Quantitative Fluorescence Measurements: An International Comparison Study

NIST and BAM (the European Commission's Federal Institute for Materials Research and Testing) organized an international comparison for quantitative fluorescence spectroscopy. The goals of this study are 1) to define the state of the art of spectral correction of fluorescence, i.e., what is the best precision and accuracy that can be achieved by a group of expert labs when they attempt to measure the true shape of a fluorescence spectrum, and 2) to identify systematic errors that can arise when calibrating different instruments. These goals are the first steps towards achieving the larger goal of standardizing instrument calibration in fluorescence spectrometry.

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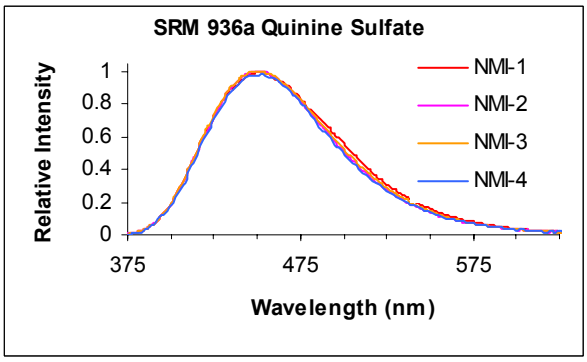
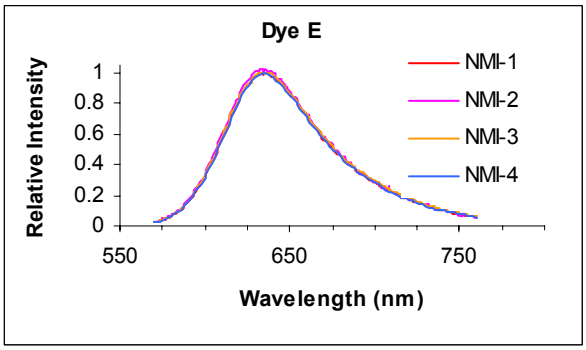
In the Fall of 2004, the European Commission's Federal Institute for Materials Research and Testing (BAM) and NIST co-organized an *Exploratory Study in Fluorescence Spectrometry*, with four participating national metrology institutes (NMIs) (BAM, National Research Council (NRC Canada), the Physikalisch-Technische Bundesanstalt (PTB, Germany), and NIST). NIST supplied SRM 936a Quinine Sulfate solutions. BAM provided solutions of dyes A, B, C, D, and E, which are undisclosed dyes that BAM intends to sell as certified reference materials (CRMs) in the near future. All dyes were supplied at two concentrations, corresponding to absorbance (A) values of A=0.04 and 0.08 at each dye's excitation wavelength, with accompanying blanks. The samples' fluorescence spanned the emission wavelength range from 310 nm to 750 nm, covering the near ultraviolet (UV), visible, and the beginning of the near infrared (NIR). This is the typical wavelength range of conventional fluorescence spectrometers. The routine calibration procedure used by each NMI to qualify their instruments for obtaining spectrally corrected or "true" fluorescence spectra was performed and the resulting routine spectral correction was applied to all spectra taken by that participant. Now that all the data have been received, the corrected spectra from all participants are presently being compared. Preliminary results show very good agreement between the spectra from the four labs. Preliminary results for two (quinine sulfate and dye E) of the dyes are shown in the figures. Quinine sulfate and dye E cover the blue and red regions of the emission wavelength range, respectively.





The agreement in the results for these two dyes is representative of that observed for all the dyes, with almost all values falling within plus or minus 5% of the average or consensus value. BAM and NIST agreed to perform statistical analyses on the data, independently, to help to eliminate the possibility of errors in the final results. In the preliminary analysis, both BAM and NIST used principles-based analysis methods, with the BAM method containing two parameters and the NIST method containing four. These parameters correspond to possible instrumental errors or bias, such as wavelength shift or intensity offset.

All data have been collected and a preliminary analysis has been completed. Very good comparability of corrected fluorescence spectra has been demonstrated for the expert lab participants. Principles-based models have been used to compare the spectra taken by each laboratory to the consensus spectra, enabling systematic errors to be identified for particular instruments and calibration methods.

Four National Metrology Institutes from US, Canada, and Germany demonstrated good comparability for quantitative fluorescence measurements in the first-ever international comparison study.

Impact: This study will inform users of fluorescence spectrometers of:

1) how close they can expect their collected and corrected spectra to be to "true" spectra,

2) systematic errors that are of concern for their particular instrument or calibration method, and

3) a principles-based model for identifying systematic errors.

This information is an important first step in standardizing calibration methods in fluorescence spectrometry, an area where calibration is often difficult and standard methods are greatly needed by users to support their quality assurance/quality control (QA/QC) claims.

Future Plans: The participants will decide on a final analysis method to apply to the data that will serve as a suggested standard treatment for data from such intercomparisons in the future. Final results and methods will be published in an appropriate journal. In further support of our measurement claims, BAM and NIST hope to expand the scope of this study by including non-NMI participants from academia and industry in a nearly identical future study that we envision organizing by the time this first study concludes.