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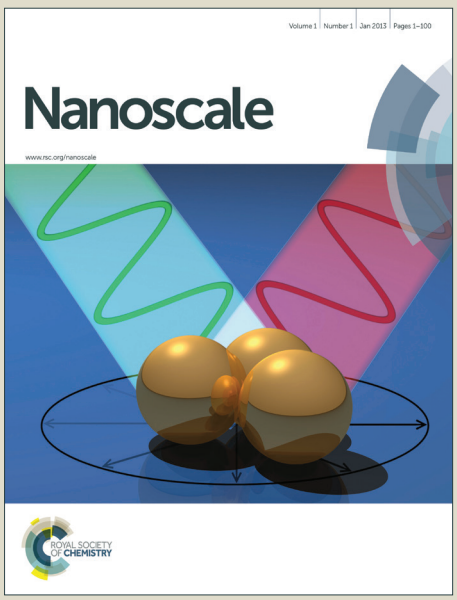





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# Playing the notes of DNA with light: Extremely high frequency nanomechanical oscillations

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ABSTRACT

We use a double nanohole (DNH) optical tweezer with two trapping lasers beating to excite the vibrational modes of single-stranded DNA (ssDNA) fragments in the extremely high frequency range. We find the resonant vibration frequency of a 20 base ssDNA to be 40 GHz. We show that the change in the resonant frequency for different lengths of the DNA strand is in good agreement with one dimensional lattice vibration theory. Thus the DNH tweezer system could distinguish between different lengths of DNA strand with resolution down to a few bases. By varying the base sequence and length, it is possible to adjust the resonance frequency vibration spectrum. The technique shows the potential for use in sequencing applications if we can improve the resolution of the present system to detect changes in resonant frequency for a single base change in a given sequence. The technique is single-molecule and label-free as compared to the existing methods used for DNA characterization like gel electrophoresis.

## Introduction

Studying the mechanical and thermodynamic properties of DNA has helped us to understand related biological processes[1, 2]. These studies have mainly been divided into two domains: study of conformational flexibility and the elastic phonon modes. Conformational flexibility of DNA has been studied using approaches such as mechanical stretching[3], fluorescence resonance energy transfer (FRET)[4], transient electric birefringence[5], thermal melting profiles of DNA hairpins[6] and atomic force microscopy[7]. The elastic dynamics of DNA have been studied using Raman scattering[8, 9], Brillouin scattering[10], far-infrared absorption[11], and sub-millimeter wave absorption spectroscopy in the range of 0.01–10 THz[12, 13]. The study of vibrational dynamics of DNA has resulted in various applications such as detecting mutations by identifying resonant modes associated with localized defect in the DNA polymer[14]. Most of these works have used double stranded DNA (dsDNA) and given insight into the dsDNA conformational flexibility and vibrational modes, while single stranded DNA (ssDNA) remains less studied. ssDNA occurs as an intermediate molecule in the DNA metabolic processes of repair, replication and transcription, which warrants further study of the associated dynamic properties of ssDNA. It is also the form of genetic information in many viruses. Here we investigate the vibrational modes of ssDNA at the single molecule level (i.e., studying individual molecules one-at-a-time) and up to the extremely high frequency electromagnetic range (>30 GHz). This is done using optical tweezers, which has been an important tool for mechanical, spectroscopic and opto-mechanical studies of nanoparticles at single molecule level[15].

Low frequency collective oscillations or resonant modes of DNA and proteins molecules have revealed many of their biological functions along with their dynamic mechanisms such as the intercalation of drugs in DNA[16, 17]. The low frequency vibrational modes correspond to the macroscopic bending, stretching, and torsion motions of the molecule, which leads to the mechanical deformation necessary for the biological processes[14]. The resonant modes of small DNA strands have been identified previously by Raman spectroscopy[18, 19] in the very far infrared region, mostly above 10 THz[20, 11]. The absorption spectroscopy of sub-millimeter electromagnetic waves has been performed on dried DNA films to extract their vibrational resonances[21]. The requirement of using dried films stems from the high absorption of water that would swamp out the signal in the aqueous phase. Recently hypersound spectroscopy was used to study phonon modes of DNA in the range of 500 GHz and above[13]. But still these studies have been unable to show the lower frequency modes in the few tens of GHz range, in part due to the requirement of sufficiently thick and large diameter films and thus lack conclusive identification of these lower frequency modes[12].

