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# Characterization of Dual Beam Optical Tweezers System Using a Novel Detection Approach

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## Abstract

*Characterization of optical tweezer system involves determining the stiffness coefficient of the trap and the range in which it behaves as a linear spring. This is often done by calculating the power spectrum of the fluctuations of the trapped bead observed on a photodetector. The detection process entails observing the deflection of either the same trapping beam or a different laser beam of very low power. We present a new approach where a laser beam from a source is split into s- and p- polarizations where s-polarization is used for trapping and p-polarization for detection. We also analyze and experimentally verify the effect of the detection beam on the trap dynamics. We further explain how to incorporate the effect of a strong detection beam, capable of trapping beads, in the photodetector calibration in a dual beam trap scenario. The novel detection method and the associated modelling sets the stage for the application of modern control approach.*

## 1. INTRODUCTION

In recent years, tools for single cells and single molecules study have become popular with the biophysicists and biochemists. Optical Tweezers [1] is one such tool that is non invasive to the biological specimen (built using an Infra red laser), has a high spatial resolution in naometers [6], measures forces in the pico-Newton range [5] and enables studying the specimen over long periods of time for in vivo [2] and in-vitro assays. Ever since the first optical tweezers was built in 1986 by Ashkin [1] it has become an essential tool for many cell biological studies [3, 4].

The construction and functioning of optical tweezers setup with a single and multiple traps is explained in [7]. For small displacements (roughly 100-300 nm) of the trapped particle from the center of the trap, the tweezers behave as a Hookeian spring characterized by a trap stiffness [7] and viscosity of the aqueous medium defines the damping. As the system is heavily damped, the inertial terms become insignificant and the trap behaves as a first order low pass filter with a characteristic frequency, $f_c$. The input to the system is thermal noise which results in the Brownian motion of the trapped particle. A direct way to reduce the effect of thermal noise is to increase the laser power and hence the trap stiffness, however this can damage the specimen. Using feedback to actuate the laser trap position can greatly reduce Brownian motion without having the adverse effect of increased laser power. This motivates the need to accurately characterize the tweezers system. A well aligned tweezer exerts a cylindrically symmetric force about the axial axis of the beam (z-axis). A complete characterization of the optical trap involves determining its stiffness constant in the transverse and axial directions. In this paper however, we have characterized the trap in only the transverse direction.

There are several optical tweezer calibration methods reported in the literature. The most commonly used is the method of power spectrum [8] that involves measuring the instantaneous position of the trapped particle as a function of the laser deflection on the photo detector. This is done by measuring the trapping beam passing through the trapped particle on the photo detector. However, implementing a feedback with such a measurement system interferes with actuation as the actuation is done to the trapping beam. This problem is overcome by using a separate low power laser beam in the visible spectrum for detection [4, 9]. In this paper we present a novel approach to use the same IR laser for the purpose of trapping and detection and also incorporate the effect of the detection beam on the trap position, which so far has been ignored. The advantage of our scheme is its relative ease of one laser as against two, one each for trapping and for detection and also does away with the need to introduce extra op-

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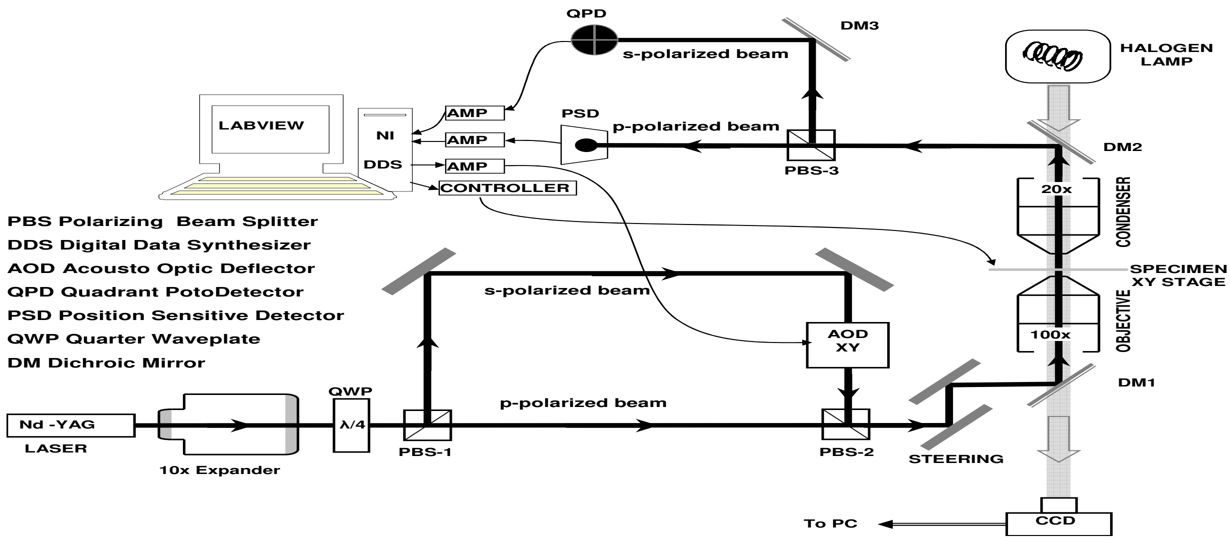


