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Convenient determination of luminescence quantum yield using a combined electronic absorption and emission spectrometer

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It is possible to measure luminescence quantum yield in a facile way, by designing an optical spectrometer capable of obtaining electronic absorption as well as luminescence spectra, with a setup that uses the same light source and detector for both the spectral measurements. Employment of a single light source and single detector enables use of the same correction factor profile for spectral corrections. A suitable instrumental scaling factor is used for adjusting spectral losses. © *2016 AIP Publishing LLC.* [http://dx.doi.org/10.1063/1.4940234]

I. INTRODUCTION

Luminescence quantum yield (Φ_F) represents the fraction of emitted photons from an ensemble of optically excited fluorophores. It is one of the essential parameters to understand the photochemistry and photophysics of natural processes driven by light. Quantum yield of a fluorophore can be measured in two different ways: relative to a fluorescent standard material with a known quantum yield or as an absolute quantity. The most commonly used method of Parker and Rees[1] for relative quantum yield measurement requires a fluorophore of known quantum yield (standard fluorophores) such as quinine bisulphate, fluorescein (Flu), and Rhodamine B. It also requires information such as absorption and luminescence intensities and refractive indices of the medium as a function of wavelength.[2] Thus, it is important to select an appropriate standard fluorophore with knowledge of instrumental response function for accurate quantum yield measurements. Determination of absolute quantum yield is necessary for the introduction of luminescence standards as well as for situations where relative quantum yields cannot be measured conveniently. Optical methods for the absolute Φ_F measurement are Vavilov reflecting surface method,[3] Weber-Teale diffusing media method,[4] and Mello's integrating sphere method.[5] These methods measure absorption information of fluorophores directly or indirectly using references (reflective surface, diffusing media, or integrating sphere) which are either reflective or diffusive, without losing the excited light.[6,7] Mello's integrating sphere method has gained wide acceptance in recent years. In this method, three measurements are made: (a) signal on direct sample excitation, (b) sample excitation by diffused light, and (c) signal from empty sphere. This eliminates the possibility of polarization effect and the need of refractive index correction. The possibility of reabsorption of emitted radiation because of multiple reflections as well as selection of perfectly reflecting inner coating to maximise "sphere efficiency" is essential for this method.[5] Ishida and co-workers modified the Mello's model using optical fibers to measure absolute quantum yield of liquid and solid samples using a monochromatic light source (LS), integrating sphere, and a diode array detector (DAD). In this design, absorption and emission spectral responses of fluorophore and reference (without fluorophore) are measured, and the ratio of corrected luminescence to that of absorption gives absolute quantum yield.[8–10] The problem of reabsorption because of multiple reflections in the integrating sphere model and the use of scattering, reflecting, fluorescing, or diffusing media as a reference are needed to be avoided for reliable and accurate quantum yield measurements. Both the relative and absolute quantum yield measurements need spectral correction with respect to the light source and detector response. In addition to the above, methods such as photoacoustic, calorimetric, and thermal blooming methods, introduced by various research groups to measure the quantum yield (Φ_F) of a fluorophore,[11] have been useful under specific circumstances.

Though a variety of methods are available for quantum yield determination in solution as well as solid/film state, many of them are either complex or inaccurate. The issues of reabsorption (even at low concentration in integrating sphere model), unavailability of standard fluorophores (relative measurement), inaccuracy in temperature or pressure based measurements (calorimetric and photoacoustic methods) still persist. Thus, introduction of a simple and elegant technique continues to be important. Luminescence emission of the transparent fluorescent samples is isotropic in nature and can be measured by a detector at any orientation with respect to the exciting light source. Luminescence quantum yield can be determined from the number of photons absorbed and emitted by the fluorophores.

Design of a spectrometer which measures both (i) the absorbed photons along the light path and (ii) the emitted photons at right angle to the light path, using a *single light source and a single detector for both the measurements*, can have significant advantages for quantum yield measurements. Such a design would help to minimize the conventional spectral corrections which are inevitable in any quantum yield measurement technique. As a consequence, collection of spectral data can be made facile, and data processing can be a single step process once the correction factor (CF) profile is created. It is to be noted that conventional quantum yield measurements with commercial instruments are done with vendor supplied correction factors and emission integral comparison to a similar absorption/emission standard with consideration of solvents' index of refraction if different. In the

present design, however, the end user can determine the correction factors with a single standard which may have different absorption/emission characteristics. The design also makes it possible to make measurements without repeated comparison to a standard. In this work, we have explored the possibility of such a technique for the determination of quantum yields of fluorophores in dilute and transparent solutions in a single-instrument mode. The present instrument design is expected to offer ease of measurement of luminescence quantum yield.

II. MATERIALS AND METHODS

A. Materials

Fluorescent molecules were purchased from Lambda Physik Goettingen-Germany (coumarin-153 and coumarin-1), S.D. Fine Chem. Ltd. Mumbai-India (9-amino acridine, acriflavine, fluorescein, Rhodamine 6G, Rhodamine B, and riboflavin), and Sigma-Aldrich Co. (anthracene, chrysene, coronene, DL-tryptophan, L-tryptophan, methylene blue, perylene, pyrene, and quinine). Ultraviolet (UV) spectroscopic grade solvents such as cyclohexane (CH), toluene, ethylene glycol, and methanol (MeOH) as well as sodium hydroxide of spectroscopic grade quality were purchased from Sisco Research Laboratories Pvt. Ltd. Mumbai-India. Commercial grade ethanol (EtOH) was purchased from Merck and was purified to the desired level. Triple distilled water (TDW) was obtained from distilled water by distilling it twice from alkaline permanganate solution.

