Manual

PSF Simulation App

(written in MATLAB)

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1 Introduction

This manual accompanies the app for interactive simulation and visualization of point-spread functions in single molecule localization microscope as presented in reference [1]. The simulations are implemented in MATLAB and based on a vectorial PSF model [2].

The app is organized as a main window with multiple subwindows (Fig. 1). The main window consists of multiple tabs that allow to set a wide range of simulation parameters and adjust further options. All windows and parameters will be listed and explained in detail in the following sections. Upon start of the application, the main window and the window showing the 2D PSF image will open up. All other subwindows will open upon selection.

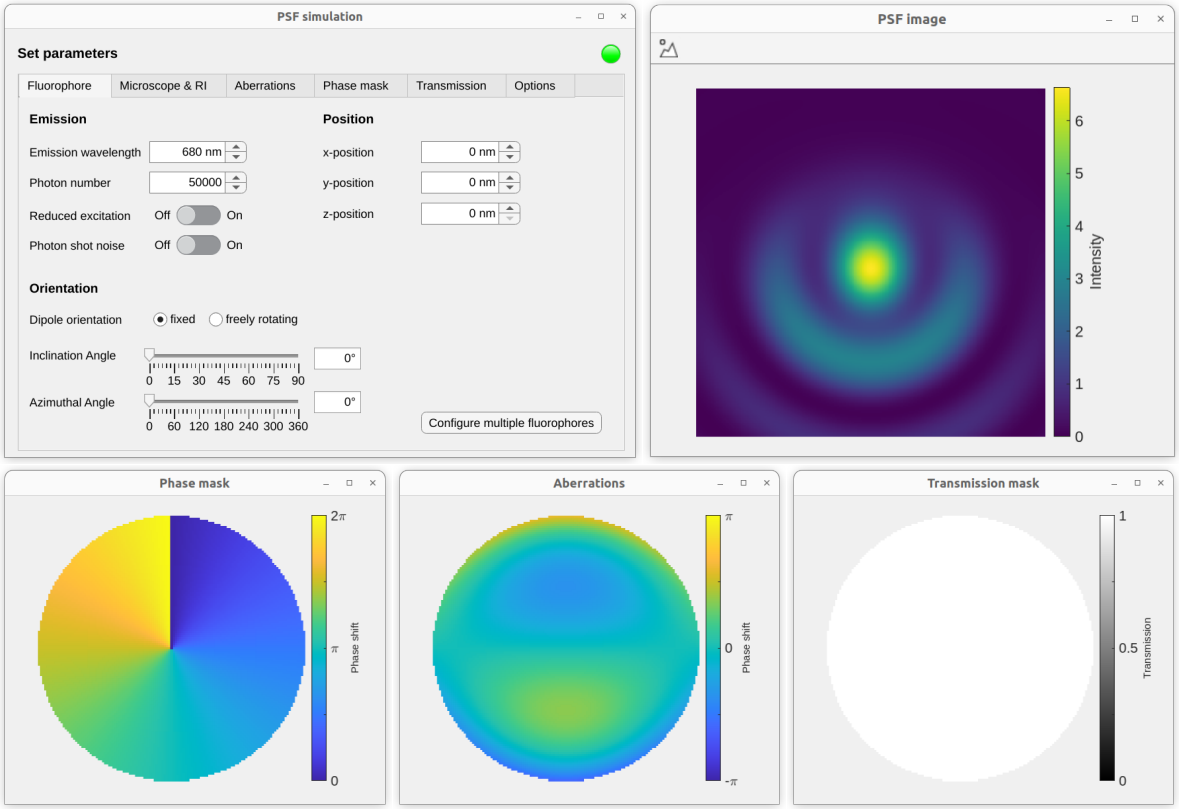

Figure 1: Overview of windows. From top left to bottom right: Main window, 2D PSF window, aberrations, phase mask, transmission mask window.

2 Fluorophores

The tab *Fluorophore* (Fig. 2) of the main window allows to specify the characteristics of the emitting fluorophore.

