

## README

Authors: R.P.J. Nieuwenhuizen, B. Rieger, and S. Stallinga.

Citation: Please cite the following publication in case you use the software:

R.P.J. Nieuwenhuizen et al., *Quantitative localization microscopy: Effects of photophysics and labeling stoichiometry*, submitted

This helps us to ensure financial support for our projects and allow further development of this software. Thank you.

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This document describes the steps needed to install and use the matlab code for estimating the number of localizations per labeled site in localization data.

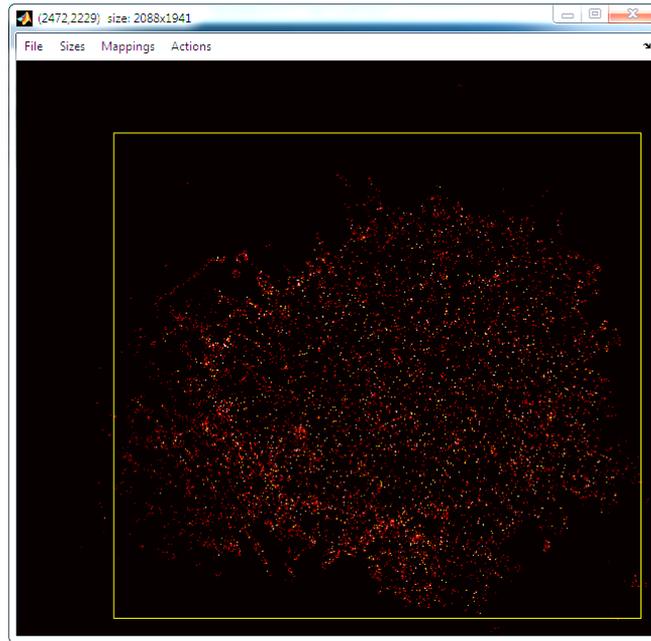
### A. REQUIREMENTS

The Qcounting algorithms require a recent installation of the software package MATLAB , as well as the toolbox DIPimage. The latter can be downloaded at <http://www.diplib.org/>.

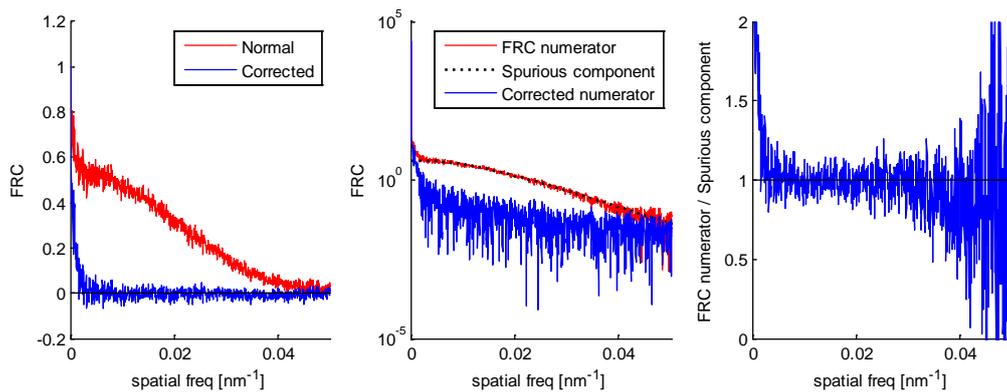
### B. USING THE SOFTWARE

The function that needs to be called to estimate the number of localizations per labeled site (e.g. epitope labeled with fluorescent antibodies) is the function Qcount.m. To see how it works in practice, run the example script testscript\_lgEdata.m.

When the function Qcount is called, it will first ask the user to select a region of interest in the field of view. The counting estimation will be done using only the localizations in this region.



