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# Goniofluorometer for characterization of fluorescent materials

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A goniofluorometer has been built that is capable of measuring in various viewing angles ranging from 10° to 90°. The incident angle can be varied from 0° to 8°. The goniofluorometer can measure bispectral luminescent radiance factors in the wavelength range of 250–800 nm. To our knowledge, there are no other reported results of similar devices capable of spectral measurements in various measurement geometries. © 2008 Optical Society of America

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## 1. Introduction

The color of a fluorescent specimen illuminated by standard illumination and viewed by a standard observer is defined by its tristimulus values. The source and observer dependent tristimulus values can be calculated from the source and observer independent Donaldson radiance factors of the specimen. The Donaldson radiance factors can in turn be estimated from the bispectral luminescent radiance factor data according to standard practice [1].

Most existing instruments, capable of measuring the bispectral luminescent radiance factors, use fixed measurement geometry [2–4]. The presently recommended geometry is 45°/0° (or equivalent 0°/45°). However, it is also recommended that the spectral and geometric conditions of fluorescent color measurements should resemble those of the anticipated use of the samples [1]. Furthermore, various fields of industry have different needs for measurement geometry, such as, e.g., 0°/10° in the biochemical analyzer industry. To meet these needs, we have developed a goniofluorometer for the calibration of fluorescent standards. The instrument can measure bispectral luminescent radiance factors in the wave-

length range of 250–800 nm in various measurement geometries.

The structure of this paper is as follows. In Section 2 the design of the goniofluorometer is introduced. The calibration and characterization of the instrument are described in Section 3. Section 4 presents an example of measurement results and a preliminary uncertainty budget. Finally Section 5 discusses the features and drawbacks of the device and compares the instrument with other existing similar measurement devices.

## 2. Measurement Setup

The goniofluorometer is designed to perform fluorescence measurements with an illumination angle between 0° and 8° and a viewing angle between 10° and 90°. The instrument consists of an excitation system for sample irradiation and excitation, a sample holder system, and a collection system to detect the radiation (Fig. 1). The setup is integrated in the existing gonioreflectometer [5,6].

### A. Excitation System

The excitation system comprises two optional light sources, a double monochromator, polarizer, and the required optics. A 450 W xenon arc light source is used at the excitation range of approximately 250–450 nm and a flat-filament 50 W quartz-tungsten-

halogen lamp can be used for excitation above 400 nm. The light from the halogen lamp is collected by a spherical mirror (focal length 125 mm) and coupled into an input slit of the double grating monochromator (DMC). Light from the xenon lamp is coupled on another input slit of the DMC, and the selection between the light sources is made by turning a mirror inside the monochromator just after the input slits (Fig. 1). Both light sources are overfilling the input slits. The halogen output is quite stable. The signal drift is measured to be 0.2% within 1 h. The xenon is considerably less stable, and the drift of the output power is measured to be approximately 1.5% within 1 h. The DMC consists of two 300 mm focal-length and  $f/4.1$  aperture-ratio monochromators in a Czerny–Turner configuration [7]. Both monochromators are equipped with a grating turret that holds three diffraction gratings, but only two are used in the fluorescence measurements. A 1800 lines/mm holographic grating blazed at 250 nm is used in the UV region, and a 1200 lines/mm ruled grating blazed at 500 nm is used for the visible region. The dispersion of the gratings are 1.8 and 2.7 nm/mm, respectively. The maximum slit width of the monochromators is 10 mm, but the slit width of 2 mm is routinely used in the measurements resulting in a 3.6 or 5.4 nm bandwidth depending on the used grating. The output slit of the second mono-

chromator is kept  $\sim 20\%$  wider for better tracking stability. The device is equipped with toroidal mirrors for better imaging properties than in [5].

