High resolution live cell imaging with electron-beam assisted (EXA) optical microscopy

Yoshimasa Kawata[1,3], Yasunori Nawa[1], and Wataru Inami[2,3]

[1]Shizuoka University, Faculty of Engineering, Johoku, Naka, Hamamatsu 432-8561

[2]Shizuoka University, Global Research Leaders, Johoku, Naka, Hamamatsu 432-8561

[3]JST, CREST

Abstract

We propose electron beam excitation assisted optical microscope, and demonstrated its resolution higher than 50 nm. In the microscope, a light source in a few nanometers size is excited by focused electron beam in a luminescent film. The microscope makes it possible to observe dynamic behavior of living biological specimens in various surroundings, such as air or liquids. Scan speed of the nanometric light source is faster than that in conventional near-field scanning optical microscopes. The microscope enables to observe optical constants such as absorption, refractive index, polarization, and their dynamic behavior on a nanometric scale. The microscope opens new microscopy applications in nano-technology and nano-science.

In conventional near-field optical scanning microscopes [1, 2] (NSOMs) capable of sub-diffraction limit resolution, confinement of optical illumination area on the specimen is realized by small aperture of metal-coated fiber probe, or aperture-less tip probe, optically-trapped gold particle, solid immersion lens, non-optically probing technique. Images are acquired by scanning the small aperture near specimen surface while keeping a constant distance from the specimen surface.

Here, we propose electron beam excitation assisted optical (EXA) microscope in which electron beam focused on a luminescent thin film excites nanometric light source near the specimen [3, 4]. The light emission is well known as cathodoluminescence. It is possible to excite a light source of a nanometric size, because electron beam can be focused to a few nanometers. EXA microscope combines scanning electron microscopy that has nanometric resolution and optical microscopy that is advantageous to dynamic observation of living biological specimens.

Figure 1(A) shows schematic diagram of the proposed EXA microscope. An electron beam is focused on a luminescent film. A specimen is put on the luminescent

film directly. The inset in Fig. 1(A) shows magnified image of the luminescent film and the specimen. Nanometric light source is excited in the luminescent film by the focused electron beam. The nanometric light source illuminates the specimen, and the scattered or transmitted radiation is detected with a photomultiplier tube (PMT). The light source is scanned by scanning of the focused electron beam in order to construct on image. Figure 1(B) shows the structure of sample holder used in EXA microscope. A square aperture of 100 × 100 μm^2 size was fabricated on silicon substrate and sealed by SiN film with a thickness of 50 nm.

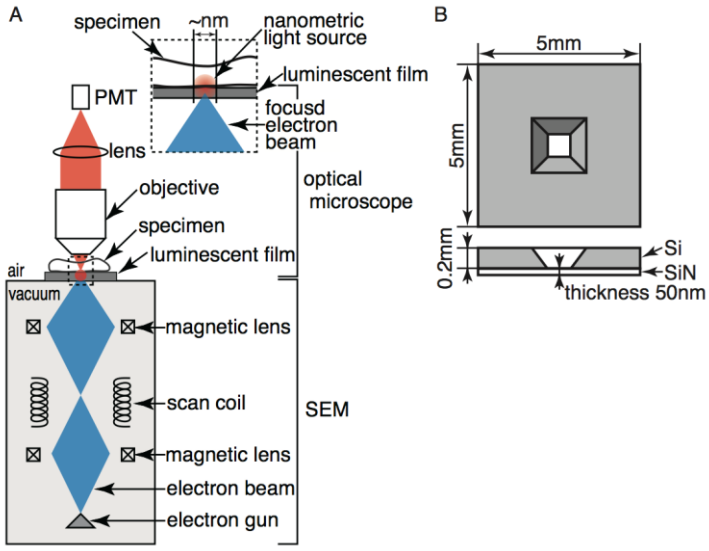

Fig. 1. (**A**) Schematic diagram of EXA microscope (A) and layout of silicon nitride membrane (B).

To verify feasibility of the high resolution EXA microscope, we used silicon nitride (SiN) film of 50 nm thickness as a luminescent material and all optical components were inserted in vacuum chamber of scanning electron microscope (SEM) (JEOL, JSM-6390). SiN film emits blue to ultra-violet (UV) light by irradiation of electron beam. We confirmed in a preliminary experiment that SiN film of 50 nm thickness had enough strength to separate 1 atm air pressure from vacuum.

In the experiment, electrons were accelerated with 10 kV voltage and focused to a 8nm diameter spot on the SiN film to excite nanometric light source. Scattered or transmitted light from the specimen was collected by a lens with numerical aperture 0.65 and detected with a photomultiplier tube (PMT) (Hamamatsu Photonics, R7400-U20). Images were reconstructed from the signal detected with raster scanning

of electron beam using a computer. We used polystyrene latex spheres dispersed on the SiN film directly as specimens for resolution veri- fication. The latex spheres were dispersed in monolayer, which was confirmed with an atomic force microscope (Seiko, SPI-3800).