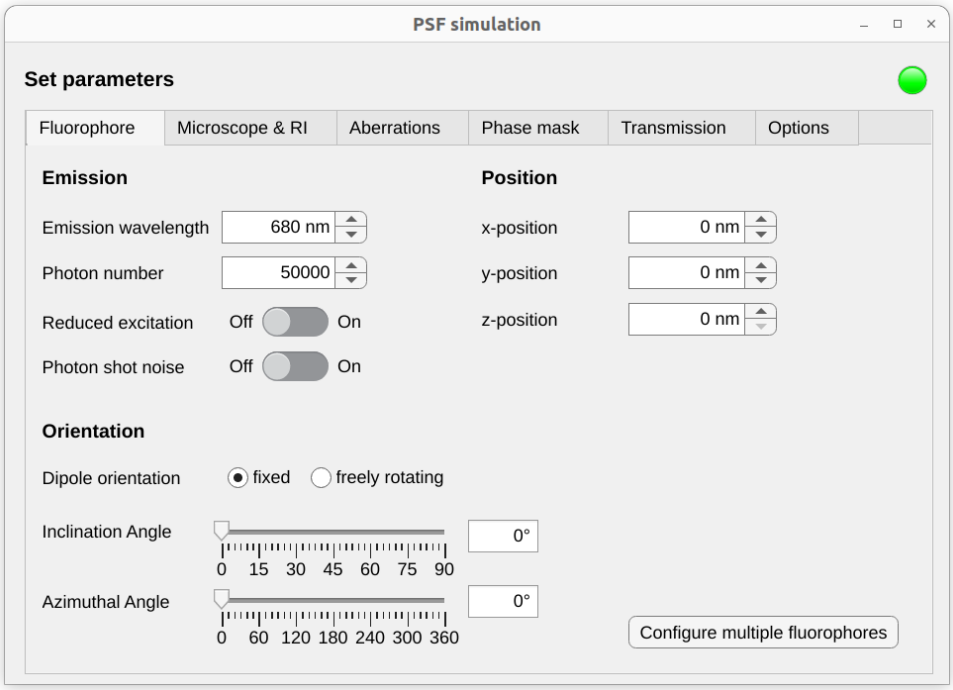

Figure 2: Fluorophore tab of main window. This tab allows to set emission parameters, the fluorophore's position and dipole orientation. The button *Configure multiple fluorophores* will open a separate window that enables to specify position and dipole orientation parameters for multiple fluorophores in the same region of interest.

Customizable parameters:

- *Emission wavelength.* The fluorophore's emission wavelength in units of nanometer. For simplicity we assumed monochromatic emission in this app.
- *Photon number.* The number of detected photons (integer number).
- *Reduced excitation.* A switch for deciding if the fluorophore's excitation dipole should be taken into account. If turned on, the number of photons is multiplied by $\sin^2(\theta)$ where θ is the inclination angle measured from the optical axis. In all our calculations, the excitation dipole is assumed to be parallel to the specified emission dipole.
- *Photon shot noise.* If turned on, photon shot noise (modelled by a Poissonian distribution) is included in the final PSF image.
- *z-position.* Position of the fluorophore on the axial axis in nanometer. The position is specified relative to the coverslip surface, i.e., a value of 0 nm corresponds to the fluorophore being on the coverslip surface. Positive values specify the distance of the fluorophore above the coverslip.

- *Dipole orientation.* Select if the fluorophore dipole is assumed to be fixed or freely rotating.
- *Inclination angle.* Inclination angle of the fluorophore dipole specified in degrees w.r.t. the positive optical axis (see Fig. 3). Only enabled if the dipole orientation is selected to be fixed.
- *Azimuthal angle.* Azimuthal angle of the fluorophore dipole specified in degrees w.r.t. the positive horizontal x-axis (see Fig. 3. Only enabled if the dipole orientation is selected to be fixed.

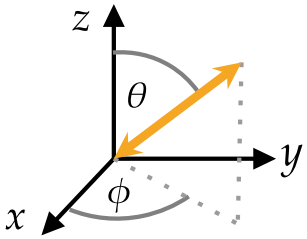

Figure 3: Schematic showing the dipole orientation of a fluorophore (yellow arrow) with inclination angle θ and azimuthal angle ϕ. The xy-plane corresponds to the focal plane, the z-axis is aligned with the optical axis.

2.1 Multiple fluorophores

Multiple fluorophores can be configured by clicking the button *Configure multiple fluorophores*. This will open a new window (Fig. 4) that allows for setting the position and dipole orientation for each fluorophore individually. An arbitrary number of fluorophores can be included in the image by adding one fluorophore at a time upon clicking the button *Add fluorophore*. This will open a new tab in the window for the new fluorophore. Any existing fluorophore can be deleted by clicking the button *Delete fluorophore*.

Customizable parameters:

- *Inclination angle.* Inclination angle of the fluorophore dipole specified in degrees w.r.t the positive optical axis (see Fig. 3. Only enabled if the dipole orientation is selected to be fixed.
- *Azimuthal angle.* Azimuthal angle of the fluorophore dipole specified in degrees w.r.t. the positive horizontal x-axis (see Fig. 3. Only enabled if the dipole orientation is selected to be fixed.
- *x-position / y-position.* Position of the fluorophore in the lateral plane specified in nanometer.

- *z-position.* Position of the fluorophore on the axial axis in nanometer. The position is specified relative to the coverslip surface, i.e., a value of 0 nm corresponds to the fluorophore being on the coverslip surface. Positive values specify the distance of the fluorophore above the coverslip.