B. Methods

The design of an optical spectrometer for quantum yield measurement used components from Ocean Optics. Components such as sample holder were designed and fabricated at Central Electronic Centre and Physics workshop of Indian Institute of Technology Madras. Details of the design are explained in Section III B. A standard JASCO V630 ultraviolet-visible (UV-VIS) spectrophotometer was used for comparing the absorbance values obtained from the designed optical spectrometer. The concentration of the fluorophores was chosen such that the absorbance is less than or equal to 0.02 at excitation maxima.[12]

III. RESULTS AND DISCUSSION

A. The conceptual basis of quantum yield measurement

Let us consider the interaction of light radiation with a fluorophore in a dilute solution. A small fraction of incident radiation (I_0) is absorbed (I_A) by the fluorophore, and most of the energy is transmitted (I_T) through the analyte. A small fraction of incident radiation is scattered (I_S) and reflected (I_R). Radiation intensity, expressed in watts per square meter, corresponds to the energy being transmitted per unit of time across a unit area that is perpendicular to the direction of propagation,[13,14]

$$I_0 = I_A + I_T + I_S + I_R + I_R'. \quad (1)$$

The reflection (I_R and I_R') contribution is not considered in the following session because it gets corrected easily by using neat solvent while measurement of absorption and fluorescence intensities in difference mode (or spectral correction mode, Equation (4)). The scattering (I_S) contribution is made negligible by proper selection of diluted, transparent samples and normal incidence (incident angle = 90°) of light radiation on the analyte solution in a standard cuvette. Thus, Equation (1) can be modified as

$$I_0 = I_A + I_T. \quad (2)$$

The absorption intensity of fluorophore can be determined from the transmission intensity (I_T), which is the intensity of light transmitted through solvent media taken in a transparent cuvette and illumination intensity (I_0) of the light source,

$$I_A = I_0 - I_T. \quad (3)$$

Transmitted light intensity of fluorophore in a particular solvent (T_F) and that of the same solvent (T_S) is measured in transmission mode. Difference of these transmitted intensities is the same as the absorption intensity of fluorophore (I_A). Absorption intensity measured in a difference mode (Equation (4)) corrects scattering and reflection contribution of the analyte. Similarly, luminescence intensities of the fluorophore were corrected with solvent as follows: the luminescence spectral response of sample (fluorophore in a solvent) was subtracted from reference (solvent),

$$T_s - T_F = I_A. \quad (4)$$

Luminescence emission (I_F) of the fluorophore can also be measured at right angle geometry along with the absorption intensity. All fluorophores in a specific medium have a particular absorption and emission spectrum. Ratio of the areas under the luminescence and the absorption spectral profiles should give Φ_F (Fig. 1).

B. Single beam spectrometer design for Φ_F measurement

Luminescence quantum yield of a fluorophore is its ability to convert absorbed photons into emitted photons.[15,16] So, it is necessary to determine the number of single photons absorbed

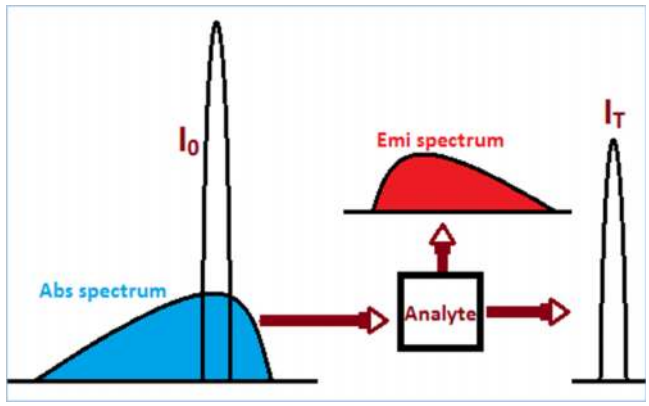

FIG. 1. Schematic representation of the rationale behind the optical spectrometer for simultaneous measurement of absorption as well as luminescence intensities.

by fluorophore molecules and the fraction of emitted photons to obtain the quantum efficiency of the fluorophore. Light radiation on the fluorophores kept in a container (preferably quartz cuvette) causes many electromagnetic interactions such as reflection, absorption, emission, and transmission. The angle of incidence of incident photons was chosen as right angle to the transparent quartz cuvette, which minimizes the possibility of reflection and refraction from the air-quartz interface.[17] The assumptions made in this measurement are (i) the internal reflections from the wall of the cuvette are the same for the transmitting as well as emitting radiations, i.e., reflection losses are the same; and (ii) since the luminescence emission is isotropic, a constant fraction of emitted radiation is always seen by detector in right angle geometry.

The one-detector design is similar to Mello's integrating sphere model without the integrating sphere. For dilute and transparent solutions, the design provides a very simple "one excitation source-one detector" combination that obviates the need of an integrating sphere. The optical design of our single beam spectrometer for the simultaneous determination of transmission/absorption and luminescence intensities of fluorophore in a particular solvent is depicted in Figure 2. The instrument components such as LS (DH-2000-BAL), monochromator (MonoScan-2000), sample holder (S), FOS-2 × 2-TTL dual switch, and a charge-coupled device diode array detector (CCD-DAD, QE65000) were assembled as shown in Figure 2. The Fiber Optic Switch (FOS) has two outputs. These outputs were coming from the sample holder, one carries the transmission and the second one carries emission information. A bifurcated fiber sequentially collects both luminescence and transmission spectral data from the sample holder and guides to the detector via FOS switch. In this way, FOS switch sequentially allows either luminescence intensity or transmission intensity to the detector (D) via bifurcated fiber. This one-way switch was used to control the light path. Optical components such as LS, monochromator, sample holder (S), and detector (D) were connected through SR fibres of 400 μm diameter.

