Drift correction, filtration, visualization, FRC resolution.

We will correct sample drift iteratively by cross-correlation, filter datasets and evaluate results visually in different representations and by FRC resolution. The proposed dataset is an extreme case of drift that is nevertheless a useful illustration of possibilities of the software.

Run SharpViSu

Leave all parameters by default

Load file "…\Data\Tub642dr\tub drift 642.ascii" to the red and the green channels. We load the same dataset to both channels in order to easily compare corrected results with the initial data.

Put Pixel size = 20 nm, Steps = 11, method: histogram in the Drift panel for the both Red and Green datasets. Check "Auto save in current folder: Drift graphs".

Press "Calculate".

After the calculation finishes, you will see the initially estimated drift (the same for both channels as we have the same data).

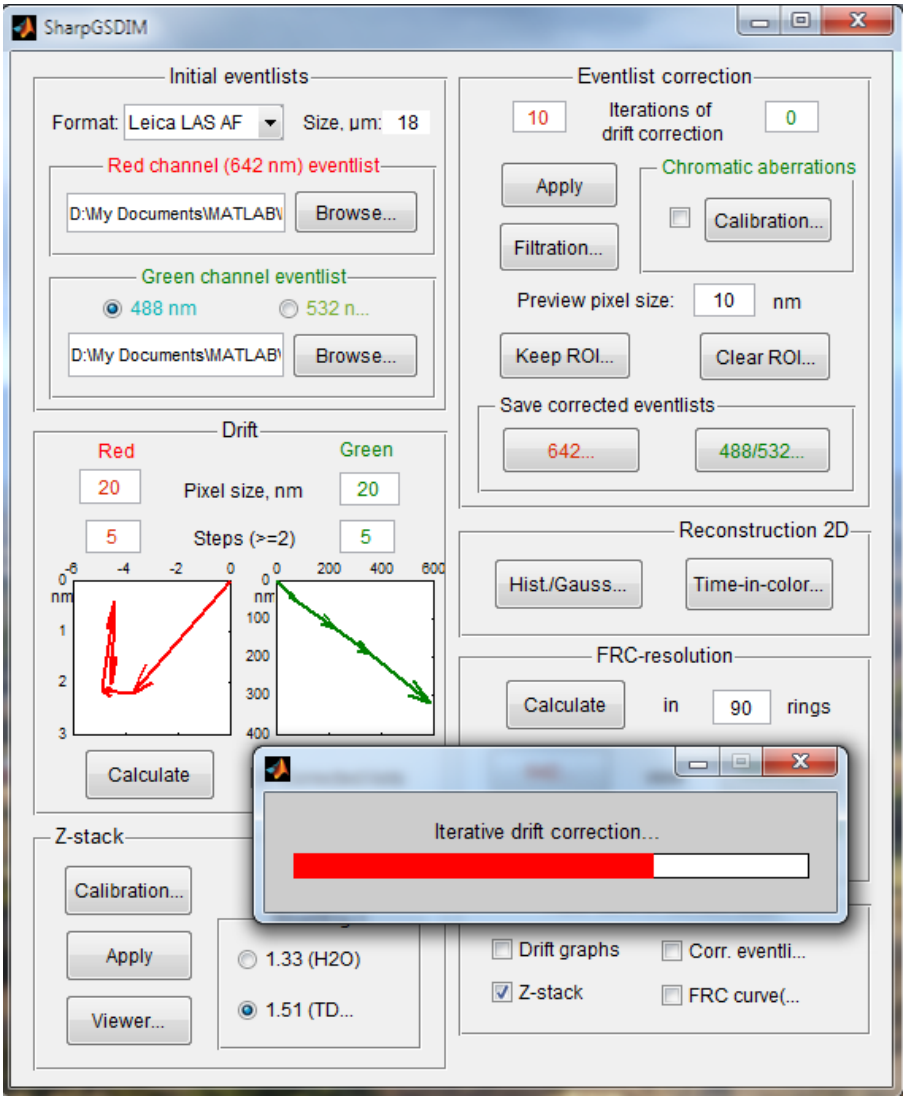
Go to the panel "Eventlist correction". Put Iterations of drift correction: 4 (red), 0 (green). Uncheck "Chromatic aberrations" (no need to correct them as we have the same data in both channels). Press "Apply". You will see a waitbar appearing with a text "Iterative drift correction…" The calculated drift in the box for the red channel will reduce with iterating the drift correction (note that the scale of drift plots changes). The picture of drift in the green channel will remain the same as we do not perform corrections there. Wait until the correction finishes. The shown drift plots will be saved in the folder "…\Data\Tub642dr\".

