Christopher, J., Donnachie, M., Rooney, L., McConnell, G., Uttamchandani, D., & Bauer, R. (2024). Using 3D printed optical elements for multifocal image scanning microscopy. Proceedings of SPIE, 12827, Article 1282702. https://doi.org/10.1117/12.3002507

Using 3D printed optical elements for multifocal image scanning microscopy

Jay Christopher[*,1], Mark Donnachie[1], Liam Rooney[2], Gail McConnell[2], Deepak Uttamchandani[1], and Ralf Bauer[1]
[1]Centre for Microsystems and Photonics, Technology and Innovation Centre, University of Strathclyde, Glasgow, Scotland
[2]Centre for Biophotonics, Strathclyde Institute of Pharmacy & Biomedical Sciences (SIPBS), University of Strathclyde, Glasgow, Scotland
[*]jay.christopher@strath.ac.uk

ABSTRACT

We present a small footprint and affordable implementation of a multifocal image scanning microscope (ISM), utilizing both a microelectromechanical system (MEMS) micromirror for flexible optical excitation control, and multifocal patterned illumination using a custom 3D printed optical quality lenslet array. We highlight the individual element performance and demonstrate its use in fluorescence imaging to allow comparison of the affordable and customizable approach with its commercial counterpart.

Keywords: 3D printing, 3D printed optics, structured illumination, mSIM, image scanning microscopy, ISM

1. INTRODUCTION

Optical super-resolution microscopy has flourished into a diverse field, with many techniques relying on the photophysical properties of fluorophores to surpass the optical diffraction limit. This dependence adds limitations when imaging thick specimens in multicolor and live-cell conditions, minimizing their accessibility within biomedical research. Structured illumination microscopy (SIM) mitigates the necessity of specialized fluorophores, instead relying on optical illumination properties to extract super-resolution information [1], [2]. One variation of SIM is image scanning microscopy (ISM) which recovers high-spatial-frequency information through pixel reassignment of a confocal detector [3]–[5]. To improve the speed of data capture, multifocal approaches have been introduced, such as multifocal SIM (mSIM), which allow the use of sensitive sCMOS cameras with virtual pinholes used for the reassignment process to achieve a doubling of the maximally obtained optical resolution [6]. However, expensive microlens arrays have so far been necessary to create the multifocal illumination necessary for virtual pinholes at fast scan rates which minimizes the impact of the technique for biomedical research globally. 3D printing has a unique potential to minimize the reliance on expensive glass optical lenslet arrays.

There are numerous methods to manufacture 3D printed optical elements, many of which are applicable to microlens array fabrication. The 3D printing techniques which can print with sufficient resolution and surface quality to negate post-processing for optical quality microlens arrays regularly use two-photon polymerization methods, such as direct laser writing [7]–[9]. The drawback of these two-photon methods is the dependence on expensive pulsed light-sources which currently are unable to provide a low-cost 3D printed optical manufacturing method. Stereolithography (SLA) techniques, which manufacture in a layer-by-layer process, have helped to address the economic gap between optical 3D printing technologies without sacrificing optical quality as seen in digital light processing (DLP) printing, which uses inexpensive LEDs in conjunction with LCD screens to provide sub-50 μm printing resolutions [10]–[13]. Using low-cost desktop 3D printers, optical quality 3D printed components have proved successful in imaging chrome lithography targets and

biological specimens [12], [13]. However, minimal observations have been made on the impact of 3D printed optical components solely within the optical excitation arm of a microscope. Given their ability to manufacture free-form geometries, 3D printing optics provides a unique potential for reducing the costs of the excitation arm within ISM and the microlens array component needed.

Conventional approaches for scannable-excitation within microscopy may often be found in galvo-scanning, piezo-scanning or digital micromirror devices (DMDs) [14]–[16]. These can be relatively simple in their implementation for single excitation beams, though when using multiple beams, as is necessary for structured illumination approaches, the alignment using these components is significantly more complex and difficult. An alternative for excitation control is microelectromechanical system (MEMS) mirrors which can offer high-frequency, three-axis scanning potential while maintaining a budget-friendly component cost, which we utilize for our ISM in conjunction with a 3D printed lenslet array.

Here we present an in-expensive ISM implementation making use of optical 3D printed lenslet arrays in combination with a 2D MEMS scanning mirror for a small footprint microscope concept.

