Spekwin32, Guidance for:
Subtract blank EEM dataset

Subtract Blank EEM dataset does:

Removes all scattering and solvent contributions by subtracting a complete EEM fluorescence dataset.

Sequence of steps:

1. Open your Blank spectral files
2. Make sure it has excitation wavelengths attributed
3. Save as *.spv file
4. Open your sample spectral files
5. Make sure it has excitation wavelengths attributed
6. Execute Blank subtraction
7. Enjoy!

Following:
Example for Blank subtraction by using example data sent in by a Spekwin32 user

Motivation

In two-dimensional fluorescence data (EEM = excitation emission matrix), measured with sensitive fluorescence spectrometers, and especially for samples with low fluorescence intensity, there are prominent scattering artifacts from Rayleigh and Raman scattering. Plus, there can be some fluorescence background originating from matrix or solvent. Sometimes it is best to measure a Blank EEM dataset and just subtract this from the sample EEM dataset before all other data analysis.
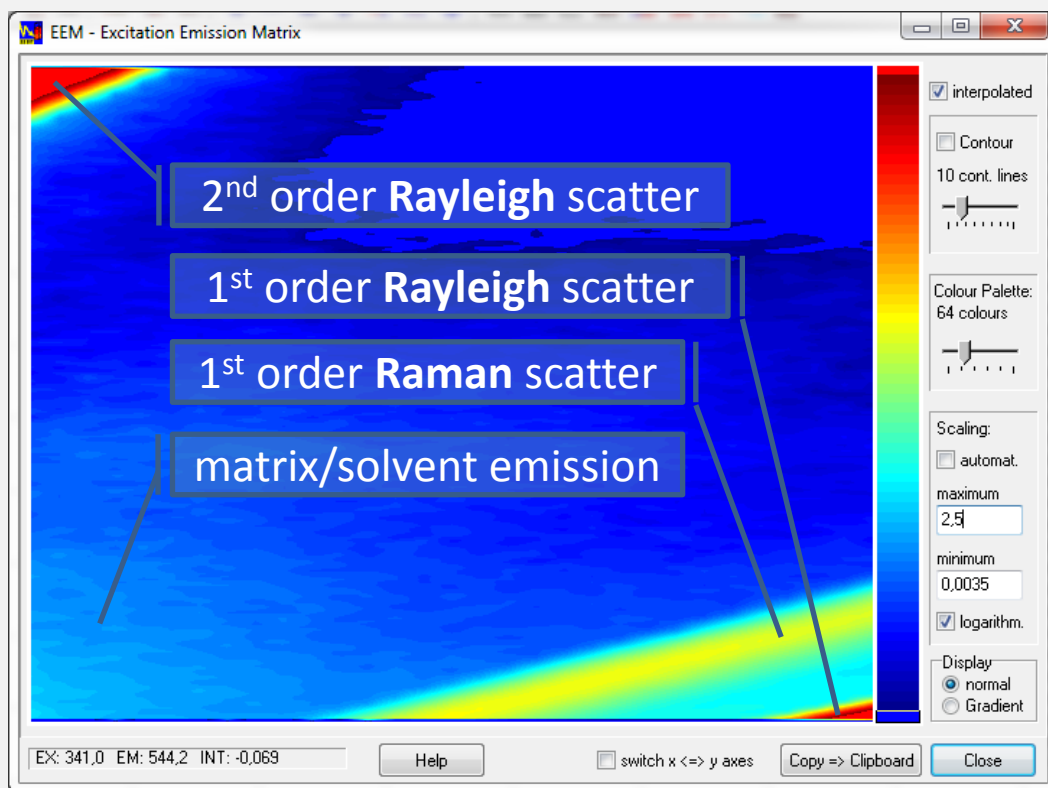
Create Blank dataset

