

Manual for the software tool

GATTAnalysis

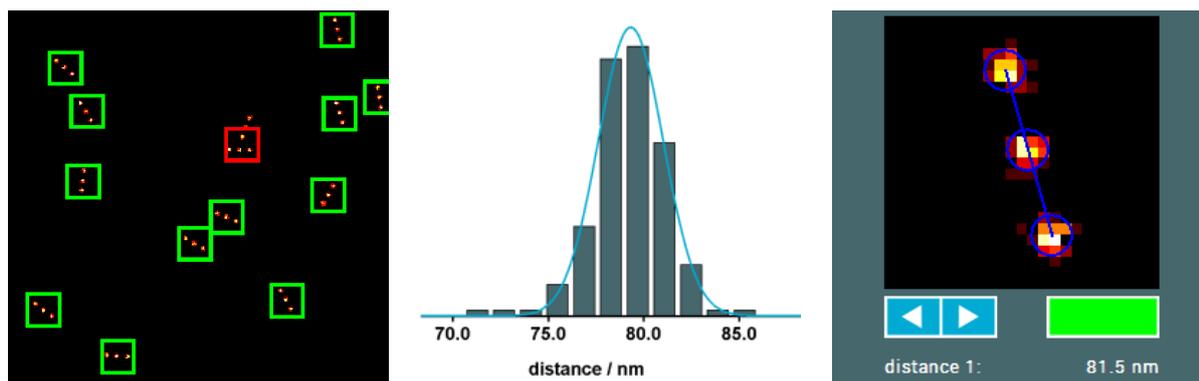


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1. About GATTAnalysis

The software tool GATTAnalysis by GATTAquant provides a fast and easy way to evaluate super-resolution data gained from measurements with DNA-origami based nanorulers. Just by a few clicks origami structures can be detected in the super-resolved image and analyzed with regard to their dye-to-dye-distance. The resulting distance values are subsequently presented in a histogram including basic statistical analyzing options and can be exported to a text file.



The analysis of data gained from microscopy with intrinsic binning (STED, SIM, etc.) differs slightly from those gained from localization based microscopy (STORM, PALM, DNA-PAINT, etc.). The processing of both data types will be presented independently in the subsequent chapters.

The software is based on Java and requires the Java SE Runtime Environment 7 or higher.

2. Microscopy with intrinsic binning

Read this section, if you would like to evaluate data from microscopy measurements with intrinsic binning like STED or SIM.

2.1 Import a data image



To load an image file click the “Import Image” button and select the file you want to analyze. Note the file type has to be either “BMP”, “PNG”, “JPEG” or “TIFF”. After loading the image will appear on the user interface. Using the mouse wheel you can zoom in and out and by moving the mouse while pushing the left button you can navigate through the image.

2.2 Open an existing project



To open an existing project click the “Open Project” button and select the respective project file (“.gatta”). This will recover the stored session with all data and configurations.

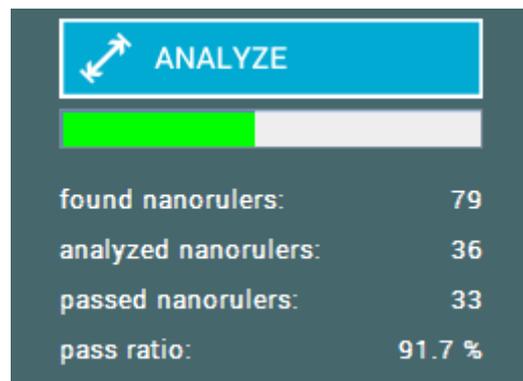
2.3 Finding nanorulers



To scan the image for nanorulers click the “Find Nanorulers” button. The spot finder marks every found structure with a white square. Furthermore the number of found structures is displayed in “found nanorulers”. If necessary, the result can be postprocessed manually by clicking into the image and doing the following: If there is a nanoruler, which was not detected by the spot finder (false negative), click on it so that an additional white square will appear (if you do not hit the center exactly but slightly aside, GATTAnalysis will assist you by snapping the square to the spot center). If the spot finder has detected an object that is definitely not a nanoruler structure (false positive), remove it by clicking into the center of the respective white square (the marker will disappear). However, in the most cases the automatic filtering in the next processing step (see next paragraph) is sufficient, so that a manual postprocessing of the spot finding is usually not essential.