The spectrometer used for the collection of spectral response is QE65000 from Ocean Optics, Inc. The QE65000 is equipped with a back-thinned FFT-CCD detector (Hamamatsu S7031-1006) with a 2-D arrangement of pixels (1044 horizontal × 64 vertical) that is responsive from 200 to 950 nm. The Hamamatsu S7031 provides 90% quantum efficiency, low noise, and low dark signals. It is a 2D array, which allows binning pixels in vertical column to acquire light from entire height of the slit. The charges corresponding to photon intensity is stored at every constant time interval (integration time) and is counted as photon intensity. The data (photon flux) captured by the CCD array are converted to digital information within the detector (using an 18-bit A/D card) and are transferred to a computer via USB port or Universal Serial Bus port and displayed. This spectral readout or the intensity of light is proportional to the amount of charge and, hence, the photons flux.[18] The combination of the spectrometer's low-noise detector and 18-bit A/D converter delivers a dynamic typical range of ~85 000:1 and a signal-to-noise (S/N) ratio of 1000:1 (at full signal). The spectral range of the detector is from 200 nm to 11 000 nm. The entrance aperture size of the spectrometer was chosen as 200 μm (SLIT-200, pixel resolution ~8.9 pixels). The grating used in our QE65000 is HC1-QE of 300 mm^{-1} grove density, and the grating has spectral range from 200 to 1100 nm. Variable long pass order sorting filter, OFLV (DET-QE-OFLV-250), is applied to the detector window to eliminate the second and third order effects. In QE65000, L2 detector collection lens is used to ensure aberration free performance. It is fixed to the detector to focus the light from the tall slit onto the detector elements. The excitation monochromator used in the work is MonoScan2000 to excite the fluorophore molecules. It captures the incoming light, transmits it via fiber to detector, one wavelength at a time. Since the MonoScan has no slit, the diameter size of the optical fiber determines the optical resolution. Since SR fiber of diameter 200 μm was used, the optical resolution of the excitation monochromator was <3 nm (FWHM ~2.5 nm). Optical fibres are connected to the optical components via SMA-905 connector with 74 UV collimating lens of focal length 10 mm, diameter 5 mm, and an entrance aperture of 200 μm. The wavelength range of the instrument is 270–900 nm. The spectral resolution of the instrument is 2.5 nm (FWHM ~2.5 nm) with boxcar of ±5 pixels and integration time of 10 s. This study mainly focuses on the molecules which are absorbing and emitting in the spectral range 270–900 nm. Instrument can be used if the fluorescence maximum is about 750–800 nm and the spectral tail cannot extend beyond 900 nm.

C. Absorption intensity measurement

Optical path of the spectrometer for absorption intensity measurement is shown in Figure 3 (solid line). In this mode, monochromatic light rays are guided through the sample (analyte) and the detector (D) which measures the transmission intensity of the sample. The absorption intensity of analyte was measured as follows. Transmission intensities of solvent with (T_F) and without fluorophores (T_s) were measured. The

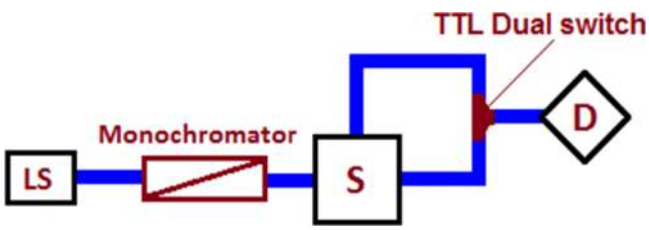

FIG. 2. Optical design of single beam spectrometer for measuring spectral intensities.

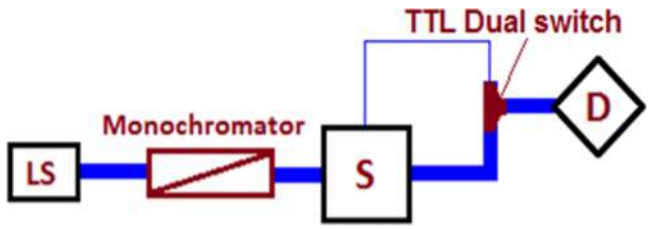

FIG. 3. Optical path of single beam spectrometer mode for absorption intensity measurement (highlighted path).

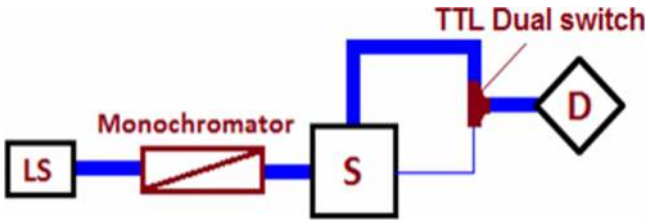

FIG. 4. Optical path of single beam spectrometer mode for luminescence measurement (highlighted path).

absorption intensity of fluorophore in a solvent was obtained using Equation (4).

