

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



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Introduction

We have previously used a stripe-array Light Emitting Diode (LED) in a conventional microscope to implement a widefield optically sectioned technique called grid-projection structured illumination. Keeping the same microscope setup, we are able to implement several other optical sectioning approaches such as line scanning confocal and multi-line scanning confocal microscopy.

Line-scanning confocal principle

Using slit illumination, optical sectioning can be achieved simply by blocking the out-of-focus light with a confocal detection slit. Normally a 2-d image is built up by scanning the object or scanning the slit image across the object. Here we use the micro-stripe LED to illuminate the object one line at a time. For each line position, an image is captured on a 2-dimensional array detector (CCD camera) and the light originating from out-of-focus regions of the object is rejected by post-processing the stack of $N=120$ images corresponding to the N line positions on our device.

Confocal detection

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

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

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.

Maximum projection detection

Similarly, a confocal 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 stack. This is represented by:

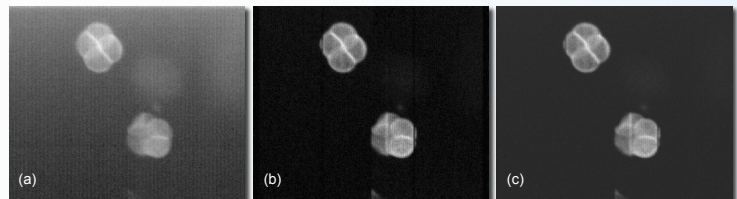
$$I_{semiconfocal} = \text{Max}_{i=1}^N [\text{img}_i(\mathbf{x})]$$

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

Sectioning ability

The sectioning strength was measured and compared with the theory.

Stained pollen grain images illustrate the sectioning ability of the single slit scanning system.



20x magnification images of stained pollen grains (a) conventional image, (b) automatic slit confocal image and (c) maximum projection confocal image.

Multi-slit optical sectioning

With no need to change the microscope setup, the acquisition time can be greatly reduced by scanning several beams on the sample at the same time instead of just a single beam. The virtual slit and maximum projection detection methods remain valid. The increased acquisition speed comes however at the expense of increased out-of-focus signal, as measured by scanning a fluorescent sheet through the focus of the microscope.

Micro-stripe microscope advantages

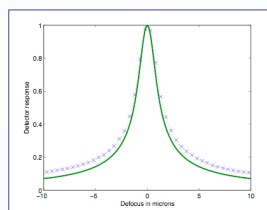
A clear advantage of our microscope design is the possibility to switch from one optically sectioned imaging technique to another without changing the microscope setup. This enables the optimum method for the practical application:

- Surface profiling or thin samples: Structured illumination has a better sectioning strength.
- Biological specimen: Line scanning has a bigger penetration depth and is less susceptible to artefacts.
- Fast biological phenomena: Multi-slit scanning is faster.

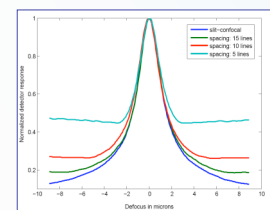
Conclusion

We have implemented several optical sectioning techniques with no moving parts using a single novel micro-structured LED as the illumination source. With no need to change the simple microscope setup, we are able to switch from one technique to the other and thus choose the most suitable confocal technique to image an object. The micro-stripe LED has a potential for optically sectioned imaging in endoscopy applications because the small grid or line pattern is embedded within the light source and does not need to be scanned mechanically.

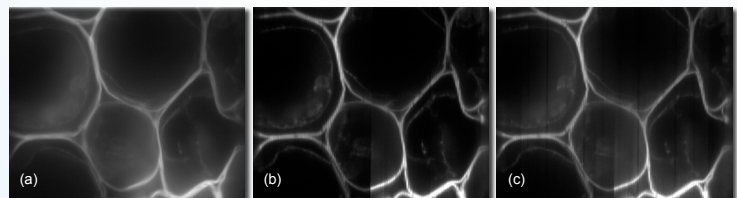
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Experimental (crossed) and theoretical sectioning strength of a 40X 0.75 NA single slit scanning system



Measured sectioning strengths for different line to line spacings



20x magnification multi-slit images of stained convalaria (a) conventional widefield image (b) single beam image (c) 12 beam image (10 stripe centre to centre spacing)