Optically Sectioned Microscopy Using Micro-Pixellated Light Emitting Diodes

Basic Technology Research Programme

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A 1000 micro-emitters per square millimetre

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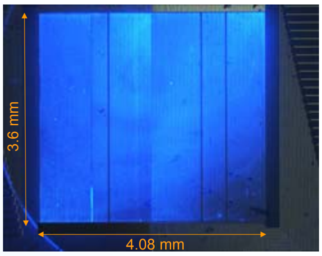
Introduction

Optical sectioning is a means of removing out of focus blur to obtain higher contrast images having enhanced axial resolution. We have used a novel micro-structured light emitting diode to implement optical sectioning in three ways: structured illumination, line scanning semiconfocal and line-scanning confocal microscopy. The resulting optical systems are compact and have no moving parts.



The LED stripe array.

Micro-structured LED array

The GaN LED consists of an array of 120 individually addressable 3600μm long 17μm wide emitters separated by 34μm. The output wavelength is 470nm.

The emitters share a common n contact and are addressed via their p contacts using a custom designed constant current driver board that is controlled using a PC via a USB port. This driver can operate at a frame rate of up to 60 000 line patterns per second.

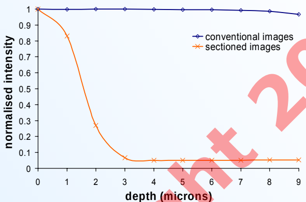
The LED stripe array driver

Structured Illumination

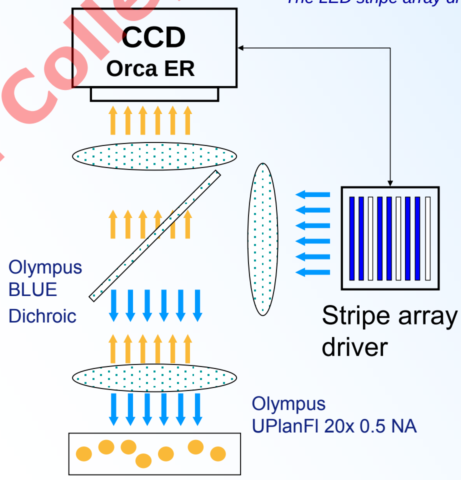
Structured illumination is a wide field optical sectioning technique. It relies on the fact that non zero frequency components are attenuated with defocus. By projecting a single spatial frequency grid onto the sample at three different phases the out of focus light can be removed. The sectioned image is given by:

$$I_{\sec} = \frac{\sqrt{2}}{3}\sqrt{(I_1 - I_2)^2 + (I_2 - I_3)^2 + (I_1 - I_3)^2}$$

A single frequency grid pattern consisting multiple periods of two adjacent stripes on and one off was projected onto the sample. The phase of the pattern could be adjusted by 2π/3 by simply turning one stripe on and one off. This is a compact alternative to the piezo-actuated grid systems that are currently used and has no moving parts.



Comparison of the axial response of the structured illumination and conventional imaging systems



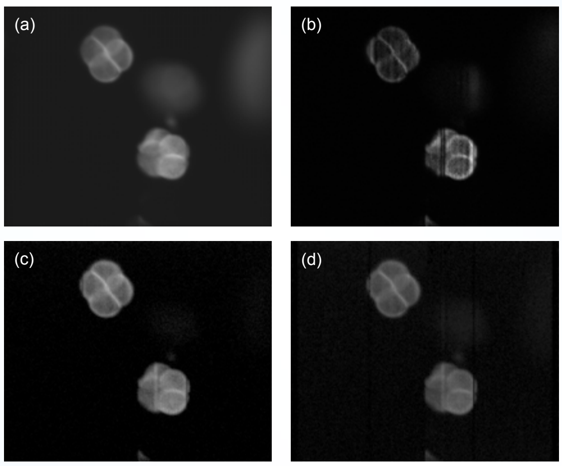
Configuration for patterned illumination

Semiconfocal imaging

A maximum or semiconfocal image can be obtained by illuminating the sample line by line and, for each pixel in the final image, selecting the maximum pixel from the image series. This can be represented as:

$$I_{semiconfocal} = \operatorname*{Max}_{i=1}^{N}\left[\mathrm{img}_i(\mathbf{x})\right]$$

This gives an image that is similar to that obtained in multipoint confocal images since the brightest pixels often correspond to the in-focus image pixels.



X20 magnification images of stained pollen grains (a) conventional image, (b) structured illumination image, (c) semi-confocal image and (d) confocal image.

Confocal line scan imaging

A confocal line scan image of the sample can be can be obtained by illuminating the sample line by line and, for each line, detecting with a slit mask. This can be represented as:

$$I_{confocal} = \sum_{i=1}^{N} \mathrm{Mask}_i \cdot \mathrm{img}_i(\mathbf{x})$$

A set of registered slit masks were generated by illuminating a thin, uniform fluorescent sheet with each stripe in the LED array, taking the fluorescence image and performing edge detection.

Conclusions

We have implemented several optical sectioning techniques using a novel microstructured LED as the illumination source. These methods yield enhanced contrast by reducing the contributions from out of focus light. Characterisation of these and other techniques is ongoing.