CHAPTER 1

Introduction

1.1  Bioluminescence— the nature of luminescence

BIOLUMINESCENCE is an interesting natural phenomenon, in which living organisms produce light by chemical means. The enchanting phenomenon of biological generation of visible light has inspired the curiosity and interest of mankind ever since the ancient times [1-3], as well as its appealing visual displays has attracted the attention of many researchers for decades. In nature, there is an amazing diversity of creatures that exhibits bioluminescence; for example, fungi, bacteria, mollusks, crustaceans, fishes, insects, marine coelenterates etc [4-7]. A vast majority of bioluminescent organisms are aquatic, and there are many in terrestrial forms too, notably luminous beetles (Coleoptera) [8], representing about 2000 species worldwide including Lampyridae (the true fireflies), Phengodidae (railroad worms) and Elateridae (clickbeetles) [4, 8, 9].

Bioluminescence is a special kind of luminescence that originates from the Greek word ‘bios’ means “living” and the Latin word ‘lumens’ means “light” [10]. As opposed to incandescence, Eilherdt Wiedeman in 1888 introduced the term luminescence as ‘luminescenz’
to mean all those phenomena that are not conditioned by the rise in temperature [11]. Unlike in incandescence which gives hot light, luminescence is all about ‘cold’ light. The former involves the conversion of vibrational energy into radiation energy as a consequence of increase in temperature [12], whereas the latter in electronic transitions [13]. Regarding the physical process necessary for light emission one may also point towards a major difference in between these two such as whether the emission of light involves transitions in electronic energy levels within atoms or molecules in luminescence, or transitions in energy levels between atoms and molecules in case of incandescence [14]. Thus, luminescence arises when electrons in excited orbitals decay to different levels of ground state with an emission of visible or near-visible radiation from the potential energy of electronic transitions among atoms or molecules [14]. Luminescent compounds can be of different kinds, organic, inorganic and organometallic [11], which upon proper excitation exhibit luminescence. According to the mode of excitation, luminescence can be classified into various types as shown in table 1.

In photoluminescence, luminescence arises due to photoexcitation [15], that is, after absorption of a photon the electronically excited species re-emit the photon with a much lower energy. Fluorescence and phosphorescence are considered to be the particular cases of photoluminescence. Similarly, in chemiluminescence, it arises from chemiexcitation, where a chemical reaction forms the excited species, and in bioluminescence, it is produced by a chemical reaction inside the living system [15]. The biochemical process responsible for bioluminescence is assumed to be a physical effect in the light-matter interaction. Figure 1 [11] reflects the position of bioluminescence in the frame of light-matter interactions.
Table 1.1 Various types of luminescence with their excitation modes [11]

<table>
<thead>
<tr>
<th>luminescence type</th>
<th>excitation mode</th>
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<tbody>
<tr>
<td>photoluminescence</td>
<td>absorption of light (photons)</td>
</tr>
<tr>
<td>electroluminescence</td>
<td>electric field</td>
</tr>
<tr>
<td>thermoluminescence</td>
<td>heating (e.g. radioactive irradiation)</td>
</tr>
<tr>
<td>chemiluminescence</td>
<td>chemical process (e.g. oxidation)</td>
</tr>
<tr>
<td>bioluminescence</td>
<td>biochemical process</td>
</tr>
<tr>
<td>radioluminescence</td>
<td>ionizing radiation (X-ray, α, β, γ)</td>
</tr>
<tr>
<td>sonoluminescence</td>
<td>ultrasound</td>
</tr>
<tr>
<td>cathodoluminescence</td>
<td>cathode rays (electron beams)</td>
</tr>
<tr>
<td>triboluminescence</td>
<td>frictional and electrostatic forces</td>
</tr>
</tbody>
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It is well known that all processes involved in between absorption and emission of light can be well understood by Jabłoński diagram [13]. A comparison of this vis-à-vis photoluminescence and bioluminescence is shown in figure 2. Usually several processes occur in photoluminescence. After absorption of a photon a typical fluorophore is excited to a higher vibrational level of the excited singlet states of either $S_1$ or $S_2$ so that the electron remains paired (by opposite spin) to the second in the ground state ($S_0$). In the excited state, the molecule loses its energy through various ways. It can lose energy through collisions with other molecules or by vibrational relaxation (VR). Additionally, the fluorophore can lose
energy by a rapid relaxation to the lowest vibrational level of first excited state \( (S_1) \) by internal conversion (IC). It may also undergo a non-radiative decay without any light emission. Finally, electron in the thermally equilibrated state, that is, from the lowest vibrational level of first excited state \( (S_1) \) returns to the ground state \( (S_0) \) by emitting a photon via a fluorescence pathway [13]. On the other hand, in the other process, phosphorescence, molecules in the excited singlet state \( (S_1) \) can undergo a spin conversion to the first excited triplet state \( (T_1) \) by inter system crossing (ISC) so that the electron in excited triplet state \( (T_1) \) has the same spin.