As shown in Fig. 1 the light emerging from the monochromator output slit is collimated by a  $90^\circ$  off-axis parabolic mirror (OPM) and is steered toward the sample with a flat mirror. Part of the light is directed to a monitor detector (MD) by a beam splitter. The MD comprises a silicon photodiode and a preamplifier in a specially made housing. Its function is to monitor possible fluctuations in the measurement beam power. The polarizer is a motorized wheel with two sheet polarizers for both UV and visible wavelength ranges. The transmission axes of the sheets are in orthogonal orientations. The extinction ratio of the polarizer sheets has been measured to be less than  $3 \times 10^{-3}$  between 300 and 450 nm for the UV sheets and between 400 and 650 nm for the sheets for visible radiation. At 700 nm the extinction ratio is still less than  $5 \times 10^{-3}$ , but above 700 nm the efficiency of the visible sheet polarizers drops, and at 800 nm their effect is negligible. Below 300 nm the transmittance of the UV sheets drops rapidly also increasing the extinction ratio. A baffle and two 10 mm iris diaphragms are used to image a collimated uniform circular  $\sim 12$  mm diameter beam on the sample surface at  $\sim 1$  m distance from the OPM so that sharp edges are formed.

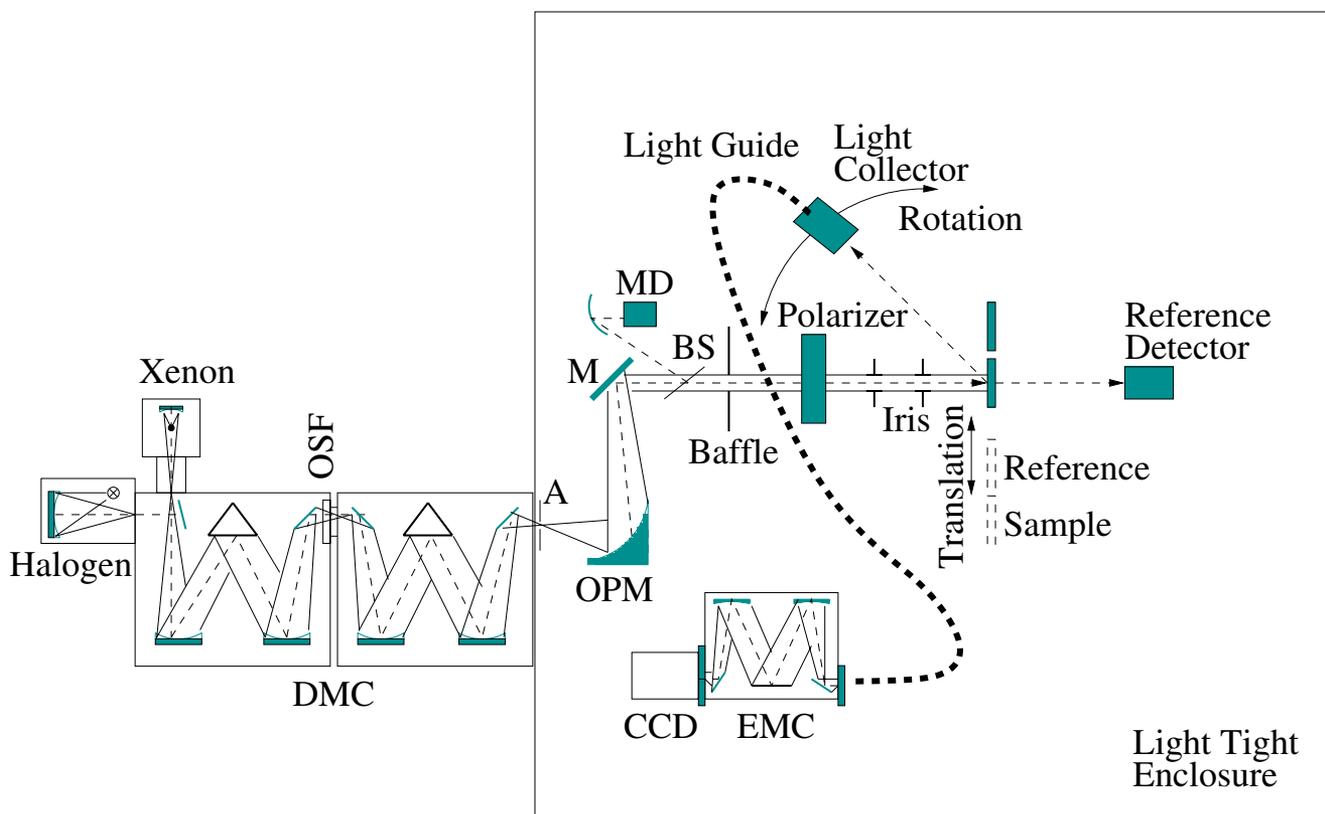


Fig. 1. (Color online) Schematic of the goniofluorometer setup: OSF, order-sorting filter; DMC, excitation monochromator; A, aperture; OPM, off-axis parabolic mirror; M, flat mirror; MD, monitor detector; BS, beam splitter; CCD, charge-coupled device; and EMC, emission monochromator.

