3D PSF Models for Fluorescence Microscopy in ImageJ

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We introduce an ImageJ application that allows one to generate various 3D PSF models. Keeping the biological practitioner in mind, few input parameters are required only (Figure 1). Our application can generate z-stacks at any size and at any lateral and axial resolution. Our implementation utilizes a multi-thread design that allows for parallel and fast computations.

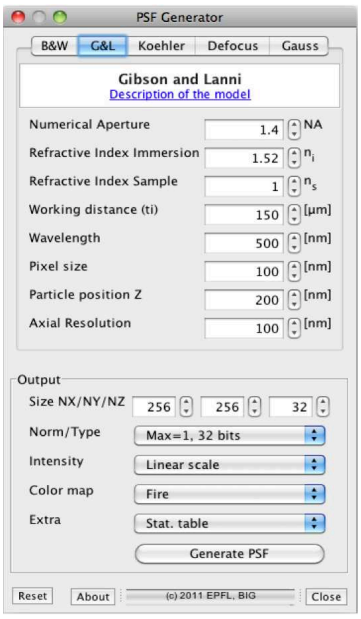

Figure 1: User interface. The "Output" section is common to all models. A detailed description of each model is provided by the interface, too, along with additional required parameters.

The current version allows for five different PSF models: The Gaussian model simulates a blurring effect by setting three different variance values. These values characterize the width of the PSF at various axial positions along the z-stack. The defocus model is simulated in the Fourier domain by a modulated Gaussian where the *sinc* modulation depends on the axial defocusing distance. Another defocusing model is due to Koehler, which also uses the Gaussian function in the Fourier domain. In this model, however, the *sinc* modulation is replaced by a linear term for the variance.

The Born & Wolf model provides yet another defocusing model for which the observed fluorophore particle is located at the focal plane of the objective lens, right beneath the coverslip. The slices of the output z-stack correspond then to different values of the microscope's stage. The Gibson & Lanni PSF model can be seen as a generalization of Born & Wolf in the sense that the fluorophore particle can be located at any depth within the sample. It also considers three optical layers (sample-coverslip-immersion) instead of two (glass-immersion). This, in turn allows for non-symmetric PSF models that originate from refractive indices mismatch (Figure 2). Both models use Kirchhoff's diffraction integral formula,

$$I(r) = \left| \int_0^1 J_0\left[k \cdot \mathrm{NA} \cdot \rho \cdot r\right] e^{j \cdot k \cdot \mathrm{OPD}(\rho)} \rho \, d\rho \right|^2,$$

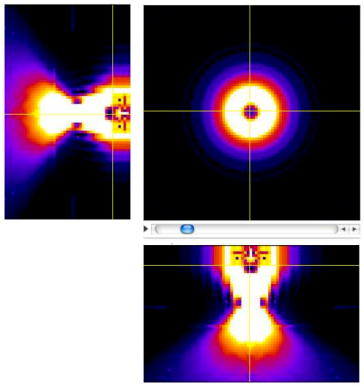

Figure 2: Gibson & Lanni 3D PSF model. Shown here is a non-symmetric z-stack due to refractive indices mismatch.

where I is the pixel value located at a distance r from the centre of the image, NA is the numerical aperture of the microscope, k is the wave number of the fluorophore, and OPD is the optical path difference described by each model. We implemented this formula by means of iterative Riemann sums, which allows one to set the accuracy of the integral approximation a-priori. The software design is modular and additional PSF models can be easily incorporated. Relevant works on this topic are the Diffraction PSF 3D ImageJ plugin [1] and the PSF LAB Matlab-based application [2]. The former relies on a simplified fourth power model for modelling spherical aberrations, and the latter relies on a detailed vectorial model in providing 2D images that may take several minutes each.

The models of the ImageJ plugin were successfully applied to fluorescence microscopy applications such as de-convolution [3], fluorescent particles tracking [4], extended depth of field estimation [5] and super-resolution 3D PALM localization. These models can also be used for validating experimental PSF measurements and to further find optimal model parameters for a given experimental data set. The plugin is available at http://bigwww.epfl.ch/algorithms/psfgenerator/.

References: [1] http://www.optinav.com/Diffraction-PSF-3D.htm [2] M. J. Nasse and J. C. Woehl "Realistic modeling of the illumination point spread function in confocal scanning optical microscopy" Journal of the Optical Society of America A 27, (2010) [3] A. Griffa *et al.* "Comparison of Deconvolution Software in 3D Microscopy. A User Point of View - Part 1", G.I.T. Imaging & Microscopy, vol. 1, 2010. [4] D. Sage *et al.* "Automatic Tracking of Individual Fluorescence Particles: Application to the Study of Chromosome Dynamics," IEEE Transactions on Image Processing, 14(9), 2005. [5] F. Aguet *et al.* "Model-based 2.5-D deconvolution for extended depth-of-field in brightfield microscopy," IEEE Trans. Image Process, 17(7), 2008.

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