

A NOVEL, COST-EFFICIENT CONCEPT FOR INTERFEROMETRIC MULTICOLOUR STRUCTURED ILLUMINATION MICROSCOPY

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Gentle sample illumination and the possibility to image in multiple colours makes Structured Illumination Microscopy (SIM) a high-resolution imaging technique which is applicable to a wide range of biological problems. SIM can achieve resolution doubling over a large field of view and allows for fast imaging, making it suitable for live cell observations [1,2].

We have developed a new concept for SIM that is fast, achromatic and easier to implement than existing solutions. The setup can be operated with arbitrary laser lines to achieve imaging speeds, which are only limited by the camera performance.

The formation of illumination patterns relies on interferometry where rotation and phase stepping are performed with a mirror galvanometer. The method works by projecting, in sequence, parallel beams of light onto three different mirror configurations. Each of the mirror pairs is individually tilted and therefore produces one of the three required pattern orientations. Pattern rotation is achieved by addressing different mirrors in the array, while phase shifts are created with small steps of the galvanometer across one mirror.

The system does not require complex synchronization, polarisation control or expensive and photon inefficient liquid crystal (LC) devices. The simple and flexible design is cost-effective and allows for extension to 3D and non-linear imaging capabilities. It is, therefore, suitable for use in a variety of biological applications and a valuable tool for the imaging of living cells.

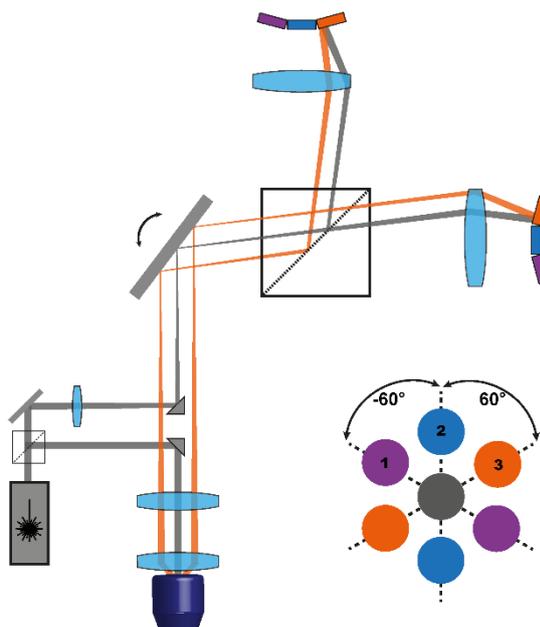


Figure 1. Schematic overview of the microscope. Two scan lenses and corresponding mirror arrangements are used to create and rotate the desired SIM patterns. Targeting one mirror in each illumination arm positions two interference spots (orange) in the back focal plane for pattern formation.

[1] Fiolka, R., Shao, L., Rego, E. H., Davidson, M. W. & Gustafsson, M. G. L. "Time-lapse two-color 3D imaging of live cells with doubled resolution using structured illumination," *Proc. Natl. Acad. Sci.* **109**, 5311–5315 (2012).

[2] Kner, P., Chhun, B. B., Griffis, E. R., Winoto, L. & Gustafsson, M. G. L. "Super-resolution video microscopy of live cells by structured illumination," *Nat. Methods* **6**,