

ANNULAR PUPIL FILTER UNDER SHOT-NOISE CONDITION FOR LINEAR AND NON LINEAR MICROSCOPY: A FREQUENCY APPROACH

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Shot-noise represents a pernicious limit in scanning fluorescence microscopy, significantly affecting and deteriorating the imaging capabilities[1,2]. Mainly adopting computational simulation methods, a detailed analysis of its influence in typical two-photon excitation (2PE) and confocal laser scanning microscope (CLSM) imaging conditions is here presented, both in the spatial and in the frequency domain. This study reveals a remarkable shot-noise influence in the high-frequency range with a relevant detriment of the high-frequencies transmission capabilities and a substantial reduction of the optical transfer bandwidth.

In order to partially retrieve the high frequencies information loss, we propose to insert an annular filter on the microscope objective lens in the illumination light pathway[3,4]. The insertion of such a filter induces an electromagnetic interference effect, resulting in an engineering of the point spread function and in a redistribution of the optical transfer function. Especially, the filtered frequency spectrum displays an enhancement in the high frequencies response, in particular along the optical axis direction [5].

The filter capability in boosting the signal to noise ratio in the high-frequency range has been exploited to partially recover the high frequencies components hampered by shot-noise and improve the imaging capability. Filter benefits in 2PE and CLSM imaging for different shot-noise contributions are reported.

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