SPACE AND FREQUENCY DOMAIN RECONSTRUCTION STRATEGIES
IN A CONFOCAL MICROSCOPE BASED ON
PATTERNED ILLUMINATION AND WIDE-FIELD IMAGE COLLECTION

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Widefield fluorescence and reflection microscopy as opposed to confocal microscopy have a very limited optical sectioning capability. By a patterned illumination of the sample in combination with widefield image detection an improved optical sectioning and in-plane resolution can be achieved.

Experimental data with the illumination performed through a regular pinhole grid will be presented. The pinholes were imaged into the microscope resulting in a ~ 2 x 2 µm. spacing (1.30 NA, 40x immersion objective) of illumination foci in the sample plane. Having collected an image at each of the different illumination pattern positions [1], confocal-like images were computed using different strategies: one strategy was to choose for each pixel the maximum intensity as given by its measured value over the series of pattern positions; better rejection of out-of-focus light was achieved by the difference between maximum and minimum; even further confocality resulted from computing the sum of maximum and minimum, then subtracting the double average.

Emulating soft-edged pinhole detectors moving conjugate to the illumination spots during image reconstruction represented an interesting alternative, which enabled the user to define the pinhole size during reconstruction as opposed to setting it during image acquisition in a standard confocal microscope.

Another reconstruction method could be achieved by calculating the degree of modulation in every pixel by evaluating the strengths of orders, when Fourier-transforming along the series direction. This scheme is related to a quadrature demodulation technique, which has previously been applied for data acquired using grating-illumination [2].

Yet a different approach relies heavily on considerations in Fourier-space [3-4]. Overlapping components of the Fourier-transformed object are identified, separated, shifted and recombined to form the reconstructed image.

All of the above methods were compared with respect to resolution, sectioning ability and linearity.