HIGH-RESOLUTION AND HIGH-SPEED ATOMIC FORCE MICROSCOPY SIMULTANEOUS TO ADVANCED OPTICAL MICROSCOPY

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Abstract

In recent years, atomic force microscopy (AFM) has become a well-established technique for single molecule studies and even sub-molecular scale research. Several new developments in terms of faster AFM imaging and imaging modes, based on the phase or frequency, have been established in order to decrease the cantilever response time and increase the AFM's scan speed, e.g., for studying molecular dynamics. The novel NanoWizard® ULTRA Speed AFM combines the latest scanner technologies and compact design allowing a full integration of AFM into advanced commercially available optical microscopy. Thus, fast AFM imaging of approximately 1 frame per second can be seamlessly combined with methods such as, fluorescence, confocal, TIRF, STED microscopy and many more. Individual molecule dynamics can now be studied with AFM and simultaneously with optical microscopy by applying JPK's tip scanner technology.With JPK's HyperDrive™ sub-molecular resolution is achieved even on soft samples imaged in liquid environments. It allows for imaging with smallest amplitudes of often approximately 0.2 nm for lowest tip-sample interaction. Topographical images of membrane proteins and DNA-origami are presented. It has been shown that the phase response in phase modulation AFM (PMAFM) is faster allowing higher imaging speeds for the study of molecule kinetics. In conjunction with JPK's NanoWizard® ULTRA Speed AFM, a dynamic biomechanical study of Bacteriorhodopsin (bR) when interacting with photons will be discussed.More than half a century after the first high-resolution electron microscopy images of collagen type I banding of 67 nm have been reported, now with the NanoWizard® ULTRA Speed AFM we could gain a high-resolution temporal insight into the dynamics of collagen I fibril formation and its characteristic 67 nm banding hallmark. The literature still abounds with conflicting data regarding the models of its fibril formation, structural intermediates, and kinetics. AFM is the only currently available high-resolution imaging technique amongst many to offer insight into the collagen I fibrillogenesis by operating in situ. The described technique could be instrumental for future studies of the structural dynamics of protein systems, etc. The systems newly gained flexibility will also be demonstrated on a study of living fibroblast cells directly imaged in their culture petri dish at 37 degrees C. Here, the dynamics of individual membrane structures is investigated with AFM while simultaneously observing the individual living cell with optical phase contrast. The unambiguous correlation between AFM and optical microscopy is achieved by the DirectOverlay™ technique.

Keywords: Atomic Force Microscopy, Optical Microscopy, fast scanning AFM, submolecular resolution, force spectroscopy

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