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Atomic force microscopy of bacteriophage T4 and its tube-baseplate complex

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Bacteriophage T4 was imaged by atomic force microscopy with the finest resolution to date with a clear image of tail fibers of an estimated diameter of 2–3 nm. T4 phages were spread on a clean surface of silicon wafer and dried under air before observation with an atomic force microscope. The head, tail and tail fibers were routinely imaged with somewhat distorted dimensions. The ease of imaging isolated phage particles with a good resolution raised our expectation for the further use of AFM in biomedical applications.

Atomic force microscopy; Bacteriophage T4; Tail fiber

1. INTRODUCTION

Atomic force microscopy has been applied to image varieties of biological specimens with the aim to reveal their atomic scale surface topographies. Successful applications have been reported on DNA [1–3], proteins [4,5], cell surfaces [6], and viruses and phages [7–9]. It is considered to be a very promising technique in the visualization of biosystems at a molecular and atomic level. The resolution at the present stage of its application to biological specimens is limited to about 5–10 nm though it is intrinsically capable of imaging individual atoms [10] and molecules in the absorbed layers of liquid crystals [11] or in synthetic lipid layers [12]. In this respect, although imaging of a bacteriophage has been reported and its head and tail parts have been identified, reliable imaging of thinner structures like tail fibers has been left to further study. In this report we describe our successful imaging of bacteriophage T4 with its head, tail and tail fibers with good reproducibility.

2. MATERIALS AND METHODS

2.1. *Bacteriophage T4*

Bacteriophages T4 were prepared according to the method in the literature [13] and diluted with deionized water shortly before application to the surface of a silicon wafer. About 20 μl phage suspension on the wafer was dried in air and used as sample for AFM observation. The tail of the phage was prepared from the growth medium of the headless mutant as described previously [14]. When the buffer solution containing the tail preparation was diluted with deionized water, proteins forming the tail sheath came off leaving a skeleton complex of the tail tube and base plate (tube–baseplate complex) to be observed by AFM.

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Abbreviations: AFM, atomic force microscope.

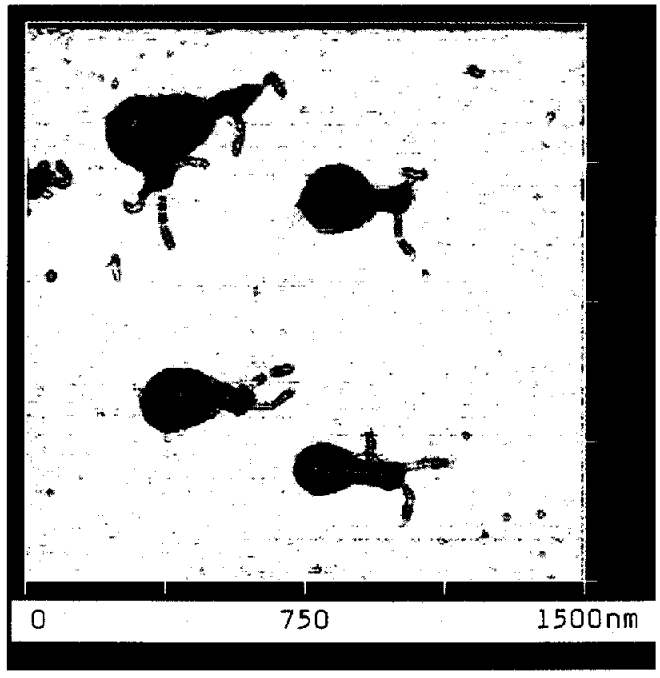
2.2. *AFM*

A NanoScope II (Digital Instruments, Santa Barbara, CA) and an SPI 3700 atomic force microscope (Seiko Instruments, Tokyo) were used with commercial probes with pyramidal tips.

3. RESULTS

Fig. 1 is an AFM image and its line section of polystyrene latex spheres with a nominal diameter of 312 nm used for calibration of the vertical scale of AFM. The horizontal scale was calibrated by imaging metal-coated or uncoated tobacco mosaic viruses [15].

Fig. 2 shows a typical AFM imaging of bacteriophage T4 with its head, tail and tail fibers. Since the three parts of the phage differ considerably in their dimensions, the vertical scale of the graphical software must be adjusted for the best view of each part. It was no surprise that heads and tails with their considerable sizes could be visualized with AFM but routine imaging of tail fibers of 2–3 nm in diameter is, to our knowledge, the best result so far for bacteriophage T4. The number of tail fibers per phage particle was very rarely 6 as expected from the best results of electron microscopy [16] but mostly between 2 and 5. This is probably due to the damage caused by the AFM tip. The apparent width of the tail fibers in Fig. 2 was approximately 10 nm, which is a considerably larger value compared with the expected width of 2–3 nm from electron microscopy [16]. This is undoubtedly due to the convolution effect between the AFM tip and the specimen [11]. The apparent widths of the head and tail sections of the phages were also larger than the expected ones for the same reason.



