This is a peer-reviewed, author's accepted manuscript of the following research article: (Eds.) (2024).
A multi-colour 2D and 3D structured illumination microscope using MEMS scanning mirrors. In I. Gregor, F. Koberling, & R. Erdmann (Eds.), Proc. S
(Proc. SPIE Progress in Biomedical Optics and Imaging). SPIE. https://doi.org/10.1117/12.3002511

A multi-colour 2D and 3D structured illumination microscope using MEMS scanning mirrors

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ABSTRACT

We present our latest results on a structured illumination microscope (SIM) implementation using individual microelectromechanical systems (MEMS) micromirrors with three-axis full angular, radial and phase control of the illumination pattern in the sample. Results of a simultaneous multi-color 2D SIM and 3D SIM implementation are shown with digital system adjustment to select the optimal imaging conditions and adapt to variable microscope objectives used in the system. Calibration and cell images of 2D and 3D samples will be shown to verify the resolution enhancement and axial sectioning potential.

Keywords: Structured Illumination Microscopy, MEMS micromirror, super-resolution, multi-color

1. INTRODUCTION

In the quest for ever higher resolving power in bio-imaging applications, optical techniques have made impressive strides in recent years, approaching the resolving power of electron-beam imaging techniques while retaining the advantages of specificity and targeted labelling. While many of the recent resolution enhancement developments require fixation of biological specimen, such as single molecule localization microscopy or expansion microscopy techniques, approaches that increase resolution for live-cell compatible investigations are of equal interest to biology, pharmacology or drug discovery researchers. Specifically approaches using structured illumination patterns for fluorescence excitation, such as STED, MINFLUX or SIM, have all shown great promise [1]. Out of these, SIM has the advantage of direct compatibility with any fluorescence image preparation pipelines and fast image acquisition due to the widefield illumination approach using 9 or 15 grating patterns allowing a resolution doubling through reconstruction [2].

While approaches using diffractive optical elements to create the multi-beam illumination pattern in SIM have exhibited fast frame rates that are only limited by camera exposure times, limitations through chromatic dependence of the diffractive elements lead to independent pattern or incidence angle requirements for each illumination wavelength to satisfy blazed grating conditions [3]. To circumvent these constraints, approaches based on reflective optical control elements have been implemented. Notably, galvanometric mirror and piezo phase shift element based approaches have been demonstrated [4], [5] as well as fiber optic or MEMS based approaches [6], [7], which both allow individual control of the multiple illumination beams required for super-resolution SIM. The complexity of alignment and control of the multiple beam paths through multiple galvo and piezo elements are however potentially a restricting factor. Our previously introduced MEMS micromirror approach [7] reduces the number of required optical control elements significantly, albeit with a constraint of the input beam size due to MEMS mirror apertures.

Here we present our latest results on integrating a combined 2D and 3D SIM system based on multiple MEMS micromirrors for beam control and show the direct possibility for multi-color imaging based on the reflective system design.

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2. MATERIALS AND METHODS

2.1 System overview

The combined 2D/3D SIM microscope implementation is schematically shown in Figure 1. Light from multiple laser modules for 405 nm, 488 nm or 561 nm excitation is coupled to a single mode fiber (Thorlabs P1-405B-FC-1) whose output is collimated by an achromatic doublet to a diameter of 5 mm. An aperture reduces the beam diameter to 2 mm to fit to the mirror diameter of the MEMS micromirror (Mirrorcle A7M20.2-2000AL) used for beam control. The MEMS-SIM unit creates 3 individually controlled coherent beams with free control over position and phase. A f = 45 mm achromat is placed in a telecentric position, with a 4f system consisting of a f = 125 mm and f = 100 mm achromat focusing the beams on the back aperture of a 40x water immersion objective (Olympus UAPON40XW340). A multiband dichroic mirror (Chroma ZT405/488/561/640rpcv2) is placed behind the objective to transmit the collected fluorescence. A further emission filter (Chroma ZET405/488/561/640mv2) and a f = 250 mm achromat acting as tube lens create an image on the industrial CMOS camera (IDS UI-3060CP). The sample is placed on a piezo flexure-translation stage (Thorlabs NFL5DP20/M) for accurate focal plane positioning and z-stack imaging.

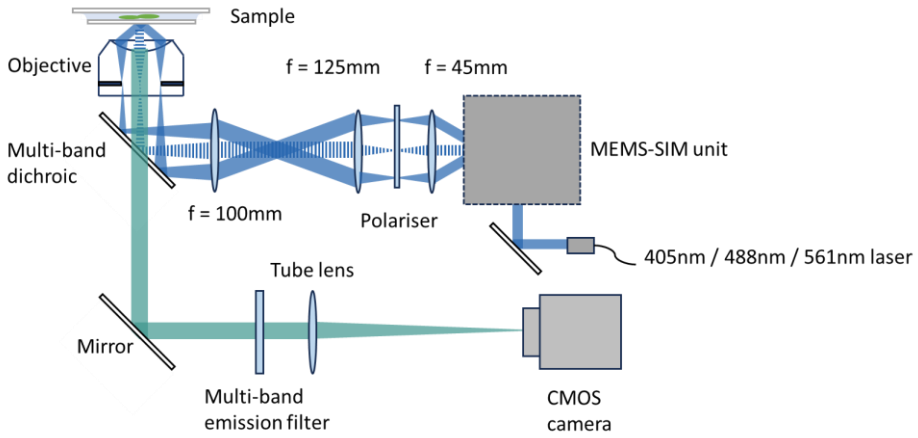

Figure 1. System schematic for the multi-color 2D and 3D SIM implementation using a MEMS-SIM unit for light control.

