



Standard Practice for Detecting Fluorescence in Object-Color Specimens by Spectrophotometry¹

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1. Scope

1.1 This practice provides spectrophotometric methods for detecting the presence of fluorescence in object-color specimens.

NOTE 1—Since the addition of fluorescing agents (colorants, whitening agents, etc.) is often intentional by the manufacturer of a material, information on the presence or absence of fluorescent properties in a specimen may often be obtained from the maker of the material.

1.2 This practice requires the use of a spectrophotometer that both irradiates the specimen over the wavelength range from 340 to 700 nm and allows the spectral distribution of illumination on the specimen to be altered as desired.

1.3 Within the above limitations, this practice is general in scope rather than specific as to instrument or material.

1.4 *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:²

[D2244 Practice for Calculation of Color Tolerances and Color Differences from Instrumentally Measured Color Coordinates](#)

[E284 Terminology of Appearance](#)

[E308 Practice for Computing the Colors of Objects by Using the CIE System](#)

[E313 Practice for Calculating Yellowness and Whiteness Indices from Instrumentally Measured Color Coordinates](#)

[E991 Practice for Color Measurement of Fluorescent Specimens Using the One-Monochromator Method](#)

¹ This practice is under the jurisdiction of ASTM Committee E12 on Color and Appearance and is the direct responsibility of Subcommittee E12.05 on Fluorescence.

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² 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.

[E1164 Practice for Obtaining Spectrometric Data for Object-Color Evaluation](#)

[E1331 Test Method for Reflectance Factor and Color by Spectrophotometry Using Hemispherical Geometry](#)

[E1348 Test Method for Transmittance and Color by Spectrophotometry Using Hemispherical Geometry](#)

[E1349 Test Method for Reflectance Factor and Color by Spectrophotometry Using Bidirectional \(45°:0° or 0°:45°\) Geometry](#)

[E2152 Practice for Computing the Colors of Fluorescent Objects from Bispectral Photometric Data](#)

[E2153 Practice for Obtaining Bispectral Photometric Data for Evaluation of Fluorescent Color](#)

3. Terminology

3.1 The definitions in Terminology [E284](#), Practices [E991](#), [E2152](#), and [E2153](#) are applicable to this practice.

4. Significance and Use

4.1 Several standards, including Practices [E991](#), [E1164](#), and Test Methods [E1331](#), [E1348](#) and [E1349](#), require either the presence or absence of fluorescence exhibited by the specimen for correct application. This practice provides spectrophotometric procedures for identifying the presence of fluorescence in materials.

4.2 This practice is applicable to all object-color specimens, whether opaque, translucent, or transparent, meeting the requirements for specimens in the appropriate standards listed in [2.1](#). Translucent specimens should be measured by reflectance, with a standard non-fluorescent backing material, usually but not necessarily black, placed behind the specimen during measurement.

4.3 This practice requires the use of a spectrophotometer in which the spectral distribution of the illumination on the specimen can be altered by the user in one of several ways. The modification of the illumination can either be by the insertion of optical filters between the illuminating source and the specimen, without interfering with the detection of the radiation from the specimen, or by interchange of the illuminating and detecting systems of the instrument or by scanning of both the illuminating energy and detection output as in the two-monochromator method.

4.4 The confirmation of the presence of fluorescence is made by the comparison of spectral curves, color difference, or single parameter difference such as ΔY between the measurements.

NOTE 2—In editions of E1247 - 92 and earlier, the test of fluorescence was the two sets of spectral transmittances or radiance factor (reflectance factors) differ by 1 % of full scale at the wavelength of greatest difference.

4.5 Either bidirectional or hemispherical instrument geometry may be used in this practice. The instrument must be capable of providing either broadband (white light) irradiation on the specimen or monochromatic irradiation and monochromatic detection.

4.6 This practice describes methods to detect the presence of fluorescence only. It does not address the issue of whether the fluorescence makes a significant or insignificant contribution to the colorimetric properties of the specimen for any given application. The user must determine the practical significance of the effect of fluorescence on the color measurement.

