

**Combining bio-AFM with advanced optical techniques;  
CLSM, FCS and tip-enhanced optics**

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The atomic force microscope (AFM) has been widely applied to imaging of biomolecules and other biological samples. Here an AFM is used that is optimized for the combination of imaging and force measurement on biological samples with advanced optical techniques. This AFM using three closed loop scan axes has demonstrated the high stability and low noise required for high resolution imaging of single molecules up to whole cells. The tip-scanning AFM system allows a combination with a stationary stage for laser scanning confocal imaging (including FCS). In addition, new software is available to allow true integration and calibration of images from AFM and light microscopy.

The AFM has here been combined with a commercial confocal laser scanning microscope (CLSM) to perform simultaneous AFM and CLSM imaging and manipulation on supported lipid bilayers and cell samples. This combination extends the information that is available from these studies by the complementary information from the optical and scanning probe techniques. In supported lipid bilayers, structures were visible in the AFM images that could not be distinguished using the CLSM. The CLSM allowed higher speed imaging of the membrane response to deliberate manipulation from the AFM tip, giving information on the dynamics of domain reorganization. The fluorescence images from the CLSM provide information on the composition of the structures seen with the AFM and this has also been extended to using fluorescence correlation spectroscopy (FCS) to study the diffusion constants of lipids in the bilayers. On cells, the CLSM allows functional interpretation of the AFM surface structures seen by fluorescent labelling for different proteins. The CLSM allows optical imaging of the topmost "slice" of the cell to isolate the molecules that are actually present in the structures seen in the higher resolution AFM images. With the calibration of the optical image, true integration allows precise comparison of images from the two different sources.

A trend in optical spectroscopy has emerged that makes use of the interaction of an AFM tip with a sample in an external optical field. Special attention has been devoted to tip enhanced Raman spectroscopy and to the manipulation of molecules and particles with simultaneous fluorescence. JPK picks up these trends and presents the Tip Assisted Optics (TAO) module as an add-on to the tip-scanning NanoWizard AFM. The TAO system consists of an additional 100x100µm X-Y-scanner for the sample, which is independently and simultaneously used with the AFM. This allows for the precise compensation of the offset between confocal image obtained with the sample scanner and the AFM topography. The tip can be positioned exactly into the optical focus while the user can navigate within the AFM image. Thus the tip-enhancement effect can be maximized and it becomes possible to do single molecule manipulation experiments within the focus of a confocal optical setup.

**KEY WORDS:** atomic force microscopy (AFM), confocal laser scanning microscopy (CLSM), fluorescence correlation spectroscopy (FCS), supported lipid bilayer (SLB)