Backfocal plane (BFP) interferometry [1,2] is a widely employed single particle tracking technique. It is typically combined with optical tweezers to precisely track three-dimensional displacements of the trapped particle from its equilibrium position. Many studies on single molecules utilize this technique, for example, to follow the transcription of DNA to RNA [3] as well as to show the stepping of molecular motors [4] or their mechanical properties [5].

The method relies on the coherent scattering of focused light at the particle to be tracked. The scattered light interferes with the unscattered excitation light and forms an intensity pattern that can be recorded to track the position of the scatterer. The interference pattern is observed in the BFP of a detection lens (DL), which forms a telescope with the focusing objective lens (OL).

A model for the formation of the tracking signals that encode the lateral displacement of a point scatterer perpendicular to the optical axis is given by Gittes and Schmidt [6]. Pralle et al. [1] present a model that also covers the formation of the axial tracking signal, which encodes the position of the scatterer along the optical axis. However, the model does not explain the dependency of the tracking sensitivity on the detection angle \( \theta \) observed in simulation and measurement [7–9].

Here, we use Fourier optics to develop a new model for the BFP interference pattern for the case of a point scatterer located on the optical axis, which helps to maximize the sensitivity of axial tracking. The model predicts a detection angle \( \theta_B \) at which the BFP intensity is independent of the particle position and thus explains the referenced observations.

The arrangement is sketched in Fig. 1. The focal electric field distribution \( E_{i2}(r) \) can be computed from the angular spectrum \( E_{i1}(k_{\perp}) = \text{FT}\{E_{i1}(q)\} \) of the incident plane wave \( E_{i1}(q) = i E_0 q \), which illuminates the BFP of the OL with a constant amplitude \( E_0 \). \( q \) and \( r \) designate the distance to the optical axis in the BFP and the FP, respectively. For scalar fields one can assume radial symmetry.

The focusing angle and thus the integration are limited by the numerical aperture \( NA_{OL} \) of the OL. Without loss of generality it is assumed that the OL and the DL have the same focal length \( f \).

The Airy disk \( E_{i2}(r) \) is obtained by rescaling \( E_{i1}(k_{\perp}) \) according to [10].

\[
E_{i2}(r) = \frac{1}{\lambda_0 f} E_{i1}(k_{\perp} = r k_0 / f), \tag{2}
\]

\[
= E_0 f NA_{OL} \frac{J_1(NA_{OL}k_0 r)}{r}, \tag{3}
\]

where \( k_0 = 2\pi / \lambda_0 \) is the wavenumber of the incident light with vacuum wavelength \( \lambda_0 \).

A second application of the described formalism yields the field \( E_{i3}(q) = -i E_0 \) in the BFP of the DL. Note that
the phase of $E_{\perp}(r)$ is zero and the phase of $E_{\parallel}(q)$ is $-\pi/2$ in agreement with the phase anomaly of focused light affecting $E_{\parallel}(q)$ and $E_{\perp}(q)$.

We use a Gaussian beam approximation \[11\] for $E_{\perp}(r)$ to estimate the phase of the focal field distribution along the optical axis. The Gaussian beam $E_{\parallel}(r, z)$ reads

$$E_{\parallel}(r, z = 0) = E_{\parallel}(r = 0)e^{-\left(\frac{z}{\omega_0}\right)^2} = E_{\parallel}(r). \tag{4}$$

By equating the second derivatives $\partial^2 E_{\parallel}(r)/\partial r^2$ and $\partial^2 E_{\parallel}(r, z = 0)/\partial z^2$ at $r = 0$, the approximation is matched to the exact solution and the beam waist $w_0$ evaluates to the following:

$$w_0 = \frac{\sqrt{2}\lambda_0}{\pi N_{\text{AOL}}} \tag{5}.$$\

The phase $\phi_g(z) = \arg\{E_{\parallel}(r = 0, z)\}$ of the focal field distribution along the optical axis is

$$\phi_g(z) = k_n z - \arctan\left(\frac{2z}{k_n w_0^2}\right). \tag{6}$$

$k_n = n_n k_0$ denotes the wavenumber inside the medium in the FP with refractive index $n_n$.

