer, S. S. Aćimović, G. Volpe, M. Correia, S. B. Petersen, M. T. Neves-Petersen, and R. Quidant, "Plasmon-assisted delivery of single nano-objects in an optical hot spot," Nano Lett. **13**(9), 4299–4304 (2013).
23. K. Wang, E. Schonbrun, P. Steinvurzel, and K. B. Crozier, "Trapping and rotating nanoparticles using a plasmonic nano-tweezer with an integrated heat sink," Nat. Commun. **2**, 469 (2011).
24. A. A. E. Saleh and J. A. Dionne, "Toward efficient optical trapping of sub-10-nm particles with coaxial plasmonic apertures," Nano Lett. **12**(11), 5581–5586 (2012).
25. Y.-T. Chen, Y.-C. Liu, W.-F. Fang, C.-J. Huang, S.-K. Fan, W.-J. Chen, W.-T. Chang, C.-H. Huang, and J.-T. Yang, "DNA diagnosis in a microseparator based on particle aggregation," Biosens. Bioelectron. **50**, 8–13 (2013).
26. D. Wang, G. Chen, H. Wang, W. Tang, W. Pan, N. Li, and F. Liu, "A reusable quartz crystal microbalance biosensor for highly specific detection of single-base DNA mutation," Biosens. Bioelectron. **48**, 276–280 (2013).
27. A. G. Cherstvy, "Detection of DNA hybridization by field-effect DNA-based biosensors: mechanisms of signal generation and open questions," Biosens. Bioelectron. **46**, 162–170 (2013).
28. X. Zhu, L. Sun, Y. Chen, Z. Ye, Z. Shen, and G. Li, "Combination of cascade chemical reactions with graphene-DNA interaction to develop new strategy for biosensor fabrication," Biosens. Bioelectron. **47**, 32–37 (2013).
29. T.-Y. Chen, P. T. K. Loan, C.-L. Hsu, Y.-H. Lee, J. Tse-Wei Wang, K.-H. Wei, C.-T. Lin, and L.-J. Li, "Label-free detection of DNA hybridization using transistors based on CVD grown graphene," Biosens. Bioelectron. **41**, 103–109 (2013).
30. H. A. Bethe, "Theory of diffraction by small holes," Phys. Rev. **66**(7-8), 163–182 (1944).
31. F. J. Garcia-Vidal, L. Martin-Moreno, T. W. Ebbesen, and L. Kuipers, "Light passing through subwavelength apertures," Rev. Mod. Phys. **82**(1), 729–787 (2010).
32. A. Roberts, "Electromagnetic theory of diffraction by a circular aperture in a thick, perfectly conducting screen," J. Opt. Soc. Am. A **4**(10), 1970–1983 (1987).
33. D. E. Smith, T. T. Perkins, and S. Chu, "Dynamical scaling of DNA diffusion coefficients," Macromolecules **29**(4), 1372–1373 (1996).
34. C. F. Bohren and D. R. Huffman, *Absorption and Scattering of Light by Small Particles* (John Wiley & Sons 1998), p. 132.
35. H. Yamakawa, *Helical Wormlike Chains in Polymer Solutions* (Springer-Verlag GmbH, 1997), p. 418.

1. Introduction

Optical trapping is an effective tool for trapping and manipulating micrometer scale objects. With a careful selection of the wavelength of the laser so that the absorption causing heat to the target object is avoided, even heat-sensitive biological specimens can be also trapped [1, 2]. The ratio between the gradient force and the scattering force is proportional to r^{-3} [3], where r is the radius of the object. This simple relation between the forces predicts stable trapping for extremely small particles. However, trapping becomes less favorable as the size of the object becomes extremely small (below 100 nanometer). The solution to this problem is to increase the trapping force by increasing the laser power. However, there is a limit to this solution due to the possible heat damage to the specimen at the high laser intensity. Recent works have overcome this problem by using the localized optical field enhancement, which can be devised by fabricating nanometer scale plasmonic structures that produces extraordinary field enhancement when illuminated by light [4–6]. This field enhancement results from the surface confined electromagnetic field that strongly couples with the free electrons on metal surfaces. This strongly confined electromagnetic field is called surface plasmons [7, 8]. The interplay of the surface waves with subwavelength nanostructures results in localized intensity that becomes several orders greater than the incident intensity [9]. This

