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Near-field fluorescence imaging with 32 nm resolution based on microfabricated cantilevered probes

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High-resolution near-field optical imaging with microfabricated probes is demonstrated. The probes are made from solid quartz tips fabricated at the end of silicon cantilevers and covered with a 60-nm-thick aluminum film. Transmission electron micrographs indicate a continuous aluminum layer at the tip apex. A specially designed instrument combines the advantages of near-field optical and beam-deflection force microscopy. Near-field optical data of latex bead projection patterns in transmission and of single fluorophores have been obtained in constant-height imaging mode. An artifact-free optical resolution of 31.7 ± 3.6 nm has been deduced from full width at half maximum values of single molecule images. © 2000 American Institute of Physics.

Scanning near-field optical microscopy (SNOM) is a promising tool to meet current and future demands for high-resolution optical characterization. Despite some spectacular demonstrations of this technique, its difficulty of operation and the often ambiguous interpretation of results have so far hindered its broad application.

In a common SNOM scheme, a subwavelength sized aperture probe is scanned over the surface at close distance. Both the properties of the probe and the ability to keep the probe-sample gap constant determine the quality of the image obtained.

Most of today’s probes used with this SNOM scheme consist of a tapered optical fiber that is metal coated so that a small aperture is formed at the tip apex. These probes typically suffer from irregular aperture shapes, which degrade the emission characteristics, and from small taper angles leading to long damping regions and therefore low transmissivities. They are generally irreproducible and expensive since they are produced on a piece-by-piece basis. The high stiffness of the fiber perpendicular to the surface requires a feedback relying on the damping of a lateral oscillation of the fiber. The physics of this “shear-force” interaction is poorly understood compared to that of the force interaction of atomic force microscopy (AFM), and its application to heterogeneous or soft samples is difficult and often subject to artifacts.

In this letter, we present the development of cantilevered near-field optical probes, which avoid most of the difficulties described earlier. The fabrication process based on microfabrication is inherently reproducible and highly parallel, i.e., inexpensive. The probe tips are mounted onto cantilevers and therefore allow all imaging modes of force microscopy. The most significant difference to similar work published before is that the tips are made of solid quartz (index of refraction 1.51). This reduces the cutoff radius for nondissipative light propagation resulting in higher transmissivities and allows the manufacturing of tips with various shapes and heights of several tens of micrometers. High resolution imaging in transmission and using fluorescent contrast is demonstrated.

The microfabricated probes (Fig. 1) are rectangular-shaped silicon layers with aluminum coated solid quartz (fused silica) tips. The spring constants of the cantilevers were calculated to be 1 and 7 N/m, respectively. Due to the high stiffness of the cantilevers we did not observe snap-in events while approaching the surface and therefore scanning at constant height was feasible. For light coupling, windows were etched into the levers at the location of the tips. The tip structuring process follows an established procedure for producing sharp quartz tips with smooth surfaces. For this work, four-sided pyramidal or conical tips were employed with heights of 25 μm. Previously a process based on reactive ion etching was utilized to form a physical aperture at the tip apex. For the probes used in the experiments shown

![Image](image-url)
coupled piezoelectric actuators. The feedback

The feedback light from the sample is scanned in the excitation light and coupled into multimode fibers non-polarizing beamsplitter and focused onto the cantilever probing active feedback range.

A dedicated microscope was developed in order to perform cantilever-based SNOM (Fig. 3). The cantilever is mounted at a 15° angle to the sample in a home-built tripod microscope head, which is placed on top of an inverted optical microscope (Axiovert 135, Zeiss). Its deflection is monitored by an optical beam deflection scheme. The sample is scanned in the horizontal directions compared to the orientation-sensitive DOP of the four-sided pyramidal tip. Figure 2(c) shows the autofluorescence spectra of a microfabricated probe with solid quartz tip (solid line) and of a conventional 20 cm long tapered optical fiber probe (dashed line). The advantage of the short transmission path length through the probe is clearly visible. The microfabricated probe with a short quartz tip contributes only minimally to the fluorescence background, whereas the spectrum of the optical fiber probe exhibits a high autofluorescence and Raman scattering of considerable strength.

A latex bead projection pattern was imaged to determine the optical resolution achievable with the probes. It is produced by evaporating a 35-nm-thick aluminum film onto a hexagonal closed-packed monolayer of 220 nm diameter latex spheres. The beads are then washed away, leaving behind a regular array of metal islands on the glass substrate. We performed constant-height scans by pulling the tip out of the optical resolution from the width of the measured edges. This would suggest an optical resolution of around 25 nm. However, as became evident after the experiment, the simultaneously acquired bending and friction signals indicate a residual force interaction between tip and sample. Since we could not achieve the expected resolution, we have to conclude that the optical resolution is limited by the mechanical properties of the probe, rather than by diffraction effects.
can quantify neither the crosstalk between topographical features and the optical signal\textsuperscript{12} nor the influence of the cantilever’s bending on the light coupling into the tip, the value for the optical resolution given earlier might be influenced by topography artifacts.

A better measure of the optical resolution is the image of a point-like light source such as individual fluorescently labeled biomolecules.\textsuperscript{13} To this purpose goat anti-rat antigens were labeled with AlexaFluor 488 (Molecular Probes) with a binding ratio of 1.5 fluorophores per protein and diluted in buffer to a concentration of 10\textsuperscript{-6} M. Then the proteins were deposited by spin coating on a glass cover slip, which was previously cleaned by dipping it shortly into 2\% hydrofluoric acid and rinsing it thoroughly in high-purity water. According to previous experiments a surface density of roughly 10 molecules per 1 m\textsuperscript{2} was expected. The sample was scanned in constant-height mode with a gap width of around 10 nm between tip and surface.

The near-field fluorescence image [Fig. 5(a)] shows approximately 85 peaks attributed to individual proteins with 1 or 2 attached fluorophores, respectively. The simultaneously acquired topography, bending, and friction signals (not shown) exhibit no variations over the whole scan confirming true constant-height operation. The maximum fluorescence count rate is \(~\sim 16\text{ kcps}\) with an excellent signal-to-noise ratio. A measure of the lateral optical resolution is the full width at half maximum (FWHM) value of the fluorescence peaks. Cross sections along the 47 brightest molecules were taken and fitted with a Gaussian distribution. An example is shown in Fig. 5(b). The four individual peaks can be reproduced by a multipeak fit revealing a resolution better than 32 nm. The average of the FWHM values for all 47 molecules results in an optical resolution of 31.7\(\pm\)3.6 nm. It is worth noting the small variation of the averaged FWHM value, which indicates that the probe did not alter during the scan.

In conclusion, we have demonstrated near-field optical imaging with a true optical resolution of 32 nm utilizing microfabricated cantilevered probes with solid quartz tips. TEM images indicate a closed aluminum layer at the tip apex. Optical characterization of the probes shows good polarization characteristics and low autofluorescence making them eminently suitable for fluorescent imaging. At this point, the explanation of the experimental findings is not clear. While the far-field measurements can be understood in terms of residual light transmission through a finite metal film, this cannot account for the high-resolution near-field data. These could be explained by coupling to modes propagating at the outside surface of the metal coating, in combination with field localization at the metal tip. A discussion will be given in a forthcoming publication.\textsuperscript{7} The accidental formation of a small aperture during tip approach seems unlikely.

We feel confident that the development of cantilevered microfabricated probes opens the door to high-resolution near-field optical imaging of delicate samples in fluid environments.

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