

GaussFit_OnSpot

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What is the plugin for?

GaussFit_OnSpot is an ImageJ plugin for fitting Gaussian profiles onto selected positions in diffraction-limited images (e.g. single molecules, protein clusters, vesicles, or stars).

The plugin performs a function fit in regions of interest (ROI) around spots marked by point selections in grayscale images. Single or multiple spots can be either selected manually with the 'Multi-point tool' or automatically with the 'Find Maxima' function.

The plugin outputs the resulting Gaussian parameters in the ImageJ Results window.

Installation

Drag and drop [GaussFit_OnSpot.jar](#) to the "ImageJ" window or download and install using the *Plugins>Install* command.

Usage

- 1- Right click onto the point selection tool icon and select 'Multi-point Tool'
- 2- Open a grayscale image and select the spots in the image with the Multi-point tool
- 3- Start *GaussFit_OnSpot* from the ImageJ plugins menu
- 4- Check and adapt the settings in the displayed parameter window and press OK

Note 1:

The calculations are performed in 16bit grayscale images. Other types of grayscale images are automatically converted into 16bit images with an intensity scaling. Color images are not supported.

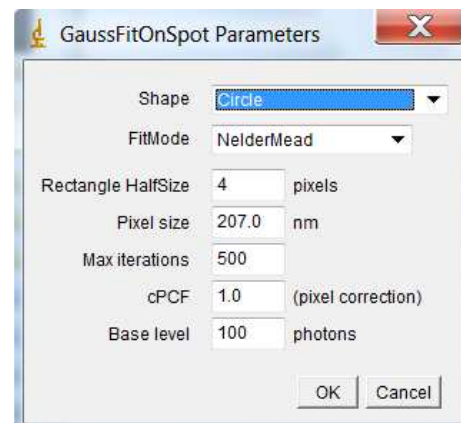
Note 2:

When the spots are marked with the 'Find Maxima' function the edge maxima should be excluded.

Settings

The following parameters can be adjusted to control the function fit:

Shape	The width of the Gaussian profile can be estimated by a circle(1), circle with varying width in x and y (2) or ellipse parameters (3)
FitMode	Fitting algorithms: 1-NelderMead 2-Levenberg Marquard
Rectangle HalfSize	Half edge length of the square ROI used for fitting
PixelSize	Pixel size in object plane in nm (= sensor pixel size / magnification)
MaxIterations	Maximum number of iterations
cPCD	Pixel correction factor (default = 1.0) (Multiplicative correction of intensity and background values)
BaseLevel	Noise background (specified in photon counts)



Result Output

The results in the ImageJ Results window are TAB separated and can be easily transferred into Excel by copy&paste.

The following result values are displayed:

Nr:	Number of the selected point
X:	X position of the selected point
Y:	Y position of the selected point
XC:	X center position of the Gaussian profile (in dimensions of the object plane /nm)
YC:	Y center position of the Gaussian profile (in dimensions of the object plane /nm)
Sigma:	Gaussian parameter σ
Theta:	Rotation angle of ellipse (in case of Shape mode 3)
Width:	Diameter of Gaussian profile (in dimensions of the object plane /nm)
A:	Ratio widthX/widthY (in case of Shape mode 2 or 3)
BGrd:	Background value (in photons above base level)
Intens:	Normalized intensity value

License

The *GaussFit_OnSpot* plugin is licensed under GPL (GPL: General Public License (latest) as specified at <http://www.gnu.org/licenses/gpl.txt>)

Reference

The *GaussFit_OnSpot* code is based on the μ Manager/ImageJ plugin 'GaussianFit' developed by Nico Stuurman.

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