#207176 - $15.00 USD Received 25 Feb 2014; revised 19 May 2014; accepted 16 Jun 2014; published 3 Jul 2014

extraordinary transmission has been studied for metallic structures of various dimensions and shapes [10, 11]. Recently, plasmonic structures have found applications in optical trapping and have led to the discovery of sub-100 nanometer sized dielectric particles trapping with only sub-10 mW μm^{-2} intensity, also known as self-induced back-action (SIBA), the term coined by Juan et al. [12]. SIBA allows the trapping of nanometer scale objects with significantly less laser intensity and the trapping of 100 nm particles for five minutes was demonstrated with only 1 mW μm^{-2} of intensity. Reducing the laser intensity is important because it reduces the danger of photo damage. Furthermore, a double nanohole made of overlapping holes drilled on a gold substrate was used for trapping nanoparticles [13] and a single protein molecule [14, 15]. This method combined with the scattering signal measurements can also work as a sensor for size based characterization of nanoparticles [16]. Shoji et al. have similarly demonstrated the trapping of lambda DNA molecules in the vicinity of the gold nanostructure using localized surface plasmons [17]. A different approach using high dielectric materials such as silicon combined with nanoscale structures that also results in a high field enhancement was also reported. Yang et al. have used a slot waveguide for trapping 75 nm particles and lambda DNA flowing in a medium [18]. The result was verified using a fluorescence imaging. Chen et al. have demonstrated similar work with silicon nitride photonic crystal resonators for trapping 10 nm protein molecules [19]. The results were also verified through fluorescence imaging. Even further, there are many researches related to trapping of nanoscale objects using different shapes of plasmonic nanostructures [20–24].

In summary, nanometer scale structures are excellent tools for enhancing the trapping force and have been used for trapping extremely small single molecules. When we narrow our scope for trapping DNA molecules, only the field enhancement using waveguides and localized surface plasmons were reported. Although waveguides and localized surface plasmons are quite capable of immobilizing a large quantity of DNA molecules, they have not been used for trapping a single DNA molecule. Furthermore, to our best knowledge, trapping of a DNA using plasmonic structures was also not reported. In this paper, we demonstrate the trapping of a single DNA molecule using the field enhancement from plasmonic structures. A nanohole is made on a gold substrate by focused ion milling. Then a PDMS chamber is created with the hole milled substrate as the ceiling. Subsequently, the chamber is filled with DNA molecules and a laser beam is incident on the gold substrate with a nanohole. The DNA is then trapped and the trapping is verified using the scattering signal collected on an avalanche photodiode. We expect that the proposed method can be used to characterize DNA. Previous biosensors detected specifically sequenced DNA by using complementary DNA coated particle aggregations [25], toehold-mediated strand displacement reaction [26], electrostatic effects taking place upon DNA hybridization in dense DNA arrays immobilized on a layer of Au nano-particles deposited on the surface of a field-effect-based DNA capacitive biosensor [27] and electrochemical techniques that detect target-regulated grapheme-DNA interaction [28, 29], etc. However, those methods have not shown to classify DNA based on its length. Length classification has mostly relied on the gel electrophoresis, which requires dyeing with invasive fluorescent chemicals such as ethidium bromide and SYBR Green, etc. In the result section, we present a new method to differentiate DNAs by their lengths using the scattering signals. This approach requires no invasive modification to the target DNA.

2. Experimental setup

Figure 1 illustrates the schematic layout of the nanoplasmonic trapping system. The incident beam from a TEM_{00} 1050 nm wavelength fiber laser (IPG Photonics, YLM-10) is delivered to the PDMS chamber from the top left corner through two achromatic lenses L1 and L2 that works as a beam expander. The expanded beam is reflected by a mirror M1 and the beam diameter is scaled down by a second beam expander formed by L3 and L4. The resulting

