TOTAL INTERNAL REFLECTION FLUORESCENCE PATTERN-ILLUMINATED FOURIER PTYCHOGRAPHIC MICROSCOPY

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1. ABSTRACT

We report a widefield super-resolution (SR) fluorescence microscopy technique termed total internal reflection fluorescence pattern-illuminated Fourier ptychographic microscopy (TIRF-piFPM). It employs pattern-illuminated Fourier ptychography (piFP) under the total internal reflection fluorescence (TIRF) mode, providing a lateral resolution of ~100 nm, reducing the background level, and correcting unknown optical aberrations. Like the total internal reflection fluorescence structure illuminated microscopy (TIRF-SIM), the illumination field of TIRF-piFPM is modulated by sinusoidal patterns generated by evanescent wave interference. It differs from TIRF-SIM in that TIRF-piFPM reconstructs the SR image by FP iteration. To demonstrate the performance of TIRF-piFPM, we compare it with TIRF-SIM by conducting simulations and experiments and prove that TIRF-piFPM can provide a better result than TIRF-SIM in terms of its robustness to noise and aberration correction ability. In addition, dynamic changes of mitochondria in U2OS cells are captured with a temporal resolution of 2 s, demonstrating its live-cell imaging capability. The addvantages may enable TIRF-piFPM to serve as an alternative to SR fluorescence microscopes in the TIRF family, with promising applications in biological and biomedical imaging.

2. EXPERIMENT RESULTS

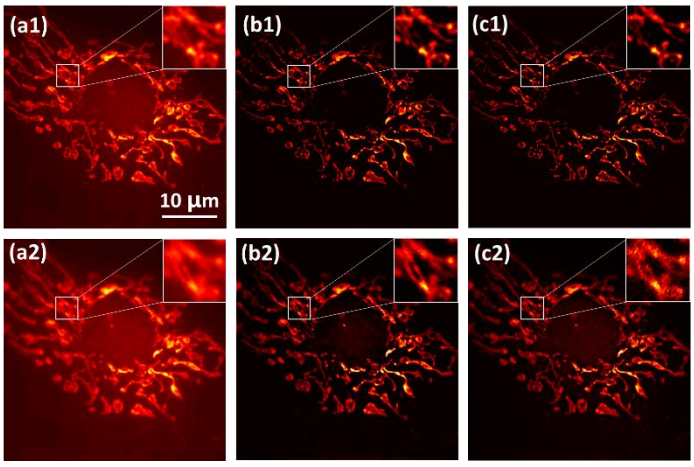

Fig. 1. Experimental results of mitochondria in fixed BPAEC. (a1)–(c1) In-focus images acquired by deconvoluting the data in the widefield, TIRF-piFPM reconstruction, and TIRF-SIM reconstruction, respectively. (a2)–(c2) The corresponding images obtained from the raw data stack when captured under 300 nm out of focus.