While measuring transmission intensity, it is necessary to avoid the detector saturation which was made possible by selecting low integration time (10 ms). Luminescence contribution in the transmission intensity of fluorophore (T_F) was also checked by measuring luminescence at right angle geometry using the same instrument parameters and was found negligible (isotropic nature of luminescence).

D. Luminescence intensity measurement

Optical path of spectrometer for luminescence measurement is shown in Figure 4 (solid line). Light rays of particular energy band illuminate the sample through the monochromator, and luminescence emission response of the analyte was collected at right angle geometry using detector, D.

Luminescence intensity of fluorophores was measured at right angle to the excitation signal. While measuring luminescence intensity, scattering contribution was corrected using solvent response in right angle mode. Luminescence measurements were made by selecting the instrument parameters such as integration time of 10 s, spectral averaging of 5, and boxcar smoothening of 5. By choosing the integration time of 10 s, the detector integrates photons for every 10 s at each wavelength. These spectral responses were averaged for five such signals (spectral averaging 5 nm) and displayed. Also, signal smoothing was done over pixels (wavelengths) by selecting boxcar averaging of ±5 pixels. The average of 11 adjacent points was measured as one data point for better smooth signals. Since the boxcar average is 5, 11 pixels (~0.48 nm) were averaged and the effective spectral resolution is about 2.5 nm at FWHM.

E. Measurement of luminescence quantum yield

The ratio of absorption intensity and luminescence intensity in right angle mode of a fluorophore leads to a quantum yield like number. The quantum yield of a fluorophore can be obtained from the ratio by introducing a CF. Since the integrated absorption/emission profile is proportional to the number of photons absorbed or emitted by the fluorophores, the quantum yield (Φ_F) can be expressed as

$$\Phi_F = \frac{\int I_F \, d\lambda}{\int I_A \, d\lambda} \times CF, \tag{5}$$

where $\int I_F \, d\lambda$ and $\int I_A \, d\lambda$ are integrated area under the luminescence and absorption spectra, respectively. The corrections required for the Φ_F measurement are (i) calibration for the solvent medium (wavelength dependency of refractive index), (ii) instrumental measurement geometry induced variations in the absorption and luminescence intensities, and (iii) non-uniform transmission efficiency of monochromatic excitation and non-uniform detector response. Non-uniformities and differences in the spectral response (sensitivity) of the spectrophotometer and spectrofluorimeter detectors are the usual problems in the relative Φ_F measurement and that can be avoided by employing the same light source-detector combination for both absorption and emission measurements.

F. Calibration for solvent: Refractive index correction

"The ratio of fluorescence intensities measured by the instrument is equal to the ratio of the absolute rates of emission of fluorescence by the two solutions because the geometrical arrangement of the specimen and optics is identical for the two measurements. A change in the refractive index of the solution results in a variation in the angles of the rays emerging from a plane cuvette-air interface.[19] Thus, if the two substances to be compared are dissolved in different solvents, the observed intensities must be corrected by multiplying by n^2." In this work, we created an instrument correction profile (ICP), using a fluorophore in water (0.1*N* NaOH). Luminescence intensity is a function of refractive index of the medium (Equation (6)), which is a function of emission wavelength.[20,21] Fluorescence intensity and, hence integrated intensity, any fluorophore in a medium other than the medium used for *ICP* needed to be multiplied by the square of the refractive index at the emission wavelength. So, this wavelength dependency of refractive index is needed to be addressed in the measurement and was used from the existing literatures (Figure 5). The refractive index of solvents, EtOH,[22] MeOH,[23] glycol,[24] CH,[22] toluene,[23] and TDW,[25] as a function of wavelengths is shown in Figure 5,

$$\int I_F \, d\lambda \propto n_\lambda^{-2}. \tag{6}$$

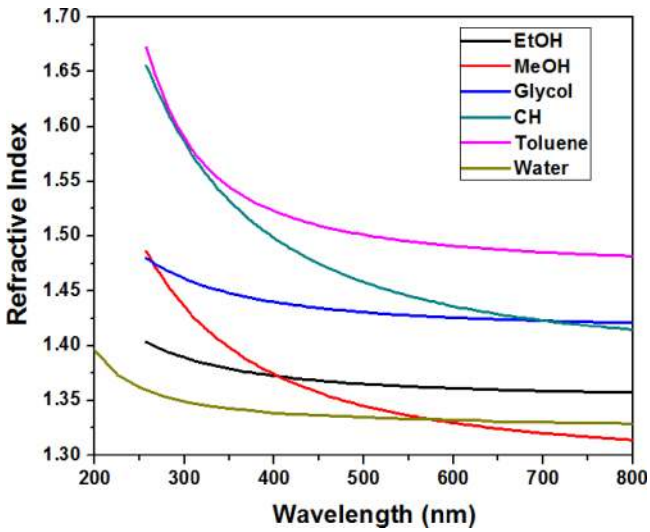

FIG. 5. The dependency of refractive index on wavelength for different solvent media.

