



Standard Guide for Fluorescence—Instrument Calibration and Qualification¹

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1. Scope

1.1 This guide (1)² lists the available materials and methods for each type of calibration or correction for fluorescence instruments (spectral emission correction, wavelength accuracy, and so forth) with a general description, the level of quality, precision and accuracy attainable, limitations, and useful references given for each entry.

1.2 The listed materials and methods are intended for the qualification of fluorometers as part of complying with regulatory and other quality assurance/quality control (QA/QC) requirements.

1.3 Precision and accuracy or uncertainty are given at a 1 σ confidence level and are approximated in cases where these values have not been well established.³

1.4 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.5 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 ASTM Standards:⁴

E131 Terminology Relating to Molecular Spectroscopy

E388 Test Method for Wavelength Accuracy and Spectral Bandwidth of Fluorescence Spectrometers

E578 Test Method for Linearity of Fluorescence Measuring Systems

E579 Test Method for Limit of Detection of Fluorescence of Quinine Sulfate in Solution

3. Terminology

3.1 Definitions(2):

3.1.1 *absorption coefficient* (α), n —a measure of absorption of radiant energy from an incident beam as it traverses an absorbing medium according to Bouguer's law, $I/I_0 = e^{-\alpha b}$, where I and I_0 are the transmitted and incident intensities, respectively, and b is the path length of the beam through the sample. **E131**

3.1.1.1 *Discussion*—Note that transmittance $T = I/I_0$ and absorbance $A = -\log T$.

3.1.2 *absorptivity* (a), n —the absorbance divided by the product of the concentration of the substance and the sample pathlength, $a = A/bc$. **E131**

3.1.3 *Beer-Lambert law*, n —relates the dependence of the absorbance (A) of a sample on its path length (see *absorption coefficient*, α) and concentration (c), such that $A = a bc$.

3.1.3.1 *Discussion*—Also called Beer's law or Beer-Lambert-Bouguer law. **E131**

3.1.4 *calibrated detector* (CD), n —optical radiation detector whose responsivity as a function of wavelength has been determined along with corresponding uncertainties (3).

3.1.5 *calibrated diffuse reflector* (CR), n —Lambertian reflector whose reflectance as a function of wavelength has been determined along with corresponding uncertainties (4).

3.1.6 *calibrated optical radiation source* (CS), n —optical radiation source whose radiance as a function of wavelength has been determined along with corresponding uncertainties (5, 6).

3.1.7 *calibration*, n —set of procedures that establishes the relationship between quantities measured on an instrument and the corresponding values realized by standards.

3.1.8 *certified reference material* (CRM), n —material with properties of interest whose values and corresponding uncertainties have been certified by a standardizing group or organization. **E131**

3.1.9 *certified value*, n —value for which the certifying body has the highest confidence in its accuracy in that all known or

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² The boldface numbers in parentheses refer to the list of references at the end of this standard.

³ Certain commercial equipment, instruments, or materials are identified in this guide to foster understanding. Such identification does not imply recommendation or endorsement by ASTM International nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

⁴ For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.