**Figure 1.** Schematic of our optical tweezers setup showing the detection and actuation mechanism. The solid black line represents laser beam path and visible light from microscope lamp is shown in gray line with the arrows indicating the direction. The laser beam consists of s and p polarized beams before entering PBS-1 and after exiting PBS-2. Both polarized beams create two traps at the specimen plane. The fluctuations of the trapped bead are measured at the two photodiodes, viz. QPD and PSD, which meausre the s and p polarized beams respectively. Only the s-polarized beam passes through AOD, which is computer controlled, thus enabling modulating the optical trap due to s-beam.

tics for the other laser. This scheme also scores over the existing method as the detection beam is in the IR region and hence, is not absorbed by biological samples. However silicon being less sensitive in IR than in visible regime results in a lower detection sensitivity. For displacement measurements, the sensitivity of the photo detector needs to be determined. The traditional method involves positioning an immobile bead stuck to the glass slide at the trap position and moving the bead by a known distance by actuating the stage. The calibration of the photo detector thus involves the beam getting deflected through the stuck bead at an axial position different from where it would be in an actual experiment [9, 10]. Also, the photodiode is calibrated not for a trapped bead that will be used in the experiment, but with a stuck bead. The cohesive force between the cover slip and the stuck bead may affect accuracy of sensitivity measurements. The accuracy suffers further as the viscosity of the fluid near the cover slip is different from viscosity at the trap position in an actual experiment. To overcome the mentioned problems, in our experiment we evaluate the sensitivity of the photo detector for the trapped bead and use the same bead for trap calibration. The trapped bead is actuated by moving the trapping beam with the AOD while the stationary detection beam is measured at the photo detector.

## 2. Model for Optical Tweezers

### 2.1. Single beam trap

For a single beam optical trap, the trap dynamics is governed by the equation

$$m\ddot{x} + \beta\dot{x} + kx = F_{th} + F_{ext},$$

where the trap center is assumed at the origin, $x(t)$ is the instantaneous position of the trapped bead, $m$ is the mass and $r$ is the radius of the bead, $\beta$ is the drag coefficient and is measured as $6\pi\eta r$ where $\eta$ is the viscosity of the medium and $k$ is the spring constant of the trap, $F_{th}$ is the stochastic Langevin force and $F_{ext}$ is any external force applied to the bead. In the simplest case where no external force is exerted $F_{ext} = 0$. This analysis is valid for small displacements from the trap position as the trap behaves as a linear spring only in a small region. The effect of inertial term is neglected as the system is highly overdamped.The equation of motion gets simplified to

$$\dot{x} + \frac{k}{\beta}x = \frac{1}{\beta}F_{th},$$

therefore the solution is

$$x(t) = \frac{1}{\beta}\int_{-\infty}^{t} e^{-\frac{k}{\beta}(t-\tau)}F(\tau)d\tau.$$

