



# Good practice guide for fluorescence measurements of solid surfaces by the two-monochromator method

*Created during the research project “xD-Reflect: Multidimensional Reflectometry for Industry”*

## 1. Introduction

Surface colour measurements are important in the production of a wide variety of manufactured goods. The appearance of many products are enhanced with fluorescent agents, which absorb light in the UV and blue region of the visible light spectrum and re-emit at longer wavelengths, causing the products to appear brighter or more saturated in colour. Thus, the appearance of a fluorescent surface does not depend only on the visible part of the spectrum of irradiating light source, but also the UV part. The bispectral nature of fluorescence makes measurements more demanding in time and instrument complexity than other spectrophotometric measurements.

The colour coordinates of a fluorescent surface can be calculated from its total radiance factor spectrum corresponding to a specified light source, which in turn can be calculated for any light source from its spectra of reflected and bispectral luminescent radiance factors. There are several methods for obtaining these quantities, however, the most accurate of them is the two-monochromator method.

This guide emphasizes various aspects involved in measuring bispectral luminescent and reflected radiance factors by the two-monochromator method in order to improve the accuracy of the measurements. The main sources of measurement errors and uncertainties are discussed.

## 2. General considerations

- The measurement geometry should conform to internationally accepted standards.
- The excitation and emission bandwidths need to be known.
- The ambient temperature needs to be recorded. As a general rule of thumb, temperature always affects measurement results.
- Samples to be measured should be at the same room temperature as the instrument.
- The measurement instrument needs to be used and maintained according to the user manual. This includes regular calibration of relevant standards.
- The measurement instrument needs to be turned on and allowed to warm up and stabilize.
- The samples being measured should be handled with care. It is advisable to wear gloves and avoid touching the surface to be measured.

## 3. Measurement errors

In this section, the spectrophotometric errors are discussed qualitatively. All of them are known to affect the measurement results, although their extent varies among instruments. It is the user's responsibility to check the instrument or to trust manufacturer's specification in this sense.



### 3.1. Absolute value of the scale

A reference white sample with calibrated reflectance values is generally used for calibrating the absolute value of the scale of the instrument. The reference panel should be highly reflecting and neutral in the whole wavelength range used and not exhibit any photoluminescence. The additional requirements for the reference material are:

- Stability – UV radiation used in fluorescence measurements may degrade the material, changing its reflectance values and leading to measurement errors.
- Uniformity – spatial changes in the reflectance of the reference sample may lead to errors if the irradiated area during the calibration of the reference is different from that during routine measurements. In addition, the spatial non-uniformity makes the alignment of the sample more critical.
- Low translucency – with high translucency, the area where the light is reflected from is larger than the irradiated area, leading to additional errors when there are differences in the field of view of the detection optics between calibration and routine measurements.

### 3.2. Spectral responsivity of the detection system

The spectral responsivity of the detection system can be calibrated using a reference detector with a known spectral responsivity, a reference source with a known spectral radiance or a set of luminescent materials. In any case, care should be taken that the spectral responsivity is calibrated for all the used detection system configurations. A change of a single element (e.g. grating, filter, fiber) will invalidate the calibration unless accounted for.

### 3.3. Non-linearity of the photodetector

The response of the detector to light is always to some extent non-linear. In the higher signal ranges the saturation of the detector or electronics may occur. In the lower signal range, the noise floor will limit the minimum measurable quantity. Also in the middle of the dynamic range there may be effects influencing the linearity of the response. This is especially important in fluorescence measurements where the signal levels between reflected and emitted light can be several orders of magnitude. A set of neutral density filters, calibrated for transmittance; reflectance panels, calibrated for reflectance; or apertures; calibrated for areas can be used to characterise the non-linearity of the response of the detector. A function based on a polynomial can be usually fitted for linearity correction over the tested range.

### 3.4. Geometry: angular alignment and distance

With bidirectional measurements, care should be taken with sample alignment. Both the angle of the sample and the distance from the detector may cause significant errors. At  $0^\circ:45^\circ$  geometry, a  $0.5^\circ$  error in the angle between the sample normal and the viewing direction of the detector can lead to about 1% error in the measured value.

There are several methods to align the sample accurately, including: using back reflection from the sample (not possible with diffusely reflecting samples), using a pre-aligned sample holder, using pre-aligned cameras and optics, using a line projector and a stereo vision camera setup, or by covering the sample with a lens paper and using a flat mirror in contact with the sample rim.