## B. Sample Holder System

The sample holder unit consists of a sample holder and a reference holder for a white nonfluorescent reference mounted on a linear translator (Fig. 2). Linear translator 1 is used to select the measurement target, the sample or the reference, and to move both targets out of the beam path for measurement of the full beam intensity or CCD dark. A sample turntable is employed to select the illumination angle, and linear translator 2 is used to align the reference sample surface at the axis of rotation of the sample turntable. Linear translator 3 aligns the fluorescent sample surface at the same plane with the reference sample surface. Linear translator 1 moves from left to right along the plane of the paper surface, and linear translators 2 and 3 move in the direction perpendicular to the plane of the paper.

## C. Collection System

The collection system comprises a detector turntable, a reference detector, a light collector, a light guide, and an emission spectrometer. The reference detector is a Hamamatsu S1337 silicon photodiode with a dedicated transimpedance amplifier. The detector is used inside a black-anodized aluminum housing including a 25 mm aperture and a fused silica lens (focal length 100 mm). The lens focuses the light in such a way that the photodiode is underfilled. The responsivity calibration of the reference detector is traceable to cryogenic radiometers via comparison to a trap detector [8]. The reference detector and the light collector are mounted on opposite sides of the cantilever on the detector turntable. At the reference position, the reference detector faces the incoming light at  $0^\circ$ , and the light collector faces the sample holder at  $170^\circ$  relative to the reference detector.

The light collector includes a 34.5 mm diameter aperture and a fused silica lens (focal length 75 mm). It is used to collect a defined cone of the emission radiation into the front end of a light guide (4 mm diameter, numerical aperture 0.22). The light collector includes a fiber adapter that is used to fix the light guide in its position. An additional holder is em-

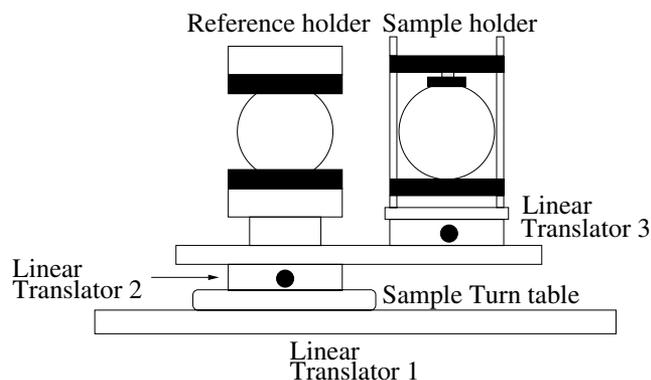


Fig. 2. Sample holder system.

ployed to fasten the light guide firmly in place so that it cannot move inside the adapter. Possible interreflection errors between the lens and the fiber front end are taken into account by the collection system calibration (see Section 3). The light guide has high spectral transmittance in the wavelength range of 250–800 nm. The rear end of the light guide is rectangular in shape (1.25 mm  $\times$  10 mm) and is mounted on the input of the emission spectrometer producing a uniform illumination of the spectrometer input slit. The length of the fiber is 2.5 m. The degree of polarization of the radiation emerging from the light collector and the light guide combination is less than 5% when the input radiation is linearly polarized. The degree of polarization has been measured between 350 and 800 nm. Most of the fluorescence emission of the samples, whose excitation and emission ranges fall in the wavelength range of our goniofluorometer, occurs between this wavelength region.

