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A DUAL OPTICAL TWEEZER FOR MICRORHEOLOGY OF BACTERIAL SUSPENSIONS

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A dual optical tweezer has been built around an inverted microscope with high numerical aperture objective (N.A 1.4). The setup is versatile and can be used both as a single and a dual tweezer, and in the dual mode, enables us to optically trap two micron-sized latex beads within a few microns from each other in solution. Using this setup, we report measurements of the microrheological parameters of *Pseudomonas fluorescens* and *Bacillus subtilis* bacterial suspensions. We study the variation of viscoelastic moduli of these bacterial suspensions as a function of their cell count in solution. A comparison with inactive bacteria of corresponding cell count enables us to characterize the activity of the bacterial samples in terms of an average force that the bacteria exerts on the trapped bead. This work paves way for studies of interesting nonlinear rheological phenomena at small length scales.

Keywords: Microrheology; optical tweezer; active bacterial suspensions.

1. Introduction

In recent years there has been a growing interest in the behavior of microorganisms owing to their uses in various industrial processes in the food and beverage industry and in the production of ethanol, enzymes, antibiotics, and insecticides. Microrheology, the study of flow and deformation of a material under stress at the micro- and nano-regime, is a technique that can reveal the mechanical properties of active suspensions. Optical tweezers are highly sensitive instruments capable of measuring forces of the order of pN, and are ideally suited for extracting micro-rheological parameters of such suspensions, thus providing us an insight into the dynamics of bacterial activity.[1]

The mechanical properties of bacterial suspensions have been characterized by a variety of techniques. Al-Ashesh *et al.*, have shown that the apparent viscosity of a bacterial suspension increases with increasing biomass concentration and decreases with the increase of temperature by using a viscometer.[2] Wu *et al.* have shown an increase in the diffusion coefficient with increase in cell density using a novel three-dimensional defocused particle tracking method.[3] Soni *et al.* have explained the method of measuring the dynamic viscosity of self-propelled active particles using an intensity-modulated optical tweezer[4] while a violation of the fluctuation−dissipation theorem in active bacterial suspensions has been demonstrated by Chen *et al.*[5]

It is imperative that any process of measurement should inflict minimal damage to the bacterial cells under investigation. This, for instance, is not assured in the case of high shear rates in a viscometer.

Here we report the construction of an optical dual trap and a study of the microrheological behavior of two bacterial suspensions: *Pseudomonas fluorescens* and *Bacillus subtilis* at a temperature of $22 \pm 0.5°C$ using a single optical tweezer. These experiments serve as a first step and forerunner to further experiments to be performed in the dual trap mode.

One-point microrheology, using a single probe particle in a trap is a sensitive technique to characterize the local environment at micro- and nano-length scales and enables one to characterize local heterogeneities, if present, in the material. However, in the absence of such heterogeneities, or where one is more concerned with measuring the bulk limit of the viscoelastic parameters of the material, differences in these parameters arising due to local variations in structures may need to be averaged. In such situations it is more advantageous to monitor the cross-correlated mean-squared displacements of two beads.[6]