2. METHODS

Manufacturing 3D printed lenslet arrays using SLA technology follows the same principles as manufacturing any other 3D printed optical element, as described in previous literature [11]–[13]. Careful consideration is required however in the design stage of the lenslet array as lenslet curvature is intrinsically limited by the voxel size of the 3D printer. Lenslet arrays were designed in Autodesk Inventor in both a ‘honeycomb’ pattern and in a rectangular pattern, where each lenslet has a 1.6 mm diameter and 3 mm radius of curvature. Each lenslet was designed to be 1200 µm center to center from its nearest neighbor which helped to minimize background illumination. In addition, a 3D printed mask was designed in Autocad Inventor with near identical properties to the lenslets, though instead with a 1000 µm diameter pinhole at each lenslet location and printed (Elegoo Mars 2 with Anycubic ABS-like black resin) before being painted black. The mask was placed onto the lenslet face so each pinhole was concentrically aligned with the corresponding lenslet to minimize background illumination further. After designing the lenslet array, it was found that the best lenslets were produced when sliced (Chitubox Pro) and printed using Anycubic High Clear resin with the lenslet faces perpendicular to the z-axis i.e. ‘upright’ or ‘side on’ and at a 10 µm z-resolution with grayscaling/anti-aliasing maximized. This ensured that the pixel intensities at the lenslet surfaces followed an intensity distribution which provided lenslet curvatures matching the design constraints.

Following printing of the lenslet arrays, a spin-coating approach was employed to coat the lenslets and planar surface as previously reported [12], [13]. The lenslets were coated with Vida-Rosa resin and spin-coated over four pre-programmed steps, starting at 1000 RPM and then 2000 RPM both for 5 s each, before a final two steps of 4000 RPM and 6000 RPM for 10 s each. Upon completion of spin-coating, the lenslet array was then immediately cured with 405 and 385 nm LED’s (Elegoo Mercury Plus 2-in-1 Wash and Cure station) for 20 minutes. The planar surface followed the same method for the planar surfaces as has been previously reported [13].

Following fabrication, the lenslet arrays could then be utilized in the ISM setup as shown in Fig.1 where the ‘MLA’ component was either a commercial glass microlens array (RPC Photonics MLA-S250-f30) or our home-produced 3D printed lenslet array.

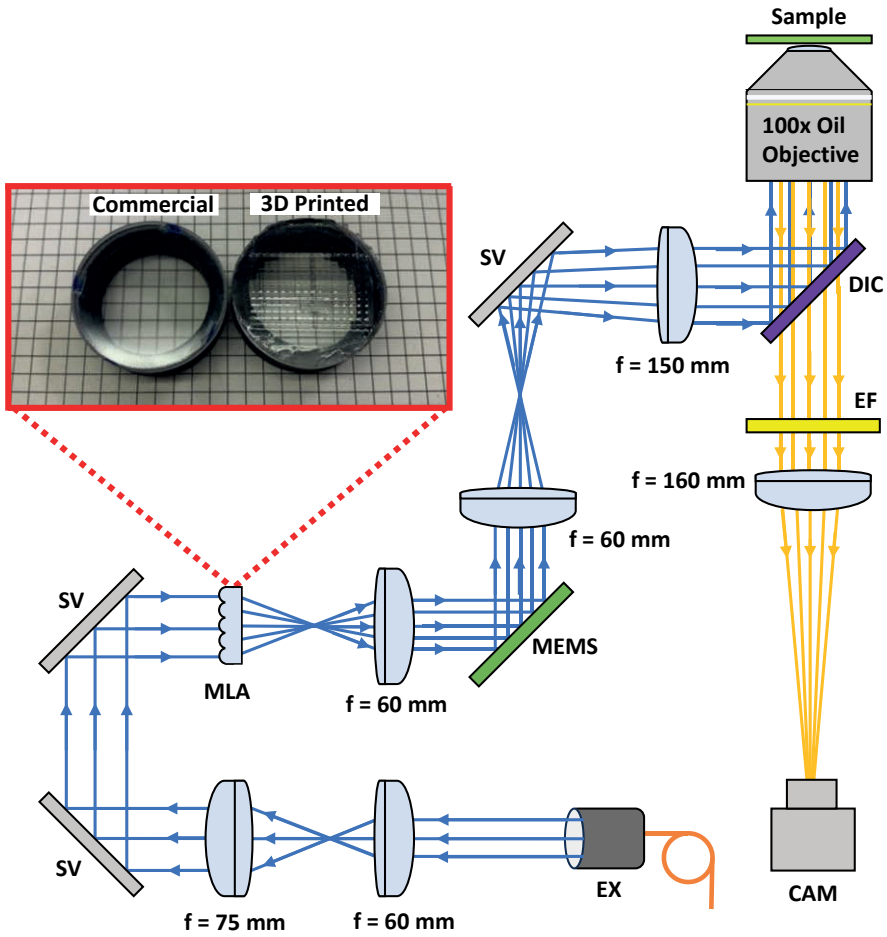

Fig.1 – Image scanning microscope schematic with reference commercial (left) and 3D printed (right) lenslet arrays. EX – 488 nm single-mode laser collimator; SV – silver mirror; MLA – microlens array, either commercial microlens array or 3D printed lenslet array; MEMS – microelectromechanical mirror; DIC – multi-band 405/488/561/640 dichroic; EF – FELH0500 emission filter; CAM – IDS camera.

