

Data Quality and Post Processing

UNIVERSITY OF
BIRMINGHAM



Data Quality and post-processing

Quality control

- Standards e.g. quinine sulphate, raman, solid fluorescence standards
- Manufacturer supplied QC routines
- Instrument correction factors

Post-processing

- Inner-filtering
- Raman or quinine sulphate equivalent units
- Removal of raman line and scatter

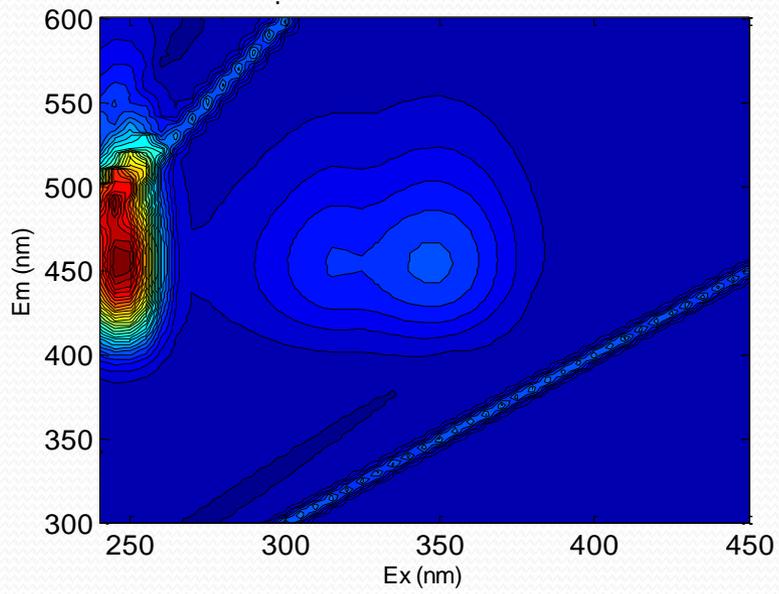
Standards

A wide range of standards exist:

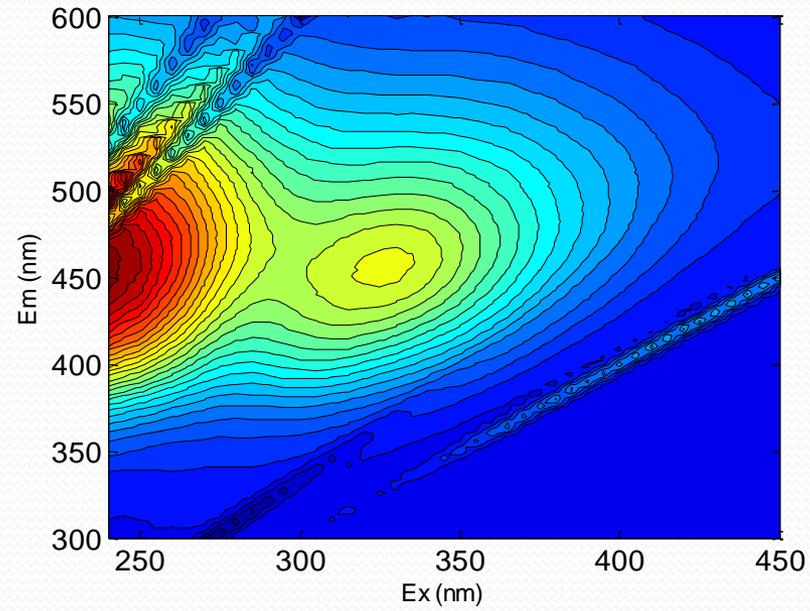
- Quinine sulphate solution
- Raman signal of (deionised) water
- International Humic Substances Society (IHSS) standards e.g. Suwanee River Fulvic Acid
- Solid fluorescence standards (e.g. Starna)

Each have advantages and disadvantages.

e.g. Quinine sulphate units are widely reported. Quantum efficiency known. But dilution series need operator skill and solutions are not stable over time.