#207176 - $15.00 USD Received 25 Feb 2014; revised 19 May 2014; accepted 16 Jun 2014; published 3 Jul 2014

beam diameter is just the right size to overfill the back aperture of the objective lens (Olympus, UPLSAPO 60XW, 1.2 NA) mounted on a three axes piezo stage (Mad City Labs Inc., Nano-LP200) sitting on a two axes motorized stage (Thorlabs, MT1/M-Z8). The combined use of the motorized and piezo stages allows the exact positioning of the nanohole drilled on the gold substrate with the resolution of 0.4 nm. The workspace is 13 mm in the lateral direction and 200 µm in the axial direction. A fast CMOS camera (Microtron, Eosens CL) is used for obtaining the bright field image. The scattering beam from the sample plane is collected by a condenser lens and reduced in the intensity using a neutral density filter and measured by an avalanche photodiodes (Hamamatsu, G8931-04) mounted on a manual stage.

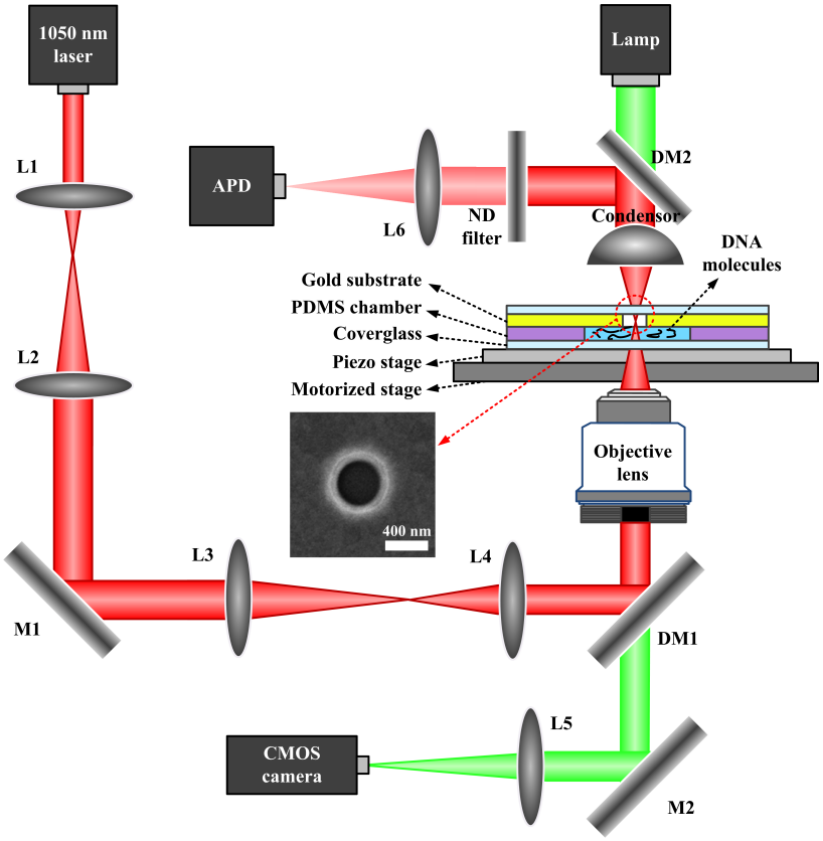

Fig. 1. Schematic of the nanoplasmonic system; Lens: L, Mirror: M, Dichroic mirror: DM, Neutral density: ND.

3. Preparation of microchamber

Figure 2 shows the process of sample preparation for the experiment. Before the DNA trapping experiments are performed, the DNA is placed on a several micrometer deep chamber. This chamber is made of Polydimethylsiloxane (PDMS) and following steps are carried out for the preparation. Firstly, a commercially available Sylgard 184A and Sylgard 184B (Dow Corning) are mixed at 10:1 ratio (Fig. 2(a)) and stirred (Fig. 2(b)). Vapor bubbles formed during the stirring is removed using an evaporator (Fig. 2(c)). The resulting mixture is spread on a coverglass (Fig. 2(d)) and spin coated so that the thickness is even and the coating covers the whole coverglass (Fig. 2(e)). The thickness of the PDMS is determined by the rotational speed of the spin coater. For 30 µm thickness, spin rotation speed is set to 3000 rpm for duration of 30 seconds. The resulting spread is still in a liquid state and needs to be solidified. For this purpose the spin coated coverglass is placed on a hot plate and baked for

