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Malmqvist, Lars; Hertz, H. M

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Two-color trapped-particle optical microscopy

L. Malmqvist and H. M. Hertz

Department of Physics, P.O. Box 118, S-221 00 Lund, Sweden

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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[1] (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.[2] Current SNOM probes include etched apertures, protrusions,[3] fluorescent tips,[4] and pulled fiber tips.[2] 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[5] (TPOM) eliminates this restriction by using a nonintrusive optical trap[6,7] 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.[8]

In the first TPOM experiments[5] 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 μm 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.[7] 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 ($\lambda = 1.064$ μm) 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

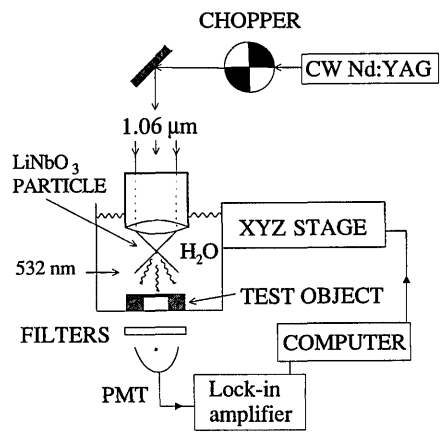

Fig. 1. Experimental arrangement for two-color TPOM.

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lithium niobate particle–water mixture is injected into the trap with a 100-μm-diameter syringe directed toward the focus. With a reasonably high particle concentration in the injected fluid, the probability of trapping a particle is high. Still the residual concentration of particles in the water cell is very low, resulting in a small probability of collecting many particles in the trap during the experiment. Routinely a particle is trapped for several minutes. The emitted visible intensity from the particles may vary by 2 orders of magnitude for different particles. This is consistent with the size range determined above with the assumption that the intensity is proportional to the sixth power of the radius.[9] Over the size range, we typically detect 0.01–1 pW of $\lambda = 532$ nm radiation from a trapped lithium niobate particle. Most measurements discussed below were performed in the 0.1–1-pW regime. One may achieve higher power by Q switching the trapping laser beam with a high repetition rate.[10] The signal-to-noise ratio is typically 20–30 for the 100-ms averaging used in the measurements, but in some instances the signal-to-noise ratio deteriorates and the power fluctuates. This might be due to rotation of the particle in the trap, with the fluctuations reflecting the large difference in the coefficients in the nonlinear optical tensor of lithium niobate.[11]

The method is experimentally demonstrated on test objects that exhibit significant absorption at visible wavelengths and low absorption in the IR. The low absorption is necessary so that we can avoid thermally induced turbulence that is due to absorption of the trapping laser beam. The objects also show resemblence to absorption properties of dyed biological objects. The first object used was lithographically produced dyed photoresist patterns on microscope glass slides. In separate spectrometer measurements the absorption of the approximately 0.8-μm-thick film was determined to be 70% at $\lambda = 532$ nm and a few percent at $\lambda = 1064$ nm. Figure 2 shows a gray-scale image of a 6-μm-wide line with 0.2 μm × 0.2 μm pixels. The total data-acquisition time for the 90 × 30 pixel image was approximately 5 min, illustrating the stability of the method. However, the resolution is low because of the large film thickness and repulsive surface forces, which result in a too-large particle–object distance. Furthermore, the resolution is limited by the edge sharpness of the test object, which was determined by electron microscopy to be 0.7–1 μm (10–90%).

