High-throughput Fluorescence Microscopy using Motion Deblurring

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High-throughput wide- eld uorescence microscopy plays a critical role in many elds (*e.g.* drug-discovery, disease screening, neuropathology). A central issue for uorescence microscopy is light-throughput, which is signi cantly reduced (compared to bright eld imaging) due to low uorophore e ciency, imaging optics, and chromatic lters. 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 [1]. The same idea was later used for illumination coding in the context of microscopy [2]. These techniques can enable higher acquisition SNR, but su er noise ampli cation during post-processing; hence, they are useful for low-light applications, but may not provide bene t over strobed imaging (illumination by single, short pulse while in motion) when low-noise sensors and a bright source is available [3]. Here, we show we show improved acquisition SNR relative to strobed imaging and stop-and-stare (static) imaging for slide-scanning uoresence 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 uorescent polystyrene beads (ThermoFisher G0300) in a commercial wide- eld uorescent 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, magni cation, motion stage velocity, illumination repetition rate, and camera noise parameters.

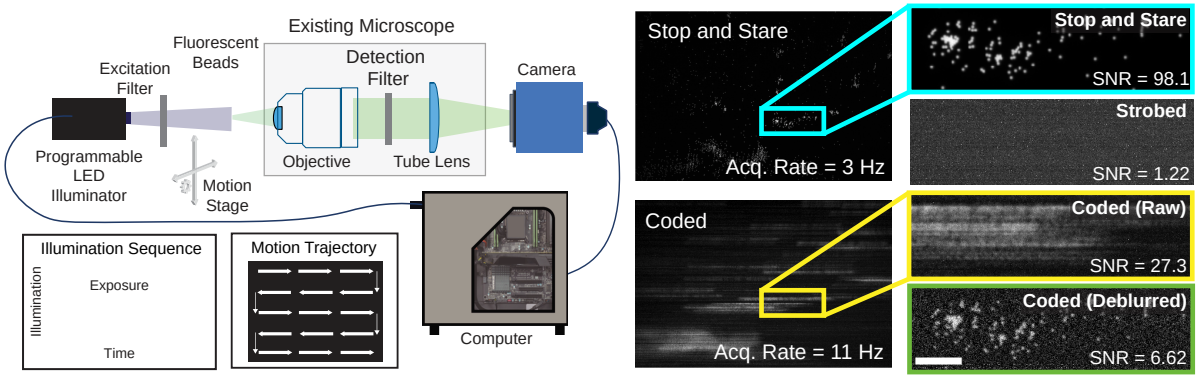

Figure 1: Multi-Frame motion deblurring for wide- eld uorescence slide scanning microscopy. **Left**: Our system is a wide- eld uorescence microscope equipped with a commercial motion stage and programmable LED illumination source. The sample is scanned continuously while the illumination is coded in time. **Right**: Comparison of experimental results for uorescent 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 *m*.

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