Assuming the process $\{x(t)\}$ to be stationary, the autocorrelation function of the displacement $x(t)$ is given by

$$R_x(\tau) = \frac{1}{\beta^2} e^{(\frac{-\tau k}{\beta})} \int_{-\infty}^{0} e^{(\frac{\tau' k}{\beta})} d\tau' \int_{-\infty}^{0} \langle F_{th}(\tau'), F_{th}(\tau'') \rangle e^{(\frac{\tau'' k}{\beta})} d\tau'',$$

where $F_{th}(\tau')$ is a random force due to the Brownian motion which is an independent and stationary process. Therefore $\langle F_{th}(\tau'), F_{th}(\tau'') \rangle = A\delta(\tau' - \tau'')$, where $A$ is the amplitude. This yields for a real stochastic function $x(t)$,

$$R_x(\tau) = \frac{A\beta}{2\nu^2 k} e^{-\frac{|\tau| k}{\beta}},$$

From the equipartition energy theorem, $k\langle x^2(t) \rangle = k_B T$, therefore

$$A = 2\beta k_B T R_x(\tau) = \frac{k_B T}{k} e^{\frac{-|\tau| k}{\beta}},$$

where $k_B$ is the Boltzman constant, $T$ is the absolute temperature. Taking the Fourier Transform of the autocorrelation function, the power spectrum of the bead displacement is given by

$$S_x(\omega) = \frac{2k_B T \beta}{(k^2 + \omega^2 \beta^2)},$$

which for a single sided power spectrum, with spring constant $k = 2\pi\beta f_c$, where $f_c$ is the cut off frequency, becomes

$$S_x(f) = \frac{k_B T}{\beta \pi^2 ({f_c}^2 + f^2)}.$$

### 2.2. Dual beam trap

The above analysis can be applied to the dual beam trap if the two traps are outside the region of influence of one another. The analysis becomes involved if the two traps are within the non linear region of field of influence of one another. We concentrate on the case where the traps are within the linear spring region of each other. In our experimental setup, the two traps created using the s and p polarized laser beams are brought very close to each other by aligning the spots through the CCD camera. If the two traps are within each others linear region and the beams do not undergo interference, the trapped bead due to both the traps should satisfy the equation

$$\beta \dot{x} + (k_p + k_s)x = F_{th}(t).$$

Thus the cut off frequency of the trap becomes the sum of individual cut off frequencies and the bead gets trapped at a position somewhere between the two trap centers. Consider the dual beam trap setup as described above. The position of the p-pol beam (detection beam) is kept fixed while the position of the s-pol beam (trapping beam) is varied (over a small distance) at a rate slower than the characteristic frequency. Let the position of the stationary trap center be the origin, the instantaneous bead position be $x_b$ and the instantaneous position of the trap due to s-polarized beam be $x_s$. The equation of motion of the bead (neglecting the effect of Brownian motion) is given by

$$\beta \dot{x_b} + (k_p + k_s)x_b = k_s x_s,$$
$$X_b(s) = \frac{k_s}{s + k_p + k_s} X_s(s).$$

## 3. Experimental Setup

Figure 1 shows the schematic of our optical tweezers setup along with the detection mechanism. A linearly polarized IR laser beam from Nd:YAG laser (CrystaLaser Inc., $\lambda$ = 1064 nm, max. power output = 500 mW) is expanded using a $10\times$ beam expander (Thorlabs Inc.) and passed through a half wave plate that rotates the linearly polarized light. A polarizing beam splitter ($PBS1$, Newport Inc.) allows the p-pol component of the beam to pass through and reflects the s-pol component at right angle, thereby creating two non interfering beams. The power in the p-pol component is kept much lower than its s-pol counterpart using the half wave plate to use the s-pol beam for creating the trap and have detection of the trapped particle as the main purpose of the p-pol beam. The PBS should ideally allow the p-component to pass through while reflecting the whole of the s-component, however, approximately 4% of the p-component gets reflected and about 0.01% of the s-component goes through that results in a small amount of coupling. It is for the significantly lower leakage of s-pol beam that p-pol beam is used for detection. The s-pol beam is passed through a dual Axis, acousto Optic Deflector, AOD (Intraction Corp. DTD-276HD6) and combined with the p-pol beam at the beam splitter $PBS2$. The combined beam is introduced into the inverted microscope (Nikon Inc., TE2000U) from the epi-fluorescence port and directed into the back aperture of the oil immersion objective (numerical aperture, NA, = 1.4) using a dichroic mirror, $DM1$. The high NA objective converges the two beams to the image plane where it creates two trap centers. The specimen is a colloidal solution of, $1.1\mu m$ in diameter, polystyrene beads. The specimen is mounted on a custom built, X-Y piezo-electric actuated, stage (Asylum Research Inc.), that can be moved manually or through a computer. The condenser, a $20\times$ objective, mounted above the sample stage on a manual X-Y-Z positioner, plays a dual role. It provides illumination for the sample by focussing the light from the lamp above and collimates the diverging laser beam from below. The collimated beam is incident upon a third beam splitter $PBS3$ via dichroic mirror $DM2$. $PBS3$ splits the beam into p- and s- polarizations. The p-polarization is allowed to directly fall on a position sensitive photodetector (Pacific Silicon Sensor Inc., DL100-7PCBA3) while the high power s-polarized beam is directed on a quadrant photodiode (Pacific Silicon Sensor Inc., QP50-6SD) by reflecting through a cold mirror. The signals from both the photodiodes are acquired using a data acquisition card (National Instruments, PCI 6133 DAQ) and LABVIEW based software. The AOD is also driven using a LABVIEW based VIs, for the purpose of beam modulation. A colored Charged Couple Device, CCD, camera is used to view the specimen on the computer. The optical setup is mounted on an air table (Melles Griot Inc.) to isolate it from external mechanical vibrations. The detection system optics are mounted on a custom made aluminum optical breadboard.

