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 Second 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.

Although AFM has been used to image live cells and probe their mechanical properties down to molecular level, it is one potential method for tracking implanted cells. However, the utility of AFM for bone regeneration from myoblasts and stem cells in a mouse injury model with high anatomical detail remains to be explored. Here we report three new red-absorbing monomeric FPs obtained to rapidly image mechanical properties of live and cross-linked fibroblasts, neurons and Human Umbilical Vein Endothelial Cells in culture media or buffer.

The nuclear pore complex (NPC) is one of the largest macromolecular assemblies in eukaryotic cells and tightly regulates the transport between the cytoplasm and the nucleus. In order to unravel the structural details of the NPC, an exact stoichiometry of its constituent proteins (NUPs) is needed. Mass spectrometry has been exploited to establish stoichiometric ratios of the NUPs, but in order to trace absolute copy numbers it is necessary to quantify the number of instances of at least one NUP. To achieve this goal, a gene replacement strategy was combined with a quantitative PALM approach: one of the core proteins of the NPC, namely NUP107, was genetically tagged with the photo-activatable protein mEos2 and expressed in HEK293 cells where native NUP107 was knocked down using micro-RNAs. For optimal signal to noise, NUP107-mEos2 containing NPCs from functional purified nuclei were placed in the evanescent field of a highly sensitive microscope. We then performed PALM imaging of mEos2-NUP107 with an optimized photo-conversion scheme for iterative activation and bleaching cycles assuring complete photo-conversion of the sample. In order to retrieve the average number of fluorophores in each pore, a computational workflow was designed 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.

We 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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