

Linking Fluorescence Measurements to Radiometric Units

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Introduction

Fluorescence techniques are among the most widely used analytical tools and detection methods in material sciences, environmental analysis and the life sciences. However, comparability of fluorescence data across instruments, laboratories, and over time is only possible with large uncertainties due to the variety of fluorescence instruments and the many possible sources of error inherent to fluorescence spectroscopy. To improve this situation, there is an urgent need for simple, internationally accepted and ideally standardized procedures for the performance of fluorescence measurements and instrument characterization in combination with suitable physical and chemical transfer standards.

In the framework of the project “Development, Characterization and Dissemination of Fluorescence Standards for the Traceable Qualification of Fluorometers” by the Federal Ministry of Economics and Labour of Germany it was our goal to develop, provide, and promote suitable calibration tools and procedures to facilitate the traceability of fluorescence spectra to radiometric primary standards and to eventually link fluorescence measurements to radiometric units for a broad range of applications.

Two approaches to traceable fluorescence measurements

Due to different aims, it is necessary to address two target groups with different strategies and different types of standards.

For **metrological or reference laboratories** equipped with high accuracy fluorescence instruments, absolute measurements and minimization of calibration and measurement uncertainty are of primary interest. Accordingly, application of physical transfer standards for both spectral radiance (see references) and spectral responsivity with their small uncertainties is the method of choice for instrument characterization. The most critical point is here to develop a calibration method for the emission channel that ensures a similar spectral radiance at the sample position for instrument characterization as well as for typical fluorescence measurement tasks. We will present necessary procedures and currently achievable uncertainties. The uncertainties result from a state-of-the-art calibration of the current reference fluorometer at BAM, a modified commercial instrument. In addition, we will illustrate our attempts to further reduce these uncertainties with a new reference fluorometer designed and constructed by BAM and a calibration scheme relying only on trap detectors and a reflectance standard. This instrument (see references) is designed for measurement and dissemination of absolute fluorescence

spectra and quantum yields with lowest possible uncertainties.

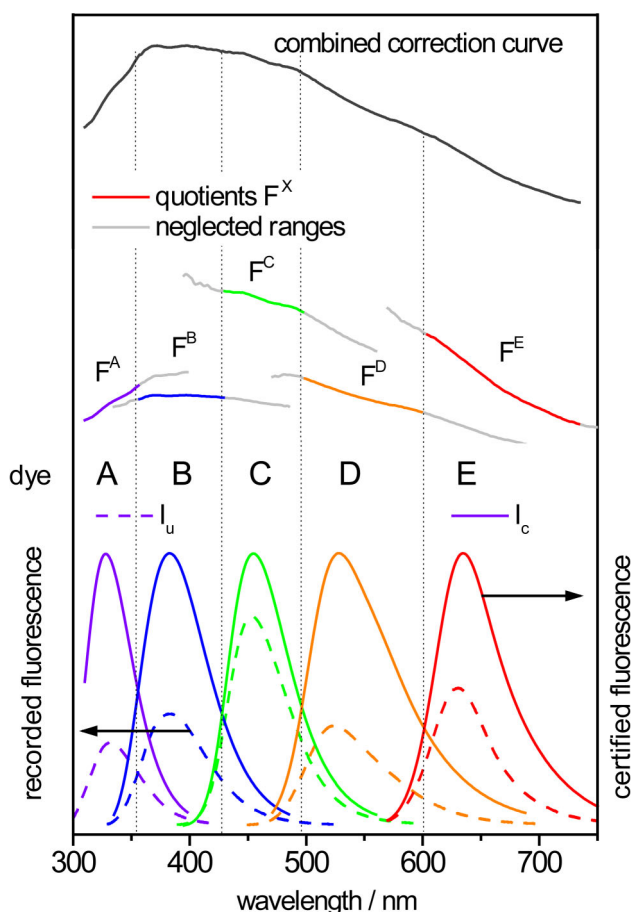


Figure 1. Determination of an emission correction curve with the liquid fluorescence standards dye A – E by division of recorded, i.e., uncorrected instrument-specific fluorescence emission spectra $I_U(\lambda_{em})$ and BAM-certified, i.e. corrected instrument-independent fluorescence emission spectra $I_C(\lambda_{em})$. A subsequent weighted combination of the individual quotients F^X results in an overall emission correction curve which equals the relative spectral responsivity of the instrument to be calibrated.

For the **broad community of users** of fluorescence techniques with instruments covering the range from spectrofluorometers over microplate readers to fluorescence microscopes, ease of use of standards has to be given priority. This is accomplished with a set of liquid spectral fluorescence standards developed by BAM and consisting of organic dyes. With these chemical standards and a simple procedure, determination of the spectral characteristics (Figure 1) and long-term performance of different types of fluorescence instruments is possible.

These standards match in an ideal way the optical and radiative properties of typical samples. Thus, many difficulties arising from the calibration with physical transfer standards can be elegantly avoided. The certified corrected fluorescence spectra of these standards are determined with the BAM reference fluorometer that was previously characterized with PTB-calibrated physical transfer standards. Accordingly, they are traceable to the primary standard of the spectral radiance realized in Germany by PTB.

Proficiency Test

The spectral fluorescence standards dye A – E (see references) and the according calibration procedure have been successfully employed for the characterization of the relative spectral responsivity of several commercial spectrofluorometers. Moreover, they have been successfully tested in a recently performed exploratory study on the state-of-the-art of the calibration of spectrofluorometers and colorimeters employing physical and chemical transfer standards. This study was organized by BAM and NIST with NPL, NRC, and PTB participating.

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References

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- The design and the realization of the BAM reference fluorometer is the topic of an additional poster presentation at the conference
- A new compact integrating sphere based spectral radiance standard with reduced radiance is also presented on an additional poster.
- The fluorescence standards dye A – E are commercially available as BAM-F001 – BAM-F005.