A versatile fiber-optic coupled system for sensitive optical spectroscopy in strong ambient light

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In this work we describe design and use of a fiber-optic based optical system for the spectroscopic studies on the samples under the presence of strong ambient light. The system is tested to monitor absorption, emission, and picosecond-resolved fluorescence transients simultaneously with a time interval of 500 ms for several hours on a biologically important sample (vitamin B2) under strong UV light. An efficient stray-light rejection ratio of the setup is achieved by the confocal geometry of the excitation and detection channels. It is demonstrated using this setup that even low optical signal from a liquid sample under strong UV-exposure for the picosecond-resolved fluorescence transient measurement can reliably be detected by ultrasensitive microchannel plate photomultiplier tube solid state detector. The kinetics of photodeterioration of vitamin B2 measured using our setup is consistent with that reported in the literature. Our present studies also justify the usage of tungsten light than the fluorescent light for the healthy preservation of food with vitamin B2. © *2009 American Institute of Physics.* [DOI: 10.1063/1.3131807]

I. INTRODUCTION

Understanding the photoinduced chemical reactions are very important for the fundamental processes of life. For example, photoinduced electron transfer is an essential step in the conversion of solar energy into chemical energy in photosystems I and II and is also frequently used by chemists to build complex molecules from simple precursor.[1] The conversion reaction is also equally important to understand our vision processes.[2,3] The efficiency of spectroscopic measurement of the photoinduced processes lies in the efficacy of detection of low optical signal in presence of strong excitation light. In order to measure the spectroscopic signature of the chemical processes, very high sensitive detector such as photomultiplier tubes (PMTs) are unavoidable. However, measurement of very low optical signal in presence of strong ambient (excitation) light is very crucial, as the ambient light often saturates the detection of the PMT detectors. Here we report a miniaturized fiber-optic based system for the measurement of light induced chemical/biological processes with spectroscopic precision. In our design we have also considered the compatibility of our designed system with other commercially available spectroscopic instruments including Shimadzu absorption spectrophotometer, Jobin Yvon spectrofluorimeter, and Edinburgh Instruments time resolved fluorimeter.

In order to demonstrate the sensitivity and usefulness of our designed system, we have studied in detail photodegradation of vitamin B2 (riboflavin), which is very important for the preservation of food in right light condition.[4] The photochemical reactions of riboflavin[5–8] are well understood. It has already been demonstrated that the intramolecular electron transfer process is responsible for the photoreduction of the chromophore, riboflavin.[9,10] It has been proposed that two photoproducts, lumichrome and lumiflavin are generated via an intermediate compound deuteroflavin during the photodeterioration of the riboflavin chromophore in presence of oxygen.[10] The role of proton transfer from the ribityl chain to the side nitrogen moiety for the generation of the photoproduct is also identified. In a recent study, it has been established that riboflavin is a potential photosensitizer, and the activated triplet state in the molecule is concluded to be responsible for the photosensitization.[8,11] It is to be noted that the photoproducts of riboflavin are also very hazardous for the biological activity as they degrade important amino acids including the tryptophan and tyrosin.[12]

II. MATERIALS, EXPERIMENTS, AND METHODS

Increased sensitivity in the signal detection (see below) in presence of strong ambient light in our experimental setup lies on the confocal geometry of the excitation and detection sides. As shown in the schematic ray diagram of the experimental setup (Fig. 1) the excitation fiber (400 μM core diameter) carrying laser light is connected to an optical coupler (Ocean Optics, USA, model: 74-UV) in order to focus excitation light at the middle of a sample holder, which is an all-side polished quartz cuvette from Starna cells (USA). The numerical aperture (NA) of the excitation fiber (NA=0.45) and focal length of the optical coupling lens (0.5 cm) systems are to achieve out-of-focus rejection by two strategies: first by illuminating a single point of the specimen at any one time with a focused beam, so that illumination intensity drops off rapidly above and below the plane of focus, and