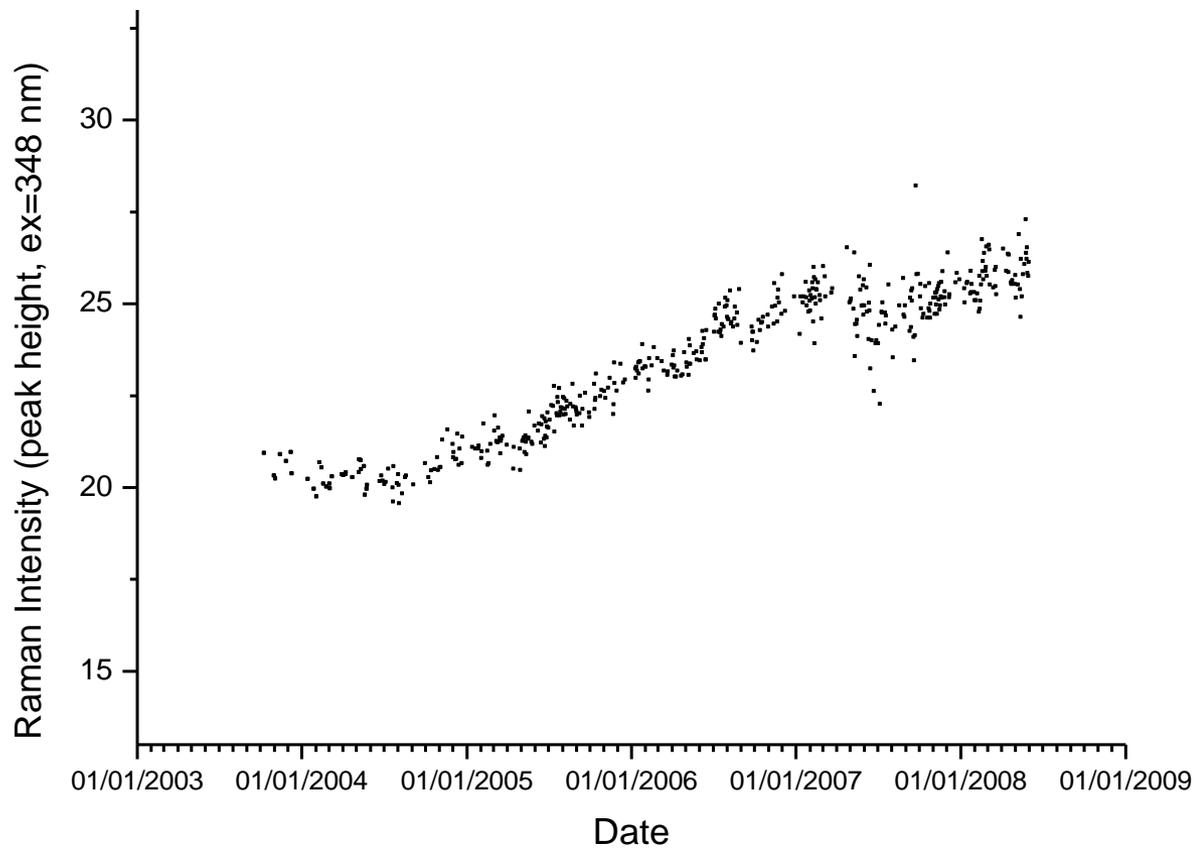


Quinine Sulphate



SRFA

Raman Intensity, Varian Cary Eclipse, 2003-2009

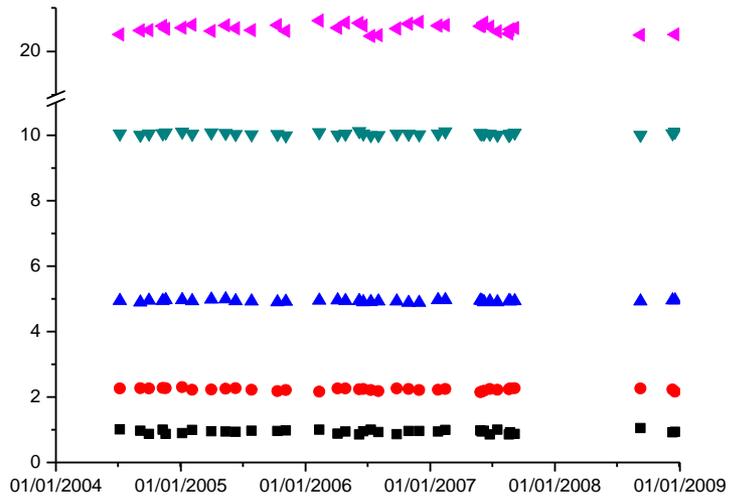


Manufacturer supplied QC routines

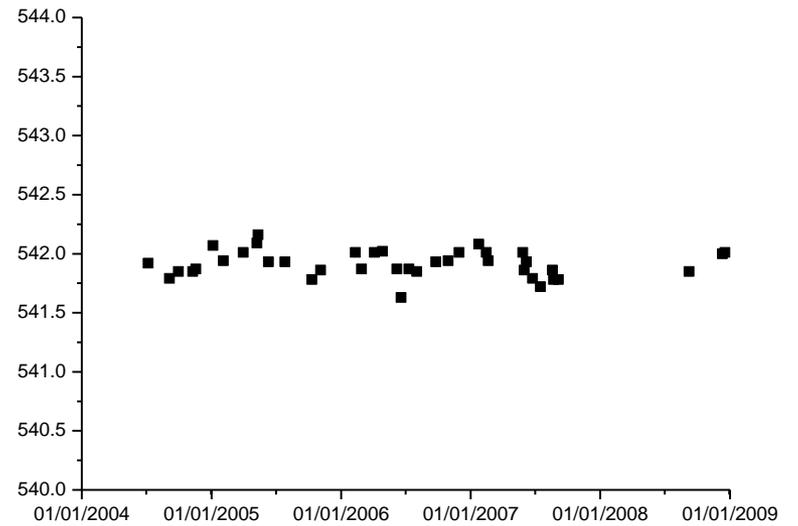
Most instruments have a wide range of validation / QC procedures.

These will check instrument parameters such as excitation and emission wavelength precision, band pass precision and signal to noise.

Recommended that these should be run regularly. They will require some accessories (often supplied with instrument)