In the focal region the phase can be linearly approximated by $\phi_g(z) = z \cdot k_\perp$. Even though the Gaussian beam model relies on a paraxial approximation and is not valid for large $N_{\text{AOL}}$, simulations show that the value

$$k_\perp = \frac{\partial}{\partial z} \phi_g(z) \bigg|_{z=0} = k_n \left(1 - \left(\frac{N_{\text{AOL}}}{2n_m}\right)^2 \right) \tag{7}$$

of the reduced axial wavenumber in the focus differs from the exact value by less than 10% for $N_{\text{AOL}} \leq n_m 0.96$.

The particle to be tracked is located on the optical axis at a coordinate $b_z$ with respect to the focal plane. It is modeled as a point scatterer that emits a spherical wave. For small displacements $|b_z| \ll \lambda_0/n_m$, the exciting amplitude can be approximated as constant while the initial phase of the scattered wave is $k_\perp b_z$.

$$E_{s1}(r, b_z) = e^{i k_\perp r} S_\perp \cdot e^{i k_\perp b_z} \cdot E_{\parallel}(r = 0). \tag{8}$$

The scattering parameter $S_\perp = -i k_\perp^3 \alpha/4\pi$ depends on the Clausius–Mossotti polarizability $\alpha = 3V(n_v^2 - n_m^2)/(n_v^2 + 2n_m^2)$ of the scatterer with volume $V$ and refractive index $n_v$.

The angular spectrum

$$\tilde{E}_{s1}(k_\perp, b_z) = \frac{i k_\perp^2 \alpha}{\sqrt{k_n^2 - k_\perp^2}} \cdot e^{i k_\perp b_z} \cdot E_{\parallel}(r = 0) \tag{9}$$

of the scattered field is given by the Weyl representation

$$\text{FT}\{\text{exp}(i k_n r)/r\} = i 2\pi/\sqrt{k_n^2 - k_\perp^2}$$

of a spherical wave \[12\].

The lower part of Fig. 1 outlines the concept to calculate the distribution $E_{s3}(Q, b_z)$ of the scattered field in the BFP of the DL. The angular spectrum $\tilde{E}_{s3}(k_\perp, b_z)$ of the scattered field in the FP is found by propagation of $E_{s1}(k_\perp, b_z)$, defined in the plane of the scatterer, by a distance $b_z$ \[13\].

$$\tilde{E}_{s2}(k_\perp, b_z) = \tilde{E}_{s1}(k_\perp, b_z) \cdot e^{-ib_z \sqrt{k_n^2 - k_\perp^2}}. \tag{10}$$

According to the Fourier transformation property of the DL, $E_{s3}(Q, b_z)$ equals $E_{s2}(k_\perp, b_z)$ apart from the necessary scaling, introduced in Eq. (2).

The phase of the scattered light depends on the axial position $b_z$ of the scatterer, enabling axial interferometric tracking:

$$\arg\{E_{s2}(k_\perp, b_z)\} = \frac{\pi}{2} + b_z \left(k_\perp - \sqrt{k_n^2 - k_\perp^2}\right). \tag{11}$$

On the optical axis ($k_\perp = 0$), the phase of the scattered light is very sensitive on $b_z$ because of the difference of the wavenumbers $k_\perp$ and $k_n$ of the incident and the scattered field.

However, Eq. (11) shows that there exists a detection direction $k_\perp = k_\perp$ at which the phase is independent of $b_z$.

$$\frac{\partial}{\partial b_z} \arg\{E_{s2}(k_\perp, b_z)\} = 0 \Rightarrow k_\perp = \sqrt{k_n^2 - k_\perp^2}. \tag{12}$$

This direction corresponds to a detection angle $\theta_\perp = \arcsin(k_n/k_\perp)$.

In the BFP of the DL, the fields $E_{s3}(Q)$ and $E_{s3}(Q, b_z)$ interfere and form an intensity pattern:

$$I(Q, b_z) \propto |E_{s3}(Q)|^2 + |E_{s3}(Q, b_z)|^2 + 2 \text{Re}\{E_{s3}(Q) \cdot E_{s3}^*(Q, b_z)\}. \tag{13}$$

The term $|E_{s3}(Q, b_z)|^2$ can be neglected, since it is small compared to the two other summands.

To assess the performance of a tracking system that exploits the variations in $I(Q, b_z)$ to track a particle moving along the optical axis, it is helpful to introduce the normalized sensitivity

$$g_z(Q) = \frac{\partial I(Q, b_z)}{I_0} \bigg|_{b_z = 0} \tag{14}$$

of the intensity on the particle position. $I_0 \propto |E_{s3}(Q)|^2$ is the intensity in the BFP of the DL for no scatterer in the beam path. For the detection angle $\theta_\perp$ corresponding to $r_\perp = f n_m \sin(\theta_\perp)$, the sensitivity is $g_z(r_\perp) = 0$ since the scattered light is independent of $b_z$.