- Open all the blank's spectral files (fluorescence emission)
- Assign the excitation wavelength for each spectrum with "*Excitation Wavelength Assignment*" function from "*Fluorescence/EEM*" menu. Fill in start wavelength + stepwidth.
- Save as Spekwin32 *.spv file.
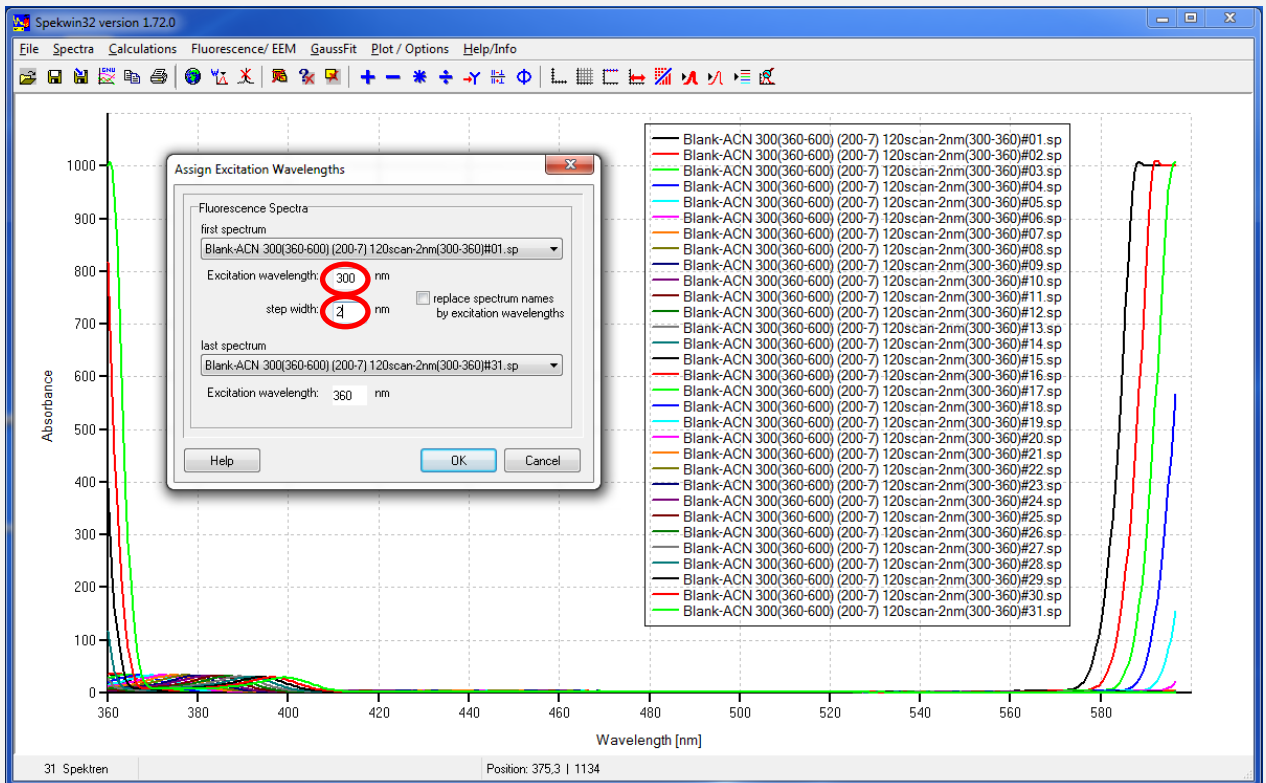
View blank dataset

- Create the EEM plot with *„Show 2D spectrum (EEM)"* from the *„Fluorescence/EEM"* menu.
- The 2-dimensional plot looks like below:
  (used logarithmic scaling to visualize the weaker parts)

EEM - Excitation Emission Matrix

2nd order **Rayleigh** scatter

1st order **Rayleigh** scatter

1st order **Raman** scatter

matrix/solvent emission

☑ interpolated

☐ Contour
10 cont. lines

Colour Palette:
64 colours

Scaling:
☐ automat.
maximum
2,5
minimum
0,0035
☑ logarithm.