2.4 Getting distances

 Start the distance measurement by clicking on the “Analyze” button. This procedure takes a period of time (several seconds, depending on the number of located nanorulers and your processing power). The analyzing progress is displayed by the green progress bar. Further the current number of analyzed spots and the number of passed spots is displayed.



The presentation of various analysis parameters has its origin in a set of specific filters, which are supposed to distinguish between proper measurable nanorulers and other detected structures. The fraction of good spots is displayed in “pass ratio”. After the process has finished the marking squares in the main image will be colored in green for passed structures or red for failed structures. The filtering can be changed manually by using one of the following options.



Use the “Select All” button to mark all found nanorulers as passed.



Use the “Deselect All” button to mark all found nanorulers as failed.

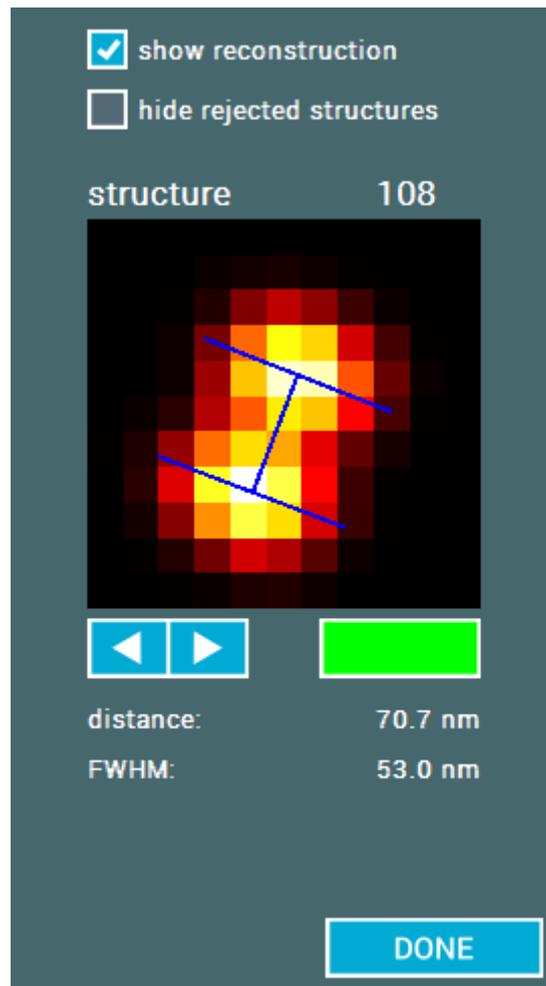
You can further change the filtering result for each nanoruler individually (see next paragraph).

2.5 Getting single spot information

When the distance measurement has finished, you can select a single nanoruler (by clicking into its square) to get further information about it. This will open an information box for the respective nanoruler.



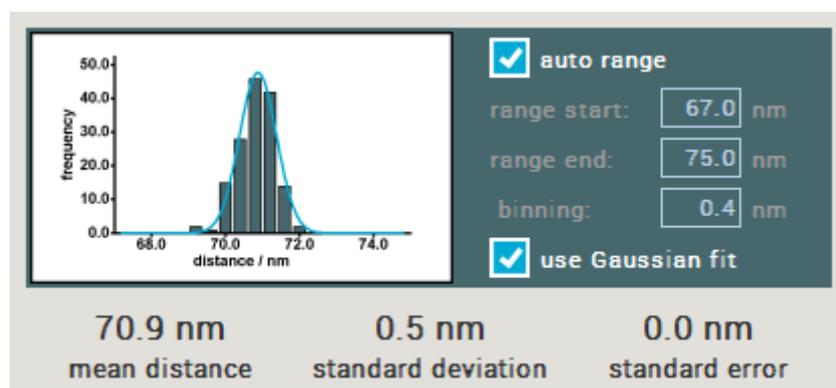
Here a magnified image of the nanoruler is displayed and the distance value is shown. By using the arrow buttons or typing the arrow keys of your keyboard you can click through all analyzed nanorulers. Thereby the filtering result for the respective nanoruler is displayed by the colored button next to the arrow buttons. This button can also be used to change the filtering result by clicking on that button and manually change the color from green to red or vice versa. Nanorulers, which did not pass the filtering, will be omitted by the arrow buttons, if the option “hide rejected structures” is activated.



Further you can use the “show reconstructions” option to display the calculated reconstruction of the selected nanoruler.

2.6 Statistical data analysis

When the distance measurement has finished, a histogram of the distances is shown right next to the main image.



