We demonstrate a three-dimensional scanning probe microscope in which the extremely soft spring of an optical tweezers trap is used. Feedback control of the instrument based on backscattered light levels allows three-dimensional imaging of microscopic samples in an aqueous environment. Preliminary results with a 2-μm-diameter spherical probe indicate that features of approximately 200 nm can be resolved, with a sensitivity of 5 nm in the height measurement. The theoretical resolution is limited by the probe dimensions. © 1999 Optical Society of America

1. Introduction

Since the resolving power of an optical microscope was shown in 1873 to be limited by diffraction to 200–300 nm, many innovative schemes have been proposed and have subsequently been implemented to overcome this limit. Examples of these are the scanning near-field optical microscope (SNOM) and the atomic force microscope (AFM). Although achieving at least an order of magnitude better resolution than the conventional diffraction limit, both these techniques have limited application to soft or living samples, as possibly damaging mechanical access to the sample is necessary. In several attempts to overcome these limitations, researchers have used a particle trapped in an optical tweezers potential to replace the SNOM and the AFM probes, as the spring constant of an optically trapped probe can be much softer than mechanical cantilevers, and no mechanical access to the specimen is needed. Better than optical resolution was achieved by use of an optically trapped probe for the SNOM and for noncontact mode scanning force microscopy.

Ghislain and Webb trapped a glass shard in an optical tweezers trap and monitored the forward-scattered laser light from the shard as it was scanned over a photoresist surface. In this demonstration of a novel type of scanning force microscope, the probe displacement was related to the changes in the forward-scattered light. Ghislain and Webb reported that they could detect features of 20 nm with this technique. Similar imaging methods with optical tweezers explored by Malmqvist and Hertz and Kawata et al. were described as near-field imaging techniques. Malmqvist and Hertz used trapped particles as a light source to illuminate a nearby surface. By trapping LiNbO3 particles between 50 and 100 nm in diameter in 1064-nm light, they were able to produce a submicroscopic light source at 532 nm, thus separating the trapping and the imaging wavelengths. This source could then be brought close to a sample to image it in the near field. Although their experimentally achieved resolution was only 500 nm, the technique is theoretically limited by the size of the probe and the amplitude of its Brownian motion. Kawata et al. trapped a 1-μm-diameter polystyrene sphere in 1064-nm laser light and imaged 100-nm spheres in a poly(methyl methacrylate) film. The surface to be imaged was illuminated by an argon laser beam in dark-field condition, and the 1-μm probe was used to convert the surface evanescent photons into propagating ones that traveled through the objective lens (the same lens as is used to form the laser trap). Detecting these photons with a photomultiplier allowed a 100-nm resolution to be achieved.

In the experiments of Ghislain and Webb and Kawata et al. described here, it is not absolutely clear whether the deflection of the probe was being monitored or whether the optical properties of the sample surface were being measured. It seems quite likely that both these effects were occurring in both experiments and that both effects can combine to allow subwavelength optical imaging. In this paper, we present a scanning probe method in which the probe is an optically trapped spherical particle and a sam-
ple is imaged through monitoring of the backscattered light from the trapped particle. Detection of the backscattered rather than forward-scattered light means that changes in the thickness or in the material of the sample have less effect on the measurement. The vertical position of the sample is maintained with a servo to obtain height information. Our results show that scanning with an optically trapped probe in combination with feedback based on a backscattered light signal yields three-dimensional imaging with suboptical resolution.

2. Experimental Design

A. Position Monitoring with Backscattered Light

Backscattered light from an optically trapped particle has been previously used to measure the force constant of an optical tweezers trap. The method relies on measuring small changes in backscattered light resulting from the thermal motion of the particle. We use the same method to measure the average position of a trapped particle. A photodiode is placed a few centimeters from the image plane of the lens used to produce the optical trap, where the backscattered light forms an expanding cone that is larger than the photodiode area (Fig. 1). The power fluctuations in the backscattered light measured by the photodiode can be shown to be almost directly proportional to the changes in the vertical position of the trapped particle. The average power measured at the detector $P_{av}$ is given by the fraction of the cone of illumination subtended by the detector multiplied by the total power $P$,

$$P_{av} = P(R_{det}/R_{im})^2,$$
where \( R_{\text{det}} \) and \( R_{\text{lm}} \) are the radii of the detector and the image. If a trapped particle moves a distance \( \Delta z \), the backscattered light image of the particle moves a distance \( \Delta Z \), given by

\[
\Delta Z = M_y \Delta z = (M_z)^2 \Delta z,
\]

where \( M_y \) and \( M_z \) are the longitudinal and the transverse magnification of the imaging system. The amplitude of the power variation \( \Delta P_z \) due to a vertical particle movement \( \Delta z \) is given by

\[
\Delta P_z = (dP_{\text{av}}/dz) \Delta z = [2P_{\text{av}}(M_z)^2/(D - Z)] \Delta z.
\]

The distance \( D - Z \) (Fig. 1) is almost constant, so the detected power variations are close to being directly proportional to the variations in the particle position. Fluctuations from lateral particle motion are negligible owing to the elliptical shape of the optical tweezer potential (the radial trapping force is typically 3 to 5 times greater than the axial trapping force) and because the longitudinal magnification of the image is the square of the transverse magnification.

