ENHANCED EXPANSION MICROSCOPY ALLOWS THREE-DIMENSIONAL AND BIOCHEMICAL MAPPING OF INTRACELLULAR SIGNALLING NANODOMAINS AT SINGLE CHANNEL RESOLUTION

Thomas M. D. Sheard[1], Miriam E. Hurley[1], John Colyer[1], Ed White[1], Yufeng Hou[2], Christian Soeller[2], Michael A. Colman[1], Izzy Jayasinghe[1]

[1]Faculty of Biological Sciences, University of Leeds LS2 9JT, UK
[2]Institute of Experimental Medical Research, Oslo University Hospital, Oslo, Norway.
[3]Living Systems Institute, University of Exeter, Devon, EX4 4QL, UK.
Email: i.jayasinghe@leeds.ac.uk

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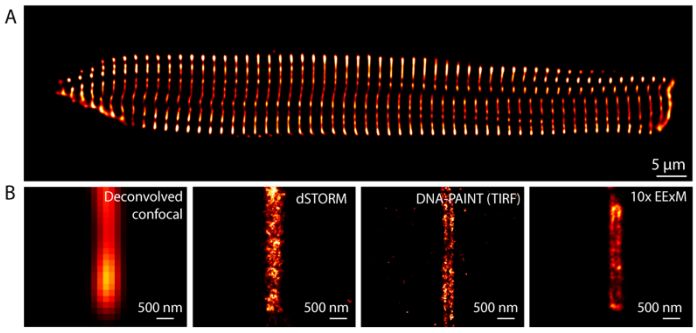

Fig 1: (A) α-actinin in cardiomyocytes imaged with (B) confocal, dSTORM, DNA-PAINT, demonstrating the resolution of EExM

Nanodomains are intracellular foci where signals are transduced between major cellular compartments. One of the most ubiquitous effectors of this signal transduction, the ryanodine receptor (RyR) calcium channel, is found tightly clustered in these nanodomains. Super-resolution techniques like DNA-PAINT have previously shown that a resolution ≤ 30 nm is required to resolve single RyRs within clusters [1]. A majority of nanodomains are however located deeper within cells and follow curved topologies which make them unresolvable with current super-resolution techniques which perform best as near-field illumination methods (e.g. DNA-PAINT using TIRF). By combining the new "X10" chemistry of Expansion Microscopy [2] with Airyscan, we have been able to achieve a working resolution ≤ 15 nm and ~35 nm, in-plane and axially deep within cells. We have demonstrated this improvement in resolution with the enhanced Expansion Microscopy (EExM) protocol, particularly beyond the conventional super-resolution techniques, by resolving the multi-lattices of the cytoskeletal protein α-actinin which spans the interiors of rat cardiomyocytes (Fig 1). With this three-dimensional (3D) imaging capability we have mapped the complex patterns and 3D topologies of self-assembled RyR arrays as well as a site-specific phosphorylation signature for each RyR. Applying the EExM protocol to examining cardiac nanodomains in rats suffering from right-ventricular failure showed nanometre-scale dispersion of RyRs and gradients of RyR phosphorylation *within* the nanodomains that were not observed before. A simulation based on these EExM data of healthy and diseased hearts allowed us to visualise the likely calcium signals arising from these nanodomains at a resolution of 10 nm and 0.1 ms. It also revealed how the natural RyR positions within the nanodomain determine the unique shapes of the local (cytoplasmic) calcium signals whilst the topography of RyR phosphorylation can 'fine tune' the temporal properties and amplitudes of the signals. Our EExM data therefore demonstrate how enhanced super-resolution techniques can bring novel structural and functional insights into healthy and pathological cell physiology.

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[2]. Truckenbrodt, S., et al., *X10 expansion microscopy enables 25-nm resolution on conventional microscopes*. EMBO reports, 2018.