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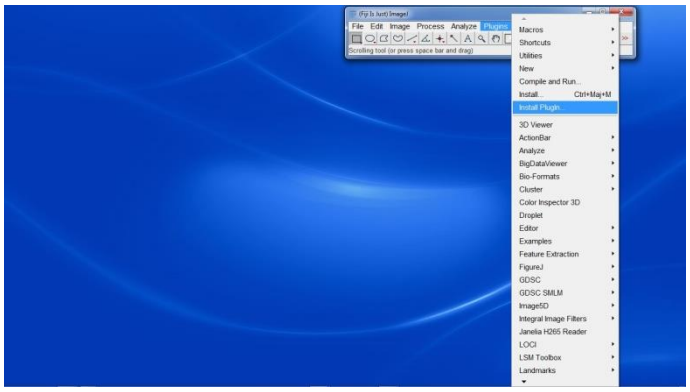
How to use sPAINT_Render plugin:

Install & run:

Download the Calib_sPAINT.jar, Fit_sPAINT.jar and Render_sPAINT.jar files and drag them one-by-one in Fiji window.



Alternative: Copy the *"*.jar"* file in the plugins folder of ImageJ (or Fiji) (on my own computer this folder is located at *E:\fiji-win64\Fiji.app\plugins*)

Launch Fiji - in the *Plugins* menu choose *Install Plugin...*

What does the plugin do?

Calib_sPAINT.jar use a stack of images of calibration TetraspeckBeads (*.tiff) and its corresponding localisation file (PeakFit output) to compute the calibration factors and to generate a calibration file.

Fit_sPAINT.jar is extracting the spectral information

sPAINT_Render use an imageJ Results table as an input and generates two corresponding super-resolution images (a localisation density image in greyscale and a spectral image in RGB).

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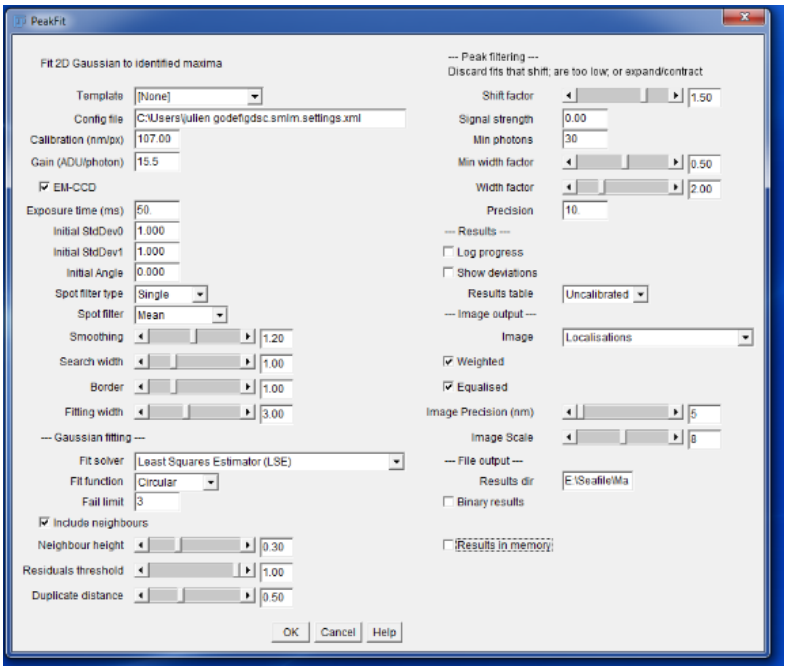
How to use it?