5. Instrumental Requirements

5.1 This practice requires instrumentation meeting the following requirements.

5.1.1 The instrument source shall provide sufficient irradiation energy at the sample port to excite fluorescent emission, if present.

5.1.2 The instrument must provide one of the following illumination/viewing combinations:

5.1.2.1 Monochromatic illumination and monochromatic viewing (that is, a two-monochromator spectrophotometer sometimes called a bispectrometer or spectrofluorimeter).

5.1.2.2 Polychromatic illumination and monochromatic viewing.

5.1.2.3 Reversible illumination/viewing to allow both polychromatic illumination with monochromatic viewing and monochromatic illumination with polychromatic viewing.

5.1.3 The instrument and associated computer software shall allow the standardization/calibration of the instrument using user modified standardization/calibration values, which is a requirement for using any of the filter methods described in this practice.

NOTE 3—Repeatable and accurate application of this practice requires specialized instrumentation. Some commercial one-monochromator spectrometers are limited in their ability to allow for the insertion of optical filters and re-standardization with the filter in place as required in this procedure.

6. Procedures

6.1 There are three general types of procedures to detect the presence of fluorescence instrumentally. Each has its advantages and shortcomings depending on the wavelength and intensity of the fluorescent emission and the instrumentation available to the user.

6.2 *Two-Monochromator Method:* This method requires a colorimetric measuring instrument that is equipped with two separate monochromators: the first, the illumination monochromator, irradiates the specimen with monochromatic light and the second, the viewing monochromator, analyzes the

radiation leaving the specimen. A two-dimensional array of bispectral photometric values is obtained by setting the illumination monochromator at a series of fixed wavelengths (μ) in the illumination band of the specimen, and for each μ , using the viewing monochromator to record readings for each wavelength (λ) in the specimen's viewing range. The resulting array, once properly corrected, is known as the Donaldson matrix, and the value of each element (μ, λ) of this array is the Donaldson radiance factor ($D(\mu, \lambda)$). The reflection values are confined to the diagonal of the matrix, and these diagonal values are equal to the spectral reflectance factor of the specimen. Therefore, the presence of fluorescence is demonstrated by non-zero off-diagonal elements. The measurement procedures for this method are given in detail in Practice [E2153](#).

6.3 *Filter Methods:* Filter methods follow the general procedure of making a measurement of spectral radiance factor using a spectrometer with broad band illumination, then adding one or more filters to remove the fluorescence-excitation energy and measuring the spectral radiance factor under the modified illumination. The comparison of the resulting spectral curves shows the presence or absence of fluorescence. If the exclusion of the excitation energy results in a difference in the remaining part of the curve, fluorescence is present and must be considered in the measurement procedures. If no difference is found, then fluorescence is not an issue in the measurement of that specimen.

6.3.1 *UV-Blocking Method*—This procedure is typically used for detecting the presence of optical brighteners, such as in white paper and textiles.

6.3.1.1 Calibrate the instrument as required by the manufacturer. (See Practice [E1164](#) and the appropriate test method for the instrument geometry.)

NOTE 4—Since the measurement will be used to detect fluorescence, it should be considered that fluorescence might be present, therefore the calibration procedure should include adjusting the instrument's illuminator to conform as closely as possible to D65 including the UV region of the spectrum. In some commercial instruments this may be accomplished by calibrating by whiteness index or the UV profile.

6.3.1.2 Measure the specimen, obtaining either a table or a graph of spectral transmittance or reflectance factor versus wavelength.

6.3.1.3 Insert a long-wavelength bandpass filter between the illuminating source and the specimen. Select the cutoff wavelength of the filter according to the color of the specimen using the recommendation in [Table 1](#) as a guide.

(a) For spectrophotometers equipped for illumination by means of an integrating sphere, the filter must be placed between the illuminating source and the illumination entrance

TABLE 1 Edge-Position and Emission Wavelengths

Sample Color	Edge-Position Wavelength, nm	Minimum Emission Wavelength, nm
White or blue	440	400
Green	510	480
Yellow	540	480
Orange	620	550
Red	650	560