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

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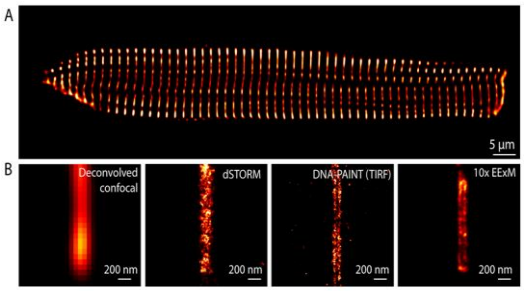

Fig 1: (A) α-actinin in cardiomyocytes imaged with (B) confocal, dSTORM, DNA-PAINT, demonstrating the resolution of EExM

Nanodomains are intracellular foci where signals are transduced between major cellular compartments. The ryanodine receptor (RyR) calcium channel which underlies the cardiac contraction, is clustered to form nanodomains. Super-resolution techniques like DNA-PAINT have previously shown that a resolution ≤ 30 nm is required to resolve single RyRs within clusters [1]. Nanodomains located deeper within cells, often with curved topologies, are unresolvable with current super-resolution techniques which perform best as near-field illumination methods (e.g. DNA-PAINT using TIRF). By combining X10 Expansion Microscopy [2] with Airyscan (termed Enhanced Expansion Microscopy (EExM)) we have been able to achieve a working resolution ≤ 15 nm and ~35 nm (in-plane and axial), to tens of microns in depth into a sample. We have demonstrated this improvement in resolution beyond conventional super-resolution techniques by resolving the multi-lattices of the cytoskeletal protein α-actinin, which spans the interior 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 nanodomains, as well as the positions of RyRs modified with site-specific phosphorylations. 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.

[1]. Jayasinghe, I., et al., *True Molecular Scale Visualization of Variable Clustering Properties of Ryanodine Receptors.* Cell Reports, 2018. **22**(2): p. 557-567.
[2]. Truckenbrodt, S., et al., *X10 expansion microscopy enables 25-nm resolution on conventional microscopes.* EMBO reports, 2018.