The plotting range can be chosen with the fields “range start” and “range end” or by activating the option “auto range”. If the option “use Gaussian fit” is activated, the distribution is fitted with a Gaussian function and its center is displayed in “mean distance”. Otherwise this field indicates the average distance value within the selected range.

This statistical data analyzing tool is also available for the measured full width at half maximum (FWHM) of each single spot.

2.7 Saving and exporting data



By clicking on the “Save” button you can save the current session to a “.gatta” file. This file will store the data and the state of analysis including all settings.



Additionally, there are some options to export your result to other file types. When you click on the “Export” button, a respective dialog will appear where you can select the kind of data you want to export.



The option “export main image” will export the main image to a PNG file. The options “export distance values” and “export FWHM values” will export the respective values to

a spreadsheet file. The options “export distance histogram” and “export FWHM histogram” will export an image of the respective histogram to a PNG file.

3. Localization based microscopy

Read this section, if you would like to evaluate data from localization based microscopy measurements like STORM, PALM or DNA-PAINT.

3.1 Open data

Localization based super-resolution data for processing in GATTAanalysis has to be available in a conventional spreadsheet file e.g. as “.txt” file. Furthermore the spreadsheet has to be formatted that way that every line corresponds to a single localization record, whereas every column contains a certain parameter (e.g. x-coordinates, y-coordinates, frame number, etc.). Besides that, GATTAanalysis is relatively flexible regarding your formatting, but of course the software has to be configured to the exact format before loading the file.



To open a data file click on the “Import Image” button and select the file to analyze. After confirming an import dialog will appear where you have to define the configuration of the spreadsheet file to open.

IMPORT SETTINGS

exclude first line (header)

