

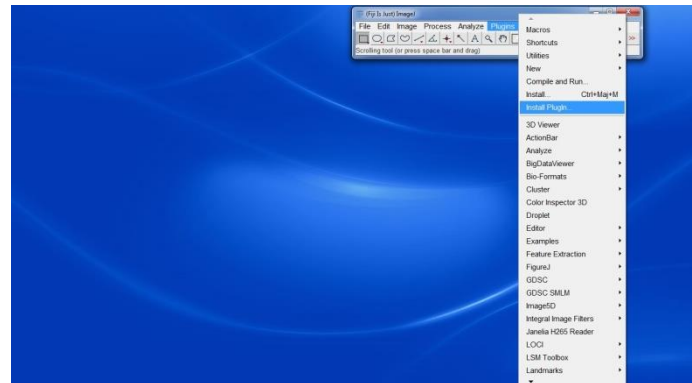
## How to use sPAINT\_Render plugin:

### 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

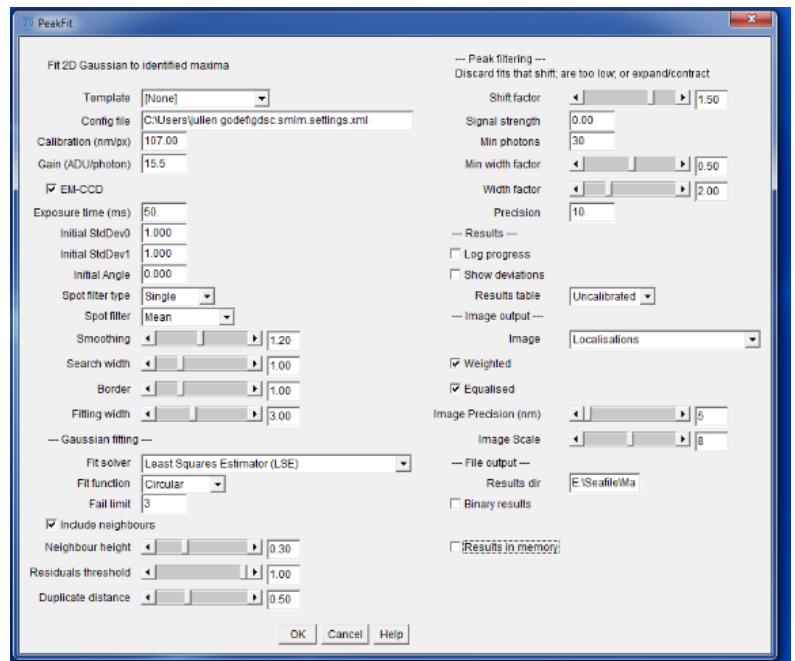
sPAINT\_Render use an imageJ Results table as an input and generates two corresponding super-resolution images (a localisation density image in greyscale and a spectral image in RGB).