The emission spectrometer comprises a 200 mm focal length,  $f/4$  aperture-ratio single grating monochromator in Czerny–Turner configuration [9] and an open electrode CCD detector [10]. The grating turret of the emission monochromator (EMC) can hold two gratings, but only one is used. It is a ruled 600 lines/mm grating with an 8 nm/mm dispersion and blazed at 300 nm. The CCD has 1024  $\times$  255 pixels (pixel size 26  $\mu\text{m}$   $\times$  26  $\mu\text{m}$ ) and a spectral responsivity range from 200 to 1000 nm. The minimum achievable dark current is 0.0017 electrons/pixel/s at  $-87.7^\circ\text{C}$  but increases with increasing temperature. The dynamic range of the CCD, defined as the pixel well depth divided by read noise, is approximately 120,000. The CCD surface is mounted on the focal plane of the output of the monochromator with a dedicated adapter. The CCD detector views a 200 nm wide spectrum, but only 100 nm can be used reliably because of the optical configuration of the present monochromator. The monochromator is supposed to be flat field as specified by the manufacturer, but in reality the focal plane has some angular dispersion. Only a range of 100 nm is focused accurately on the CCD surface, and the rest of the spectrum, 50 nm on both sides, must be discarded.

The detector turntable [11] is used to select the viewing angle at the light collector (angular resolution  $0.01^\circ$ ). The sample turntable (Fig. 2) is positioned so that the axes of rotation of both the detector and the sample turntables coincide. When the sample turntable is turned (angular resolution  $0.1^\circ$ ), linear translator 3 is used to move the sample surface back to the axis of rotation of the sample turntable. The range of linear translator 3 is 17 mm, which at present limits the illumination angles to approximately  $0^\circ$ – $8^\circ$ . The viewing angles are only limited by the size of the light collector so that it does not shadow the incident light beam.

## 3. Measurement Procedure

The alignment procedure of the goniofluorometer is described first. Then the calibration of the device is

discussed, and finally the measurement procedure is presented briefly.

#### A. Alignment

The alignment of the goniofluorometer system is done very much along the lines described in [5]. A two-beam alignment laser is used to define the optical axis of the system to pass through the axis of rotation of the detector turntable. The laser, linear translator 1 (Fig. 2) and a horizontal slide are used to align the axis of rotation of the sample turntable to coincide with the axis of rotation of the detector turntable and a point on the optical axis. The horizontal slide is used to provide movement for the sample turntable in the direction of the optical axis. Linear translators 1 and 2 (Fig. 2) and the laser are used to align the plane of the reference standard surface to lie on the rotation axis of the sample turntable. The fluorescent sample surface is aligned in the same way, but using linear translator 3 instead of 2. The reference detector is aligned at the reference position so that the optical axis of the system crosses through the center of the aperture in the reference detector system.

The light collector is aligned for  $170^\circ$  relative to the reference detector. This is achieved by rotating the detector turntable  $170^\circ$  clockwise relative to the reference position. This means that the reference detector faces the sample holder at  $-170^\circ$  relative to the reference position. After that, the light collector at approximately 300 mm distance from the sample is aligned to look directly at the incoming light ( $0^\circ$  incidence), with the help of the alignment laser.

#### B. Collection System Calibration

The wavelength and responsivity calibrations are done by illuminating a nonfluorescent reference standard for which the reflectance is known and by measuring the reflected beam with the emission spectrometer. Responsivity calibration is done separately for each sample characterization session.

In the wavelength calibration the spectral dispersion of the emission spectrometer is measured separately for all desired EMC grating positions. This is done by setting the EMC at the desired wavelength and scanning the excitation monochromator (DMC in the source system) for 120 nm near this wavelength in 20 nm steps. For each DMC wavelength setting, the signal corresponding to the reflected beam from the reference standard is read from the CCD, and the peak position of the signal is determined by fitting a quadratic polynomial to the measured data points. Spectral dispersion (nm/pixel) is calculated from the measured peak positions and the DMC wavelength settings. This is done for all desired EMC positions. Finally the spectral dispersion is plotted against the EMC positions, and spectral dispersion is calculated as a function of the EMC wavelength. The measured spectral dispersion was found to be the same as when calculated from the manufacturer given specifications of the CCD and EMC

(0.208 nm/pixel). The absolute wavelength scale of the monochromators is calibrated against known mercury spectral lines. Except for the absolute calibration of the monochromators, the wavelength calibration procedure is fully automated and controlled by a personal computer.

