

## A Study of Shot Noise in Wide Field Structured Illumination Microscopy

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Structured illumination is a cheap and flexible technique used with wide-field microscopes to optically section a sample [1]. The illumination system is modified in order to project a single-spatial frequency grid pattern onto the sample. The projected pattern can be resolved only in the focal plane and so by looking for changes as the grid is moved, an image of the parts of the in-focus parts of the object can be calculated.

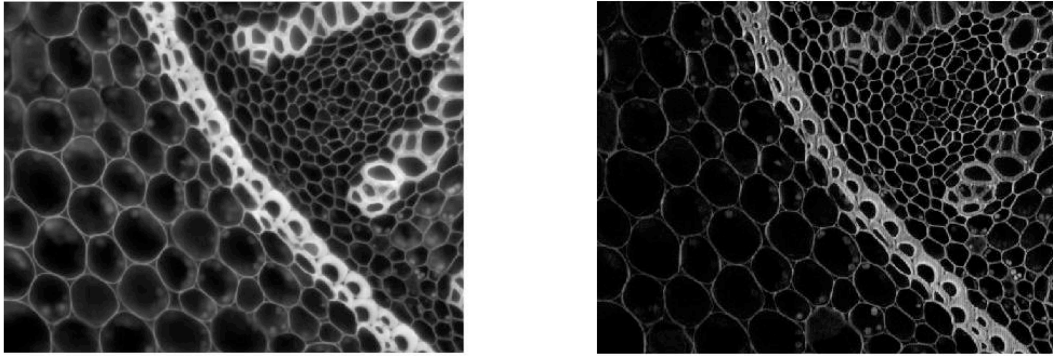


Fig1: Images of lily-of-the-valley stem: conventional (left) and sectioned (right).

Artefacts due to photobleaching, fluctuations in illumination and inaccurate grating shifts have been investigated previously [2]. Another significant issue is the shot noise in the detected images. As this technique is looking for changes between images as the grid is moved it becomes impossible to differentiate between these changes and those due to shot-noise. We have calculated the propagation of Gaussian distributed shot noise through to the sectioned image and show that it has an expected intensity distributed according to the probability function.

$$P(I_s) = \frac{1}{\sqrt{3\pi(2I_b + I_f \cos(3k.r))}} \exp \left[ \frac{(I_s - 3I_f/\sqrt{2})^2}{3(2I_b + I_f \cos(3k.r))} \right]$$

where  $I_b$  is the out-of-focus intensity,  $I_f$  is the in-focus intensity and  $k$  is the spatial frequency of the grid. Noise is thus dependant on both position and out-of-focus intensity. These calculations have been verified experimentally in a structured illumination microscope with a blue LED light source and a piezo controlled grid.

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2. L. H. Schaefer, D. Schuster and J. Schaffer, "Structured illumination microscopy: artefact analysis and reduction utilizing a parameter optimization approach", *Journal of Microscopy* 216, **165-174**, (2004)