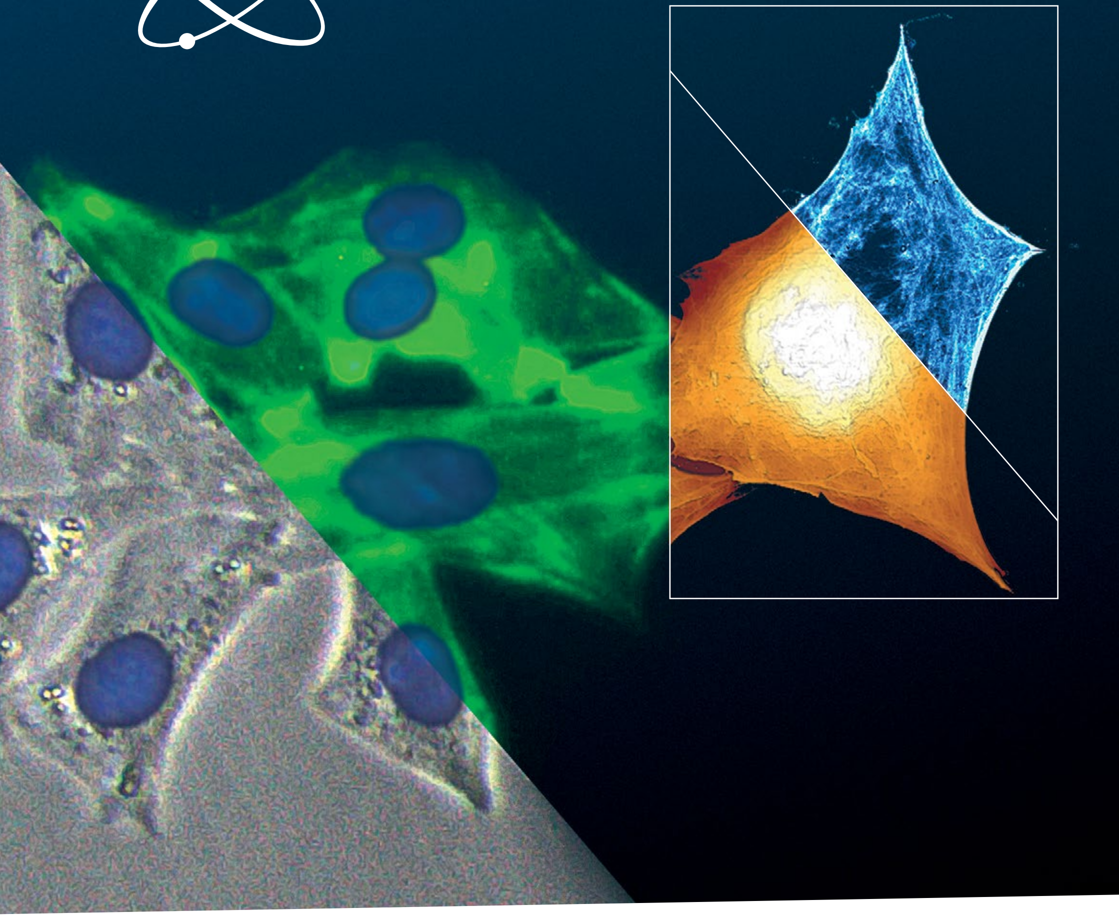

ATOMIC FORCE MICROSCOPY

Correlative AFM and Optical Microscopy in Life Science Research

A Brief Overview

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Introduction

The intricate details of small objects, invisible to the human eye, have always fascinated humankind. Historically, understanding biological complexity relied on the principle of "seeing is believing". Recent decades have seen substantial advancements in the field of microscopy, driven by the desire to unravel the relationship between structure and function in cells, organelles, and biomolecules.

Conventional light microscopy provides an enlarged, two-dimensional view of a sample by illuminating it with visible light and viewing it through an optical lens system. However, the resolution is limited by the wavelength of the light used, known as the Abbe diffraction limit, whereby only objects larger than approx. 250 nm can be sharply imaged.[1] In 1986, atomic force microscopy (AFM) emerged as a new three-dimensional approach,[2] quickly becoming a standard for the high-resolution structural analysis of samples ranging from single molecules to complex macromolecular systems.

AFM uses a sharp tip to scan the surface of an object, generating three-dimensional topographical images with nanometer resolution. In addition, it enables the multiparametric, quantitative characterization of a sample's nanomechanical properties. AFM can be combined with other microscopy techniques, such as optical microscopy, to reveal additional aspects of a sample, providing multi-level analysis and a more comprehensive understanding of the sample.

By combining AFM with fluorescent microscopy, specific molecules or features carrying immunochemical information can be directly targeted.[3] Both AFM and light microscopy can be operated under ambient environmental conditions, enabling live-cell imaging and leveraging the advantages of both techniques. The ability to obtain real-time, correlative data sets is particularly relevant in life science research.

Novel super-resolution microscopy approaches have emerged in light microscopy that surpass the diffraction limit of light,[4] such as structured illumination microscopy (SIM),[5] single-molecule localization microscopy (SMLM),[6,7] and stimulated emission depletion microscopy (STED).[8] Combining AFM with these advanced optical techniques is a powerful approach for investigating biological samples at the nanoscale that delivers profound new insights into molecular and cellular mechanisms and the relationship between structure and function.