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Diagnostic

Atomic force microscopy as a tool of inspection of viral infection

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Abstract Here we present a short review of application of atomic force microscopy (AFM) for investigation of viruses, accompanied by examples of high-resolution AFM images of different viral particles. The possibility of using AFM to identify viruses is discussed.

Key words: Atomic force microscopy; Bionanotechnology; Viruses

Development of atomic force microscopy (AFM) technique has significantly increased the number of tasks that can be carried out in biological and medical investigations. Operation of AFM is based on the raster scanning of a sharp probe that interacts locally with the surface of a specimen [1]. The most remarkable feature of AFM, which is high-resolution imaging of soft living objects, allows the study of single agents of infection—viral particles.

Viruses cause infectious diseases in plants, animals, and humans, and they can reproduce only in living cells. Different viruses vary in shape. Many bacteriophages have a head and a tail; moreover, viruses may have rectangular, spherical, rodlike, filamentous, multifaceted shapes. Their sizes also vary from 20 to 400 nm.

The use of AFM has made it possible to study mechanical properties of single viruses [2], their interaction with each other and different surfaces [3] and with living cells, degradation processes [4,5], growth of viral crystals [6], and packing of viral DNA [7], among others.

Protease treatment can achieve degradation of viral particles, as Day et al. observed, when condensed RNA was evident after several hours of such treatment of satellite tobacco mosaic virus (sTMV) [8]. Partially uncoated TMV particles with protruding RNA molecule from one or both ends were also imaged with AFM after stripping of tobacco mosaic virions with mild alkali or urea and dimethylsulfoxide [4]. Release of RNA from human rhinovirus was examined with AFM by Kienberger et al. [9].

Many viruses represent very convenient systems for studying crystallization processes in solution. AFM in liquid currently allows one to observe the dynamics and growth peculiarities of crystals from different icosahedral plant viruses within the time range of as long as several days [6,10]. Maximum resolution (down to capsomeres) is achieved specifically on crystal surfaces [10].

Investigation of adsorption and adhesion of viruses on model surfaces is important for study of the interaction between viral particle and host cell, detection of viruses, and their purification. Adsorption of viruses onto model surfaces with properties similar to the properties of cell membranes, can mimic the initial stage of "virus-host" interaction.

We should emphasize the importance of the immobilization procedure of viruses for AFM, including the choice of substrate, type of its modification (if needed), and application technique. For example, the ability of a highly oriented pyrolytic graphite (HOPG) surface to absorb TMV particles is several times greater than that of a mica surface. In addition, HOPG may affect TMV particles, leading to their partial destruction [11].

So, the immobilization procedure was proved to be specific for each virus. Thus, for every particular virus one has to seek the appropriate method of sample preparation for acquiring adequate AFM images of that virus.

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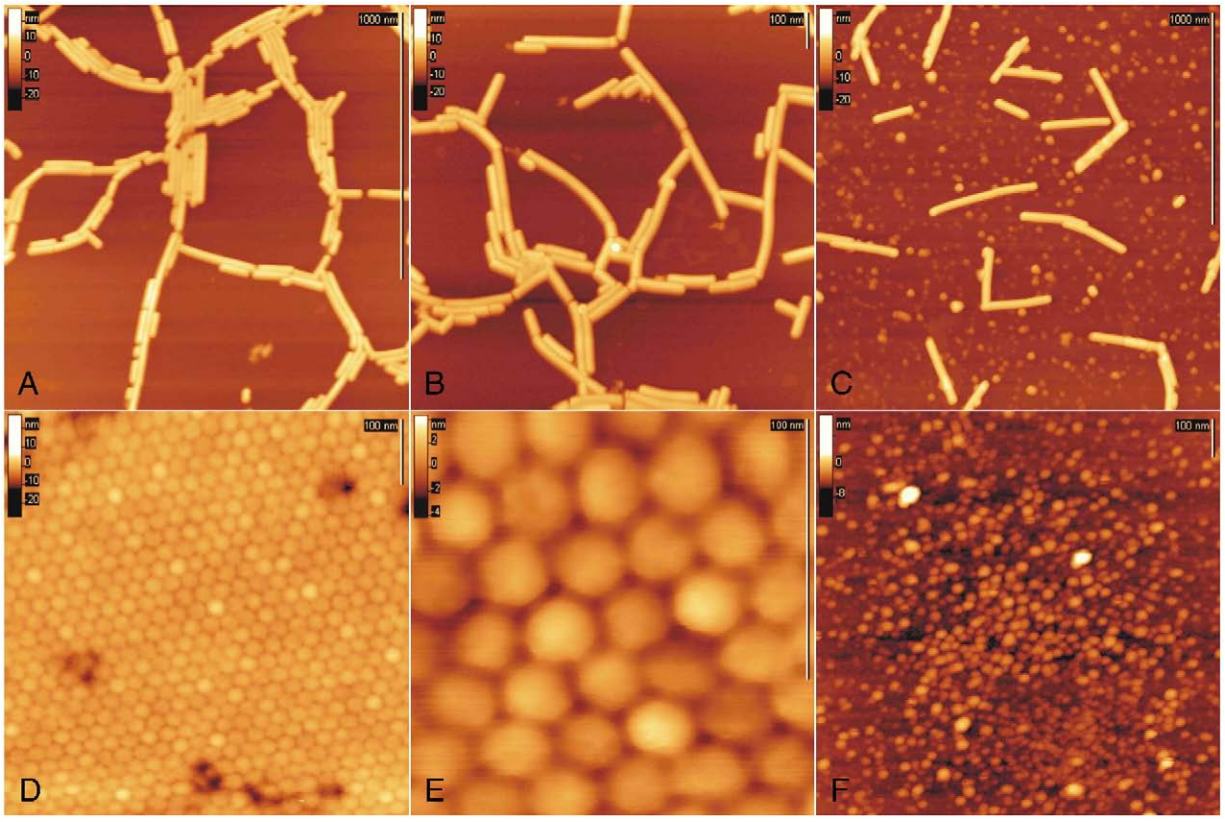

