

# Fourier ring correlation as a resolution criterion for super resolution microscopy

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## FRC MANUAL

## FRC from SRM stand-alone Labview installation and operation instruction

1. This program requires installation of the Labview runtime environment 2011 as well as the NI Vision runtime environment 2011, which can be downloaded from the Ni.com homepage. You will need to register with NI.
  - a. Please go to <http://joule.ni.com/nidu/cds/view/p/id/2534/lang/en> and follow the instructions to download and install the Labview 2011 runtime environment (select “standard”). This runtime environment is freeware.
  - b. Please go to <http://joule.ni.com/nidu/cds/view/p/id/2599/lang/en> and follow the instructions to download and install the Ni Vision 2011 runtime environment. This runtime environment is free to trial for 30 days.
2. Unzip “FRCfromSRMexe.zip”
3. Start “FRCfromSRM”
4. The program will start and prompt for a User Input

Select the unit of the input coordinates. If you select pixels, a sub window will open (see bottom box).

Select the number of rings for the FRC calculation. More points will lead to a smoother (higher sampling) but also a noisier curve. 40 is a good starting value.

If you selected pixels as unit insert the pixel size

Insert the desired super-resolution image pixel size. The pixel size should be at least  $\frac{1}{4}$  of the expected resolution, if the pixel size is too big, the FRC will not reach zero. A too small pixel size will increase the computation time and introduce artifacts (see troubleshooting section). For a real image a pixel size of 5 is a reasonable starting value, for the included simulation

## 5. When you press OK, you will be asked to select an input file

The input for the program needs to be a tab delimited files with two columns containing respectively the x and y coordinates of the detected fluorophores. You will find example coordinates in the folder termed "ExampleData", all the examples contains coordinates in nm. For all the simulation the suggested super-resolution pixel size is 2 nm. For the Nuclear Pore Images a super-resolution pixel size of 8 nm is recommended.

File name	Description	Technical resolution FWHM [nm]	Average one dimensional drift [nm]	Biological Drift	Labeling Density [part/nm <sup>2</sup> ]
Sim_10nmfwhm.txt	Simulation from High Res Image	10	0	0	5
Sim_20nmmoddrift.txt	Simulation from High Res Image	10	20	0	5
Sim_20nmmoddrift.txt	Simulation from High Res Image	10	50	0	5
Sim_20nmbiodrift.txt	Simulation from High Res Image	10	0	20	5
Sim_underlabeled.txt	Simulation from High Res Image	10	0	0	0.01
mEos2_tagged_NPC.txt	Real data: PALM Imaging of mEos2 labeling the Nucleoporin 107	-	-	-	-

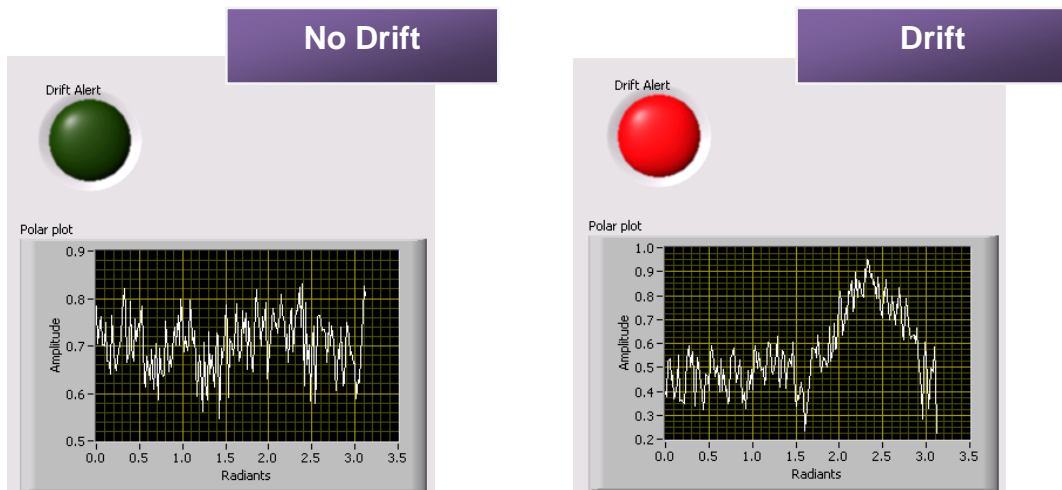
## 6. The program will now run. Please give it a few seconds to complete.

