A sample preparation workflow for dSTORM.

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Direct stochastic optical reconstruction microscopy, or dSTORM, is a single-molecule localisation based super-resolution technique and is a powerful tool used for viewing cellular structures at a resolution unattainable by conventional widefield epifluorescence microscopy. The resolution that can be achieved using dSTORM does not present without technical difficulties. In particular, sample preparation differs from conventional epifluorescence microscopy and should be well understood before considering super-resolution microscopy. Here, alpha-tubulin immunostained COS-7 cells (derived from kidney tissue of African Green Monkey) were used to create a workflow model that may be used as a sample preparation guide for dSTORM. Using measurements of background fluorescence, fluorescence intensity of microtubules, and full width at half maximum (FWHM) of microtubules, we examined and directly compared different methods of coverslip cleaning, a range of fixation techniques, as well as the duration in which samples may be stored prior to viewing. In addition to this, we also examined the possibility of removing hazardous chemicals such as nitric acid (HNO_3) and sodium borohydride ($NaBH_4$) from coverslip cleaning and sample fixation procedures. While our results indicated that there was no significant difference in FWHM measurements of microtubules between the different fixation methods, they did show that quenching with sodium borohydride as part of the fixation process significantly increased the intensity of microtubule fluorescence and significantly decreased background fluorescence. Unsurprisingly, we found that samples viewed immediately after fixation and staining performed far better than those stored for 14 days prior to viewing. This workflow model is a guide for optimal sample preparation and also offers fixation alternatives which may eliminate the use of hazardous chemicals for users of dSTORM.