Fig 1. AFM images of (**A**) PSLV, (**B**) BSMV, (**C**) TMV, (**D** and **E**) BMV, and (**F**) AMV. Atomic force microscope Nanoscope IIIa (Digital Instruments) was used in all experiments. AFM measurements were established in air in tapping mode using commercial silicon nitride cantilevers. A droplet containing 5 μL of virus-containing suspension was placed on the surface of freshly cleaved mica, then left for 30 minutes in a wet atmosphere for adsorption, and then dried in a vacuum desiccator just before imaging. Scale bar corresponds to 1 μm (**A** and **C**) and 100 nm (**B**, **D**–**F**).

The aim of this work was to obtain AFM images of different-shaped viruses from several families (to create their image library) and to compare their properties so as to demonstrate the potential for virus identification using AFM.

Materials and methods

All measurements were performed with a Nanoscope IIIa multimode scanning probe microscope (Digital Instruments, Santa Barbara, CA) in contact and tapping modes. For tapping mode measurements, we used commercial silicon 125-μm cantilevers (NanoProbe, Neuchatel, Switzerland) with a spring constant of 42 N/m and 280–310 kHz resonant frequencies and 100 μm noncontact cantilevers with 200–300 kHz resonant frequencies (State Research Institute for Problems in Physics, Zelenograd, Moscow, Russia). For contact mode measurements we used silicon nitride cantilevers (Nanoprobe) with a spring constant of 0.06 and 0.32 N/m. FemtoScan Online software was used for the image processing [12].

For sample preparation we deposited a droplet containing 5 μL of colloidal solutions of viruses onto a freshly cleaved piece of mica and left this assembly to stand in a humid chamber for 30 minutes. Substrates with adsorbed virus were rinsed with triply distilled water and dried in a vacuum desiccator just before imaging.

Results

Because it makes possible the imaging of single viruses in liquids, AFM opens new perspectives on methods of creating rapid medical testing systems. One of the challenges of this important task is the development of an AFM image library of viruses. As an example, here we present AFM images of five plant viruses (Figure 1): tobacco mosaic virus (TMV), brome mosaic virus (BMV), *Poa* semilatent virus (PSLV), barley stripe mosaic virus (BSMV), and alfalfa mosaic virus (AMV).

The differing shapes and sizes of viruses allow the discrimination of various viral strains in the AFM images. For example, Figure 2 presents the length distributions of TMV and BSMV. Distribution maximums fit the values 300 nm for TMV and 135 nm for BSMV. The presence of viral particles whose length is smaller than the most probable value indicates their partial destruction, and the

