

CORRELATIVE STORM/PALM AND ATOMIC FORCE MICROSCOPY: INVESTIGATING THE STRUCTURE AND MECHANICS OF CELL ADHESIONS

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ABSTRACT

Fluorescence localization microscopy techniques (STORM/PALM) and atomic force microscopy (AFM) are both capable of imaging subcellular features in physiological buffers at resolution well below the diffraction limit. The type of information obtained by these techniques is very different: AFM provides height and/or mechanical property maps of the sample surface, and STORM/PALM the location of labelled biomolecules inside the cell.

We have build a setup for combined localization microscopy and AFM for functional imaging of cell adhesions. In macrophages the formation of podosomes — micron-sized circular adhesion/invasion structures on the cell surface — is linked to cell motility and pathologies such as cancer and chronic inflammation. To build a dynamic molecular model of podosomes, we employ STORM/PALM to image the location of fluorescently labelled podosome components, while at the same time mapping the spatial variation of stiffness with AFM.

The combination of AFM with STORM was previously hindered by the need to change buffers between imaging modes. By using a new fluorescent dye that enables STORM imaging in a buffer that is also compatible with AFM,[1] we demonstrate correlative AFM and STORM on fixed cell samples without the need to change buffers, see Fig 1. We have also created cDNA constructs to label different podosome components with photoswitchable fluorescent protein mEOS3.2, which enables localization microscopy in living cells (PALM).

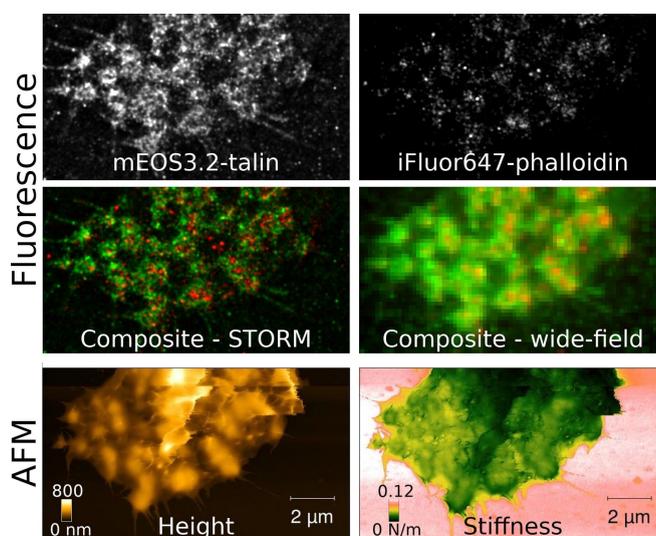


Fig 1: Podosomes in a fixed, unroofed THP1 cell imaged with correlative 2-colour STORM and AFM.

References:

- [1] L. M. Hirvonen & S. Cox, “STORM without enzymatic oxygen scavenging for correlative atomic force and fluorescence superresolution microscopy,” *Methods Appl Fluoresc*, **6**, 045002 (pp.1-8), 2018.