2. Experimental Details

2.1. *Construction of dual optical tweezer*

Figure 1 is a schematic of the optical dual trap built around an inverted microscope. It consists of a continuous wave Ytterbium fiber laser (1064 nm, 5 W, IPG, Germany) used for trapping micron-sized particles in a viscous fluid and a diode laser (980 nm, Thorlabs, USA) used for tracking the position of the trapped particle in two dimensions. The beams from these two lasers are merged into the same optical path using a dichroic mirror (DM1) and then split into two using a polarizing beam splitter (PBS1). The two split beams from PBS1 recombine at a second polarizing beam splitter PBS2 after undergoing reflections from a plane mirror (M1) and a $\theta_x \theta_y$ tilting mirror, respectively. A second dichroic mirror (DM2) reflects this beam toward a high numerical aperture oil-immersion microscope objective (1.4 NA, Olympus, Japan), which strongly focuses the beams to form two optical traps. The sample is placed in a sample holder made by a rubber "O" ring on a clean cover-slip mounted on a nanometer precision three-axis (XYZ) piezoelectric transducer stage (TRITOR 102 SG,
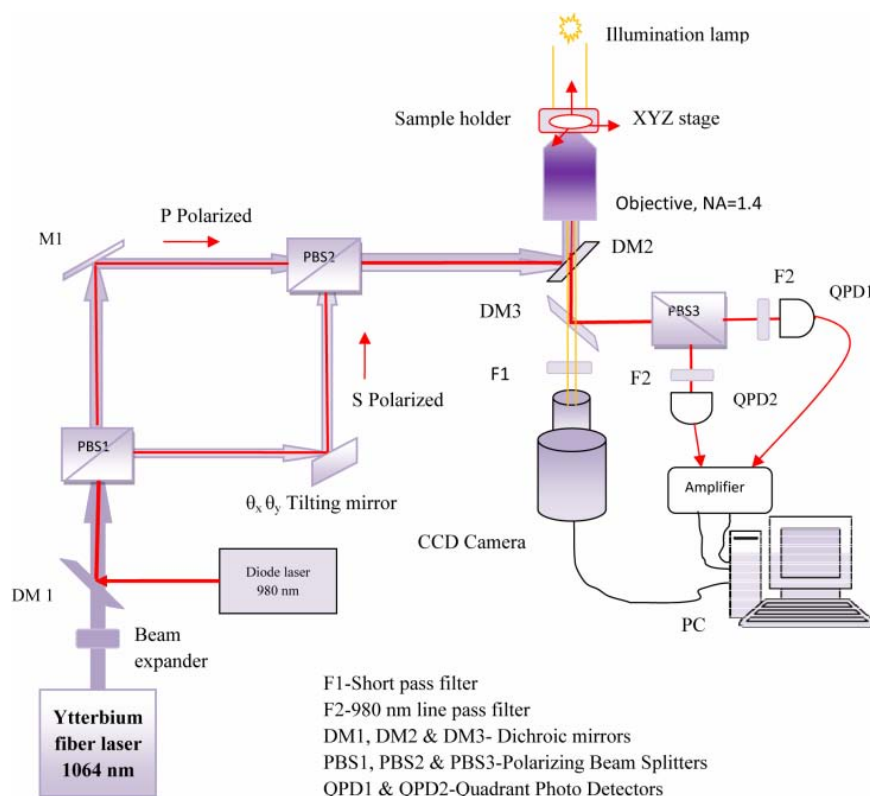

Fig. 1.   Dual optical trap — A schematic view.

Piezosystem Jena, GmbH, Germany). The back-scattered laser beam (980 nm) from the two independently trapped beads is reflected on a third polarizing beam splitter (PBS3). The resulting separated S-polarized and P-polarized laser beams are then incident on two separate Quadrant Photo Detectors (EOS, USA), QPD1 and QPD2 fixed on *XY* stages. These detectors record the position information of the two trapped beads. Data acquisition is through an eight-channel, 16-bit, 250 kS/s/ch DAQ card (PCI 6143, NI, USA). There is a provision for video-imaging by a high-speed CCD camera (200 frames/s, Voltrium, Singapore) and fluorescence imaging by yet another CCD camera (CoolSNAP.EZ, Princeton Instruments, USA) (not shown in figure).

The entire setup is built on a vibration isolation optical table (TMC, USA). All optics used here are from Thorlabs, USA, and Casix Inc., China while the optomechanical components are from Holmarc, India.

2.2. *Preparation of bacterial suspensions*

Bacillus subtilis is a beta-hemolytic Gram-positive bacterium, and includes both free-living and pathogenic species. *Pseudomonas fluorescens*, a Gram-negative bacterium belongs to the family of pseudomonads and is an opportunistic human pathogen.