Figure 2(A) and 2(B) show observation images acquired with the SEM and EXA microscopes, respectively. The two images were acquired successively. The scale bars in the images represent 100 nm length. The acquisition time of EXA image was 16 minutes at 512 by 512 pixels. The resolution and signal-to-noise ratio of the SEM image in Fig 2A is poor because the SEM image was acquired through the SiN membrane. Each latex sphere of 100 nm diameter was observed clearly and its position in the EXA image was identified with that in the SEM image. This allows us to conclude that EXA microscope has resolution higher than the diffraction limit [23].

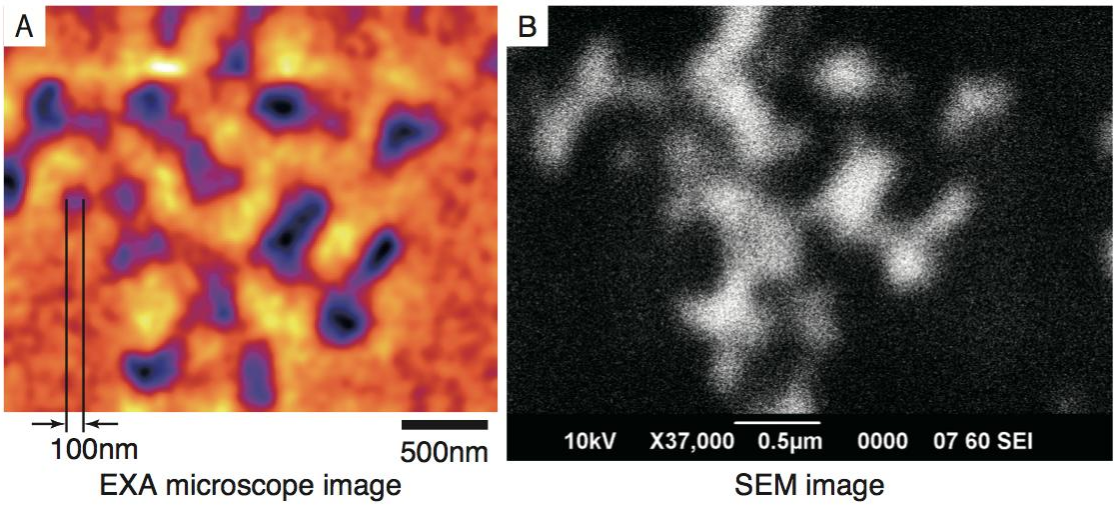

Fig. 2. (A) EXA microscope image of 100 nm latex spheres and (B) SEM image of the same area as in (**A**).

Figure 3(A) shows observation results of latex spheres of 50 nm diameter with EXA micro- scope. Figure 3(B) shows intensity distribution on the solid line indicated in Fig. 3(A). It is recognized that EXA microscope can resolve aligned spheres of 50 nm diameter clearly. We believe that the developed EXA microscope has a potential to achieve a few tens nanometer resolution because electron beam can be focused in a few nanometers.

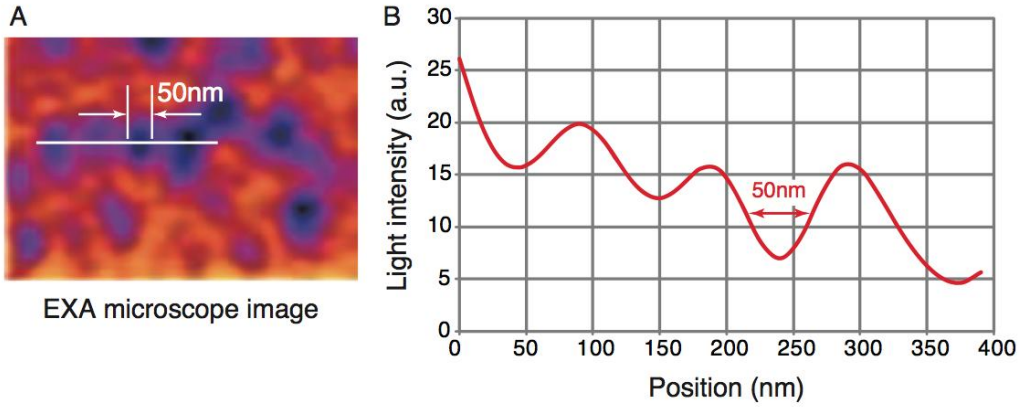

Fig. 3. (**A**) Observation image of 50nm diameter latex spheres with EXA microscope and (**B**) intensity distribution on the solid line indicated in (**A**).

We proposed a new type near-field optical microscope and demonstrated its resolution higher than 50 nm. The concept of EXA microscope is an integration of a high-resolution SEM and an optical microscopy suitable for observation of living biological specimens and spectroscopic analysis of materials. Due to the conversion of the electron beam to light source, specimens can be placed in air or liquids, as well as vacuum. Since electron beam can be scanned at a fast speed, it is possible to observe dynamic behavior of specimens with fast frame rate. We expect that the EXA microscope can resolve the scanning speed limitation of conventional NSOMs due to low signal-to-noise ratio at higher resolution, and difficulty of maintaining the probe-specimen distance.

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