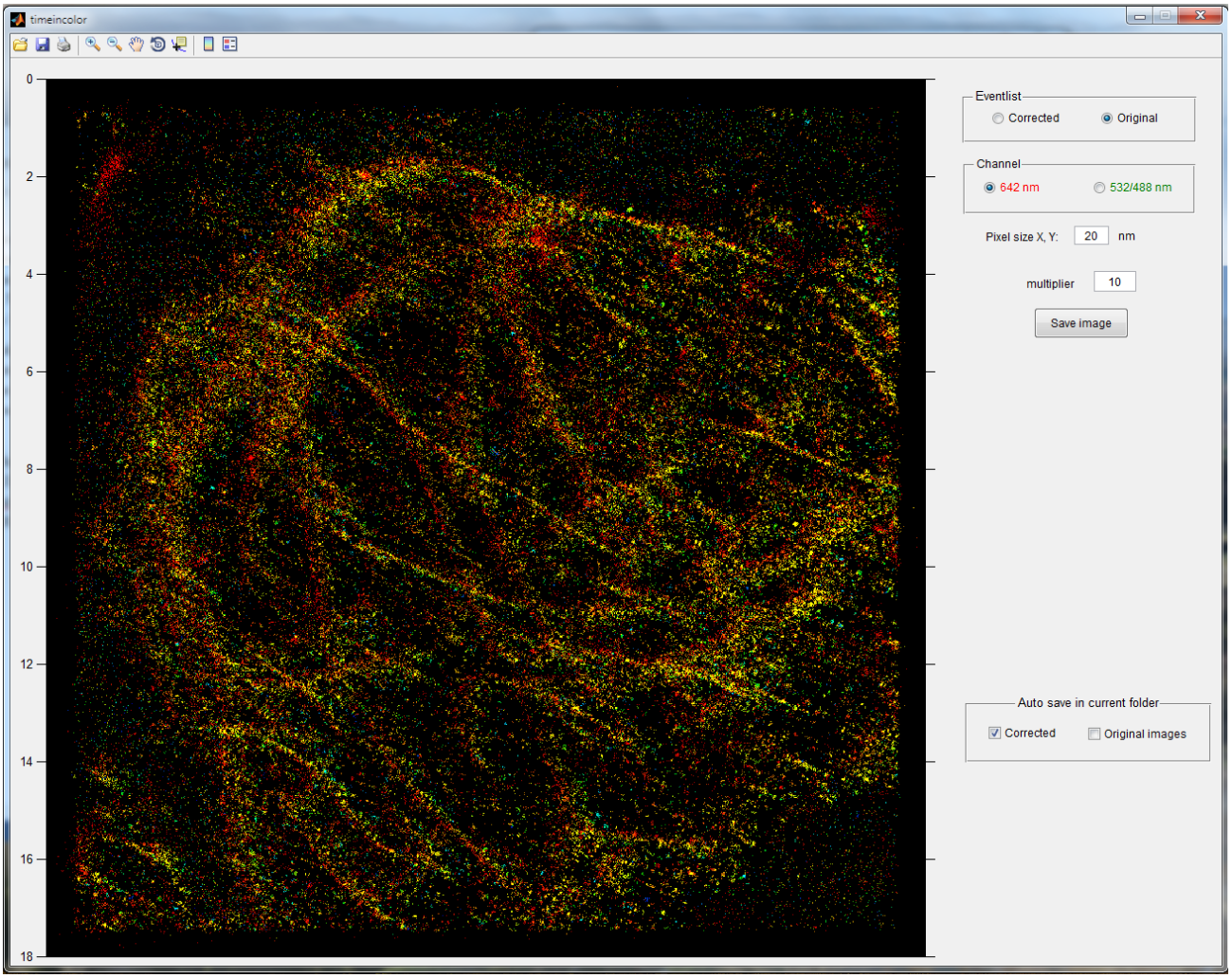
Press "XY…" in the panel "Reconstruction 2D". The viewer window will appear. By choosing red or green channel you will see the corrected or the original data, correspondently. You can also change other parameters here. Note that the calculation of Gaussian and Voronoi representations can be slow. If an image is too dark on the screen, increase the multiplier. Leave checked "Auto save in current folder: "Corrected"" and close the window. The pictures – overlay of green and red channels will be saved in the folder "…\Data\Tub642dr\", for each representation that you have viewed.

