

MULTI-FOCUS MICROSCOPY OF $N \times N$ FOCAL PLANES FOR FAST LIVE IMAGING

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Multi-focus microscopy is a 4D wide-field imaging technique designed for fast, live biomicroscopy applications where both temporal and spatial resolution is critical. The concept of multi-focus microscopy is to instantaneously record an entire focal series of the sample, eliminating the need for mechanical refocusing and multiple exposures. The recorded 3D movie is fast (limited only by exposure time and camera read-out) and simultaneous (all focal sections are recorded in the same instant). Multi-focus microscopy has previously been applied in one direction (1x3 images) [1, 2]. We have designed a multi-focus system capable of producing a 2D array of $n \times n$ images with very good light efficiency. The fluorescence emission light is manipulated by a diffractive element placed in the pupil plane. This produces an array of equal-intensity images with different focus on a large-field-of-view CCD camera. The images are then combined computationally to form the 3D image. We have implemented the system with $n=3$ (9 focal sections) and $n=5$ (25 focal sections).

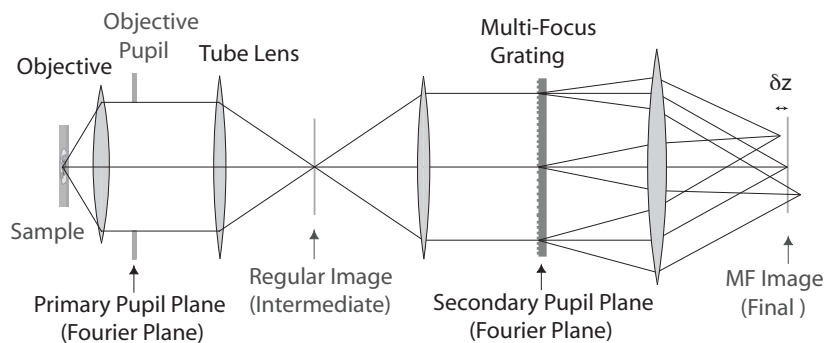


Fig. 1: Conceptual sketch of our multi-focus microscope in 3x3 configuration. The grating is chirped in two directions (one seen in figure) to produce an instant focal-series of nine focal sections of the sample (three seen in figure).

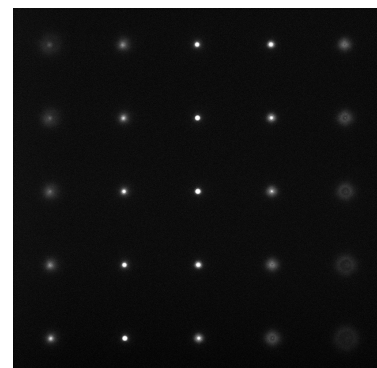


Fig. 2: Experimental Point Spread Function for 5x5 grating.

References:

[1] A. Demuro and I. Parker, "Multi-dimensional resolution of elementary Ca^{2+} signals by simultaneous multi-focal imaging". *Cell Calcium* 43:367-374 (2007).

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