Correlative atomic force and super-resolution fluorescence microscopy: a novel tool for characterization at the nanoscale

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Abstract

Fluorescence microscopy is an essential tool in many fields of science, particularly in biological and biomedical research. However, its spatial resolution is limited by light diffraction to about 200 nm, which precludes its application to the study of subcellular structures and its wide implementation in nanoscience. Currently, approaches for improving the spatial resolution in fluorescence microscopy are experiencing a spectacular expansion and recognition, including the recent award of the Nobel Prize in Chemistry 2014. Several imaging schemes have been successful in breaking the diffraction limit and achieving a spatial resolution of tens of nm [1]. One alternative for super-resolution imaging is photoactivated-localization microscopy (PALM), also termed stochastic optical reconstruction microscopy (STORM). PALM, STORM and related techniques rely on the combination of photoswitchable fluorescent labels, a wide-field fluorescence microscope with single-molecule sensitivity, and image post-processing. These techniques are in continuous development, and new fluorescence labelling and image analysis methods need to be tested. Both labelling and post-processing analysis are prone to imaging artifacts, therefore new tools that allow robust validation of super-resolution images are needed. For that purpose, we have implemented a novel correlative microscope that allows sequential *in situ* imaging of the same sample area by atomic force microscopy (AFM) and PALM/STORM [2]. The technical aspects of the correlative microscope, including image alignment and sample preparation requirements will be discussed, as well as its application in optimizing DNA super-resolution imaging. The combination of super-resolution and AFM is not only a useful tool to improve current nanoscopy methods but also to answer new biological questions.

References

[1] S. W. Hell, Nat. Methods, **6** (2009) 24.

[2] A. Monserrate, S. Casado, C. Flors, ChemPhysChem, 15 (2014) 647.