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Two-color trapped-particle optical microscopy

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We demonstrate a method for nonintrusive scanned near-field optical microscopy. The microscope utilizes an optical trap to position accurately a 50–100-nm-diameter lithium niobate particle. The infrared trapping beam is frequency doubled in the particle, resulting in a visible microscopic optical probe. By separation of the trapping and detection wavelengths, objects that are transparent in the infrared (e.g., biological) may be positioned close to the particle, resulting in high resolution. The current experimental resolution is limited to approximately 500 nm by the properties of the test objects. The theoretical resolution is less than 100 nm.

Scanned near-field optical microscopes (SNOM's) have demonstrated imaging with an order-of-magnitude better resolution than the 200–300-nm conventional diffraction limit by scanning an optical probe in immediate proximity to the studied object. The SNOM has been used primarily to study dry surfaces, but biophysical applications are receiving increasing attention. Current SNOM probes include etched apertures, protrusions, fluorescent tips, and pulled fiber tips. Because the positioning of these probes requires mechanical access to the object, samples with intervening membranes or rough surfaces (e.g., biological) are not always accessible for such studies. Trapped-particle optical microscopy (TPOM) eliminates this restriction by using a nonintrusive optical trap to position and scan the microscopic light source. In addition to its nonintrusive character, the elastic nature of the TPOM probe permits probe-sample collisions without damage. Should a probe particle be accidentally lost, it is easily replaced by a new one since many particles are available for injection into the trap. In this Letter we describe an increase in the resolution of TPOM by separating the trapping and detection wavelengths, using a microscopic lithium niobate crystal as the optical probe.

In the first TPOM experiments scattered light from 290-nm dielectric particles trapped by an argon-ion laser ($\lambda = 514$ nm) was used for the imaging. However, the resolution was limited to approximately 2 $\mu$m since the minimum particle–object distance was a few micrometers. At smaller distances, the scattering by the object is significant compared with the scattering by the particle, resulting in a low signal-to-noise ratio. We eliminate this by trapping a nonlinear crystal particle with an IR beam and using the emitted visible frequency-doubled light for the microscopy (two-color TPOM). By detection of only the visible light, the scattering problem is circumvented, and smaller particle–object distances may be used, resulting in higher resolution. Furthermore, the IR trapping wavelength exhibits a low absorption in most biological materials, thereby minimizing thermally induced turbulence and damage to the studied object. It has been shown that approximately 100 mW of trapping power may be used without damaging biological cells. Finally, the second-harmonic power shows no sign of the bleaching known to occur in many fluorescent probes.

Figure 1 shows the experimental arrangement for two-color TPOM. The optical trap consists of a mechanically chopped cw TEM$_{00}$ Nd:YAG laser beam ($\lambda = 1.064 \mu$m) focused by a N.A. = 1.25, 100× water immersion objective into a water cell. In most of the experiments described below, the average power at the focus was approximately 50 mW. A lithium niobate particle is trapped just below the focus, and $\lambda = 532$ nm frequency-doubled light is emitted. This light is collected with a N.A. = 0.12 microscope objective to an optical fiber, and through interference and IR-blocking filters it is detected by a low-noise photomultiplier tube (PMT) and a lock-in amplifier. The IR laser light scattered perpendicularly to the beam is monitored by an IR video camera. The test objects are mounted in the water cell, which may be positioned accurately with a piezoelectric stage. The data acquisition and the piezoelectric stage are controlled by a personal computer.

We produce the lithium niobate particles by grinding a crystal in a mortar. The particles are then mixed with water. After a few days of sedimentation the top layer of the water-particle mixture is used for the trapping. With electron microscopy the size range of the used particles was determined to be 50–100 nm in diameter. The

![Fig. 1. Experimental arrangement for two-color TPOM.](image-url)
light source is approximately Gaussian (see below), this corresponds to a resolution of approximately 0.45 μm.