Emission bandpass



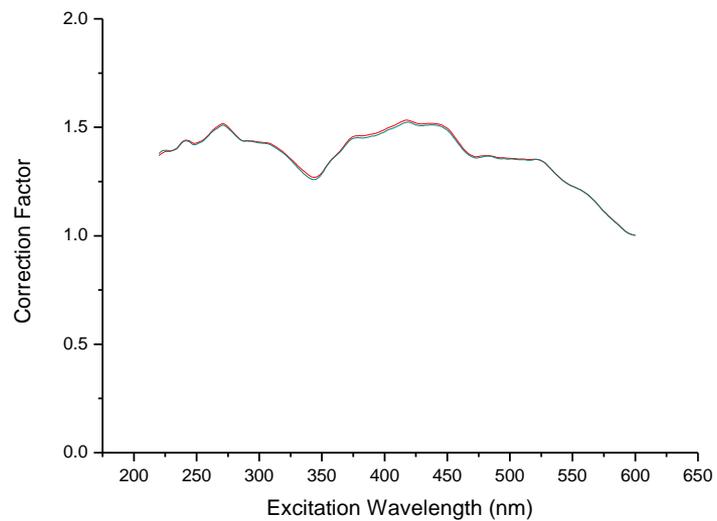
Instrument correction factors

Correction factors can be run to correct excitation and emission spectra for instrument specific characteristics e.g. Variations in lamp emission at different wavelengths.

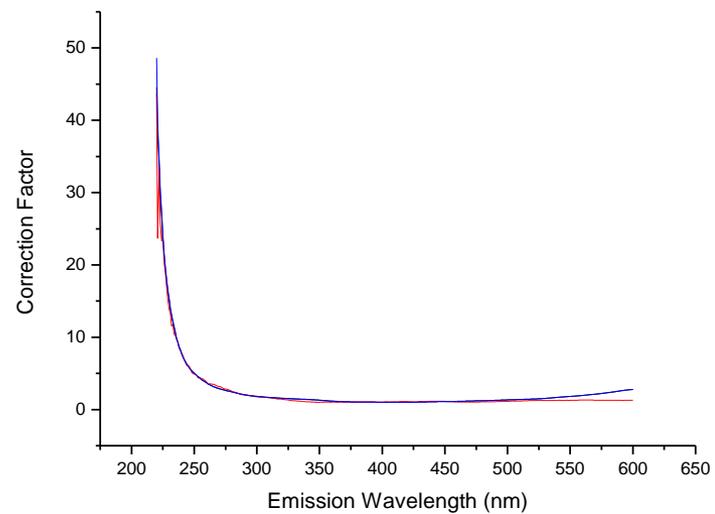
Standards are needed and the procedure isn't trivial. Instrument correction factors might not be available for all wavelengths of interest.