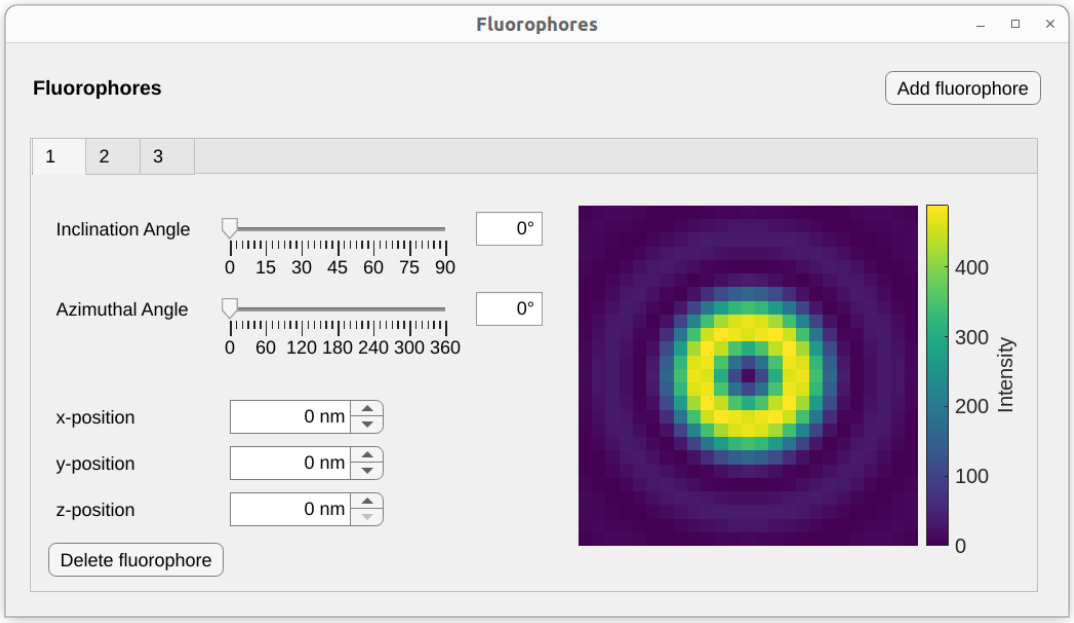

Figure 4: Subwindow for configuration of multiple fluorophores. Each fluorophore has a separate tab that allows to adjust the fluorophore's position and dipole orientation, and shows its 2D PSF.

3 Microscope

The tab *Microscope* (Fig. 5) of the main window allows to specify the microscope parameters, including parameters for the objective and tube lens, refractive indices and the camera.

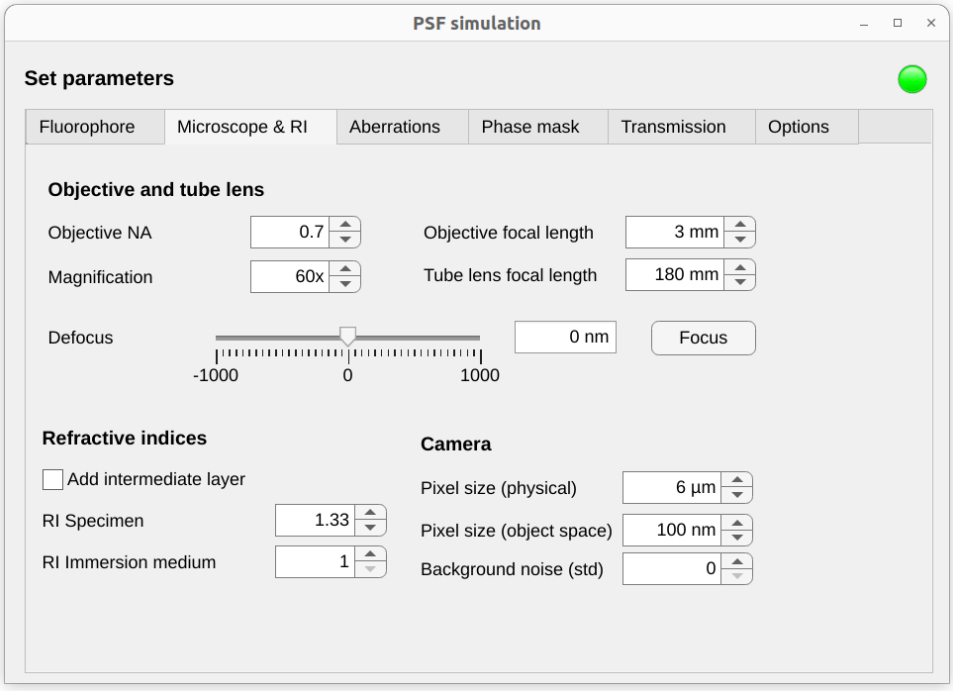

Figure 5: Microscope tab of main window. The tab allows to set parameters for objective and tube lens, refractive indices and camera.

Objective and tube lens parameters:

- *Objective NA*. Numerical aperture of the objective.
- *Magnification*. Magnification of the objective.
- *Objective focal length*. Focal length of the objective given in millimeter.
- *Tube lens focal length*. Focal length of the tube lens given in millimeter.
- *Defocus*. The defocus of the objective with respect to the coverslip plane given in nanometer. A negative (positive) value corresponds to the objective moving closer to (away from) the sample. Clicking the button *Focus* sets the defocus value to 0 nm.

Note that the magnification depends on the focal lengths of objective f_{obj} and tube lens f. Changing either will automatically update the magnification according to the equation

$$M = \frac{f}{f_{\mathrm{obj.}}}. \tag{1}$$

In case the magnification value is changed, the value of f_{obj} is updated according to Eq. 1, while f is left unchanged.

Refractive index parameters:

- *RI specimen.* Refractive index of the sample layer.
- *RI immersion medium.* Refractive index of the immersion medium.
- *RI intermediate layer.* Refractive index of an optional intermediate layer. (only enabled if *Add intermediate layer* is selected.)
- *Intermediate layer height.* Height of the optional intermediate layer given in units of micrometer. Default 0 µm. (only enabled if *Add intermediate layer* is selected.)

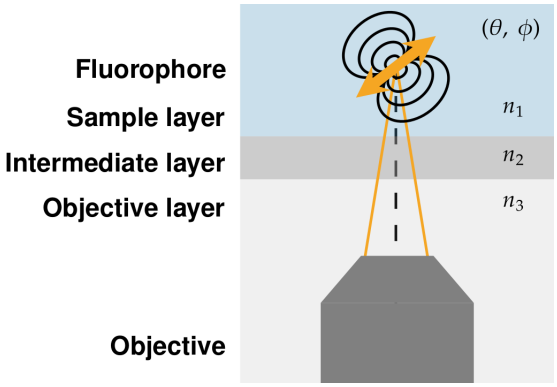

Figure 6: Schematic of the assumed refractive index layers.

