Accurate Estimation of Refractive Indices of Organic Microparticles in Dual-Beam Optical Trap

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Abstract— **The Refractive Index (RI) is an important parameter of characterizing optical properties of particles. In a dual-beam optical trap, two counter-propagating laser beams are used to trap micro-particles suspended in an aqueous medium. When a ray of light passes from one medium of lower RI (e.g. aqueous suspension medium) to another medium of higher RI (e.g. suspended particle), its momentum changes which exerts a proportional trapping force on the surface of the particle. Thus, accurate knowledge of RI of the particles and the surrounding medium is needed to determine the behavior of particles in an optical trap. The RI of micro-sized beads can be experimentally measured using traditional optical methods such as absorption microscopy. We developed an alternative theoretical method to estimate the RI of trapped particles based on non-contact optical trapping experimental outcomes. In our study, a theoretical model was formulated based on the experimentally measured minimum trapping powers for polystyrene and polyethylene beads using a dual-beam optical setup. The tendencies of trapping power-RI curves predicted by our model agreed very well with those measured experimentally. Our technique provides an alternative approach to determining the RI of a certain micro-size particle regardless of its size or density. Our method is especially advantageous over traditional methods to determine RI of biological particles which exhibit significant variations based on physiological and environmental conditions.**

Index Terms— **Optical trapping, refractive index estimation, dual-beam optical tweezers, mathematical modeling**

I. INTRODUCTION

Optical parameters of biological cells such as absorption, reflection and refraction can determine how light propagates in cells. For characterizing biophysical and mechanical properties of cells, one of the most important parameters is cell's refractive index (RI). It has been reported that precise information about cell's RI can help to characterize important properties of the cell such as mass, constituents' expressions, membrane elasticity, density, etc. [1]. Recently, RI of cells was accurately estimated using digital holographic microscopy while performing optical diffraction tomography for a pollen grain [2]. Several other models of cell RI have been developed in the last several years, which include (1) the average RI of a cell population suspended in a medium; (2) the effective RI of a single cell; and (3) the 2D and 3D RIs of a single cell. The effective RI of a single cell is more precise as compared to the average RI of a cell population. However, neither of the models is sufficient in providing enough information for biological applications with only a single RI value to represent a cell. More sophisticated and complex optical systems have been developed to measure the 2D RI profile in a surface layer and, more recently, the 3D RI profile of a single cell [3]. Both models provide more in-depth RI information down to a sub-micron resolution. With such in-depth and precise RI information, biologists and biomedical researchers can perform advanced biophysical research in order to obtain vital insights into the mechanisms and diagnosis of diseases.

In this study we used a dual-beam optical trapping (OT) setup to estimate the RI of optically trapped microparticles based on minimum trapping power measurements. In OT the small particles in the light path stay still and are manipulated by laser with no contact or support by the radiation pressure of a focused laser beam [4]. In our study we implemented a dual beam OT using two counter propagating, diverging, and identical laser beams to trap or even stretch microscopic objects [5]. As light travels from one medium to another of different RI, it changes its velocity and direction. This change is accompanied by a subsequent momentum change of the light at the interface of the two media which results into a proportional force. As the light moves from an optically dense medium to an optically denser medium (e.g. trapped particle) the surface of the optically denser medium gains momentum in the opposite direction of the light propagation. As the light exits the optically denser medium, the surface gains momentum in the direction of light propagation. In this situation the overall net force on the particle points in the direction of light propagation [6]. If a second identical counterpropagating light beam is used, these forces interact with each other resulting into a trap. The efficiency of such a trap depends on the power of the lasers, the wavelength of light used, the size of the particle and the relative refractive indices of the two media under consideration. Recently, OT has found widespread applications in studying the mechanoelastic properties [7] of biological cells which can be used in applications such as cell sorting [1], cell fusion, cell characterization [8], and disease identification [9].

Our goal of this study is to build a theoretical model formulated based on the experimentally measured minimum trapping powers for polystyrene beads of specific diameter (15μm) using a dual-beam optical setup. Then to match the tendencies of trapping power-RI curves predicted by our model with those measured experimentally for polyethylene beads. Our method provides an alternative non-toxic and label-free approach to determining the RI of micro-size particles regardless of their size or density.