Display
◉ normal
○ Gradient

EX: 341,0 EM: 544,2 INT: -0,069   Help   ☐ switch x <=> y axes   Copy => Clipboard   Close

Now, create the sample dataset

- Open all the blank's spectral files (fluorescence emission)
- Assign the excitation wavelength for each spectrum with "*Excitation Wavelength Assignment*" function from "*Fluorescence/EEM*" menu. Fill in start wavelength + stepwidth.
- Save as Spekwin32 *.spv file.

NOTICE: For some file formats, it may already be assigned. The function works also for a set of excitation spectra. Then, it is their detection wavelengh that gets assigned)

View sample dataset

- Create the EEM plot with „*Show 2D spectrum (EEM)*" from the „*Fluorescence/EEM*" menu.
- The 2-dimensional plot looks like this:
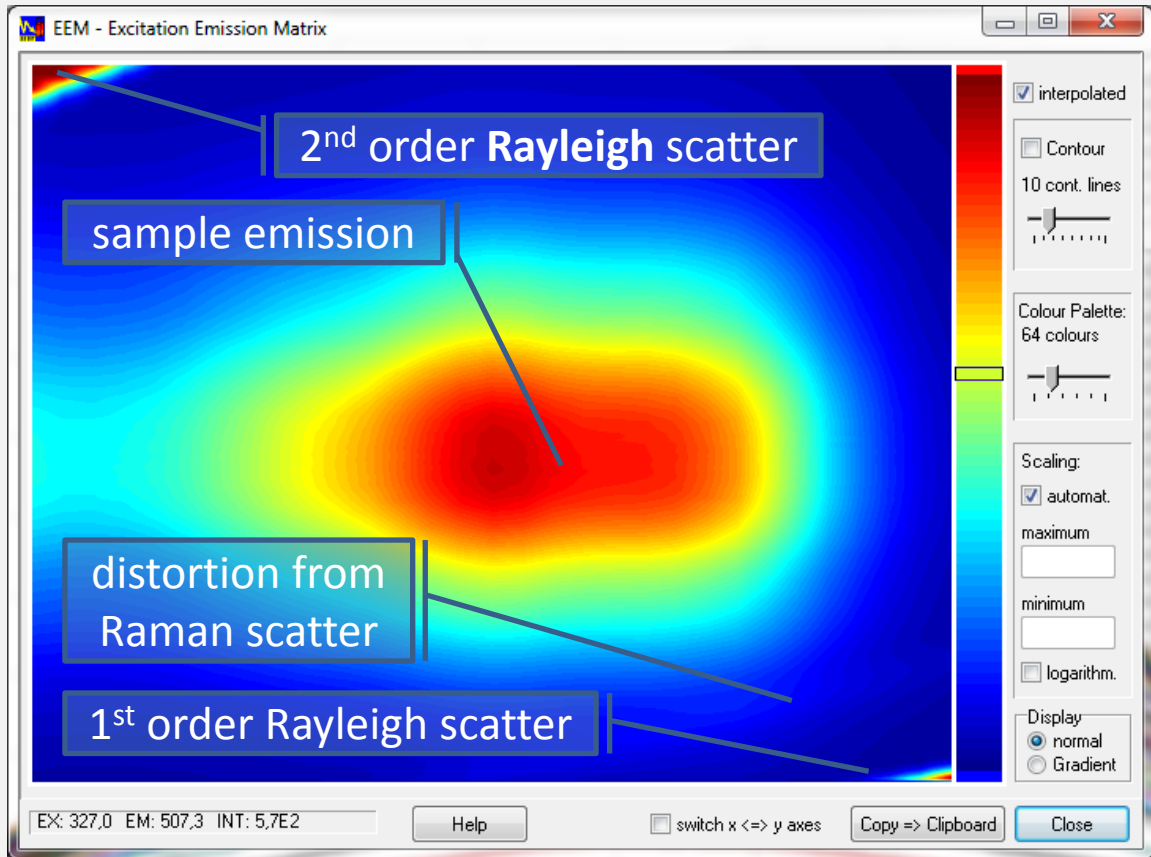
Final step: Blank subtraction

- Use the "*Subtract Blank EEM dataset*" function from the *Fluorescence/EEM* menu.
- In the upper part, properties of the current sample dataset are displayed (dimensions + stepwidth).
- Click the "**Load Blank dataset**" and select the spv file with Blank data (created before).
- Finished! Blank is subtracted.

