EXTENDED-RESOLUTION VIDEO MICROSCOPY OF LIVING CELLS BY STRUCTURED ILLUMINATION

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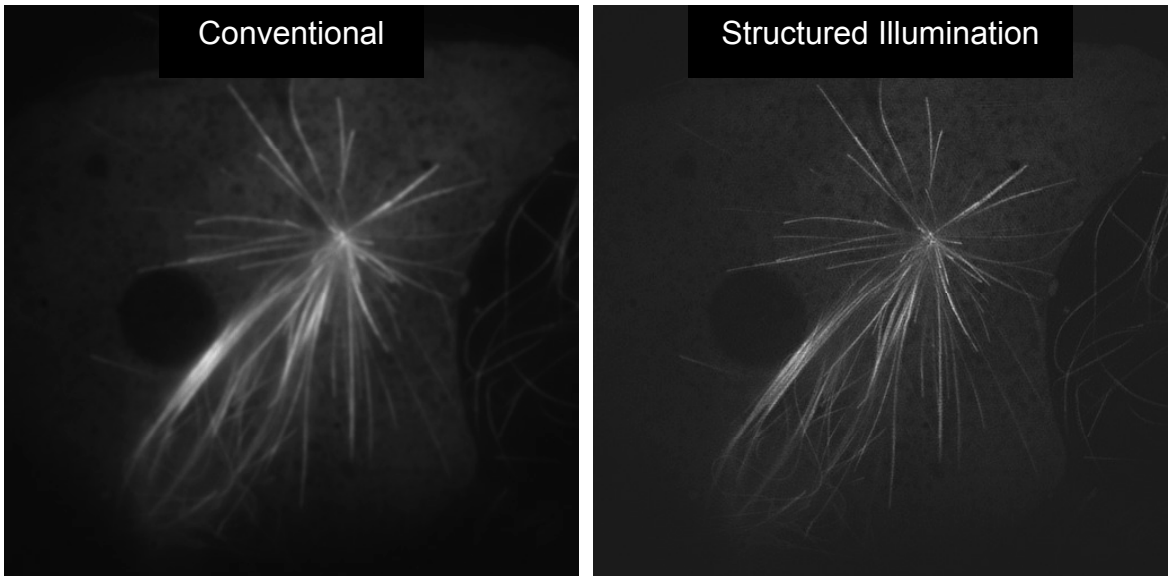
Periodically structured illumination light can extend the resolution of fluorescence microscopy beyond the classical limit through spatial frequency mixing. The amount of resolution extension, set by the spatial frequency of the illumination pattern, is normally about a factor of two, because the pattern frequency is limited by the diffraction in the same way as the conventional resolution; dramatically greater resolution extension is possible if a nonlinearity can be introduced between the incoming illumination intensity and the outgoing emission rate.

Structured-illumination microscopy typically uses data reconstruction algorithms that assume that the entire data set represents a single unchanging structure. It has therefore been largely confined to fixed, unmoving samples. If a data set can be acquired in a time that is short compared to sample motions, however, live imaging becomes possible. Here we present live imaging with ~100 nm lateral resolution at multi-Hz rates for several hundred time frames, using linear structured illumination with a rapid pattern-generating system in the TIRF mode.



One frame from a TIRF microscopy video of GFP-α-tubulin dynamics in a living S2 cell