The spectral responsivity calibration of the collection system is done in two steps. In the first step spectral responsivity (CCD counts/W) is measured for center pixel columns of the CCD. This means that the DMC and the EMC are set at the same wavelength, and the signal reflected from the known reference is measured at the CCD. Full intensity of the beam is measured by the reference detector whose spectral responsivity is known. The DMC and EMC are scanned synchronously over all desired wavelengths. The CCD center pixel column responsivities relative to the responsivity at 500 nm are plotted in Fig. 3 as a function of the DMC and EMC wavelengths (center wavelength). The difference between the responsivity for two polarizations at 500 nm is approximately 10%. For clarity, error bars are shown only for  $p$  polarization. The uncertainty estimate includes the emission monochromator wavelength uncertainty and the standard deviation of multiple measurements.

The second step of the responsivity calibration accounts for possible differences between responsivity of individual pixel columns. Now the DMC wavelength is fixed, and the EMC is scanned over the desired range of the CCD. Signals are recorded at the reference detector and at the CCD for each EMC position, and the responsivity is calculated for each pixel column. The CCD responsivity at different pixel columns with the same DMC setting are compared with the responsivity of the center pixel (DMC and EMC at the same wavelength setting). A relative correction is calculated with the center pixel normalized to 1. This procedure is repeated for all desired DMC wavelengths in 10 nm steps. The calibration results in a relative correction matrix for all emission and excitation wavelengths. Together with the responsivity of the center pixel columns, calculated in the first step, the correction matrix gives the relative spectral responsivity of the emission system. Responsivities relative to the center wavelength for a few DMC wavelengths are plotted as a function of the EMC wavelength in Fig. 4. The values in the figure are for  $p$  polarization, but within uncertainties they are the same for  $s$  polarization. The other polarization has been left out of the figure for clarity. The uncertainty of the calibration is approximately 2% ( $k = 1$ ), and it includes the emission monochromator wavelength uncertainty and standard deviation of multiple measurements.

#### C. Fluorescence Measurements

The measurement procedure is depicted in Fig. 5. The DMC and the EMC are set at the first excitation wavelength, and the reflected radiance is measured at the CCD from the fluorescent sample and from the

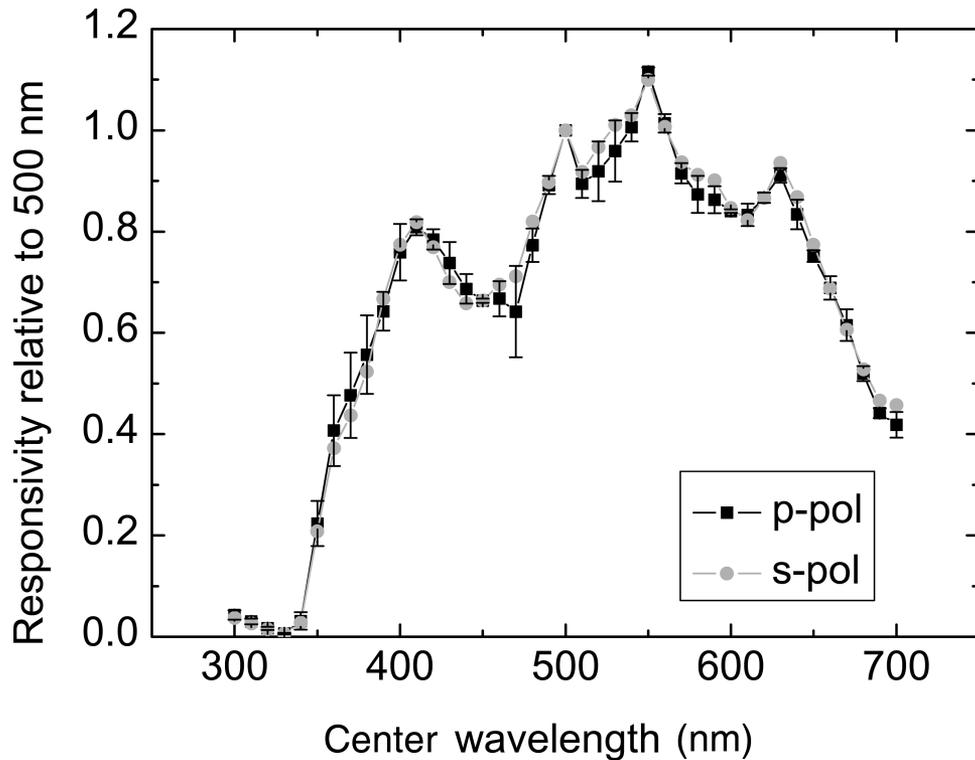


Fig. 3. Collection system responsivities at the center wavelength (both monochromators at the same wavelength) relative to the responsivity at 500 nm.