## How to use it?

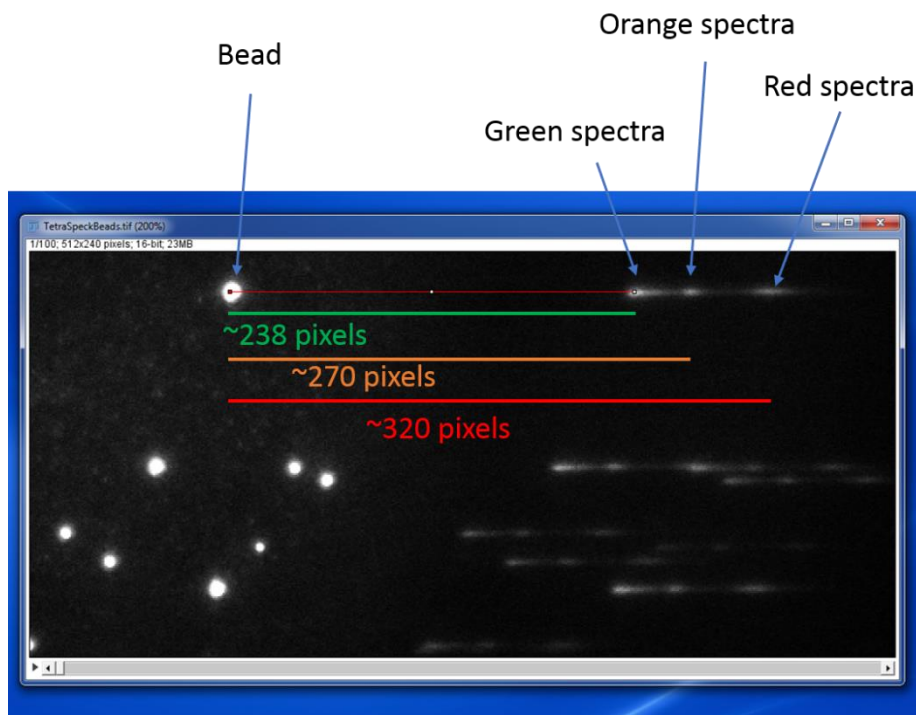
### 1. Calibration

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

Pixel Size .107 $\mu$ m  
EMGain = 100 (Gain (ADU/photon) = 15.5)  
ExposureTime = 50ms



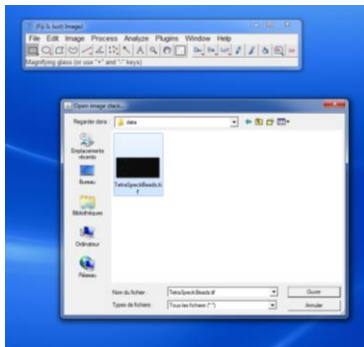
On the image stack: estimate the distances (in pixels) between a bead and its corresponding spectras



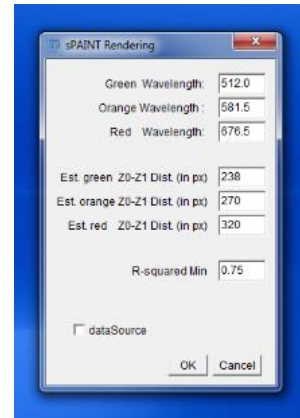
You can close the stack...

Launch calibration\_sPAINT plugin:

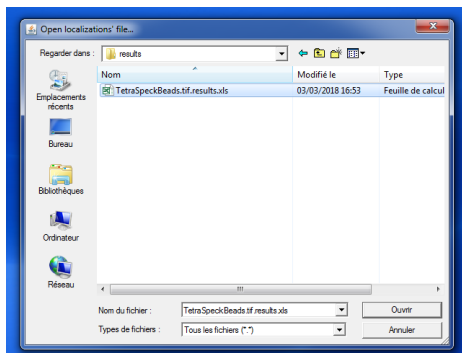
- Open the image Stack



- Fill the user GUI form with the appropriate values

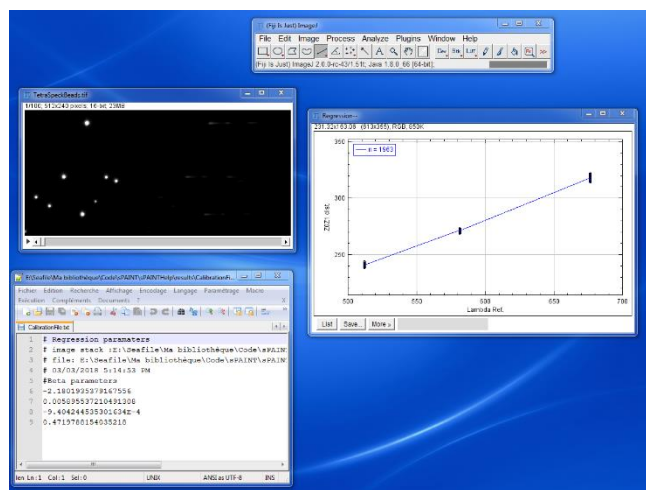


- Then open the localisation file



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.