The program automatically splits the data into two random sets as detailed in the Supplementary Information. The two input images will be displayed as well as the FRC curve. The resolution is determined as the crossing of the FRC curve with the 2-sigma curve which predicts the noise level at a determined frequency. The crossing point is the last frequency for which the signal is higher than the noise. A final filtered Image will be generated, in which the data has been filtered with a Gaussian at the determined frequency cutoff.

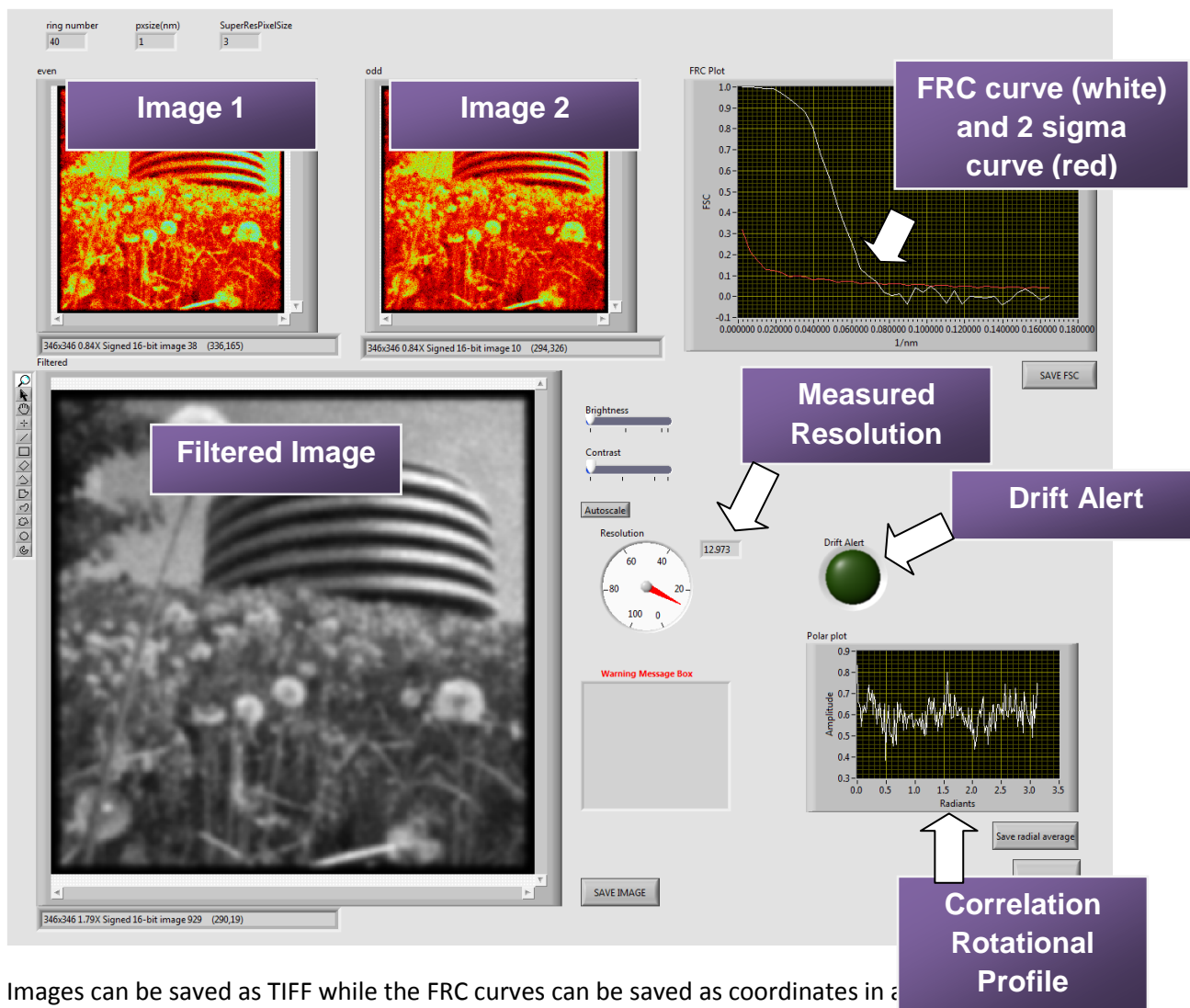
### Drift detection

A linear drift in the image (usually caused by stage drift) will result in a correlation varying with the angle, to detect for such an effect a plot of the correlation as a function of the angle is displayed. If there is no drift the profile will look noisy, in case of drift it will show a dependency (see figure below).

The drift alarm turns red if the software detects a trend in the profile, as detailed in the Supplementary Information and in **Supplementary Fig. 7**.



