Correlative live cell STED and atomic force microscopy identify changes of cytoskeletal organisation and cell physical properties during polarised migration of astroglia.

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Astroglia are motile signalling elements which undergo significant morphological maturation during development. Morphological changes, such as polarised migration, are thought to be related to dynamic reorganisation of the cytoskeleton and to changes of the physical properties of the membrane, affecting cell stiffness. Here, atomic force microscopy (AFM) and stimulated emission depletion (STED) super-resolution imaging are combined to investigate cytoskeletal organisation and membrane properties during polarised migration. AFM permits measurement of cell topography and mechanical properties and optical microscopy identifies the underlying cytoskeletal elements [1]. Here, super-resolution microscopy is required to enhance the resolution of the dense cytoskeletal structures [2] and has the advantage that it can be carried out on living cells.

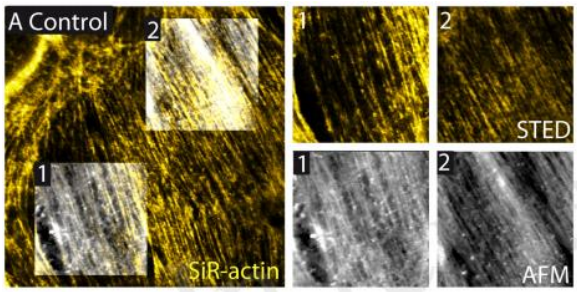

Figure 1: Correlative STED/AFM image of astroglia. Actin (yellow) is super-resolved using STED. AFM topography image is directly overlaid.

Super-resolution imaging of live astrocytes is performed under basal conditions and during polarised migration. A custom STED super-resolution microscope is used to image the actin or tubulin cytoskeleton. AFM height and modulus images are acquired on the same field of view and images are directly overlaid (figure 1). In control conditions, AFM height images reveal a homogenous distribution of stiff filaments which correlate with actin fibres revealed by STED. Furthermore, depolymerising actin using cytochalasin D leads to a decrease in cell stiffness. During induced migration, we show an increase in stiffness and actin organisation at the leading edge of migration relative to the cell basis. Thus, combining STED and AFM on live cells permits the investigation of the role of nanoscale structures on the physical properties of the cell and can be applied to the study of cell migration during development or tumourogenesis.

References

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