II. METHODS

A. Theoretical Formulation

We use ray optics approach to clearly describe the forces generated from a single beam. This approach is valid only

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when the size of the object under consideration is large compared to the wavelength of light used for trapping. Consider a ray of light traveling in a medium with RI of n_1 is incident on a spherical microparticle, with a higher RI of n_2, in the light path. Once hitting the particle, some of the light is reflected back while some of it is transmitted through (see Fig. 1). Once exiting the particle, some of the ray is transmitted to medium with RI of n_1 while some is reflected back depending on the particle's RI.

Photons contain momentum P which changes with direction or velocity variations. The ray's direction and velocity change when passing from one medium to another medium with different RI. This change in velocity and direction causes a change in the momentum of the photons at the interface. However, due to the law of conservation of momentum, part of the momentum is transmitted to the object while some has to be reflected. According to Newton's second law of motion, the momentum change causes a force, which points away from the object while the surface gains momentum in the direction of light propagation. The momentum P will cause a force in the backward direction,

$$P = \frac{n \cdot h}{\lambda} \quad \textbf{(1)}$$

where n is the effective RI, h is the Plank's constant and λ is the wavelength of light. As the light is incident on the particle surface, the change in momentum, ΔP, can be found by computing the incident momentum (P_i), the reflected momentum (P_r) and the transmitted one (P_t) as:

$$\Delta P = P_i - (P_r + P_t), \quad \textbf{(2)}$$

As the light beam exits the object, it will again lose momentum. This is because there is a drop in the velocity of light and scattering at the interface of the two media due to change in the RI. Since there is the conservation of momentum, the surface will gain a proportional momentum. A net force F is exerted on the surface that points towards the direction of propagation.

$$F = m \cdot a = \frac{m \cdot \Delta V}{t} = \frac{\Delta P}{t} \quad \textbf{(3)}$$

This net force is derived based on the fact that the reflected momentum is negligible compared to the transmitted momentum [2]. In order to satisfy this assumption, the RI of the object should be larger than that of the surrounding medium to confine the beam within a proper angle. The force will produce stress σ on the cell [10] as given by:

$$\sigma = \frac{\Delta P}{A \cdot \Delta t} = \frac{P_i - (P_r + P_t)}{A \cdot \Delta t}$$

$$= \frac{n_1 \cdot}{c} \frac{W}{A}\left[\overrightarrow{a_i} - \left(\frac{n_2}{n_1} T\overrightarrow{a_t} + R a_r\right)\right] = \frac{n_1 \cdot W}{c \cdot A} \overrightarrow{Q} = \frac{F}{A}$$

$$P = \frac{F \cdot c}{n_1 \cdot Q} \quad \textbf{(4)}$$

where W is the power of the light, c is the speed of light, A is the area of the particle illuminated by light, Q is momentum transfer vector, or the trapping efficiency.

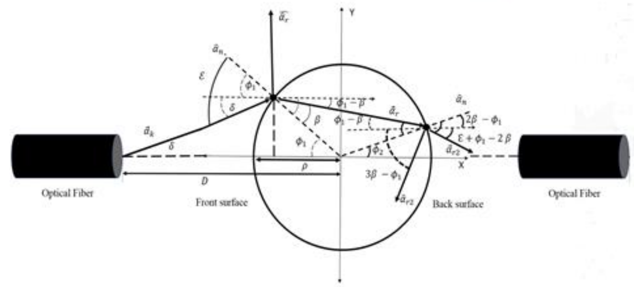

Figure 1: Representation of incident, reflected and transmitted rays on a spherical object trapped in the path of two counter-propagating beams carried by optical fibers. Note that for simplicity the rays are shown only for one beam.

The reflection and transmission coefficients will decide the trapping efficiency, i.e. how efficient the particle can be trapped by the OT. The RIs of the medium and the particle influence the incidence, refraction and transmittance angles (see Fig. 1).