**Figure 1.1** Position of bioluminescence in the frame of light-matter interaction.
Figure 1.2 Jablonśki diagram for (a) bioluminescence: excited state product is formed from a biochemical reaction inside a living body; and its decay to different levels of ground state ($S_0$) produce visible light emission (b) photoluminescence: excitation of molecules is initiated by absorption of a photon, whereas their transition from excited singlet state ($S_1$) to ground state ($S_0$) gives fluorescence; and that from excited triplet state ($T_1$) to ground state ($S_0$) is accompanied by emission of phosphorescence.
orientation as the ground state ($S_0$) electron. In excited triplet state ($T_1$), the electron changes its spin and consequently returns to the ground state ($S_0$) with an emission of a photon. In addition to these, there is also a possibility for an electron in excited triplet state ($T_1$) to return to ground state ($S_0$) by a non-radiative decay [13]. Similar processes occur in bioluminescence (figure 2a), and it is quite indistinguishable from the phenomenon of fluorescence except that the excitation of molecules is caused by a biochemical reaction in the former case, which in turn forms the excited singlet state, and whose subsequent decay to the ground state ($S_0$) produce the bioluminescence emissions.

Bioluminescence is generally classified into two classes based on the type of biochemical reactions. In the first type, the total amount of light emitted from a biochemical reaction is directly proportional to the amount of an organic substrate (luciferin) present in the organisms, whereas the second type involves a different chemical reaction, which is exhibited by photoproteins only [10].

1.1.1 Quantum yield

Quantum yield is essentially an important characteristic that can be used to measure the efficiency of a light emitting system. In luminescence, it is basically defined for a compound as a fraction of molecules emitting photon after excitation by a source [16]. From photochemistry point of view, the quantization of light absorption by matter is primarily concerned with ‘Stark-Einstein’ law, which states that the light absorption by a molecule is a one quantum process in which sum of all the primary process quantum yields must be unity, and can be applied to all photochemical systems. Thus, the quantum yield ($\Phi$) in the photo-conversion of reactants into photoproducts [17] is given as
Φ = molecules of photoproducts formed per unit volume per unit time/quanta of light absorbed per unit volume per unit time.

In bioluminescence or in chemiluminescence, it is simply defined as the efficiency to produce a photon from a single reactant molecule [18]. Strict to bioluminescence, it is the number of luciferin molecules that oxidized [19]. Thus, the overall efficiency is defined as

\[ Q = \Phi_p \Phi_e \Phi_f \]

where \( \Phi_p \) is the fraction of a reaction producing light emitter (oxyluciferin), \( \Phi_e \), the fraction of oxyluciferin formed in the excited state and \( \Phi_f \) (oxyluciferin fluorescence yield) is the fraction of excited state producing light [19].

It is suggested that the quantum yield value could be of great assistance in order to understand the mechanism associated with the brightness of bioluminescence or chemiluminescence reactions [20], which may vary from organism to organism in the wide bioluminescence diversity.