#207176 - $15.00 USD Received 25 Feb 2014; revised 19 May 2014; accepted 16 Jun 2014; published 3 Jul 2014

30 minutes at 80 °C (Fig. 2(f)). Subsequently, the PDMS is cut in a rectangle whose dimensions are equal to that of the gold substrate using a cutter (Fig. 2(g)). Finally, the chamber is filled with a DNA solution being careful of not overfilling (Fig. 2(h)) and covered with the gold substrate (Fig. 2(i)). Nanoholes were drilled with a focused ion beam on a 100 nm thick gold substrate which is made from depositing Au (EMF Corp., TA124) on a glass with 5 nm thick Ti that works as the adhesive layer between the two. The purpose of fabricating this chamber is to slow down the quick evaporation of the DNA solution during the experiment.

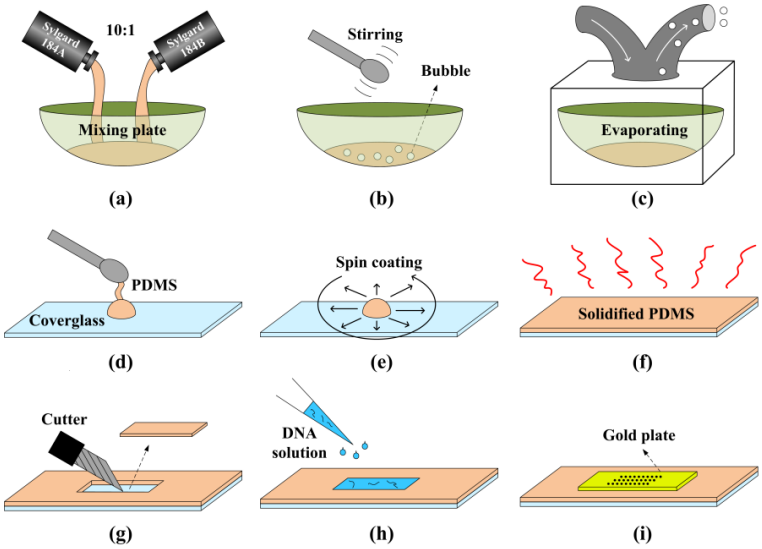

Fig. 2. Process of sample preparation for experiment.

4. Transmittance of laser according to size of holes

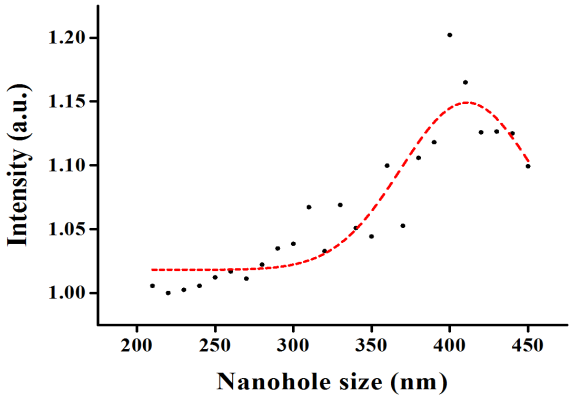

Fig. 3. Transmission intensity vs. nanohole size drilled on 100 nm thick gold substrate.

Bethe has done the theoretical work of a light transmission through a sub-wavelength nanohole [30]. He derived the mathematical equation of a light transmission through a circular hole on a zero thickness perfect conductor. His theory predicts the total radiation transmitted through this hole as a function of the hole diameter [31]. His theory only works for the ideal plate of zero thickness. On the contrary, Roberts [32] proposed a numerical calculation on the transmission coefficient dependence on $k_0 a$. $k_0 (= 2\pi / \lambda)$ and a are wave number and radius of aperture, respectively. The calculations were only done for h (thickness

#207176 - $15.00 USD Received 25 Feb 2014; revised 19 May 2014; accepted 16 Jun 2014; published 3 Jul 2014

of conducting screen) values, zero, a, $5a$ and $10a$. However, in case of our experiment, h (= 100 nm) is smaller than a (= 200 nm) thus direct comparison is not possible. If we let the thickness of the screen be equal to the radius, the peak value is 368 nm when 1050 nm wavelength laser is used. The authors also showed that the transmission is increased up to a cut off limit and then drops down and plateaus. To maximize the trapping force we need to find this peak hole diameter of 400 nm. We have conducted experiments with varying hole sizes to find this diameter. Later, trapping was done using this diameter.

