Monday, February 21
1:30 PM – 3:00 PM
Esplanade, Room 157
Bruker

Application of Large Area Mapping AFM for Automated Structural and Mechanical Analysis of Developing Cells and Tissues

Active forces in biological systems define the interactions between single molecules, growing cells and developing tissues. Further development of novel biomaterials for tissue engineering is driven by the biomechanical and molecular cues provided to cells by their environment which are crucial parameters that influence motility, behavior, and the fate of progenitor cells.

AFM can be successfully applied for comprehensive nano-mechanical characterization of single molecules, cells and tissues, under near physiological conditions. Currently, the trend is to extend this by studying the mechanobiology of living cells while evaluating their structure and the interaction with their cell culture substrates. In particular, it is interesting to understand how cell behavior is driven by the cytoskeletal dynamics and cell mechanics in typical cell culture scaffold scenarios. We will introduce the concept of automated large area multiparametric characterization of densely packed cell layers and highly corrugated tissue samples, where full automation, smart mechanical sample analysis, multiple scanner technology, and optical integration is critical for data throughput and reliable correlative microscopy. We will discuss how these developments, in combination with advanced optical and super-resolution microscopy techniques, can overcome the inherent drawbacks of traditional AFM systems for characterizing challenging biological samples.

Cells adapt their shape and react to the surrounding environment by a dynamic reorganization of the F-actin cytoskeleton. We will demonstrate how cell spreading and migration in living KPG-7 fibroblasts and CHO cells, can be studied with high-speed AFM and associated with spatially resolved cytoskeletal reorganization events. We will further extend this with high-speed mechanical mapping of confluent cell layers, which in combination with optical tiling can be applied to automated analysis of large sample areas.

External mechanical stress is known to influence cell mechanics in correlation to the differences in actin cytoskeleton dynamics. As a tool for analyzing the complex cellular mechanobiology, we went beyond purely elastic models, and performed sine oscillations (up to 1 kHz, amplitude 5-60 nm) in Z while in contact with the surface to probe the frequency-dependent response of living fibroblasts. We will further discuss how to calculate the viscoelastic properties, characterized by the dynamic storage and loss modulus (E’, E’’) distribution in such samples.

In the past, investigating large and rough samples such as tissues and hydrogels using AFM was challenging due to the limited z-axis of the AFM. Using osteoarthritic cartilage as an example, we will demonstrate how a newly developed hybrid of a motorized and piezo stage enables multi-region AFM probing over a large, rough sample area while providing additional correlative optical data sets.

Speaker

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