

## FRC Analysis

We provide 10 Matlab scripts. The *Image Processing* and *Signal Processing* toolboxes are required. The script *Demo\_FRC.m* is used to perform FRC analysis on the provided test data, consisting in a couple of CLSM 0.2 A.U. images, a couple of CLSM 1.4 A.U. images and a couple of ISM images from the very same dataset. The demo outputs the FRC plots for each couple of images, and allows to quantitatively appreciate the resolution improvement when considering the ISM images. The time necessary for running the demo is in the order of one second.

Alternatively, we also provide the script *IIT\_FRCAnalysis.m* to perform analysis on any couple of 2D or 3D images. Input arguments are:

- **im1**: 2D or 3D matrix of double containing photon counts of the first measurement;
- **im2**: 2D or 3D matrix of double containing photon counts of the second measurement;

Optional input arguments are:

- **pixelSize** (default 0.025  $\mu\text{m}$ );
- **driftCorrection**: if true, performs drift correction before FRC analysis (default false);
- **meanFilterWidth**: radius of the mean filter used to perform smooth the FRC curve (default 3 frequency bins);
- **maxIterations**: maximum iterations for the gaussian fitting performed when applying drift detection algorithm (default 200);
- **roiRadius**: radius of the crosscorrelation function considered for gaussian fitting when applying drift detection algorithm (default 5 pixels);
- **theta**: if set, for each ring split the FRC analysis for pixels in the frequency domain for which  $-\theta < \text{angle} < +\theta$  (0 rad).  $0 \leq \theta \leq (\pi/2)$ ;
- **title**: the title of the displayed image

Output:

- **result**: Matlab structure containing all parameters and result of the FRC analysis.

## Pixel Reassignment and Deconvolution of ISM data

We provide an executable file, Matlab runtime is needed. It allows to load a .tiff containing a stack of 25 images, each related to a different element of the SPAD array, and to display the results of ISM analysis: CLSM 0.2 A.U., CLSM 1.4 A.U., ISM, ISM<sup>++</sup>. It is then possible to save results as.tif images or as a Matlab structure. We provide a file for testing in the folder DataForTest. The time necessary for running the analysis with supported data and 8 deconvolution iterations is in the order of ten seconds.

## Phasor Analysis

We provide an executable file, Matlab runtime is needed. It allows to load a .tiff file containing the time-resolved measurement, and shows the temporal decay of the field of view. It is thus possible to calculate and show the phasor plot of the analysis. We provide a file for testing in the folder DataForTest. The time necessary for running the analysis with supported data is in the order of one second.