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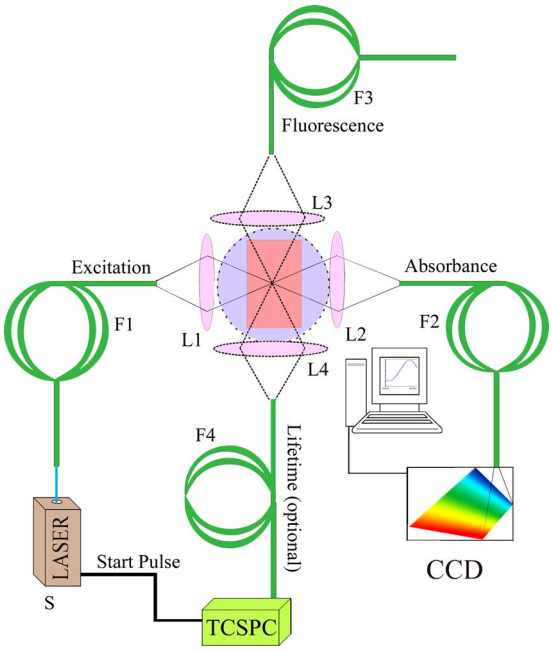

FIG. 1. (Color online) Schematic diagram of the universal setup. S is the source, F1–F4 are the optical fibers, L1–L4 are the fiber coupler lenses, and the violate circle represents the ambient light around the sample (reddish rectangle). CCD is the photosignal detector, connected with computer.

second by the use of optical fiber as a collecting unit with similar coupling lens so that light emitted away from the point in the specimen being illuminated is blocked from reaching the detector. The schematic ray diagram of the experimental setup is shown in Fig. 1. As shown in the Fig. 1, the collection fiber is arranged in I or L geometry and connected with the commercially available *Shimadzu spectrophotometer (UV-2450)* or *Jobin Yvon (Fluoromax-3)* fluorimeter, respectively, in order to measure absorption and photoluminescence, respectively, of the sample of interest. More flexibility in the measurement can also be achieved by using charge-coupled device (CCD) based miniaturized spectrograph (e.g., Ocean Optics, USA, model: HR4000). The spectrograph used in our setup has reasonably higher sensitivity (130 photons/count at 400 nm; 60 photons/count at 600 nm).

In order to establish the fact that the absorption and photoluminescence of a sample can be measured in presence of strong ambient light effectively, we have placed the quartz cell containing aqueous sample on a home-made UV bath (60 W). We have used the same setup in order to investigate the photodeterioration of vitamin B2. The stray-light rejection efficiency of the setup is so efficient that collecting fiber can also be coupled to a microchannel plate photomultiplier tube (MCP-PMT), Hammamatsu, Japan make (solid state PMT) based time resolved (picosecond resolution) spectrofluorimeter from *Edinburgh Instruments, UK*, as shown in Fig. 2. The sensitivity of the MCP-PMT detector with associated electronics is designed to detect single photon from the sample. Insignificantly small offset in the fluorescence transients (see below) measured in this setup justifies our claim of efficient stray-light rejection. The observed fluorescence transients are fitted by using a nonlinear least square

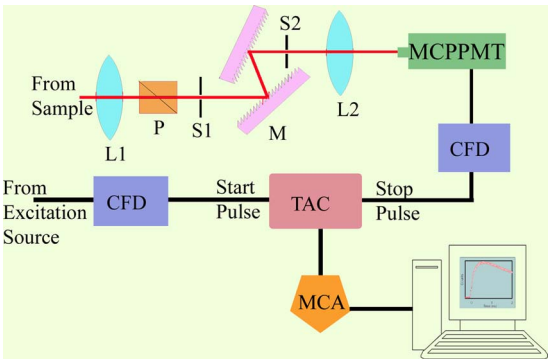

FIG. 2. (Color online) Schematic representation of the time resolved measurement with this universal setup. L1 and L2 are the lenses. P is the polarizer, M represents the monochromator, S1 and S2 are variable slits, MCP-PMT (Hammamatsu, Japan) is microchannel plate photomultiplier tube, CFD is constant fraction discriminators, TAC is the time to amplitude converter, and MCA is the multichannel analyzer (see Ref. 14 for details).

fitting procedure to a function $[X(t)=\int_0^t E(t')R(t-t')dt']$ comprising of convolution of the IRF $[E(t)]$ with a sum of exponentials $[R(t)=A+\Sigma_{i=1}^{N}B_i e^{-t/\tau_i}]$ with pre-exponential factors (B_i), characteristic lifetimes (τ_i) and a background (A).[13] Relative concentration in a multiexponential decay is finally expressed as $C_n=B_n/\Sigma_{i=1}^{N}B_i\times 100$. The quality of the curve fitting is evaluated by reduced chi-square and residual data.

