Mapping cell mechanical properties down to molecular level can both refine our knowledge about cellular structures and reveal information about biological functions like cell adhesion, proliferation and survival. Atomic force microscopy (AFM) is a powerful method to probe materials at the nanoscale. Although AFM has been used to image live cells and probe their mechanical properties, its resolution on cells is much lower than on stiffer materials. To address this, we adapted Microsecond Force Spectroscopy to rapidly image mechanical properties of live and cross-linked fibroblasts, neurons and Human Umbilical Vein Endothelial Cells in culture media or buffer. Mechanical properties images showed cellular features as small as 50nm, representing an unprecedented resolution over a wide variation of cell types. The enhanced resolution and speed of our method allowed the identification of dynamic changes in elastic modulus of fine cellular structures, which did not appear to be reflected in optical images of fluorescently-labeled actin, acquired from the same cells. These changes might reflect a loss of tension in the actin network underneath the cell membrane. Preliminary data further suggest that our platform might allow the label-free recognition of stress fibers, retraction fibers, Weibel-Palade bodies and microvilli solely based on nanomechanical contrast. These developments extend the cell imaging capabilities of AFM and highlight the value of mechanics in the delineation of cellular physiological states. The compatibility of our method with human cells suggests that it can be further developed as a diagnostic tool for the detection of disease-specific cellular mechanical responses.


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Cancer Diagnostics by Multiparameter Fluorescence Image Spectroscopy: A Bioinformatic Classifier Trained on Cultured Immunostained Cells

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We have developed a new approach for multiplex imaging of immunostained cells to improve breast cancer diagnosis. For detection of fluorescence signal we use a confocal laser scanning microscope with custom extension for multi-parameter fluorescence image spectroscopy (MFIS)[1]. For the detection of tumor biomarkers a six fold parallel immunostaining protocol was developed, combining indirect immunolabeling with antibodies as well as direct labeling with novel affinity binders (Affibody molecules). To allow for separation of fluorescence photons from different markers, dyes were selected that can be discriminated by both their spectral emission bandwidth and their fluorescence decay time using three patterns maximum entropy fit. The massive amount of information contained in the identified spatial intensity features from the six markers and their correlations thereof necessitate advanced bioinformatics to fully exploit the available information. We used an analysis workflow to process the MFIS-data with the aim of training a classifier for cancer diagnosis.

The method provided promising results to discriminate non-malignant breast- and different breast cancer cell lines forming a basis for the analysis of individual cells in clinical fine needle aspirates (FNA) from the breast. Applied to FNA samples MFIS can demonstrate its full potential in extracting the necessary information for diagnosis out of a very limited number of cells. In a further perspective, our approach offers a more objective alternative to the currently common procedure of visual inspection of classical core biopsy histological staining. Moreover, it can be transferred to different samples and markers without major adaption of setup and analysis workflow to diagnose different cellular diseases.


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Metabolic Imaging of Colon Cancer Tumors In Vivo by Phasor Fluorescence Lifetime Microscopy of NADH

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