CHARACTERISATION OF GFP AND DERIVATIVES FOR SUPER-RESOLUTION CRYOGENIC SINGLE MOLECULE LOCALISATION MICROSCOPY

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The application of single molecule localisation microscopy (SMLM) at cryogenic temperatures is limited by the number of available fluorophores which retain their photoswitchable and photoactivatable properties at low temperature. Although important photophysical properties of both fluorescent dyes [1] and fluorescent proteins [2] are well-characterised at room temperature, no such comprehensive study currently exists for cryogenic temperature. We quantify the photoblinking properties, such as the average photon budget and on-time per blink, for GFP-derivatives, including EGFP and mEmerald. High photon budgets help to improve localisation precision in SMLM [3], whilst shorter blink times can reduce the chance of localisation errors due to overlapping fluorophores being detected simultaneously. We aim to screen green fluorescent proteins to determine which gives (on average), the highest photon budget and shortest blink time, whilst functioning at the lowest laser power possible.

We show that our tested members of the GFP-family have the ability to photoblink at cryogenic temperatures, making them compatible for cryo-SMLM studies. Initial results suggest that mEmerald outperforms EGFP, with an average blinking on-time 2.3-fold shorter, but with a similar photon budget. Preliminary results also suggest that mEmerald begins to blink at significantly lower power densities than that of EGFP, making it more gentle on cryogenically-fixed samples.

By measuring the photophysics of more fluorescent proteins, we hope to provide a solid reference for the community of cryo-SMLM researchers, allowing them to choose the optimum fluorescent probe for their studies.

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