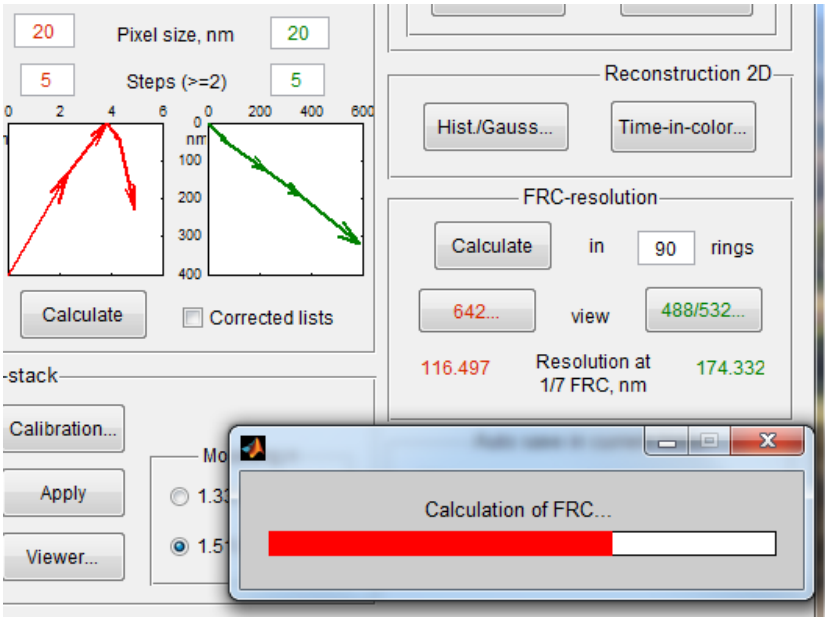
Press "XYt…" in the panel "Reconstruction 2D". After a delay for calculation, a new window will appear. You will see the data with the time showed in HSV colormap. Choose "Eventlist: Original", "Channel: 642 nm" (or "Eventlist: Original", "Channel: 532/488 nm", or "Eventlist: Corrected", "Channel: 532/488 nm") to see the drift visualized by color. You can increase the pixel size to 20 nm to increase contrast and improve speed of calculations. You can save the images and close the window.

Press "Filtration" in the panel "Reconstruction 2D". In the opened window, leave all parameter by default and press "Apply" in the top "Merge consecutive events" panel. After the calculation finishes, you will see different number of events and different values on the graphs and histograms. Close the window.

Go to the panel "FRC-resolution", choose method "histogram" and press "Calculate". You will see a waitbar appearing twice (for each of the channels). After the calculation, you will see the estimated resolution: 105 nm for the corrected dataset (red) and 177 nm for the original one (green). If "Auto save in current folder: FRC curve(s)" is checked, the curves of FRC will be saved in the current folder. You can also see the FRC curves by pressing "View: 642… or 488/532…"