delimiter

x values

y values

z values

width x

width y

frame numbers

photon counts

filter imported data

length unit nm

length unit (z) nm

binning nm

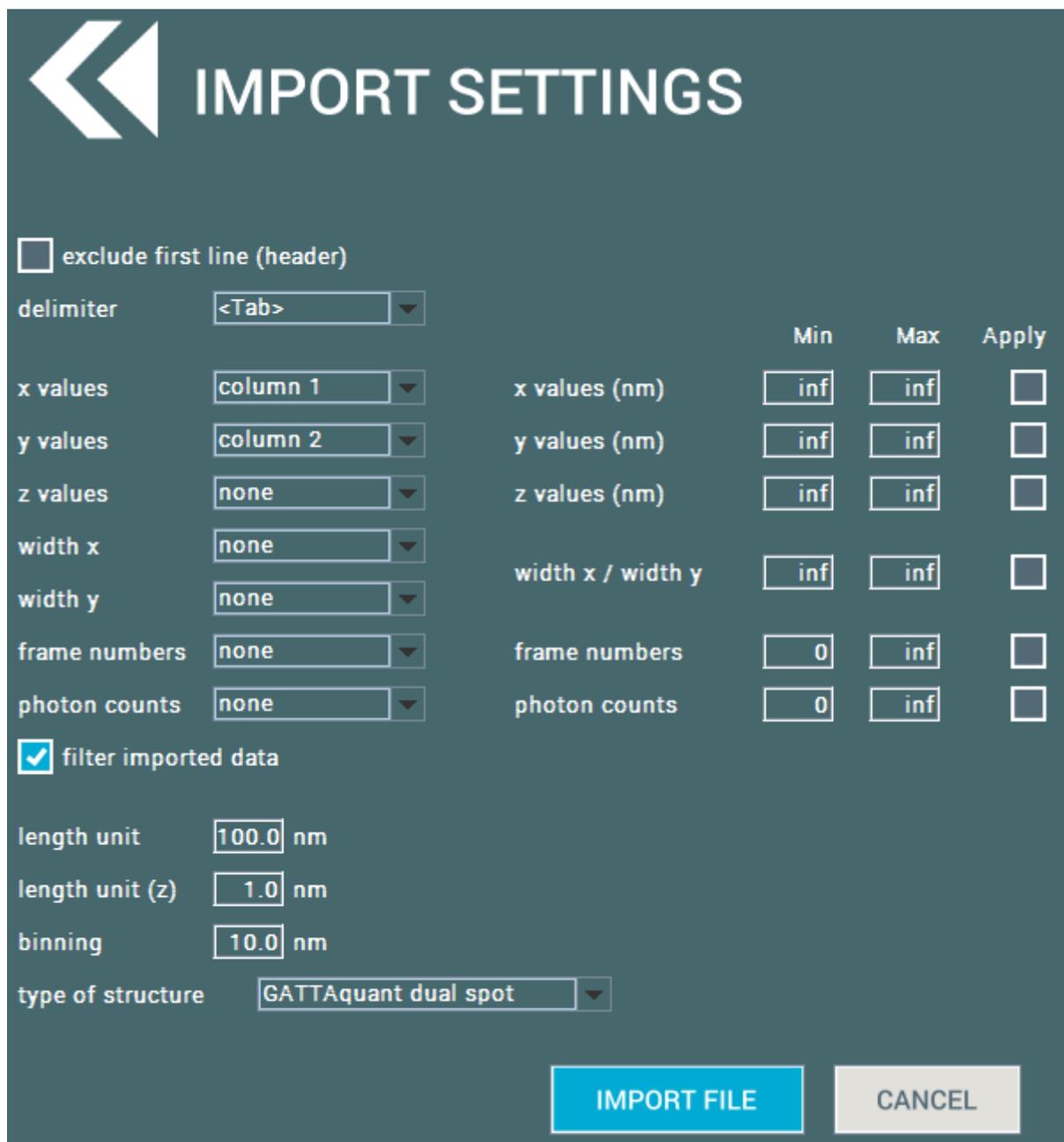
type of structure

IMPORT FILE **CANCEL**

Activate the option “exclude first line (header)”, if the data file has a header line. The delimiter which separates the individual columns can be chosen out of <Tab>, <Space>, “,” or “;”. After specifying this, the different data parameters have to be assigned to the columns in which they are located in the file. To assign a column for a certain parameter click on the drop down menu next to the parameter and choose a column. Repeat that for all parameters you want to include. Furthermore the length unit has to be defined (e.g. if all the data is scaled in nanometers, this value has to be equal to 1). The parameter “binning” indicates the size of a pixel of the image which will display the loaded data. Be aware that a low value for the binning will lead to a

high consumption of memory. The result of the data analysis does not depend on the binning value. Very important for the data analysis is a correctly chosen “type of structure” which provides the options “GATTAquant dual spot” (nanoruler with two fluorescent marks) and “GATTAquant triple spot” (nanoruler with three fluorescent marks).

If the option “filter imported data” is activated, the data will be filtered concerning to filter criteria, which can be defined in the appearing table on the right side of the dialog.



IMPORT SETTINGS

exclude first line (header)

delimiter

		Min	Max	Apply
x values	<input type="text" value="column 1"/>	<input type="text" value="inf"/>	<input type="text" value="inf"/>	<input type="checkbox"/>
y values	<input type="text" value="column 2"/>	<input type="text" value="inf"/>	<input type="text" value="inf"/>	<input type="checkbox"/>
z values	<input type="text" value="none"/>	<input type="text" value="inf"/>	<input type="text" value="inf"/>	<input type="checkbox"/>
width x	<input type="text" value="none"/>	<input type="text" value="inf"/>	<input type="text" value="inf"/>	<input type="checkbox"/>
width y	<input type="text" value="none"/>	<input type="text" value="inf"/>	<input type="text" value="inf"/>	<input type="checkbox"/>
frame numbers	<input type="text" value="none"/>	<input type="text" value="0"/>	<input type="text" value="inf"/>	<input type="checkbox"/>
photon counts	<input type="text" value="none"/>	<input type="text" value="0"/>	<input type="text" value="inf"/>	<input type="checkbox"/>

filter imported data

length unit nm

length unit (z) nm

binning nm

type of structure

IMPORT FILE **CANCEL**

Here an acceptance range for every parameter can be chosen. If the corresponding option “Apply” is activated, only data points in which the respective parameter’s value is contained by the specified range will pass the loading process.

3.2 Open an existing project



To open an existing project click the “Open Project” button and select the respective project file (“.gatta”). This will recover the stored session with all data and configurations.