B. Scanning Microscope Setup

Measurement of the height of surface features of a sample can be achieved by feedback control of a piezodriven stage based on the measurement of backscattered light from an optically trapped probe. Averaging of the backscattered light signal avoids measurement of thermal motion, and only small changes in the average particle position are measured. As the probe is scanned over a specimen, a change in the averaged detector signal results from a change in the specimen height. This signal is amplified and used to produce an error signal to feed back to the piezocontrolled \( xyz \) stage on which the specimen rests. Use of the servo to maintain the vertical position of the sample while scanning and recording the resulting voltage applied to the piezocontroller yields a three-dimensional image of the specimen. Figure 2 illustrates the principles of the feedback system. The method relies on the scattering surface of the probe being inside the region of the beam waist, so that changes in the probe position result in changes in the backscattered light level of the appropriate sign. The diameter of the beam waist, \( 2w_0 \), in water can be as small as 0.8 \( \mu \text{m} \) for 1064-nm light brought to a focus with a lens of numerical aperture (NA) 1.3. The length of the beam waist is equivalent to the Rayleigh range

\[
z_R = \frac{\pi w_0^2}{\lambda},
\]

so the beam waist could be as short as 0.63 \( \mu \text{m} \).

We constructed our optical trap by focusing an intensity-stabilized Nd:YAG beam of 150 mW using a 100 \( \times \) NA 1.3 oil-immersion microscope objective. The optically trapped probe and specimen to be scanned are viewed with the same 100\( \times \) objective used to produce the trap and with a CCD camera. Fifty percent of the backscattered light is directed to a small-area photodetector placed a few centimeters beyond where the image of the trapped particle is formed. The photodetector signal is amplified and averaged with an integrating amplifier before being input to the \( xyz \) translation stage piezocontroller.

The specimen to be scanned, the trapped probe is first brought close to the specimen while the backscattered light level is monitored. The dc offset of an integrating amplifier is adjusted so that when turned on, the servo locks the stage to that position. The scan is then initiated, and the servo system ensures that the stage is raised and lowered so that the probe is always maintained in the same vertical position. At each step of the scan, the error signal driving the \( z \)-axis piezoactuator is digitally sampled, averaged, and then recorded. The \( xyz \) stage has 25-\( \mu \text{m} \) travel in each direction. Scanning of the stage in the \( xy \) directions and recording of the stage \( z \) position is by a 12-bit, 0–10 V analog-to-digital—digital-to-analog conversion board. This limits the step size of the scan to 6.1 nm, which is of the order of the stage resolution.

3. Resolution Limit of the Method

For these preliminary studies, we used a 2-\( \mu \text{m} \)-diameter polystyrene sphere as the scanning probe, because this allows us to calculate the theoretical resolution of the scanning probe for comparison with our experimental results. Although higher resolution would be expected with a pointed probe, the use of a spherical probe removes all uncertainty over the shape and orientation of the probe particle. We consider the case of a sphere of radius \( r \) passing over a deep, straight-sided depression [Fig. 3(a)]. The path followed by the sphere as it remains in contact with the surface of the feature is a circle of radius \( r \). For a sphere passing over a steep-sided well [the case
depicted in Fig. 3(a), a horizontal displacement of $x$ results in a vertical probe displacement

$$y = -r + (r^2 - x^2)^{1/2}. $$

If the probe diameter is smaller than the width of the well, the probe can detect the true depth of the well, but the edges of the feature will be distorted. For the case of a probe with diameter greater than the width of the depression [Fig. 3(b)], it is still possible to detect the existence of the feature although its real dimensions will not be clearly resolved. Detection of the true depth of the well is not possible for this case. The width of the depression $w$ thus determines the detectable depth

$$d = r - (r^2 - w^2/4)^{1/2}. $$

For example, a 1-$\mu$m-radius sphere measures the depth of a depression of width 200 nm to be a maximum of 5 nm deep.

A sphere passing over a straight-sided protrusion [Figs. 3(c) and 3(d)] can detect the height of the feature correctly, within the limits of the detector equipment, but the edges are distorted owing to the circular path of the probe, as in the case of a depression. For example, the width of a 5-nm-high feature is always measured to be greater than 200 nm. The practical limit of the feature height detection depends on the signal to noise of the apparatus. To test the limits of our detection, we monitored the backscattered light from a 1-$\mu$m-radius sphere attached to a glass slide that was mounted on the stage, while sinusoidally modulating the $z$-axis piezoeartuator of our translation stage. We modulated the stage at 16 Hz with a peak-to-peak amplitude of 30 mV. From the stage specifications, this corresponds to a peak-to-peak modulation of 5 nm in the $z$ axis. The stage modulation was clearly present in the backscattered light signal (Fig. 4), showing that with our experimental setup, the resolution of the height measurement is less than 5 nm. Features of this height could be detected with lateral resolution of 200 nm.