nonfluorescent reference sample. Then the EMC is scanned in 100 nm intervals, and the luminescent radiance from the sample is read by the CCD for the whole emission range. The dark signal is measured after each signal reading of the CCD by moving the sample and reference out of the beam path. Dark is automatically subtracted from the corresponding measurement signal reading. This procedure is repeated for all desired excitation wavelengths. The

100 nm regions are measured partly on top of each other, and they are merged together by averaging the outermost 5 nm of the adjacent regions.

#### 4. Measurement Results

The Donaldson radiance factor,  $D(\mu, \lambda)$ , is approximately equal to the ratio of the specimen radiance within the rectangular waveband of width  $\Delta\lambda$  centered at  $\lambda$  to the radiance of the perfect reflecting diffuser when each is irradiated over the rectangular waveband of width  $\Delta\lambda$  centered at  $\mu$  [1]. The Donaldson radiance factors can be estimated from the bispectral radiance factors that are a combination of reflected and luminescent radiance factors. The bispectral luminescent radiance factor,  $\beta_{L\mu}(\lambda)$ , is defined as the ratio of fluorescence radiation at  $\lambda$  from the sample per unit emission bandwidth when illuminated at excitation wavelength,  $\mu$ , to the radiation from a perfectly reflecting (nonfluorescent) diffuser illuminated and viewed identically to the fluorescent

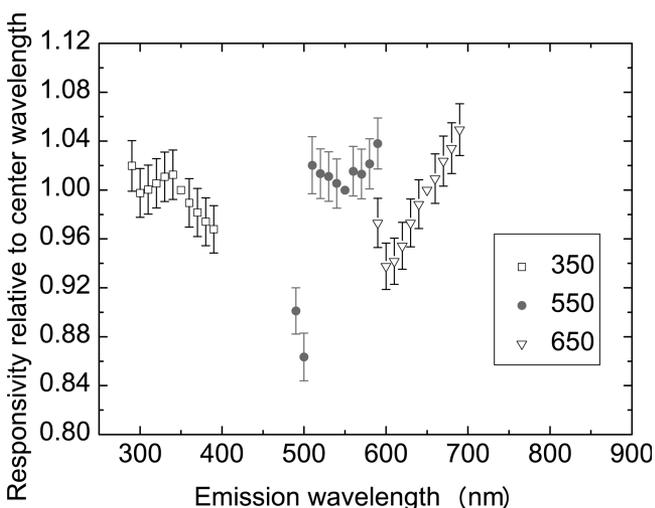


Fig. 4. Responsivities relative to the center wavelength (both monochromators at the same wavelength) for *p* polarization as a function of the EMC wavelength. The symbols 350, 550, and 650 indicate the excitation monochromator wavelength in nanometers.

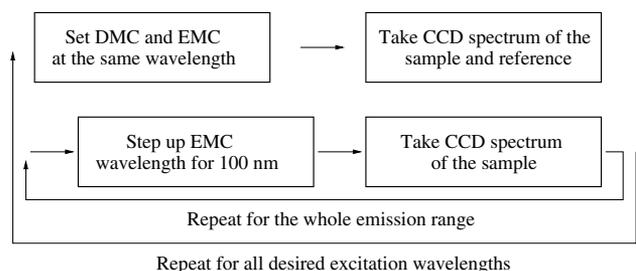


Fig. 5. Measurement procedure.

