

ANALYSIS OF RELATIVE POSITIONS IN 3D PALM DATA PROVIDES HIGH-RESOLUTION INFORMATION ON ORDERED BIOLOGICAL COMPLEXES

Alistair P. Curd, Ruth E. Hughes, Michelle Peckham

Faculty of Biological Sciences, University of Leeds, Leeds LS2 9JT, United Kingdom

Email: a.curd@leeds.ac.uk, r.e.hughes@leeds.ac.uk, m.peckham@leeds.ac.uk

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‘Super-resolution’ microscopy techniques now provide the ability to precisely locate specific molecules within cells and cellular structures, to within ~10 nm. However, macromolecular complexes contain internal organisation at length scales that are still very difficult to access. Single-particle averaging techniques adopted from electron microscopy show some promise, but are of limited use in many fluorescently-labelled samples. As well as limits on precision for the location of each detected molecule, low labelling density of a sample, and heterogeneity between and internal to macromolecular complexes, pose significant problems. We recently found that we can address these and allow the investigation of such structures with an alternative method, PERPL (Pattern Extraction from Relative Positions of localisations) [1].

We apply this technique to 3D PALM data on proteins in the cardiomyocyte Z-disc, a complex crucial for muscle structure and contractility, which has an inhomogeneous, ~20-nm tetragonal lattice structure [2]. We imaged target proteins with fluorescent protein (mEos) tags in 3D PALM, and were able to extract known structural features of α -actinin-2 (ACTN2) organisation on the tetragonal lattice, despite high background, low labelling and detection efficiency of the labels, and average localisation precision comparable with the lattice length scales. This analysis was verified against previous 3D dSTORM data, using anti-ACTN2 Affimers labelled with Alexa Fluor 647 [1]. We proceeded to study two other less-understood proteins (myopalladin and LIM-nebulette, or LASP2) in 3D PALM, tentatively finding novel organisational features and a new model for the localisation of LASP2. As well as continuing to advance understanding of the Z-disc, we expect this analysis to be applied to study regularity and symmetry in many other complexes.

[1] Curd, A. P., et al. (2020), "Nanoscale Pattern Extraction from Relative Positions of Sparse 3D Localizations," *Nano Lett.* (2020). Published online ahead of print, DOI:10.1021/acs.nanolett.0c03332.

[2] T. Burgoyne, E.P. Morris and P.K. Luther, "Three-Dimensional Structure of Vertebrate Muscle Z-Band: The Small-Square Lattice Z-Band in Rat Cardiac Muscle," *J. Mol. Biol.*, **427**, 3527-3537 (2015).

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