Per default, only the objective layer and intermediate layer are considered, while the intermediate layer height is set to zero. This reflects a system where the coverslip, immersion medium and objective are refractive index matched. The intermediate layer can represent a coverslip coating with different refractive index, or the coverslip itself in case the immersion medium is not refractive index matched.

Camera parameters:

- *Pixel size (physical).* Physical width of a pixel of the camera given in micrometer.
- *Pixel size (object space).* Width of a camera pixel in object space. Specified in nanometer.
- *Background noise (std).* Standard deviation of the background noise in each pixel. The background noise is assumed to follow a Poissonian distribution.

The physical width p_{cam} of a camera pixel and the pixel size p_{im} in the object space are related via the magnification M of the microscope:

$$p_{\mathrm{im}} = 10^3 \, p_{\mathrm{cam}}/M, \tag{2}$$

with p_{im} given in units of nanometer and p_{cam} given in units of micrometer. Changing one of the values will update the other one accordingly.

4 Aberrations

The tab *Aberrations* (Fig. 7) of the main window allows to add custom aberrations to the PSF. The aberrations are defined via Zernike modes. In order to specify the aberrations, one can choose between the following two options:

- *Select common aberrations.* Choose from a set of given Zernike aberrations and specify the coefficients (in RMS wavefront error given in units of the wavelength).
- *Specify Zernike indices (Noll) and coefficients via input vector.* Specify the Zernike indices (using Noll convention [3]) and coefficients as input string using the format `index:coefficient;index:coefficient`. Alternatively, the input vector can be loaded from a file using the button *Load*. The input can be saved to a file via the *Save* button.

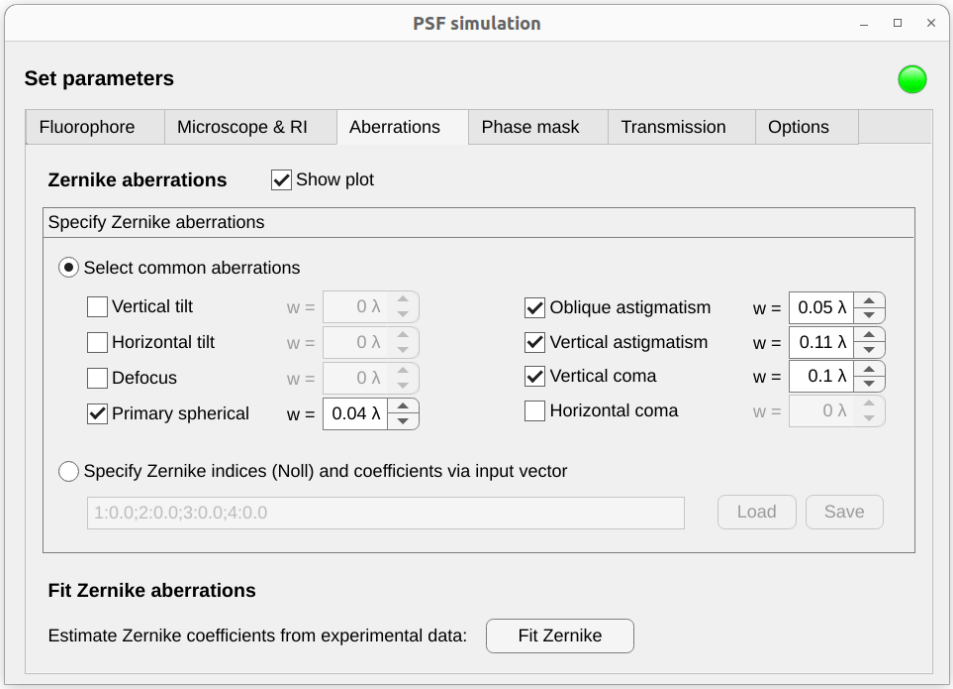

Figure 7: Aberrations tab of main window. Aberrations can be specified in terms of Zernike modes either via selecting predefined modes or by input of custom Zernike modes and coefficients. Parameters can also be loaded from or saved to a file.

Selecting the checkbox *Show plot* opens a new window (Fig. 8) that shows the aberration as phase shift in the back focal plane.

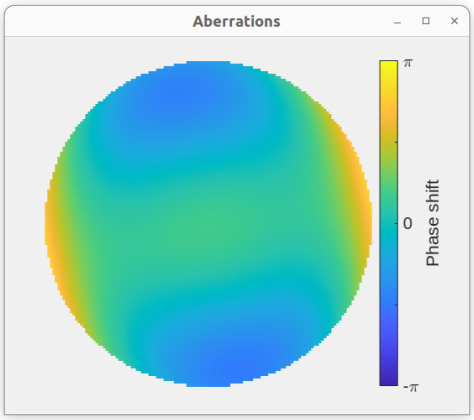

Figure 8: Subwindow showing the phase shift induced by aberrations.

5 Phase mask

The tab *Phase mask* (Fig. 9) allows to add a custom phase mask in the back focal plane. The phase mask can be selected from a list of predefined phase masks. Alternatively, a custom phase mask can be loaded by selecting the option *Custom* and then clicking the button *Load*. The options for predefined phase masks include astigmatism, a vortex phase mask, sector and opposing sectors, pyramid phase mask and double helix phase mask. Many of the phase mask options provide additional parameters for adjusting the phase mask, described in the following.