Instrument correction allows inter-laboratory comparison, but wouldn't be necessary for single instrument applications.

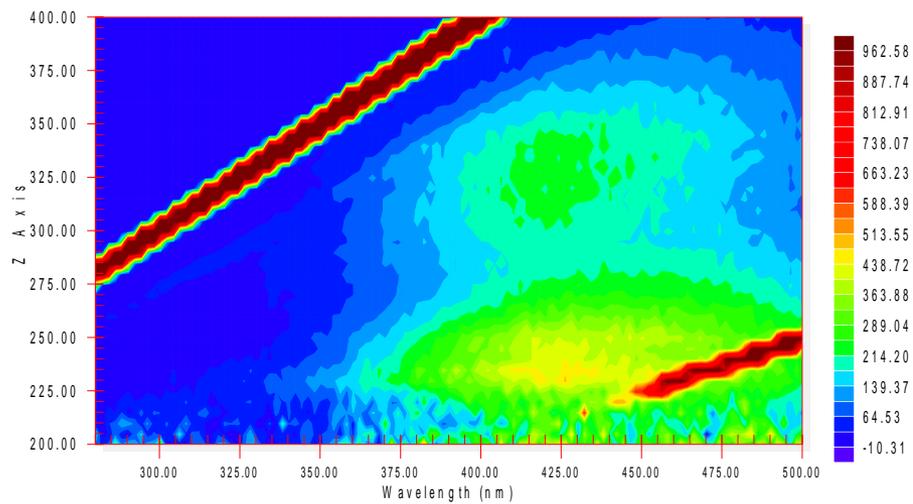
Excitation Correction



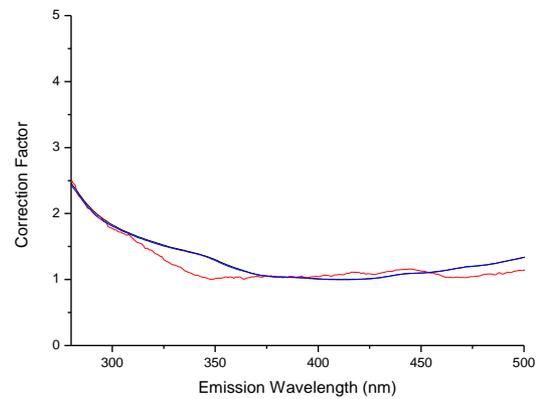
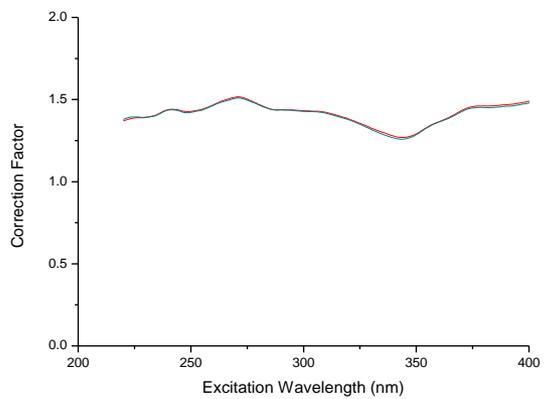
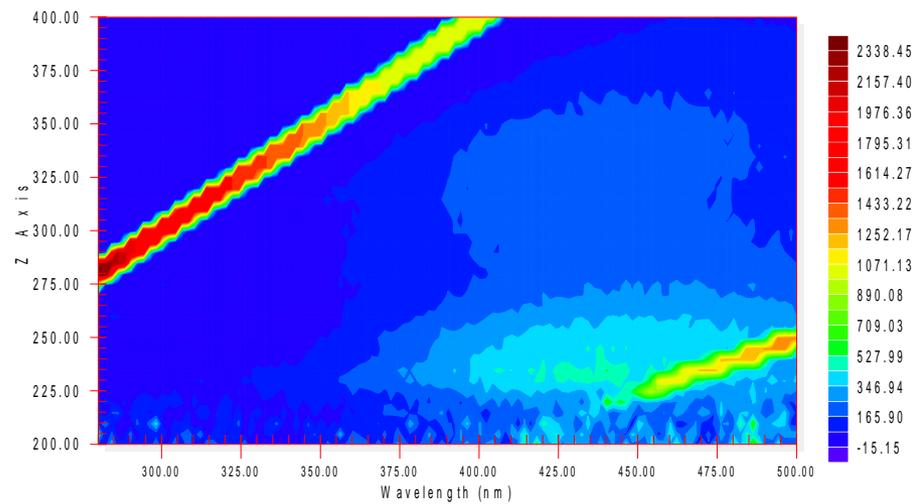
Emission Correction