## 4. Experimental Procedure

### 4.1. Calibration

**4.1.1. Measurement of Trap Stiffness using Power Spectrum Method.** In this method, the power spectrum of the fluctuations of a trapped bead of known radius, $r$, is measured [7]. The model of trapped bead dynamics is given in section 2. The characteristic frequency, $f_c$, is obtained by fitting the Lorentzian to the experimentally obtained power spectrum. In our setup with both the trapping beam and detection beam active, the bead movement is captured on both the photodiodes. The PSD captures the variations in the p-pol beam and the QPD captures the variations in the s-pol beam. Trap stiffness due to single beam is determined by blocking the other beam.

**4.1.2. AOD Calibration.** The AOD is calibrated to obtain a relationship between the input to AOD and the trap translation. The computer screen is first calibrated using a diamond ruled precalibrated slide. To calibrate the AOD a Radio Frequency (RF) input signal is applied and the movement of the trapped bead on the computer screen is measured. The procedure is repeated for several input frequencies and the average is taken. In our setup, the trap spot moved by $3\mu m$ for a change in input frequency of 1 $MHz$.

**4.1.3. Photodetector calibration using Power Spectrum method.** Let the photodiode sensitivity be $S$ (in $Volts/nm$). The experimentally obtained power spectrum, $P_v(f)$ as described above, is a plot of $V^2$ versus $Hz$. Therefore

$$P_v(f) = S^2 P_x(f) = S^2 \frac{k_B T}{\beta \pi^2 (f_c^2 + f^2)},$$

and from the DC value, sensitivity is

$$S = \pi f_c \sqrt{\frac{P_v(0)\beta}{k_B T}}.$$

**4.1.4. Photodetector calibration using Stuck bead method.** This is the widely used method of photodiode calibration for its ease of use [7]. A bead stuck to the coverslip is moved to the center of the trap and oscillated by a known amount in x or y direction, by applying a signal to X-Y piezo stage and the corresponding output signal from the photodetector is recorded. The ratio of these two signals gives sensitivity.

**4.1.5. Photodetector calibration using AOD.** This method offers the advantage of determining sensitivity for the bead used in the actual experiment. The trapped bead is oscillated along a known distance in the x-y plane by actuating with the AOD, while the detection beam stays stationary. As the trapped bead moves, the deflection of detection beam on the photodiode changes. In our experiment, a triangular wave input is applied to the AOD in one of the axis, which drives the trap with a constant velocity along the waist of the detection beam. A S-shaped sensitivity curve is obtained using this method when a large input amplitude drives the AOD. The output is triangular if the displacement is within linear region of trap stiffness and the slope (volts/time) divided by bead velocity (nm/time) gives photodiode sensitivity (volts/nm).

