Visualising immunological signalling

A team of researchers at University of New South Wales are developing a novel single molecule microscopy technique that will allow for three-dimensional, super resolution imaging of proteins that are essential in immunological signalling. We are looking for partners to drive the integration of the computational analysis and visualisation into a microscope platform.

Benefits over existing Imaging techniques:

- Ability to quantify the number of molecules within a region of interest
- Ability to obtain 3D spatial and temporal information of immunological signalling events

Background

Photo-Activated Localization Microscopy (PALM) is a single-molecule localization (SMLM) technique that relies on the stochastic activation of fluorescence to switch individual fluorophores to a bright state, which are then imaged and bleached. Repeating this cycle of activate-measure-bleach multiple times allows us to temporally separate very closely spaced molecules that would have otherwise been spatially indistinguishable.

However, molecules can blink or go into a long-lived dark state in which they behave as if re-activated, leading to an over-estimate of the number of molecules present. In addition, detection of fluorophores is close to 50% efficient[1].

T cell receptors, for instance, are known to form clusters as an essential step in their pathway. Many others transiently form clusters or higher-order structures dynamically in many essential processes such as caveole formation or perforin complexation in killer T cells. How, when and why these clusters form remain open problems in biology that are difficult to impossible to observe through other means within intact tissues.

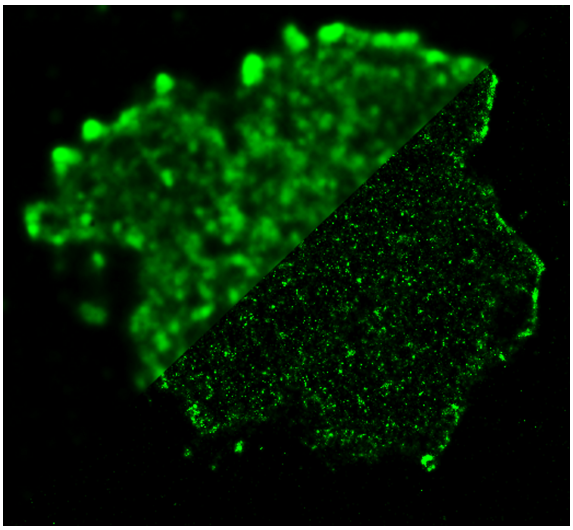

Illustration of the gain in resolution that can be obtained by SMLM. Top left shows a conventional fluorescence image and bottom right shows a SMLM image

The opportunity

Our team has developed a computational analysis method that can robustly determine the number of molecules within a cluster detected by SMLM methods such as PALM[2]. The approach is based on the photophysics of the underlying fluorophores and treats the SMLM data in a manner analogous to particle tracking data with both spatial and temporal information of individual detections taken into account.

The team has made recent advances in visualising the clustering of T cell receptors (TCRs) with to understand what causes TCR triggering that leads to an immune cascade.

The Centre for Advanced Molecular Imaging and the University of New South Wales are looking for partners to:

(a) drive the integration of the computational analysis and subsequent visualisation into a suitable microscope platform, and
(b) provide biological systems that can be used to show the efficacy of this technique in a high impact scenario.

Our team of researchers have deep expertise in programming, and the ability to build and use a wide range of optical and fluorescence microscopes and components.

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1. Durisic, N. et al. (2014) Single-molecule evaluation of fluorescent protein photoactivation efficiency using an in vivo template. *Nature Methods* 11, 156-162.

2. Pandzic, E. et al. (2015) Tracking molecular dynamics without tracking: image correlation of photo-activation microscopy. *Methods Appl. Fluoresc.* 3, 014006.

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