SharpGSDIM
Initial eventlists
Format: Leica LAS AF
Size, µm: 18
Red channel (642 nm) eventlist
D:\My Documents\MATLAB\
Browse...
Green channel eventlist
488 nm
532 n...
D:\My Documents\MATLAB\
Browse...
Drift
Red
Green
20
Pixel size, nm
20
5
Steps (>=2)
5
Calculate
Z-stack
Calibration...
Apply
Viewer...
1.33 (H2O)
1.51 (TD...
Eventlist correction
10
Iterations of drift correction
0
Apply
Chromatic aberrations
Calibration...
Filtration...
Preview pixel size:
10
nm
Keep ROI...
Clear ROI...
Save corrected eventlists
642...
488/532...
Reconstruction 2D
Hist./Gauss...
Time-in-color...
FRC-resolution
Calculate
in
90
rings
Iterative drift correction...
Drift graphs
Corr. eventli...
Z-stack
FRC curve(...


timeincolor
Eventlist
Corrected
Original
Channel
642 nm
532/488 nm
Pixel size X, Y:
20
nm
multiplier
10
Save image
Auto save in current folder
Corrected
Original images

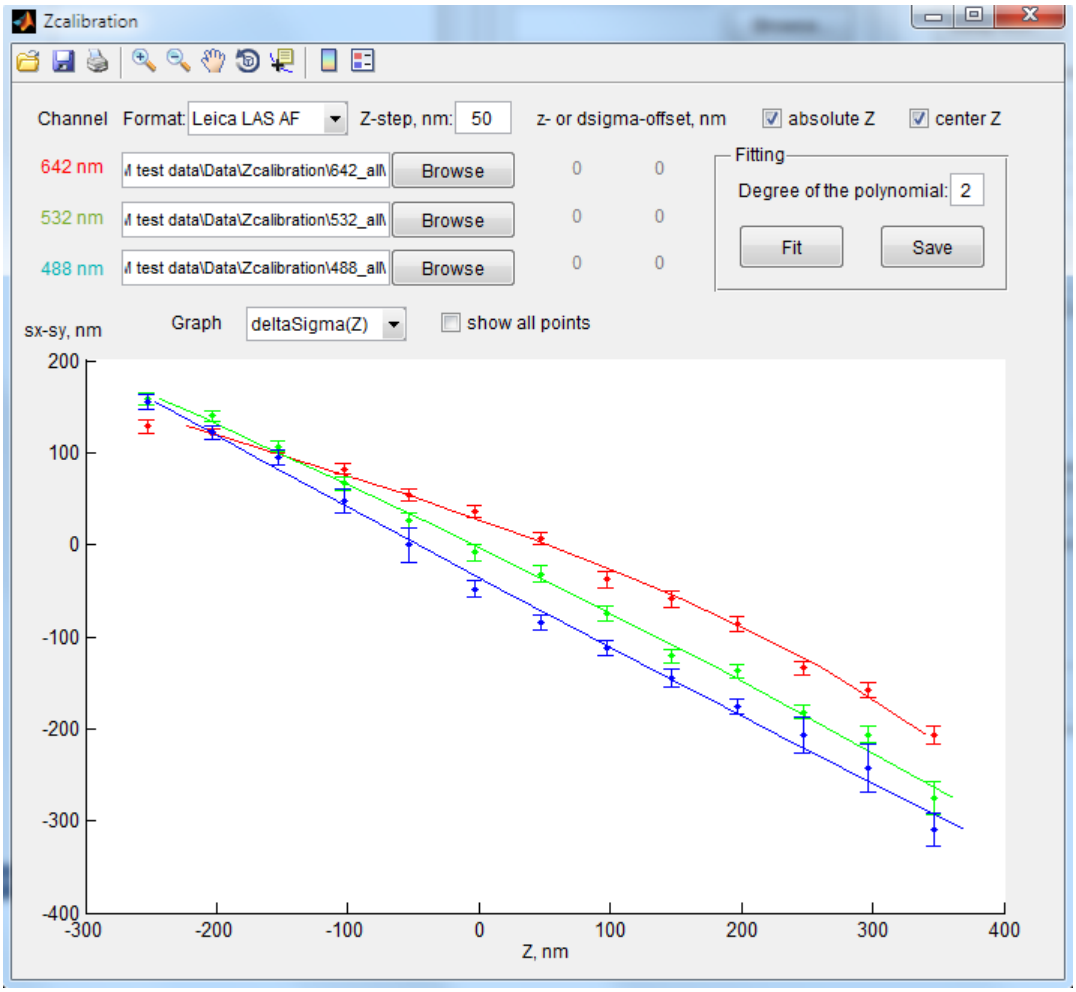

20
Pixel size, nm
20
5
Steps (>=2)
5
Calculate
Corrected lists
Reconstruction 2D
Hist./Gauss...
Time-in-color...
FRC-resolution
Calculate
in
90
rings
642...
view
488/532...
116.497
Resolution at
1/7 FRC, nm
174.332
-stack
Calibration...
Apply
Viewer...
Calculation of FRC...

Calibration of astigmatism

Run SharpViSu