### 4.2. Measuring the distance between the two trap centers

The stiffness constant of the trapped bead due to the two traps is determined by the power spectrum method. This is compared with the stiffness constants of the same trapped bead due to individual traps. If the two trap centers are within the linear region of each other the determined stiffness constants satisfy the model described in section 2.2. If it doesnt, the two traps are realigned and brought closer and linearity is established. The bead position in dual trap is measured by averaging the normalized voltage signal obtained at the PSD. The sensitivity of the PSD is determined using the method

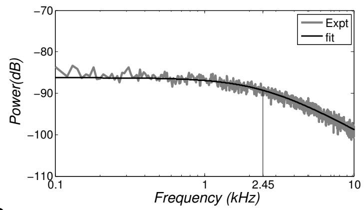


**Figure 2.** The figure shows the power spectrum of the trapped bead fluctuations and its Lorentzian fit. The cut off frequency of the trap from the Lorentzian is found to be 2.458 kHz.

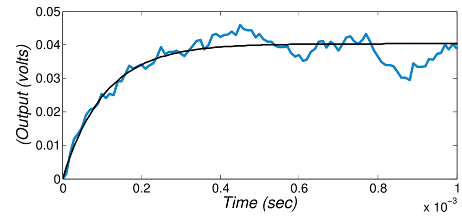


**Figure 3.** The figure shows the rise time of the PSD signal when the trapped bead was moved in a step by applying a step signal to the AOD.

described in section 4.1.5. The trapping bead is blocked and the bead shifts to a new position which coincides with the position of the detection trap center in the transverse plane. The change in signal on PSD is measured from which the distance between the two trap centers is calculated using the sensitivity. The position of the trapping beam trap center can then be evaluated by balancing the spring forces.

### 4.3. Spatial Resolution with the detection beam

To determine the spatial resolution of our setup, a trapped bead is oscillated at a known frequency, by applying a triangular wave input to the AOD. The power spectrum of fluctuations in detection beam are observed on PSD. If the spatial resolution of the setup is better than the amplitude of bead oscillation, peaks are observed in the power spectrum at the input frequency.

## 5. Results and Analysis

The power spectrum of the bead fluctuations in the dual trap is shown in Figure 2. The bead fluctuation data is captured as described in section 4.1.1. The Lorentzian fit to the power spectrum (Figure 2) gives the cut off frequency of the dual trap system to be at 2458 $Hz$, which for a 1.1 $\mu m$ diameter bead corresponds to a trap stiffness of 0.067 $pN/nm$. The trap exhibits a 20 $dB$ per decade roll off beyond the cut off frequency, which corresponds to free diffusion. This value of stiffness constant of the trap and the DC gain of the system yields the -1.8in sensitivity to be 0.61 $mV/nm$(refer sec 4.1.3). From the expression $\frac{1}{2}k\langle x^2\rangle = \frac{1}{2}k_bT$, the rms displacement of the bead in the trap is found to be 7.73 $nm$. The spring constant for the trap was also determined by applying an input step to the trapping beam trap position. Figure 3 shows the plot of voltage signal on PSD when the bead is moved in a step by applying a step input equivalent of 150 $nm$ translation of trapping beam center. The time constant, $\tau$, from the plot, is found to be 0.107 $ms$ and the stiffness constant, $k = \beta/\tau$, is evaluated to be 0.097 $pN/nm$. The probable reason for the difference in the evaluated spring constants from the two methods described above can be attributed to the large step movement of 150 $nm$ of the trapping beam and thus the two traps may have been outside of each others linear region. Also, the stiffness of the trapping beam trap would have changed as the symmetry of the beam changed on translation. Other reasons for the anamoly in spring constant needs to be investigated further.

The voltage versus time plot obtained, when a trapped bead is oscillated by applying a triangular input signal to the AOD (refer sec 4.1.5), is shown in Figure 4. A triangular output is observed when the trapping beam is oscillated about its original position with an amplitude of 100 $nm$ at a frequency of 10 Hz. The sensitivity of the photodetector, from the slope, is found to be 0.53 $mV/nm$. The sensitivity of the photodetector determined from the stuck bead method was found to be 0.35 $mV/nm$ (refer sec 4.1.4).

