THREE-DIMENSIONAL MULTI-COLOUR SINGLE MOLECULE LOCALISATION MICROSCOPY AT CRYOGENIC TEMPERATURES

Benji Bateman[a], Lin Wang[a], Laura C. Zanetti-Domingues[a], Amy N. Moores[a], Sam Astbury[a], Christopher Spindloe[a], Michele C. Darrow[b], Maria Romano[c,d], Sarah R. Needham[a], Konstantinos Beis[c,d], Daniel J. Rolfe[a], David T. Clarke[a] and Marisa L. Martin-Fernandez[a]

[a]Science and Technology Facilities Council, Central Laser Facility, Rutherford Appleton Laboratory, Didcot OX11 0QX, United Kingdom

[b]Diamond Light Source, Harwell Campus, Didcot OX11 0DE, United Kingdom

[c]Department of Life Sciences, Imperial College London, London SW7 2AZ, United Kingdom

[d]Research Complex at Harwell, Rutherford Appleton Laboratory, Didcot OX11 0FA, United Kingdom

E-mail: benji.bateman@stfc.ac.uk

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Cryo-vitrification is an established technique allowing the preservation of the ultrastructural morphology in biological specimen and is superior to artefact-inducing chemical fixation commonly used in fluorescent sample preparation at room temperatures [1]. Cryogenic temperatures also reduce the photodecomposition rate of fluorescent molecules leading to an improved localisation precision and hence resolution in single-molecule localisation microscopy (SMLM) [2]. The advantages outlined would only be achievable through the use of high-numerical aperture (typically NA > 1.4) oil immersion objective lenses which are not compatible at cryogenic temperature due to freezing. In a recent paper [3] we describe the use of a solid immersion lens (*super*SIL) coupled to a dry objective (0.55 NA) to effortlessly achieve an effective NA = 2.17 at cryogenic temperatures. This enables us to routinely acquire SMLM images with localisation precision of ~8 nm and lateral resolution of <20 nm on plunge-frozen bacterial and yeast cells in liquid nitrogen vapour. We have subsequently extended this technique to enable simultaneous two-colour SMLM imaging of multiple proteins in cells. Furthermore, we have modelled and applied Point Spread Function (PSF) engineering to the *super*SIL microscope to characterise axial distance in the ~250 nm depth of field.

We believe this method paves the way for significant improvements to cryo-correlative light and electron microscopy (cryo-CLEM) research and associated applications.

References

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