1.2 Objective of the thesis

The thesis is an attempt to apply novel spectroscopic and optical techniques to investigate the various aspects of the bioluminescence emissions of fireflies. The species of firefly selected for this thesis work is *Luciola praeusta* Kiesenwetter 1874 (Coleoptera: Lampyridae: Luciolinae), an Indian one. The selected methods applied here are steady-state and time-resolved emission spectroscopy. Several instruments were used for this purpose including a spectrometer, spectrograph, photomultiplier tube, diffraction grating, Michelson and Young two pin-hole interferometers.
Firefly bioluminescence is a very efficient light producing system, and that is where its importance lies. The objective of this thesis primarily lies in highlighting many spectral characteristics of firefly bioluminescence emissions. It has also been a long desire for electro-optical physicists to generate laser light by efficient chemical means. Keeping these in mind, the steady-state spectroscopy is used to record the emission spectra of the firefly light. The coherence properties of this light are also investigated under steady-state observations. Time-resolved measurements are generally more informative than steady-state ones, and are used in this thesis work to study the nature of firefly flashes at various temperatures. Through all these studies, interesting conclusions have been drawn as well as hypotheses put forward. On the basis of results presented in this thesis, it is expected that it will certainly offer a great potential in future bioluminescence research.

It is to be mentioned here that quite a few studies have been carried out on the other species of fireflies related to their behavioral patterns or to biochemistry of bioluminescence. Its various applications are also shown in bioanalytic chemistry, biomedicine, and biophysics. A survey of literature indicates that studies on the coherence properties as well as flashing patterns at different temperatures for this Indian species of firefly have not been carried out.

1.3 Firefly bioluminescence

Fireflies are the most common organisms in our surrounding exhibiting bioluminescence, and among all bioluminescent systems they are the best studied and characterized ones. In nature, there exist approximately 2000 species of fireflies in 100 different genera, including both diurnally active and nocturnal species [21-24]. Fireflies are also known as lightning bugs or glow worms that usually prefer temperate and tropical zones for their habitat. They are frequently found in moist areas like wet wooded areas, marshes, near ponds, and streams, especially in warm summer evenings [25]. Fireflies normally span a three year life cycle from
larval stage to adult. They are popularly known as insects, belonging to the Lampyridae family. The scientific classification of fireflies [26] is given as,

- **Kingdom:** Animalia  
- **Class:** Insecta  
- **Order:** Coleoptera  
- **Phylum:** Arthropoda  
- **Infraclass:** Neoptera  
- **Family:** Lampyridae

The Indian species of firefly that used in the present works is known as *Luciola praeusta* Kiesenwetter 1874 (Coleoptera: Lampyridae: Luciolinae). This species is identified by Dr. L. Ballantyne, School of Agriculture and Wine Sciences, Charles Sturt University, Australia and Dr. P. Mukhopadhyay, Coleoptera Section, Zoological Survey of India, Kolkata [27]. It is to be mentioned here that this species of firefly is found available in this part of the country during March to early November.

### 1.3.1 Firefly anatomy

In its basic anatomy, it has an exoskeleton and a body having three parts— the head, thorax and abdomen. Usually fireflies are small in size ranging from 2 mm up to approximately 3 cm, and are mostly black or brown in their appearances. They have two pairs of wings that concealed under wing covers on back side. The male specimens are more likely to have full wings, and are smaller in size as compared to the female ones. Fireflies have two compound eyes, two antennae and six joined legs [26]. The adult male fireflies have two abdominal segments, while the female has only one. The appearance of the Indian species of firefly is shown in figure 3. The adult male specimen of this firefly has a usual body length, which ranges from approximately 8 mm up to 1 cm, and a width of approximately 3 mm. The average body weight of the Indian firefly is approximately 9 mg.

The adult fireflies produce bioluminescent signals in the specialized abdomen of their body part [28, 29]. It is widely reported that the light organ of the firefly is a slab-like
tissue that composed of a ventral photogenic layer and a dorsal reflector layer [30-34]. Bioluminescence from fireflies occurs in the photogenic layer [31-34]. It has a thickness of 40 µm, and possesses a net like structure [35]. The photocytes—light producing cells [36] are arranged in rosettes around channels (usually cylinders), each of which contains the tracheal branch system and lantern nerve. Each photocyte touches at least two cylinders through which it can receive tracheal and nervous inputs at both ends. These regions of photocytes contain all of the cell’s mitochondria as well as differentiated zone granules (commonly known as DZ granules) [33]. The mitochondria are grouped in the cell periphery, positioning in between the tracheolar air supply and the bioluminescent reactants requiring oxygen access [36]. Apart from nucleus, the cell’s interior is completely filled with high concentration of peroxisomes. These organelles in the photogenic layer contain the organic substrate and enzyme, which are responsible for firefly light reaction [33].

