LOOKING THROUGH A MISMATCHED SAMPLE: WHEN THE EMISSION AND EXCITATION PSF ARE NO LONGER THE SAME

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The Point Spread Function (PSF) of a Confocal Laser Scanning Microscope (CLSM) is the product of the Excitation and Emission PSFs. The former can be computed directly from the properties of the microscope objective lens as the image of a point source of light. The latter is classically considered equivalent to an image of the point detector in the sample [1]. In order to take the finite size of the pinhole into account, the Emission PSF is convolved with the de-magnified image of the pinhole [2]. This approach, in which the two PSFs are (nearly) identical works exceedingly well for matched samples. With increasing depth in a *mismatched* sample, however, this classical approach starts to fail and predicts that along the optical axis secondary peaks of the Emission PSFs (see Fig. 1) become larger than the central maximum, which leads to a broad profile of the total confocal PSF despite the use of a pinhole and an otherwise ideal imaging system.

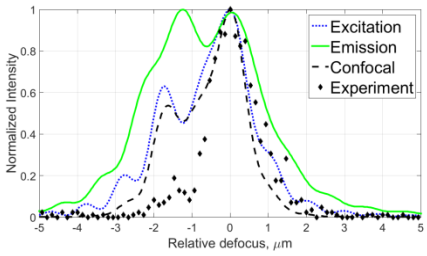

Figure 1: Comparison of the experimental axial PSF profile with classical theory

Here, we introduce a subtly different approach for the calculation of the Emission PSF, which agrees much better with experimental measurements in mismatched samples, which occur unavoidable in industrial setting or in integrated light and electron microscopy applications. To us, the pinhole is not an ideal detector but rather an obstacle to light propagation. This small change leads to a method that reproduces PSF axial profiles very similar to experimental ones (see Fig 2) at a given depth in the mismatched sample and coincides with the classical description mentioned above when the aberrations induced by the refractive index mismatch are small.

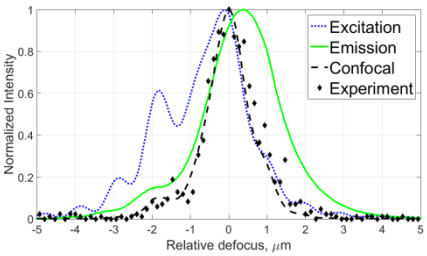

Figure 2: Comparison of the experimental axial PSF profile with proposed theory

[1] S. Hell, G. Reiner, C. Cremer and E.H.K. Stelzer "Aberrations in confocal fluorescence microscopy induced by the mismatches in refractive index," *Journal of Microscopy,* **169**, 391-405 (1993).

[2] C.J.R. Sheppard and T. Wilson, "Image Formation in Scanning Microscopes with partially Coherent Source and Detector," *Optica Acta,* **25**, 315-325 (1978).