The Effect of Immersion Oil in the Optical Tweezers

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In this paper, we present a theoretical study on the effect of refractive index of immersion oil on the position of optimal depth and optical trap quality in optical tweezers. Using simple numerical calculation presented here, one can study the optical trapping in a realistic setup. The electric field and intensity distribution in sample medium is derived. Our calculations is in very good agreement with the previous reported experimental results.

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1. Introduction

Optical Tweezers are used as micromanipulation tools in many scientific areas, from biology[1–4] to nanotechnology[5–8]. A typical Optical tweezer (OT) consists of a Gaussian laser beam tightly focused through a high Numerical Aperture (NA) objective lens producing a 3-D Gaussian intensity profile at the focus. An object with the refractive index greater than that of the surrounding medium experiences a Hookean force towards the focus[9]. The strength of the trap can be regarded as the spring constant. OTs are widely used as non-contact micromanipulator using a micron (and nano)-sized object as a handle. For biological applications a near-infra-red laser beam is hired to minimize the damage to the sample [3]. Nanometer spacial resolution along with sub-Megahertz temporal resolution have turned OT to a widely desired tool in many scientific communities. OT are normally implemented into an optical microscope in order to visualize the specimen under manipulation. It is experimentally shown that refractive index discontinuity in the optical pathway of OT will introduce considerable amount of spherical aberrations. To compensate for such an aberration, different methods are proposed[16–19] among which the changing the refractive index of the immersion medium[17] seems to be more feasible. In this letter we present a theoretical calculation which gives rise to the refractive index of the immersion medium at which the optimal trap occurs. Trapping in water and air are considered as examples. The theoretical results are in very good agreement with the previously available experimental results.

2. The effect of immersion oil on the intensity distribution in focal region

If the image space in an aplanatic system is homogeneous, then electric field at point P located around the focus(origin located at the focus) can be written as[10]:

\[ \vec{E}(P) = -\frac{ik_1}{2\pi} \int \int_{\Omega} \frac{\vec{a}(s_x, s_y)}{s_z} \exp \left( ik[\Phi(s_x, s_y) + \vec{s}.\vec{r}_P] \right) ds_1 dx_2 ds_1y \]

(1)

Where \( k \) is wavenumber, \( \vec{s} = s_x \hat{i} + s_y \hat{j} + s_z \hat{k} \) is a unit vector along a typical ray, \( \Omega \) denotes the lens aperture, \( \vec{r}_P \) is position vector of point P, \( \Phi \) is aberration function of the lens and finally \( \vec{a} \) is the electric strength vector. A similar equation can be written for magnetic field. It can be shown that if there has been a refractive index mismatch in image space, namely we have a planar interface between two media with refractive indices \( n_1 \) and \( n_2 \), then assuming the lens is aberration free(or has a constant aberration) the electric field on this
boundary \(z = -z_f\) and on the side of second medium can be written as\cite{14}:

\[
\vec{E}_2(x, y, -z_I) = -\frac{i k_1}{2\pi} \int_{\Omega} \int \mathbf{T}^{1\rightarrow 2} \mathbf{W}_e(s_1) \exp \left( i k_1 (s_{1x} x + s_{1y} y - s_{1z} z_I) \right) ds_{1x} ds_{1y}
\]  \tag{2}

Where \(k_1\) is wavenumber in the first medium, \(\Omega\) is denoting the surface of objective lens, \(\mathbf{W}_e = \frac{\vec{\alpha}(s_{1x}, s_{1y})}{s_{1z}}\) and \(\mathbf{T}^{1\rightarrow 2}\) is an operator that describes the changes in electric field on crossing the boundary and it is a function of \(n_1, n_2\) and incidence and refraction angles at this interface\cite{14}. Equation (2) can be extended to the general case when there are \(m\) different media with \(m-1\) interfaces. Assuming that the refractive indices of these media are \(n_1, \ldots, n_m\), and a linear polarized Gaussian beam \(\vec{E} = E_0 e^{-\rho^2/2w_0^2}\) \((w_0\) is the beam waist and \(\rho = \sqrt{x^2 + y^2}\)) incident on the front aperture of the objective lens, it can be shown that the electric field inside the \(m\)-th medium after transforming to spherical coordinates can be written as:

