

# Improvements in high-speed AFM and observation of membrane protein dynamics

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High-speed atomic force microscopy (AFM) is a unique tool to investigate the dynamic behaviors of proteins at works. Owing to the efforts for improving the scanning speed and feedback performance, an imaging rate of 50ms/frame has been achieved and then it is now possible to routinely observe dynamic actions of proteins such motor proteins. However, the imaging rate would be still insufficient to capture faster movement. Also the information we can take from the images was limited to only structure although tapping-mode AFM has a capability to sense chemical and mechanical properties of surface through detecting the phase difference of the cantilever oscillation. Here, we present some improvements to overcome these current limitations regarding the imaging rate and the phase imaging. Also, we show the recent results which show one application of high-speed AFM to observe dynamic behavior of membrane proteins.

## ***I. Direct control of tip-surface distance using photo-thermal actuation of a cantilever.***

An intensity-modulated infrared laser beam was used to photo-thermally deflect small cantilevers. The slow response of the photo-thermal expansion effect was eliminated by inverse transfer function compensation. By regulating the laser power and hence regulating the cantilever deflection, the tip-sample distance was controlled, which was made much faster than conventional piezoactuator-based z-scanners because of the very high resonant frequency of the cantilevers. Using this control, video-rate imaging of protein molecules in liquids was achieved for the scan range of 250nm.

## ***II. Fast phase imaging on polymer surface***

We developed a fast phase detector which can detect the phase difference between the cantilever oscillation and the excitation signal at each oscillation cycles. The phase-shift images clearly revealed the compositional heterogeneities in styrene-butadiene-styrene block copolymer films even at an imaging rate of more than 100 ms/frame.

## ***III. Dynamic observation of purple membrane***

We applied high-speed AFM to image purple membrane (PM) in a buffer solution. We observed fluctuations of the crystal structures at the edge of PM due to desorption of bacteriorhodopsin (bR) trimers, diffusion of bR trimers in lipids, and decrystallization process of PM induced by photo bleaching. We also succeeded in imaging bR trimers faster than video rate. These results will open up new possibilities for studying membrane assembling processes and protein-protein interactions in membrane.