A structured illumination microscopy module using two micro-electro-mechanical system scanning micromirrors

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ABSTRACT

We present the development and application of a novel structured illumination microscope (SIM) in which the grating pattern is generated using two optical beams controlled via two micro-electro-mechanical system (MEMS) 3D scanning micromirrors, each having static angular and piston control. This arrangement enables the generation of a fully controllable spatial interference pattern at the focal plane by adjusting the positions of the beams in the back-aperture of a high numerical aperture (NA) microscope objective. The utilization of MEMS micromirrors to control angular, radial and phase positioning for the structured illumination patterns has advantages of flexible control of the fluorescence excitation illumination, with achromatic beam delivery through the same optical path, reduced spatial footprint and cost-efficient integration.

Keywords: Structured illumination microscopy, MEMS, super-resolution microscopy, life science imaging

1. INTRODUCTION

Widefield fluorescence microscopy is now an integral and fundamental tool for the life sciences. However, due to the well-known optical diffraction effects it is typically not possible to resolve objects smaller than 250 nm laterally and 600 nm axially using widefield microscopy [1,2].This diffraction limit of light has led to the development of many new types of fluorescence microscopy which yield far-field imaging below the optical resolution limit, so termed super-resolution microscopy. These can broadly be categorized as being either localization-based or structured illumination-based approaches. Structured illumination-based techniques operate based on the manipulation of the very structure of the imaging point spread function to achieve super-resolution, as for example in stimulated emission depletion microscopy [3–5].

One of the increasingly popular approaches for achieving super-resolution, without relying on intensive computation, is structured illumination microscopy (SIM). SIM is a technique which enables widefield spatial resolution doubling in 2D or 3D through the application of optical interference effects [6,7]. This can be achieved whilst retaining typical widefield low light doses and high temporal resolutions, and without stringent requirements on sample sparsity, morphology or usage of special labels which are typically characteristic of other techniques [8,9].

SIM imaging requires the projection of a fine, high-contrast sinusoidal interference grating onto a fluorescence specimen, which itself can be described as a high spatial frequency grating object. The superposition between these two grating patterns results in a Moiré pattern which shifts high frequency information into a lower frequency pattern which can be detected in the optical transfer function of the imaging optics [8,10].

The optical interference required for 2D SIM is generally obtained by placing a fixed diffraction grating, or more recently a configurable digital micromirror device (DMD) or spatial light modulator with a programmed grating pattern, in the excitation path and focusing the 1st orders of the resulting interference pattern onto the back aperture of a microscope objective, thereby generating high resolution stripe patterns within the fluorescently labelled specimen [10,11]. When using a fixed diffraction grating the temporal resolution is limited by the speed of mechanical manipulation of the grating [12] , while when using a DMD the system is typically restricted to using only a single color, as the excitation must impinge upon the

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DMD at a wavelength-dependent blaze condition, making it difficult to image at different excitation wavelengths using the same system [11].

In this work we demonstrate an alternative method to achieve the SIM excitation grating pattern in the specimen by using a pair of commercially obtained silicon MEMS scanning micromirrors. We have adapted the micromirrors to develop a SIM module which allows us to generate a controllable spatial interference pattern at the specimen plane of the constructed SIM system, and manipulate the phase and angular rotation of the pattern by only applying control voltages directly to the MEMS