\[
\vec{E}_m(x, y, z) = -\frac{i k_1}{2\pi} E_0 \sqrt{\frac{n_1}{n_2}} \int_0^\alpha \int_0^{2\pi} \frac{E_{\text{sample}}}{s_{1z}} \exp \left( i k_0 [n_1(t_2 + t_3 + \ldots + t_m) \cos \phi_1 - n_2 t_2 \cos \phi_2 - \ldots - n_m t_m \cos \phi_m] \right) \exp [i n_m k_0 z \cos \phi_m] \exp [i n_1 k_0 \sin \phi_1 (x \cos \theta + y \sin \theta)] \sin \phi_1 \cos \phi_1^{1/2} d\theta d\phi_1
\]  \tag{3}

Where \(\alpha\) is the convergence angle of the objective given by the Numerical Aperture of the objective \((NA = n \sin \alpha, \) with \(n\) being the refractive index of the immersion medium), \(t_k (k = 2, 3, \ldots, m)\) is the thickness of \(k\)-th medium, and the angles \(\phi_i, (i = 2 \ldots m)\) are refraction angles in different media. \(E_{\text{sample}}\) is the electric field strength vector inside the sample given by:

\[
E_{\text{sample}, x} = \prod_{l=1}^{l=m-1} \tau_{pl} \cos^2 \theta \cos \phi_m + \prod_{h=1}^{h=m-1} \tau_{sh} \sin^2 \theta , E_{\text{sample}, y} = \prod_{l=1}^{l=m-1} \tau_{pl} \cos \theta \sin \theta \cos \phi_m - \prod_{h=1}^{h=m-1} \tau_{sh} \cos \theta \sin \theta \cos \phi_m \text{ and } E_{\text{sample}, z} = -\prod_{l=1}^{l=m-1} \tau_{pl} \cos \theta \sin \phi_m.
\]

Normally for optical trapping applications there would be 3 discontinuity in the refractive indices (\(m=4\), with media being the objective \((n_1 = n_{\text{obj}} = 1.518)\), immersion oil \((n_2 = n_{\text{imm}}, t_2 = Y, Y\) is variable), coverslip \((n_3 = n_g = 1.518, t_3 = 170\mu m)\), and sample \((n_4 = n_s, t_4 = d=\text{probe depth})\). Note that when the objective’s top lens, coverglass, and the immersion medium are index matched \((n_{\text{obj}} = n_g = 1.518)\), there would be only one interface (coverslip-water). Considering that the restoring force of optical tweezers is proportional to the intensity gradient\cite{20}, one can use equation 3 to find the optimal parameters (such as refractive index of the immersion medium\cite{?}) for a desired optical trapping experiment. In the following sections the results for the two most popular cases will be presented.
A. Trapping in water

For the popular case of trapping inside water using an oil immersion objective, one can consider $n_s = 1.33$, and $n_{imm} = 1.518$. Figure 1 shows the resulted axial intensity profiles at different depths (a) as well as the calculated average intensity gradients (b) for a 1$µm$ polystyrene bead trapped using an objective with NA=1.3 and working distance (W.D) of 200$µm$ through a coverglass of 170$µm$ thick.

![Intensity distributions in the axial direction for different immersion oils.](image)

Figure 1 illustrates that: (1) for $n = 1.518$, where the system is supposed to be aberration-free, the optimal trap occurs just in the vicinity of the coverglass inner surface. (2) The trapping strength significantly decreases as the trapping depth is increased. (3) By increasing the refractive index of the immersion medium, the optimal trapping depth (minimum spherical aberration) shifts towards the deeper axial positions. (4) The maximum trapping strength decreases slightly by increasing the $n_{im}$ which implies the slight increase in the residue of spherical aberration and reflection at boundaries. Table 1 quantitatively summarizes the optimal conditions using different immersion oils.

It can be deduced from table 1 that 0.01 increment in the refractive index of the immersion medium results in 3 – 3.5$µm$ shift for the optimal trapping depth which is in very good
Fig. 2. Intensity distributions in the lateral direction for different immersion oils.