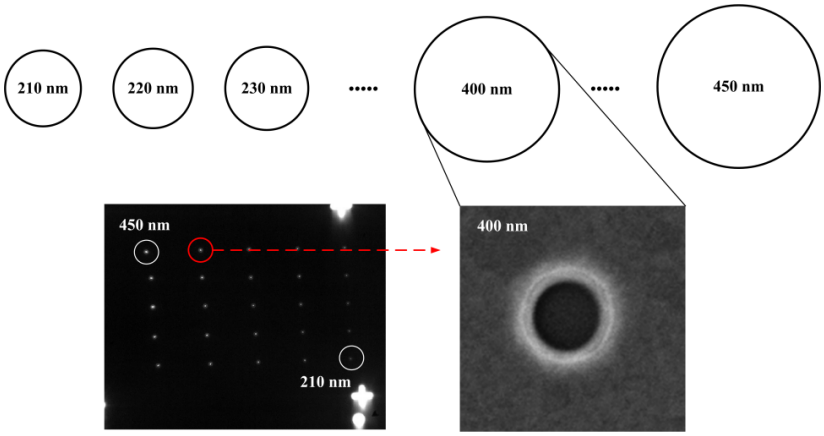

Fig. 4. Microscope and SEM images of nanoholes fabricated on the gold substrate.

We have fabricated holes ranging from 210 nm to 450 nm diameters at 10 nm increments and measured the transmission intensity resulting from illuminating each hole with a focused laser. Because we trapped a single DNA molecule in deionized water, the measurement was conducted with a sample chamber filled with deionized water. Figure 3 plots the transmission measurements on a diameter varying nanoholes for an incident beam of 7.8 mW. We can verify that from 210 nm to 400 nm, the transmission increases as the hole diameter increases. The rate of the increase also increases rapidly and peaks at 400 nm. Afterwards, from 400 nm to 450 nm, we see an abrupt drop of the transmission. This experiment follows the trend of the transmission calculations as a function of hole diameter reported by Roberts [32]. Although we have not measured beyond 450 nm, it is conceivable that it will plateau as predicted by Roberts. We would also like to make a note that Juan et al. have chosen 310 nm for the optimally strong field enhancing diameter [12] but our experiment shows 400 nm is best. Figure 4 shows the optical microscope image of nanoholes ranging from 210 nm to 450 nm on a gold substrate and a SEM image of a 400 nm nanohole.

5. Experimental results and analysis

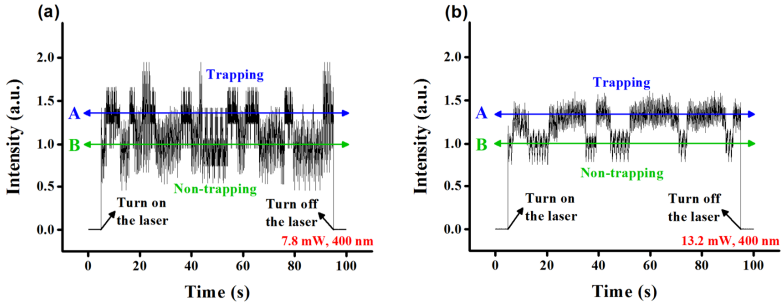

Fig. 5. Measurement of transmission intensity by trapping of a plasmid DNA using laser power of (a) 7.8 mW and (b) 13.2 mW, all intensity values are normalized by the original intensity value of B and this is why B is 1.0.

#207176 - $15.00 USD Received 25 Feb 2014; revised 19 May 2014; accepted 16 Jun 2014; published 3 Jul 2014

We have used two types of DNA for the trapping experiments. The first is a 4.7 kbp plasmid DNA (pAcGFP-C1), and the other is a 48 kbp lambda DNA. 1 bp is about 0.34 nm when stretched. Both DNA solution concentrations are same as 50 ng/μl. Figure 5 shows the result of intermittent trapping of a plasmid DNA for 90 seconds by illuminating the 400 nm hole with a focused laser beam. Figure 5(a) shows the result of using a 7.8 mW laser for trapping plasmid DNA where the trapping duration totals to 38 seconds and Fig. 5(b) is the same with the laser power raised to 13.2 mW and the total trapping duration is 61 seconds. After the laser is turned on the transmission intensity rises up to line B and then rises for the second time to line A. Afterwards, the measurement oscillates only between the two lines. We can deduce that the higher value at line A is due to the increase in the transmission with the help from the DNA scattering the incident beam.