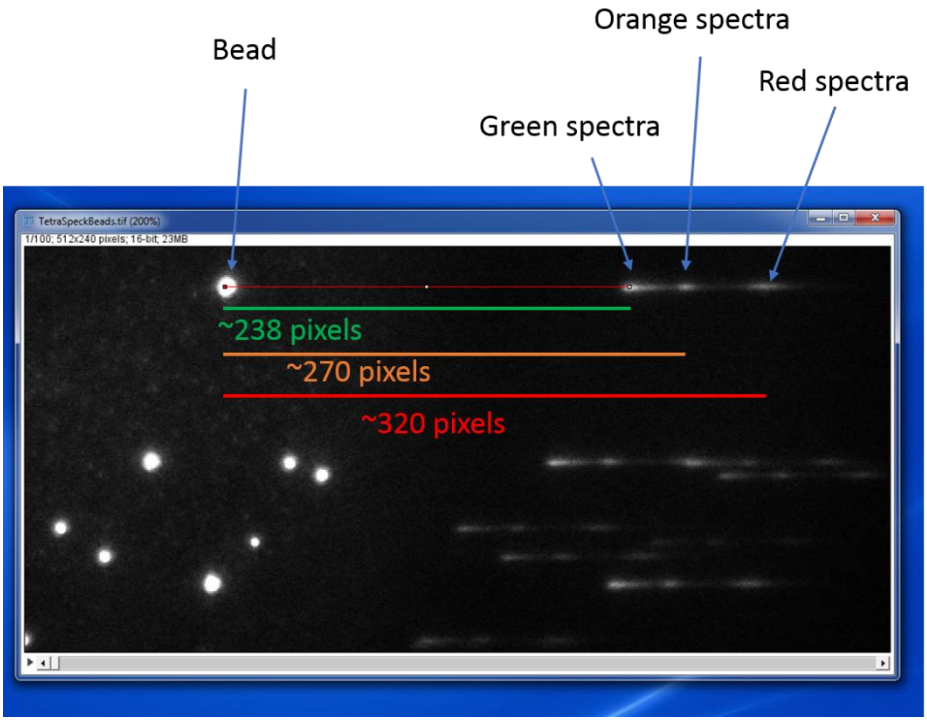
1. Calibration

Open the TetraspeckBeads.tiff file and run PeakFit to extract the localisations (save the output localisation file).

Pixel Size .107µm
EMGain = 100 (Gain (ADU/photon) = 15.5)
ExposureTime = 50ms



On the image stack: estimate the distances (in pixels) between a bead and its corresponding spectras



You can close the stack…

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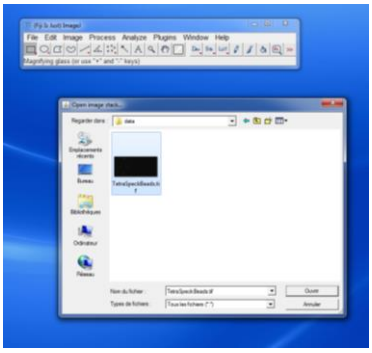
Launch calibration_sPAINT plugin:

- Open the image Stack



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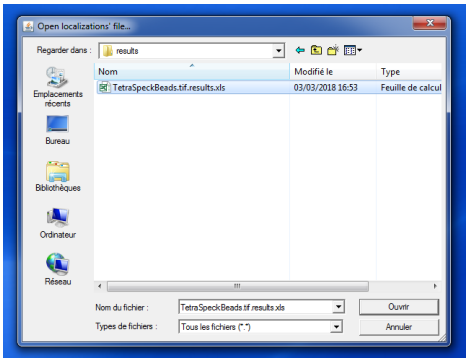
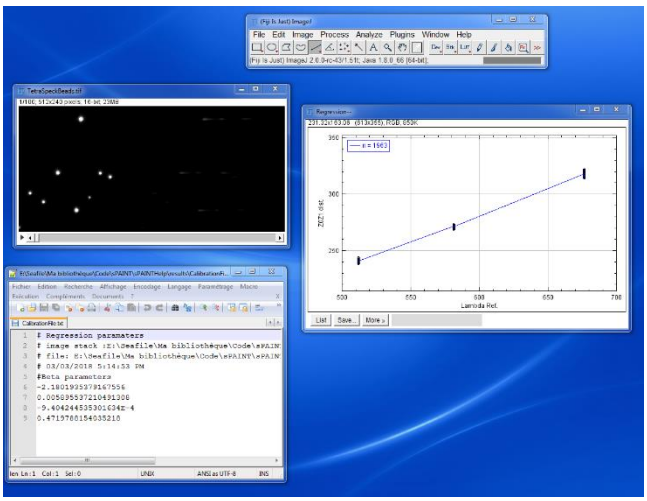
- Fill the user GUI form with the appropriate values