Parameters for phase mask adjustment:

- *Inner ring radius*. The central part of the phase mask can be cut out, i.e., the phase for low frequencies remains unaltered within a circular disk around the center. The inner ring radius defines the relative radius of this inner disk with respect to the radius of the phase mask.
- *Rotate phase mask*. Rotate the phase mask with the given rotation angle.
- *Sector*. Enabled for *Sector* and *Opposing Sector*.
- *Number of facets*. Enabled for *Pyramid*. Specifies the number of pyramid facets.
- *Max. shift*. Enabled for *Pyramid*. Maximum phase shift at the edge of the pyramid.

Selecting the checkbox *Show plot* opens a new window (Fig. 10) that shows the aberration as phase shift in the back focal plane.

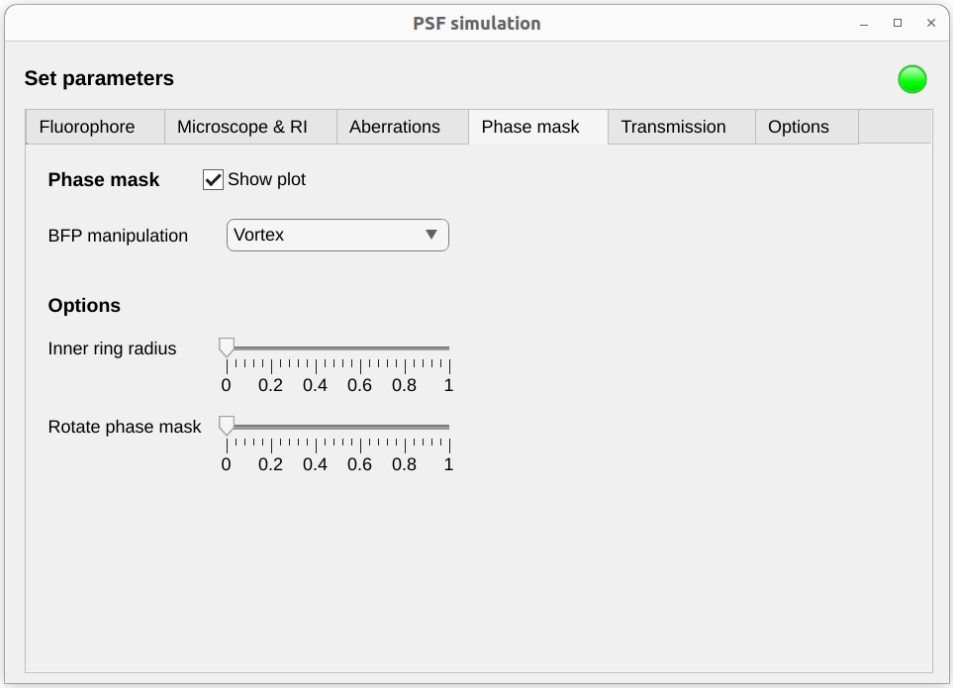

Figure 9: Phase mask tab of main window. The applied phase mask can either be selected from a predefined list, or a custom phase mask can be loaded from a file. Selecting one of the options will show additional parameters for customizing the phase mask.

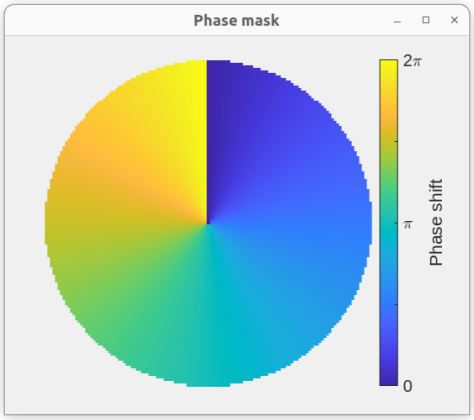

Figure 10: Subwindow showing the selected phase shifts induced by the selected phase mask.

6 Transmission

The tab *Transmission* (Fig. 11) of the main window allows to add a custom transmission mask in the back focal plane. The default is a transmission mask corresponding to the aperture of the objective (i.e., full transmission within the aperture and no transmission outside of the aperture). A custom transmission mask can be loaded by selecting the option *Custom* and then clicking the button *Load*. Note that the loaded transmission mask will be multiplied with the aperture. Selecting the checkbox *Show plot* opens a new window (Fig. 12) that shows the transmission in the back focal plane.