The emission intensity correction with respect to solvent medium can be easily obtained from the dependency plots as shown in Figure 5. Thus, this correction can be introduced in the quantum yield measurement equation as well as in the determination of CF. Thus, the equation could be represented as

$$\int I_F d\lambda = k \times \left(\frac{L_\lambda \times A_\lambda \times \phi_F}{n_\lambda^2} \right) = k \times \left(\frac{L_\lambda \times \varepsilon_\lambda \times c \times l \times \phi_F}{n_\lambda^2} \right). \quad (7)$$

So, $\phi_F = \frac{\int I_F d\lambda \times n_\lambda^2}{L_\lambda \times \varepsilon_\lambda \times c \times l \times k}$,

$$\phi_F = \left(\frac{\int I_F d\lambda}{\int I_A d\lambda} \right) \times n_\lambda^2 \times \left(\frac{2.303 \times I_0}{k \times L_\lambda} \right), \quad \because \varepsilon_\lambda \times c \times l = \frac{\int I_A d\lambda}{2.303 \times I_0}, \quad (8)$$

$$\text{or} \quad \phi_F = \left(\frac{\int I_F d\lambda}{\int I_A d\lambda} \right) \times n_\lambda^2 \times ICP,$$

where $ICP = \frac{2.303 \times I_0}{k \times L_\lambda}$, *ICP* is instrument correction profile, ε_λ is the molar absorption coefficient at excitation wavelength, k is a proportionality constant (evidently correction required in the measurement), n_λ is the refractive index of the medium at emission wavelength, L_λ is the excitation intensity (lamp intensity at excitation wavelength), l is the pathlength of the medium, and c is the concentration of the fluorophore.

The proportionality constant, *ICP*, can be determined experimentally and is dependent on the instrumental measurement geometry induced anisotropy in the absorption-emission intensities and non-uniformity in the excitation intensity of light source, transmission efficiency of monochromator, and response of detector.

G. Instrument calibration profile for quantum yield measurement

1. Selection of instrument parameters

Absorption spectra were measured ($I_A = T_s - T_F$) in transmission mode and very low integration time was chosen in order to avoid the detector saturation. Since molecules uniformly emit emission radiation throughout the space (isotropic), detector at the right angle measurement captures only a fraction of emitted radiation. So, a proper correction is required for this large loss of the emission intensity. Instrument parameters such as integration time, spectral averaging, and boxcar smoothening were chosen in such a way so that S/N is greater than 150 for all absorption and emission intensity measurements. The parameters spectral averaging (ave: 5) and boxcar smoothening (boxcar: 5) were kept constant for the absorption as well as emission measurement. The effective spectral resolution is about 2.5 nm at FWHM. The intensity saturation of QE65000 is 64 000 units; in all our measurements, the highest intensity of absorption is kept low as 5% of saturation (maxima of 3500 for very low emitting methylene blue). This was observed as an integration time of 10 s. Fluorescence intensity maxima were about 100, which were observed at 10 ms integration time. Such a low absorption and emission intensities detector shows good linearity in the integration parameter (int. time). The integration time (10 s) was not long enough to use a significant portion of the detector well depth (only 5% of saturation), and hence, the measured intensities did not need any non-linearity correction. The optimized integration times for the measurement of luminescence as well as absorption intensity are 10 s and 10 ms, respectively.

This spectrometer design was also needed to be calibrated with respect to detector response, transmission efficiency of monochromator, and lamp excitation profile. The *ICP* required for such calibration was obtained using any standard fluorophore. In this work, Flu was chosen as the standard fluorophore and its luminescence quantum yield is known at six different excitation wavelengths, viz., 313 nm, 366 nm, 436 nm, 460 nm, 488 nm, and 521 nm.

a. Determination of ICP. Flu in 0.1*N* NaOH was taken as a standard to measure the ratio of luminescence intensity to absorption intensity ($\frac{\int I_F d\lambda}{\int I_A d\lambda}$). *ICP* for the excitation at 488 nm was measured as follows. Absorption intensity of 0.31 μM Flu (Optical Density (OD) = 0.02 at abs maxima) was obtained by subtracting transmission intensity of Flu in 0.1*N* NaOH from that of only 0.1*N* NaOH. Luminescence intensity of Flu at 488 nm excitation was measured in right angle mode in the entire wavelength range. The measured absorption and the scattering subtracted emission intensities of Flu are shown in Figure 6.

Integrated area under the absorption and emission intensities was measured. The ratio of corrected emission ($\int I_F d\lambda$) to the absorption energies ($\int I_A d\lambda$) multiplied by 0.72 to get Φ_F of Flu, i.e., *ICP* at 488 nm excitation is 0.72. The refractive index dependence of the quantum yield is also included as a function of emission wavelength, Equation (8) (n^2 at emission wavelength, 515 nm is 1.78).

Similarly, *ICP* for different excitation wavelengths [factor for the Φ_F] was calculated and is tabulated in Table I. *Absorption and emission* intensities of the same solution were

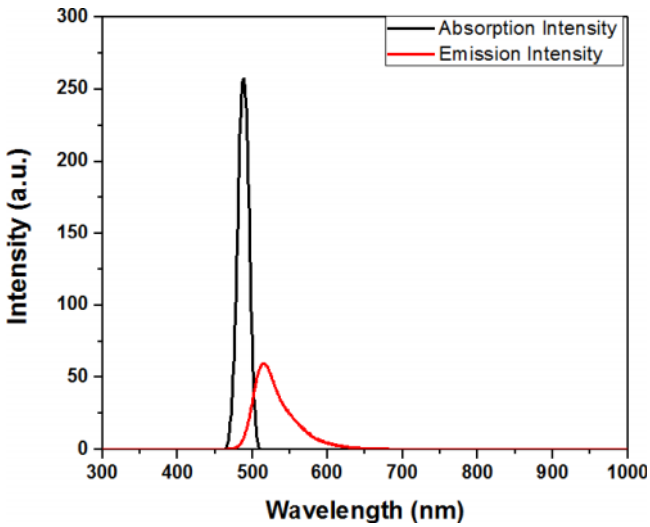

FIG. 6. Absorption and emission intensities of Flu in 0.1*N* NaOH (OD ≤ 0.02) measured at narrow band excitation of 488 nm.