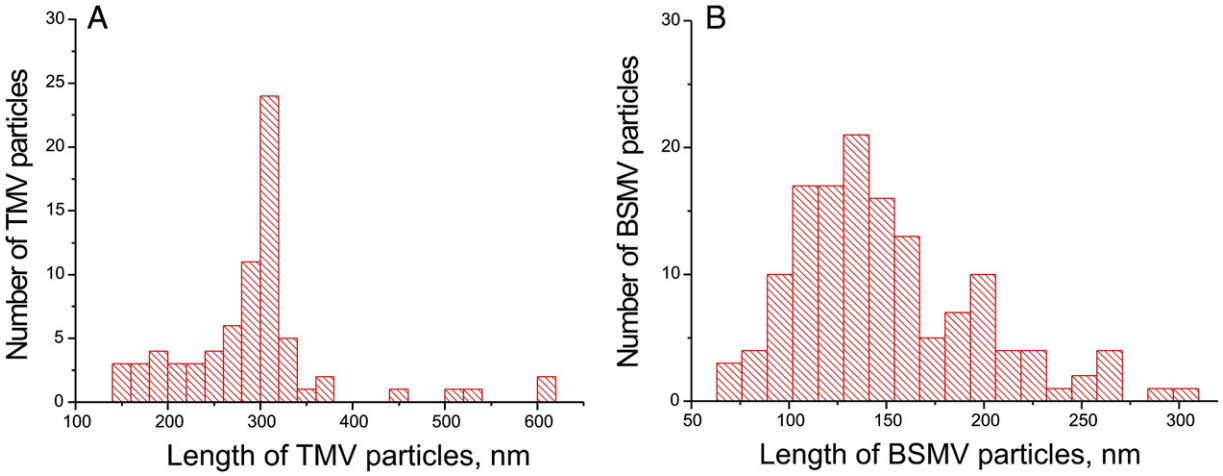

Fig 2. Length distributions of (**A**) TMV and (**B**) BSMV particles, built from the AFM images.

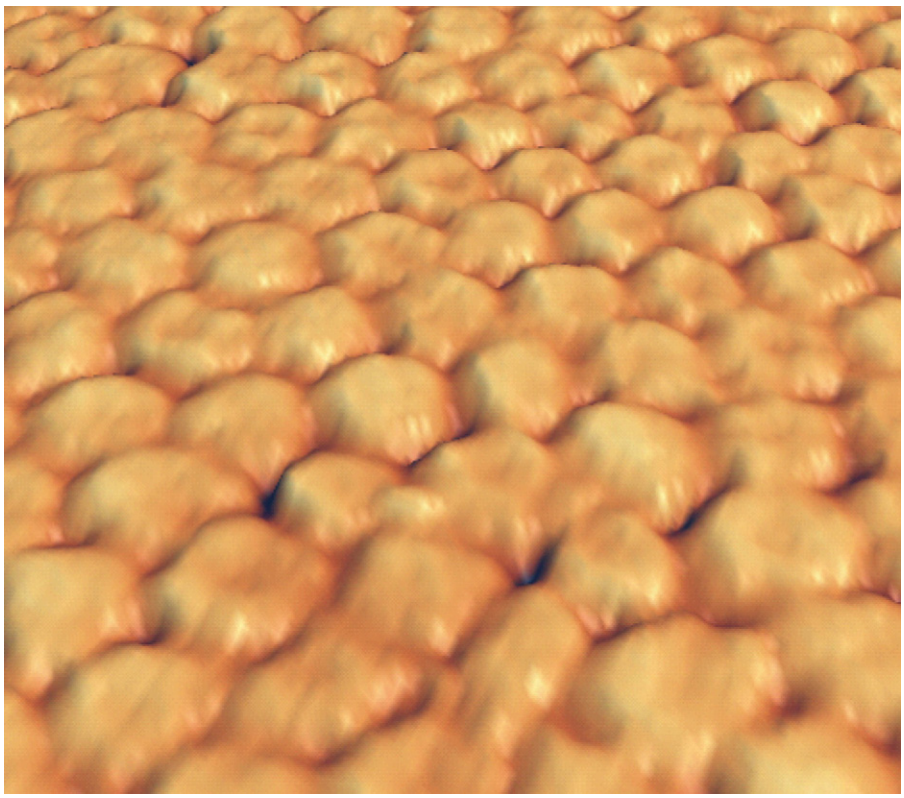

Fig 3. High-resolution 3D AFM image of BMV. Image size is approximately 300 × 300 × 5 nm^3.

presence of longer particles indicates the ability of virions to join in an end-to-end manner.

Discussion

It is remarkable that as a rule viruses have fixed dimensions that do not vary from particle to particle (as is the case, for example, for cells). In this regard viruses can be used as biological nanotemplates for calibration purposes [11]. Length, height, and diameter of viral particles can serve as their identification parameters.

Topographic features are the main but not the only criterion for detection and identification of viruses with AFM. They can be also differentiated by their properties: local frictional and adhesion forces, elastic and viscous properties of outer surfaces with high spatial resolution, and also by the mutual interaction of viral particles. An example of the latter can be clearly seen in Figure 1, *D–F*; even though they belong to the same *Bromoviridae* family, BMV (Figure 1, *D*, *E*) and AMV (Figure 1, *F*) have differing ability to adhere to each other, leading to different structures on the surface. If AMV particles accidentally adsorb onto

the mica surface, BMV forms a 2D crystal. As we have already mentioned, maximal AFM resolution is achievable on crystal surfaces. A high-resolution 3D AFM image of a BMV crystal is shown in Figure 3. The period of this crystal is 28.4 ± 0.3 nm.

Creation of the AFM image library of viruses together with investigation of their properties on the nanometer scale is an important step in their rapid identification. We demonstrate here, using the example of plant viruses, that as a result of both the development of AFM technique and the peculiarities of viruses as the object for AFM, such identification has now become possible.

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