2. MEMS SIM DESIGN AND CHARACTERIZATION

2.1 SIM module design

The SIM module schematic is shown in Figure 1 and uses a $\lambda = 473$ nm solid state laser (CNI OEM-I-473) as excitation source, which was free-space coupled to a 50/50 beam splitter directing the split light at 45 degrees to the two 2 mm diameter MEMS micromirrors (fabricated by Mirrorcle Inc) mounted in 3D printed adaptors on a 5-axis holder. The two beams were magnified through a 4f configuration consisting of two f = 30 mm achromatic lenses, a knife edge prism (KEP) and a f = 125 mm achromatic lens. A f = 75 mm achromatic lens then focuses the two beams in a telecentric manner onto the back aperture of a 100X/1.25 microscope objective (100X ZEISS A-Plan Oil Immersion Objective, Zeiss). The two beams undergo interference at the specimen plane, generating an excitation sinusoidal interference grating with frequency and orientation dependent on the beam positions. Resulting fluorescence emission was collected by the objective before passing through a dichroic (DMLP490, Thorlabs) and longpass filter (FELH0550, Thorlabs) and finally being imaged onto a CMOS camera (UI-3060CP-M-GL Rev.2, IDS) using a f = 150 mm achromatic tube lens. The specimens are placed onto a custom 3-axis stage that comprises of both 3D printed and off-the-shelf components utilizing a piezo controlled focusing axis. The entire footprint of the system fits onto a 300 mm x 450 mm breadboard and makes use of-off-the shelf elements keeping the component cost for the entire microscope below £7000.

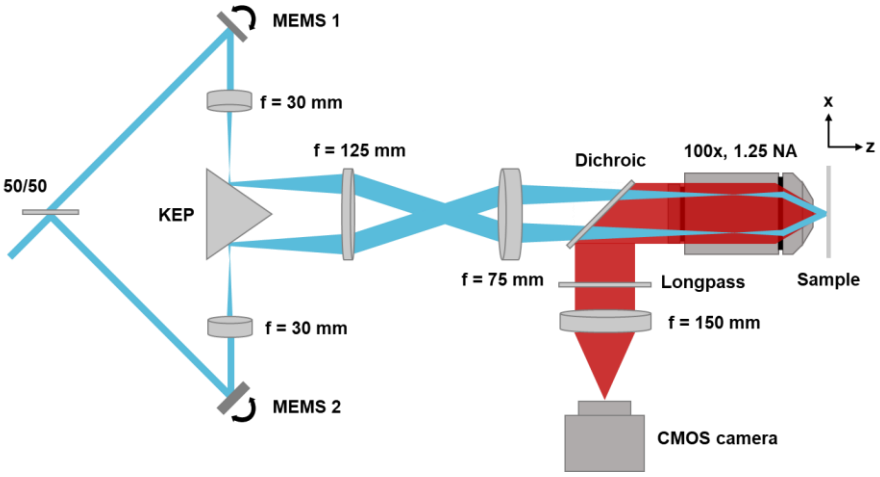

Figure 1. Schematic of the MEMS SIM module demonstrating the excitation path in blue and the resulting fluorescence emission in red.

2.2 MEMS mirror characterization and beam positioning

Prior to testing the SIM module, the two MEMS mirrors were characterized for their angular and piston movement. To enable piston movement of the commercially available mirrors, a minor modification to the bond-wires of one movement axis was undertaken, leading to a reduction of movement angle in one of the otherwise identical axes. In order to assess the angular movement along both axes a laser was reflected off one of the MEMS onto a spot on the wall. Both angular

movement response axes are shown in Figure 2A, detailing a ±10 ° angular range at the full Y-axis and a ±5 ° angular range for the reduced X-axis.

The piston displacement will translate into a phase change between the two interfering beams, allowing generation of the minimum 3 phase steps necessary for SIM reconstruction. This was measured using a microscope coupled vibrometer with only a limited actuation voltage range due to the sub-wavelength phase changes required for SIM. A plot of this characterization can be seen in Figure 2B, showing a 15 µm piston movement with a 10 % voltage range on the MEMS scanner actuators.