Figure 5 shows the power spectrum of the bead fluctuations obtained for the three cases, viz. trap due to both trapping beam and detection beam, only trapping beam, only detection beam. The cut off frequencies for the three cases are found to be 3023 $Hz$, 1729 $Hz$ and 1247 $Hz$ respectively, which correspond to stiffness

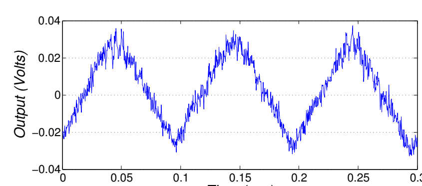


**Figure 4.** This figure shows the time data of the detection beam measured on PSD when a trapped bead is oscillated about the stationary detection beam trap, by moving the trapping beam along the Y-axis with the AOD. The amplitude of oscillation of the trapping beam spot is 100 nm. The linear variation indicates the trap behaves as a linear spring in this region.

constants of 0.082 $pN/nm$, 0.047 $pN/nm$ and 0.034 $pN/nm$. The cut off frequencies of the two single beam traps add to 2976 $Hz$ which is in good agreement with the condition of linearity as described in Section 4.2. The position of the two traps in different transverse planes can affect the stiffness constant measurements. Therefore for a complete analysis stiffness in the axial direction should also be considered. Another possible source of difference could be measurement of the bead fluctuations on PSD due to detection beam while measuring fluctuations due to single beam trap due to the trapping beam on QPD. To determine the distance between the two trap centers the experiemnt was repeated by trapping another bead at a different axial position. The cut off frequency for this trap was found to be 2458 $Hz$ (refer fig 2) and the difference in the PSD signal was 6 $mV$. For a sensitivity of 0.53 $mV/nm$ the distance between the trap centers is evaluated to be 19.7 $nm$ (refer sec 4.2) that, again, corroborates the claim that the two trap centers are within the linear region of each other. The power spectrum obtained, when the trapping beam is oscillated with an amplitude of 2 $nm$ at 70 $Hz$ by applying a triangular signal to the AOD (refer sec 4.3), is shown in Figure 6. This corresponds to bead movement of 1.16 $nm$ for the stiffness constants of the trapping beam and detection beam traps to be 0.047 $pN/nm$ and 0.034 $pN/nm$ respectively. As expected the power spectrum shows peaks at 70 $Hz$ and at its harmonics. The peaks may be observed because of leakage of s-polarized beam by PBS3 even in the absence of detection beam, however with the detection beam the peak gets accentuated by approximately 20 $dB$. The coupling between the two polarized beams can be further reduced by using special optical filters that block one polarization and allow the other to pass through.

## 6. CONCLUSION AND FUTURE WORK

This paper describes a new method of characterizing optical tweezer system by using non interference property of s and p polarizations of a linearly polarized laser beam thereby making the setup suitable for feedback applications by using same set of optics. This method will find merit in the investigation of biological samples as the detection beam has IR wavelength. The spatial resolution of 1.16 $nm$ of bead displacement measured with our setup compares well with the earlier methods. An analysis of the effect of the detection beam on the trap dynamics (within the linear region) is presented and verified experimentally. This results in a better position and force measurement when using a high power detection beam to increase sensitivity. The setup described is an open loop system with a sensing mechanism. Further work needs to be done on closing the loop by actuating the AOD, based on the fluctuations of the trapped bead measured by the sensor.

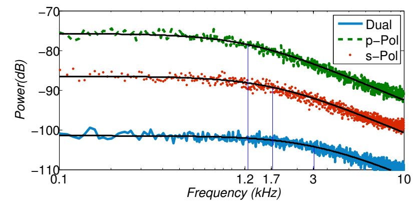


**Figure 5.** The figure above shows three power spectrums of the bead in a trap formed with both trapping and detection beams (dual), only detection (p-pol) and only trapping (s-pol) with cut off frequencies of 3.023 kHz, 1.247 kHz and 1.729 kHz resp. The plots are modified by adding constant gains to avoid overlapping.

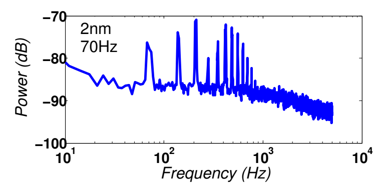


**Figure 6.** The figure shows power spectrum of fluctuations of a trapped bead when the trapping beam is oscillated about the detection beam spot with a triangular input of amplitude 2 nm at 70 Hz. The peaks in the spectrum are at 70 Hz and its higher harmonics indicating detection of 1.16 nm movement.

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