sample. It can be calculated from the measurement results of our goniofluorometer by

$$\beta_{L\mu}(\lambda) = \frac{L_{f\mu}(\lambda)/\Delta\lambda}{L_r(\mu)/\beta_r(\mu)} = \frac{L_{f\mu}(\lambda)\beta_r(\mu)}{L_r(\mu)\Delta\lambda}, \quad (1)$$

where  $L_{f\mu}(\lambda)$  is the fluorescent radiance from the sample at emission wavelength  $\lambda$  when irradiated at excitation wavelength  $\mu$ ,  $\Delta\lambda$  is the bandwidth of the collection system,  $L_r(\mu)$  is the reflected radiance from the nonfluorescent reference sample at excitation wavelength  $\mu$ , and  $\beta_r(\mu)$  is the known radiance factor of the reference sample. All these quantities [except for the radiance factors,  $\beta_r(\mu)$ ] are measured during the measurement procedure after the calibration of the collection system as described in Section 3. The radiance factors  $\beta_r(\mu)$  of the reference sample are measured separately for the desired geometries with our gonioreflectometer [6].

Figure 6 presents an example of the bispectral luminescent radiance factors,  $\beta_{L\mu}(\lambda)$ , measured with the goniofluorometer. The measurements have been made in  $0^\circ/10^\circ$  geometry with 5 nm bandwidth. The measured sample is a Spectralon standard [12] with inorganic fluorophores. In the figure, some values from the beginning of the spectrum seem to be missing for higher excitation wavelengths. This is due to the fact that fluorescence is not measured for the first 50 nm above the excitation wavelength. The uncertainty of the values in Fig. 6 is approximately 3.8% based on the uncertainty evaluation given in Table 1.

The first component in the table represents the reproducibility of the measurements. Random noise is

the average noise in the CCD output signal above 400 nm. The next two components, namely, CCD linearity and CCD response uniformity, are uncertainty estimates related to the CCD performance as given by the manufacturer. The response uniformity represents the deviation from the average response of the CCD in fully binned operation, when illuminated with uniform white light. The uncertainty estimate for incident power includes the uncertainty of the responsivity calibration of the reference detector [8] and the effect of light spilling around the aperture in front of the reference detector [6]. The uncertainty in the linearity of the reference detector comprises the nonlinearity of the photodiode and the amplifier. It has been estimated by use of the detector in our transmittance measurement setup [13]. The known transmittances of absorption filters have been measured with various power levels of the beam and various gains of the detector. The uncertainty of the radiance factor of the reference sample applies for all measurement geometries of the instrument using  $0^\circ$  incidence. The illumination and viewing angle uncertainty comes from the precision of the sample and detector turntables and their alignment. The uncertainty caused by stray light has been estimated by comparing the CCD reading of reflection from a nonfluorescent reference standard to the CCD reading when the emission monochromator is set far above the excitation monochromator wavelength, and no signal should be visible. Finally, the wavelength scale uncertainty is determined in the wavelength calibration against mercury lines. A correction polynomial is calculated for the emission monochro-

