

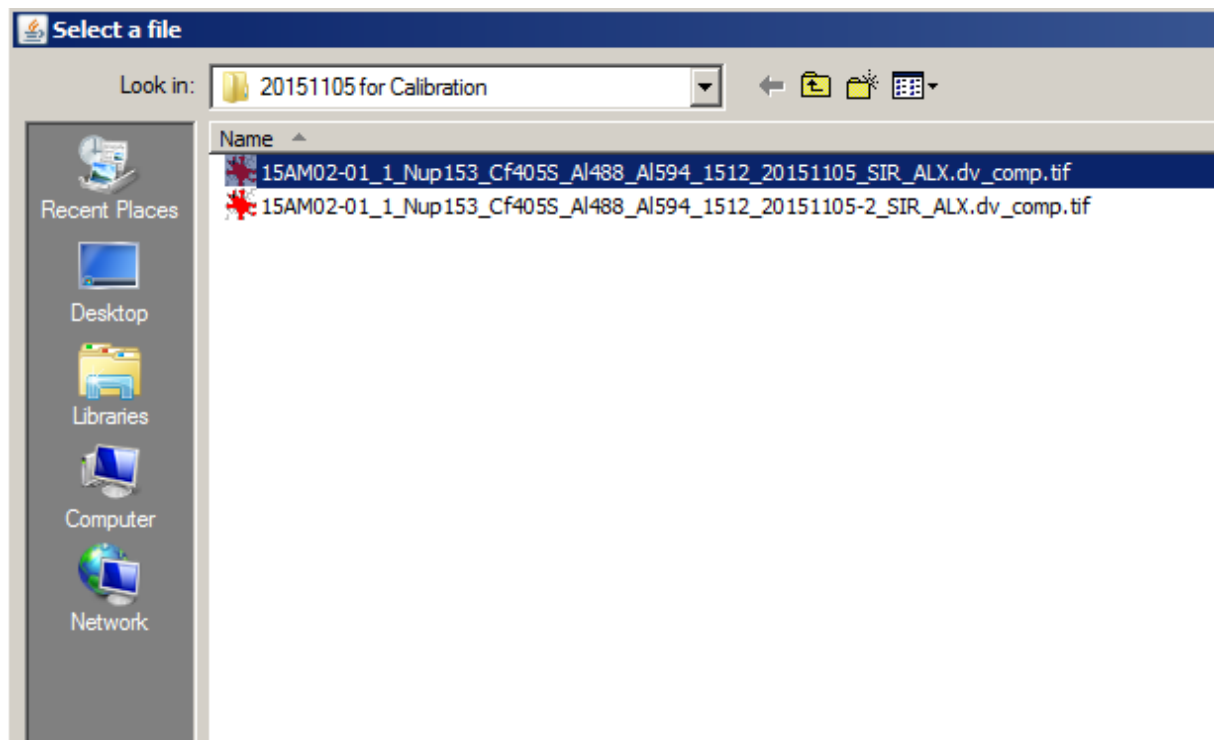
## OMX\_Aberration\_Correction\_Calibration and OMX\_Aberration\_Correction Macro

The OMX\_Aberration\_Correction\_Calibration and OMX\_Aberration\_Correction macros were written to automatically remove both natural and technical aberration errors in OMX microscopy images. They are primarily based on the already existing Multiview Reconstruction Plugin.

### OMX\_Aberration\_Correction\_Calibration

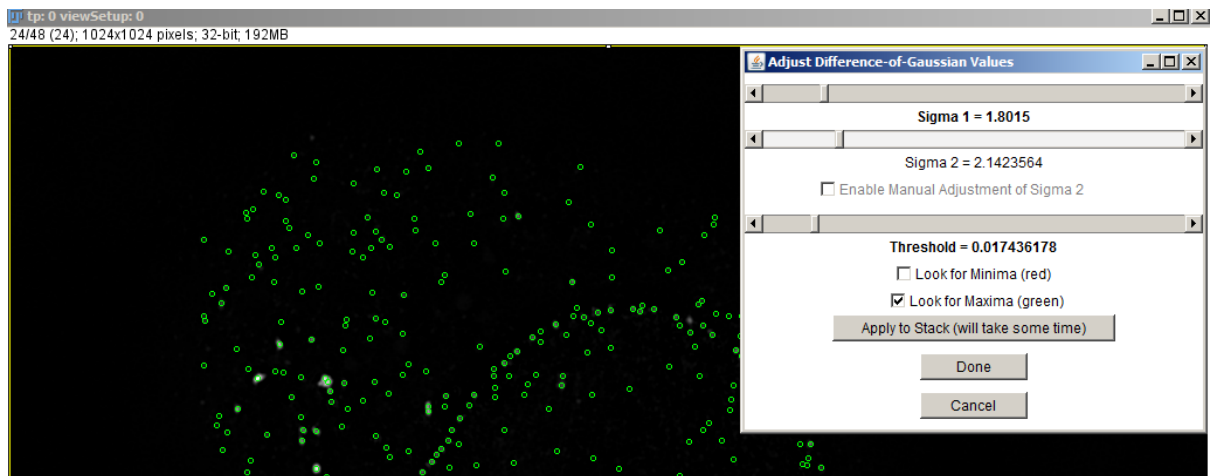
This macro enables the generation of an XML file in which all relevant values are stored to perform the alignment (which is also needed for the second macro).