Figure 1.3 (a) An adult male specimen of the Indian species of firefly *Luciola praeusta* (b) Ventral side of the firefly.

The reflector layer, on the other hand, is a kind of specialized tissue, which has a thickness of about 200 µm [35]. Functionally, this layer increases the bioluminescence
intensity through reflection, and is formed with a group of cells, which are filled with white opaque granules [31-34], namely spherical uric acid granules with a regular diameter of 700 nm [35].

1.3.2 Firefly light production

It is a well established phenomenon that fireflies produce their own light through a chemiluminescence reaction that take place within the photocytes of their lower abdominal light organ. The chemistry regarding firefly light emission is extensively studied and reported many times with physical descriptions [37-43]. The generally accepted mechanism (3) of firefly light production is the one in which visible light is produced as a consequence of conversion of chemical energy of an organic substrate into excited electronic state (product*), energetic enough to emit a photon.

\[
\text{Substrate} + O_2 \rightarrow \text{Product*} \rightarrow \text{Product} + h\nu \quad (3)
\]

As shown in figure 4, the reaction uses an organic substrate luciferin, Mg-ATP and molecular oxygen to excite electronically, catalyzed by an enzyme luciferase [4, 42].

\[
\text{Luciferin} + \text{ATP} \xleftrightarrow{Mg^{2+}} \text{Luciferyl\textbullet\textnormal{AMP} + PP_i}
\]

\[
\text{Luciferyl\textbullet\textnormal{AMP} + O_2} \xrightarrow{[\text{Oxyluciferin}]} \text{[Oxyluciferin]}^* + \text{CO}_2 + \text{AMP} + \text{H}^+
\]

\[
\text{ATP-adenosine triphosphate}
\]

\[
\text{Luciferyl\textbullet\textnormal{AMP-Luciferyl adenylyl}}
\]

\[
\text{PP}_i\text{-pyrophosphate}
\]

\[
\text{Oxyluciferin} + h\nu
\]

**Figure 1.4** Luciferase catalysed firefly bioluminescence reaction.

However, the light producing mechanism can be simply described in three steps. In the first step, luciferase converts luciferin molecules into the corresponding enzyme-bound
luciferyl adenylate intermediate and by-product PP\textsubscript{i} in the presence of ATP and Mg\textsuperscript{2+}. In the next step, recruitment of luciferase amino acid residues promotes the addition of O\textsubscript{2} to yield an electronically excited-state oxyluciferin molecule and other products such as CO\textsubscript{2}, AMP and H\textsuperscript{+}. Finally, in the last step, visible light is produced as the excited-state oxyluciferin molecule rapidly loses its energy via a fluorescence pathway [4]. From an understanding point of view, this enzyme mediated reaction mechanism underlining firefly light emission is primarily concerned with ‘Chemical Initiated Electron Exchange Luminescence’ (CIEEL) mechanism [19].

In contrast to many chemiluminescent enzymes, which preferably give ultra-weak chemiluminescence, luciferases are considered as unique because they possesses the ability to produce short-lived singlet excited-state (<10\textsuperscript{9} sec), and may decay preferentially by emitting visible light. It may also provide active-site microenvironments to the biochemical reaction, which is favorable to emissive decay rather than other photochemical and photophysical processes [8]. The photo-emitter system of firefly is known for its extremely high quantum yield (QY) value [44-46]. In a recent finding, it is reported that the green light-emitting luciferase of Brazilian click beetle of species \textit{Pyrearinus termitilluminans} exhibits the highest quantum yield value of 0.61 [20].

