Correlative Super-Resolution/AFM for Investigating Cellular Ultrastructure Modifications

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The cytoskeleton of cells is predominantly comprised of actin and microtubules, which both have key roles in maintaining cell shape and transporting macromolecules throughout the cytoplasm[1]. Due to these functions, many viruses have developed mechanisms to utilise or disrupt the cytoskeletal architecture. However the sub-diffraction size of these viruses the protein structures that they are manipulating limits the extent to which these processes can be visualized using conventional fluorescence microscopy. Fortunately, with the advent of single molecule super-resolution (SR) microscopy, we can now circumvent the diffraction limit of light and visualize cellular substructure at spatial resolutions as good as 10 nm[2] . To date, only a handful of correlative topographical approaches for probing the nanometer scale ultrastructural landscape of cells have been described. Such methods are exceptionally attractive for studying cytoskeletal changes because topographical mapping either by atomic force microscopy (AFM) or electron microscopy (EM) would provide a detailed map of the entire actin/microtubule network, while SR imaging would allow specific highlighting of the architectural subunit of interest as well as any cytoskeleton binding proteins including molecular motors and structural modifiers.

To enable such applications we have devised assays for directly correlating topographical techniques (AFM/EM) with super-resolution imaging of the internal ultrastructure of individual cells. Unroofing of cells was required for the topographical techniques to probe the cytoskeletal ultrastructure and careful optimization was needed to balance preservation of structure with successful removal of the cell membrane. Multiple unroofing mechanisms were considered including the use of surfactants to dissolve lipids within the membrane[3], and sonication to peel off the membrane[4] . The effect of dehydration and rehydration was also investigated to resolve whether desiccated cytoskeletal structures could be preserved without significant artifacts. This was a critical determination because topographical techniques tend to produce higher quality images of dehydrated structures. Monolayer graphene was also utilised as a method of sealing wet cells under a conductive and impermeable membrane, allowing for both scanning-EM and AFM to be used on the dry layer of graphene while the cell remained fully hydrated. The optimized correlative protocol was applied to investigate cytoskeleton perturbation caused by anticancer drugs such as, colcemid which is known to cause depolymerisation of the microtubule network (See Figure 1). This correlative approach enables a diverse range of cytoskeletal modifications to be investigated, providing greater insight into the mechanisms by which they occur.

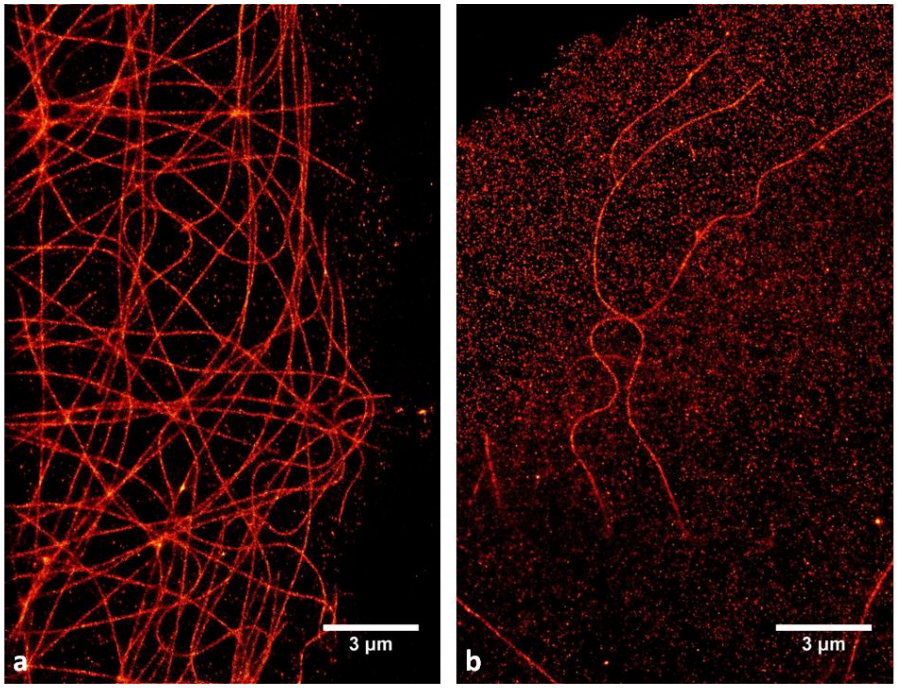

Figure 1: *d*STORM image of microtubules immunolabelled with Alexa Fluor 647 in a healthy COS7 cell (a), and a COS7 cell treated with 50 ng/mL Colcemid (b).

References

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