III. RESULTS AND DISCUSSION

The efficacy of the designed instruments is established through our studies on the photodeterioration of the vitamin B2 (riboflavin) in aqueous phase. The ~50 μM riboflavin solution (from Sigma-Aldrich, USA) is prepared using millipore water (resistivity, 18 MΩ). Figure 3 represents the

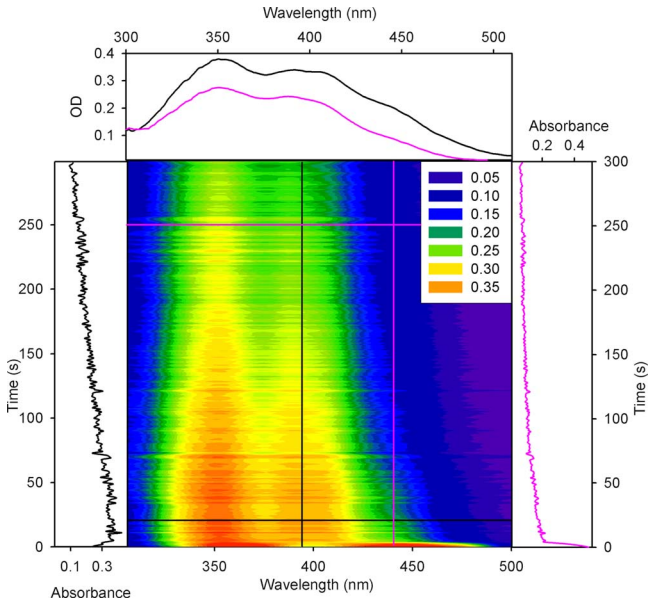

FIG. 3. (Color online) Wavelength (nanometer) vs time (second) contour diagram of the absorbance of riboflavin in presence of UV light. Upper panel of the contour is the full absorbance spectrum at two different times. The black one is at 25 s and the pink one is at 250 s. The right hand side of the contour diagram is the temporal decay spectrum of the species having absorbance of ~450 nm. The left hand side of the contour diagram is the temporal decay spectrum of the species having absorbance of ~390 nm.

time dependent absorption spectra of the sample solution indicating photodamage of riboflavin in presence of UV light (60 W; normally used for water purification). In our studies we have used CCD based spectrograph (Ocean Optics, HR4000). Here we have measured the absorption spectra in the wavelength range of 200–1100 nm with 500 ms interval using SPECTRA SUITE software supplied by Ocean Optics. The minimum time interval for the kinetics measurement (full wavelength measurement) in our present experimental setup is 3 ms. Note that in the present study we are interested to explore the long time photodeterioration of vitamin B2 (for several hours). Thus we set the averaging time for each data point to be 500 ms in order to obtain good signal to noise ratio. However, we have also confirmed that no faster kinetics (around 3 ms) is involved in the photodeterioration process. The two dimensional (2D) plot consisting of wavelength (nanometer) along *X*-axis and time (second) along *Y*-axis is generated in Microcal ORIGIN-7 software. From the Fig. 3 (right side of the contour) it is clear that the absorbance at ~450 nm is decreasing very rapidly with time. This is due to the deterioration of riboflavin to its photoproducts.[10] The rise in the 390 nm component with time clearly indicates the formation of different species.[8] After ~50 s, the decrease in the absorbance at 390 nm again implies the instability of the photoproduct. The spectral property of the photoproduct is consistent with that of lumiflavin. The indication of the generation of lumichrome[10] is reflected in the absorbance ~350 and ~385 nm.[10] The observed rate of formation of lumiflavin is measured to be 0.39 s^{-1}. In presence of light, all the photoproducts initially get distributed in different proportions and finally get degraded with different rates.

