High-speed atomic force microscopy for directly capturing dynamic biomolecular and cellular processes on video

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Proteins are dynamic in nature and function at the single molecule level. Therefore, directly observing individual molecules at high spatiotemporal resolution must be a straightforward approach to understanding how proteins function. However, the functional mechanism of proteins has been studied by techniques that can obtain their detailed but static structures or single-molecule optical techniques that detect the dynamic behaviors of optical markers attached. Therefore, these traditional approaches only provide indirect information of how proteins really work. Although atomic force microscopy (AFM) allows us to directly observe individual molecules in physiological solutions, its imaging rate is too low to reveal their dynamic action. To overcome this limitation of AFM, various efforts have been carried out in the last two decades and consequently high-speed AFM has now come of age. It can film protein molecules in action at sub-100 ms temporal and sub-molecular spatial resolution without disturbing their function. Various application studies carried out recently on protein systems have provided not only corroborative "visual evidence" for previous inferences of their molecular processes but also resolved long-standing questions that have previously been difficult or impossible to address by traditional approaches. Therefore, the filmed images have successfully provided more and deeper insights into how the proteins operate to function. High-speed AFM techniques have now being further advanced in various directions: wide-area/fast scanning, sample manipulation during imaging, tip-scan mode HS-AFM for large samples, and combination with light microscopy techniques. Some of these developments have already made it possible to visualize dynamic events occurring in live cells. Thus, high-speed AFM will become a more useful and indispensable tool for biological research in the near future.

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