Near field laser tweezers in biophotonics

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ABSTRACT

Lasers have opened up numerous opportunities for biomedical sciences. Optical tweezers are one of the key biophotonic techniques. They employ the forces of radiation pressure of light to trap and manipulate microscopic particles, and have enormous applications in various disciplines ranging from physics to biology. The trapping volume of the far field optical tweezers is diffraction limited with an elongated axial size. While one deals with very small biological specimens like single cells or molecules, a reduced trapping volume is desirable, which would ideally be provided by a near field trap. Near-field trapping employs the evanescent field to manipulate microparticles, and hence reduces the axial trapping volume down to tens of nanometres. In this lecture, I will introduce a near-field trapping technique using focused evanescent illumination produced by a high numerical aperture objective, obstructed by an opaque disk whose size satisfies total internal reflection condition.

Keywords: Trapping, near-field tweezers, focused evanescent illumination, morphology dependent resonance

1. INTRODUCTION

Optical tweezers employ the forces of radiation pressure of light to trap and manipulate microscopic particles. Since the pioneering work of Arthur Ashkin in 1970\textsuperscript{1}, optical tweezers have emerged as a powerful tool with enormous applications in physics and biology. The capabilities of optical traps have greatly evolved, incorporating different advanced techniques resulting in a wide range of applications\textsuperscript{2-10}. In addition to being used as a tool to manipulate microscopic particles and molecules, theoretical and experimental work on the fundamental aspects of optical trapping are also being explored, providing new insights and information day by day.

Even though the far field optical tweezers could be remotely accessed and easily controlled, it is greatly desirable to reduce the trapping volume in far field while trying to study single molecule dynamics. One possible method would be to utilize the optical near field\textsuperscript{11}. By using probes of dimensions significantly smaller than the illuminating wavelength, that is, the near field probes, one can monitor the non diffractive interaction of light with a sample in the immediate near field of probes used. The most popular near field probes used are the nano-aperture and the metallic tip. Around the year 2000, a few proposals were made to use the aperture type and the aperture less probes to construct near field laser tweezers\textsuperscript{12-14}. Achieving near field tweezers using such geometries is practically challenging due to various reasons. The first and foremost challenge would be to position the sample accurately within the near field of the probe. It would be difficult to access the sample without damaging the probe. When a metallic tip is used as the near field probe, the heat generated could affect the stability of trapping. Even if we could construct such a near field trap, it would give only a low throughput.

An alternate method of generating a near field would be by using total internal reflection at the interface of two media. If light is incident from a denser to rarer medium, all the light incident at angles higher than the critical angle for the pair of media, would be total internally reflected and generate evanescent field. Recently we have demonstrated the construction of near field tweezers using a focused evanescent wave generated using a high numerical aperture objective\textsuperscript{15}. Here the evanescent field is produced by a ring beam illumination. The size of the obstruction disk is chosen such that all the rays incident at angles less than the critical angle are cut off, resulting in a pure evanescent field. In this case, the gradient forces trap the particle laterally due to the ring nature of the beam and the axial forces push the particle towards the
interface, resulting in a three dimensional trap. The advantage of this method is that a microparticle can be thus confined to the center of the evanescent focal spot and therefore the manipulation of a trapped particle is possible with great flexibility and ease compared to that of using other near field probes. Due to the focused illumination, the evanescent wave strength is stronger than the evanescent field generated using total internal reflection on a prism. Also this does not generate any heating unlike in the case of using metallic probes. In addition to being used in near field tweezers, the focused evanescent wave has enormous applications in different fields like near field scanning imaging, non-linear microscopy, polarization microscopy, and superresolution.

2. THE FOCUSED EVANESCENT WAVE

In order to perform evanescent trapping, the NA 1.65 objective was chosen, as a reasonably wide ring beam was available to generate the near field. The objective used a special immersion oil of refractive index 1.78 and hence when water was used as the medium to suspend the microparticles, the critical angle for total internal reflection was 48 degrees and the maximum angle of convergence was 68 degrees, resulting in a sufficiently wide ring beam and high power to perform near field trapping (Figure 1a).

![Figure 1](image_url)

(a) Focused evanescent field generated for trapping the particle in near field. (b) The mapping of the intensity distribution in the focal region of an unobstructed beam. (c) The intensity distribution of a pure evanescent focus

A linearly polarized He–Ne laser beam was coupled into a high NA objective (Olympus, NA=1.65, 100X). A SNOM head (NTMDT) with an aluminium coated fiber probe vertical to the interface was placed on top of a cover glass. The tightly focused field was directly mapped with the fiber probe scanning in a transverse plane. Figure 1b shows the intensity distribution at the focus of an unobstructed beam. The focal spot is elongated as expected from the theoretical predictions and experiments. The direction of the incident polarisation of the beam is as indicated by the arrow. Figure 1c shows the intensity distribution at the focus of a pure evanescent field generated by using an obstruction disk of size 0.8, normalized with respect to the objective back aperture, where 0.6 is the critical obstruction size for total internal reflection at an air-glass interface. We can clearly see that the intensity distribution has two identifiable peaks along the direction of incident polarisation. The peak intensity was measured at different distances from the interface and it was shown that the intensity showed an exponential decay with distance from the interface and the decay constant measured was in close agreement with the theoretical value of decay constant. This confirmed the evanescent nature of the field.