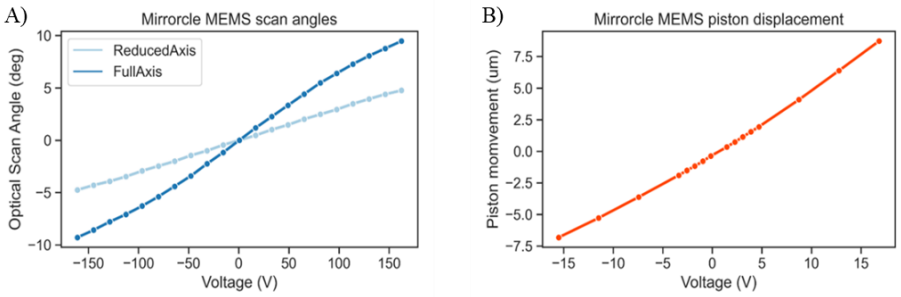

Figure 2. A) Measured full optical scan angle of the identical 2-axis MEMS with the reduced axis being the in-plane X axis and the full axis being the out-of-plane Y axis of the setup. B) A measurement of the piston movement taken around the mirror center position.

To conduct 2D SIM imaging, one typically requires at least three angular positions of the interference fringes on the specimen. This is due to the resolution improvement only being obtained in the direction of the grating, and a single grating only covering around one third of the Fourier domain. As such, the grating pattern must be rotated at least two times in 60 degree steps to obtain an isotropic resolution improvement [10].

We first reimaged two beam spots that are brought to focus at the location of the back aperture of the objective using a CMOS camera (DCC1514M-GL, Thorlabs) equipped with a C-mount imaging lens with a demagnification of 1.65X. The first beam positions, which we defined as our first grating orientation, only made use of the X scan of the MEMS mirrors. The voltages applied to the two X axes actuators were for both MEMS, 88 V. Using the image coordinates and the knowledge that our other grating orientations were to be set at ± 60 degrees, we were able to use trigonometry to define the image coordinates the beams are necessary to be at. Then by changing the voltages applied to the mirrors in X and Y we were able to move the two beams spots to those image coordinates and take note of the applied voltages. The beams in these positions can be seen in Figure 3.

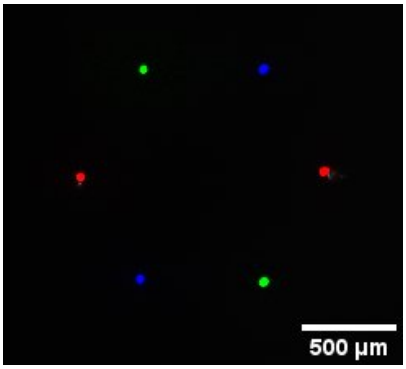

Figure 3. Reimaged beam spots from the back aperture location. The different beam angular orientations are color coded for each pair with the ones in red only utilizing the X displacement. The green and blue excitation spots located at ±60° utilize both X and Y mirror displacement.

3. SIM IMAGING OF FIXED CELL SPECIMEN

A fixed specimen of BPAE cells with the F-actin labelled with Alexa Fluor 488 phalloidin (F36924 FluoCells™ Prepared Slide #1, Invitrogen) was placed on a custom-made specimen stage and imaged using the high NA oil immersion objective. The separation between the two beams in the zero grating orientation was first adjusted by translating the KEP along the optical axis until both beams were passing through the 4.1 mm back aperture of the objective. A 3 mm aperture was placed at the focus between the f = 125 mm and f = 75 mm lenses to remove any scattered light from the MEMS.

Using the X and Y coordinate voltages determined in the MEMS characterization the three different beam rotations were applied to the MEMS using a custom labview script. The excitation laser power at the specimen plane was set to between 2 and 2.5 mW/cm^2 per beam. The specimen was imaged using an exposure time of 30 ms, no camera binning and an analogue gain set to 100 using µManager [13]. The resulting images and Fourier transform of the images can be seen in Figure 4.

