HIGH-SPEED, MULTICOLOR, STRUCTURED ILLUMINATION ENABLED BY AN ELECTRO-OPTICALLY CONTROLLED FIBER ARRAY

Taylor Hinsdale, Sjoerd Stallinga, Bernd Rieger
Department of Imaging Physics, Delft University of Technology
Lorentzweg 1, 2628 CJ Delft, The Netherlands
E-mail: t.a.hinsdale@tudelft.nl

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Structured illumination microscopy (SIM) has proven an instrumental technique for studying in vitro cellular processes that occur below the diffraction limit; however, the need to capture multiple raw frames to form a single SIM reconstruction precludes imaging highly dynamic phenomena. This speed limitation is further exacerbated as most current systems rely on diffractive optical elements to generate patterned excitation in the sample plane. These are usually slow to manipulate, sensitive to wavelength, or both. Here we introduce a novel high-speed, multicolor, structured illumination microscope that utilizes a fiber array for pattern generation and electro-optics for fast pattern manipulation. The use of broadband polarization optics, electro-optics, and fibers allows for efficient illumination over an extensive wavelength range, 400-700 nm, with greater than 10 kHz pattern manipulation capabilities. Combining these elements enables structured illumination frame rates of over 100 frames per second, well exceeding the frame rates of commercially available microscopes such as the OMX system. Figure 1 shows a schematic of the fast fiber SIM system. We demonstrate the system on fluorescent bead monolayers, fixed and multi-color stained Cos-7 cells, and live YFP stained chromosomes in e coli.

Figure 1 - (a) shows a 3D rendering of the inverted microscope construction. The fiber array is collimated by relay lens RL 1 and then rotated by a polarization rotator. Lens pair, (R L 2,3), then relay the excitation beam to the compensation dichroic mirror (DiM 1). (RL 4) then focuses the light to the back focal plane of the objective after reflecting off of the imaging dichroic (DiM 2). The orientation of the pattern is determined by which pairs of fibers (1a,b, 2a,b, or 3a,b) are emitting light. The Fourier plane of the emission light is relayed by the tube lens and first deformable mirror lens (DiM L 1) to the deformable mirror. The aberration corrected emission light is then imaged onto the sCMOS camera via (DiM L 2). (b) shows how the excitation lasers are phase shifted and switched between different fiber pairs [1].

References