2.2 MEMS mirror

At the heart of the MEMS-SIM unit highlighted in Figure 1 are multiple 2D MEMS micromirrors with 2 mm diameter reflective surface. A small modification on the mirror allows 3-axis movement for accurate static or dynamic positioning of tip, tilt and piston. The mirrors are controlled using custom 8-channel DAC and amplifier units, with the 16-bit DAC allowing control of the MEMS angle with precision of 8 µrad, and its piston position with 38 nm accuracy. This leads to a phase shift accuracy of around 2° of the sinusoidal interference grating created in sample space by the interfering 2 or 3 beams for 2D or 3D SIM usage respectively.

2.3 SIM processing and sample preparation

To create 2D or 3D SIM imaging 3 angles and 3 or 5 phase steps each are used. The MEMS position is calibrated to lead to 1.3, 1.6 or 1.9 times nominal resolution enhancement. The collected images are post-processed for super-resolution SIM using HiFi-SIM [8] with the basic settings.

To demonstrate the system performance, nanobead samples and fixed BPAE cells with multiple fluorescence labels are used. The bead samples are based on 175 nm PS-Speck beads (Invitrogen P7220) which are airdried on Poly-L-lysine coated cover slips. The BPAE cells are from a fixed cell slide sample (Invitrogen F36924).

3. RESULTS

To characterize the 2D and 3D SIM modalities, the 175 nm bead sample was imaged using 405 nm, 488 nm and 561 nm illumination. For 2D SIM two of the MEMS controlled beams are telecentrically focused into the back focal plane of the illumination objective and images for 3 orientations and 3 phases are taken with 30 ms exposure time. A z-stack is created by moving the piezo stage in 400 nm steps through the sample. For 3D SIM the same approach is taken, but a

third illumination beam is added at the center of the beam paths, with images for 3 orientations and 5 phase steps each being taken. A resulting bead image for 488 nm excitation with a set point for a resolution enhancement of 1.6 is exemplary shown in Figure 2, consisting of both a widefield image through summing of all illumination orientations and phases and a HiFi processed SIM image. Exemplary single bead xy and xz sections for point spread function evaluation are also included, leading to a FWHM of 350 nm and ~2 µm in xy and xz for widefield, 220 nm and 1.9 µm for 2D SIM and 220 nm and 1.2 µm for 3D SIM.

In conclusion, we demonstrate a compact 2D and 3D multi-wavelength structured illumination microscope using several MEMS micromirrors working together to precisely and repeatably generate the illumination patterns needed in SIM.

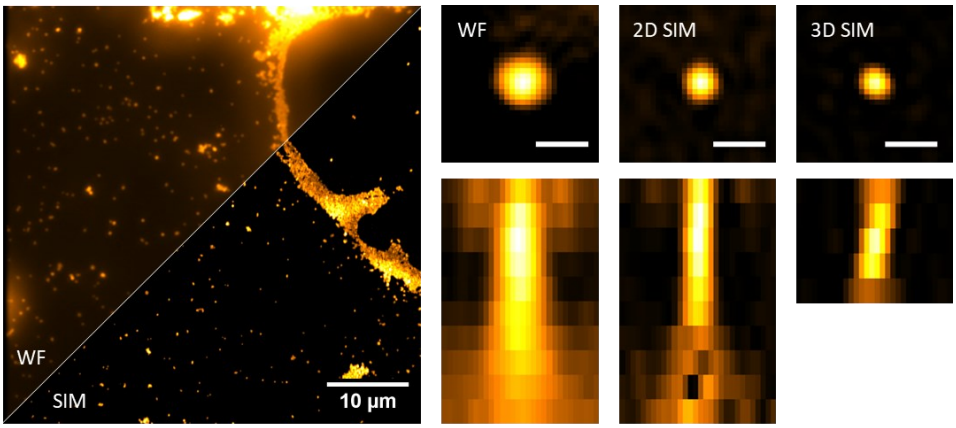

Figure 2. Bead images for reconstructed widefield (WF) and 2D SIM image, including individual bead cross-sections for WF, 2D SIM and 3D SIM. Scale bars for individual beads are 500 nm.

ACKNOWLEDGEMENTS

The authors want to acknowledge funding from the UK Engineering and Physical Sciences Research Council (grant no EP/S032606/1, studentship EP/T517938/1, and Impact Accelerator Account R220416-149), and internal funding by the University of Strathclyde.

P.T. and R.B. have filed a patent application based on the structured illumination generator.

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