Uncorrected



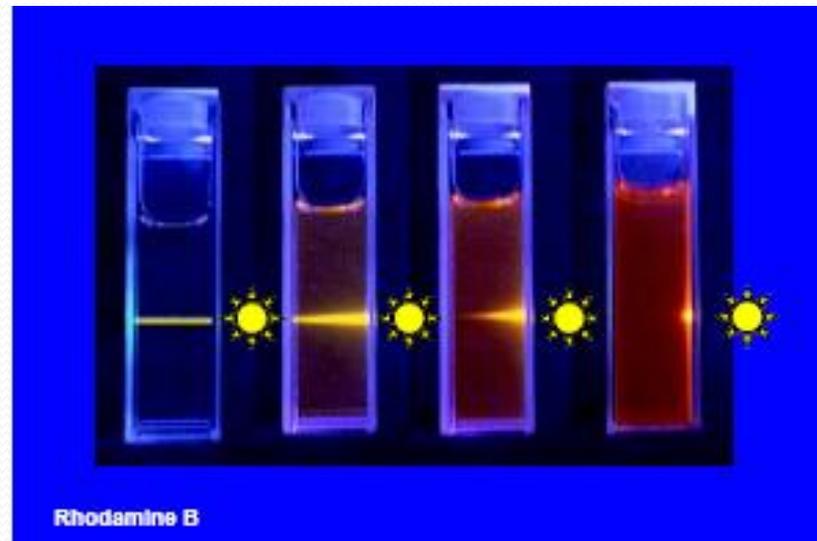
Corrected

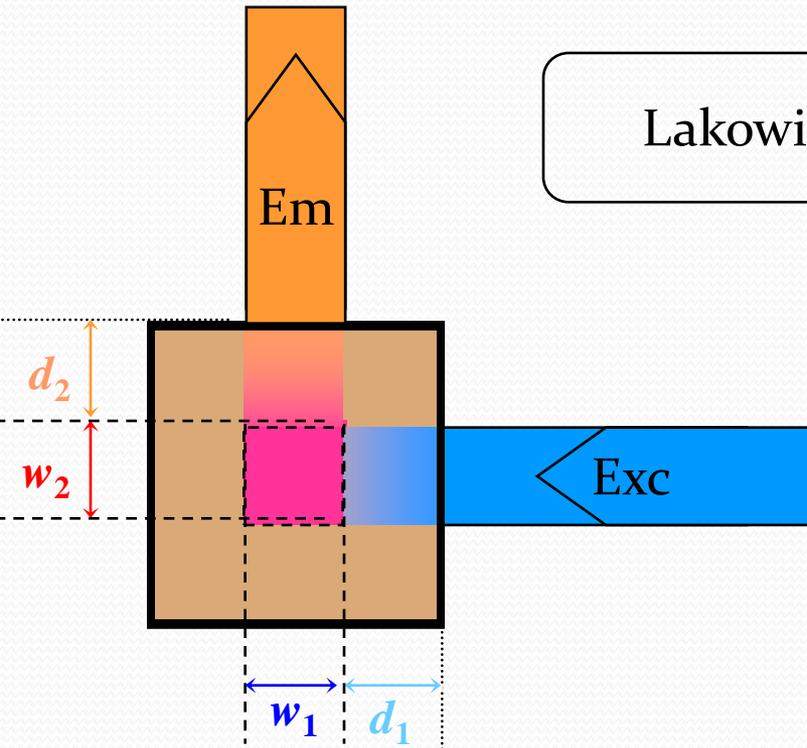


Post Processing

'Inner Filter Correction' is the most common post-processing technique to be needed.