The screen should look like



Images can be saved as TIFF while the FRC curves can be saved as coordinates in a

### Stop and rerun the program.

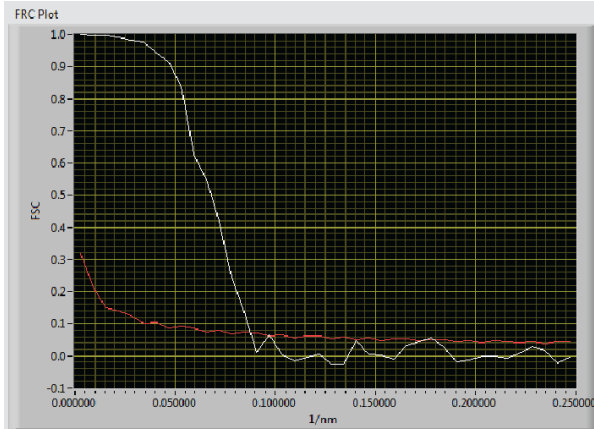
You can STOP the program anytime by either hitting the STOP button, or the red dot in the Labview taskbar. To restart the program, please press the arrow (→) in the Labview taskbar.

In case of detected drift: try to reload the dataset to see if the detection is systematic, the correlation test can always give false positive, which will not be systematically reproducible. A visual inspection of the correlation rotational profile is also helpful to distinguish between false positive and true drift. Drift will always result in distinguishable pattern in the plot profile (see figure above). In case of true one dimensional drift the resolution detected by FRC provides only limited insights, as it is only reflects the average of anisotropic resolution; the data should then be corrected for drift prior to analysis.

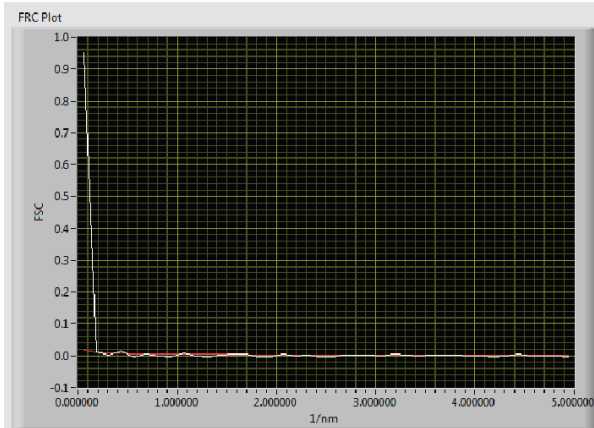
Enjoy.

