Kinesin-1 Particle Tracking Imaged with a Non-Destructive Readout Camera

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Improvements in camera technology and microscopy techniques have enabled the optical detection and localization of single molecules. The quality of single molecule localization is dependent on the signal to noise ratio and to improve accuracy, the signal must be increased or the noise reduced[1].

Most light microscopes currently use electron multiplied charged coupled devices (EMCCDs) or complementary metal-oxide semiconductor (CMOS) cameras and both camera types have high quantum efficiencies above 95%. Many biological applications require low light imaging to protect samples from photo-toxicity and to prevent photobleaching. Therefore, for biological applications, improving data quality is highly dependent on noise reduction.

We present particle tracking data of kinesin motor proteins captured using a form of CMOS camera called non-destructive readout (NDR). In NDR the removal of electrons from the pixel is suppressed allowing "analogue, multiple-frame integration", meaning we repeatedly view the image on the sensor without additive read-noise. In this work we interrogate the chip several thousand times per second to build up a high-speed image of the sample. Each NDR image can be considered a temporal sub-sample of a normal CMOS image, allowing high speed accurate single particle tracking of biological samples. We have previously used NDR technology for STORM localisation microscopy of *Staphylococcus aureus*[2].

Kinesin-1 is the major anterograde motor protein for axonal transport of cargo along microtubules from the negative end at the cell centre to the positive end at the cell periphery. Defects in axonal transport are associated with many neurodegenerative diseases, such as motor neurone disease, making research into kinesin activity crucial for future treatments. The kinesin protein molecules used have a HaloTag with a Janellia 646nm fluorophore attached. Hilyte-488 Tubulidentata microtubules were illuminated and the fluorescent kinesin molecules were imaged with a 647nm laser in a TIRF setup and analysed using custom scripts and tracking software to follow the molecules in post processing.

[1] R. Thompson; D. Larson, and W. Webb, "Precise nanometer localization analysis for individual fluorescent probes." Biophys J. 82: 2775–2783 (2002).

[2] Barnett, S.F.H., M. Snape, C.N. Hunter, M.A. Juárez, and A.J. Cadby. 2017. A Novel Application of Non-Destructive Readout Technology to Localisation Microscopy. Sci Rep. 7: 42313.