Overnight cultures were diluted in sterilized nutrient broth, which is a mixture of 3.0 g beef extract, 5.0 g peptone, 5.0 g NaCl, and 1 L distilled water. The cultures in the nutrient broth were then grown at 37°C until the desired cell counts were achieved. The experiment was performed immediately following the culture growth for active cell studies. To the well-grown cultures, about $1-2 \mu L$ of $3 \mu m$ polystyrene beads were added. During the measurements care was taken to maintain a constant distance of $30 \mu m$ between trapped bead and cover-slip to avoid surface effects.

Next, the well-grown cultures were made inactive by UV treatment for 45 min duration. The inactive/dead cultures were subcultured in a new nutrient broth medium at 37°C for 36 h to confirm that no viable organisms remained in the sample. These cells are used for comparison with the active cell cultures.

3. Theory

To characterize the bacterial activity, we monitor the changes in the power spectral density of a laser-trapped bead in the presence of an active bacterial suspension.

The Langevin equation for the motion of a bead in an optical trap and in the presence of fluctuating forces may be written, in the limit of low Reynolds number, as

$$\gamma \frac{dx}{dt} + kx = F(t), \tag{1}$$

where $\gamma = 6\pi\eta a$ is Stoke's drag coefficient, η is the viscosity of the medium, and "a" is the radius of the bead. $F(t) = F_s(t) + F_B(t)$, $F_s(t)$ is the ideal thermal noise and $F_B(t)$ is the force due to bacterial activity. $k = 2\pi\gamma f_c$ is the trap stiffness and is proportional to the corner frequency f_c of the optical trap.

$F_S(t)$ and $F_B(t)$ have a time average of zero. Taking the Fourier transform of Eq. (1),

$$\gamma(2\pi i f)x(f) + kx(f) = F_s(f) + F_B(f), \tag{2}$$

where $x(f) = \int_{-t/2}^{t/2} x(t')e^{2\pi i f t'} dt'$.

Taking the complex conjugate of Eq. (2) and multiplying by the original, gives

$$x^2(f) = \frac{|F_s(f) + F_B(f)|^2}{4\pi^2\gamma^2(f_c^2 + f^2)}. \tag{3}$$

The quantity $x^2(f)$ is the two-sided power spectrum of the random Brownian motion of the particle.

The one-sided power spectral density (PSD) can be written as

$$S_x(f) = x^2(f) + x^2(-f) = 2x^2(f) \quad \text{for } 0 < f < x. \tag{4}$$

In Eq. (3) the time average of $|F_s|^2$ is given by

$$|F_s|^2 = 2\gamma k_B T,$$

where k_B is the Boltzmann constant and T is the solution temperature.

Equation (4) gives us the PSD of the bead:

$$S_x(f) = \frac{(\sqrt{2\gamma k_B T} + F_B(f))^2}{2\pi^2\gamma^2(f_c^2 + f^2)}. \tag{5}$$

This is the expression for PSD in presence of bacterial force and this force along with the corner frequency or "knee" of the PSD can be extracted by fitting the data using Eq. (5). We assume that the bacterial activity at low concentrations does not modify appreciably the viscosity η of the solution.[4] In fitting Eq. (5) to the data (Fig. 2), we restrict the upper frequency to about 30 Hz, since the high-frequency data corresponds to "free" bead's micromotion, where the bacterial "kicks" do not come into play.

The microrheological parameters of the material viz., storage $G'(f)$ and loss $G''(f)$ moduli are calculated following standard techniques.[7,8]

4. Results and Discussion

We have recorded position information data of a thermally fluctuating trapped bead at a scan rate of 20 kHz. For each set of data, PSD is calculated.[7,8]

Figure 2 shows the PSD values of the trapped particles embedded in (a) Bacillus subtilis-active cells suspension and (b) Pseudomonas fluorescens-active cells suspension at different cell counts. The data shown in this plot for both samples are a normalized average of 25 data sets collected at the same temperature and the same environment.