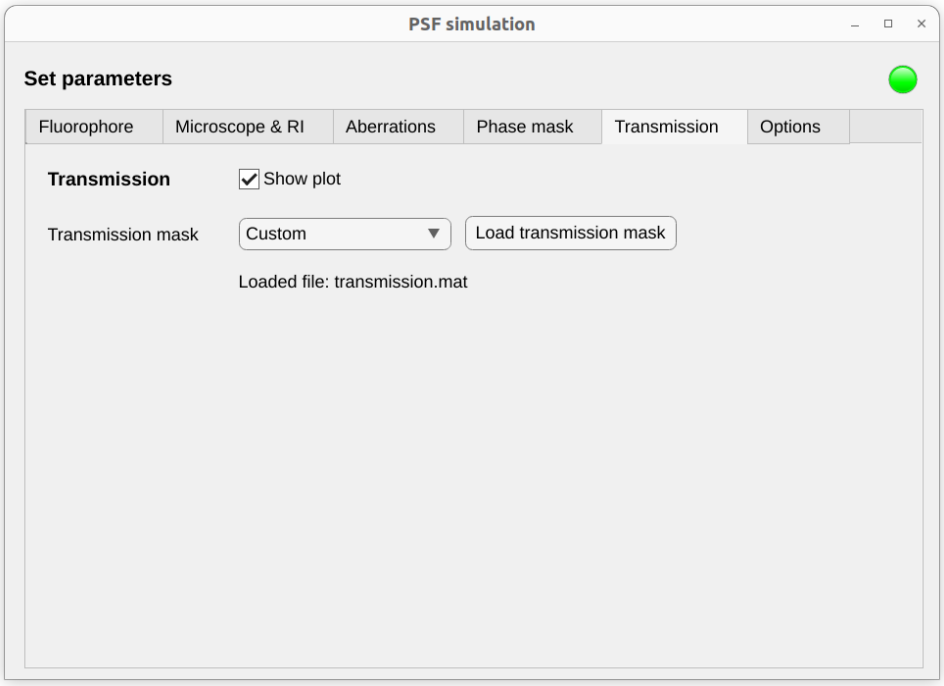

Figure 11: Transmission tab of main window. The tab allows to load a custom transmission mask from a file.

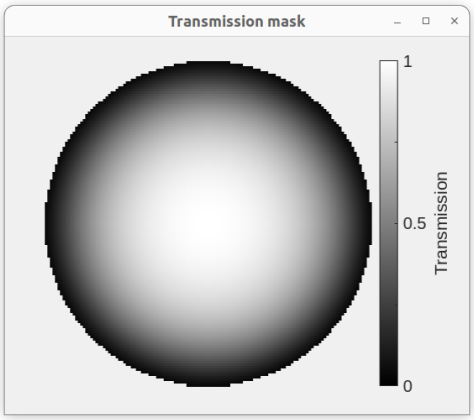

Figure 12: Subwindow showing the transmission of light. For values of 1, light is fully transmitted, while for values of 0, the light is fully blocked.

7 Options

The tab *Options* (Fig. 13) of the main window allows to select the visualization options for the PSF, export the 2D and 3D PSF data, adjust various plot settings, and calculate the Cramér-Rao bound for the current parameter settings.

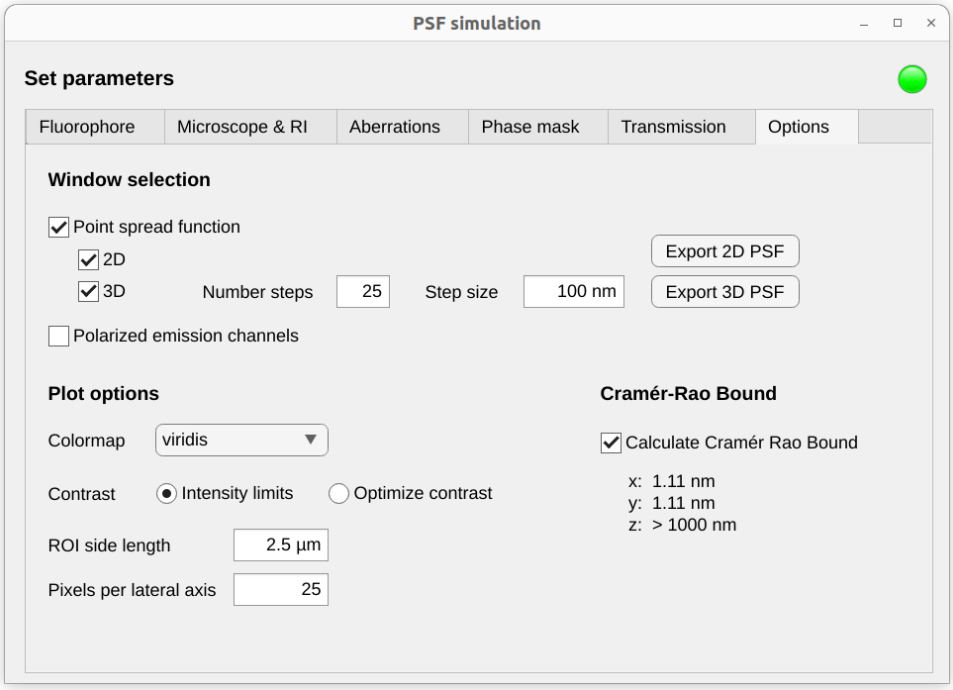

Figure 13: Plot options tab of the main window. This tab allows to set the window selection, plot options and calculate the Cramér-Rao bound.

First, the section *Window selection* allows to select if the PSF should be created as a 2D or 3D plot. Both the calculated 2D and 3D PSF data can be exported using the export buttons. By default, the 2D PSF plot is selected. Additionally, a 3D PSF plot can be created by selecting the option *3D*. This opens a new window for the 3D plots (Fig. 15). This window includes a lateral projection view of the PSF (xz-view), and an isosurface plot. For the isosurface, one can select any value from 0 to the maximum pixel intensity value of the 3D PSF for which the isosurface should be displayed via a slider. Note that these values do not directly correspond to the values in the projection plot, as the projection is a sum of the pixel values over all y-slices. The transparency of the isosurface plot can be set with the transparency spinner. The transparency values range from 0 (fully transparent) to 1 (fully solid). When rotating the plot, the lighting can be updated by clicking the button *Update lighting*.

Further, clicking the option *Polarized emission channels* splits the emission into an x- and y-polarized channel and plots the respective 2D PSFs (Fig. 16). Further, the section *Options* allows to customize various plot parameters:

- *Colormap*. The colormap that is chosen for all PSF plots. The available options include: viridis, gray, parula, hot, jet, turbo. Default: viridis.

