



## **Tutorial I:**

### **AFM for Life Sciences - Introduction**

This session will provide an introduction to atomic force microscopy with a focus on enabling research in biology. A live demonstration will cover practical hands-on aspects, while a review of the tip-sample interaction at the core of this technique will provide the theoretical underpinnings needed to understand imaging modes and their benefits and limitations in biological applications. The focus will be on imaging in aqueous solution, addressing the nondestructive, in situ interrogation of biological samples. The discussion will include the investigation of dynamics in such processes as protein unfolding using force spectroscopy, the natural complement to AFM imaging.

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## **Tutorial IIa:**

### **High Resolution Imaging**

This tutorial focuses on the ability of the AFM to image biological samples far beyond the diffraction limit of optical microscopy. AFM can provide spatial resolutions of a few nanometers and below. Actual achievable resolution depends on the size of the tip (can be as small as 1 nm in radius) and the mechanical properties of the biological sample. Highest resolutions providing submolecular details have been achieved on flat and stiff (non-motile) biological membranes.

It was possible to construct atomic models of supramolecular assemblies from topographical images, in the case of photosynthetic proteins or channel proteins. Furthermore, great progress has generally been made in cell imaging: On these soft and dynamic samples structural details in the order of tenth of nm can be imaged.

In addition, cellular machines have been studied at work at the single molecule level, like the RNA polymerase or channel proteins. AFM measurements can be carried out in liquid environment opening the possibility to study structure and function under relevant physiological conditions.

While in principle there is no need for sample staining, there are a number of important aspects for sample preparation that will be discussed, e.g. the selection of suitable substrates and buffer solutions.

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## **Tutorial IIb:**

### **Probing sample properties on a molecular level - Force Spectroscopy**

This tutorial will cover the unique capability of the AFM to study properties of biological and biomaterial samples on a molecular level and look at its potential to give better insights to biological functions. The AFM can probe forces existing between tip and sample with high spatial resolution. One can measure adhesion or elasticity properties by nano-indentation using bare tips; functionalized tips offer the possibility to investigate intermolecular affinities, e.g. in ligand-receptor binding studies. A striking force spectroscopy application is related to the unzipping or unfolding of individual bio molecules like membrane proteins or DNA. Dependent on environmental conditions molecules being stretched exhibit different (un)folding behavior due to specific properties of *intramolecular* bonds. Repetitive experiments give insight into the energy landscapes of individual molecules, thus revealing kinetic parameters of the process.



### **Tutorial III:**

#### **Multimodal imaging: Combining AFM and advanced optical microscopy methods**

Modern BioAFMs offer the ability to combine AFM with optical microscopy. We will take a closer look at the implementation and applications of the two methods, and show how correlation of data does open entirely new research approaches for structural and functional studies in Life Sciences.

AFM and optical microscopy are simultaneously used to probe dynamic functional properties and investigate their interdependencies. Consequently measurements of structural information (morphology, topography) can be associated to functional analysis (e. g. physiological and mechanical). New image overlay functionality of software allows correlation of optical/ fluorescence data with AFM topography and mechanical information.

The tutorial will explore use of transmitted light imaging techniques (e.g. Phase contrast, DIC) as well as fluorescence microscopy for visualizing samples, both in wide field mode (CCD based imaging) as well as confocal laser scanning microscopy, which can be utilized for advanced fluorescence imaging and analysis methods, such as FCS, FRET and FRAP.

While optical imaging hits a barrier with respect to lateral and axial resolution in the range of (half) the wavelength of illuminating light, AFM can deliver resolution down to several Nanometers laterally as well as axially, also allowing for quantitative topography and volume measurements.

New software functionality allows registering both microscopy techniques and execute multimodal imaging. We will explain and show how it is possible to select regions of interest of acquired optical images for examination with either via higher resolution AFM imaging or force measurements, thus allowing the correlation of structure and functional sample parameters including mechanical properties (e.g. physiological parameters and cell elasticity). Furthermore we discuss analyses of the impact of mechanical stress on biological samples monitored via fluorescence imaging.

Furthermore we will discuss aspects of configuration of combined systems for optical and atomic force microscopy.

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