

TRABI 1.1

Installation Guide and How to Use

Installation (Figure 1):

1) Download and install ImageJ or Fiji (<http://imagej.nih.gov/ij/> or <http://fiji.sc/Fiji>), update to version 1.51k or higher.

2) Two options for the installation can be used.

- a) For temporary use install TRABI as macro in ImageJ/Fiji (**Figure 1**, (1.1)), select the file 'TRABI_v1.1.ijm' ('TRABI_v1.1.ijm' might be copied into the macro folder of ImageJ/Fiji).
- b) In order to install TRABI permanently copy 'TRABI_v1.1.ijm' into the plugins folder of ImageJ/Fiji (suggestion: 'Fiji.app\plugins\Analyze'). When starting ImageJ the macro automatically appears in *Plugins>Analyze>TRABI_v1.1* (2). A very helpful feature is assigning a keyboard shortcut to TRABI using *Plugins>Shortcuts>Add Shortcut...*

Optionally: Copy the settings file named 'TRABI_v1.1_settings.txt' to the macro folder of ImageJ/Fiji ('Fiji.app\macros'). The settings file contains editable start values when launching TRABI. Note Boolean variables (0 or 1 for false or true, respectively) in line 2, 4, 5, 8, 11, 12, 18.

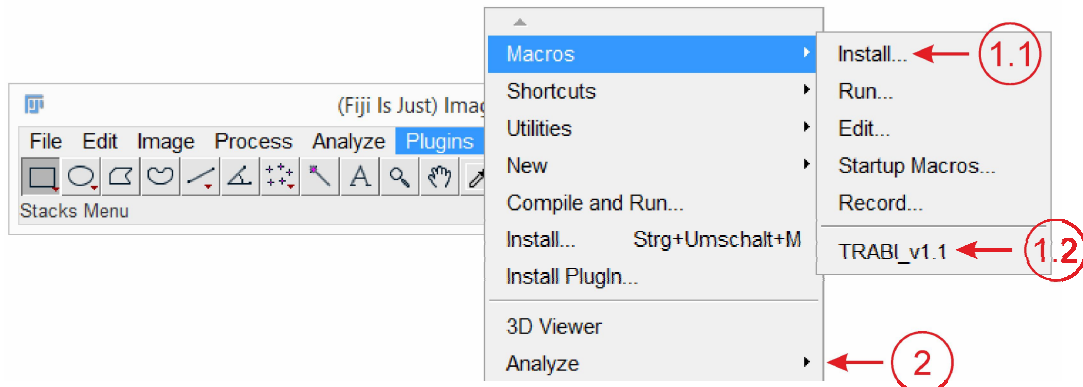


Figure 1. Install TRABI either temporarily (1.1) as executable macro (1.2), or permanently by copying TRABI into the plugins folder of ImageJ/Fiji, e.g., 'Fiji.app\plugins\Analyze' where it will always be selectable after starting ImageJ (2).

3) Load TIF-stack into ImageJ (skip if batch process is desired).

4) Run TRABI (**Figure 1**, (1.2) or (2)).

5) Select settings in GUI (**Figure 2**), press 'OK'.

TRABI GUI (Figure 2)

The GUI consists of three parts: input options, output options and TRABI settings.

Batch Processing Option

If activated TRABI will process multiple files sequentially. In order to work properly, create a folder that only contains image stacks to be processed with their corresponding localization files. The stem file name of both should be identical (e.g., 'dSTORM.tif' and 'dSTORM.txt', 'measurement_1.tif' and 'measurement_1.txt'). After pressing 'OK' another dialog window appears where the file extensions of image stacks and localization files must be defined (e.g., '.tif' and '.txt'). After selecting the folder,

TRABI will execute all files using the settings specified below, while the TRABI output files will be saved in a subfolder ('folder\TRABI'). Eventually, double-check the Log window for proper analysis.

I. Input options

Loc-file. Specify the type of localization file that should be used for the TRABI analysis. TRABI features selection of localization files from rapidSTORM, ThunderSTORM and PeakFit. It also allows reanalyzing TRABI output files ('TRABI_output'), as well as any localization file when reducing its content to x-, y-coordinates, frame number (starting with 0), and intensity, using space as delimiter and changing the header to #X Y Frame Intensity ('Basic'). If batch processing was deactivated, a single-molecule image stack (TIF-stack) must be opened in ImageJ before starting TRABI.

Free PSF fit. Activate if free PSF fit was used and parameters should be saved (needs output file 'Basic+FWHM' or 'Complete').

Coordinates in nm & Pixelsize. The dialog 'Coordinates in nm' must be activated and a pixel size stated, if the coordinates in the SMLM localization file are in nm, too. For instance, rapidSTORM always exports coordinates in nm, but PeakFit allows to store coordinates as pixel or nm coordinates. The pixel size must be exactly the same as used in the SMLM software, otherwise spot intensities are not properly analyzed.

