Structured illumination microscopy using micro-pixellated light emitting diodes

V. Poher, G. T. Kennedy, D. S. Elson, P. M. W. French and M. A. A. Neil

Blackett Laboratory, Imperial College London, South Kensington Campus London SW7 2AZ, UK

H.Z. Zhang, E. Gu, Z. Gong, C. Griffin, J. M. Girkin, and M. D. Dawson

Institute of Photonics,University of Strathclyde, Wolfson Centre, 106 Rottenrow, Glasgow G4 0NW, UK

vincent.poher@imperial.ac.uk

Abstract. We describe a novel implementation of structured illumination microscopy using programmable micro-structured light emitting diodes. This compact system has no moving parts can be operated at frame rates in excess of 1000 frames per second.

1. Introduction

Structured illumination is a flexible and economical method of obtaining optical sectioning in wide-field microscopy [1]. In this technique the illumination system is modified to project a single-spatial frequency grid pattern onto the sample [2, 3]. The pattern can only be resolved in the focal plane and by recording images for different transverse grid positions (or phases) an image of the in-focus parts of the object can be calculated.

Light emitting diodes (LEDs) are becoming increasingly popular for lighting and illumination systems due to their low cost, small dimensions, low coherence, uniform illumination, high efficiency and long lifetime. These properties, together with recent developments in high brightness, ultraviolet operation and microstructured emitter design offer great potential for LEDs as light sources for microscopy.

In this paper we demonstrate a novel structured illumination microscope using a blue micro-structured light emitting diode as the illumination source. The system is potentially very compact and has no-moving-parts.

2. Micro-pixellated LED source

The micro-structured GaN light emitting diode [4] consisted of 120 17 µm wide 3600 µm length individually-addressable stripes having a centre-centre separation of 34 µm, resulting in a total emitting area of 7.34 square millimeters. The emission wavelength was centred at 470 nm. An image of the device is presented as Figure 1.

In this prototype device several stripes were inoperative and a pair of stripes was shorted together. It is anticipated that further developments to optimize the stripe for structured illumination microscopy will result in a brighter and more uniform stripe device which will yield improved sectioned images with reduced artifacts.

The device was driven by an in-house designed programmable constant-current driver sourcing up to 35mA per stripe. This allowed any stripe pattern to be generated at frame rates in excess of 1 kHz

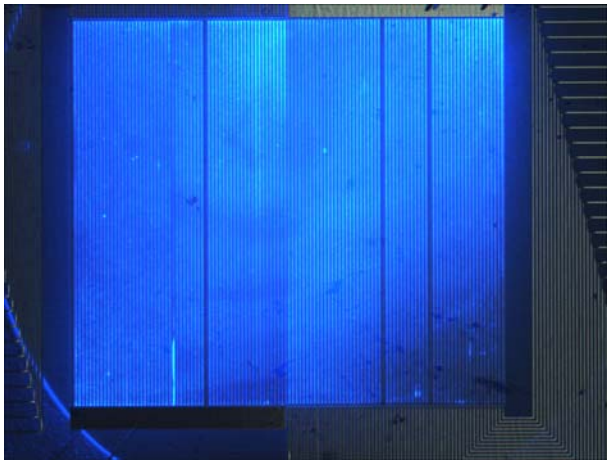


Figure 1: picture of the micro-structured LED

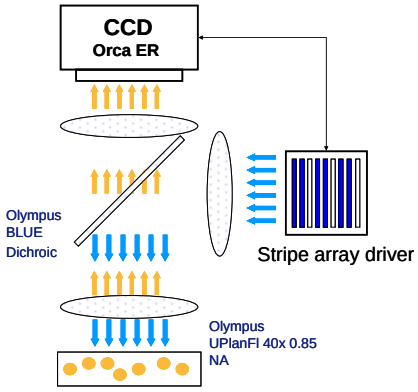


Figure 2: schematics of the microscope setup

3. Microscope configuration

The LED was incorporated in a critical illumination configuration into a home-built microscope consisting of a 180mm Olympus tube lens, an Olympus blue fluorescence filter cube and an Orca ER camera (Fig 2). A 34 µm period grid pattern was obtained by turning 2 stripes on and 1 off at a time. To obtain the three grid positions required for structured illumination, a scanning scheme was implemented by shifting the grid pattern by one stripe as shown in Figure 3. The camera was synchronised with the array driver to avoid any unnecessary illumination of the sample.

