THE POINT SPREAD FUNCTION OF OPTICAL MICROSCOPES IMAGING THROUGH LAYERED AND HETEROGENEOUS MEDIA

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The optical microscope has proven to be an invaluable tool in biology, because of its unique capabilities of 3-D imaging of living specimens. However, it is only recently that accurate descriptions of the imaging process by a high NA objective has been available [1,2], to model the classical, confocal or multiphoton microscope.

These vectorial models however operate with input parameters that are not practical for every day's biological use. The Gibson and Lanni scalar model [3] proposes a convenient ray-tracing method for computing the aberration function and explicitly introduces the design conditions of use of the objective, as recommended by the manufacturer, and the actual ones. We show how to combine both approaches to propose a rigorous and easy-to-use method to model imaging through a stratified medium, which is a very common situation in microscopy (presence of a cover-glass). We discuss the accuracy of the model for biology, crystallography or wafer inspection [4], and also show that considering the illumination and the detection PSFs as identical is an assumption, which may fail in some cases [2,4].

In this approach however, the specimen itself is considered as homogeneous. We first extend the model for specimens, which are not homogeneous along the z-axis only, more precisely, specimens which can also be considered as layered, as for example the sandwich composed of a membrane cell (made out of lipids), the intra-cellular liquid (assimilated to salty water), and the nucleus (composed of proteins). This model may for example be used to describe the illumination and detection PSFs of less common configurations, like 4Pi microscopy or STED microscopy, which have proven to deliver an unsurpassed resolution [5-7]. They are for this reason very sensitive to aberrations, but also because these methods rely on the precise interference of two counter-propagating illumination beam (4Pi), or on the precise superposition of an excitation beam and a STED beam (STED microscopy), which have different wavelengths, and are therefore affected by a different amount of aberrations.

Biological specimen however often exhibits 3-D spatial variations of their refraction index. We show how in some cases one could combine our vectorial diffraction/ray tracing model to the case of imaging through a heterogenous medium. Our method therefore resembles that of Kam *et al.*[8]. We discuss the validity of this model compared to other, more rigorous methods.

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