Single Particle Tracking Performed with A Non-Destructive Readout Camera.

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Over the last few decades, improvements in camera and microscopy technologies have enabled the optical detection and localization of single molecules. The quality of single molecule localization data is defined by the signal to noise ratio. To improve localization accuracy, the signal must be increased or the noise must be reduced[1]. Current cameras are usually either electron multiplied charged coupled devices (EMCCD) or complementary metal-oxide-semiconductor (CMOS), both of which have quantum efficiencies above 95%. Therefore, improving data quality is currently highly dependent on noise reduction.

We present particle tracking data captured using a novel form of sCMOS called non-destructive readout (NDR) technology. In NDR the removal of electrons from the pixel is suppressed allowing "analogue, multiple-frame integration" meaning we can repeatedly view the image on the sensor without additive read-noise. In this work we interrogate the chip several thousand times per second without incurring a readout, 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, without incurring the associated read noise. This is essential for high speed accurate single particle tracking.

The data presented in this work was taken using an NDR camera running at 1000 fps. Fluorescent FluoSpheres™ Carboxylate-Modified Microspheres were imaged with a 488nm laser in a TIRF setup using a custom built Nikon Ti2 microscope. We utilized the correlation between each frame within the block to reduce the noise associated with imaging single molecules for tracking purposes. The 1000 fps NDR data is processed first by a CDS correction and then the difference between every ten frames is taken to recreate data at 100 fps. Finally, we apply a number of noise reduction algorithms, capitalizing on the known temporal correlation, which will allow for greater resolution and precision in single particle tracking applications.

References:

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