Press “Calibration…” in the panel “Z-stack”. Leave all parametes by default. Press “Browse” for 642 nm and choose the content of the directory “…\Data\Zcalibration\642_all” (choose all the .ascii files in the window “Please select eventlists of the Z-stack” and press “Open”). Repeat for the 532 nm and the 488 nm channel (use content of corresponding folders).

Check “absolute Z” and “center Z”. Put a desired degree of polynomial or leave 2 by default. Press “Fit”. You will see the calibration curve appeared. Check/uncheck “show all points” and choose “Graph: deltaSigma(Z)/Z(deltaSigma)” to appreciate different representations of data. Calibration files from these datasets are installed by default, no need to save this fit if you did not perform any calibration after installation. If you did, copy your calibration files (‘Z488.dat’, ‘Z532.dat’, ‘Z642.dat’) from the installation folder of the software (‘%Program Files%\SharpViSu\application’ by default) to another location and press “Fitting: Save”. The calibration files in the installation folder will be overwritten with new ones corresponding to the performed fit.



Calibration of chromatic aberrations

Run SharpViSu

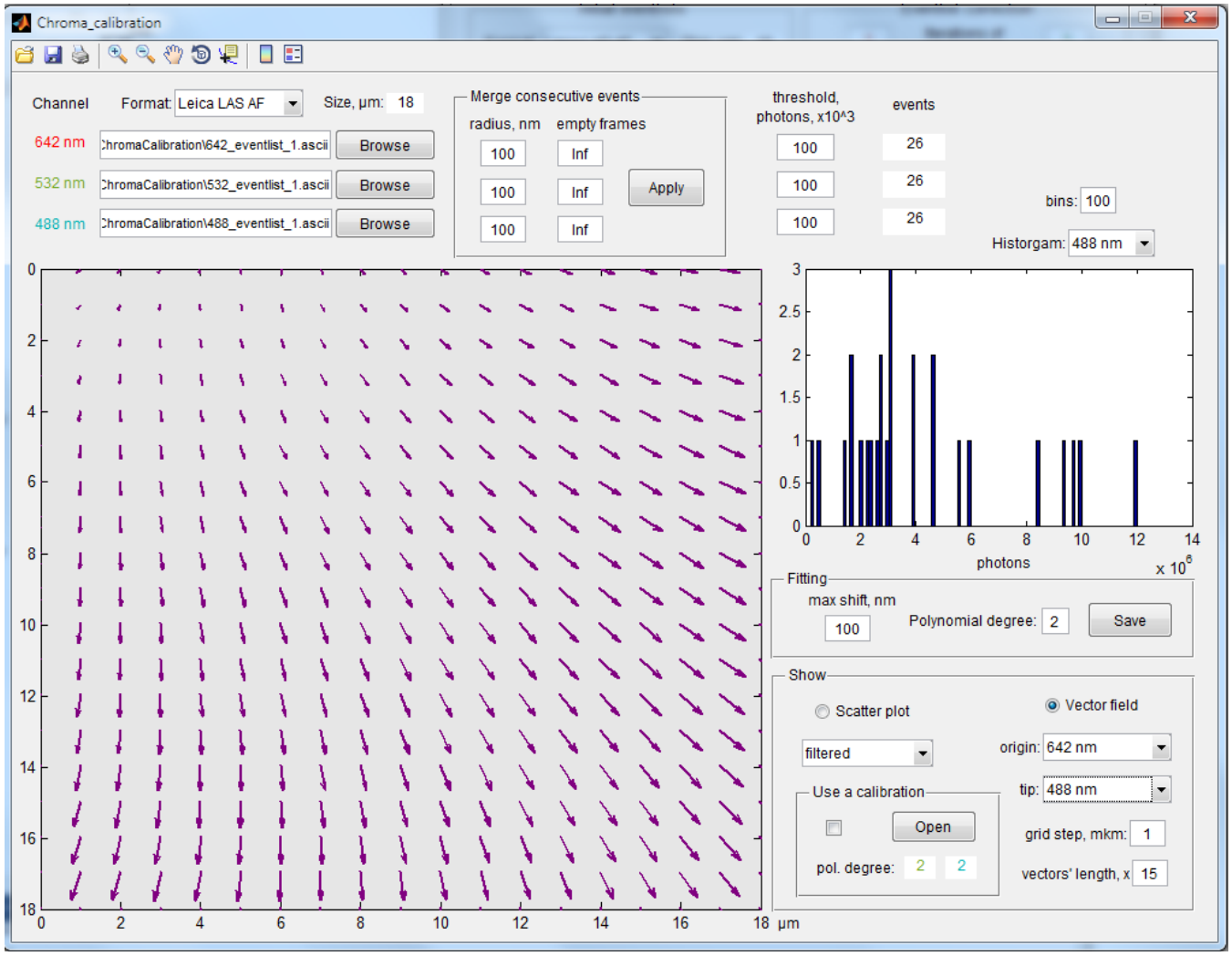
Press “Calibration…” in subpanel “Chromatic aberrations” of panel “Eventlist correction”

