# Spectral Correction for Fluorescence Spectrophotometers

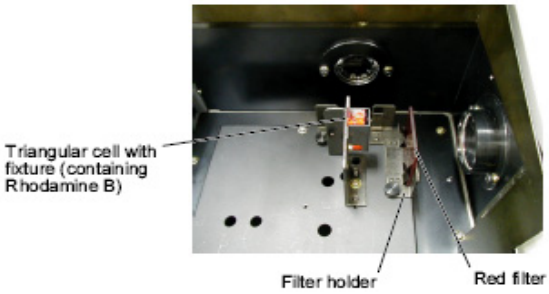
Hitachi High Technologies America, Inc.

In fluorescence spectroscopy, the spectra obtained may be distorted due to instrument component characteristics. Diffraction gratings change efficiency as a function of the wavelength; lamps have their own emission intensity spectra and detectors have wavelength sensitivity characteristics. Spectral correction is particularly important when comparing data against data measured in another instrument or published data.

Hitachi has simplified this procedure by implementing a 4-step procedure and software to perform spectral correction in the full extended range of the instrument, in both the excitation and the emission sides.

### Step 1. Excitation Spectral Correction in the Low Range

Items required: Triangular Cell, Rhodamine B quantum counter (3g/L in ethylene glycol) and Red filter




The measurement of spectral correction factors is performed and stored automatically by the instrument. With rhodamine B solution in a triangular cell and a red scattering filter placed in the sample compartment, a fluorescence excitation scan is measured with the emission wavelength fixed at 640 nm. Correction factors at each wavelength are determined directly from the fluorescence intensity of the quantum counter. Fluorescence intensity $I_{FL}$ of the counter is proportional to the intensity of the excitation beam.

$$I_{FL} \approx I_{ex} * Q * A * K_{ex} * K_{em}$$

where: $I_{ex}$ is the intensity of the excitation beam
$Q$ is the emission quantum yield
$A$ is the absorbance
$K_{ex}$ is the correction factor on the excitation side
$K_{em}$ is the correction factor on the emission side

Rhodamine B has a narrow width emission spectra, so influence by the detector wavelength characteristics is nominal. In addition, quantum yield, absorbance, and optimum emission wavelength of rhodamine B are unaffected in the range of 200 to 600 nm. Corrected excitation spectra is given by the following relationship:

$$I_{FL}\text{ (corrected)} = \frac{I_{FL}\text{ (uncorrected)}}{I_{FL}\text{ (quantum counter)}}$$

### Step 2. Emission Spectral Correction in the Low Range

Items required: Quartz diffuser

Correction factors for each emission wavelength are then obtained using the corrected excitation monochromator. In order to perform emission spectral correction, the quartz diffuser is mounted in the sample compartment so the scattered excitation beam is passed to the emission side. Emission intensity is measured while scanning both monochromators in synchronization. Emission intensity is proportional to the product of correction factors on the excitation and emission sides. The formula below is used to calculate the Emission Correction Factors:

$$\text{Emission-side correction factor} = \frac{\text{Product of correction factors on Ex and Em sides}}{\text{Excitation-side correction factor}}$$

The emission correction factors are stored in the computer memory for performing automatic correction via software. Figure1 shows emission spectra for quinine sulfate before and after correction.

### Step 3: Emission Spectral Correction in the Long Range

Items required: Substandard Light Source

In this case the lamp light is directed from the sample compartment to the emission monochromator. The readings at each wavelength are divided by the known emission values for the lamp, generating the correction factors for the long range of the emission monochromator. These factors are combined with the emission factors for the short range, affording spectrum correction in the full range of the instrument (200 to 900 nm) for the emission side.

### Step 4: Excitation Spectral Correction in the Long Range

Items required: Quartz diffuser

In order to generate spectral correction factors for the long wavelength range of the excitation monochromator (< 600 nm), we read the instrumental response in the excitation monochromator in the long range with reference to the instrumental response obtained in the long wavelength range in the emission side.

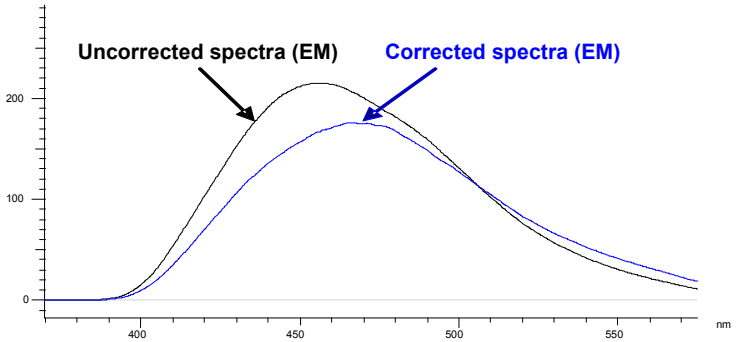



**Figure 1:** Quinine sulfate solution before and after spectral correction

**References:**

1 – Technical Data No. 24, Hitachi High Technologies Corporation.

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