
High-speed AFM for Visualizing Biomolecular Processes

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Single-molecule observation by fluorescence microscopy provides us with information of translational or rotational motion of the fluorescent spots emitted from individual molecules labeled with a fluorophore. Such information is valuable for dissecting the dynamic behavior of the labeled biomolecules at work. However, we still feel as if we are trying to scratch our foot through our boot, because we have to infer from the observed behavior of the fluorescent-spots how the labeled molecules are behaving in fact. If possible, we should observe directly single molecules themselves at nanometer and millisecond resolutions, which is much more straightforward to understanding biomolecular behavior rather than observing fluorescent spots.

AFM is a unique microscope that made it possible for the first time to observe the nanometer-scale world in liquids. Although it can visualize the structure of unstained biomolecules under physiological solution conditions, it takes minutes to get an image, which is too slow to observe dynamic biomolecular processes. This slow imaging rate originates in that AFM employs mechanical scanning to detect sample height at every pixel. It is quite difficult to move very fast a mechanical device with macroscopic dimensions at sub-nanometer accuracy without producing unwanted vibrations. Various efforts carried out in the past decade have been improving the imaging rate of AFM. Key devices for high-speed imaging are (1) small cantilevers with a high-resonant frequency and a small spring constant, (2) a high-speed scanner with a high resonant frequency, (3) active damping techniques to suppress mechanical vibrations of the scanner, (4) fast feedback control. The current high-speed AFM can capture images on video at ~30 frames/s for the scan range of ~240 nm and 100 scan lines, without significant disturbance of weak biomolecular interaction. Recent studies demonstrated that this new microscope could reveal biomolecular processes such as myosin V walking along actin tracks and association/dissociation dynamics of chaperonin GroEL-GroES that occurs in a negatively cooperative manner. These demonstrations clearly indicate that high-speed AFM has a great potential of giving straight and quick answers to how and what structural changes of individual molecules progress while their physiological function is being produced.

One of the dreams that biological science had longed for was materialized at last to some extent. However, the force exerted between the oscillating tip and sample is in the level just small enough not to break weak protein-protein association. In order for high-speed AFM to become truly useful to studies on a wide variety of biological systems, reduction in the force is essential. If the non-contact condition is realized in high-speed imaging, small cantilevers that have higher stiffness and hence higher resonant frequencies can be used for bio-imaging, which leads to higher imaging rates. In addition, it allows us to study biomolecular processes that occur on living cell membranes, which tremendously expands the usefulness of high speed AFM in biological science. We need to carry out efforts further to exploit this difficult yet quite dreamful challenge.

References

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