The expressions for the components of Q on the front and back surfaces are given by:

$$Q_{front\,X} = [\cos(\delta) - n \cdot T(\varepsilon) \cdot \cos(\phi_1 - \beta) + R(\varepsilon) \cdot \cos(2\varepsilon - \delta)], \quad \textbf{(5)}$$

$$Q_{front\,Y} = [\sin(\delta) - n \cdot T(\varepsilon) \cdot \sin(\phi_1 - \beta) + R(\varepsilon) \cdot \sin(2\varepsilon - \delta)]. \quad \textbf{(6)}$$

$$Q_{back\,X} = T(\varepsilon) \cdot \left[n \cdot \cos \begin{matrix} (\phi_1 - \beta) + n \cdot R(\beta) \cdot \cos(3\beta - \phi_1) \\ +T(\beta) \cdot \cos(\varepsilon + \phi_1 - 2\beta) \end{matrix}\right], \quad \textbf{(7)}$$

$$Q_{back\,Y} = T(\varepsilon) \cdot \left[n \cdot \sin(\phi_1 - \beta) \begin{matrix} +n \cdot R(\beta) \cdot \sin(3\beta - \phi_1) \\ +T(\beta) \cdot \sin(\varepsilon + \phi_1 - 2\beta) \end{matrix}\right]. \quad \textbf{(8)}$$

Where R is the reflection coefficient and T is the transmission coefficient, The angles of incidence, reflection and refraction at various interfaces are shown in Fig. 1.

For simplicity we neglected the Gaussian beam correction from Reference [2] and assumed a ray optics paradigm. For example, we set NA=0.11, n_1=1.335, n_2=1.37 and D=39.9um. In [2] it was shown that the larger the beam-particle ratio, the smaller the emitting angle it produces. When the particle is small enough or the beam is close to a plane wave, the emitting angles get the smallest number of each incidence.

By using Eqns. (5) through (8), the expression for total Q is

$$Q_{tot} = Q_{front} + Q_{back}, \quad \textbf{(9)}$$

$$Q_{tot} = \sqrt{\left(Q_{front\,X}(\phi_1)\right)^2 + \left(Q_{front\,Y}(\phi_1)\right)^2} + \sqrt{\left(Q_{back\,X}(\phi_2)\right)^2 + \left(Q_{back\,Y}(\phi_2)\right)^2} \quad \textbf{(10)}$$

To determine the ratio of the incident beam that is either reflected from the particle or transmitted to the particle, the optical properties of the particle are considered. For our study, we assumed all the incident and emitting angles as zero because our setup uses two counter-propagating beams. So, the simplified Q is given as,

$$Q = [n + Rn - (1 - R)](1 - R) - [1 + R - n(1 - R)] \quad \textbf{(11)}$$

n in the above equation is the ratio of the RI of particle and the RI of the surrounding solution. Thus, if we know the Q number and the RI of the solution, we can theoretically predict the RI of the particle. The trapping power p_{tr} can be represented as

$$p_{tr} = \frac{6\pi r v \mu}{1-\frac{9}{16}\left(\frac{r}{d}\right)+\frac{1}{8}\left(\frac{r}{d}\right)^3-\frac{45}{256}\left(\frac{r}{d}\right)^4-\frac{1}{16}\left(\frac{r}{d}\right)^5} \quad \textbf{(12)}$$

where d is the distance between the particle and the substrate. r and v are the radius and velocity of the bead, respectively. μ is the viscosity of the medium.

$$\nu = \frac{\mu}{\rho} \quad \textbf{(13)}$$

Where ν is the kinematic viscosity (momentum diffusivity) and ρ is the density of the medium. So far it is clear that the minimum trapping power p_{tr} in equation (12) is mainly a function of medium density and relative RI, assuming that the particle radius, particle velocity and kinematic viscosity are known. Thus, by measuring the minimum trapping power p_{tr}, we can determine the precise RI of the trapped particle under certain circumstances.

B. *Experimental setup*

We used a dual-beam OT setup to trap the polystyrene beads and polyethylene beads of certain size (15 μm diameter). This setup utilizes two multi-axis positioning stages to position the optical fibers directly counter-propagating from one another with < 5 μm accuracy in the XY plane as seen under the KH 1300 Microscope as shown in Fig. 2. Two high power laser sources (975 nm wavelength) were used to derive the optical beams through each fiber. An optical power meter with an accompanying photodetector was used to measure the output power of cleaved and polished optical fibers before use. The sample stage along with fiber ends was observable on screen through a digital microscope image acquisition.