This corrects for changes in fluorescence due to strongly coloured / absorbing solutions. A range of techniques are available, but often recommended is an empirical correction based on the absorbance of the solution.





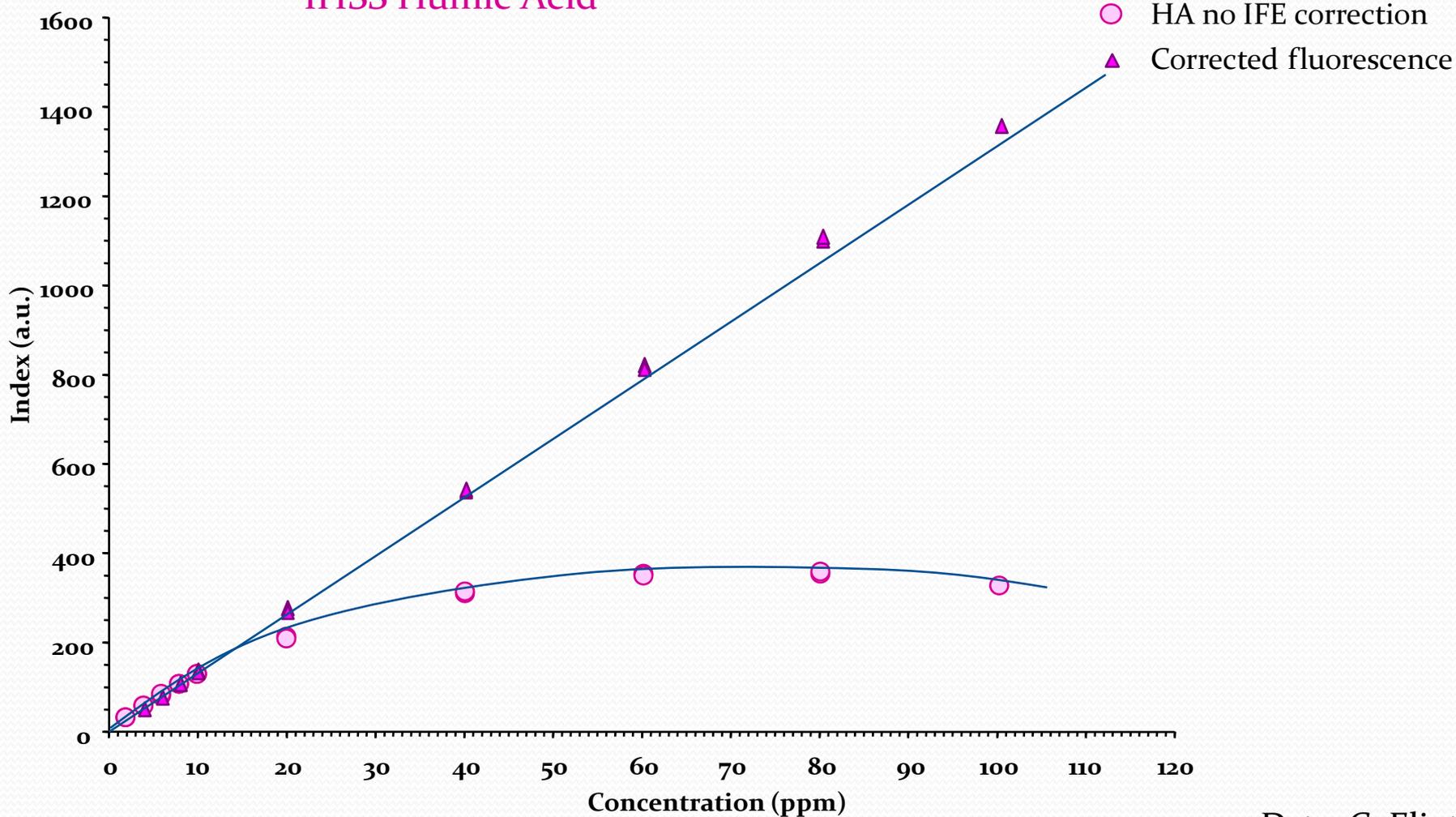
Lakowicz (1983)

$$\text{CL} : \frac{F_{corr}}{F_{obs}} = 10^{[(A_{exc} + A_{em})/2]}$$

Parker and Barnes
(1957)

$$\text{CP} : \frac{F_{corr}}{F_{obs}} = \frac{2.3 w_1 A_{exc}}{10^{-d_1 A_{exc}} (1 - 10^{-w_1 A_{exc}})} \times \frac{2.3 w_2 A_{em}}{10^{-d_2 A_{em}} (1 - 10^{-w_2 A_{em}})}$$