Here we excite and measure the resonant vibrational modes of small ssDNA molecules (tens of bases) in the 10-100 GHz range using a DNH optical tweezer. The vibrational modes are excited by the interaction of an extremely high frequency (EHF) beating

electromagnetic field and ssDNA molecule. The EHF range defined by the radiofrequency designation is actually an extremely low frequency from a molecular spectroscopy point of view, representing low frequency long range modes of macromolecules. The same technique was recently used to measure the Raman-active vibrations of isolated nanoparticles in the 0.1 $cm^{-1}$ to 10 $cm^{-1}$ range. It is to be noted that those measurements were performed on polystyrene nanoparticles and roughly spherical globular proteins, and not related to the study of ssDNA, which is a linear chain, performed here[22]. We show the tuning of the resonant mode with the change in the length of the ssDNA. The DNH tweezer provides a better way of identifying short strands of DNA called oligonucleotides compared to present techniques that require dyes and fluorescent markers[23,24], and therefore can play an important role in genetic testing, forensics and DNA amplification. The DNH tweezer provides label-free, real-time oligonucleotides characterisation by their resonant modes in the tens of GHz range, even in aqueous environment and at single molecule level, in contrast to the above mentioned past investigations.

Our previous work has shown the trapping of a 20 base ssDNA using DNH tweezers. The DNH tweezer is able to unzip a hairpin-DNA and also shows interaction of ssDNA with transcription protein at the single molecule level[25]. The present work, however, studies the dynamic behavior of the ssDNA in terms of its collective vibrational modes in the tens of GHz range. Until now, this range of low frequency modes could not be observed in real systems due to viscous effects of the medium[26, 27]. But the high sensitivity of the detected signal in our system to the overall macromolecular motion makes it possible to observe these vibrational modes in a real system with aqueous media, all at the single molecule level. The significant amplitude of these low frequency vibrational modes and their consequent importance to the overall motion of DNA is a key enabling feature of this work[26, 28]. The resonant vibrational mode corresponding to larger overall motion of the DNA strand in the optical trap is detected by measuring the increase in the intensity fluctuation of the transmission signal through the aperture. This increase is observable as a peak in the root-mean squared (RMS) deviation of the optical transmission through the DNH when the beat frequency matches the vibrational resonance of the ssDNA: that is, we measure an increase in the noise amplitude of ssDNA motion due to resonant heating. The overall motion of the DNA due to the low frequency vibrational modes is closely related to global dynamic properties such as bending and stretching rigidity of the DNA molecule[26].

## Experimental set up

In the past, we have used a simple double nanohole (DNH) tweezer with inverted microscope geometry and the laser focused on the DNH[29]. The laser transmission through the DNH aperture is collected by a condenser lens and measured as a voltage using an avalanche photodiode (APD). The small gap between the two cusps of DNH has high local field intensity, which enables the trapping of small particles in the range of a few nanometers[30]. The transient jump in the transmission signal is indicative of the