The theoretical value of $g_z$ on the optical axis ($Q = 0$) can be determined by inserting the field

$$E_{s3}(Q = 0, b_z) = \frac{1}{\lambda_0} \tilde{E}_{s2}(k_\perp = 0, b_z). \tag{15}$$

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into Eq. (13) and replacing $k_\perp$ according to Eq. (7):
\[ g_z(Q = 0) = N_{0,OL}^4 \frac{\alpha \pi^2}{\lambda^4} . \]

The strong dependence of \( g_z \) on the focusing numerical aperture \( N_{0,OL} \) arises from the fact that both the amplitude of the focal field \( E_{12}(r = 0) \) exciting the scatterer and the reduced focal wavenumber \( k_r \) depend on \( N_{0,OL} \).

Figure 2 shows the results of a measurement that serves to illustrate the described effects. A polystyrene particle (\( n_s = 1.57 \)) is attached to a glass coverslip and moved through a focus of \( \lambda_0 = 1064 \) nm laser generated with a focusing numerical aperture \( N_{0,OL} = 1.2 \) in water immersion (\( n_m = 1.33 \)). For every axial position \( b_z \) of the particle, the intensity in the BFP of a detection lens (\( NA = 0.9 \)) is recorded with a camera. The pixels of each frame are then binned according to their distance \( q \) to the optical axis and normalized to the intensity \( I_0 \), which is obtained without the particle in the beam path.

The displayed data show that the correlation between small displacements \( |b_z| \leq 500 \) nm and the BFP intensity is positive for low detection angles \( \theta \) and negative for high detection angles. The line profiles of \( I(Q, b_z) \) presented in Fig. 2(a) further illustrate this well-known interrelation [8,9].

From the line profile at \( q_0 = 0.5 \) mm the experimental on-axis sensitivity \( g_z(Q = 0) \) can be estimated. It is a factor of 0.94 below the theoretical value 73.5 mm\(^{-1}\) obtained from Eq. (17). This deviation can be attributed to two effects. Because of the scalar theory used to determine \( E_{12}(r) \), the focal field is overestimated by approximately 10% since a significant fraction of the incident focused light is polarized along the optical axis and does not contribute to the interference. Furthermore, a Gaussian intensity profile was used to illuminate the BFP of the OL in the experiment instead of a plane wave \( E_{11}(Q) = 1E_0 \), reducing the effective focusing \( N_{0,OL} \).

Figure 2(c) shows the experimental course of \( g_z(Q) \). It has a zero crossing at \( \theta = 34.2^\circ \), which is close to \( \theta_0 = 37^\circ \) predicted by the theory introduced here.

In a typical tracking system, a circular detector located in the BFP of the DL integrates the central part of \( I(Q, b_z) \) and thereby generates a tracking signal encoding the axial position \( b_z \) of the tracked particle. For practical applications, a high value of \( g_z(Q) \) is desirable to achieve a strong signal compared to noise arising from fluctuations of the incident light power.

The results of this study show that the numerical aperture of the tracking system should be smaller than \( N_{\Theta} = n_m \sin(\theta_0) \), so that only the region with positive sensitivity \( g_z(Q) > 0 \) is recorded (see right side of Fig. 1). Inserting Eq. (7) into Eq. (12) allows one to derive the convenient first order approximation

\[ N_{\Theta} = \frac{N_{0,OL}}{\sqrt{2}} \]

for the crucial detection numerical aperture.

In conclusion, we presented a theoretical approach that explains the previously observed [7,8] dependency of the axial tracking sensitivity on the detection angle. The sensitivity of tracking systems can be optimized by selecting detection angles smaller than the derived angle \( \theta_0 \), at which the sensitivity is zero. If exclusively detection angles \( \theta > \theta_0 \) are used, a negative sensitivity is obtained that allows axial tracking as well. Recording the intensity pattern at detection angles \( \theta = \theta_0 \) yields a measure for the incident laser power, irrespective of the position of the tracked particle. This measure can be used as a reference, similar to the approach in [14]. The listed applications of the presented results enable more precise three-dimensional tracking of small particles.

References

12. L. Mandel and E. Wolf, Optical Coherence and Quantum Optics (Cambridge Univ., 1995), Chap. 3, p. 120.