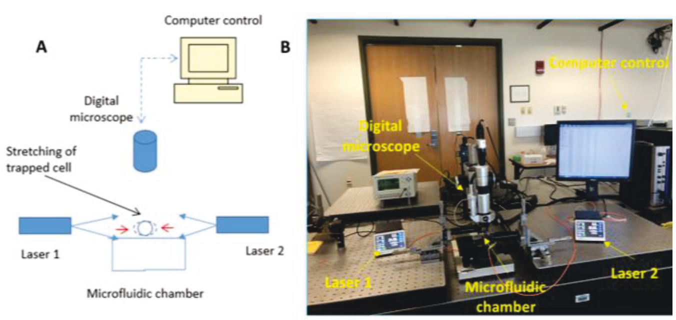

Figure 2: The experimental setup for the optical trapping experiments.

As discussed in the previous section, the minimum trapping power is only dependent on the RI and the density of the medium. Therefore, we used four different types of solutions to investigate how the minimum trapping power of the 15 µm polystyrene beads changes with the RI and density of the surrounding medium. Fig. 3 shows the RI vs. density plots for four different types of solutions: NH_4Cl, sucrose, NaCl and Na_2CO_3. Among these, the NH_4Cl has the largest slope which means its RI changes the fastest with density, whereas Na_2CO_3 has the smallest slope which indicates that its RI changes the slowest with density. Based on these plots, three sets of experiments were designed: 1) using the four solutions

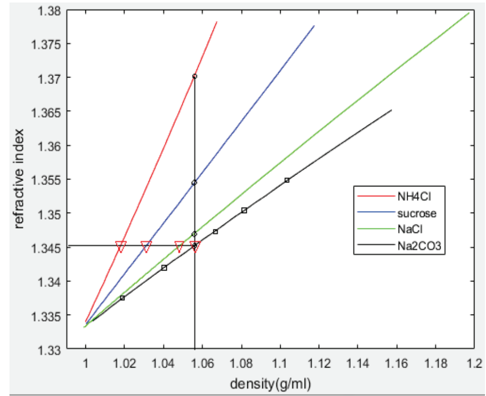

Figure 3. Plots of RI-density relations for 4 solutions used in trapping experiments

types with the same RI values, but different densities (shown by triangles in Fig. 3), 2) using the four solution types with the same density values, but different RIs (shown by the circles in Fig. 3), and 3) using the Na_2CO_3 solutions with different values of density and RI (shown by diamonds in Fig.3). In each case, minimum trapping powers were measured by trapping the 15 µm polystyrene beads in our dual beam OT setup described before. To validate our results, we performed similar set of experiments using polyethylene beads of same size (15 µm), but different RI (1.49). In each experiment, the beads were trapped at higher power and then gradually the power was decreased until the beads lose the trap, which is recorded as the minimum trapping power.

III. Results & Discussion

Figure 4A shows a comparison of theoretically calculated (blue) and experimentally measured (yellow) minimum trapping powers for polystyrene beads suspended in the four solutions exhibiting different RIs but the same density. It can be observed that the minimum trapping power increases monotonically with the increase in RI of the medium at the same density of 1.0556g/ml. The theoretical calculations and experimentally measured powers match with each other remarkably. This implies that the minimum trapping power is a function of medium's RI and we can predict the RI with measured minimum trapping power if the density of the medium is known.

Figure 4B shows similar comparison of minimum trapping powers for solutions with the same RI but different densities. As expected, the minimum trapping power increases monotonically with the increase in density at the same RI of 1.3453. Consequently, the minimum trapping power is a function of medium's density and one can predict either the RI or density with measured minimum trapping power if the other quantity is known.

From Figure 4C we can observe that the minimum trapping power increases monotonically with the increase of RI of medium (Na_2CO_3) with varying density theoretically and experimentally. It means that the minimum trapping power is a function of both, medium's RI and density. It is worth noting that in all the three cases, the experimental results are in remarkable agreement with the theoretical predictions. Above all, if we know any three parameters out of the four—