- *Contrast.* Select between *Intensity limits* and *Optimize contrast*. The option *Intensity limits* sets the colorbar range from 0 to the maximum intensity value occurring in the image. The option *Optimize contrast* sets the colorbar limits to the minimum and maximum intensity value occurring in the image.

- *ROI side length.* The side length of the region of interest specified in micrometer. Default: 2.5 µm.

- *Pixels per lateral axis.* The number of pixels per lateral axis. The total number of pixels in the image is the square of this number. Default: 25.

Note that the parameters for the side length s_{ROI} of the region of interest and the number of pixels n_{px} per lateral axis are related via the pixel size p_{obj}:

$$s_{\mathrm{ROI}} = 10^{-3}\, p_{obj}\, n_{px}, \tag{3}$$

where the region of interest is given in units of micrometer and the pixel size (object space) in units of nanometer. Updating either s_{ROI} or n_{px} will change the other value accordingly. Updating the pixel size p_{obj} will leave the size of the region of interest unchanged, but update the number of pixels n_{px}.

The section *Cramér-Rao Bound* allows to calculate the Cramér-Rao bound, i.e., the theoretical limit for the best localization precision in the x-, y- or z-axis that can be achieved by an unbiased estimator [4, 5]. Note that in some cases the PSF does not provide enough information to estimate a particular parameter. In this case, numerical errors lead to unstable results. Therefore, we do not provide an exact value for the CRB if the calculated number is greater than 1000 nm, i.e., much greater than the diffraction limit of around 200 nm.

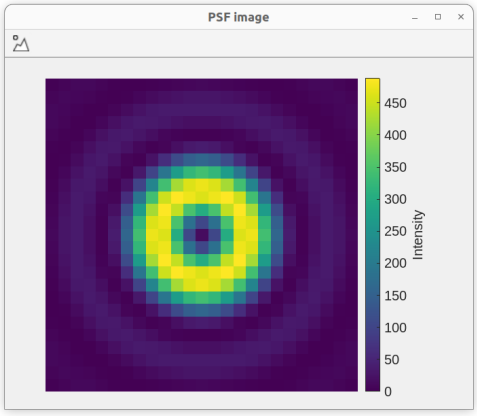

Figure 14: Window showing the 2D PSF.

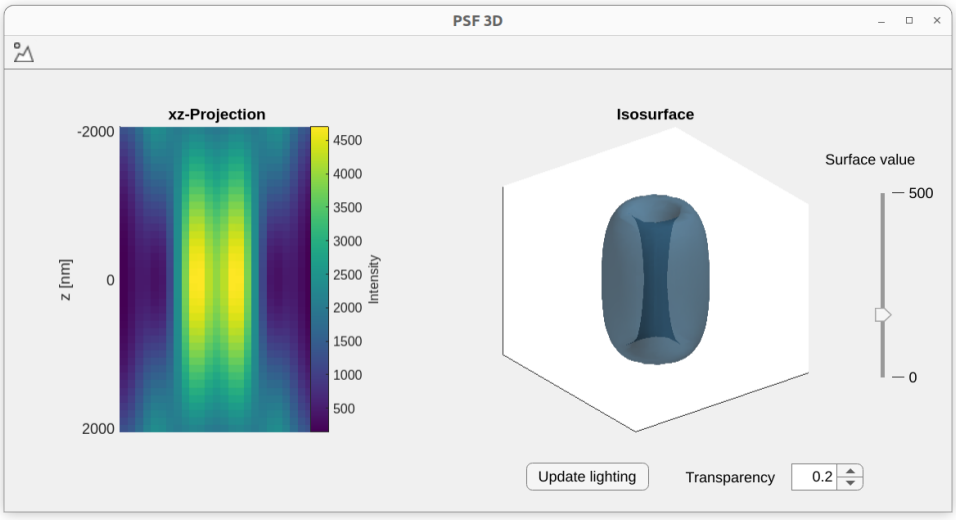

Figure 15: Window showing the 3D PSF. Left: xz-projection of the PSF. Right: Isosurface of the 3D PSF. The slider allows to change the isosurface value to any value from 0 to the maximum pixel intensity value of the 3D PSF.

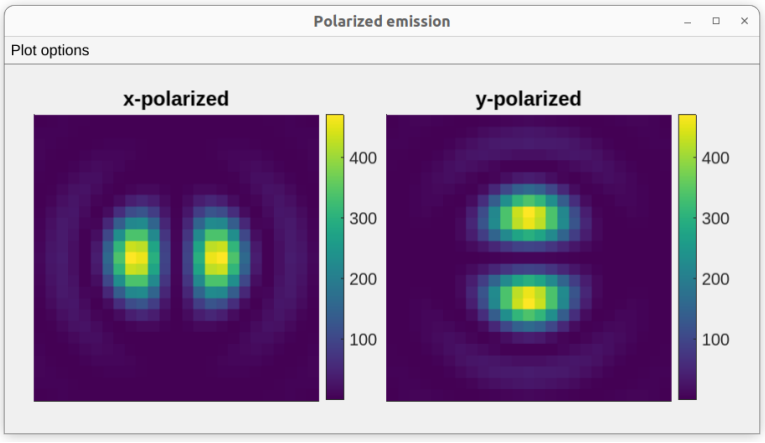

Figure 16: Window showing the 2D PSF split into polarized emission channels.

