

## Point Spread Function Software for Confocal Microscopy OTT ID#1136

### TECHNOLOGY

The point spread function (PSF) of an optical imaging system such as a microscope describes the optical characteristics of the system, including inherent diffraction effects, possible additional blurring imperfections, or aberrations. The PSF can be understood as the fundamental building block of the imaging process. The resulting blurred image is the convolution, point by point, of the real object with the PSF of the system. **PSF Lab** is a software program that allows the calculation of the illumination PSF of a confocal microscope under various imaging conditions. The calculation of the electric field vectors and the associated light intensity distribution is based on a rigorous, vectorial model that takes polarization effects in the near-focus region and high numerical aperture microscope objectives into account

The polarization of the input beam (assumed to be collimated and monochromatic) can be chosen freely (linear, circular, or elliptic). Furthermore, a constant or Gaussian shaped input beam intensity profile can be assumed. On its way from the objective to the focus, the illumination light passes through up to three stratified optical layers, which allows to simulate an immersion oil/air (layer 1) objective that focuses light through a glass cover slip (layer 2) into the sample medium (layer 3). Each layer is characterized by its (constant) refractive index and thickness. **PSF Lab** can also simulate microscope objectives that are corrected for certain refractive indices and cover slip thicknesses (design parameters). Thus, any deviations from the ideal imaging conditions for which the objective was designed for are properly taken into account.

While it is possible to measure the PSF of a microscope experimentally, it is difficult, and might have to be repeated for different experimental conditions. A good theoretical calculation of a PSF is very important as the quality of the PSF impacts directly on the quality of the deconvolved image. The quality of PSFs currently used in commercial imaging varies widely due to the complex underlying theory. The **PSF Lab** software represents the most complete software package for calculating PSFs available today and should greatly improve the quality of deconvolved images.

### FEATURES/BENEFITS

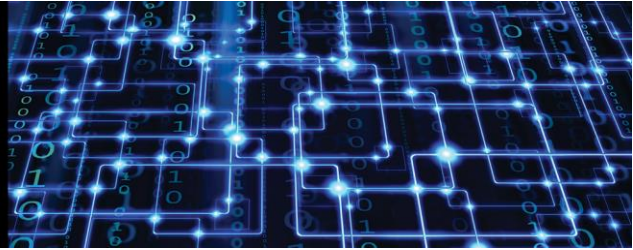
- **More versatile** – Allows for taking a Gaussian or constant input beam intensity profile into account
- **Refractive Index Settings** – Model includes possible mismatch of immersion medium, glass cover slip, and specimen medium; simulates objectives corrected for certain cover glass refractive index *and* thickness
- **Babinet-Soleil compensator** – Incorporated in the illumination path, which allows for calculation of polarization effects during sample illumination
- **Improved image quality** – Rigorous and complete theoretical model
- **More input parameters** – Model allows input of numerous experimental parameters

### INTELLECTUAL PROPERTY

Copyrighted Software; This technology is available on an exclusive or non-exclusive basis.



# Technology Overview



## MARKETS

Almost all commercial imaging software sold today proposes the use of post-acquisition image deconvolution algorithms in order to obtain deblurred images that recreate the imaged object at high resolution and with much more detail than in the experimental image.

**PSF Lab** will be useful in the optical engineering industry in order to predict the expected PSF for a certain configuration or in academia for optics and nanoscale imaging research. The main application is as a plug-in or standalone program providing high quality PSF as an input for commercial microscopy imaging software packages used for image deconvolution. The PSF generated by **PSF Lab** could also be used as a starting PSF for iterative deconvolution techniques, such as “blind deconvolution” and would lead to a much faster convergence of these types of algorithms.

## INVENTOR(S)

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*Dr. Jörg Woehl* is a Professor in the Department of Chemistry and Biochemistry at the University of Wisconsin-Milwaukee. He obtained his Ph.D. in 1996 at the University of California at Riverside in the Department of Chemistry and conducted post-doctoral work at C.N.R.S.-University of Bordeaux, France. Prior to obtaining a position at UWM, Dr. Woehl was a faculty member in Physics (1999-2004) at the University of Grenoble, France. *Dr. Michael Nasse* is an Assistant Scientist in the Department of Physics at the University of Wisconsin-Milwaukee and at the Synchrotron Radiation Center, University of Wisconsin-Madison. He earned his Ph.D. in 2004 from the University Joseph Fourier in Grenoble, France. He performed post-doctoral work both at the University of Wisconsin-Milwaukee and the Synchrotron Radiation Center, University of Wisconsin-Madison.

## PUBLICATIONS

Nasse M. J., Woehl J. C. (2010). Realistic modeling of the illumination point spread function in confocal scanning optical microscopy. *J. Opt. Soc. Am. A* **27** (2): 295–302.

Nasse, M.J., Woehl, J.C., and Huant S. (2007). High-resolution mapping of the three-dimensional point spread function in the near-focus region of a confocal microscope. *Applied Physics Letters* 90 (3).

Software Download Available At: <http://onemolecule.chem.uwm.edu/software>

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