TABLE I. Φ_F of Flu in 0.1N NaOH (OD = 0.02) was measured from the ratio of absorption area to emission area at different excitations such as 313 nm, 366 nm, 436 nm, 460 nm, 488 nm, and 521 nm (n^2 at emission wavelength, 515 nm is 1.78).

λ_{ex} (nm)	$\int I_F d\lambda$	$\int I_A d\lambda$	Ratio	*ICP*	Φ_F (in this work)	Φ_F (Literature)
313	619.6	2569.5	0.2411	1.99	0.86	0.86[26]
366	181.6	498.5	0.3644	1.44	0.93	0.94 ± 0.02[4]
436	438.7	890.6	0.4926	1.04	0.92	0.92 ± 0.04[4]
460	1978.7	2987.5	0.6623	0.74	0.88	0.88 ± 0.03[27]
488	3333.6	4760.5	0.7003	0.72	0.90	0.90[28]
521	549.3	889.2	0.6177	0.84	0.92	0.92 ± 0.04[7]

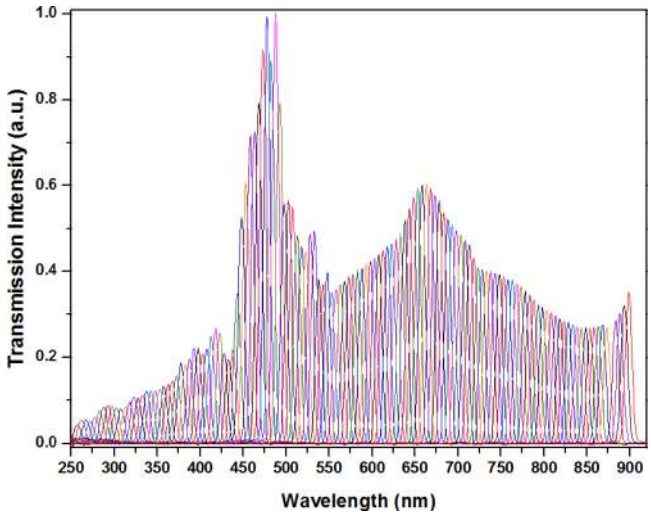

FIG. 7. A plot of normalized transmission intensity against excitation wavelength due to the non-uniform intensity of excitation at excitation wavelength, non-linear transmission efficiency of the monochromator, and spectral response of detector.

measured at five other excitation wavelengths such as 313 nm, 366 nm, 436 nm, 460 nm, and 521 nm and are shown in Fig. S1 of the supplementary material.[29] Correction with respect to excitation wavelength is needed to be generalized for entire wavelength range in order to measure the quantum yield of any fluorophores on different excitations.

2. Wavelength dependence of the instrument response due to light source-monochromator-detector (LMD) combination

The observed luminescence intensity is a function of the three parameters, viz., intensity of excitation at excitation wavelength, transmission efficiency of monochromator, and spectral response of detector. Monochromatic excitation profiles in the spectral range from 260 nm to 900 nm were measured at different excitations. The non-uniformity in the spectral response of a particular LMD combination is shown in Figure 7.

The reciprocal of spectral response of a particular light source-monochromator-detector combination can be used to correct the non-uniformity in the excitation efficiency of light source, transmission efficiency of monochromator, and spectral response of detector (Figure 8). The reciprocal file is called LMD profile, LMD (λ).

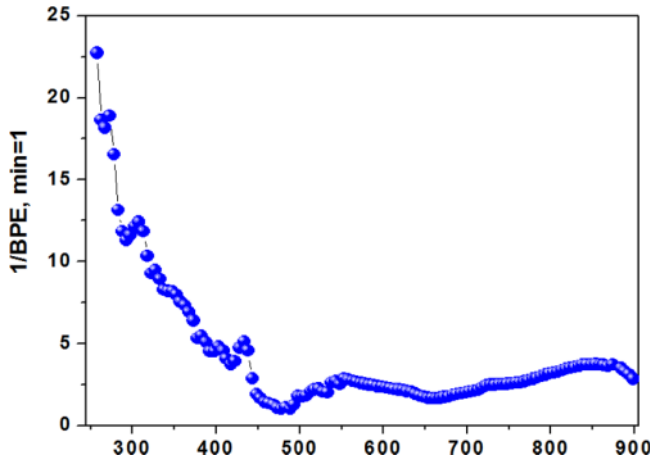

FIG. 8. The profile for transmission intensity correction obtained from the reciprocal transmission intensity, LMD profile (LMD (λ)).

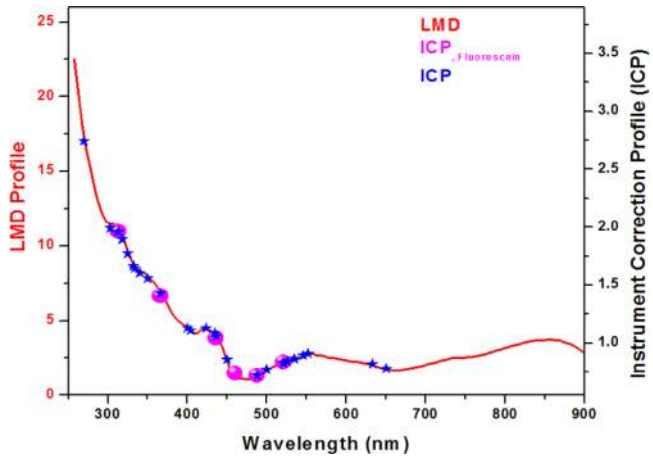

FIG. 9. Correlation of *ICP* from the LMD profile.