The fluorescence spectra of the vitamin B2 solution is also measured in the interval of 500 ms. We have used 409 nm laser diode (Picoquant, Germany) as an excitation source for riboflavin. Figure 4 represents the contour plot of the fluorescence of riboflavin in water under UV-exposure. As evidenced from the figure, the emission peak maxima (~530 nm) are slightly blueshifted in the experimental time window. It is to be noted that the emission of riboflavin is not very simple as the fluorescence is associated with the multiple systems e.g., lumichrome[14–16] and lumiflavin.[16] The small blueshift in the fluorescence spectrum in Fig. 4 may indicate the formation of lumichrome (λ_{max}=500 nm). A potential use of the fluorophore riboflavin as a useful photosensitizer[17] can also be justified through this observation. Figure 5 shows fluorescence transients of riboflavin in absence and presence of UV light. From the figure it is clear that the lifetimes of the species in dark and in light conditions are comparable (~4 ns).[18] The observation further implies that the intermediate photoproducts of vitamin B2 have insignificant contribution in the excited state dynamics. It is worth mentioning that in order to establish the data reproducibility; we have performed the whole experiment five times on different days with same experimental condition and found a variation of 2%–5% from one to the other. We have also studied the photodeterioration of vitamin B2 under the light from tungsten lamp of similar power (60 W). No evidences of photodamage of the vitamin are observed under the light exposure for about 2–3 h. This observation clearly

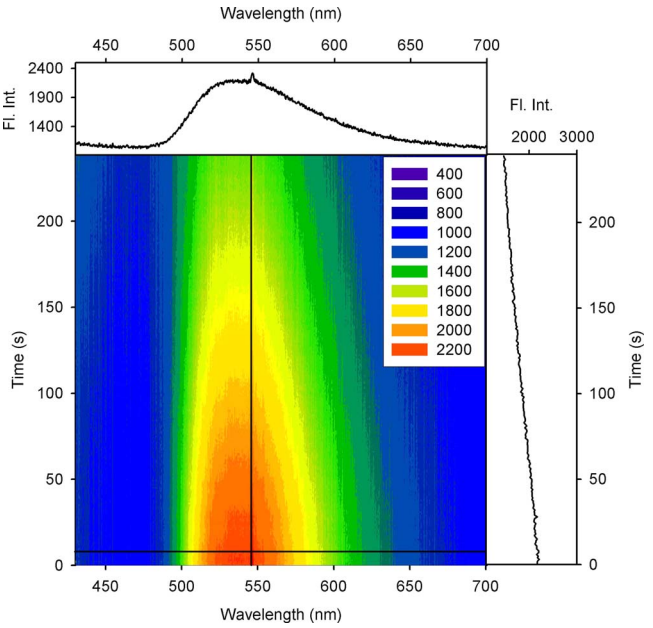

FIG. 4. (Color online) Wavelength (nanometer) vs time (second) contour plot of riboflavin in presence of UV-light. The above panel of the contour diagram is the full emission spectrum at time ~10 s. The right hand side of the contour is the time dependent decay spectrum at ~540 nm.

indicates that light from tungsten lamp is more desirable than the light having UV component (fluorescent lamp) for the preservation of food product, particularly foods containing vitamin B2. In principle, the setup can be used to study other photoinduced chemical reactions including excited state charge transfer kinetics.[19]

IV. CONCLUSION

In summary, we have designed a fiber-optic based setup for the spectroscopic measurement of various samples in cuvette under strong ambient light. An efficient rejection of stray light is achieved by the confocal geometry of the excitation and detection channel in the setup. We have showed that even low optical signal from the sample under strong UV light exposure can reliably be detected by high sensitive MCP-PMT detector. In order to prove the viability of the

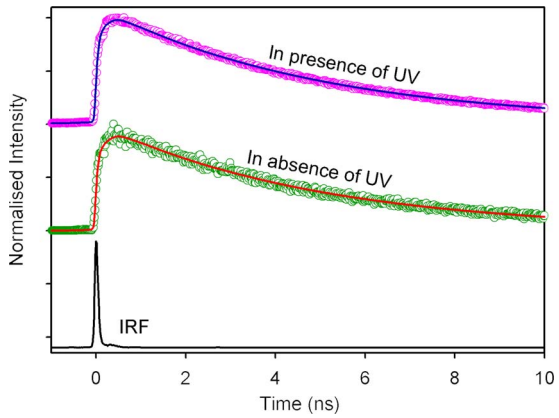

FIG. 5. (Color online) The nanosecond fluorescence transient decay of riboflavin, excited at 409 nm, observed at 540 nm, in presence (upper) and in absence (lower) of UV-light.

setup, we have studied the UV damage of an important biomolecule vitamin B2. Our observations of the photodeterioration kinetics of the vitamin are consistent with that in the literature. Our studies also indicate the potential danger of using fluorescent light (having UV component) for the preservation of food containing vitamin B2.

ACKNOWLEDGMENTS

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