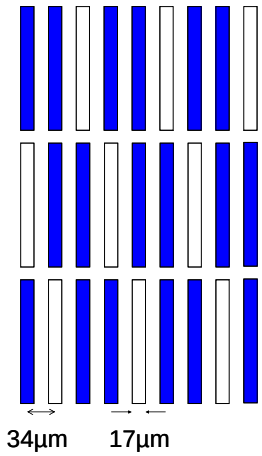


Figure 3: grid patterns

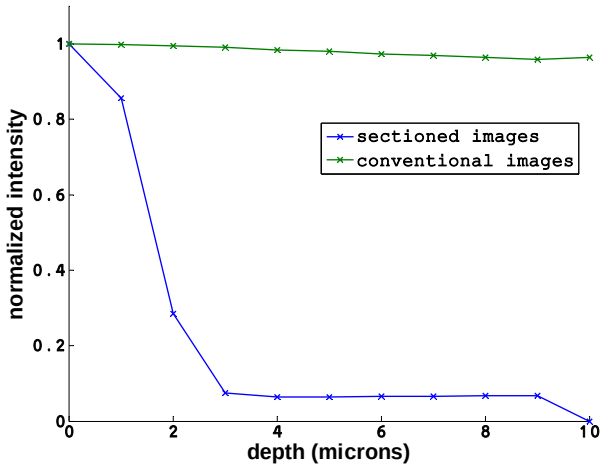
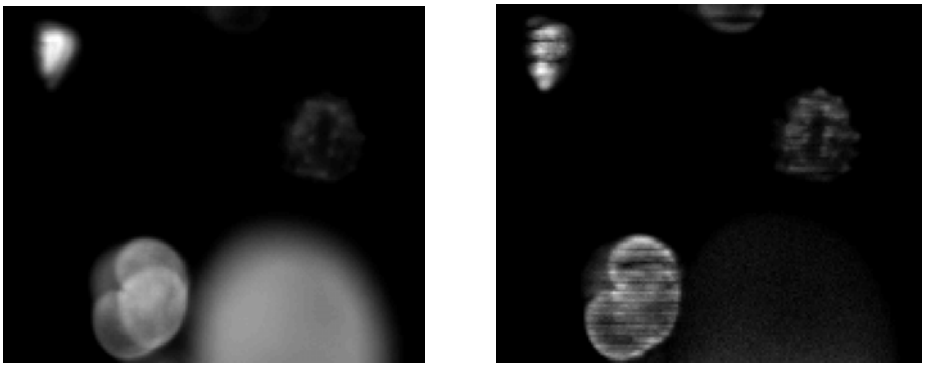


Figure 4: measured axial responses of the system

The axial resolution was measured using a 40X 0.85 NA UPlanApo Olympus dry microscope objective and a plane mirror together with a calibrated axial stage. The resulting axial responses for both sectioned images and conventional images are presented as Figure 4. A sectioning strength of 2.5 μm was measured.



(a) (b)

Figure 5: Fluorescence image of stained pollen grains (a) conventional image, (b) sectioned image

Fluorescence images of a cluster of stained pollen grains were acquired using a 20x 0.5 NA objective lens. Figure 5(a) shows the reconstructed conventional image in which a strong out-of-focus blur is clearly present due to a large pollen grain above the focal plane. Figure 5(b), on the other hand, shows the calculated sectioned image where the out-of-focus blur has clearly been removed. Some artifacts are, however, present in the sectioned image, probably due to second harmonics present in the grid pattern and non-uniformities in the device emission.

4. Conclusion

In conclusion, we have presented a novel structured illumination microscopy system based on a blue micro-structured light emitting diode. This compact, reliable illumination system has no moving parts and can operate at high frame rates. Further developments of the LED device will enhance the image quality and will enable other advanced microscopy techniques to be implemented.

Acknowledgement

This work was funded by the RCUK Basic Technology Research Programme, “A Thousand Micro-Emitters Per Square Millimetre: New Light on Organic Materials & Structures.”

References

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