The spectacular flashing displays from fireflies play important ecological and evolutionary roles. Different species of fireflies emit different kind of flash patterns as a mode of communication for various biological and chemical functions. It is that property which helps them in survival and adaptations. It can also act as a warning to potential predators. In a recent review, it is suggested that the firefly flash signals are shaped by the dual evolutionary processes of both mate choice and predation [28]. Some species emit short flashes, while some
long, continuous or many flashes in a row, exhibiting the nature of species specific communication.

1.3.3 Firefly flash control mechanism

Light in a firefly is produced in the specialized organ. The biochemistry is well understood, while the ‘on-off’ mechanism that regulates the luciferase catalyzed luciferin bioluminescence is a matter of long standing puzzle. Several speculations and hypotheses were put forwarded in this regard. It is proposed that fireflies have the ability to control the rate at which they emit flashes. This can be simply explained by two theories— ‘the oxygen control theory’ and ‘the neural activation theory’. The former explains how the fireflies regulate the amount of oxygen that they intake, thereby control the flashing rate. It is suggested that the oxygen availability for firefly light production is controlled by mitochondria such that the ‘on’ signal might depend on inhibition of mitochondrial oxygen consumption that allows delivered oxygen to pass through mitochondrial zone to reach peroxisomes [36]. Regarding the mechanism responsible for discrete flashes in adult fireflies, Timmins et. al. further concluded that the flash is controlled by gating of oxygen to the photocytes by modulating the levels of fluid in the tracheoles, providing a variable barrier to oxygen diffusion [47].

On the other hand, ‘the neural activation theory’ explains how the fireflies initiate the biochemical reaction inside their lanterns through neural stimulation. It is widely believed that the Octopamine is the neurotransmitter, which is responsible for induction of luminescence in the firefly lantern [48, 49]. As oxygen triggers firefly light production, it is proposed that NO (nitric oxide), a ubiquitous signaling molecule plays a key role on firefly flash control by transiently inhibiting mitochondrial respiration in the photocytes, thereby increasing $O_2$ levels in peroxisomes [50]. The model of NO control on firefly flash mechanism is shown in figure 5 [51]. In the quiescent or inactivate mode, little oxygen from tracheoles reaches peroxisomes as
the maximum amount is consumed by respiration in mitochondria. ATP produced by oxidative phosphorylation promotes the formation of reaction intermediate product catalysed by luciferase. In the absence of oxygen no light producing oxidation takes place.

**Figure 1.5** NO control model for firefly flashing.

However, in a flash mode, a central nervous system (CNS) signal causes octopamine release that transiently inhibits lantern nitric oxide synthase (NOS). The NO that produced from NOS rapidly inhibits mitochondrial oxygen consumption so that oxygen from tracheoles is free enough to diffuse past mitochondria to the peroxisomes and trigger firefly light reaction with intermediate product. It is also suggested that by reversing NO inhibition of mitochondrial oxygen consumption a flash itself contributes to its rapid off kinetics [51].

Regarding the shape of firefly flashes, it is hypothesize that those are shaped by neural impulses generated in the brain, which impinge on the lantern tissue, thereby assumed that it is possible to shape any kind of flash by adjusting the frequency and duration of stimulus activating the lantern nerves [52]. In the same report, it is also speculated that the firefly flashes could be regulated by calcium. However, in case of oxygen supply mechanism, very recently, it
is reported that the flashing mechanism uses a large portion of the oxygen consumed by mitochondria, which exceeds the amount that diffused through tracheal system to the photocytes: and, it is concluded that the firefly flashing could be controlled by passivation of the mitochondria functions using nitric oxide as well as switching of the oxygen supply from mitochondria to photoluminescence [53].

1.4 Review of literature

1.4.1 Steady-state observations

The spectral spreads of firefly bioluminescence is considered to be a subject of many investigations for over a century. The existence of distinct groups of bands in a few species of fireflies is reported quite a few times [54-56]. The two smallest values of full-width-at-half-maximum (FWHM) found till now, using photographic spectrometry, as reported by Coblentz in 1912 are 33.3 nm and 46.7 nm for the North American species Photinus pyralis and Photinus consanguineus, respectively [57]. The general characteristic of the firefly light spectra is that it is an intensity envelope having a singular peak and asymmetric in nature by extending to higher wavelength sides. Investigating the bioluminescence spectrum of few species of fireflies, it is indicated that the transition involves in the emission is an n-π* type [58, 59].