To evaluate better the method's potential resolution, we needed an object with sharper edges. For this purpose free-standing silicon bridges were manufactured. The 1-mm-long 10-μm-thick bridges were produced by anisotropic etching in KOH.[10] Because of the 1.11-eV band gap of silicon, the test objects absorb strongly at visible wavelengths and a few percent at $\lambda = 1064$ nm. The edge sharpness was determined by electron microscopy to be $< \pm 50$ nm. To reduce repulsive surface forces (see below) we performed the experiments in a 0.01 M sodium chloride solution. Figure 3 shows the recorded TPOM signal from the silicon edge. The 10–90% sharpness is 0.5 ± 0.05 μm. Assuming that the effective 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 $\lambda = 1.06$ μm radiation is 40 nm. Here $\omega_0 = 350$ 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 $\lambda = 800$ 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,[2] 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

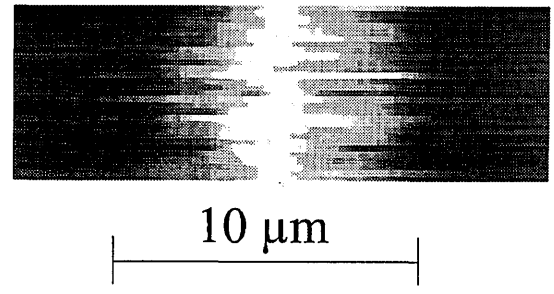

Fig. 2. Image of a dyed photoresist test object.

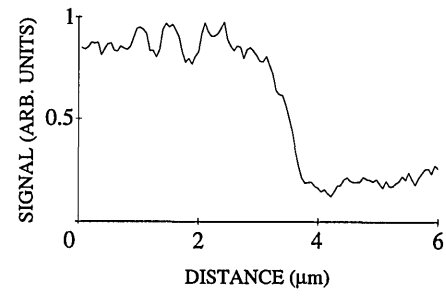

Fig. 3. One-dimensional scan over an etched silicon edge.

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} N), these forces may be exceeded by attractive and repulsive surface forces[13,14] 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 1.5×10^{-1} M (isotone) sodium chloride solution the Debye length[13] is 0.8 nm, resulting in negligible electrostatic forces at distances larger than a few nanometers. Attractive van der Waals forces may not be screened in a similar way. Because of the high refractive index of the silicon bridge ($n = 3.58$) these forces are equal to or larger than the trapping forces up to approximately 50 nm away from the surface and may dominate over the repulsive surface forces. 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,[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,[13] 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,[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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References

1. D. W. Pohl, in *Advances in Optical and Electron Microscopy,* C. J. R. Sheppard and T. Mulvey, eds. (Academic, London, 1991), Vol. 12, pp. 243–312.
2. E. Betzig and J. K. Trautman, Science **257,** 189 (1992).
3. U. Ch. Fischer and D. W. Pohl, Phys. Rev. Lett. **62,** 458 (1989).
4. A. Lewis and K. Lieberman, Nature (London) **354,** 214 (1991).
5. L. Malmqvist and H. M. Hertz, Opt. Commun. **94,** 19 (1992).
6. A. Ashkin, J. M. Dziedzic, J. E. Bjorkholm, and S. Chu, Opt. Lett. **11,** 288 (1986).
7. S. M. Block, in *Noninvasive Techniques in Cell Biology,* J. K. Foskett and S. Grinstein, eds. (Wiley, New York, 1990), pp. 375–402.
8. S. Sato and H. Inaba, Electron. Lett. **28,** 286 (1992).
9. M. Kerker, *The Scattering of Light* (Academic, New York, 1969), Chap. 3, p. 33.
10. H. M. Hertz, L. Malmqvist, L. Rosengren, and K. Ljungberg, "Optically trapped nonlinear particles as probes for scanning near field optical microscopy," Ultramicroscopy (to be published).
11. A. Yariv and P. Yeh, *Optical Waves in Crystals* (Wiley, New York, 1984), Chap. 12, p. 513.
12. S. Chandrasekhar, Rev. Mod. Phys. **15,** 1 (1943).
13. J. Israelachvili, *Intermolecular and Surface Forces* (Academic, San Diego, Calif., 1992), Chaps. 11 and 12, pp. 176–259.
14. W. A. Ducker, T. J. Senden, and R. M. Pashley, Nature (London) **353,** 239 (1991).
15. Y. Leviatan, J. Appl. Phys. **60,** 1577 (1986).
16. E. Betzig, P. L. Finn, and J. S. Wiener, Appl. Phys. Lett. **60,** 2484 (1992).