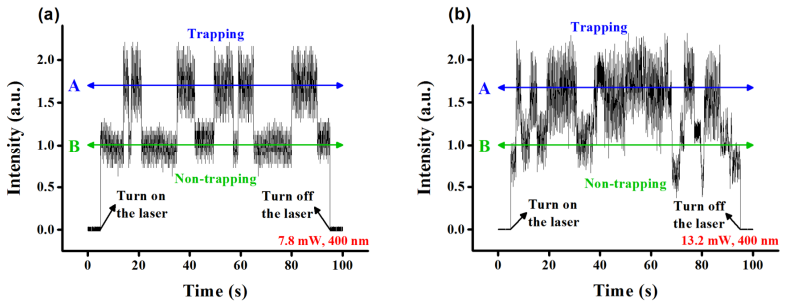

Fig. 6. Measurement of transmission intensity by trapping of lambda DNA using laser power of (a) 7.8 mW and (b) 13.2 mW, all intensity values are normalized by the original intensity value of B and this is why B is 1.0.

We have also witnessed similar results for trapping lambda DNA. Figure 6 shows trapping of a lambda DNA using a 400 nm nanohole. Figure 6(a) and 6(b) shows lambda DNA trapped for the duration of 38 and 65 seconds, respectively. There is also a similar oscillation between two lines A and B, and A is again higher than B because of the increased transmission resulting from the scattering of DNA when trapped. We can also compare the transmission intensity when plasmid DNA and lambda DNA are trapped with the same power. Lambda DNA results in higher intensity and this is due to the fact that lambda DNA is longer and bigger than the plasmid DNA resulting in stronger scattering signal. We think that DNA of different length can be differentiated by scattering signal.

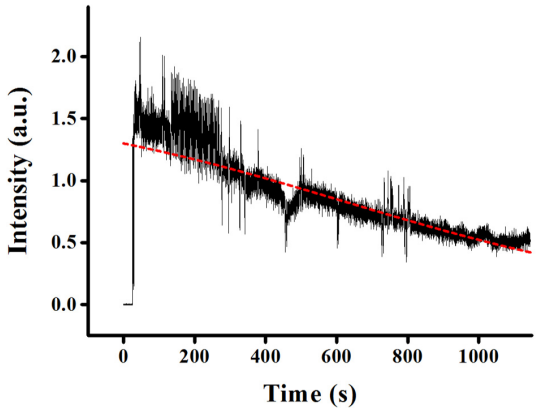

Fig. 7. Transmission intensity as a function of medium height that decreases as time increases.

#207176 - $15.00 USD Received 25 Feb 2014; revised 19 May 2014; accepted 16 Jun 2014; published 3 Jul 2014

