Review of: "Development of ultrafast camera-based imaging of single fluorescent molecules and live-cell PALM"

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Potential competing interests: The author(s) declared that no potential competing interests exist.

In this work, the authors combined ultra-fast CMOS camera with image intensifier that achieves high-speed and high SNR, which enables ultra-fast single molecule imaging and fast live-cell PALM. This method provides up to 30 kHz single molecule imaging, which was used to detect the fast hop diffusion of membrane molecules on the PM. This method also enables ultra-fast PALM imaging with the time resolution increased to 0.33 s for 330 frames with 1kHz data acquisition speed. This method opens a new window for the high-speed process in the cell, which is a useful tool for the study of the membrane molecule and the single molecule localization super-resolution microscopy with higher time resolving ability.

There're still some concerns need to be addressed which listed in the following:

1. The fluorescent signal was converted to electrons on the photocathode, then amplified by the microchannel plate, converted to photons on the phosphor screen and transmitted by the optical fiber bundle before converted and digitized by the CMOS chip. Does such a long conversion procedure affect the image quality or introduce image distortion? Consideration is also on the coupling between the image intensifier and the CMOS chip, is there pixel math problem among the image intensifier, the optical fiber bundle and the CMOS chip?

2. The pixel size of the system was 55 nm, but usually for single molecule localization, the optimal pixel size should be about 100 - 150 nm (such as Russell E, 2002), the reason for the pixel size needs to be addressed.

3. The full-well capacity of the CMOS results in a saturation photon density of about 3 photons/pixel, will this low saturation photon density restrict the further application of this methods?

4. Previous work used gold nanoparticles based SPT for single molecule tracking and announced that gold particle labeling will not affect the behavior of the molecule in 100-ms window (Takahito Fujiwara, 2002). Fluorescent molecule was used for labeling in this work which is a much smaller probe. Although the

results have been compared with previously reported data, but there should be some experimental data that directly compare the diffusion behavior of membrane molecules labeled with gold nanoparticles and dye molecules, respectively. Whether there are differences in the diffusion behavior of molecules under different labeling and the degree of the differences needs to be addressed in more detail.