High-throughput Fluorescence Microscopy using Motion Deblurring

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High-throughput wide-field fluorescence microscopy plays a critical role in many fields (e.g. drug-discovery, disease screening, neuropathology). A central issue for fluorescence microscopy is light-throughput, which is significantly reduced (compared to brightfield imaging) due to low fluorophore efficiency, imaging optics, and chromatic filters. One potential avenue to maximizing the signal is to blur multiple measurements together via multiplexing. Early work in computational photography proposed a motion deblurring technique for recovering a static scene from a blurred measurement captured with shutter coding \cite{raskar2006}. The same idea was later used for illumination coding in the context of microscopy \cite{ma2015}. These techniques can enable higher acquisition SNR, but suffer noise amplification during post-processing; hence, they are useful for low-light applications, but may not provide benefit over strobed imaging (illumination by single, short pulse while in motion) when low-noise sensors and a bright source is available \cite{cossairt2013}. Here, we show we show improved acquisition SNR relative to strobed imaging and stop-and-stare (static) imaging for slide-scanning fluorescence microscopy.

We propose a multi-frame motion deblurring technique where the sample is illuminated by a temporally-coded illumination sequence during each frame, while the sample is simultaneously raster scanned by a mechanical stage. This introduces structured motion blur into each measurement, which is removed using a deconvolution algorithm. Unlike previous single-frame techniques, we use a multi-frame acquisition strategy to serially image the moving object, enabling high-content imaging of very large objects. In Fig.1, we compare our method with conventional techniques by imaging fluorescent polystyrene beads (ThermoFisher G0300) in a commercial wide-field fluorescent microscope (Nikon TE300) with a motion stage (Prior H117) and programmable LED illumination source (Thorlabs M470L3). Through both theory and experiment, we show that under realistic conditions our method is faster than stop-and-stare imaging and provides greater SNR than a single strobed pulse. Further, we provide an analysis of when our method is economical in terms of system hardware parameters such as illumination power, magnification, motion stage velocity, illumination repetition rate, and camera noise parameters.

![Figure 1: Multi-Frame motion deblurring for wide-field fluorescence slide scanning microscopy. Left: Our system is a wide-field fluorescence microscope equipped with a commercial motion stage and programmable LED illumination source. The sample is scanned continuously while the illumination is coded in time. Right: Comparison of experimental results for fluorescent beads using stop-and-stare, strobed, and motion coded acquisitions. Our deblurred reconstruction (bottom right) has higher SNR than an equivalent strobed acquisition while maintaining the same acquisition rate. Scale bar is 50\textmu m.](image)