- Then open the localisation file

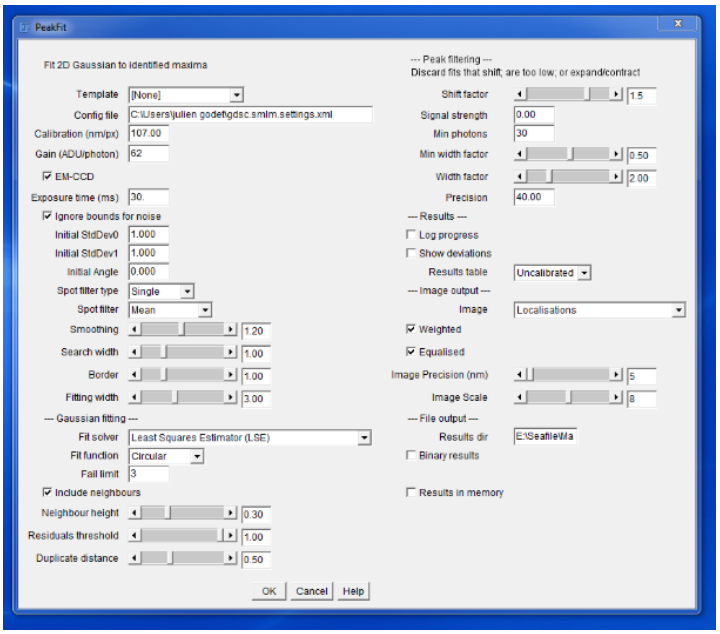


The calibration file is then being processed.

Once finished, a regression graph is shown to check for linearity and a CalibrationFile.txt has been created in the folder containing the localisation file.



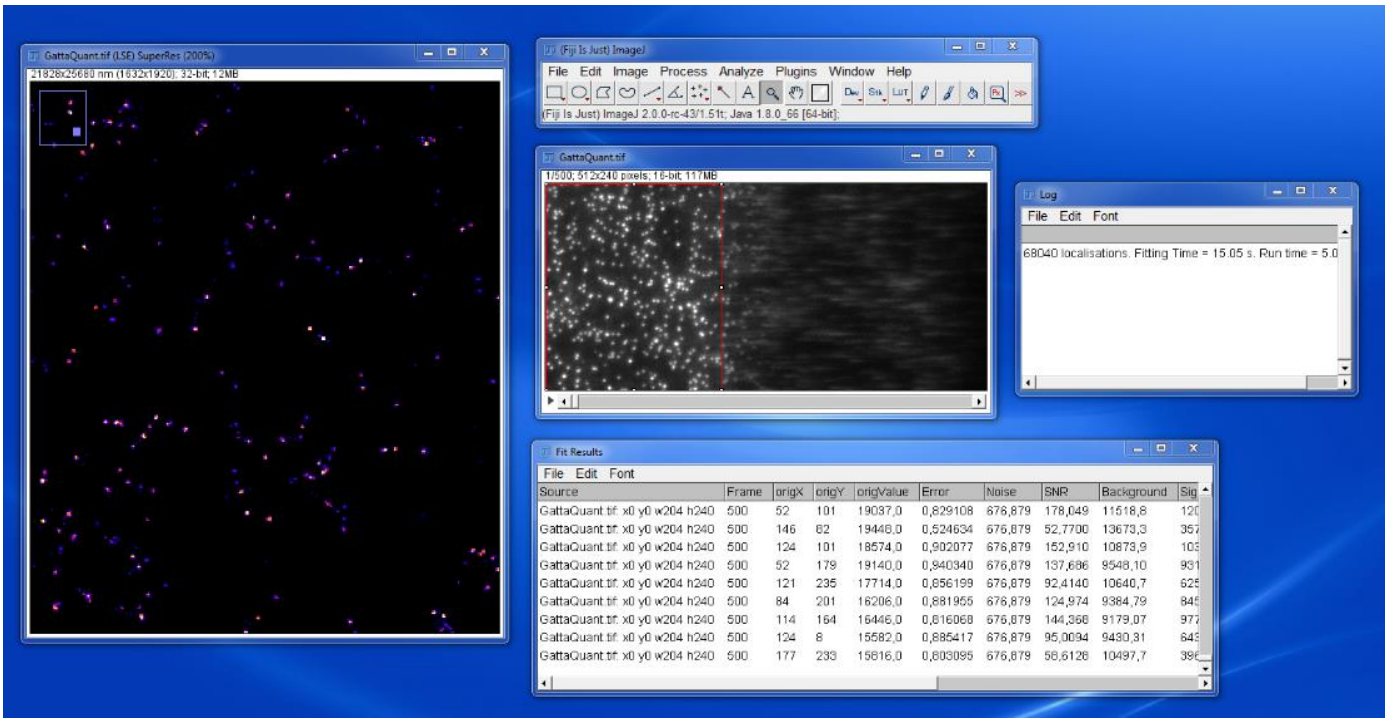
2. Fitting the Data



1. Fit the localisations

Open the TetraspeckBeads.tiff file and run PeakFit to extract the localisations (save the output localisation file). You can select the spatial domain of the stack (left part) to save fitting time.