8 Fitting of aberrations

The button *Fit Zernike* under the tab *Aberrations* of the main window opens a new window that provides the option to characterize the Zernike aberrations of a microscope system from an experimentally recorded 3D image (*z-stack*) of a small fluorescent bead. The z-stack should comprise about 10 widefield images ranging from -1 to 1 µm. It is important that the signal to noise ratio in these images is as good as possible to prevent erroneous fits. The window allows to input the parameters of the calibration sample and the microscope. The microscope parameters are prefilled with the currently set values from the main window.

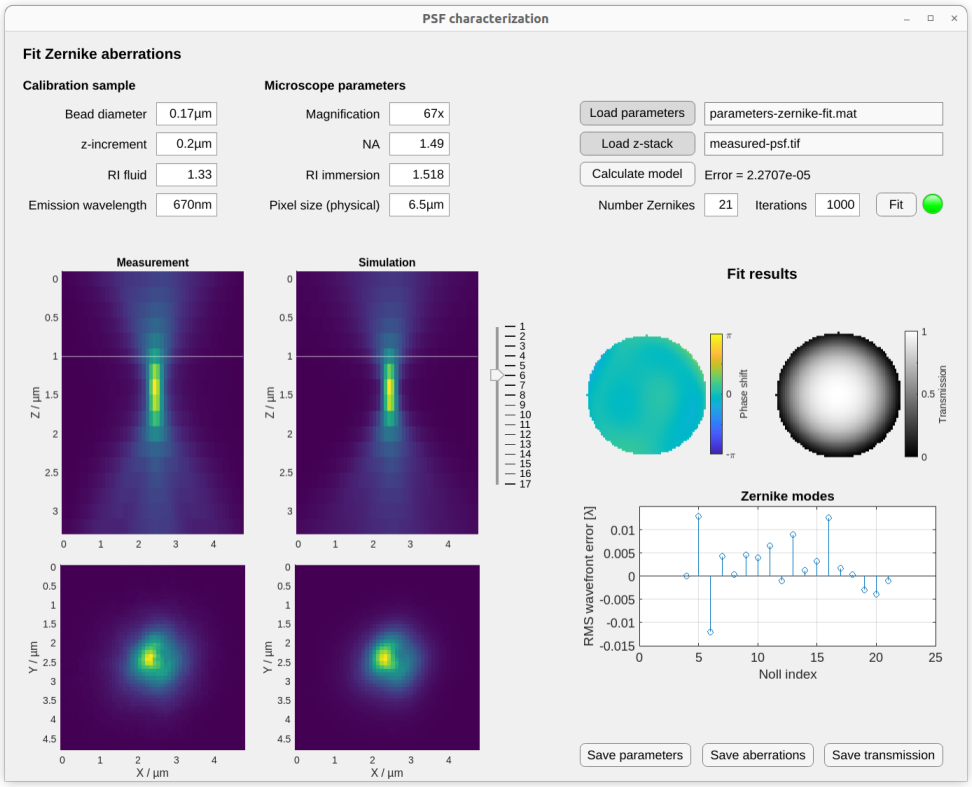

Figure 17: Window for fitting of aberrations. On the top left of the window, the parameters for the calibration sample and the microscope can be set. Microscope parameters are prefilled with the current values from the main window at the time of opening the subwindow. The buttons on the top right allow to load parameters and the experimental input data from files. The button *Calculate model* calculates the simulated PSF from the set parameters. The button *Fit* fits the aberrations present in the experimental data and outputs the aberrations, transmission and Zernike mode coefficients. The number of considered Zernike modes and the maximum number of iterations allowed for the fitting procedure can be adjusted by the user. On the bottom left, xz-projections and xy-slices of both the experimental data (measurement) and calculated model (simulation) are shown. The slider allows to select the xy-slice for display. On the bottom right, the results of the fit are shown. Buttons allow to save the current parameter settings, and the aberrations and transmission results.

Customizable parameters:

- *Bead diameter*. Diameter of the imaged bead given in micrometer. Default: 0.17 µm.
- *z-increment*. Axial step size between two adjacent frames of the z-stack.
- *RI fluid*. Refractive index of the sample layer
- *Emission wavelength*. The emission wavelength in units of nanometer.
- *Magnification*. Magnification of the objective.
- *NA*. Numerical aperture of the objective.
- *RI immersion medium*. Refractive index of the immersion medium.
- *Pixel size (physical)*. Physical width of a pixel of the camera given in micrometer.

References

[1] Magdalena C Schneider et al. "Interactive simulation and visualization of point spread functions in single molecule imaging". In: *arXiv* (2023). DOI: 10.48550/arXiv.2312.14356.

[2] Daniel Axelrod. "Fluorescence Excitation and Imaging of Single Molecules Near Coated Surfaces: A Theoretical Study". In: *Journal of microscopy* 247 (2012), pp. 147–60. DOI: 10.1111/j.1365-2818.2012.03625.x.

[3] Robert J. Noll. "Zernike polynomials and atmospheric turbulence". In: *J. Opt. Soc. Am.* 66.3 (1976), pp. 207–211. DOI: 10.1364/JOSA.66.000207.

[4] Steven M. Kay. *Fundamentals of Statistical Signal Processing: Estimation Theory*. Upper Saddle River, NJ, USA: Prentice-Hall, Inc., 1993. ISBN: 0-13-345711-7.

[5] Jerry Chao, E Sally Ward, and Raimund Ober. "Fisher information theory for parameter estimation in single molecule microscopy: Tutorial". In: *Journal of the Optical Society of America A* 33 (2016), B36. DOI: 10.1364/JOSAA.33.000B36.