The in vivo steady-state spectrum recorded for the Indian species of firefly Luciola praeusta has revealed the peak wavelength at 562 nm, with a full-width-at-half-maximum value of 55 nm, being one of the narrowest reported till date, extending from 537 to 592 nm [27]. The green emitting luciferase of the Japanese firefly Pyrocoelia miyako also shows an approximately equal in vitro FWHM value [60]. Coloured emission spectra of the light of Indian firefly show three colours— green, yellow and red, among which the red is not observable under normal conditions because of the Purkinje effect [61]. In the same report, it is
inferred that firefly light emission has a tendency for spectral narrowing in the narrow yellow-sector of its emission. The diffraction and interference patterns of firefly light are also shown in the same communication. It is suggested that different firefly species emit in slightly different spectral regions due to slight change in their enzyme structures [40]. In an *in vivo* bioluminescence study of 55 species of North American fireflies, it is found that 23 out of 32 dark-active species emit green light (\( \lambda_{\text{max}} \leq 558 \text{ nm} \)) and 21 out of 23 dusk-active emit yellow light (\( \lambda_{\text{max}} \geq 560 \text{ nm} \)) [62].

### 1.4.2 Time-resolved observations

Investigations on the aspect of flashing of different species of fireflies reveal that a single flash varies from around 70 ms [63] to a few hundred milliseconds [64-67] up to an approximately a couple of seconds [68]. Investigating the flash patterns in different species of Jamaican fireflies, it is found that some fireflies emit flash in a long-continued flow with fluctuating intensity, while some emit single concerted flash of duration of 75 to 100 ms with a regular flash separation of 2-6 seconds, and some emit quick flickers consisting of 4 to 20 or more short flashes at frequencies of 10-18 per second in every few seconds [69]. In the same communication, it is also reported that a given species of firefly usually emit simpler type of flash in the captive mode than when in flight. There are also quite a few reports on the different aspects of synchronous flashes in a few species of fireflies [70, 71]. It is also observed that some specimens of firefly inhabitant of Coastal reef in New Guinea emit bimodal, ‘crescendo’ or stepped flashes [72].

For the Indian firefly *Lucila praeusta*, a typical flash is of duration of about 100 ms consists of about 30,000 \( \mu \text{s} \) pulses, and the flashes are separated from one another by an average value of 800 ms with a minimum separation of 150 ms [27]. It is also suggested that the nature of tiny microsecond pulses in the continuous light of this firefly manifest an
oscillating chemical reaction like the B-Z (Belousov–Zhabotinsky) reaction in the anaesthetized firefly lantern [73], and that exhibits both pulse amplitude modulation (PAM) and pulse width modulation (PWM) [74].

1.4.3 External factors on firefly bioluminescence

Numerous studies were carried out on the effect of external factors like temperature and pressure on firefly bioluminescence emission, and consequently arrived at various conclusions. It is reported that in different species of Luciola fireflies, the flash period decreases with an increase in temperature [65]. An investigation on the influence of temperature on flash intervals of Japanese species of firefly Luciola cruciata at different sites in Japan, it is concluded that the flash intervals were negatively correlated with air temperatures [75, 76]. It is reported that pressure antagonizes anesthesia, as reversed to this contention, it is suggested that pressure does not show any kind of effect on initial flash intensity of the purified lipid free firefly luciferase [77]. In a study of measurement of the effect of high pressure on the enzyme kinetics of firefly luciferase, it is found that the maximum light intensity is observed at about 22.5 °C, while it shows a negligible effect at 20 — 25 °C [78].

