CORRELATIVE AFM/STED AND AFM/FLIM IMAGING FOR THE INVESTIGATION OF MECHANICAL AND FUNCTIONAL MATERIAL PROPERTIES

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Fluorescence and super-resolution microscopy techniques are being used with great success in biological and materials research. A key question in the field is how the functional information obtained through such methods can be related to the mechanical properties of imaged specimens. To address this question, we have developed novel imaging platforms, which combine Atomic Force Microscopy (AFM) with Stimulation Depletion Emission (STED) microscopy and with Fluorescence Lifetime Imaging (FLIM). We demonstrate the versatility and potential of such correlative techniques to provide functional, structural and mechanical information simultaneously.

AFM and STED provide resolution on the nanometre scale, and correlative AFM/STED provides insights into both physical and biochemical information of the sample. We addressed challenges of accurately registering images obtained with the two techniques and achieve reliable image overlay. We present data from the nano-mechanical mapping of specimens using this correlative method. We also developed a correlative AFM/FLIM platform that relates mechanical and functional properties of imaged samples. FLIM provides functional information on the molecular environment of the targeted emitter, which is complemented by mechanical and morphometric data obtained by AFM.

We present the application of both techniques to a variety of samples, and discuss advantages and potential, but also challenges and limitations. In one example we report on the observation of the plasticity of cytoskeletal structure in live astrocytes during polarised migration and correlate this information with physical properties of the membrane [1]. In an *ex-vivo* approach, we applied AFM/STED to α-Synuclein fibrils interacting with synaptic vesicles, to elucidate changes in the mechanical properties of the vesicles upon interaction with the monomeric or fibrilar forms of the protein. These measurements provide insights in the context of neurodegeneration. We have used AFM/FLIM to observe changes in the nuclear stiffness of SHSY5Y cells as a response to FUS protein aggregation. In non-biological applications, we have used combined AFM/FLIM for the characterisation of thin perovskite films in research to develop novel luminescent probes. The combined mechanical and functional information reveals how structural defects on perovskite grains affect the photoluminescence lifetime of the material. We discuss future potential and aims for further development of correlative AFM / fluorescence imaging techniques.

1. Curry N, Ghézali G, Kaminski Schierle GS, Rouach N, Kaminski CF. Correlative STED and Atomic Force Microscopy on Live Astrocytes Reveals Plasticity of Cytoskeletal Structure and Membrane Physical Properties during Polarized Migr ation. *Front Cell Neurosci*. 2017;11:104.