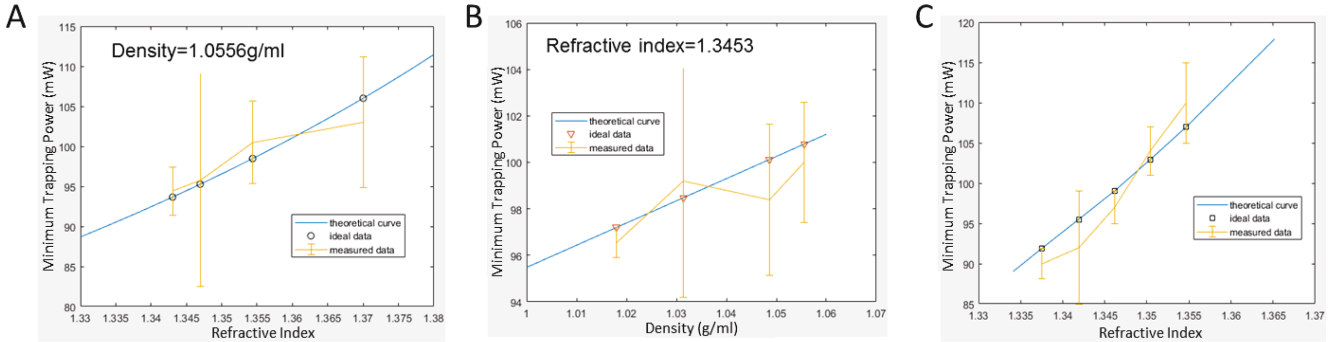

Figure 4 Experimental (yellow) and theoretical (blue) plots of A) minimum trapping power vs. RI for a constant medium density, B) minimum trapping power vs. density for a constant RI of medium, and C) minimum trapping power vs. RI for varying densities.(n=5 for each plot).

minimum trapping power, density of the medium, solution type, and RI of the medium, we can easily predict the fourth.

To validate our formulation, we replaced polystyrene beads with polyethylene beads to repeat the experiment but in a inversed way: measuring the minimum trapping power and predicting the RI using our model formulation. The RI of polyethylene bead is 1.49 which is close to polystyrene's 1.5732, so it makes the experiment practical and comparable. By measuring the minimum trapping power in experiments, we can inversely calculate the relative RI or RI of the medium since the RI of the bead is known and given the minimum

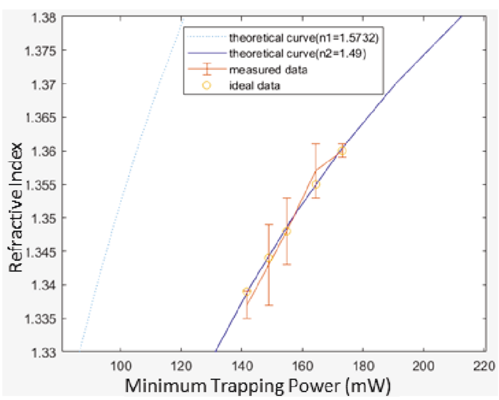

Figure 5. Theoretical (blue) and experimental (red) plots of minimum trapping power vs. effective RI for polyethylene beads (n=5).

trapping power we can also inversely calculate the density of the medium. In Fig. 5, the dotted line is the theoretical prediction of the relation between RI and the minimum trapping power of the polystyrene beads. The solid line is the theoretical prediction of the relation between the RI and the minimum trapping power of the polyethylene bead. The RI of the medium and the density of the medium varies together proportionally given the same type of chemical (Na_2CO_3) so it makes it possible to study the relation between density, RI and the minimum trapping power. As we can predict from the model, the minimum trapping power is higher in the same trapping medium for the polyethylene beads because the RI and density are lower which makes them more difficult to trap. Fig. 5 shows that the experimental data is in remarkable agreement with the model predictions. More importantly, these experimental results using polyethylene beads validate our theoretical formulation. The model can be used to accurately predict the RI of any microparticles trapped in a dual-beam OT. As such, our theoretical model offers quick, reliable and affordable means to accurately estimate the RI of biological cells through optical trapping experiments.

IV. Conclusion

We worked out a theoretical model to accurately predict the refractive index of optically trapped particle in a dual-beam optical trap based on the minimum trapping power and known refractive index or density of the medium. The predicted data points of the minimum trapping power and RI or density match remarkably well with the experimental results using polystyrene beads. The minimum trapping power is mainly a function of the density of the medium and both the RIs of the medium and that of the trapped particles. The model was validated by another set of experiments using polyethylene beads in which the model predicted the RI/density of medium accurately based on the known RI of the beads. This study can be extended to precisely estimate the RIs of biological cells which has been a challenge. Our model provides a convenient and affordable means to characterize microparticles in OT based on their RI profiling.

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