3.3 Adjusting the image display

After loading the super-resolution image will appear on the user interface. Using the mouse wheel you can zoom in and out and by moving the mouse while pushing the left button you can navigate through the image. Additionally, the display mode and properties can be further adjusted. A right click on an arbitrary point of the image will open the “image settings” window.

binning 10.0 nm

image mode number of localizations

autoscale

scale minimum 0.0 nm

scale maximum 10.0 nm

scale mode single gradient

color 1 [black]

color 2 [red]

use cosmetic filter

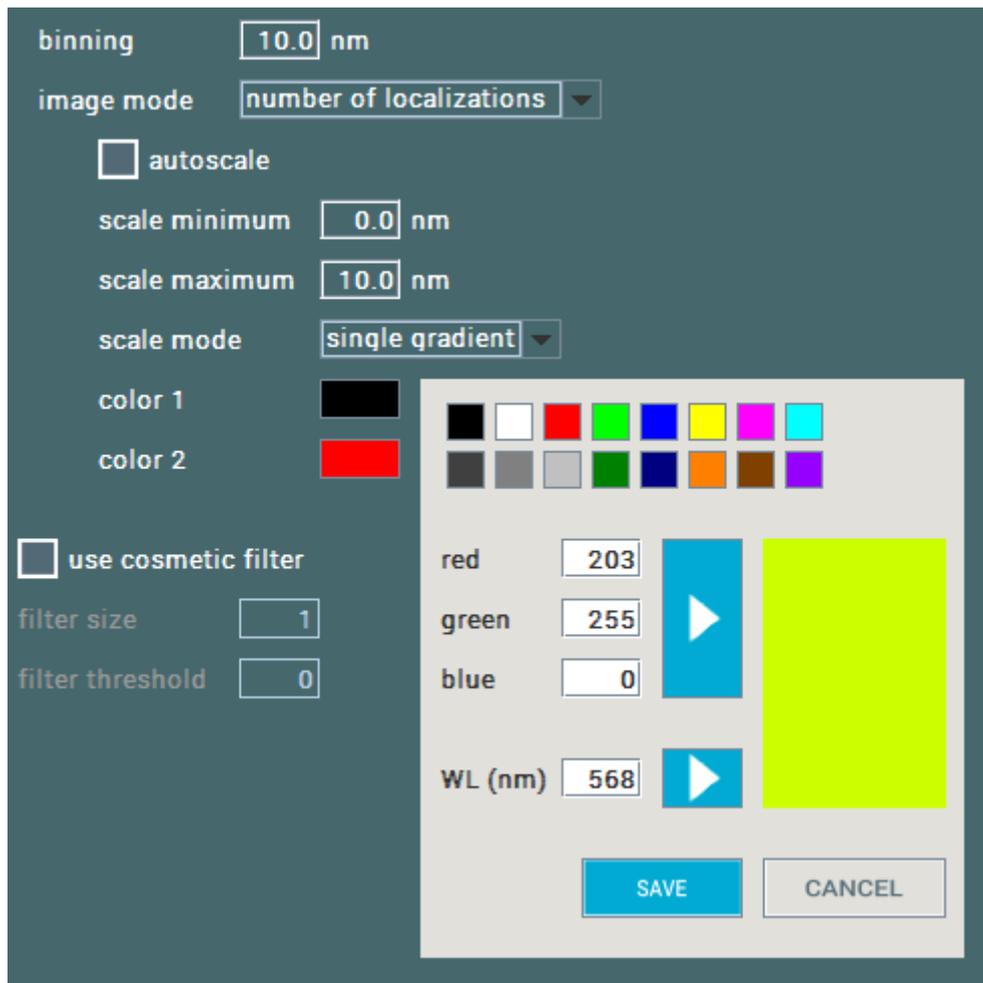
filter size 1

filter threshold 0

SAVE CANCEL

With the field “binning” the pixel size of the displayed image can be adjusted. The type of the image can be specified in “image mode” which provides the three different options “number of localizations”, “average z-value” and “average frame number”. If the image is in the “number of localizations” mode, the color of each image pixel is calculated based on the total number of localization events within this pixel. If the image is in the “average z-value” or “average frame number” mode, the pixel color is calculated based on the average of the z-values or the frame numbers of all localization events contained by the pixel.

In every mode the scale can be adjusted either by using the fields “scale minimum” and “scale maximum” or by activating the option “autoscale”. For the scale mode the three options “single gradient” (custom colors), “temperature” (black – red – yellow – white) and “rainbow” (red – yellow – green – blue – purple) are available. The upper and lower edge colors for the “single gradient” option can be specified by clicking on the respective colored button which will open a color selection.



This provides the option to choose a standard color or define a color by its RGB code or by the corresponding light wavelength.

