

ORTHOGONALLY POLARIZED EXCITATION LATTICES FOR SINGLE-SHOT VOLUMETRIC MICROSCOPY

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ABSTRACT: Existing microscopy methods suffer from background haze produced by emitters residing outside the image plane, which reduces contrast and must be avoided to obtain a sharp image. Common approaches to achieve this are confocal microscopy, where light is physically rejected from out of focus planes by a pinhole, or light sheet microscopy where fluorophores are excited in a single plane and no out of focus signal is generated [1]. Hence, by design, these approaches require the translation of the image point or plane along at least one direction to create a fully sectioned volume. This reduces imaging speed and can lead to motion artefacts, a severe problem in live cell microscopy.

We report a new optical concept to circumvent this problem, which permits the recording of depth resolved information in single camera exposures [2]. Optical sections can be recorded in single acquisition frames, making use of all fluorophores in the sample, without the requirement for physical rejection of out-of-focus light to obtain contrast.



Figure 1: Recovery of depth information from the missing cone enables optical sectioning.

Our approach is based on the simultaneous illumination of a sample with two orthogonally polarized excitation lattices. Spatial frequencies in the lattices beat with sample frequencies and the resulting patterns encode depth information. We provide a rigorous theoretical framework for the method and derive a non-iterative algorithm for the computational recovery of axially resolved images of high quality. As the lattices are translationally invariant along the optical axis, an intriguing possibility exists for the recording of entire volumes at an instant via multi-focus microscopy [3].

We perform extensive simulations and experiments to validate the technique. It offers potential to image dynamic events in living cells, for example the transport of proteins or organelles throughout the cell volume in real time.

REFERENCES

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