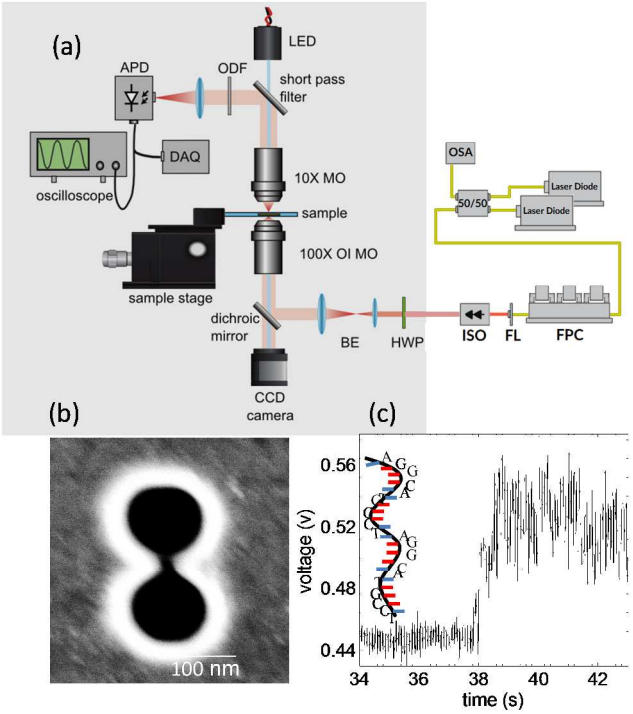


Figure 1 (a) Schematic of the experimental setup of dual-laser DNH tweezer setup. Abbreviations: optical spectrum analyzer (OSA). fiber coupler (50/50), fiber polarization controller (FPC), fiber launcher (FL), optical isolator (ISO), half wave plate (HWP), 45 degree mirror (MIR), dichroic reflector (DI), optical density filter (ODF), avalanche photodiode (APD), CCD camera (CCD). (b) SEM image of DNH nanostructure on gold film. (c) A typical trapping event showing the step change in the aperture transmission signal.

trapping and the intensity fluctuations correspond to the dynamics of the molecule in the optical trap, which have been extensively discussed in some of our previous works[31, 32]. Recently, using statistical techniques on the trapped transmission signal, we studied the binding kinetics of protein-small molecule interaction and estimated their disassociation constant using the DNH tweezer[33].

For exciting the vibrational modes of the molecule of interest in addition to trapping, we use a slightly modified tweezer setup as shown in Figure 1a. The single laser is replaced by two trapping lasers shifted in wavelength by tens of gigahertz and coupled using a 50/50 coupler to produce a single laser beam with amplitude modulation through interference beating. The beating is achieved by slightly detuned lasers. The beating frequencies were measured using on optical spectrum analyzer and found to be stable to within 0.4 GHz. Figure 1b shows the SEM image of DNH used for trapping of the ssDNA and Figure 1c shows a typical trapping event. After the ssDNA is trapped, we collect the transmission signal for different beat frequencies and use it to calculate the spectrum of the RMS deviation of the transmission signal. Using this setup, a wide range of beat frequencies between 10 GHz to 0.3THz with resolution of 0.4 GHz is possible, but for our experiment we limit the range to 10 GHz-60 GHz, which is the range of interest for the ssDNA modes studied. This was done by keeping the wavelength of one laser constant and sweeping the

wavelength of the other laser by temperature tuning. The peak in the resulting spectrum corresponds to the resonant beat frequency. Further details of the experiment are provided in the Methods section. The above experiment can be further extended to >10 THz by replacing one of the lasers with a widely-tunable external cavity laser, which could be exploited to study dynamics in the terahertz region.

## Results and Discussion

Figure 2 shows the intensity fluctuations of the transmission signal due to the trapped ssDNA for two different regions of the spectrum. Part a and b shows the intensity fluctuations of the transmission signal and the corresponding Gaussian distribution for beat frequencies corresponding to 13-15 GHz (non-resonant frequencies) and 38.5-40.5 GHz (resonant frequency). The first part corresponds to the non-resonant frequency range where the width of the Gaussian distribution corresponds to the small RMS value in Figure 3. The second part corresponds to the resonant frequency range where the larger RMS width of the Gaussian distribution corresponds to maximum RMS values or the peak in Figure 3. The Gaussian fit of the transmission signal at resonant frequency shows a 46.6% increase in the width of the Gaussian signal corresponding to resonant heating (as compared to off-resonant excitation). This is seen as a peak in the RMS deviation of the fluctuating transmission signal at the resonant beat frequency. The ssDNA is coupled to the electromagnetic field due to the charge distribution on the DNA strand. The resulting electrostriction force excites the low frequency vibrational modes in the ssDNA molecule. This appears as collective motions of the DNA strand resulting in an accordion–like motion or stretching of the ssDNA. The electrostriction force is modulated by the change in the beat frequencies produced by the two trapping laser beams. At the beat frequency corresponding to the natural frequency of the ssDNA, the DNH tweezer excites the resonant vibrational mode of the ssDNA to heat it up. As a consequence, the Brownian motion of the ssDNA increases which increases the intensity fluctuations of the transmitted signal through the aperture. The increase in RMS fluctuations occurs when the beat frequency matches the vibrational frequency of a Raman-active acoustic vibration, as we have confirmed by comparison with Lamb's theory in a recent work[22].