3. Evolving ICP from the LMD profile

The *ICP* for the Φ_F measurement shows a wavelength dependency similar to the LMD profile for the monochromatic excitation (Figure 9). Both these profiles, *ICP* and LMD profiles, were carefully analyzed, and thus, an empirical relationship was made between *ICP* and LMD profiles.

The empirical relationship between *ICP* and LMD (λ) was made as follows and $ICP(\lambda)$ was used for the Φ_F measurement (r^2 = 0.9979 and standard deviation = 0.0083):

$$\mathrm{LMD}(\lambda) = 7.7760 \times ICP(\lambda) - 4.2939, \quad (9)$$

$$ICP(\lambda) = 0.1286 \times LMD(\lambda) + 0.5522. \quad (10)$$

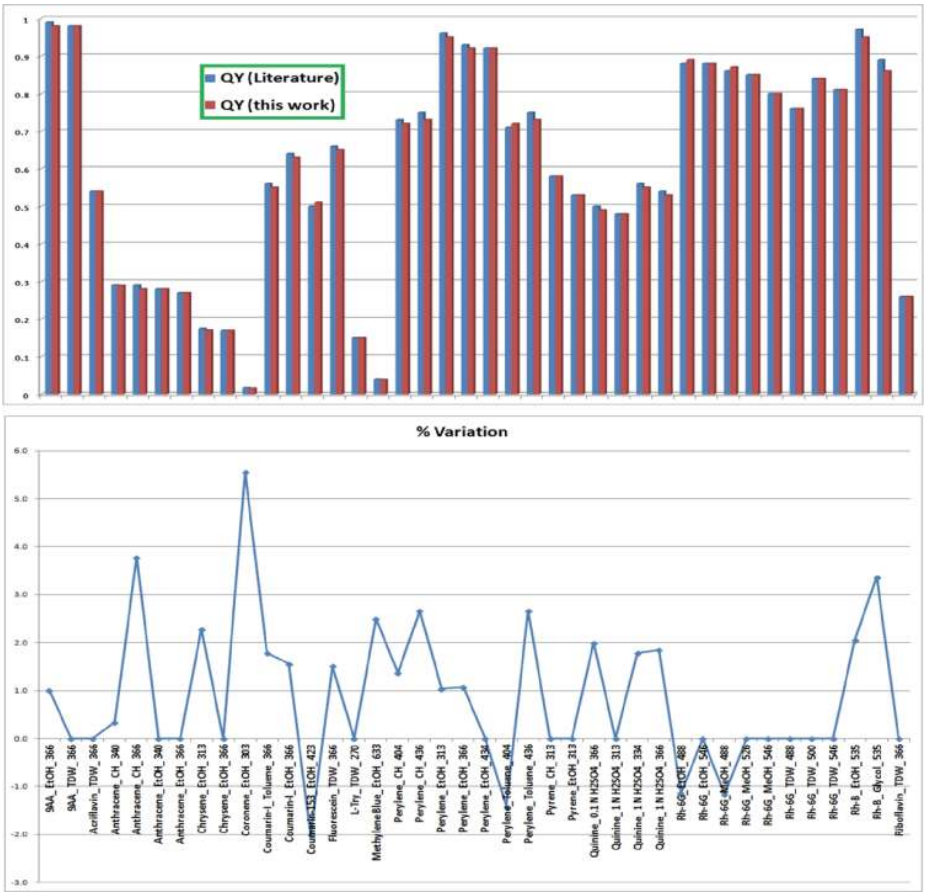

FIG. 10. A statistical comparison of quantum yield measured (this method, red bar) against the literature value (blue bar) is displayed in the top panel, and the percentage variation of quantum yield from its literature value is mentioned in the lower panel.

By comparing Equations (5), (8), and (10), we would reach to our expression (Equation (11)) for the determination of quantum yield. The quantum yield Φ_F is related to the *ICP*, refractive index, absorption, and luminescence intensities as follows:

$$\Phi_F = \frac{\int I_F d\lambda}{\int I_A d\lambda} \times CF = \frac{\int I_F d\lambda}{\int I_A d\lambda} \times n_\lambda^2 \times (0.1286 \times \mathrm{LMD}(\lambda) + 0.5522). \quad (11)$$

Since the quantum yields of different fluorophores are to be measured in a variety of solvent media, correction factor profile was made such that the dependency of refractive index is taken out from the CF and considered as a separate factor. It increases the viability of the method in any solvent medium, provided we must know the refractive index profile (against wavelength) of solvent. In short, this method requires only one *ICP* for any fluorophore in a particular solvent. Also, this empirical relation is required to be validated using a series of fluorophores in different solvent environment at different excitation wavelengths.

