

# The Measurement of Surface and Volume Fluorescence at NPL

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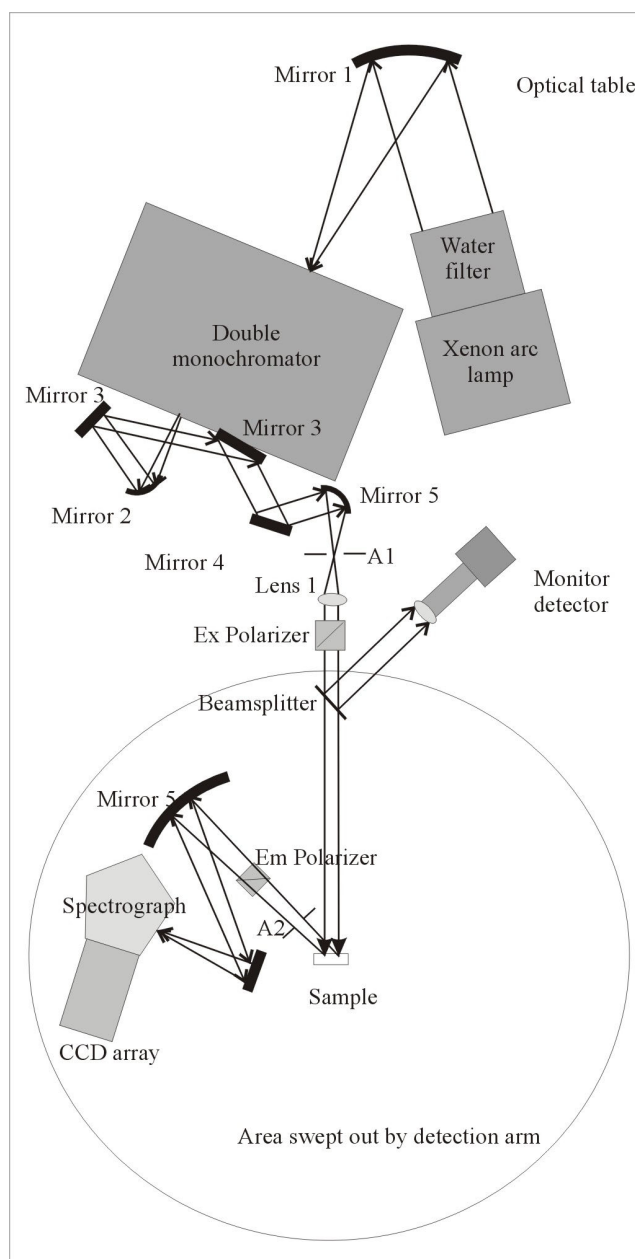
**Abstract.** A spectrofluorimeter for quantifying the fluorescent emission from liquids and transmitting solids as well as opaque surfaces has been developed at the National Physical Laboratory (NPL). The system incorporates a goniometric detection system which allows the measurement of the fluorescent emission and scattered light at any angle to the incident radiation. This paper describes the optical design of the instrument and outlines methods used to calibrate the excitation and detection systems to allow determination of the normalized emission spectrum or total radiance factor and associated colorimetry of a fluorescent material. Uncertainty budgets for the measurements are also presented.

## The NPL Reference Spectrofluorimeter

New measurement requirements, particularly in the biotechnology sector, have led NPL to extend its fluorescence measurement capability. To meet these needs a new reference spectrofluorimeter has been developed to allow analysis of fluorescent emission and scattering in multiple geometries over a range of wavelengths from the UV to NIR [1, 2].

The NPL Reference Spectrofluorimeter (RSF) (figure 1) has a high power xenon source, the output from which is focused onto the entrance slit of a double grating monochromator operated in additive mode. The beam exiting the monochromator is collimated by an off axis parabolic mirror and then steered by three plane mirrors onto a second off axis parabolic mirror which forms an image of the exit slit at a limiting aperture. The width of this limiting aperture sets the bandwidth of the light incident on the sample (usually 5 nm FWHM). A magnified image of the limiting aperture is produced at the sample plane by a lens. Using this optical arrangement the incident beam converges to a focus at the sample plane to form a rectangular patch. The polarization of the incident light, usually set at 45° to the vertical, is fixed by a Glan Taylor polarizing prism in a rotating mount. The last optical component before the sample is a silica window that acts as a beamsplitter, reflecting approximately 8% of the beam to a silicon photodiode trap detector [3] which is used to monitor the optical power incident on the sample.