Figure 3 shows the normalized RMS variation in the transmitted intensity through the DNH as a function of the beat frequency between the lasers for a 20 base ssDNA. The peak RMS is found to be 39.8 GHz. The same peak was observed with variation within 1 GHz around the mean value for different scans done for multiple trapping events.The vibrational resonance modes are expected to be broad due to strong damping by the aqueous surrounding medium.The point of interest is the beat frequency corresponding to the peak in the RMS deviation within the frequency range of interest. It should be emphasized that the RMS deviations are occurring at much lower frequencies (in the kHz range typical for thermal motion in optical tweezer systems[31, 34]); the increased RMS corresponds to local heating and not coherent light scattering in the extremely high frequency range. Higher frequency harmonics are also observed for larger frequency range sweeps (see for example Figure 4); however, we

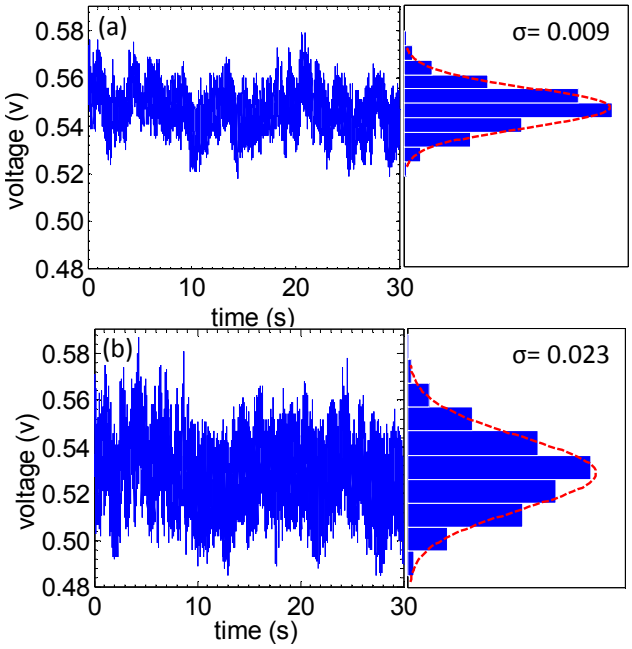


Figure 2 Transmission signal fluctuations of the trapped 20 base ssDNA and the Gaussian fit (red) to the corresponding histogram at a) f=13-15 GHz b) 38.5-40.5 GHz

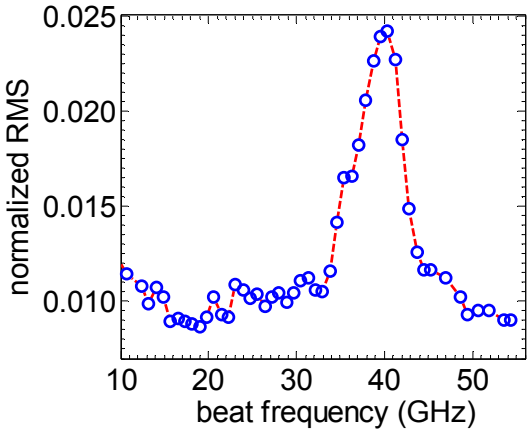


Figure 3 Normalized root mean squared (RMS) deviation of the scattering signal for a 20 base ssDNA for frequency sweep of the beating lasers.

do not study these in detail here.We also studied the influence of the size of ssDNA on the resonant vibrational frequency by repeating the above measurements for different lengths of ssDNA ranging from 20 to 40 bases. More information regarding the base sequence and concentration of DNA solutions are provided in the Methods section of the paper. Figure 5 shows a decrease in the resonant vibrational frequency with the increase in the length of DNA strand and was found to be in good agreement with the resonant frequency calculated using the 1-D lattice vibration theory. The continuous lines show the variation in resonant frequency of ssDNA molecule for a poly-G (red) and poly-C (blue) ssDNA molecule having the average masses of 150.14 Da and 110.11 Da. Thus the DNH tweezer could characterize very short DNA strands based on their different resonant frequency with resolution down to few bases. A recent work has used terahertz cavity to excite the self-resonant modes of small DNA strands , which showed different resonant frequencies for 50 and

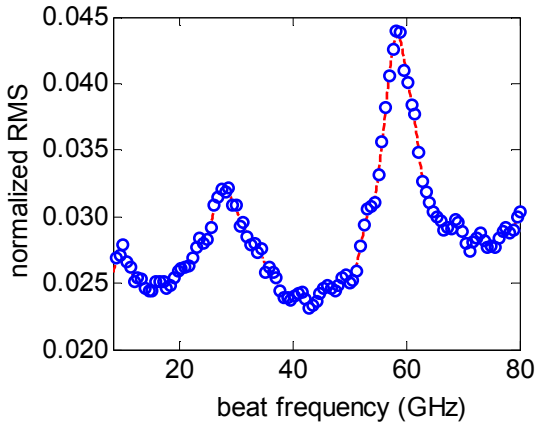


Figure 4 Normalized root mean squared (RMS) deviation of the scattering signal for a 30 base ssDNA showing the fundamental resonant frequency (f=28.3 GHz) and a second order harmonic (f=57.6 GHz).