By starting this macro, you will first be asked to select a file. In this case you should always choose a 3-channel image, in which the order of channel colors is RGB. Signals from all three channels should arise from the same locations within the image, otherwise no alignment parameters can be calculated.



During the process of generating the XML file and an aligned version of your image, files will be generated and later deleted in the folder in which your chosen file is stored. **It is important not to delete or open any files generated during this process.**

After several seconds, channel 1 will open together with a Adjust Difference-of-Gaussian Values window.



Here you can adjust Sigma1 and the Threshold. The green circles show the interest points. Sigma 1 marks the size of the interest points that will be registered. If decreased, smaller structures will be recognized as interest points. The threshold determines how high the intensity of each interest point has to be to get registered. The rectangular ROI can be expanded to the full size of the image, to show all interest points that will be registered.

This process has to be repeated for channel 2 and channel 3.

It might be necessary to adjust brightness and contrast during this step in order to have a clear view of the signals.

After successful generation of the newly aligned image, a colocalization analysis is performed. A results table will open during this process, in which values corresponding to the alignment success will be stored. It is important not to close this window during the operation. It will close automatically after the macro is finished.

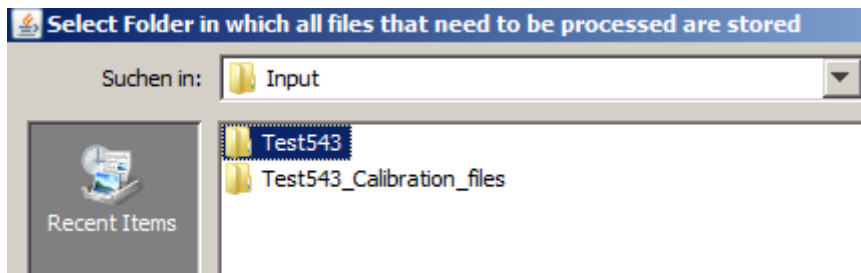
15AM02-01_1_Nup153_Cf405S_AI488_AI594_1512_20151105_SIR_ALX.dv_comp_interestpoints	13.11.2015 14:25	File folder	
15AM02-01_1_Nup153_Cf405S_AI488_AI594_1512_20151105_SIR_ALX.dv_comp.tif	06.11.2015 15:12	IrfanView TIF File	294,938 KB
15AM02-01_1_Nup153_Cf405S_AI488_AI594_1512_20151105_SIR_ALX.dv_comp.tif_ALIGNED.tif	13.11.2015 14:26	IrfanView TIF File	294,938 KB
15AM02-01_1_Nup153_Cf405S_AI488_AI594_1512_20151105_SIR_ALX.dv_comp_CALIBRATION.xml	13.11.2015 14:25	XML Document	7 KB
15AM02-01_1_Nup153_Cf405S_AI488_AI594_1512_20151105_SIR_ALX.dv_comp_CALIBRATION.xml~1	13.11.2015 14:25	XML~1 File	6 KB
15AM02-01_1_Nup153_Cf405S_AI488_AI594_1512_20151105_SIR_ALX.dv_comp_CALIBRATION.xml~2	13.11.2015 14:25	XML~2 File	5 KB
15AM02-01_1_Nup153_Cf405S_AI488_AI594_1512_20151105_SIR_ALX.dv_comp_CALIBRATION.xml~3	13.11.2015 14:25	XML~3 File	4 KB
15AM02-01_1_Nup153_Cf405S_AI488_AI594_1512_20151105_SIR_ALX.dv_comp_CALIBRATION_COLOC_results.txt	13.11.2015 14:26	Text Document	1 KB
15AM02-01_1_Nup153_Cf405S_AI488_AI594_1512_20151105-2_SIR_ALX.dv_comp.tif	06.11.2015 15:11	IrfanView TIF File	73,754 KB

Once the macro is finished, you will find the following files in your folder. You will find one new folder, which has the same name as the file selected for processing with the addition of "\_interestpoints". In this folder all the numerical values for interest point registration of the selected files are stored. Directly under the original file is the new file with the ending "\_ALIGNED.tif". You will also find 4 different XML files of which only the top XML file without any extensions beyond ".xml" is of relevance for the OMX\_Aberration\_Correction macro. The last additional file is a text file in which the colocalization results are stored.

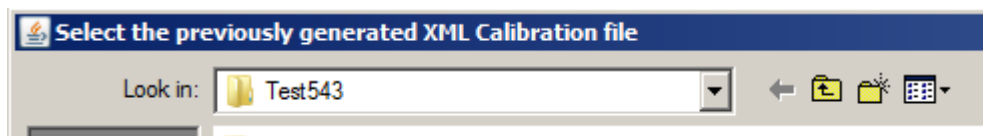
## OMX\_Aberration\_Correction

Once you have generated your XML calibration file, you can use the OMX\_Aberration\_Correction macro.

After starting the macro you will be asked to select the folder where all your files, which need to be processed are stored.



In the next open window you will be asked to select the XML calibration file, which you want to use for your alignment of all images stored within the previously selected folder.



The macro will automatically align all images stored within the selected folder, unless the images end with "RGB.tif" or "ALIGNED.tif" (this ending will be added to all aligned image names). The macro also works with 2-channel images and images in which the order of colors is not RGB.

As with the Calibration macro, during the process of aligning the images, new files will be generated in order for the macro to function and **it is important not to delete any of these files during the process**. They will automatically be removed at the end.