Sometimes sample surfaces, which are expected to be flat, are slightly curved. This may cause angular errors in the measurements when the irradiated area of the sample is changed.



### 3.5. Polarisation

A spectrophotometer's detection system may contain polarisation sensitive components, such as mirrors or diffraction gratings. In this case, the measurement is affected by polarisation changes induced by the sample. The fluorescence emission from the sample is unpolarised, however, the reflected component is generally partially polarised, especially when the sample is glossy. This effect is minimised with the sphere geometries, but may affect the measurement results with bidirectional geometries unless accounted for.

### 3.6. Zero reading and stray light

The detector registers a signal even with no reflectance or emission from the sample. Thus, the dark reading needs to be subtracted from every detector reading. The main component in the detector dark reading is stray light, which is the light scattered from different optical elements on the optical path and making its way onto the detector. The stray light should be characterised as a function of both excitation and emission wavelengths.

### 3.7. Wavelength error

Wavelength errors will lead to errors in the absolute values of the measured quantity, so the instrument's wavelength scale should be checked. Several wavelength standards are available: spectral lamps, reflection tiles or transmission filters. The user must use the most appropriate for the instrument type.

### 3.8. Spectral resolution and bandwidths

The spectral bandwidth used in recording the fluorescence spectrum has an effect on the acquired spectral values, as it broadens and flattens spectral variations. The fluorescence emission spectrum is generally broad and thus the instrument bandwidth effects are small. The bandwidth of the emission monochromator of the instrument should be equal to or smaller than the bandwidth of the excitation monochromator in order to minimise the errors in measured reflectance values in the overlap area of the excitation and emission ranges of the sample.

The instrument bandwidths need to be taken into account in the calculation of the bispectral luminescent radiance factors. Furthermore, the slit scattering functions should be known and accounted for. Bandwidth should be consistent with spectral sampling interval and 1 nm bandwidth should be used for the most accurate measurements. However, for most practical purposes, measurements with 5 nm or 10 nm interval and bandwidth are sufficient.

### 3.9. Instrument induced fluorescence

Some optical components e.g. order sorting filters are known to be slightly fluorescent. This error can be a significant contribution when relatively low fluorescent signals are being measured. A non-fluorescent sample can be used to characterise these effects.

### 3.10. Sample related errors

Samples are not 100% uniform and isotropic across their surface; therefore, the measurement result is an averaged value within the area covered by the irradiated area. To reduce this effect, it is advisable to average readings at different areas and to test whether rotating the samples around its surface normal produces different readings.



#### 4. Representation of measurement data

The data describing the reflectance and fluorescence emission of a sample should be presented as a matrix, which tabulates the bispectral radiance factors as a function of excitation and emission wavelengths. The excitation wavelengths should be tabulated in the vertical direction and the emission wavelengths in the horizontal direction. The reflected radiance factors are the diagonal elements, where excitation and emission wavelengths are equal, and the bispectral luminescent radiance factors are the off-diagonal elements.

#### 5. Uncertainties

A measurement result is not complete without an accompanying statement of its uncertainty. At least the following contributions to the uncertainty budget should be evaluated:

- white standard uncertainty,
- linearity uncertainty,
- dark level and stray light uncertainty,
- wavelength uncertainty,
- bandwidth and slit scattering functions,
- repeatability,
- uncertainty of geometry,
- spectral responsivity,
- polarisation.

The combined uncertainty should be evaluated as the contribution of all these components. The determination of total radiance factor uncertainties can be carried out by following the current guidelines for uncertainty evaluation in metrology (Guide to the expression of Uncertainty in Measurement, GUM).

#### References and further reading

1. CIE 182:2007 *Calibration Methods and Photoluminescent Standards for Total Radiance Factor Measurements* (Commission Internationale de l'Eclairage, Geneva, Switzerland)
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3. Donaldson R 1954 Spectrophotometry of fluorescent pigments *Br. J. Appl. Phys.* **5** 210–4
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5. Zwinkels J, DeRose P and Leland J 2014 *Spectrophotometry: Accurate Measurement of Optical Properties of Materials* ed Germer T, Zwinkels J and Tsai B (Elsevier) pp 221–90
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7. ISO 2469:2014, Paper, board and pulps – Measurement of diffuse radiance factor (International Organization for Standardization, Geneva).