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**PhD project title:** Single-molecule super-resolution microscopy (STORM) of actin based cytoskeleton in spines and dendrites.

University of Registration: Leicester

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**Project outline**

1. Project outline describing the scientific rationale of the project

Actin cytoskeleton plays a pivotal role in various cellular functions, including myosin based intracellular transport, cell division, cell surface based movement and the ability of cells to adopt a variety of shapes. The actin cytoskeleton is made up of actin filaments decorated by a variety of actin-binding proteins. Actin-binding proteins play a fundamental role in the architecture of the actin based cellular structures and provide a potential interface between the actin cytoskeleton and various cell signalling pathways. Knowledge of the intracellular architecture of actin-based cytoskeleton is critical for understanding how actin-binding proteins are integrated in the cytoskeleton and how they affect the cytoskeletal structures and their dynamics during various cellular processes. Despite this, our understanding of how actin-binding proteins orchestrate the organisation and command the function of actin subcellular structures remains limited. A major challenge has been the lack of imaging tools with sufficient spatial resolution, because accuracy of ~10 nanometres (corresponding to the diameter of a single actin filament) is required to unequivocally assign interactions between molecules of actin-binding proteins and an actin filament. Over the last decade, the development of super-resolution imaging methods has finally allowed visualization of cellular nano-structures, which paved the way for studying actin subcellular structures and dynamics. Most recently, for the first time, actin-spectrin intracellular complexes have been visualized at ~10 nm resolution in neuronal axons (Xu et al 2013 Nature, 339, 452) using stochastic optical reconstruction microscopy (STORM).

In this proposal we aim to study the role of the actin cytoskeleton in neurons, in particular, in dendrites and dendritic spines. We aim to use cutting-edge super-resolution microscopy (STORM) to investigate the molecular architecture of actin-based cellular structures and the precise localisation of three actin binding proteins: caldesmon, calponin and tropomyosin in these structures. Caldesmon, calponin and tropomyosin are actin-binding proteins that are widely expressed and play a role in stabilisation of actin filaments and regulation of actin-based myosin motile function. Previous studies have shown the occurrence of large actin-dependent conformational changes in dendritic spines over periods of seconds to minutes (Fisher et al 1998, Neuron, 20, 847). In addition, caldesmon and calponin localisation in dendritic spines has been demonstrated (Agassanet al 2000 Brain Res 887, 444). Finally, the level of calponin has been shown to dramatically increase following dendritic spine remodelling in the hippocampus (Ferhat et al, 2003, Hippocampus 13, 845). However, conventional diffraction-limited microscopy cannot resolve individual actin filaments, which have a diameter of ~10nm, and therefore, cannot be used to reveal the detailed molecular organisation of...
actin, caldesmon, calponin and tropomyosin inside dendritic spines.

This proposal has three specific aims:

1. Optimize STORM imaging methodology to visualize actin filaments in axons of cultured neurons. In this aim, we will adapt a four-colour single-molecule imaging microscope (currently being built in the AR lab under BBSRC grant RM31G0318) to visualize actin filaments in neurons. No additional modification to the instrument will be required to achieve STORM imaging. We will benchmark the resolution of the instrument by visualizing actin rings in axons, which has been previously achieved by the Zhang lab (Xu et al 2013).

2. Reveal the molecular architecture of actin filaments in dendrites and dendritic spines of cultured neurons (new information).

3- Localization of calponin, caldesmon and tropomyosin using immunostaining of neurons and STORM imaging.

Timing:

Year 1: Adapt an existing single-molecule imaging instrument for STORM imaging of actin filaments arrangement in neuronal axons, dendrites and dendritic spines, and develop data analysis pipeline to assign thousands of single actin-binding molecules to individual actin filaments.

Year 2: Determine the spatial localization of calponin and caldesmon molecules with respect to actin filaments.

Year 3: Determine the spatial localization of tropomyosin molecules with respect to calponin- and caldesmon-containing actin filaments.

Relevant BBSRC Strategic Research Priority: World Class Underpinning Bioscience.

Techniques that will be undertaken during the project.

- Culture of dissociated hippocampal neurons.
- Rhodamine phalloidin in vivo labelling of actin filaments.
- Total-internal-reflection fluorescence microscopy.
- Single-molecule imaging.
- Programming and STORM data analysis (MATLAB, C++) - Confocal microscopy.
- Actin, myosin, caldesmon, calponin and tropomyosin purification (Cell extract, ammonium sulfate precipitation, Chromatography).
- Labelling of proteins with fluorescent dyes (maleimide, succinimide) - Gel electrophoresis - Immunoblotting and immunostaining.

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