3.4 Finding spots

 To scan the image for nanorulers click the “Find Nanorulers” button. The spot finder then marks every found structure with a white square. Furthermore the number of found structures is displayed in “found nanorulers”. If necessary, the result can be postprocessed manually by clicking into the image and doing the following: If there is a nanoruler which was not detected by the spot finder (false negative), click on it so that an additional white square will appear (if you do not hit the center exactly but slightly aside, GATTAanalysis will assist you by snapping the square to the spot center). If the spot finder has detected an object that is definitely not a nanoruler structure (false positive), remove it by clicking into the center of the respective white square (the marker will disappear). However in the most cases the automatic filtering in the next

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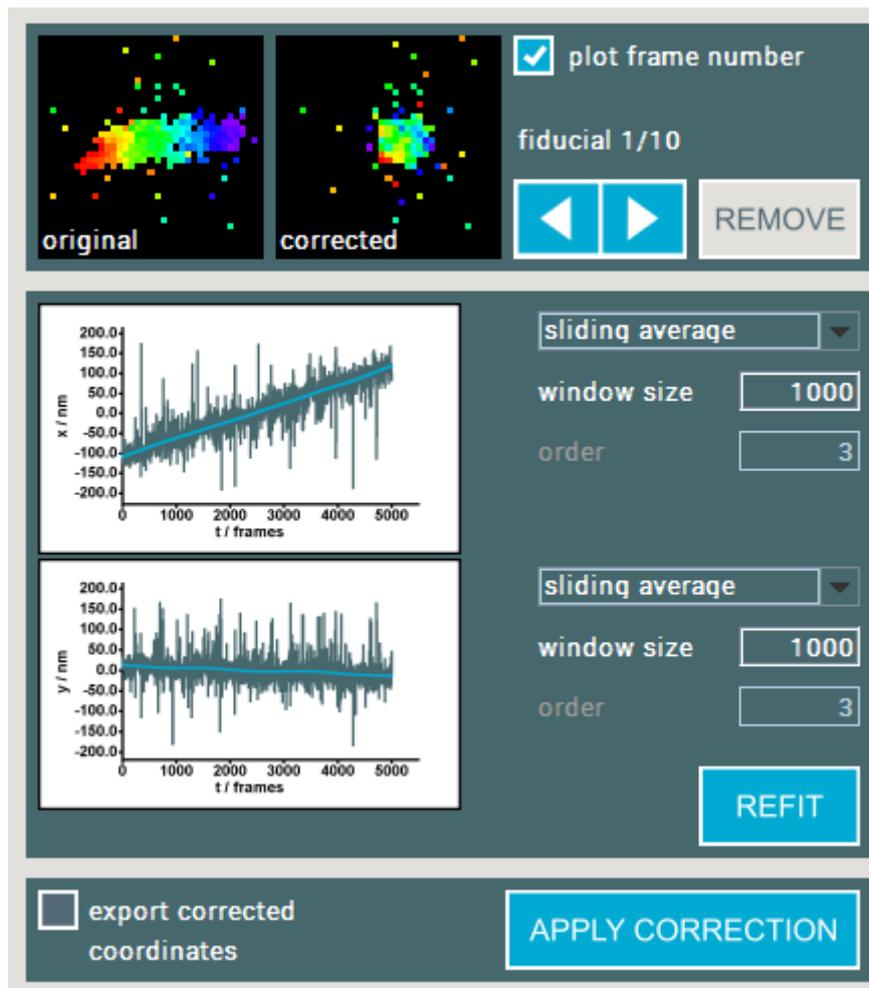
3.5 Drift correction

A common issue in super-resolution microscopy is lateral drift leading to decrease of resolution of the gained image. If you do not have this problem, you can continue directly with paragraph 3.6.

GATTAanalysis offers a tool to correct for these drift based image errors. This tool is available, if the imported localizations contain temporal information, that means the parameter "frame number" has to be connected to the right column of the imported data file (see paragraph 3.1).

In the first step for drift correction fiducial markers have to be defined. This can be done automatically by clicking on the "Find Fiducial Markers" button or manually by a right click into a white square marking a structure (see paragraph 3.4). A further right click undoes the selection.

If at least one fiducial marker was defined, in the box "Drift Correction" on the right side of the screen further controls for drift correction appear.



Here in the upper subbox the fiducial markers (left) and their correction previews (right) are shown. The arrow buttons allow navigation through the individual fiducials. Also they can individually be removed by clicking on the “Remove” button.