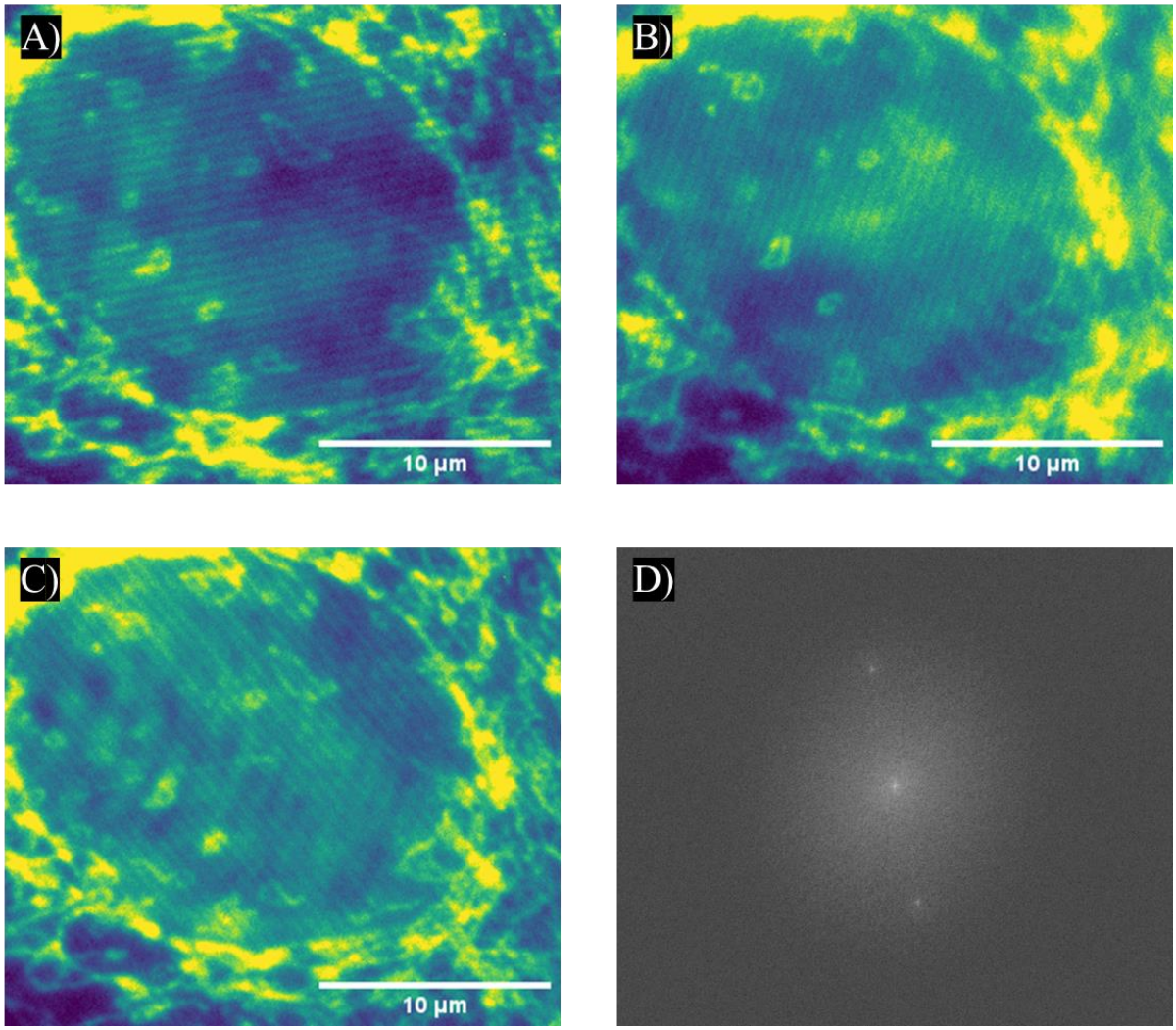

Figure 4. Cropped and contrast adjusted images of the SIM sinusoidal grating pattern projected onto a fluorescent BPAE specimen with the grating pattern set to A) 0 degrees B) + 60 degrees and C) – 60 degrees. D) A representative Fourier transform of panel A) acquired using the SIMcheck plugin[14].

It can be seen from the images that the beam position changes translate into a change in the angle of the interference grating. However, angular rotation is only half of the requirement for SIM, as three phase changes per rotation are also required.