As shown in Fig. 2, as the cell counts of active cells increase there is a slight elevation in the PSD values. Figure 3 shows fits to the data following Eq. (5). Table 1 lists the values of the corner frequencies and corresponding bacterial force exerted on the bead at different cell counts.
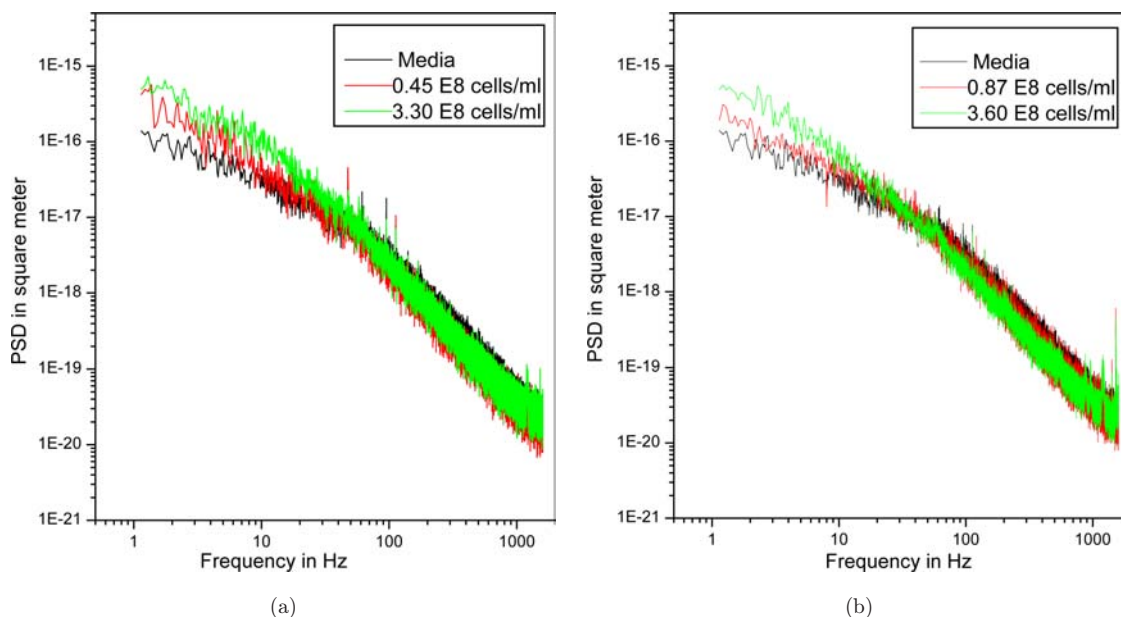

(a)   (b)

Fig. 2.   PSD data in (a) *Bacillus subtilis* and (b) *Pseudomonas fluorescens* active suspensions.

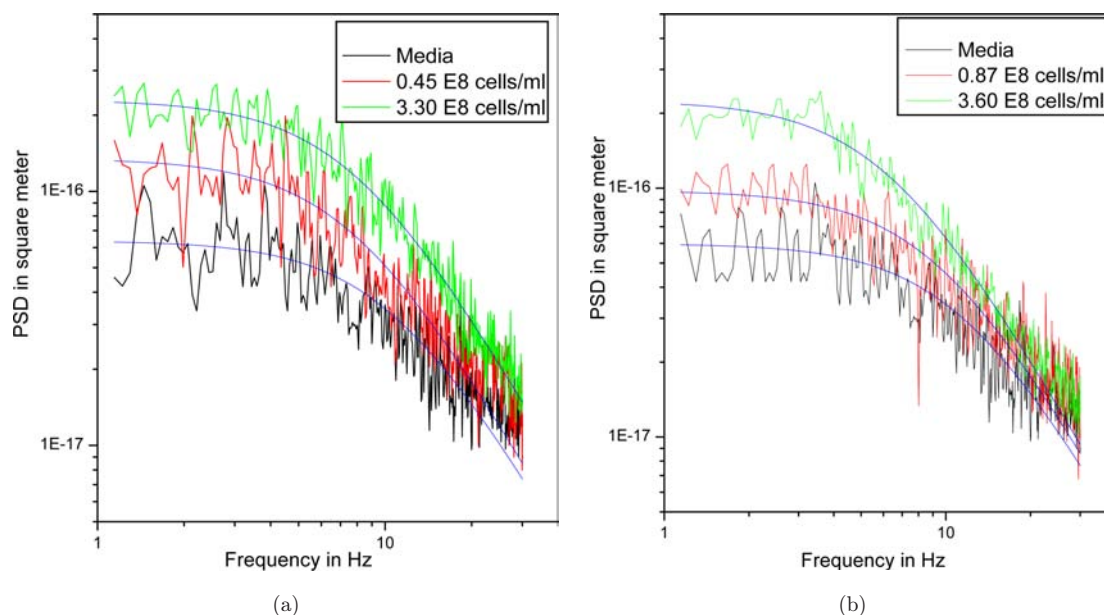(a)   (b)