The system has two different sample mounting stages, both incorporate a reflectance standard that can be measured along with the sample. Opaque reflecting samples are mounted on a linear stage which can be translated vertically to move either the sample or the reference standard into the excitation beam. When measuring volume fluorescence, cuvettes or similarly sized solids are mounted at one end of a rotating arm and the calibrated reflectance standard at the other. Rotating the arm by 180° switches between the sample and reference standard.



**Figure 1.** The NPL Reference Spectrofluorimeter configured for a 0°/45° measurement of surface fluorescence

The detection system is built around an arm that rotates about the point where the sample plane and the incident beam intersect. Light emitted by the sample into a fixed solid angle defined by an iris aperture is focused onto the entrance slit of a single grating spectrograph. The spectrograph entrance slit is imaged onto a Peltier cooled 1024 element CCD array which allows simultaneous measurement of the light spectrum across a 500 nm wavelength band. The range of wavelengths detected by the CCD array can be adjusted by rotating the grating; for

visible measurements this is usually set to be from 300 nm to 800 nm.

## Calibration

In order to determine the normalised fluorescent emission from a sample it is necessary to determine both the relative spectral irradiance at the sample and the relative spectral responsivity of the detection system. Both of these properties are determined using physical transfer standards which have been calibrated at NPL.

The relative spectral irradiance at the sample is determined by calibration of the relative spectral responsivity of the monitor detector. A silicon photodiode of known spectral responsivity [5] is positioned in the sample plane such that it is underfilled by the incident beam. For each excitation wavelength the signal from the calibrated photodiode is compared to the signal from the monitor trap, this ratio is multiplied by the responsivity of the photodiode to give the responsivity of the monitor trap. The voltage from the monitor channel can then be used directly to determine the optical power incident on the sample at any time.

The light spectrum measured by the CCD array is the product of the spectral radiance of the sample under investigation and the spectral responsivity of the detection system (which is itself a product of the throughput of the collection optics, the efficiency of the diffraction grating and the responsivity of the array pixels). By illuminating the detection system with a source of known spectral radiance it is possible to calculate its spectral responsivity and hence normalize any measured sample spectrum. A non-fluorescent Spectralon plaque of measured spectral reflectance [4] is placed at the sample position and the wavelength of the excitation monochromator is scanned across the spectral band over which the detection system is to be calibrated. The detector responsivity is then determined by recording the monitor voltage and the signal at the CCD at each excitation wavelength.

With both excitation and emission parts of the spectrofluorimeter calibrated it is possible to measure the normalized fluorescent emission and reflected scatter from a sample. Other properties can then be determined by calculation as required.

## Volume Fluorescence

Fluorescence measurements are used extensively in biotechnology and pharmaceutical applications, such as drug discovery and biological imaging, diagnostics and research where the fluorescent species is often in solution or a bulk volume. In such cases the fluorescent emission is often measured in a  $0^\circ/90^\circ$  geometry as this configuration minimizes the amount of scatter at the exciting wavelength which reaches the detector.

The bispectral radiance factor of a sample is measured by scanning the excitation monochromator and recording the spectrum of the fluorescent emission at each wavelength. The fluorescent intensity from liquid samples tends to be considerably lower than that from

surfaces (which are often designed to be highly fluorescent) and consequently longer detector exposure times are necessary. Such spectra often contain significant spurious signals due to cosmic rays striking the CCD array and methods have been developed to remove these features.

## Surface Fluorescence

Measurements of surface fluorescence are important in a variety of industries including paper, high visibility clothing and signage. In most cases it is important to characterize the material in terms of how it is perceived by a human observer under different lighting conditions. The total radiance factor of a fluorescent surface is the sum of the reflected and luminescent radiance factors which are measured simultaneously. Measurements are usually made in  $0^\circ/45^\circ$  geometry with excitation and detection over the range 320 nm to 780 nm. Once a full bispectral scan has been carried out the total radiance factor and associated colorimetry can be calculated for any illuminant spectral power distribution.

## Uncertainties

Full uncertainty budgets have been developed to cover the range of measurements made using the RSF. The total combined uncertainties depend upon the nature of the sample being measured and the spectral region of interest. In particular, uncertainties tend to increase in the short wavelength region of the UV. Typically normalized fluorescent emission spectra and total spectral radiance factor can be measured to an uncertainty of better than  $\pm 3\%$  ( $k = 2$ ). There are several dominant components contributing to the overall uncertainty including the linearity of the CCD detector, stray light in the CCD spectrograph and calibration of the transfer standards. Work is ongoing to better understand and correct for these effects and reduce the total combined uncertainty.

## Acknowledgments

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## References

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