High-speed Scanning Near-field Optical Microscopy

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1. Introduction

Scanning near-field optical microscopy (SNOM) has been recognized as a powerful technique for super-resolution optical imaging [1]. It has been even more unique compared with other super-resolution fluorescent imaging techniques because it utilizes near-field light at a metallic tip as a nano-light-source, which realizes super-resolution not only in fluorescence but also in any other optical signals such as Raman scattering, infrared absorption and photoluminescence. Such a strong advantage has been stimulating biological fields as a new class of analytical techniques. However, it has been yet highly challenging to apply SNOM for biological samples. Although there have been several issues for biological applications, one of the major problems is its low imaging speed. Because it requires to physically scan a metallic tip, the imaging rate was typically limited to several minutes per frame, which is too slow to capture dynamic biological samples.

In this work, we developed high-speed scanning near-field optical microscopy (HS-SNOM) using a scanning technique of high-speed atomic force microscopy (HS-AFM) [2]. HS-AFM achieves high speed scanning (*i.e.* high temporal resolution) below sub-second per frame owing to the high speed scanner, micro-cantilever tips, and dynamic PID feedback. By scanning a metallic tip with this technique, we achieved an imaging rate of 3.5 seconds per frame in SNOM in a liquid environment, which would open a door for SNOM bio-imaging to reveal biological dynamics at nanoscale.

2. Results and Discussions

In order to construct HS-SNOM, we utilized a tip-scanning type HS-AFM [3], which is a stand-alone system so that it is easily mounted on an ordinary inverted optical microscope, as shown in Fig. 1(a). It is therefore possible to place an objective lens as close as possible to a metallic tip to maximize the signal detection efficiency with a high-NA oil-immersion objective lens. Total internal reflection illumination, an avalanche photodetector, and many other techniques were also included to increase the detection efficiency,

Since a micro-cantilever tip is also important for high-speed scanning, it is required to fabricate a metallic tip based on the micro-cantilever tip. We developed a fabrication method of metallic nano-rod structures at the tip apex to efficiently excite near-field light at the tip apex through localized plasmon resonance, as shown in Fig. 1(b). We first fabricated an amorphus carbon tip via electron-beam deposition. It was then smoothly coated with silver or gold by sputtering

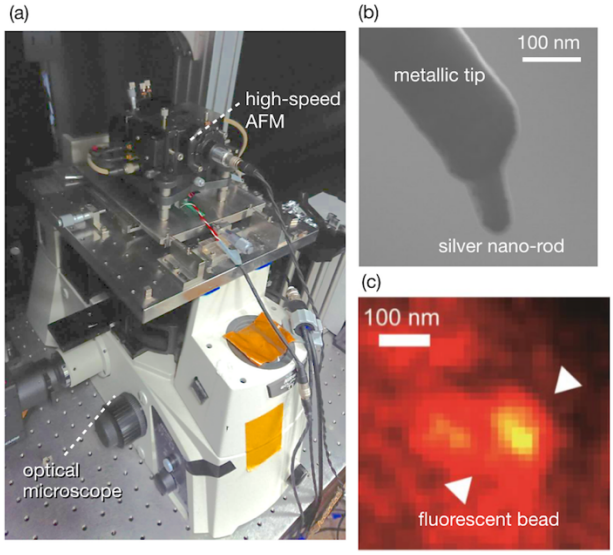

Figure. 1 (a) Constructed high-speed scanning near-field optical microscopy (HS-SNOM). (b) SEM image of a micro-cantilever metallic tip. (c) HS-SNOM image of a fluorescent bead obtained in 3.5 seconds.

to form nano-rod structure. It is possible to control the plasmon resonant wavelength by changing the length of amorphus carbon tips. We confirmed through numerical simulations that the fabricated tip structure generated strong near-field light through plasmon resonance.

We then demonstrated HS-SNOM imaging with a fluorescent bead in liquid. We successfully achieved imaging rate of 3.5 seconds per frame with the nanoscale spatial resolution (Fig. 1(c)). It is around 100 times faster than typical imaging rates of SNOM. To confirm that the HS-SNOM is available for biological applications, we further applied it for DNA labelled with fluorescent molecules. We succeeded in high-speed SNOM imaging of DNA with 10 seconds per frame in a physiological condition, which indicates a great potential of HS-SNOM for analysis of biological nano-dynamics. Through further developments in this technique, we believe that HS-SNOM becomes an effective imaging method of biological samples with label-free super-resolution features.

References

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