In order to determine whether the piston displacement on the mirrors also shifts the phase of the projected grating pattern the same specimen and imaging parameters that were used to acquire the data in Figure 4 was used with two exception; instead of rotating the grating pattern we used only the first grating position with a voltage of 88 V on all X mirror actuators; and to record the effect of the piston movement an image each with a differential voltage of ± 5.3 V on the X axis actuators of M1. The resulting phase images at a small area of the camera field of view are shown in Figure 5 A-C. To determine if there was a phase shift in the grating pattern observed the images underwent a difference operation using the image calculator in Fiji. This difference operation will aid in identifying a phase change between the images as only the locations at which two grating patterns do not overlap will be obtained.

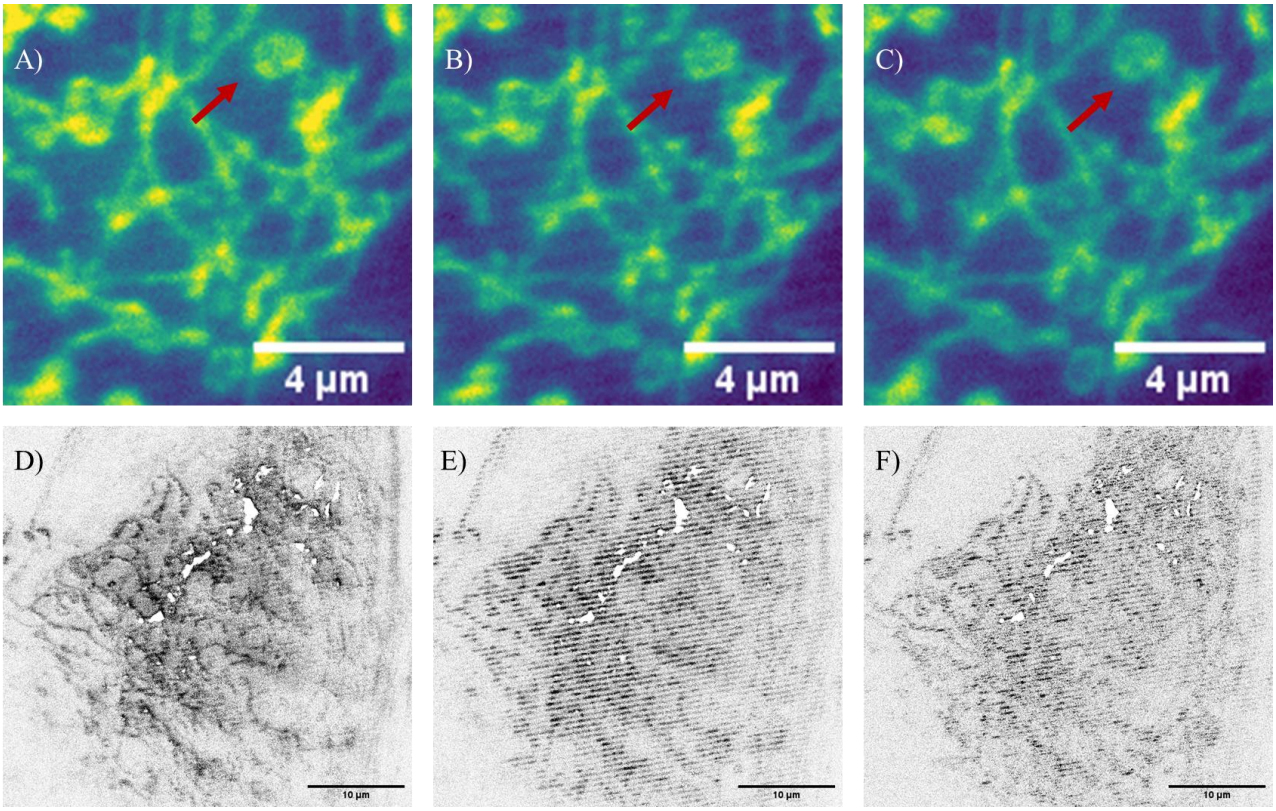

Figure 5. Cropped and contrast adjusted SIM grating images of BPAE cells with the piston set at A) – 5.3 V B) 0 V and C) +5.3 V. D) is the result of a difference operation applied between a full field of C) and A). E) shows the result of a difference operation applied between a full field of B) and A). F) shows the result of a difference operation between a full field C) and B).

