Flexible Optically Sectioned Microscopy Using Stripe-Array Light Emitting Diodes I

Basic Technology Research Programme — RESEARCH COUNCILS UK — *A 1000 micro-emitters per square millimetre*

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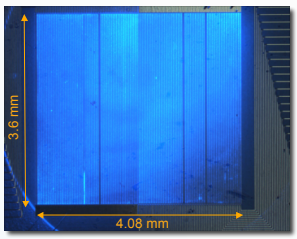
Introduction

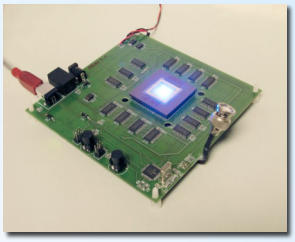
Optical sectioning is where images of only the in-focus parts of the object are produced: out of focus blur is rejected to increase contrast and true 3-dimensional resolution is achieved. We have used a novel micro-structured light emitting diode to implement optical sectioning in three ways: grid-projection structured illumination, line scanning confocal and multi-line scanning confocal microscopy. The resulting optical systems are compact and have no moving parts. This poster presents the grid-projection structured illumination technique.



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 from a PC via a USB port. This driver can operate at a frame rate of up to 50 000 patterns per second and can display arbitrary line patterns.



The LED driver

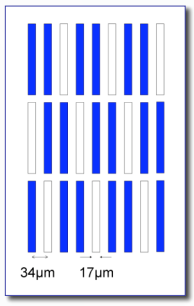
Grid-projection structured Illumination principle

Grid-projection structured illumination is a wide field optical sectioning technique. It relies on the fact that non zero spatial-frequency components are attenuated with defocus. By projecting a single spatial frequency grid onto the sample at three different relative positions (phases) the in-focus signal can be detected and the out-of-focus light removed. After acquiring 3 images I_1, I_2 and I_3, with the grid shifted laterally by one third of the grid pitch, the sectioned image is calculated from:

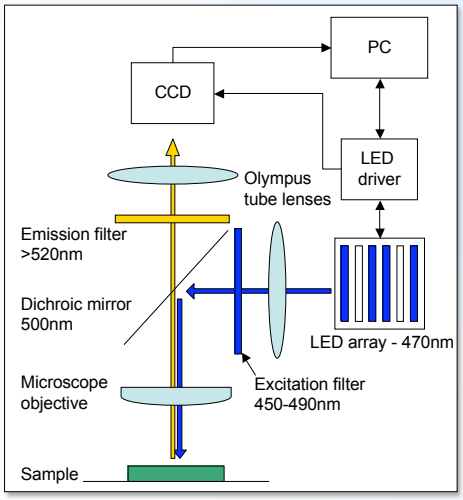
$$I_{sec} = \frac{\sqrt{2}}{3}\sqrt{(I_1 - I_2)^2 + (I_2 - I_3)^2 + (I_1 - I_3)^2}$$

LED scanning scheme

A single frequency grid pattern consisting of 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.



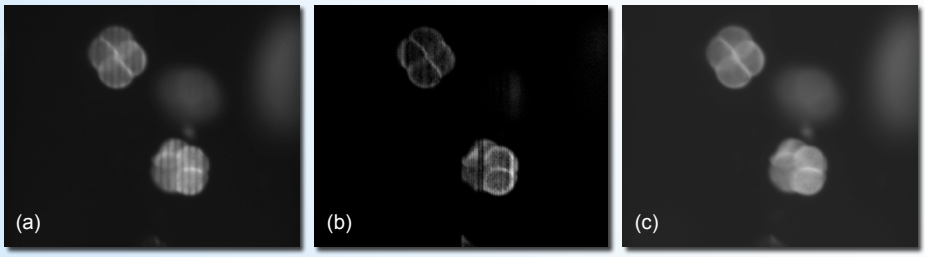
LED array scanning scheme



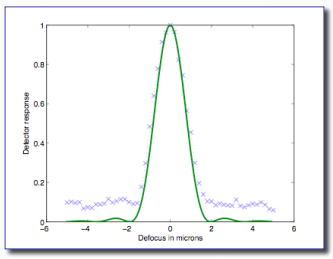
Microscope setup

Optical sectioning ability

Pictures below illustrate the structured illumination technique applied to optically section stained pollen grain. The pollen grain images were acquired using a 20x 0.5NA Olympus objective, with 500ms exposure per image and 3mA current per grid position. In order to demonstrate and quantify the sectioning ability of our microscope, we compared simulated and measured signals from the sectioned images as a thin fluorescent sheet was scanned through the focus of the microscope objective.



20x images of stained pollen grains acquired with grid-projection structured illumination. (a) Modulated raw image, (b) Sectioned image, (c) Conventional image



Experimental (crossed) and theoretical sectioning strengths using a 40X 0.75 NA objective

Conclusion

We have implemented the grid-projection structured illumination technique with no moving parts using a novel microstructured LED as the illumination source. This method yields enhanced contrast by reducing the contributions from out of focus light. The system was characterised and the sectioning ability was found comparable with the theory. Artefacts are still present in the confocal image due to missing stripes but will be removed in the next generation devices.