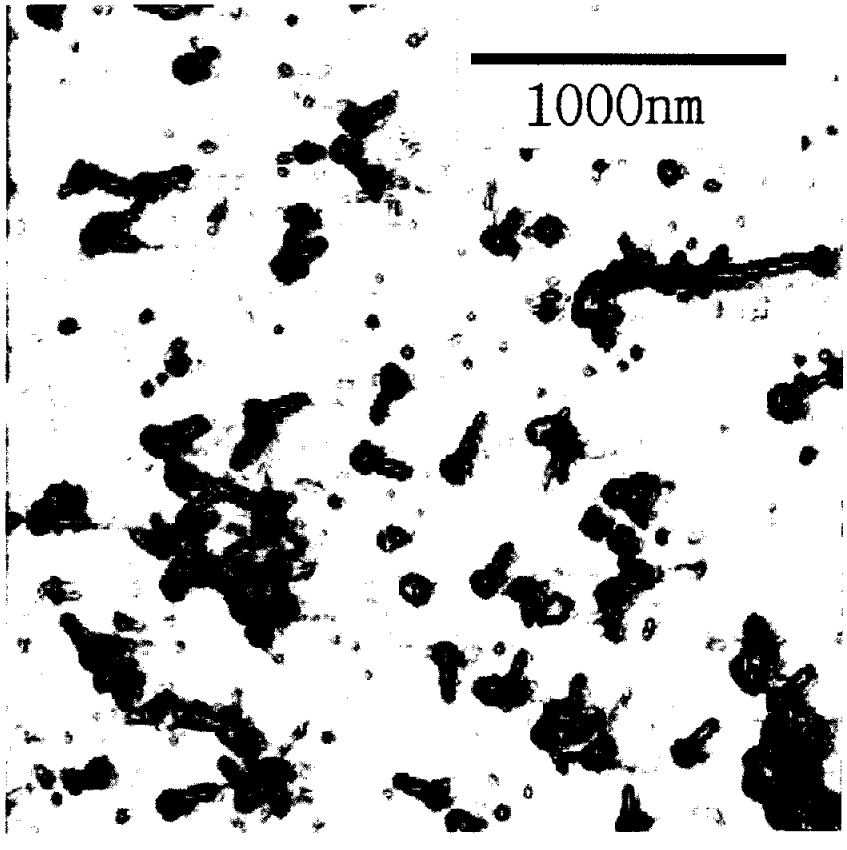


Fig. 1. (top left) AFM image of a cluster of polystyrene latex spheres of a nominal diameter of 312 nm.
Fig. 2. (top right) AFM image of bacteriophage T4 dried on a silicon wafer. The head, tail, and tail fibers are clearly imaged. This result was obtained by NanoScope II but similar images were routinely obtained also by SFI 3700. Imaging area = 1,500 nm × 1,500 nm.
Fig. 3. (bottom) AFM image of tube–baseplate complexes. The thinner bar on the T-shaped complex is the tube and the oblate bulge on one end of the complex is the baseplate. Imaging area = 2,800 nm × 2,800 nm.

The tail fibers in Fig. 2 have a characteristic kink in the middle attesting to only a limited displacement of the sample during scanning. The height of the individual parts was obtained by graphical sectioning of the images. The height of the head was 50–60 nm in average and that of the tail was 17–20 nm. The expected height of the normal head being 80 nm [16], the measured height of the sample phages was considerably lower. This was probably due to (1) flattening during the drying process on the silicon surface, and (2) flattening due to a squashing effect of the tip and cantilever. The flattening effect is apparently most pronounced for the head and less so for tail and tail fibers. The tail and tail fibers are probably more compact and resistant to the flattening effects. Some of the heads were as thin as 20–30 nm indicating that they were devoid of nucleic acid which normally fills the head.

Fig. 3 contains numerous images of tail–baseplate complexes. One end of the complex is lifted up by the presence of the circular base plate, and the structure is considered to be highly susceptible to flattening effects.

4. DISCUSSION

AFM was successfully applied to the routine imaging of proteinaceous structure as thin as 2–3 nm in diameter. So far this is one of the best resolutions in the biological application of AFM and undoubtedly the best one in imaging bacteriophage T4. In the application of AFM to the imaging of the fine structure of isolated biological specimens, it has been recognized that one of the crucial factors for obtaining good resolution is stable adsorption of the samples to the substrate surface and scanning with a sharp tip with minimal force application to the sample. In this report we used dried samples on silicon wafer with the sole purpose of getting best resolution in the imaging of fine structure of bacteriophage T4 and have been successful in visualizing its tail fibers 2–3 nm in diameter. Since a similar degree of resolution was expected but not achieved in the imaging of surface topography of either the head or the tail, our next goal is to obtain finer details on the surface of the bacteriophage T4 and other biological specimens.

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