Now, view the blank-subtracted sample dataset
- Create the EEM plot with *„Show 2D spectrum (EEM)"* from the *„Fluorescence/EEM"* menu.
- The 2-dimensional plot looks like this:
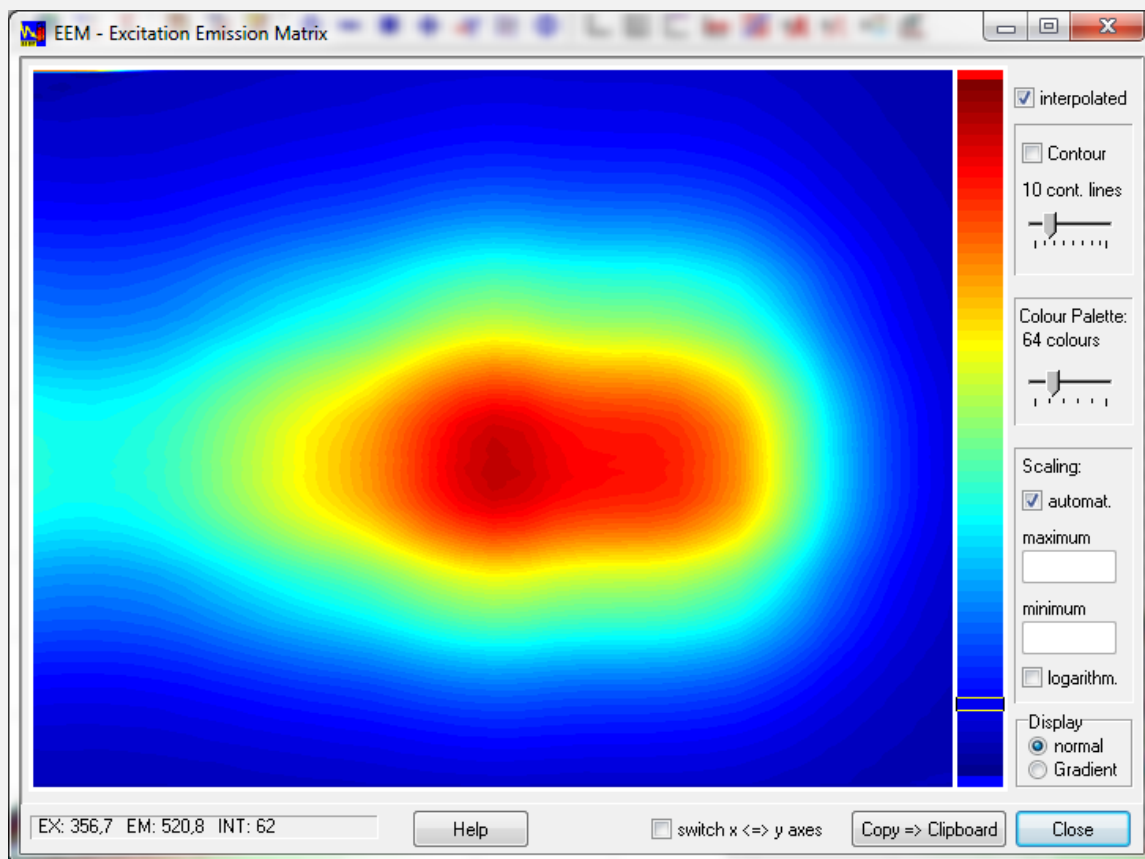
All distortions are gone. Only pure sample emission remaining!

Summary:
Automated removal of Blank datasets can significantly improve EEM fluorescence data quality!