Fig. 3.   Fits of the above PSD data using Eq. (5).

Table 1.   Corner frequency and bacterial force as a function of cell counts.

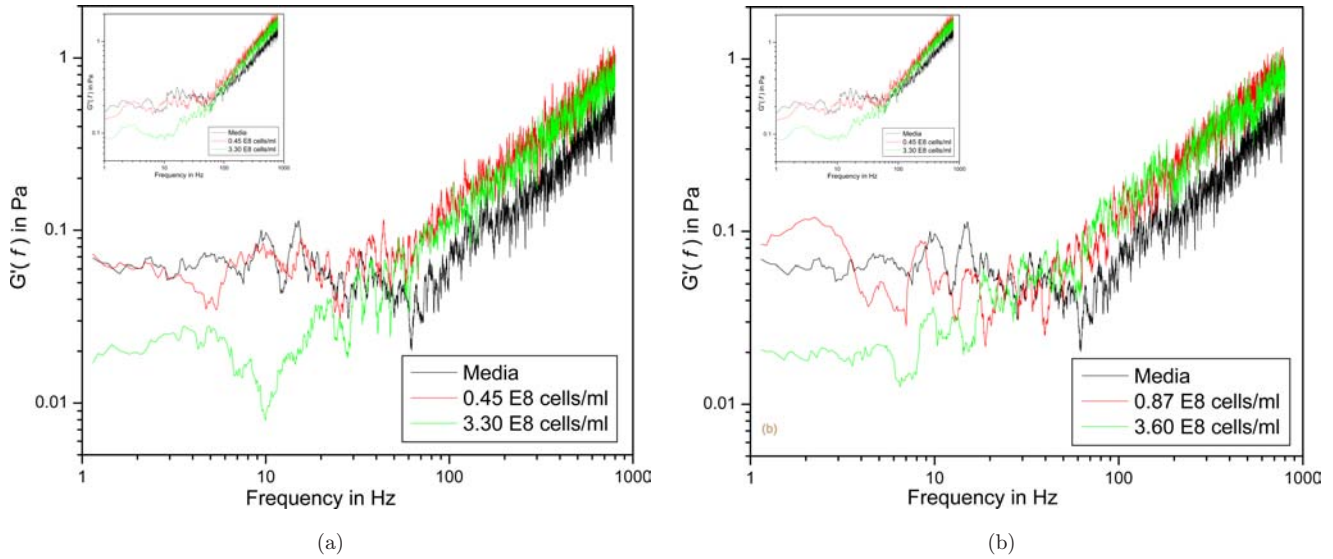| Name of the bacteria and their cell counts in the media (Nutrient broth)		Corner frequency in Hz	Bacterial force in fN
Media with zero cell counts		10.85	—
Bacillus subtilis	0.45×10^8 cells/ml	7.87	5.03
	3.30×10^8 cells/ml	7.78	14.45
Pseudomonas fluorescens	0.87×10^8 cells/ml	9.32	5.61
	3.60×10^8 cells/ml	6.24	6.09

(a) (b)