3. RESULTS

A test sample consisting of di-8-anepps crystals was illuminated using a 3D printed rectangular patterned lenslet array and the rectangular commercial microlens array for comparison. The resulting single position fluorescence response of the illumination distribution and a raster-scanned summation of all illumination positions is shown in fig. 2. The commercial lenslet array shows an expected higher density illumination pattern due to the lenslet center position spacing of 0.25 mm compared to 1.2 mm for the 3D printed array. At the same time a stronger aberration influence is visible in the commercial implementation, as seen in the zoomed sections of fig. 2A. The 3D printed implementation shows low energy blurry spots next to the lenslet focus, originating from diffraction orders of the lenslet element due to the layering process in the direct print fabrication. As we use a virtual pinholing approach for the ISM reconstruction these diffraction orders are not impacting imaging performance. As can be seen in fig. 2B, by laterally shifting each excitation spot across the sample and summing the images, we can construct an image using the 3D printed lenslet array with comparable detail and image contrast as with the commercial glass microlens array. However, more scan positions and therefore more time is required to acquire a single image frame with the 3D printed lenslets. The commercial glass optics cost >£500 per microlens array, while the 3D printed lenslet array cost <£0.10. Given the similarities between the resulting images, this shows considerable

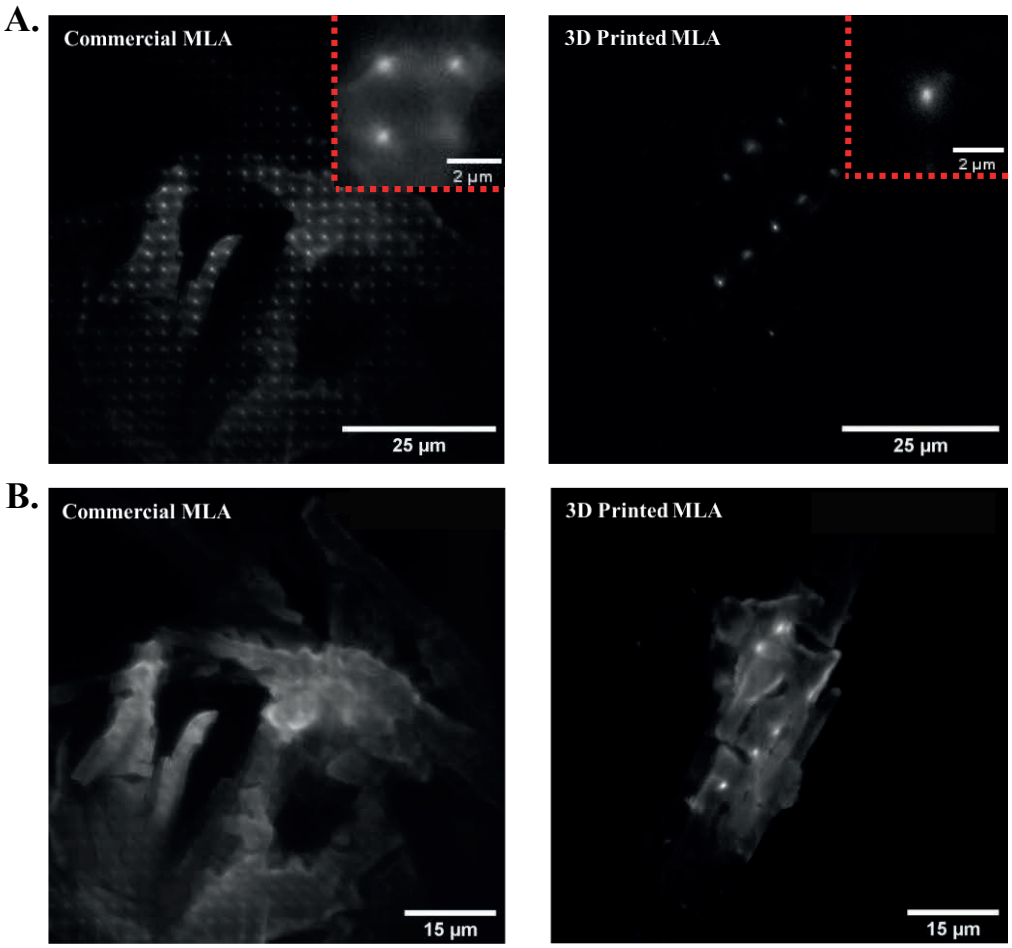

Fig.2 – Fluorescing Di-8-ANEPPS crystals under lenslet array excitation. (A) – Individual spots illuminating the Di-8 crystal with the commercial lenslet array (left), and 3D printed lenslet array (right). Zoomed in regions are shown in the red dotted box; (B) – Resultant image from the commercial lenslet array (left) and 3D printed lenslet array (right) obtained by raster-scanning the spots across the Di-8 crystal and summing the images.

promise for low-cost ISM, especially by using honeycomb patterned illumination in the element fabrication to minimize the distance between illumination spots further.

