

IMAGING QUALITY AND PERFORMANCE ASSESSMENT OF A WHITE-LIGHT WIDE-FIELD MICROSCOPE USING A LINEAR SENSOR

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We built a laboratory prototype of a wide-field microscope with white-light illumination using a CMOS linear image sensor. We aim at developing a digital microscope with optical sectioning ability. Through the scanning along one lateral axis it acquires two-dimensional (2D) images (optical sections) of the specimen containing substantial contributions from out-of-focus portions. This haze will be removed or significantly diminished using a computational method based on the definition of a model for the overall process of image formation and recording using a linear sensor. As usual a compromise should be taken between a more accurate model providing better images or a simplified and lower computational processing time one.

The output performance from these computational methods is extremely dependent on the imaging quality of an imaging system. Thus we addressed two types of measurement to assess the imaging quality of this microscope prototype: quantitative assessment such as modulation transfer function (MTF) and qualitative assessment such as images of specific objects of known dimensions. We chose the scanning method of MTF measurement that is to measure the change of contrast when a object is a sinusoidal grating. This method described in literature [1] was made suitable to linear image sensor. Owing to the practical fact that even a very high spatial frequency square wave grating is easily fabricated the contrast transfer function (CTF) was measured and converted to MTF [1]. Thus the MTF was obtained from images of an USAF resolution target that acts as a square-wave grating. Discrete spatial frequencies range from 1 LP/mm to 228 LP/mm. These images were used for qualitative assessment as well as to evaluate lateral resolution. In fact this is a very attractive object since specimen pitch range from tens down to approximately 2 μm . For this incoherent imaging system the line spread function (LSF) can be used as an estimate of the cross section of the point spread function (PSF). The line spread function can be easily calculated by deriving the edge response function [2].

Axial discrimination or optical sectioning will be evaluated along with the development of computational methods. Nevertheless axial response was also measured and two different methods of measurement were used [2,3]. A planar mirror was scanned along z-axis with 1 μm steps by the stepper motor driven translation stage (T-LA28, Zaber Technologies, USA). We also imaged a semiconductor device. A three-dimensional (3D) image reconstruction is presented. It is built from 2D images at three different depths using a simple algorithm to find the best-in-focus image through the determination of maximum intensity.

References

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