Regarding the causes of colour change in firefly bioluminescence the following factors are considered to be the key factors that influence the spectroscopic properties of ‘oxyluciferin’— the light emitter of firefly system: (a) pH [45, 79], (b) temperature [80], (c) metal ions [81], and (d) protein structure [42]. In an investigation of the effect of temperature on firefly bioluminescence, very recently, in vitro quantitative bioluminescence spectra were studied for the firefly Photinus pyralis at temperatures 15 — 34 °C, and it is found that the spectra were temperature sensitive at pH 7.0 and 8.0 above photon energy 2.0 eV, and insensitive below 2.0 eV: the intensity of green component is only found to be temperature sensitive quantity, while the other two components red and orange were quite robust, or
insensitive to environments [80]. In the same report, it is also noted that the temperature-dependent colour change of the in vitro bioluminescence is due solely to the intensity change of the green Gaussian component. In order to observe the effect of pH on firefly emission colour changes, Y. Ando et. al. successfully decomposed bioluminescence spectra into pH-sensitive and pH–insensitive components, and it is found that there is an intensity variation of the pH-sensitive Gaussian component at 2.2 eV [45]. On the effect of metal ions on firefly bioluminescence, it is reported that an increase in the amount of metal ions such as Zn$^{2+}$ and Cd$^{2+}$ on lucerin-luciferase reaction decreases the quantum yield value, as well as changes the bioluminescence spectrum from yellow-green to red, and it is proposed that the colour change caused by metal ions are due to the effect of enzyme luciferase surrounding the excited state oxyluciferin during the radioactive decay [81]. In a recent study of structural basis associated with the spectral difference in luciferase bioluminescence, it is indicated that the degree of molecular rigidity of excited-state of oxyluciferin species is controlled by the transient movement of the hydrophobic side chain of Ile 288, and it is suggested that this movement determines the colour of bioluminescence during the emission reaction [42].

In recent times, firefly bioluminescence were observed in both pulsed and static magnetic fields, and it is speculated that magnetically induced current caused by the pulsed magnetic field inside the fireflies affected their nervous system or the photochemical processes in their light producing organ [82], while the diamagnetic torque and Lorentz force induced by the high intensity static field of 10 Tesla affected the behavior and neural activity of the firefly, respectively [54, 68]. Additionally, the steady-state emission spectrum of the Japanese firefly Luciola lateralis in the optical range of 540 — 580 nm shows a reversible red shift under the strong static field [82].
1.5 Applications of firefly bioluminescence

It has been a long way since the beginning of the use of firefly luciferase emitting ‘yellow-green’ light in the sensitive applications of biochemistry like in ATP measurement. It is used to analyze biological microbial contamination, to monitor biomass, to assay enzymes and to assess cell viability [12]. The success of firefly luciferase cloning also opens a new area of research in the expression of gene reporters in living cells and tissues [8]. In recent days, firefly bioluminescence is found to have a widespread use in bio-imaging applications [83]. The bioluminescence imaging technique (BLI) is a process of molecular imaging at the molecular and cellular levels in any human or living bodies [84]. In this technique, an image is produced from the emitted light by living entities and after visualizing the emitted profile of the light one can gain much biological information [85, 86] about the normal and pathological conditions of the living cells, embryonic development, and among many other applications [87].

From the biomedical and bioanalytic application point of view, nowadays it is extensively used in the study of progression and regression of bacterial as well as viral diseases, for example mycoplasmas and HIV [88, 89], and in cell tracking and as an efficient method for drug screening in Pharmaceutical industry, respectively. Most importantly, firefly luciferase bioluminescence has also been shown in practical applications; for example, as biosensors to detect environmental pollutants like lead, arsenite, mercury, phenols and agrochemicals, which may act as endocrine disrupters to nature [90, 91]. In recent days, several analytic applications have been developed based on the principle of Bioluminescence Resonance Energy Transfer (BRET). Apart from these, a high demand has also increased for firefly luciferase emitting other colours, particularly red, for the purpose of its use in mammalian cells and tissues, which are rich in hemoglobin and can absorb shorter wavelength light [92].
It is believed that in the very near future the improvement of bioluminescence imaging technique could lead the biomedical engineers to provide a new tool in the cancer therapy. It is expected that inclusions of more physicists and researchers would certainly pave the way for a significant development and extend the range firefly bioluminescence applications in different area.
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