Now we come to the reason of the normalization of the scattering signal by the value B. When we measure the scattering signal of the chamber without the DNA being trapped, the value varies based on the incident laser power and more importantly, the value was found to be very sensitive to the medium height. Because the chamber only contains small amount of medium and the fact that we are putting heat through the laser irradiation, we found gradual decrease of the scattering signal as a function of time. This is shown in Fig. 7 where we have monitored the change of the scattering signal when the laser was incident to the chamber without DNA in the medium. As can be seen from the plot, there is a gradual and noticeable drop of the scattering signal. To remove the effect of the chamber height decrease due to the evaporation of the medium, we have normalized all signals by the signal value pertaining to the signal when there is only medium present in the plasmonic trapping region. This in effect also cancels the effect of the laser power, allowing us to compare directly even between scattering graphs coming from different incident power. For example, at the incident power of 7.8 mW, the trapped signal value subtracted by the normalization 1.0 of B gives 0.326 (Fig. 5(a)) and 0.714 (Fig. 6(1)) for the plasmid DNA and lambda DNA, respectively. Also for 13.2 mW laser, we obtained 0.317 (Fig. 5(b)) and 0.639 (Fig. 6(b)). Those subtraction values are named simply the delta values that is obtained by subtracting the trapped signal with the reference signal pertaining to the situation when there is no trapping but only medium present at the focus. Moreover, we have demonstrated trapping of DNAs using different incident laser powers. In case of plasmid DNA, the delta values are 0.281 and 0.349 (Fig. 8(a)) at 9.2 and 16.7 mW incident power, respectively. Similarly, in case of lambda DNA, the delta values are 0.676 and 0.662 (Fig. 8(b)) at the same incident powers, respectively. The beauty of this normalization allows us to compare directly these delta values irrespective of the medium chamber height and the incident laser power. The errors (standard deviation) of these two values are only less than 7% and 4% for plasmid DNA and lambda DNA, respectively. We believe that this simple normalization allows us to discern between DNAs of variable lengths. Additionally, we could also confirm the trapping duration of two DNAs linearly increases depending on the incident laser power. In case of plasmid DNA, the trapping duration is 42.0, 47.7, 68.0 and 72% at 7.8, 9.2, 13.2, 16.7 mW incident power, respectively (Fig. 9(a)). In case of lambda DNA, the trapping duration is 42.0, 52.2, 72.0 and 86.7% at the same laser powers (Fig. 9(b)).

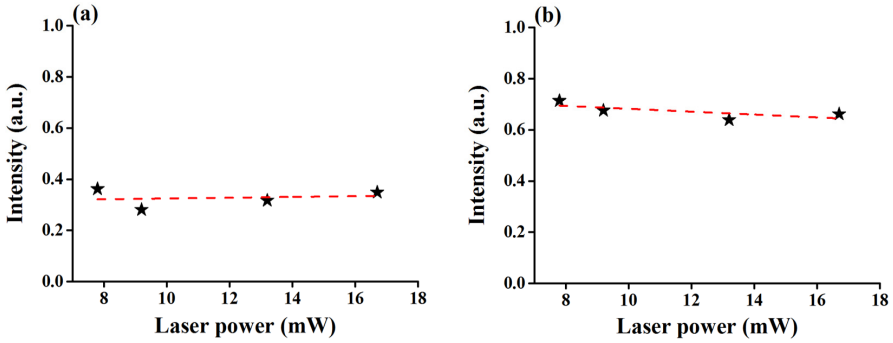

Fig. 8. Difference between average intensity value of line A and B. (a) Trapping delta value of plasmid DNA, (b) Trapping delta value of lambda DNA.

#207176 - $15.00 USD Received 25 Feb 2014; revised 19 May 2014; accepted 16 Jun 2014; published 3 Jul 2014

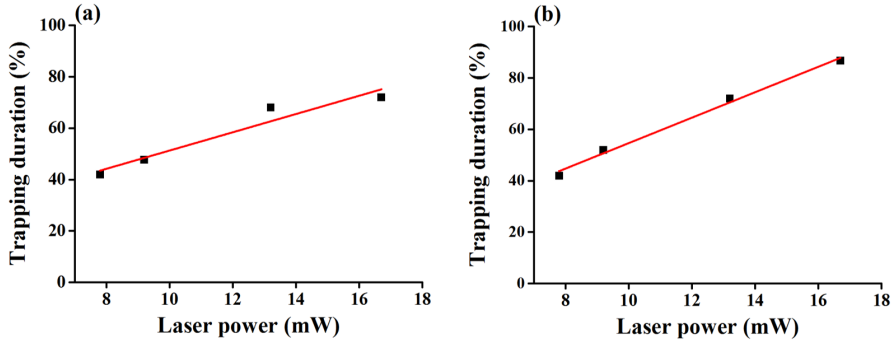

Fig. 9. Trapping duration of (a) plasmid DNA and (b) lambda DNA during 90 seconds.