100 bp DNA strands[35].The method works in the terahertz frequency domain and is not a single molecule method as compared to the present technique. Since the experiments were done for dsDNA, which has different mechanical characteristics than ssDNA, we expect different results. Compared to that work, our method provides better resolution of few base pairs to distinguish between different oligonucleotides length sequence.

To test the variation in resonance frequency with changes in base sequence, we choose three different sequences (Table 1) of 30 base ssDNA. The resonance frequencies of these ssDNA molecules were measured and compared with the analytic theory (Figure 6). This shows the ability to predictably distinguish between sequences with significant mass variations (of the order of hundreds of Daltons).

| S. No. | Sequence | Total Weight | Average mass |
|---|---|---|---|
| 1 | GGGCGGGGAGGGGGAAGGGAGAGGGAAGA | 4336.2 Da | 144.54 Da |
| 2 | CAGCACACACACGGAAGGGAGACACAACAC | 3895.9 Da | 129.86 Da |
| 3 | CCCGCCCCTCCCCCTTCCCTCTCCCTTCTC | 3463.4 Da | 115.44 Da |

Table 1 Different sequences of 30 base ssDNA with correpondng masses used in the experiment.

We calculate the resonant modes of ssDNA using simple one dimensional lattice vibration theory[36]. Within this theory, the ssDNA is considered as a linear chain of atoms with the atomic groups replaced by average mass, $M$, of the DNA sequence connected by effective springs with spring constant $\kappa$. Since the sequences were roughly equally distributed among the bases so using an average is reasonable. The solution of the classical equation of motion for the ssDNA using this model gives the resonant vibrational frequency as (within the harmonic approximation)

$$\omega_r \approx \frac{\pi}{N_b}\sqrt{\frac{\kappa}{M}}$$

where $N_b$ is the number of bases in the ssDNA, $k_B$ is the

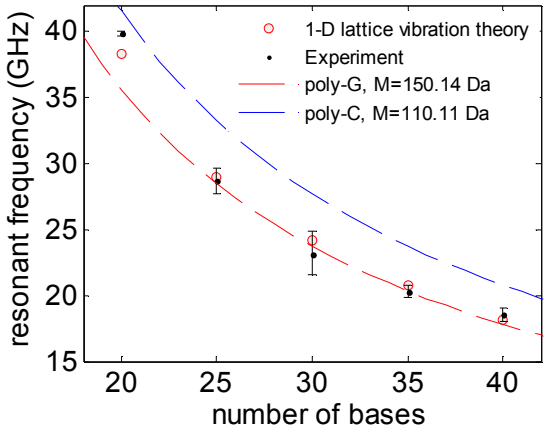


Figure 5 Resonant mode frequency as a function of number of bases of ssDNA molecule. The line (black) shows the mean resonant frequency along with the standard deviation found experimentally. The circle (red) is the resonant frequency calculated by modelling ssDNA using the 1-D lattice vibration theory. The continuous lines are the resonant frequency variation with number of bases for poly-G and poly-C sequences.

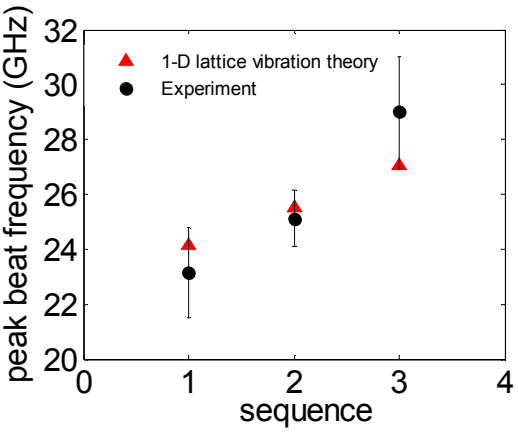


Figure 6 Resonant frequencies of three different sequences of 30 base ssDNA molecules. The line (black) shows the mean resonant frequency along with the standard deviation found experimentally. The triangle (red) is the resonant frequency calculated by modelling ssDNA using the 1-D lattice vibration theory.