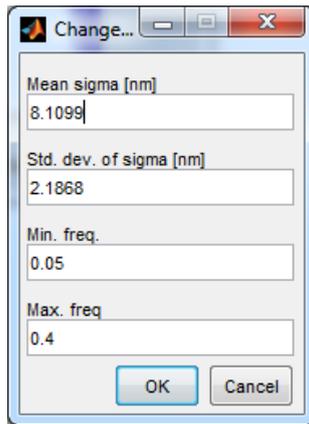
Subsequently, the algorithm will attempt to estimate the spurious correlation parameter  $Q$ . The user is then asked to judge if this attempt was successful based on three plots.



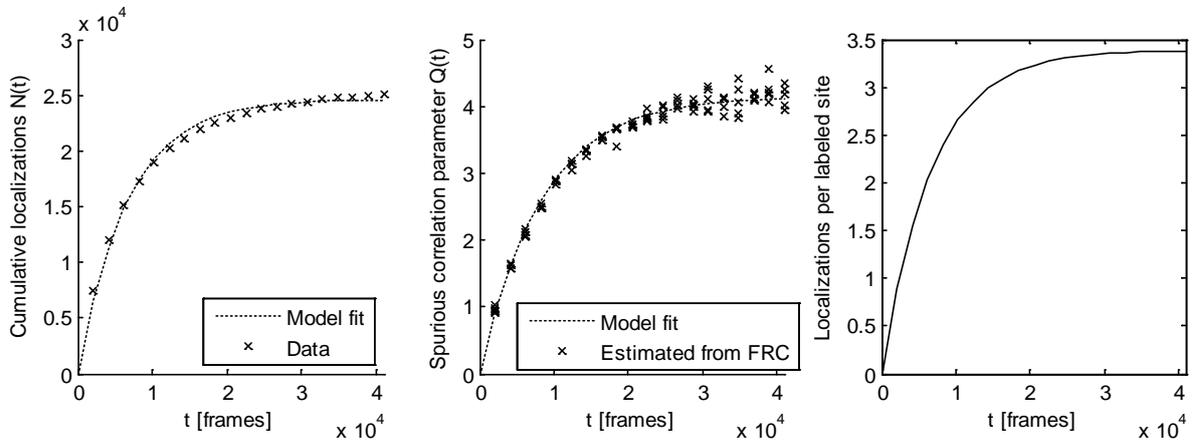
The first plot shows the Fourier Ring Correlation curve. Typically this curve should show a monotonous decline to zero and then remain approximately zero up to the Nyquist frequency of the image.

The second plot shows the numerator of the FRC curve. This numerator should decline rather rapidly at low spatial frequencies and then switch to another regime for intermediate spatial frequencies for which it is dominated by correlations due to localizations of the same labeled site. In this regime, the numerator is proportional to a Gaussian function of the spatial frequency. This Gaussian function is fitted to the FRC's numerator. The fit is good if it closely follows the FRC's numerator at these intermediate frequencies but not at low frequencies. Finally, the third plot shows the ratio of the FRC's numerator and the fitted decay. This ratio should decline initially and then remain constant at a value of 1 as long as possible. The ratio should typically not undercut the plateau value of 1 before converging towards it.

If the user is not satisfied with the obtained estimate of the spurious correlation component, a pop-up box enables them to change the parameters for the estimation.



After this, the algorithm continues until all outputs have been computed and a figure is generated showing the fits to the cumulative localizations and spurious correlation parameter  $Q$  as a function of time.



Check both plots to see if the fits are in order.

#### Inputs of Qcount.m:

- coords:** An Nx3 array of the x,y position and time stamp of localizations. x,y positions are assumed to be specified in camera pixels.
- FOV\_size:** Size in camera pixels of the field of view from which the localizations have been obtained.
- Zoomfactor:** The algorithm renders images of the localizations during the computation. Zoomfactor is the ratio of the camera pixel size to the pixel size in these intermediate rendered images. It is highly recommended to choose zoomfactor such that intermediate images have pixel sizes equal to or smaller than the localization precision.
- autoQplateau:** This Boolean variable indicates the mode in which the algorithm is operated. If TRUE, the algorithm will estimate the mean and standard deviation of the distribution of precisions over all localizations from the data.
- Qparameters:** Structure containing parameter for the estimation of the spurious correlation component of the Fourier Ring Correlation (Nieuwenhuizen2013).