Fundamentally we are determining the length of the DNA through the light scattering. If we approximate the DNA as a small ball of having the radius value equal to the radius of gyration, r_G. This value is 0.73 μm [33] for the lambda DNA and even smaller for the plasmid DNA. Since the ratio of the radius to the wavelength of the laser (1050 nm) is less than 10%, we can approximate the scattering intensity using the Rayleigh equation [34] which is in the order of r_G^4, that equation is

$$I = \frac{128\pi^5 r_G^4}{3\lambda^4}\left|\frac{\varepsilon_r - 1}{\varepsilon_r + 2}\right|^2, \tag{1}$$

where λ is the wavelength of the incident laser, and ε_r is the ratio of the effective DNA permittivity to that of the medium. The relation between r_G and the number of base pairs is complex. In the literature for the linear DNA, the equation is

$$r_{G,linear} = a\left[\frac{L}{3a} - 1 + \frac{2a}{L} - 2\left(\frac{a}{L}\right)^2\left(1 - e^{-L/a}\right)\right]^{1/2}, \tag{2}$$

where a is the persistence length and L is the DNA contour length that can be calculated by multiplying the number of base pairs with the base pair unit length (0.34 nm). The expression is different for circular DNAs as shown below.

$$r_{G,circular} = \left[\begin{array}{l}\left(2a + \frac{22a^2}{3L}\right)\left(\frac{L}{12} - \frac{2\alpha^2 a^2}{L} + \frac{8\alpha^3 a^3}{3L^2}\right) - a^2 \\ + \frac{4\alpha a^3}{L} + \frac{8\alpha^3 a^3}{L}\left(\frac{1}{3} - \frac{\alpha}{6} + \frac{k_2\alpha^2}{5} + \frac{k_3\alpha^3}{6}\right)\end{array}\right]^{1/2}, \tag{3}$$

where α, k_2, k_3 are functions of L as detailed by Yamakawa [35]. If we can assume that $L \gg a$ then $r_{G,linear}$, $r_{G,circular} \sim \sqrt{L}$ and by substituting r_G with $\sqrt{L}$ in Eq. (1), we can state that $I \sim L^2$. Although we should not have directly compared the intensity of the linear DNA with circular DNA, we justify this because L of the linear DNA was an order (x10) longer than the circular DNA. Hence, the intensity signal difference was quite large. We postulate that the proposed method can distinguish the length difference as $(L + \Delta L)^2 - L^2 = 2L\Delta L + (\Delta L)^2 \approx L\Delta L$. In other words, the intensity signal difference for a pair of DNAs that are comparatively longer will be stronger. In summary, we can differentiate

#207176 - $15.00 USD Received 25 Feb 2014; revised 19 May 2014; accepted 16 Jun 2014; published 3 Jul 2014

the difference between two DNAs in the order of the difference times the shorter base length. However, because we have conducted the experiments using only two lengths of DNAs, we could not verify this sensitivity.

The hole depth and diameter is smaller than the radius of gyration of the DNA and thus the DNA at the nanohole will undergo a motion that would be vastly different from conventional optical trapping that occurs in an open space. The intensity fluctuation when entering and exiting the trapped state could be an invaluable source for understanding what is going on. However, we don't have clear understanding of the trapping motion since it involves the understanding of the behavior of a worm-like-chain model in an optical trap. Nevertheless, we are quite confident that the trapping happens along the edges of the nanohole because this is where the intensity is maximum as shown by other calculations in the literature [12].

6. Conclusion

In this paper, we have demonstrated the trapping of a single DNA using a plasmonic nanohole. The trapping was verified through the measurement of a transmission signal that oscillates between the two states. Based on the scattering theory we can deduce that the higher state is when a scatterer is trapped as opposed to when there is no scatterer. We have conducted the experiment with a 4.7 kbp plasmid DNA and a 48 kbp lambda DNA. We have shown that the increased laser power results in longer trapping, and the longer lambda DNA results in stronger scattering signal. Transmission signal can also work as a barometer for distinguishing DNA of various lengths potentially opening new sensor applications such as DNA fractionations. In summary, this paper reports for the first time of using plasmonic structure for trapping a single DNA and proposes new possibility of using the proposed method for characterizing DNA based on its length.

Acknowledgments

This work was supported by Mid-career Researcher Program through National Research Foundation of Korea (NRF) (2012R1A2A2A03045928) grant funded by the Ministry of Education, Science and Technology (MEST) and by the Basic Research Project through a grant provided by GIST.