IHSS Humic Acid



Abs. 254 nm
(cm^{-1})

↑

↑

0.30

0.59

↑

1.17

↑

2.99

Data: C. Elliott-Laize, CEH, UK

Inner-filter corrections

For most samples, fluorescence intensity – concentration relationship is linear up to 10 ppm.

Empirical corrections simple to implement, but have to be remembered are empirical.

Precise absorbance measurements are necessary or errors will be introduced.

For many applied uses, a non-linear fluorescence intensity vs concentration relationship might be perfectly acceptable.

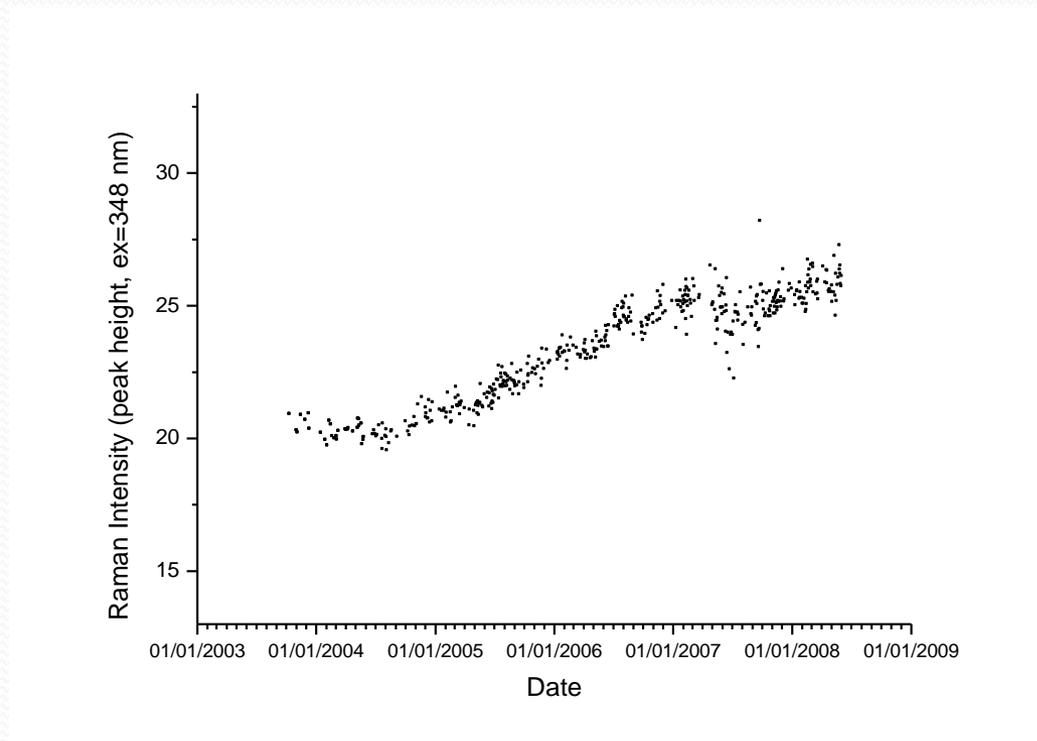
Other correction techniques are possible, e.g. Dilution, dual pathlength, etc..

No one answer – the choice should fit the application.

Conversion to raman or QS units

Fluorescence intensity is measured in arbitrary units. These can be converted to QSU if a quinine sulphate standard solution is analysed.

Similarly, the raman intensity of water can also be used to standardise the fluorescence intensity.



Removal of scatter lines, raman line, etc.

Necessary for some statistical and mathematical treatments (see later presentation). Otherwise, there is no reason to remove these data, and for many applications, both scatter and raman features can be used to calibration / validation.