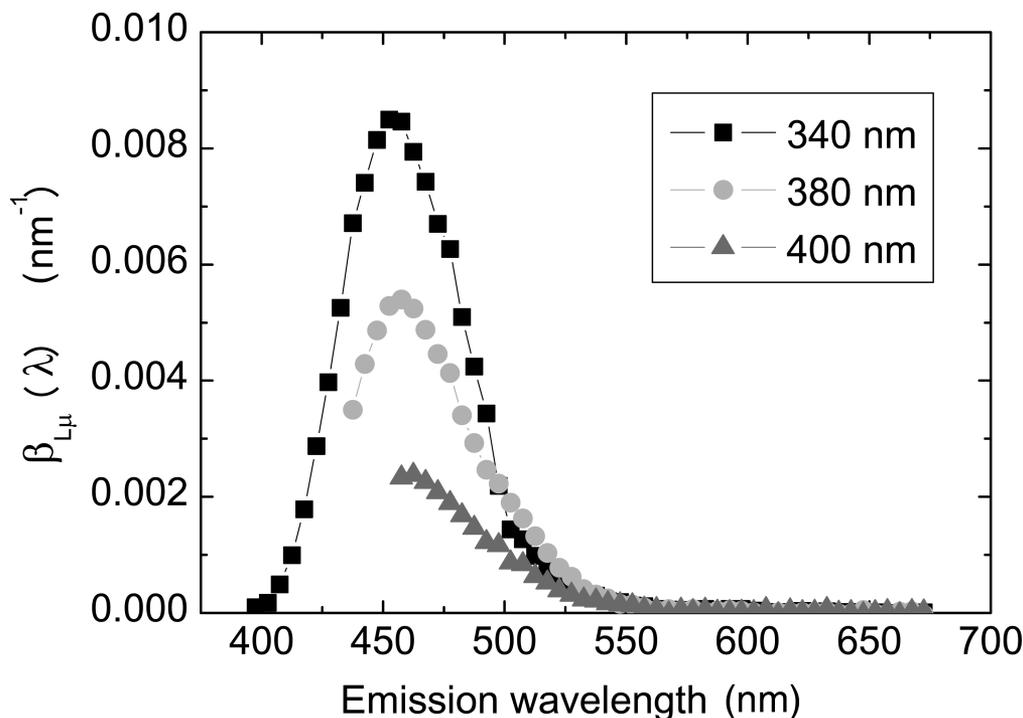


Fig. 6. Bispectral luminescent radiance factors for three excitation wavelengths measured in  $0^\circ/10^\circ$  geometry.

Table 1. Relative Standard Uncertainties in the Measurement of the Bispectral Luminescent Radiance Factor,  $\beta_{L\mu}(\lambda)$ , in Fig. 6

Source of Uncertainty	Type of Uncertainty	Standard Uncertainty	Relative Uncertainty in $\beta_{L\mu}(\lambda)$ (%)
Instrument stability	A	0.15%	0.15
Random noise	A	1.2%	1.2
CCD linearity	B	0.1%	0.1
CCD response uniformity	B	1.6%	1.6
Incident power	B	0.5%	0.5
Reference detector nonlinearity	B	0.04%	0.04
Radiance factor of the reference	B	0.3%	0.3
Illumination and viewing angles	B	0.2°	0.05
Stray light	B	2.5%	2.5
Wavelength scale			
excitation	B	0.1 nm	0.3
emission	B	1.5 nm	2.0
Combined standard uncertainty			3.8

mator, and the uncertainty value estimates the uncertainty in the wavelength when the correction polynomial is used. No correction polynomial is used for the excitation monochromator.

## 5. Discussion

The goniofluorometer can measure bispectral luminescent radiance factors in various measurement geometries within the wavelength range of 250–800 nm. It has several advantages compared to other reported similar devices. For example the device developed for investigation of surface-bound-fluorophore orientation by Barritault *et al.* [14] is capable of goniometric fluorescence detection but is limited to one excitation and emission wavelength only. The reference spectrofluorimeter at National Physical Laboratory (NPL) [15] can only use one incident angle, and the detection angle must be set manually, whereas our goniofluorometer detector turntable is automated with high accuracy in the angle selection. Furthermore, our goniofluorometer uses a lens and a fiber guide to collect and guide a part of the emitted light into the emission spectrometer, whereas the NPL reference spectrofluorimeter uses several mirrors, which would seem to make the alignment process somewhat more problematic. Nevertheless, the NPL approach may increase the possible viewing angles near the incident beam since it avoids the use of a large detection device shadowing the incident radiation. A disadvantage of our goniofluorometer relative to the reference spectrofluorimeter at the NPL is that presently we are limited to measuring only solid samples. However, work is ongoing to expand our measurement capabilities to liquid samples as well. At the same time the range of possible incident angles in the measurements will be increased.

The uncertainty of the goniofluorometer and the speed of measurement can be improved by better choice of equipment and correction procedures. At present the most significant components to the uncertainty come from the EMC wavelength selection and stray light. With a better monochromator the wavelength accuracy and repeatability can be improved close to the level of the excitation monochro-

mator. Also with a proper flat field monochromator the whole CCD active pixel area can be used. This would reduce the measurement time, since a larger area of the spectrum could be seen at the CCD at a time. Also the effect of stray light and reflection over-spill can be better taken into account with a more reliable emission monochromator.

In the near future the goniofluorometer will be tested for fluorescence quantum yield measurements, and the measurement procedure will be changed so that fluorescence is measured also near the excitation wavelength and not only after 50 nm above it. Also thorough validation of the instrument by intercomparison measurements is under preparation.

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