**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 $\sigma$
- **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

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**License**

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

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**Reference**

The GaussFit_OnSpot code is based on the µManager/ImageJ plugin ‘GaussianFit’ developed by Nico Stuurman.

The original code is licensed under the following terms:

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