Simultaneous advanced microscopies for cellular dynamics

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Abstract

Atomic Force Microscopy (AFM) has been combined with different fluorescence microscopy techniques [1]. However, the vast majority of the proposed set-ups does not enable simultaneous observation of the same spatial volume. This severely limits the utility of combing both image techniques as all dynamical information is lost.
Here, we discuss approaches and tools to minimize the mechanical and thermal noise, which are the major culprits hindering simultaneous spatiotemporal data acquisition with AFM and fluorescence microscopy. We have successfully circumvented existing problems and demonstrated simultaneous operation of aperture correlation microscopy through a Differential Spinning Disk (DSD) approach and nanomechanical mapping AFM [2,3]. We present the integration of a DSD imaging platform and an advanced bioscience AFM system capable of Quantitative Imaging (QI). This platform enables the collection of registered optical sectioning fluorescence and nano-mechanical mapping information of U2Os cells. The DSD illumination light fluctuates at time-scales much faster than the AFM cantilever movement providing a near-constant AFM cantilever illumination. Depending on the cantilever under consideration, this has the potential to avoid involuntarily induced cantilever bending by the fluorescence excitation light, which is an important problem when integrating far field fluorescence microscopy techniques with AFM.

References

[1] Moreno Flores, S. & Toca-Herrera, J. L. Nanoscale, 1 (2009), 40–49.
[2] Miranda, A., Martins, M. & De Beule, P. A. A. Rev. Sci. Instrum. 86 (2015).
[3] A. Miranda, M. Martins and P. A. A. De Beule, European Patent Office, EP15179924 (2015).

Figures

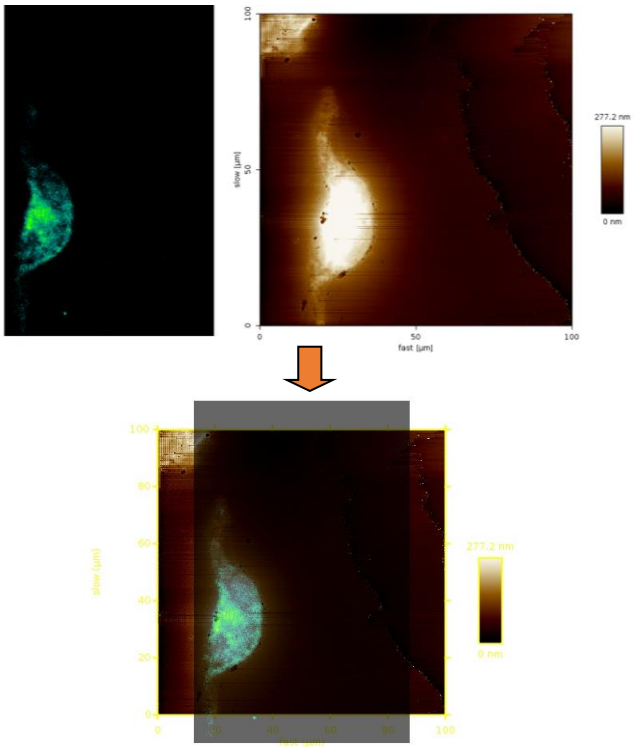

Figure 1: A fluorescence optical sectioned image and AFM QI™ height extended map and the corresponding image overlay of an U2Os cell labelled with GFP. For the AFM image we used triangular MSCT cantilevers. The scan was obtained over an area of 100x100 µm with 512x512 pixels.