## Trouble shooting

- 1- Images are not formed: check the formatting of the input data.
- 2- Wrong sampling artifacts (see figure below):

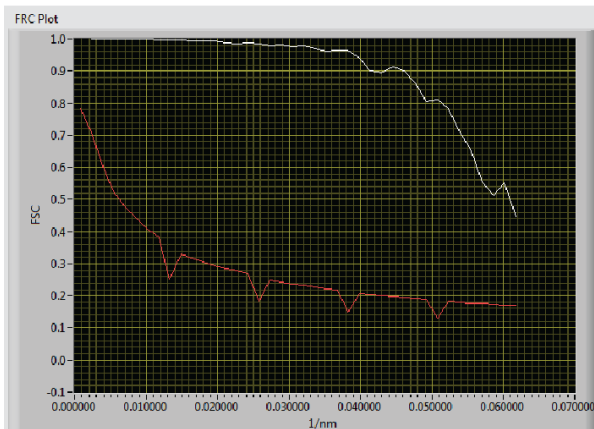


**GOOD  
SAMPLING**



**OVERSAMPLING**

**repeat increasing  
pixel size**



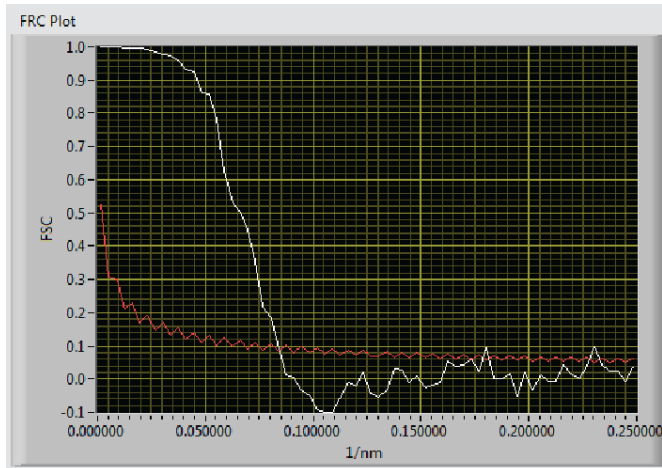
**UNDERSAMPLING**

**repeat reducing  
pixel size**

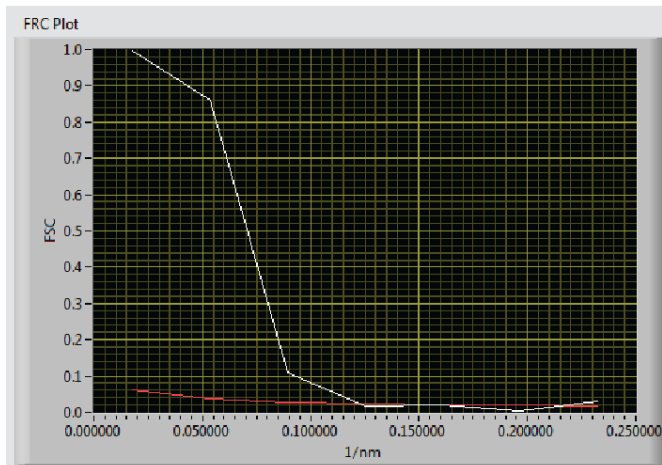
**Warning Message Box**

Crossing between two sigma and FRC not found. Try to reduce the super-resolution pixel size

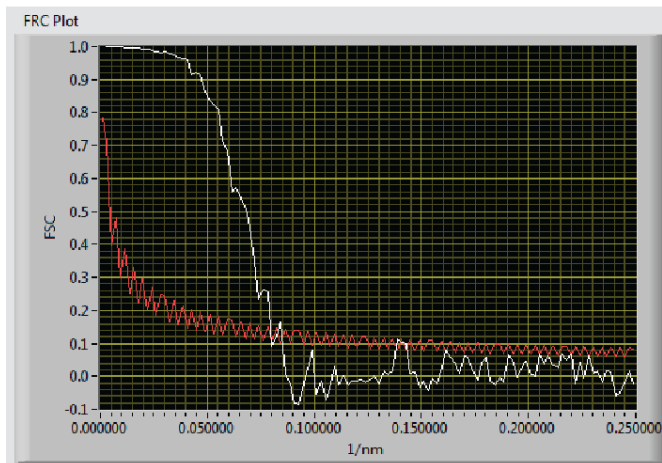
3- Wrong “ring number” artifacts (see figure below)



**GOOD**  
Ring Number  
(smooth FRC  
curve)



**LOW**  
Ring Number  
(undersampled  
FRC curve)



**HIGH**  
Ring Number  
(noisy FRC  
curve)