Another significant feature of the focused evanescent wave is the enhancement of the depolarization effect at the focus as a result of using a ring beam. There is a considerable enhancement of the longitudinal component of polarisation when we use the ring beam. Hence the incident and the longitudinal components dominate over the orthogonal component deciding the shape of the focal spot, resulting in a split-focus.
3. THEORETICAL MODELS FOR FOCUSED EVANESCENT TRAPPING

We have developed two theoretical models for focused evanescent trapping, one based on ray optics\textsuperscript{26} and another based on electromagnetic theory\textsuperscript{27}. The latter is based on the vectorial diffraction theory and the steady state Maxwell stress tensor analysis. The electromagnetic model was used to calculate the trapping efficiency for a small particle ($a = 0.25 \mu m$) and a big particle ($a = 1 \mu m$) along the direction of incident polarization. Figure 2 shows the trapping efficiency mapping, when a small and large polystyrene particle is transversally scanned in the X-direction across the focused evanescent field distribution, generated by placing a central obstruction ($e = 0.85$) perpendicularly to the path of an incoming laser beam, where $e = 0.8$ is the critical obstruction size to generate evanescent field. The trapping efficiency is related to the trapping force and power by $Q = \frac{F \epsilon}{n^2 P}$, where $c$ is the speed of light in vacuum, $n_2$ is the surrounding medium refractive index, $F$ is the trapping force and $P$ is the incident power.

![Figure 2](image1)

Figure 2  Trapping efficiency mapping for a small and a large polystyrene particle of radius $a$, scanned in the X-direction (light polarization direction) across the focused evanescent field. NA = 1.65, $\lambda = 532$ nm, $e = 0.85$, $n_1 = 1.78$ and $n_2 = 1.33$.

The calculations showed that the axial trapping efficiency was stronger for a big particle and also the decay rate of the axial trapping efficiency was less for a big particle, compared to the small particle. But, the transverse trapping efficiency was relatively stronger for a small particle than a big particle.

4. EXPERIMENTAL DETERMINATION OF THE TRANSVERSE TRAPPING EFFICIENCY

![Figure 3](image2)

Figure 3  A schematic diagram of an evanescent-field trapping system.
The transverse trapping efficiency was determined experimentally using a TEM$_{00}$ beam. The experimental setup used is schematically shown in Figure 3. The collimated laser beam of wavelength 532 nm was directed onto the high NA objective, where it underwent total internal reflection and generating an evanescent field which was used to trap the 2µm beads. The trapping process was monitored by a CCD camera and the piezoelectric scanning stage for the sample holder was controlled electronically$^{28}$.

The trapping force $F$ is derived from the measured maximum translating velocity at which a trapping particle falls out of the trap and estimated by the Stokes law $F = 6\pi \eta a v$. Here $a$ is the radius of a trapped particle, $v$ is the maximum translation speed, and $\eta$ is the viscosity of the surrounding medium. The dependence of transverse trapping efficiency on the size of obstruction disk was analyzed (Figure 4) by scanning the particle along directions parallel and perpendicular to the incident polarization. The maximal TTE decreases with the size of obstruction disk due to the decreasing contribution from propagating components. The TTE decreases at a rate very close to what predicted by the theory for both the directions. Also the maximal TTE for an unobstructed beam for the direction parallel to the incident polarization is higher than the other by about 12%.

For the 2 µm bead there was a deviation from the prediction of theory in the near field regime. This could be attributed to factors such as multiple reflections from the interface, Brownian motion and the strong axial force which are not accounted for in the theory. Experiments were done for calculating the TTE for a TEM$_{01}$ beam$^{28}$, which showed good agreement with the calculations, but the TTE for an unobstructed beam was 1.4 times less than that for plane wave illumination.

![Figure 4](image)

Figure 4 The calculated and measured maximal TTE of a polystyrene particle of 1 µm in radius as a function of the obstruction size $\varepsilon$ for $P$ and $S$ scanning directions under plane wave illumination.

5. CONCLUSION

In summary, near field optical tweezers is a promising tool for optical nanometry. We have performed the characterization of the focused evanescent wave and determined the near field trapping efficiency for microscopic dielectric particles using TEM$_{00}$ and TEM$_{01}$ beams. We have also developed two theoretical models, a ray optics (RO) Model and an electromagnetic (EM) Model to study the near field tweezers using a focused evanescent wave. The far field tweezers have already been able to monitor movements of molecules like myosin, kinesin and DNA with very high resolution. By using near field tweezers it would be possible to further improve the optical micromanipulation resolution.
to nanometer scale. By incorporating the far field and near field trapping geometries together, one would be able to create a micromanipulation setup with great flexibility and accuracy. Further, by incorporating techniques like superresolution, morphology dependent resonance (MDR) and femtosecond pulsed lasers, near field tweezers could be advantageous for near field sensing and characterization of microfluidic channels.

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REFERENCES