The above analysis assumes the sphere to be in contact with the surface. For small distances between the probe and the surface, the probe may act as

Fig. 5. Images of SiO$_2$ structures. (a) and (b) are images produced by use of scanning with an optically trapped stylus. (c) Same structure imaged with a 100× objective. (d) Scanning electron microscope picture of a SiO$_2$ structure from the same batch.
a force sensor, and the lateral resolution is then determined by the probe–surface interaction area.

The sensitivity of the method could also be affected by backscattered light from the test surface. By observing the amplitude of our photodetector signal with and without a probe in the trap, we estimate the level of backscattered light from the test surface under the probe to be of the order of a few percent of the signal from the probe itself. This signal is a dc offset to our error signal but does not affect the measurement, except to slightly decrease the signal-to-noise ratio.

4. Results and Discussion

Our test specimen was a SiO₂ cross structure microfabricated with a double lift-off technique, 11 9 μm across and 0.5 μm thick, chosen because its dimensions were reasonably well known. The thickness (height) of the structure was measured with a quartz crystal microbalance during the fabrication process to be 0.5 ± 0.05 μm. For these preliminary studies, we used a 2-μm-diameter polystyrene sphere as the scanning probe. A scan of the SiO₂ structure is shown in Fig. 5. In Figs. 5(a) and 5(b) the scan data is plotted in three and two dimensions. In the x axis the step-size used for the scan is 6.1 nm; however, in the y axis the scanning step was 300 nm, to reduce the time taken for the scan. The data-acquisition time was of the order of 30 minutes.

Figure 5(c) is an optical image of the same specimen obtained with a 100× objective. The probe scan appears to show that the cross has slightly raised edges and that it is not as flat as the microscope slide on which it sits. Scanning electron microscopy images of SiO₂ shapes produced by the same method [Fig. 5(d)] also show raised edges. The presence of these features indicates that the scan of the structure is yielding information that cannot be obtained from the optical microscopy image.

Also present in the probe scan are large edge effects where the probe is near the sides of the structure. A reasonable explanation for this is that attractive forces between the probe and the surface become large as the probe approaches the surface and at some point are large enough for significant displacement of the probe particle laterally within the trap. This would result in less backscattered light being detected but not in response to a change in the particle's vertical position. So the servo fails at this point, and the stage moves in the wrong direction.

Figure 6(a) is one line from the scan, confirming that the measured height of the structure (as determined from calibration curves for the piezoactuators and the recorded voltage) is of the expected magnitude. Scans of a small section of the surface [Fig. 6(b)] reproducibly show features ~200 nm in size. These scans demonstrate that the method can certainly obtain suboptical resolution laterally and can image some topographical features of a specimen in three dimensions.

We also scanned a polystyrene surface with a 2-μm-diameter polystyrene spherical probe. A drop of water containing 4-μm-diameter spheres was placed on a microscope slide; then the slide was heated to dry the sample and to induce the spheres to melt onto the surface. Figure 7(a) shows two consecutive scans in the same direction over the polystyrene surface by an optically trapped 2-μm polystyrene sphere. Both scans show the same behavior of the probe particle as it scans the sample. The scan is dominated by steplike features that may be the result of a strong attraction between the two polystyrene surfaces. Since the stage moves in response to the movement of the probe, the scan can in some sense be considered a record of the probe dynamics. The steplike features then most probably represent the probe particle's response to frictionlike forces, as it exhibits classic stick-slip behavior while moving over the surface. A full scan of the deformed 4-μm sphere was performed and is shown in Figs. 7(b) and 7(c). The features seen in these scans indicate that this method could be used to investigate probe–surface interactions. The data-acquisition time for this image was of the order of 30 minutes.

5. Summary

In summary, we have imaged microscopic structures in water in three dimensions. Suboptical resolution (~200 nm) was achieved with a 2-μm spherical probe.
particle trapped with 1064-nm light to scan a SiO$_2$ structure. The height of surface features of the structure was measured by use of the backscattered light signal as an error signal to feed back to the height of the $xyz$ translation stage holding the sample. One could further improve the resolution by scanning with a sharper probe or by separating the backscattered light of the probe from that of the sample, possibly by using different wavelengths for trapping and detection. The method could also be improved by stabilization of the piezostage to avoid height drift and to counter the hysteresis of the piezoactuators. The ultimate resolution limit of the method is likely to be similar to that of SNOM methods. Scans of a polystyrene surface by a polystyrene probe show interesting probe dynamics and indicate that the method could also be used to study surface interactions.

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References