INCREASING ACQUISITION SPEED IN STRUCTURED ILLUMINATION MICROSCOPY AND ITS LIMITS

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ABSTRACT: Structured illumination microscopy, SIM [1], is an optical superresolution technique, which excels in live cell imaging due to its low phototoxicity and fast acquisition rates. Superresolution is achieved by illuminating a fluorescent sample with a patterned excitation light field. The resulting beat pattern of illumination and sample encodes fine, usually lost, sample details. The frequency mixing thus opens the possibility to increase resolution and provide optical sectioning. In conventional SIM imaging, this procedure requires between 9 and 15 raw frames for a single superresolution image. A reconstruction from less than 9 raw frames is hence an elegant way to increase SIM framerate without the need for hardware adaptions.

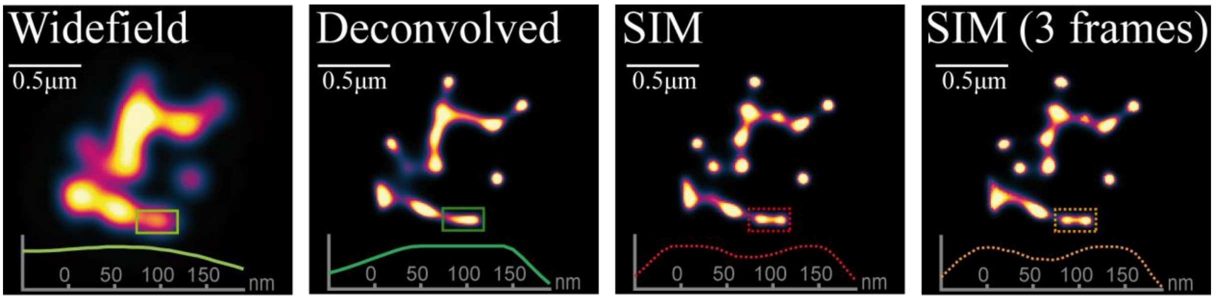

Figure 1: Comparison of different widefield and SIM modalities. SIM from just 3 raw frames offers resolution equal to conventional SIM, which requires 9 raw frames.

Here, we present both the theory and implementation of a novel reconstruction approach, which uses a joint Richardson-Lucy algorithm [2] to lower acquisition times far below the conventional performance by greatly reducing the amount of required raw data for image reconstruction, while still being able to resolve features below the diffraction limit. We validate the procedure experimentally and benchmark the achievable performance for fast imaging *in silico* to compare our novel approach against established microscopy techniques. Furthermore, we link the number of raw frames to the maximally recordable sample velocity and the achievable reconstruction quality [3]. Our results show that there exists a limit above which deconvolution microscopy becomes superior to SIM in both acquisition speed and resolution. This is due to motion artefacts, which compromise the image quality of SIM reconstructions severely, but are far less pronounced in deconvolved widefield images.

REFERENCES

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