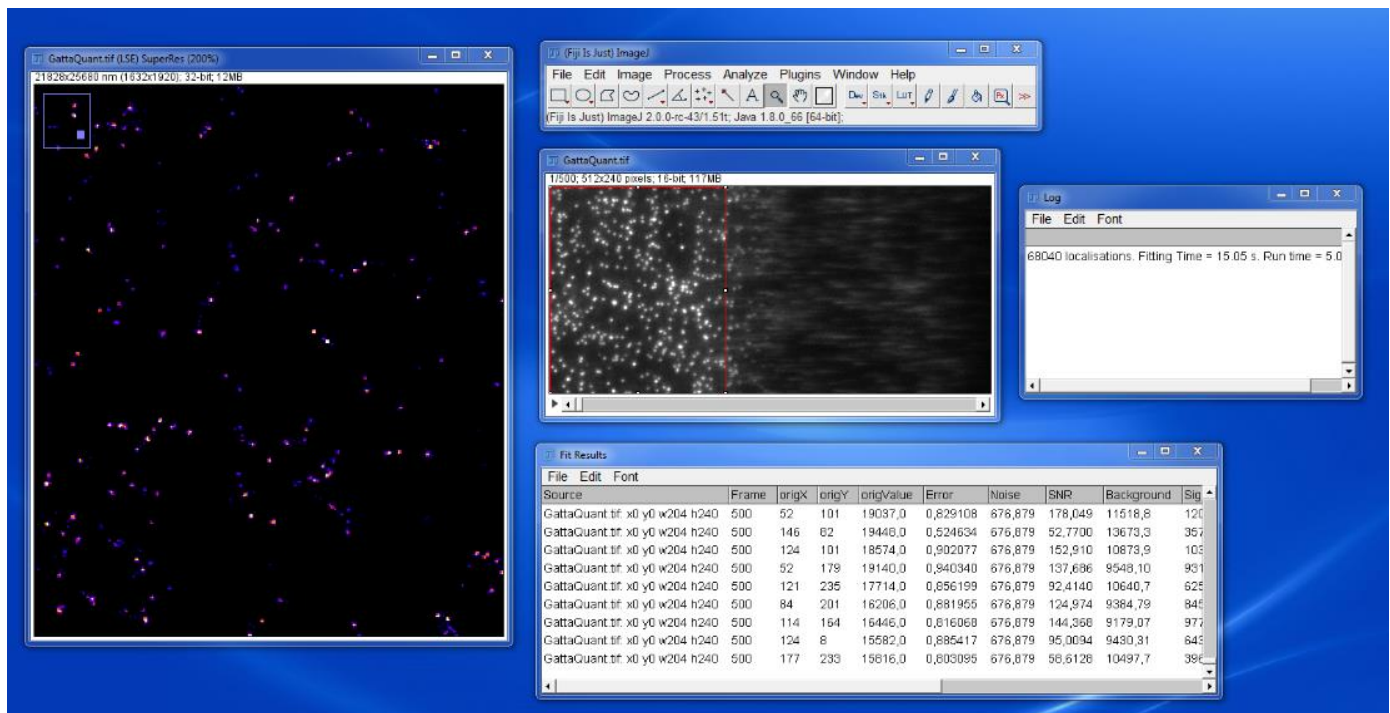
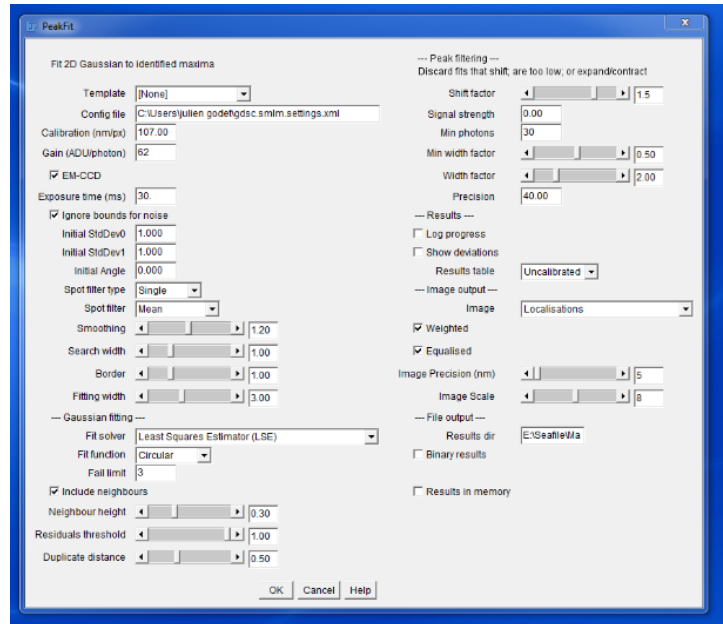