H. Validation of the proposed method

1. Selection of fluorophores

The new mode of luminescence quantum yield (Φ_F) measurement was validated by measuring Φ_F of a series of fluorophores. Fluorophores were selected such that they absorb and emit in the ultraviolet-visible region (200 nm–900 nm). Selected fluorophores are 9-amino acridine, anthracene, chrysene, coumarin-I, coumarin-153, methylene blue, perylene, pyrene, quinine sulphate, Rhodamine 6G, Rhodamine B, acriflavine, DL-tryptophan, fluorescein, L-tryptophan, quinine, riboflavin, and coronene. The concentration of fluorophores was selected such that their maximum absorbance does not exceed 0.02 at different excitation wavelengths. At this concentration, the chances of re-absorption are negligible. The concentrations and UV-VIS electronic absorption spectra of the selected fluorophores are shown in Table S2 and Fig. S3 of the supplementary material.[29]

2. Determination of quantum yield

The absorption and luminescence intensities were measured as mentioned in Sections III C and III D, and those absorption and emission spectral data are shown in Fig. S4 of the supplementary material.[29] The *ICP* file created from the lamp-monochromator-detector correction was multiplied by the product of emission/absorption ratio file $\left(\frac{\int I_F\, d\lambda}{\int I_A\, d\lambda}\right)$ and square of refractive index at the emission wavelength (n_λ^2) to obtain Φ_F. The obtained quantum yields for each fluorophores are averaged for two different measurements and are tabulated in Table S5 of the supplementary material.[29] Quantum yield measured using newly developed instrument design shows

a promising output in comparing with the literature values (shown in Table S5 of the supplementary material[29]).

The fluorescence quantum yield of fluorophores in different media at various excitation wavelengths was measured using the proposed method and is tabulated in Table S5 of the supplementary material.[29] A relative statistics is also given in the last two columns of Table S5 of the supplementary material[29] and in Figure 10. The Φ_F values are very comparable with the reported values. Consider the fluorescence quantum yield of quinine (quinine bisulphate in 1*N* sulphuric acid). It has been widely used as a standard in relative quantum yield measurements. In quinine, there is no significant overlap between its absorption and fluorescence spectra. The most commonly used Φ_F values for quinine were given by Melhuish 0.546 at infinite dilution in 1*N* H_2SO_4. The Φ_F value at infinite dilution using integrating sphere was 0.60. Our value (0.56) for the quantum yield measurement of quinine is in good agreement with the value reported by Melhuish. Likewise, a series of molecules' quantum yield were measured in different media. Some of the molecules have vibronic spectral signature, while others are perfect Gaussian, and many of them have overlapping absorption and emission spectra. The quantum yields of probes are also measured at different excitation wavelengths in order to avoid the excitation-emission overlaps. Though the molecules have perfect spectral overlap, we do obtain a reliable quantum yield, which is consistent with the reported values. A statistical comparison of the measured quantum yield with the literature value is shown in Figure 10. The percentage variation of quantum yield from the literature value is also measured and displayed in Figure 10 (lower panel). A few molecules' quantum yield varied a maximum of 6%, but this variation is within the measurement error (±0.03). Rest of the measured quantum yield values are very close to the literature reported values.

In addition to the simplicity in measurement and accuracy in the measured quantum yield values, our method has some merits over existing experimental methods. Conventional Rees method (relative quantum yield measurement) requires refractive index at the emission wavelength, UV-VIS absorption, and luminescence emission spectra of both fluorophore and reference (suitable standard fluorophore). The selection of appropriate standard fluorophore as reference is one of the key steps in Rees method. Also, the spectral measurements were made in different instruments (different optics, light source, and detector response); so, multiple corrections are required, which was avoided by introducing a combined spectrometer. Luminescence quantum yield of fluorophores absorbing and emitting in the different spectral windows requires different references in Rees method. Present method creates an instrument calibration profile, unique to the particular instrument configuration and solvent media, which eliminates the necessity of different references and multiple corrections by introducing a single correction factor file (*ICP*). The knowledge of the ratio of luminescence to absorption intensity, refractive index of medium, and *ICP* of the instrument could easily determine the luminescence quantum yield in a facile manner. Once the correction factor file is created for an instrument, it is valid for any fluorophores in a particular solvent. Though present method uses a fluorophore (any) to create spectral correction factor (*ICP*), which is necessary only when we change any of the instrument components (light source, monochromator, or detector).

IV. CONCLUSION

An optical spectrometer was designed for the determination of the luminescence quantum yield of fluorophores in dilute transparent solutions from the absorption and luminescence spectra followed by the application of experimentally determined CF. The correction factor has two main components: (i) sample contribution (refractive index correction) and (ii) instrument contribution (*ICP*). *ICP* was created to correct the non-uniformity in the illumination profile, transmission efficiency of monochromator, and detector response. *ICP* was obtained as a function of wavelength and is correlated to the LMD profile, which facilitated accurate measurement of quantum yield of fluorophores (Φ_F) absorbing and emitting in the spectral range from 270 nm to 900 nm. It was shown that an empirical relation can be formulated for a particular instrument design for quantum yield measurements. Also, the validity of the design and measurement was verified using a series of fluorophores of known quantum yield which absorb and emit in ultraviolet-visible region. The simplicity in the accurate measurement of quantum yield and low cost of the spectrometer make it reliable as existing methods, perhaps preferred over Rees method and integrating sphere model. The fibre optic compatibility of the design will have additional advantages such as simplicity and low cost in the design.

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[29]See supplementary material at http://dx.doi.org/10.1063/1.4940234 for absorption and emission spectra of fluorescein in 0.1*N* NaOH at various monochromatic excitations measured using single beam UV-VIS spectrometer, concentrations, and UV-VIS spectra of selected fluorophores, absorption, and emission spectra of selected fluorophores at monochromatic excitation measured using single beam UV-VIS spectrometer and measured quantum yield of the fluorophores in different media.