The window “Chroma_calibration” will open. You should see there in arrows the default calibration of chromatic aberration located in the installation folder (or your calibration, if you have already calibrated your system). Leave all parameters by default. Press “Browse” and load calibration eventlists for each channel: “…\Data\ChromaCalibration\642_eventlist_1.ascii” for the 642 nm channel, etc. Histogram will appear, where you can see the distribution of photon counts for each of the datasets.

Press “Apply” in panel “Merge consecutive events”. Put “100” in each field “threshold, photons, x10^3”. You shoud have 26 events left for each channel. The data are fitted automatically with parameters from panel “Fitting”.

Go the panel “Show”. Uncheck “Use a calibration”. Now you visualize the data that we have just fitted.

The calibration from this data is installed with the software by default. If you calibrated the software for you system, copy the calibration files (‘488.mat’, ‘532.mat’) from the installation folder of the software (‘%Program Files%\SharpViSu\application’ by default) and press “Save”. The calibration files in the installation folder will be overwritten with new ones corresponding to the performed fit.



Correction of drift and chromatic aberrations, reconstruction and visualization of 3D data

Here we will use the calibration files created in the two previous steps. Make sure you have the default or created at the previous steps calibration files in the installation folder (files '488.mat', '532.mat', 'Z488.dat', 'Z532.dat', 'Z642.dat' in the folder '%Program Files%\SharpViSu\application' by default).