## 2. Fitting the Data

### 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 .107 $\mu$ m  
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)

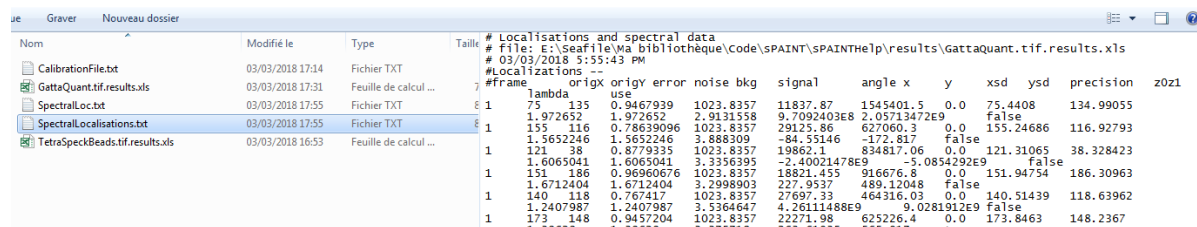
provide the Beta parameters value (or check Load Caclibration file)

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



Nom	Modifié le	Type	Taille
CalibrationFile.txt	03/03/2018 17:14	Fichier TXT	
GattaQuant.tif.results.xls	03/03/2018 17:31	Feuille de calcul ...	
SpectralLoc.txt	03/03/2018 17:55	Fichier TXT	
<b>SpectralLocalisations.txt</b>	<b>03/03/2018 17:55</b>	<b>Fichier TXT</b>	
TetraSpeckBeads.tif.results.xls	03/03/2018 16:53	Feuille de calcul ...	

#	frame	lambda	use	origx	origy	error	noise	bkg	signal	angle	x	y	xsd	ysd	precision	z0z1
1	75	135	0.9467939	1023.8357	11837.87	1545401.5	0.0	75.4408	134.99055							
1	155	116	0.972652	2.9131558	9.7092403E8	2.05713472E9	false									
1	155	116	0.78639096	1023.8357	29125.86	627060.3	0.0	155.24686	116.92793							
1	156	124	1.5652246	3.888309	-84.55146	-172.817	false									
1	121	38	0.8779335	1023.8357	19862.1	834817.06	0.0	121.31065	38.328423							
1	1	6065041	1.6065041	3.356395	-2.40021478E9	-5.0854292E9	false									
1	151	186	0.96960676	1023.8357	18821.455	916676.8	0.0	151.94754	186.30963							
1	167	12404	1.6712404	3.2998903	227.9537	489.12048	false									
1	140	118	0.767417	1023.8357	27697.33	464316.03	0.0	140.51439	118.63962							
1	1	2407987	1.2407987	3.5364647	4.26111488E9	9.0281912E9	false									
1	173	148	0.9457204	1023.8357	22271.98	625226.4	0.0	173.8463	148.2367							
1	1	30670	1.30670	3.375718	763.61035	665.017	true									

### 3. Rendering the data

Launch the render\_sPAINT plugin

Open the localisation file (SpectralLocalisations.txt)

Choose and define the appropriate parametres

