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Measuring the sizes of nanospheres on a rough surface by using atomic force microscopy and a curvature–reconstruction method

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Abstract

One of the advantages of atomic force microscopy (AFM) is that it can accurately measure the heights of targets on flat substrates. It is difficult, however, to determine the shape of nanoparticles on rough surfaces. We therefore propose a curvature–reconstruction method that estimates the sizes of particles by fitting sphere curvatures acquired from raw AFM data. We evaluated this fitting estimation using 15-, 30-, and 50-nm gold nanoparticles on mica and confirmed that particle sizes could be estimated within 5% from 20% of their curvature measured using a carbon nanotube (CNT) tip. We also estimated the sizes of nanoparticles on the rough surface of dried cells and found we also can estimate the size of those particles within 5%, which is difficult when we only used the height information. The results indicate the size of nanoparticles even on rough surfaces can be measured by using our method and a CNT tip.

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Keywords: Atomic force microscope; Gold nanoparticle; Carbon nanotube; Rough surface; Curvature–reconstruction method

1. Introduction

The atomic force microscope (AFM), invented by Binnig et al. in 1986 [1], is a powerful instrument for studying the structures and properties of nanoscale molecules. Often used to study biological systems because it does not require the sample surface to be electrically conductive and can be used in liquid as well as gaseous environments, it has been used to obtain high-resolution images of cells, proteins, and nucleic acids [2–8].

Although the AFM has several advantages over the conventional field emission scanning electron microscope (FE-SEM)—simpler setup, smaller size, and higher spatial resolution—it has not yet found application in what is one of the most common and useful applications of electron microscopy, the immunostaining of living tissues and cells. That is, it has not been used to determine the spatial distribution of gold-labeled antibodies attached to the surfaces of target tissues and cells. This might be because the conventional AFM measurement method cannot distinguish the probe gold nanoparticles from the surrounding rough tissue surfaces.

Moreover, to image the round shape of gold nanoparticles so that nanoparticles of different sizes labeling different probe antibodies or probe DNA fragments can be distinguished when the antibodies or DNA fragments are attached to tissue surfaces, the conformation of the very end of the AFM tip should be taken into account. The conformation of the AFM tip usually makes the horizontal dimensions of a structure in an AFM image appear to be larger than the actual dimensions of the structure. Consideration of the tip conformation is especially important when one is measuring

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the sizes of nanoscale targets such as molecules and investigating the shapes of high-aspect-ratio materials because the radius of an AFM tip is usually of the order of 10 nm and those shapes are circular and conical.

Generally applying the conventional procedure, to quantify the size of the target when using its height as a parameter, one needs to have an atomically flat substrate, like a fleshly cleaved mica surface, in order to keep the baseline flat. The sizes of target samples on rough surfaces, such as cell surfaces, however, cannot be quantified by using their heights as parameters because of the difficulties of establishing a suitable baseline.

Here we report a novel method for quantifying the size of nanometer-scale target on rough surfaces. In this method, called the curvature–reconstruction method, the size of a spherical target is calculated from its curvature instead of its height. It can be used to estimate the sizes of target on rough surfaces because it does not require the establishment of a flat baseline. As the spherical gold nanoparticles are the established well known markers used to label target molecules in or on cells in electron microscopy, they could be also used for visualization of receptor distribution by AFM measurement for the practical tool for immunobiological studies if we could identify those gold nanoparticles on the rough surface of cells.

In this study, thus, we measured the sizes of gold nanoparticles on smooth and rough surfaces using the curvature–reconstruction method and discussed the ability of this method for practical application of immunostaining.

2. Materials and methods

2.1. Preparation of dried cells

SAOS-2 osteoblast cell line was obtained commercially (Dainippon Sumitomo Pharma, Osaka, Japan) and cultivated in McCoy's 5A medium (Invitrogen, Tokyo, Japan) supplemented with 10% fetal bovine serum (Invitrogen) and 1% penicillin–streptomycin (Invitrogen). Before the measurement, cells were removed with 0.25% trypsin–EDTA (Invitrogen) and were cultivated on a 22×22-mm cover slip (Matsunami Glass, Osaka, Japan) at a concentration of about 10^4 cells/ml. After the cells became attached to the cover slip, they were fixed with 4% formaldehyde (Wako, Osaka, Japan) in phosphate-buffered saline (PBS) for 30 min and were washed with PBS. The cells were then rinsed with water to remove salts and dried for 2 h.

