Multi-modal Super-Resolution Microscopy through Super-Resolution Radial Fluctuations (SRRF)

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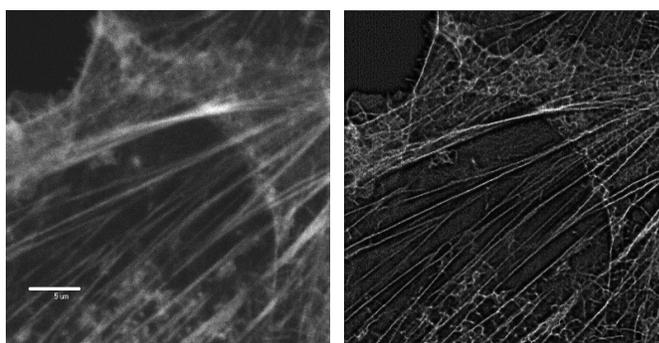

Figure 1. Images of AlexaFluor 488 Phalloidin labelled F-actin in BPAE cells. Left: one raw confocal image frame from burst of 100 frames. Right: SRRF image processed to 4X lateral resolution. Scale Bar: 5µm.

Super-resolution radial fluctuations (SRRF) is a synthesis of temporal fluctuation analysis and localization microscopy [1]. One of the key differences between SRRF and other super-resolution methods is its applicability to live-cell dynamics because it functions across a very wide range of fluorophore densities and excitation powers. SRRF can be applied to data from many different imaging modes including widefield, TIRF and confocal, where short frame bursts (e.g. 100 frames) can be processed to deliver spatial resolution enhancements similar to or better than structured illumination microscopy (SIM). On the other hand, with sparse data e.g. direct STORM, SRRF can deliver resolution similar to Gaussian fitting localization methods. Thus SRRF could provide a route to super-resolution without the need for specialized optical hardware, exotic probes or very high power densities. We present a fast GPU-based SRRF algorithm and apply it to imagery from our new multi-modal imaging platform, Dragonfly. The new implementation is >300 times faster than the current CPU version running on an Intel Xeon 3.5GHz 4 core processor, and 8 to 18 times faster than the NanoJ GPU implementation, while also being integrated with acquisition for real time use. We explore the image resolution and quality with EMCCD and sCMOS cameras, and various fluorophores including fluorescent proteins and organic dyes.

[1] Gustafsson, N., Culley, S., *et al.*, et al. (2016) Fast live-cell conventional fluorophore nanoscopy with ImageJ through super-resolution radial fluctuations. *Nat Commun* 7(12471):12471.