II. Output options

Output file. The output file of TRABI is a text file containing basic information of the original localization file, e.g., coordinates, frame and intensity obtained by fitting (I_{fit} , output as e.g. $I_{\text{rapidSTORM}}$) and new information such as corrected intensity (I_1) and P value. The header of the output file also contains information about TRABI settings, e.g., pixel size, basejump and number of background frames (n_{BG}). The file is saved in the folder of the SMLM localization file (if batch processing was activated files will be saved in a new subfolder).

The complexity of the output file can be varied between basic information ('Basic'), i.e., coordinates, frame number, corrected intensity (I_1) and P value; 'Basic+FWHM' and 'Basic+BG' add FWHM and background information to the output, respectively, whereas 'Complete' contains basic, FWHM and background information.

P-filter activated. When activated this filter allows confining P in the output file using P-min and P-max as thresholds, e.g., from 0 – 100%.

BG-Averaging: save frame info. The temporal information of the background estimation, i.e., the frame numbers of the individual background frames, can be saved in the final TRABI output file by activating the dialog (only saved for output options 'Basic+BG' and 'Complete').

Save settings to file name. Settings information are part of the filename when activated.

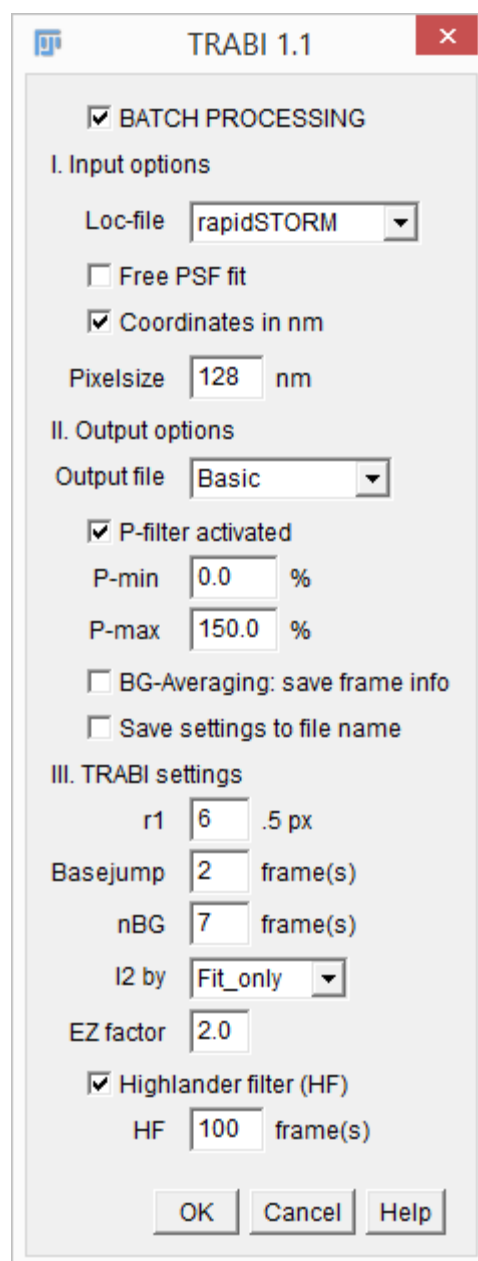


Figure 2. TRABI GUI

III. TRABI settings

All values except EZ-factor are integers (see Supp. Information (SI) of the manuscript).

r1. The radius for the intensity estimation in TRABI (I_1).

Basejump. The dialog 'basejump' can be used to skip $\#basejump-1$ frames directly after the localization. A basejump of 2 (default value) implies that the first frame after the localization is skipped. A value of 1 deactivates the basejump.

nBG. The number of frames used to average the background intensity.

I2 by. By default ('Fit_only') I_2 is taken from the localization file (I_{Fit}). Select 'Fit+Circle' if I_2 should be also characterized by a second circular aperture in TRABI. After selection and pressing 'OK', another dialog appears, where r_2 must be defined. Besides I_{Fit} , I_1 , P etc., the output file is then supplemented by I_2 and P_2 . Please note that BG information is only output for r_1 .

EZ factor. The factor being multiplied with r_1 defines the exclusion zone (EZ). A value of 2.0 is the recommended value to work with, especially if accurate single-molecule intensities are required. As a large EZ in combination with higher spot densities might lead to a loss of localizations in the TRABI output file, it can be helpful to adapt the EZ, however, at the risk of corrupted intensity values.

Highlander filter. Activate to reject spots, which reside permanently in the fluorescent on-state by using a threshold (in frames). For more details, see SI of the manuscript.

If you find TRABI useful, please cite our work: Christian Franke, Markus Sauer, Sebastian van de Linde.

Photometry unlocks 3D information from 2D localization microscopy data. *Nat. Methods*, **14**, 41–44 (2017).

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