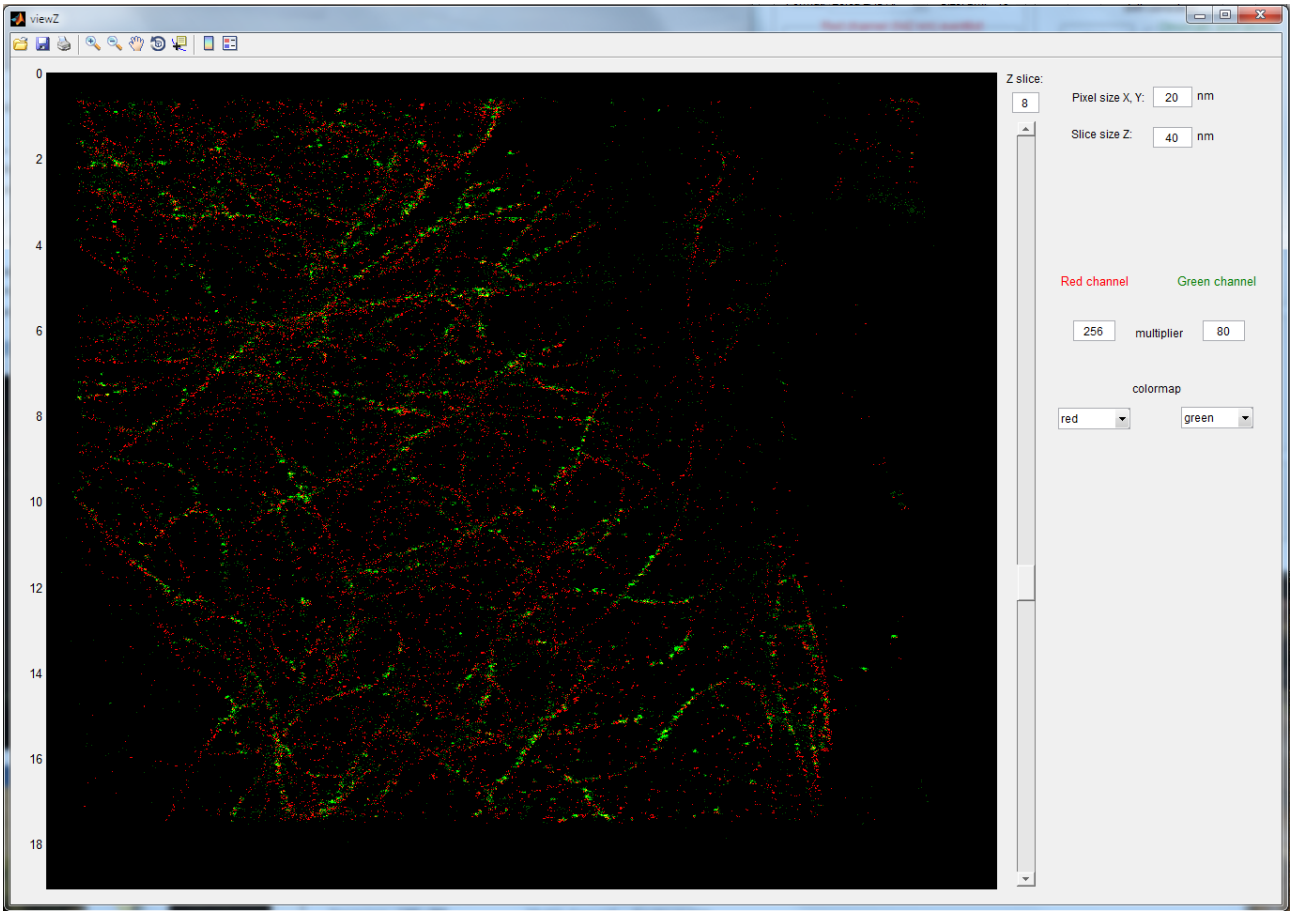
Run SharpViSu

Load file "…\Data\Tub3D\642_el_1.ascii" as a 642-nm eventlist and file "…\Data\Tub3D\488_el_2.ascii" as a 488-nm eventlist.

Put in the panel Drift: pixel size = 20 nm, steps = 6 (same for both colors). Press "Calculate". Put in the panel "Eventlist correction": Iterations of drift correction = 4 for both colors. Leave checked "Chromatic aberrations". Press "Apply". You will see an iterative drift correction similar to the first demonstration.

After the calculation finishes, go to panel "Z-stack". Choose "Mounting n = 1.33 (H2O)". Press "Apply". Press "Viewer…". The Z slice viewer will open (possible delay for calculations).

In the viewer you can already see the data with default parameters but we recommend to change parameters to the following: Slice size Z = 40 nm, Red channel multiplier = 255, Green channel multiplier = 80, the other parameters by default. You may have a delay upon changing this parameters. Then you can use slicer to go through the Z-stack.



Close the window. As the "Auto save in current folder: Z-stack" was checked, each of the slices will be saved in a separate .tif file in an automatically created directory "…\Data\Tub3D\stack".