Fig. 3. Average Intensity Gradient in the axial direction for different immersion oils, trapping medium is water.
Fig. 4. Average Intensity Gradient in the lateral direction for different immersion oils, trapping medium is water.

Table 1. The optimal trapping depth ($d_{\text{opt}}$), equivalent probe depth ($d$; distance traveled by the objective) and the focus shift ($\Delta f$) for trapping inside water using different immersion oils.

| $n_{\text{imm}}$ | 1.518 | 1.53 | 1.54 | 1.55 | 1.56 | 1.57 | 1.58 | 1.59 | 1.60 |
|------------------|-------|------|------|------|------|------|------|------|------|
| $d_{\text{opt}}$ ($\mu\text{m}$) | 0     | 3.91 | 7.31 | 9.9  | 12.85| 15.37| 17.77| 19.83| 26.49|
| $d$ ($\mu\text{m}$)     | 0     | 4.5  | 8.5  | 11.5 | 15.0 | 18.0 | 21.0 | 23.5 | 32.5 |
| $\Delta f$ ($\mu\text{m}$) | 0 | 0.59 | 1.19 | 1.60 | 2.15 | 2.63 | 3.23 | 3.67 | 6.01 |
| $d_{\text{opt}}$, lateral direction | 0 | 4.28 | 7.67 | 10.62| 13.50| 16.44| 19.19| 24.88| 27.21|
| $d$ ($\mu\text{m}$), lateral direction | 0 | 5.0  | 9.0  | 12.50| 16.0 | 19.5 | 23.0 | 30.5 | 33.5 |

agreement with the previously reported experimental results [17]. To estimate of the optimal conditions for the lateral trap, same calculations were repeated for the lateral intensity distributions. Figure 2, as an example, shows the lateral intensity distributions at different depths for $n_{\text{imm}} = 1.518$ and $n_{\text{imm}} = 1.58$. 
Fig. 5. Intensity distributions in the axial direction for three different immersion oils, trapping medium is air.

B. Trapping in Air

Optical tweezers have been used for trapping micro-objects in the air[? ]. Same calculation can be repeated using $m = 4$ and $n_{sample} = 1$ to find the optimal conditions for the aerosol trapping. It is worth mentioning that in this case, the total internal reflection may occur at the coverglass-air interface for some incident angles (marginal rays). Therefore, there would be an upper limit for the numerical aperture. Figure 3 shows typical intensity distributions in the axial directions using 3 different immersion oils.

Table 2 summarizes the calculation results for trapping in the air. It can be deduced that 0.01 increment in the refractive index of the immersion medium shifts the optimal trapping depth by $0.3\mu m$ which is very small compared to the previous case. Therefore, to get a reasonable shift in the optimal trapping depth higher refractive index liquid is required.

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Fig. 6. Intensity distributions in the lateral direction for three different immersion oils, trapping medium is air.

Table 2. The optimal trapping depth ($z_{opt}$), equivalent probe depth ($d$; distance traveled by the objective) and the focus shift ($\Delta f$) for trapping inside Air using different immersion oils.

| $n_{im}$ | 1.518 | 1.53 | 1.54 | 1.55 | 1.56 | 1.57 | 1.58 | 1.59 | 1.60 | 1.70 |
|----------|-------|------|------|------|------|------|------|------|------|------|
| $d_{opt}(\mu m)$ | 0 | 0.31 | 0.55 | 0.87 | 1.13 | 1.31 | 1.61 | 1.86 | 2.02 | 4.07 |
| $d(\mu m)$ | 0 | 0.6 | 1.0 | 1.6 | 2.0 | 2.2 | 2.8 | 3.2 | 3.4 | 6.4 |
| $\Delta f(\mu m)$ | 0 | 0.29 | 0.45 | 0.73 | 0.87 | 0.89 | 1.19 | 1.34 | 1.38 | 2.33 |
| $d_{opt, lateral direction}(\mu m)$ | 0 | 0.4 | 0.64 | 0.95 | 1.22 | 1.57 | 1.78 | 2.03 | 2.37 | 4.9 |
| $d(\mu m, lateral direction)$ | 0 | 0.8 | 1.2 | 1.8 | 2.2 | 2.8 | 3.2 | 3.6 | 4.2 | 8.4 |

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