ULTRA-STRUCTURAL IMAGING IN CLEARED MOUSE CNS USING

ANTIBODY-FUCNTIONALISED NANOPARTICLES

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Background: Recent advances in tissue clearing methods such as CLARITY, whereby whole organs become optically transparent and permeable to macromolecules, have allowed us to visualise structural and functional relationships at the cellular level using traditional fluorescently-labelled antibodies, without the necessity of multiple slicing, staining, imaging and subsequent 3D digital reconstruction.

Although these methods have allowed us to visualise with higher resolution smaller, non-neuronal structures of the mouse brain, photo-bleaching and limited range of available emission wavelengths of organic-dye fluorophores, has limited our ability to multiplex using traditional immuno-fluorescent techniques.

Nanoparticles (such as Nanodiamonds, Nanorubies and SuperDots™) are specialised inorganic particles with tunable monodisperse sizes, ranging from 10 nm up to a few microns, that can be coated with a bio-molecule such as an antibody, which can then bind to specific targets within tissue and be visualised using scanning microscopy or fibre optics. The benefit of these nanoparticles in a biological setting include; enhanced luminescence signal, reduced background and are more stable than traditional organic-fluoro dyes. Furthermore, the broad emission spectra and lifetime barcodes of SuperDots™, which allows excitation of multiple SuperDot™ populations at a single wavelength at either 800 nm or 980 nm, far removed from their respective emissions, allows for multiplexing of up to 10 targets simultaneously. However, fluorescence labelling using nanoparticles has yet to be demonstrated in a biological system such as cleared mouse brain and spinal cord tissue.

Through the initiatives of the ARC Centre of Excellence in Nanoscale BioPhotonics, collaborations between Physicists and Neuroscientists is enabling a new generation of nanoscale sensors to be developed.

Aim: To develop a technique allowing deeper, ultra-structural fluorescence imaging of proteins in cleared mouse whole brain and spinal cord tissue, using functionalized Nanodiamonds, Nanorubies and SuperDots™.

Methods: Our group is currently undertaking CLARITY clearing of mouse brain and spinal cord tissue, which we can use to label using targeted functionalised nanoparticles against various cell types that will be visualised at high resolution using specialised microscopy developed through the ARC Centre of Excellence for Nanoscale BioPhotonics.