Fig. 4.   Storage and loss moduli (inset) of active (a) *Bacillus subtilis* and (b) *Pseudomonas fluorescens* suspensions.
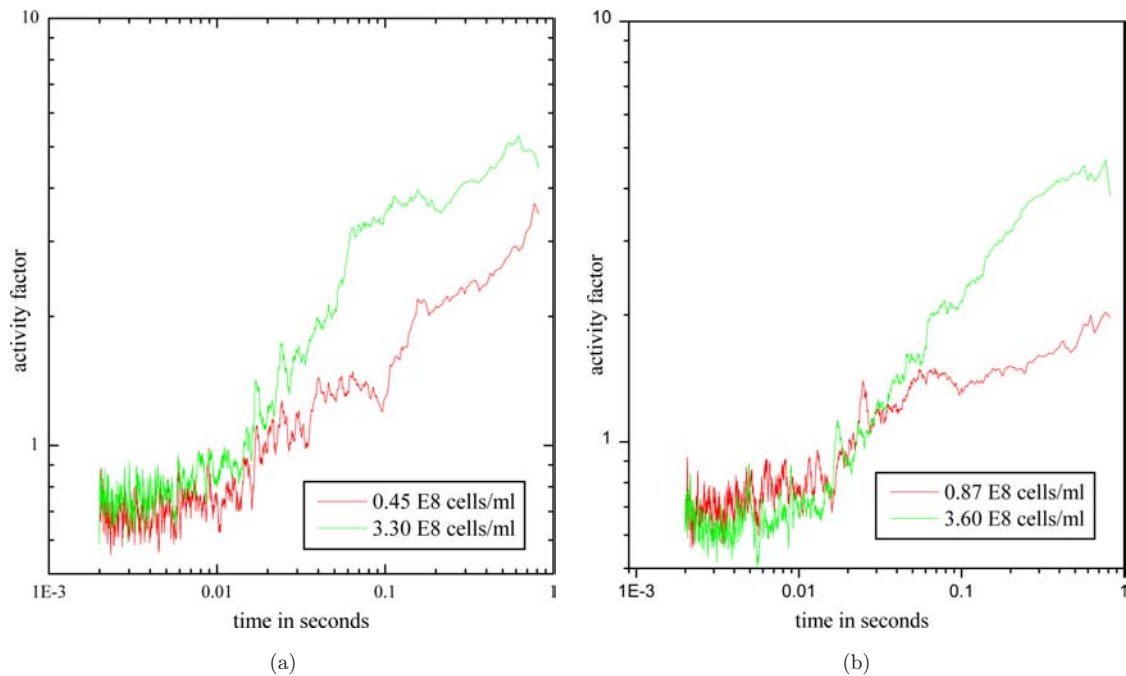

(a) (b)

Fig. 5.   Activity factors of active (a) *Bacillus subtilis* and (b) *Pseudomonas fluorescens* suspensions.

It is observed that f_c decreases with increase in bacterial cell concentration. We have verified that this activity increase indicated by the rise in the value of PSD at lower frequencies is clearly above the background (medium).

The results of storage moduli and loss moduli are shown in Fig. 4. The storage moduli values plotted have been corrected for trap stiffness.[8] The data below 10 Hz show little variation with frequency, perhaps due to Fourier transform of limited data sets. Above 10 Hz both moduli increase with frequency with a slope of 0.7, a signature of the viscoelasticity of the suspension.

Further investigations involving comparisons with the values for the nutrient broth alone as well as in the presence of inactive bacteria are currently under way. Our measurements here are restricted to 1000 Hz as the noise increases beyond this frequency.

Bacterial activity at different concentrations is characterized by defining an activity factor, viz., the ratio of PSD of the bead in the presence of active bacterial cells to the PSD of the bead in the presence of inactive bacterial cells at the same cell count. Figure 5 shows this activity factor as a function of the time period of fluctuations in the bead's position. As can be seen in Fig. 5, the bacterial force on the bead is felt at fluctuation time scales of 0.1−1 s. It also indicates that *Bacillus subtilis* shows greater activity factor than *Pseudomonas fluorescens.*

Acknowledgment

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