4. CONCLUSION

We have shown that by fabricating lenslet arrays using a low-cost desktop 3D printer, we can create an ISM implementation and obtain comparable images to commercial glass microlens arrays. With iterative improvement, this holds significant potential for 3D printed optics within super-resolution imaging, which could significantly improve the accessibility of super-resolution imaging to low-budget research.

ACKNOWLEDGEMENTS

We acknowledge funding from the UK Engineering and Physical Sciences Research Council (grant EP/S032606/1 and doctoral training partnership EP/T517938/1) and UK Royal Academy of Engineering (Engineering for Development Fellowship scheme RF1516/15/8).

REFERENCES

[1] Gustafsson, M. G. L., "Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy," *J Microsc*, vol. 198, no. 2, pp. 82–87, doi: 10.1046/j.1365-2818.2000.00710.x. (2000)

[2] Gustafsson, M. G. L., *et al.*, "Three-dimensional resolution doubling in wide-field fluorescence microscopy by structured illumination," *Biophys J*, vol. 94, no. 12, pp. 4957–4970, doi: 10.1529/biophysj.107.120345. (2008)

[3] Müller, C. B. and Enderlein, J., "Image scanning microscopy," *Phys Rev Lett*, vol. 104, no. 19, pp. 1–4, doi: 10.1103/PhysRevLett.104.198101. (2010)

[4] Sheppard, C. J. R., Mehta, S. B., and Heintzmann, R., "Superresolution by image scanning microscopy using pixel reassignment," *Opt Lett*, vol. 38, no. 15, p. 2889, doi: 10.1364/ol.38.002889. (2013)

[5] Ward, E. N., and Pal, R., "Image scanning microscopy: an overview," *J Microsc*, vol. 266, no. 2, pp. 221–228, doi: 10.1111/jmi.12534. (2017)

[6] York, A. G. , *et al.*, "resolution doubling in live, multicellular organisms via multifocal structured illumination microscopy," *Articles nAture methods* |, vol. 9, no. 7, p. 749, doi: 10.1038/nmeth.2025. (2012)

[7] Wu, S., Serbin, J., and Gu, M., "Two-Photon Polymerisation for Three-Dimensional Micro-Fabrication," *J Photochem and Photobio (2006)*

[8] Thiele, S., Arzenbacher, K., Gissibl, T., Giessen, H., and Herkommer, A. M., "3D-printed eagle eye: Compound microlens system for foveated imaging," *Sci Adv*, vol. 3, no. 2, pp. 1–7, doi: 10.1126/sciadv.1602655. (2017)

[9] Weber, K. *et al.*, "Tailored nanocomposites for 3D printed micro-optics," *Opt Mater Express*, vol. 10, no. 10, p. 2345, doi: 10.1364/ome.399392. (2020)

[10] Berglund, G. D., and Tkaczyk, T. S., "Fabrication of optical components using a consumer-grade lithographic printer," *Opt Express*, vol. 27, no. 21, p. 30405, doi: 10.1364/oe.27.030405. (2019)

[11] Vaidya, N., and Solgaard, O., "3D printed optics with nanometer scale surface roughness," *Microsyst Nanoeng*, vol. 4, no. 1, doi: 10.1038/s41378-018-0015-4. (2018)

[12] Rooney, L. M., *et al.*, "Printing, Characterising, and Assessing Transparent 3D Printed Lenses for Optical Imaging," bioRxiv doi: 2023.11.22.568002 (2023)

[13] Christopher, J., *et al.,* "Low-cost 3D printed lenses for brightfield and fluorescence microscopy," bioRxiv 2023.11.22.568227 (2023)

[14] Dan, D., *et al.*, "DMD-based LED-illumination Super-resolution and optical sectioning microscopy," *Sci Rep*, vol. 3, pp. 1–7, doi: 10.1038/srep01116. (2013)

[15] Yuan, Y., *et al.*, "Dual-color simultaneous structured illumination microscopy based on galvo-mirrors," *Opt Commun*, vol. 511, no. November 2021, p. 128012, doi: 10.1016/j.optcom.2022.128012. (2022)

[16] Gong, D., Cai, C., Strahilevitz, E., Chen, J., and Scherer, N. F., "Easily scalable multi-color DMD-based structured illumination microscopy," *Opt Lett*, vol. 49, no. 1, p. 77, doi: 10.1364/ol.507599. (2024)