High aspect ratio SNOM probe fabricated using Electron Beam Deposition and Focused Ion Beam

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1. Introduction

Fluorescence in situ hybridization (FISH) [1] is a primary method for visually mapping specific base arrangement on DNA fibers and chromosomes. This FISH method allows observing the target base arrangement that stands out as a fluorescent spot. The authors have developed a Scanning Near-field Optical Microscope (SNOM) for observing the detail of fluorescent spots high-lighted on chromosomes and DNA’s. The wave length of the illumination light limits the resolution with optical microscopes due to optical refraction. Near-field light in the neighborhood of small apertures or scattering objects, in contrast, lacks this refraction property. SNOM takes advantage of such near-field light for its resolution that does not depend on the wave-length. A number of suggestions and tests have been reported about different types of SNOM [2]. The SNOM we used for our study was a type that scans a vibrating probe over the illuminated specimen surface and periodically disperses the near-field light that is then locked in for producing an image. For further enhancing the SNOM resolution, we produced a scattering probe with high aspect ratio and observed specimen with fluorescent surface.

2. Developing the scattering probe with high aspect ratio

The scattering type SNOM uses a cantilever (OMCL-HA100WS by OLYMPUS Optical Co., Ltd.) for the scattering probe, on the atomic force microscope (AFM), that scans the specimen surface. The low aspect ratio with the pyramid shaped conventional AFM cantilever causes such problems e.g., a blurred image that hinders observing small structures due to scattered rays from the probe other than its tip when the specimen has mixed size structures (Fig.1-(a)). Our study, to counter this problem, proposes a scattering probe with high aspect ratio, as Fig.1-(b) shows, produced using the electron beam deposition [3] inside an electron microscope. Electron beam deposition is a phenomenon that a small amount of residual gas, e.g., hydrocarbon, getting excited and decomposing within the electron microscope specimen chamber, and the decomposed material adhering and accumulating on the specimen surface. Fig.2 shows a SEM image of the scattering probe with high aspect ratio that we actually produced. The tip of the AFM cantilever has an approximately 1µm long needle, grown by electron beam deposition, with a tip diameter of about 20nm. The next chapter explains the detail of our method for producing the probe.

Fig. 1 Effect of probe aspect ratio: (a) scattering from area off the tip, (b) scattering only from the tip

Fig. 2 SEM image of the high aspect ratio scattering probe
3. Evaluating the high aspect ratio scattering probe

For testing the performance of the high aspect ratio scattering probe, we made two different types of observations; one with a conventional AFM cantilever (low aspect ratio scattering probe), and the other with the high aspect ratio scattering probe. It is common to use an optical filter to block the illumination for fluorescent observation. The scattered light from a fluorescent source, however, is much weaker than the scattering from the illuminating light source, and thus we directly measured the scattering from the illumination without an using an optical filter. The specimen was a mixture of polystyrene beads with diameters of 20nm and 200nm. A semiconductor laser with a wavelength of 670nm served as the illuminating source. The AFM cantilever made of silicon nitride passes all the illuminating semiconductor laser. This will illuminate undesired area on the specimen generating noise for the scattering light measurement. To suppress such noise, we produced the high aspect ratio scattering probe with the following procedure (Fig.3): First, we deposited Al (film thickness approximately 80nm) on the entire surface of the cantilever and about 10nm of Au to prevent oxidization. Next, with electron deposition machining, removed the Al film from the tip of the cantilever to make an opening with a diameter of about 2nm to illuminate only the target area on the specimen surface. Then electron beam deposition grew a high aspect ratio tip from this.

![Fig. 3 Steps to produce high aspect ratio scattering probes for semiconductor laser](image)

![Fig. 4 Image of mixed φ20nm/φ200nm beads using a low aspect ratio probe](image)

![Fig. 5 Image of mixed φ20nm/φ200nm beads using a high aspect ratio probe](image)
opening. We sputtered a small amount of Au on the tip to increase the scattering intensity. We controlled the height by varying the stage elevation to keep a constant AFM interatomic force. We simultaneously measured the signal image (optical image) of the scattering light intensity from the near-field light, and the regular AFM stage height signal image (shape image) for reference.

Fig. 4 (a) and (b) are the images from observing with the low aspect ratio scattering probe. (a) is the optical image and (b) the shape image acquired together. The optical image (a) shows the φ200nm beads, however, not those of the φ20nm beads found on the shape image (b) in the lower center of the picture. This is due to scattering lights from the needle body other than its tip with the low aspect ratio scattering probe. Fig. 5 shows (a) optical image, and (b) shape image made with the high aspect ratio scattering probe. The probe successfully produces images of both the φ20nm and φ200nm beads. Furthermore, the boundaries of the φ200nm beads are clearer in the optical image compared to that from the low aspect ratio scattering probe. These experiments verified that our high aspect ratio scattering probe is effective for high resolution observation.

4. **Fluorescent observation with the high aspect ratio scattering probe**

For verifying the feasibility of fluorescent observation with SNOM, we observed φ200nm beads with excitation/fluorescent wave lengths of 458/540nm. He-Cd laser with a wave length of 442nm illuminated the beads to excite fluorescent light from them. The He-Cd laser illumination does not pass through the cantilever material of silicon nitride. Thus, to illuminate the specimen surface, we applied the following steps to produce a high aspect ratio scattering probe with a small opening (Fig. 6). First, we sputtered an Au film of about 10nm on the cantilever surface for Focused Ion Beam (FIB) observation, and formed an approximately 2µm square opening at the tip of the cantilever. Then we formed a high aspect ratio tip with electron beam deposition and finished by sputtering a small amount of Au on the very end. Fig. 7 shows a SEM image of the probe we produced. An optical filter of wave length 540nm was installed on the photomultiplier tube to block the background light and allow measuring a signal image of the fluorescent light only (fluorescent image). At the same time, we measured the signal image at the regular AFM stage height (shape image) for reference.

![Fig. 6 Steps to produce high aspect ratio scattering probes for He-Cd laser](image-url)
Fig. 8 (a) shows the fluorescent image and (b) the shape image. The fluorescent image (a) was processed through a 15th degree averaging filter. The shape image (b) shows fluorescent beads in the left center and upper right area of the picture, above the fluorescent beads concentration layer, and they are also identified in the fluorescent image (a). The fluorescent image (a), however, is unclear and does not show other fluorescent beads concentration. This was due to the weak fluorescent signal lost in the noise from the instrumentation and the background light. We further need to improve the S/N ratio to obtain clear fluorescent images to identify fluorescent beads concentrations using the FISH method. We believe that we can reduce the noise and improve the S/N ratio by acquiring tens to hundreds of data from 1 dot, instead of the current 1 datum from 1 dot, and averaging the data.

5. Conclusion

We developed a high aspect ratio scattering probe for near-field optical microscopes and tested its performance. The probe succeeded in obtaining an optical image of φ20nm beads, formerly unavailable with a cantilever on an interatomic force microscope, to demonstrate that high aspect ratio scattering probes are effective in high resolution observation. We also conducted an experiment of fluorescent observation and showed the feasibility of applying near-field optical microscopes for high resolution fluorescent observation.

References