# Combining Tip-Scanning AFM with Super-Resolution Optical Imaging towards Multiparametric Correlative Microscopy

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The last three decades have established atomic force microscopy (AFM) as an indispensable tool for the high-resolution structural analysis of specimens ranging from single molecules to complex biological systems[1]. AFM currently offers premium spatial resolution of the analysed samples, while simultaneously being able to correlate topography and mechanics at near native/ physiological imaging conditions. In turn, the combination with advanced/customised optics leverages the advantages of immunolabelling techniques for true correlative microscopy. Recording the stimulated emission depletion (STED) microscopy fluorescence delivers a multi-colour image with a 6-10 times enhanced spatial resolution compared to conventional optical methods and, therefore, reaches the same order of magnitude as the spatial resolution of AFM[2]. Furthermore, structured illumination microscopy (SIM) offers a unique possibility to go below the optical diffraction limit while simultaneously operating and acquiring AFM images.

We will demonstrate how AFM imaging and super-resolution 2color easy3D STED measurements can be combined, and will show results of co-localized imaging and sample manipulation with a precision far below the diffraction limit. This can be applied for the comprehensive investigation of biological samples and allow for the immunological assignment of the high-resolution cytoskeletal filaments in living fibroblasts. The mechanical stimulation of microtubules and actin filaments with AFM in living cells while performing STED experiments will be presented. We will also show examples of the accuracy of registering/overlaying AFM and optical images on commercially available DNA origami structures with dimensions below the diffraction limit.

Correlating data from different microscopy techniques has the potential to discover new facets of signalling events in cellular biology. We have recently demonstrated for the first time a hardware set-up capable of achieving simultaneous co-localized imaging of spatially correlated, far-field super-resolution fluorescence microscopy and AFM[3]. We will demonstrate the system performance using sub-resolution fluorescent beads and a test sample of human bone osteosarcoma epithelial cells with plasma membrane transporter 1 (MCT1).

[1] M. W. Amrein and D. Stamov, 'Atomic Force Microscopy in the Life Sciences', in *Springer Handbook of Microscopy*, P. W. Hawkes and J. C. H. Spence, Eds. Cham: Springer International Publishing, 2019, pp. 1469–1505.
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[3] A. I. Gómez-Varela *et al.*, 'Simultaneous co-localized super-resolution fluorescence microscopy and atomic force microscopy: combined SIM and AFM platform for the life sciences', *Sci. Rep.*, vol. 10, no. 1, p. 1122, Dec. 2020, doi: 10.1038/s41598-020-57885-z.