Boltzmann constant, $\kappa$ is the spring constant or stretch modulus and $T$ is the temperature.The freely jointed chain model of the ssDNA molecule used to determine the stretching elastic constant of the ssDNA has been studied extensively[37]. We use this stretching elastic constant value for calculation of resonant frequency. The spring constant of the ssDNA is given as $\kappa = 123.5\ k_BT$ /nm$^2$, which is the generally accepted value of the stretch modulus of the sugar phosphate backbone of ssDNA molecule irrespective of the base sequence in the given approximation[37,38]. Also, previous studies have shown that changing the salt concentration does not significantly influence the elastic stretch modulus[39,40] and therefore should have little impact on the resonant frequency.The mass M was not fitted to the data; rather it is the average mass which is uniformly distributed along the ssDNA chain. It is obtained by dividing the

total mass of the ssDNA (sum of mass of individual base A, T, G, C in the specimen) by the total number of bases. Since the sequences were roughly equally distributed among the bases, using an average is reasonable. The value of this average mass for all the DNA sequences used is given in the Methods section. The results of the calculations are also shown in Fig. 5 and Fig.6, with good agreement with the experimental observations.

## Methods

### Slide preparation

The single strand DNA consisting of 20, 25, 30, 35, 40 bases were ordered from Integrated DNA Technologies. The DNA sequences used in the experiment are as follows: 5'-AGG CAT GCC TAG GCA TGC CT-3'( 20 bases, M=129.93 Da), 5'-GGG CGG GGA GGG GGA AGG GAG AGG G-3'( 25 bases, M=145.34 Da), 5'-GGG CGG GGA GGG GGA AGG GAG AGG GAA GAG-3'( 30 bases, M=144.54 Da), 5'-GGG CGG GGA GGG GGA AGG GAG AGG GAA GAG AGG GA -3'( 35 bases, M=144.43 Da), 5'-GGG CGG GGA GGG GGA AGG GAG AGG GAA GAG AGG GAG GGG G -3'( 40 bases, M=145.14 Da). The ssDNA oligonucleotides were re-suspended using a Tris-acetate (TEC) buffer with a pH 8.0 to form a solution of concentration 0.1 % w/v. Approximately 10-20μL of ssDNA oligo solution is then dropped in the well formed on a thin 24×60 glass coverslip (EMS 032414-9) using an adhesive slide spacer (Grace Bio-labs GBL654002). The DNH structure milled on a 100 nm thick gold test slides (EMF corp.) is then placed on top such that the solution is sandwiched between the DNH and the glass coverslip.

### Experiment

The prepared slide containing the ssDNA solution and DNH structure is placed on a piezo stage and the laser is focused on the DNH of interest using a CCD camera. The transmission through the DNH is maximized by aligning the polarization of the laser beam long the axis of the cusp of DNH using the fiber polarization controller and the half wave plate. Once the ssDNA particle is trapped, which is seen as a step change in the voltage level of the APD signal and is visible on an oscilloscope, we acquire the transmission signal data for different beat frequencies. The trapped signal data is recorded using a data acquisition card for about 10-30 seconds for every beat frequency in the desired range of 10-60 GHz. The beat frequency is recorded by recording the wavelength difference between the two lasers using a fiber coupled optical spectrum analyzer (OSA). The transmission signal collected for every beat frequency is processed using a windowed root mean square deviation filter and then normalized to plot the normalized RMS spectrum with beat frequencies of interest. The beat frequency corresponding to maximum value of RMS deviation of transmission signal corresponds to the resonant vibrational frequency for the lowest order mode of the ssDNA.

## Conclusions

In conclusion, we have shown that the DNH tweezer can be used to excite and detect the resonant vibrational modes of ssDNA containing tens of bases. The DNH tweezer was able to do this at the single molecule level and in the frequency range of tens of GHz, which has not been accessible through other scattering techniques, especially in an aqueous biological environment. The approach also shows the ability to characterize small ssDNA strands with resolution of few base pairs and has the potential to be extended further for exact base sequence determination, with future improvements in resolution to resolve the different base masses. The technology could have wide implications ranging from biosensing, protein-DNA interactions to biomolecular materials.

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