2.2. Preparation of gold nanoparticles

For the measurements of nanoparticles on mica surfaces, colloid suspensions of 15-, 30-, and 50-nm gold nanoparticles, respectively containing 1.4×10^{12}, 2×10^{11}, and 4.5×10^{10} particles/ml (EMGC15, 30, and 50, British BioCell International, Cardiff, UK) were dropped onto the freshly cleaved mica surface and were dried for 2 h. After the samples were dried they were washed with water in order to remove salts and dried again for 2 h. For the measurements of nanoparticles on dried cells, 30-nm nanoparticles were applied to the prepared dried-cell surface the same way they were applied to the mica surface.

2.3. AFM observation of gold nanoparticles

An SPI4000/SPA400 scanning probe microscope system (SII NanoTechnology Inc., Tokyo, Japan) was used, and all of measurements were made in air at room temperature. All the images were obtained in the cyclic contact mode with a conventional silicon tip obtained from Olympus, Inc., Tokyo, Japan, the OMCL-AC160TS (spring constant = 42 N/m, tip radius <10 nm, and resonance frequency = 300 kHz) or with a carbon nanotube (CNT) tip

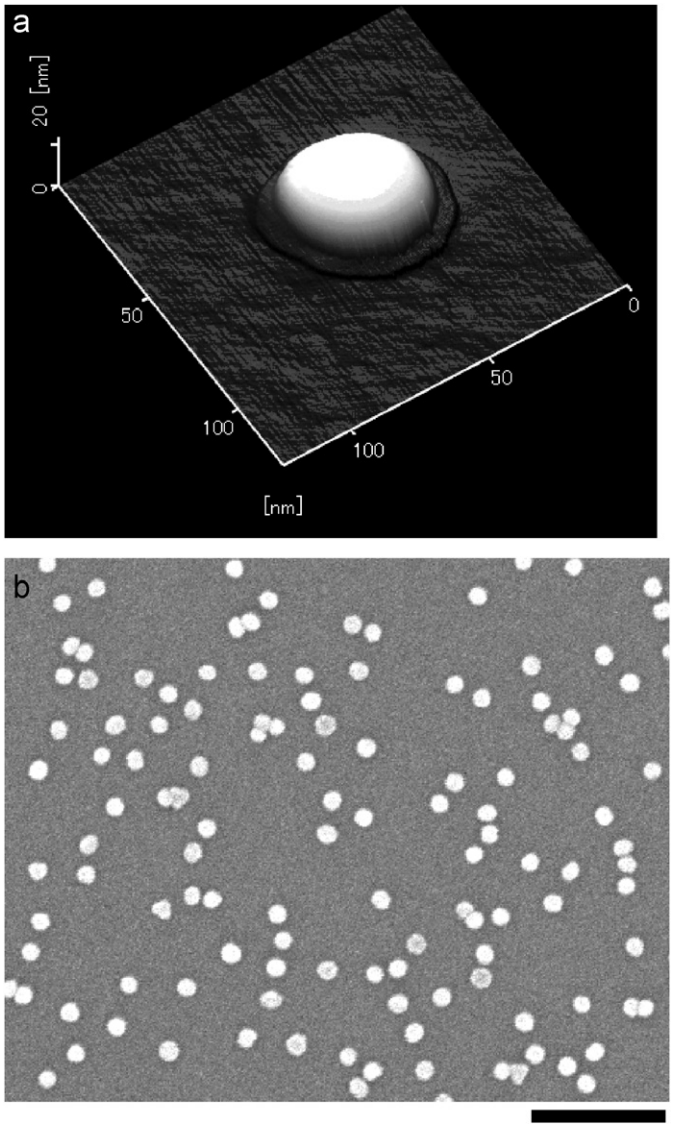

Fig. 1. AFM and SEM images of 30-nm gold nanoparticles. (a) AFM image of a 30-nm gold nanoparticle. The image was obtained with a normal Si tip. (b) SEM image of 30-nm gold nanoparticles. Bar: 100 nm.