Innovative buffer for long-lived fluorescence imaging for 2D and 3D dSTORM

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Direct stochastic optical reconstruction microscopy (dSTORM), developed in the last decade, has revolutionised optical microscopy by enabling scientists to visualise objects beyond the resolution provided by conventional microscopy (200 nm). dSTORM is based on the localisation of individual fluorophores that stochastically oscillate between an ON (bright) and OFF (dark) state, obtained in a special buffer containing a thiol reducer and a low oxygen concentration. Although efficient, this enzymatically deoxygenated buffer is short-lived and must be replaced every 2-3 h, which is both time- and buffer-consuming.

Our current study aimed at developing a simple strategy to maintain an efficient long-term blinking phenomenon within an aqueous buffer to keep high compatibility with most dSTORM imaging modalities. The alternative buffer obtained (named Eternity [1]), resulted in the photostability of our 2D dSTORM imaging for up to several weeks. Furthermore, the in-house designed 1 µm in diameter fluorescently-labelled (Alexa 647) LipoParticle, enabled us to evaluate this novel buffer independently of biological variability, demonstrating (i) the stability of the LipoParticle/Eternity buffer combination even at a pH as low as 5.0 and (ii) the quality of our 3D dSTORM image reconstruction. Finally, the Eternity buffer revealed 2D dSTORM images of centrosomes *in cellulo* over a period of 17 days enabling the detection of individual appendages and demonstrated a localisation precision within the 10 nm range for *in cellulo* 2D imaging of centrosomes, as well as their reliable reconstruction in 3D dSTORM.

[1] A. Provost, C. Rousset, L. Bourdon, S. Mezhoud, E. Reungoat, C. Fourneaux, T. Bresson, M. Pauly, N. Béard, L. Possi-Tchouanlon, B. Grigorov, P. Bouvet, J-J Diaz, C. Chamot, E-I Pécheur, C. Ladavière, M-T Charreyre, A. Favier, C. Place, K. Monier, *Innovative particle standards and long-lived imaging for 2D and 3D dSTORM,* Scientific Reports, Final revision, 2019.