Pixel Size .107µm
EMGain = 400 (Gain (ADU/photon) = 62)
ExposureTime = 30ms



2. Fit spectral information

Launch Fit_sPAINT plugin

Open the image Stack,

choose to Fit spectral data or Fit & render data

adjust the approximative Z0Z1 (in pixels) (here 265)

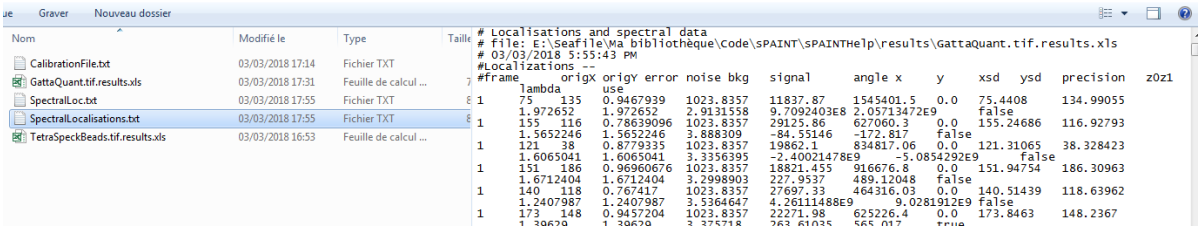
provide the Beta parameters value (or check Load Caclibration file)

If Load Caclibration file is checked, then open the file

Open the localisation file (GattaQuant.tif.results.xls)

Be patient … the progress bar is telling you how patient you have to be…

Once finished, a SpectralLocalisations.txt file is generated

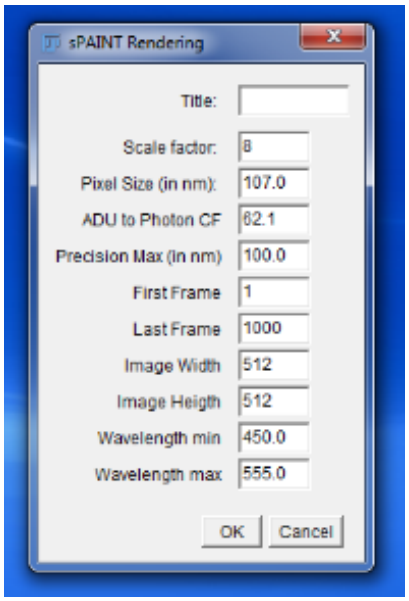
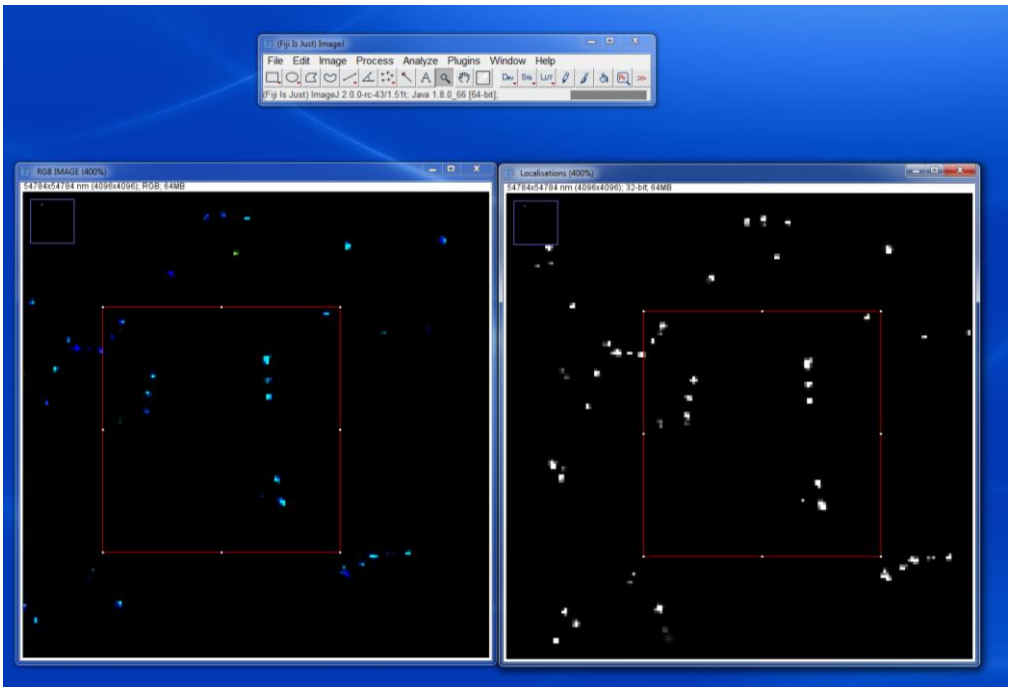


3. Rendering the data

Launch the render_sPAINT plugin

Open the localisation file (SpectralLocalisations.txt)

Choose and define the appropriate parametres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