#### Fields of Qparameters:

- minq:** Lowest spatial frequency where spurious correlations dominate the FRC, in units of 1 per pixel in the rendered superresolution images. (optional)
- maxq:** Highest spatial frequency for which the FRC is nonzero, in units of 1 per pixel in the rendered superresolution images. (optional)
- Qpoints:** Number of time points for which the correlation parameter Q is estimated. (optional)
- reps:** Number of times the correlation parameter Q is estimated. (optional)
- nblocks:** Number of time blocks into which localizations are subdivided for the computation of the Fourier Ring Correlation. (optional)
- noCheck:** If TRUE, the algorithm does not ask the user whether the fit to the FRC curve is acceptable. (optional)
- noROI:** If TRUE, the algorithm does not ask the user to select an ROI in the field of view but instead uses the all localizations for computation. (optional)
- sigmas:** Only used for autoQplateau = TRUE. Nx1 array of localization precisions corresponding to the localizations in coords. If this

input is not specified then it will be assumed that coords has a fifth column containing said localization precisions.

sigma\_mean: Only used for autoQplateau = FALSE. Average localization precision of localizations, specified in camera pixels.

sigma\_std: Only used for autoQplateau = FALSE. Standard deviation of the localization precisions of localizations, specified in camera pixels.

pixelsize: Camera pixel size in nm.

stoich\_calibration: This variable specifies the stoichiometry of the number of fluorophores per labeled site in a 2 element array [mu,S\_av]. Here S\_av is the mean of the number of fluorophores S per site and  $\mu = \text{mean}(S^2)/\text{mean}(S)-1$ . If S is not specified, then  $S_{av} = \mu/(1-\exp(-\mu))$ .

Outputs of Qcount.m:

Q: The estimated spurious correlation parameter at a number of time points.

t: Time points for which Q was computed. t has the same units as the time coordinates in the third column of the input coords.

N: Number of localizations acquired up to time t.

M: Estimated number of localizations per labeled site.

Fit\_switching: Outcomes ( $M_\infty, k_{bl}, \mu$ ) of the curve fit to Q and N with the theoretical model functions.  $M_\infty$  is the average number of activations of a single fluorophore before photobleaching and  $k_{bl}$  is the effective bleach rate of single fluorophores in units of 1 per frame.

Fit\_sigmas: If autoQplateau = TRUE, these are the estimates sigma\_mean and sigma\_std of respectively the mean and standard deviation of the effective localization precisions.

FRC\_output: Structure containing the FRC curve corrected and uncorrected for the spurious correlations with corresponding spatial frequencies (in 1/nm), FRC resolution at times t and estimated uncertainties in the resolution estimates (both in nm).

ROI\_output: Specification of the ROI for which results have been computed by means of the (x,y) coordinates of the topleft corner of the ROI and the dimensions, both specified in camera pixels.

### C. LIST OF FUNCTIONS

frc_tapermask:	Generates a mask for FRC computation that high resolution artefacts due to the edges of the image.
freresolution:	Computes the resolution from a FRC curve.
masklocalizations:	Remove localizations that do not fall inside the region of interest specified by a mask image.
Qcorrection_ims_auto:	Automatically estimates the spurious component in the FRC curve and corrects for its effects.
Qcorrection_ims_manual:	Estimates the spurious component in the FRC curve and corrects for its effects using the user specified mean and standard deviation of the distribution of localization precisions.
Qcount:	Main function.
Qfit:	Fits the cumulative localizations N and spurious correlation parameter Q to obtain the number of localizations per labeled site.
selectlocalizations:	Allows selection of localizations in a rectangular region of interest.
showlocalizations:	Generates an image based on an array of localization data.