The result of the difference operations is shown in Figure 5 D, E and F demonstrates that a different piston position on the MEMS translates into a shift of the phase of the grating patterns projected onto the specimen.

4. CONCLUSIONS

We have demonstrated the development and characterization of a MEMS SIM module that has a low spatial footprint and allowed for a spatial and phase controllable SIM grating pattern to be projected onto a fixed fluorescent biological specimen. The module consists of two 3-axis MEMS scanning mirrors as functional elements, controlling the position and phase of the 2-beam interference necessary for 2D SIM.

Future work consists of the full evaluation of phase stability and minimum incremental phase changes, as well as full automation of the system and characterization of its possible temporal resolution. An additional excitation source will also be integrated to achieve multi-color imaging and make use of opensource software such as FairSIM to fully reconstruct our SIM data in an accessible image processing pipeline.

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REFERENCES

[1] Petty, H.R., "Fluorescence microscopy: Established and emerging methods, experimental strategies, and applications in immunology," in Microscopy Research and Technique 70(8), pp. 687–709 (2007).
[2] Cremer, C., and Masters, B.R., "Resolution enhancement techniques in microscopy," The European Physical Journal H 38(3), 281–344 (2013).
[3] Hell, S.W., and Wichmann, J., "Breaking the diffraction resolution limit by stimulated-emission - Stimulated-emission-depletion fluorescence microscopy," Optics Letters 19(11), 780–782 (1994).
[4] Klar, T.A., and Hell, S.W., "Subdiffraction resolution in far-field fluorescence microscopy.," Optics letters 24(14), 954–6 (1999).
[5] Blom, H., and Widengren, J., "Stimulated Emission Depletion Microscopy," Chemical Reviews 117(11), 7377–7427 (2017).
[6] Gustafsson, M.G.L.L., "Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy," Journal of Microscopy 198(2), 82–87 (2000).
[7] Gustafsson, M.G.L., Shao, L., Carlton, P.M., Wang, C.J.R., Golubovskaya, I.N., Cande, W.Z., Agard, D.A., and Sedat, J.W., "Three-dimensional resolution doubling in wide-field fluorescence microscopy by structured illumination.," Biophysical Journal 94(12), 4957–4970 (2008).
[8] Heintzmann, R., and Huser, T., "Super-Resolution Structured Illumination Microscopy," Chemical Reviews 117(23), 13890–13908 (2017).
[9] Markwirth, A., Lachetta, M., Mönkemöller, V., Heintzmann, R., Hübner, W., Huser, T., and Müller, M., "Video-rate multi-color structured illumination microscopy with simultaneous real-time reconstruction," Nature Communications 10(1), (2019).
[10] Gustafsson, M.G.L., "Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy" (2000).
[11] Sandmeyer, A., Lachetta, M., Sandmeyer, H., Hübner, W., Huser, T., and Müller, M., "DMD-based super-resolution structured illumination microscopy visualizes live cell dynamics at high speed and low cost," bioRxiv797670 (2019).
[12] Lu-Walther, H.W., Kielhorn, M., Förster, R., Jost, A., Wicker, K., and Heintzmann, R., "FastSIM: A practical implementation of fast structured illumination microscopy," Methods and Applications in Fluorescence 3(1), (2015).
[13] Edelstein, A., Amodaj, N., Hoover, K., Vale, R., and Stuurman, N., "Computer Control of Microscopes Using μManager," Current Protocols in Molecular Biology 92(1), 14.20.1-14.20.17 (2010).

[14] Ball, G., Demmerle, J., Kaufmann, R., Davis, I., Dobbie, I.M., and Schermelleh, L., "SIMcheck: a Toolbox for Successful Super-resolution Structured Illumination Microscopy OPEN," Nature Publishing Group (2015).