If the particle–object distance is very small, the resolution of TPOM is ultimately determined by the effective size of the trapped particle, i.e., the particle’s physical size convolved with its displacement that is due to Brownian motion in the trap. In addition to the effective size in the radial r direction, the displacement in the axial z direction has to be considered since this influences the distance between the particle and the object and, thus, the resolution. Assuming that the optical trap is a harmonic potential well, we may calculate the rms displacement from the scattering and gradient radiation forces by following the Gaussian beam model in Refs. 5 and 12. Typically the rms displacement in the z direction for a 75-nm-diameter lithium niobate particle trapped by 100 mW of λ = 1.06 μm radiation is 40 nm. Here ωz = 350 nm is used in the model, in order to produce focal intensity gradients equivalent to those in our strongly truncated Gaussian beam. In the radial r direction the rms displacement is a factor of 2 smaller owing to the stronger gradient forces in this direction. Convolving the physical size with the FWHM of the rms displacement results in a Gaussian-like light source with an approximately 80-nm FWHM. Using a λ = 800 nm trapping beam, which also exhibits low absorption in biological material, results in a 25% reduction in the FWHM since smaller particles may be used. The numbers clearly indicate the potential for subdiffraction-limit imaging with the TPOM. Near-field enhancement may result in higher resolution.

Although measurement of edge sharpness is an insufficient test of resolution in the near-field region, the experimentally determined resolution is clearly less than the theoretically calculated resolution and the conventional diffraction limit. In the case of the dyed photoresist, the slope of test object and

![Fig. 2. Image of a dyed photoresist test object.](image-url)

![Fig. 3. One-dimensional scan over an etched silicon edge.](image-url)
large particle–object separation are the limiting factors. For the silicon edge experiments, the resolution is limited primarily by surface forces, resulting in a too-large separation between the particle and the object. Because of the small magnitude of the radiation forces trapping the particle (typically $10^{-12} - 10^{-13} \text{ N}$), these forces may be exceeded by attractive and repulsive surface forces\(^\text{12,14}\) at small particle–object distances. Repulsive double-layer electrostatic forces may be of the order of the radiation forces up to a micrometer away from the surface in pure water. However, these forces can largely be eliminated by use of a sodium chloride solution instead of pure water, which is also consistent with experiments on biological objects. With a $1.5 \times 10^{-1} \text{ M}$ (isotone) sodium chloride solution the Debye length\(^\text{13}\) is 0.8 nm, resulting in negligible electrostatic forces (isotone) sodium chloride solution the Debye length13 is 0.8 nm, resulting in negligible electrostatic forces (isotone) sodium chloride solution the Debye length\(^\text{13}\) is 0.8 nm, resulting in negligible electrostatic forces (isotone) sodium chloride solution the Debye length\(^\text{13}\) is 0.8 nm, resulting in negligible electrostatic forces.

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In this case, the probe particle may be lost by adsorption to the surface. For the oscillating particle not to be adsorbed, we estimate that the particle should be another five times the rms displacement away from this point. This results in a particle–object distance of approximately 250 nm in the experiments described above. At this distance the recorded FWHM of the source should be approximately 500 nm,\(^\text{15}\) which is consistent with our measurements.

The attractive forces are reduced a factor of $\approx 30$ compared with silicon for low-refractive-index objects such as biological samples (average refractive index $\approx 1.37$). Combining this method with a sodium chloride solution to reduce the long-range double-layer repulsion, we expect to reach particle–object distances of tens of nanometers, resulting in subdiffraction-limited resolution. The lower attractive forces result in a thin (a few nanometers) remaining repulsive potential barrier at the surface,\(^\text{16}\) which should prevent the adsorption of the probe particle. The barrier also would make it possible to scan the probe particle in the vicinity of rough surfaces without loss of the particle. Other potential factors that increase the effective particle–object distance are mechanical vibrations and thermally induced turbulence; careful engineering and test objects similar to biological objects with low IR absorption, respectively, should make these factors a lesser problem.

The experiments were performed without probe–sample distance regulation, and the distances derived above were determined from the edge response as the probe was moved closer to the sample. Clearly, in a practical microscope the probe–sample distance must be controlled. In tip-based SNOM’s this is often achieved with shear-force systems,\(^\text{16}\) which are not applicable to the nonintrusive probe. We are currently developing a capacitive distance control system, which will permit well-defined TPOM measurements on flat or slightly rough surfaces.

In summary, we have demonstrated a method for performing nonintrusive scanning near-field optical microscopy with an optically trapped probe. This research is motivated primarily by the possibility of performing nonintrusive optical near-field studies of living biological material with high resolution. The experimental resolution is significantly improved over that in earlier research and is limited mainly by the surface forces that are due to the high refractive index of the silicon in the test objects. Our future research will focus on increasing the resolution by using low-refractive-index objects and obtaining higher power by pulsing the trapping laser beam.

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