LOCALIZATION MICROSCOPY IMAGING OF ENDOCYTIC PATHWAY

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Super-resolution techniques based on the spatial localization of single isolated molecules can provide images with a lateral resolution of 10-20 nm. Several fluorescent proteins and organic dyes have been proposed and applied both in vitro and in cell measurements. We have used direct stochastic optical reconstruction microscopy (dSTORM) [1,2], which is a relatively simple approach using conventional fluorescent dyes. Optimization of system parameters (laser intensity, switching buffer, etc.) is essential for capturing images with structures below the optical resolution limit. We have presented our first experimental results using two almost identical dSTORM systems built at Cambridge University and at the National Physical Laboratory. The performance of the systems was tested using Alexa 488 and Alexa 647 dye labelling of clathrin, epidermal growth factor (EGF), and actin filaments. These are useful test samples as they are well characterised structures by electron microscopy. EGF labelling at the cell surface can be predominantly seen in forming pits and vesicles. These are typically 50 – 100 nm in diameter and are often clustered close together. Clathrin is known to form a variety of structures at the plasma membrane (imaged with TIRF), with a distribution of small vesicle-sized objects and larger flat sheets typically of 500 nm in size. Actin filaments can be imaged in vitro and are known to have a diameter of 7 nm. We show here how they can be used to detect sample drift and potentially provide a useful multicolour test system. Optimal measurement conditions and the possibility of multicolour imaging are also discussed.

REFERENCES