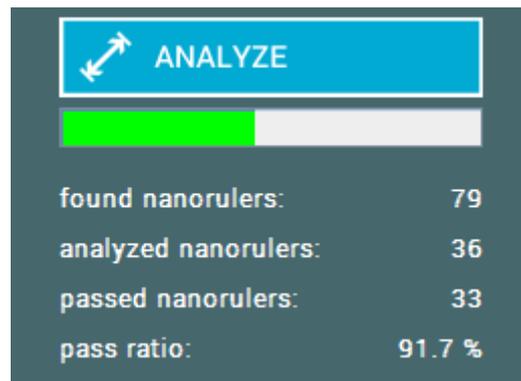
The center box shows the time traces of the x- and y-coordinates averaged over all defined fiducials. The drift correction functions calculated from these traces are plotted in blue. If the correction function does not fit well, its calculation can be optimized by using the controls next to the diagram. Hereby one can choose between the calculation modes “sliding average” and “polynom fit” and configure them via the parameters “window size” or “order”. For a recalculation of the correction function with changed parameters the “Refit” button has to be clicked.

When all configurations are done, the drift correction function can be applied to the whole image by using the “Apply Correction” button. If the option “export corrected coordinates” is activated, the new calculated coordinates will be exported to a text file.

3.6 Getting distances



Start the distance measurement by clicking on the “Analyze” button. This procedure takes a period of time (up to several minutes, depending on the number of located nanorulers and your processing power). The analyzing progress is displayed by the green progress bar. Further the current number of analyzed spots and the number of passed spots is displayed.



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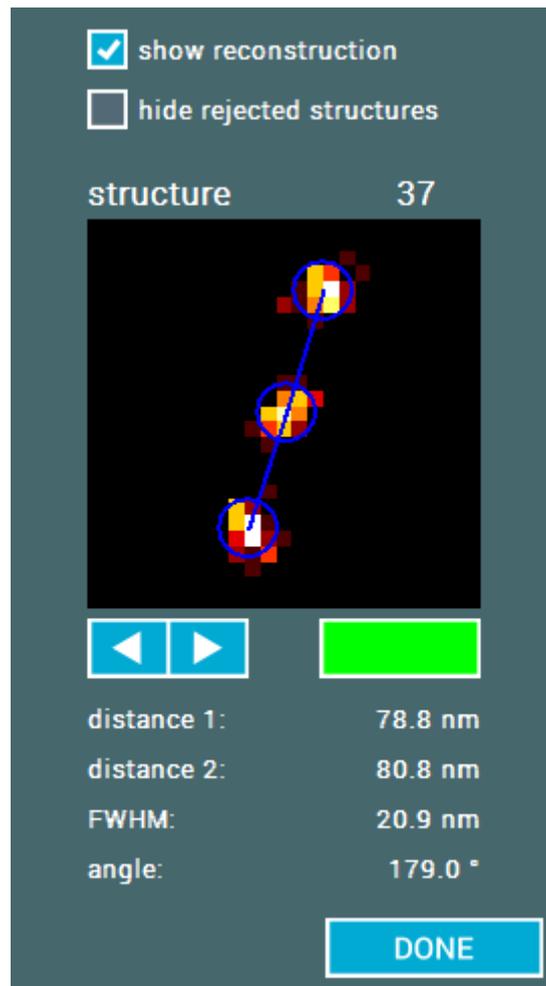
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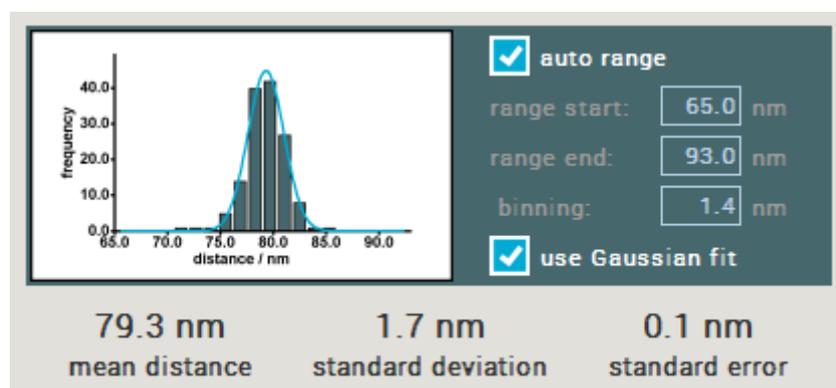
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