Bioluminescence emissions of the firefly *Luciola praeusta* Kiesenwetter 1874 (Coleoptera : Lampyridae : Luciolinae)[#]

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We recorded the *in vivo* emission and time-resolved spectra of the firefly *Luciola praeusta* Kiesenwetter 1874 (Coleoptera : Lampyridae : Luciolinae). The emission spectrum shows that the full width at half maximum (FWHM) value for this particular species is 55 nm, which is significantly narrower than the *in vivo* half-widths reported till now. The time-resolved spectrum reveals that a flash of about 100 ms duration is, in fact, composed of a number of microsecond pulses. This suggests that the speed of the enzyme-catalysed chemiluminescence reaction in the firefly for the emission of light is much faster than was previously believed.

[Barua A G, Hazarika S, Saikia N M and Baruah G D 2009 Bioluminescence emissions of the firefly *Luciola praeusta* Kiesenwetter 1874 (Coleoptera : Lampyridae : Luciolinae); *J. Biosci.* **34** 287–292]

1. Introduction

Bioluminescence is an enchanting process by which living organisms convert chemical energy into light. Fireflies are common organisms that exhibit this process. The enzyme luciferase catalyses the bioluminescence reaction, which uses luciferin, Mg-ATP and molecular oxygen to yield an electronically excited oxyluciferin species. Visible light is emitted during relaxation of excited luciferin to its ground state (figure 1). The emission of light by fireflies has been of considerable interest to naturalists and biochemists due to the complicated chemical reactions involved, and to electro-optical physicists due to the desire to generate laser light by efficient chemical means. It has also been of interest in biomagnetics, due to the effect of magnetic fields on enzymatic activities (Iwasaka and Ueno 1998).

The spectral distribution of bioluminescence has been the subject of numerous investigations. The existence of distinct groups of bands in a few species of firefly has also been reported (Iwasaka and Ueno 1998; Biggley *et al.* 1967; Bora and Baruah 1991). In this report, we present an *in vivo* emission spectrum of a firefly *Luciola praeusta*, where the full width at half maximum (FWHM) is significantly narrow. This value was consolidated by another spectrum of the firefly emitting continuous light under the influence of ethyl acetate. This firefly is a flashing firefly belonging to the subfamily Luciolinae, where the light organs are on the ventral surface of the terminal two abdominal ventrites.

Fireflies have a remarkable flash communication system involving precisely timed, rapid bursts of bioluminescence. A comprehensive synthesis of the past two decades' work on firefly signal evolution, mate choice and predation is provided in a review by Lewis and Cratsley (2008). Females of a firefly species were shown to discriminate between males on the basis of variation in the flash rate of male patterns (Branham and Greenfield 1996). It has

Keywords. Firefly; emission spectrum; FWHM; time-resolved spectrum; pulse width; pulse duration

Abbreviations used: FWHM, full width at half maximum; HWHM, half width at half maximum; NO, nitric oxide

[#]A version of this article has already appeared in an archive *Nature Precedings*; *see http://precedings.nature.com/documents/1351/version/2/html*

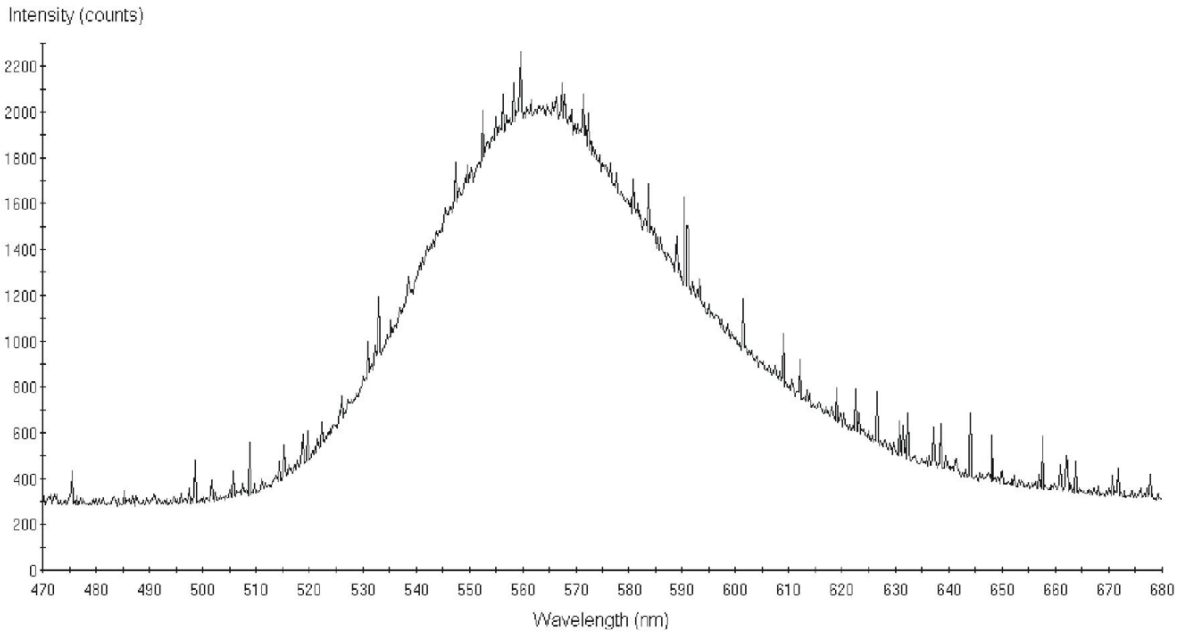
Figure 1. The firefly *Luciola praeusta* (left) and its flash (right) in the spring season at the campus of Gauhati University, Assam, India.

been reported (Vencl and Carlson 1998) that female *Photinus pyralis* fireflies prefer flashes of greater intensity and precedence, suggesting that flash 'synchronisation' is a competitive display. Nitric oxide (NO), a ubiquitous signalling molecule, has been found to play a fundamental and novel role in controlling firefly flash (Trimmer *et al.* 2001). It has also been suggested that firefly flash could be regulated by calcium (Carlson 2004). The term *flash* has been used synonymously with the term *pulse* till now. The duration of a single pulse/flash has been reported to vary from about 70 ms (Branham and Greenfield 1996) to a few hundred milliseconds (Buck *et al.* 1963; Barry *et al.* 1979; Saikia *et al.* 2001). Lloyd (1973) recorded flashing signals of 22 species of Luciolinae belonging to the genera *Luciola* and *Pteroptyx*, and one can see from the thirty-nine oscilloscope traces published that the duration of the flashes varies from about 70 ms to about 500 ms, the average duration being roughly 100 ms. The time-resolved spectrum presented in this article is clearly in disagreement with these values.

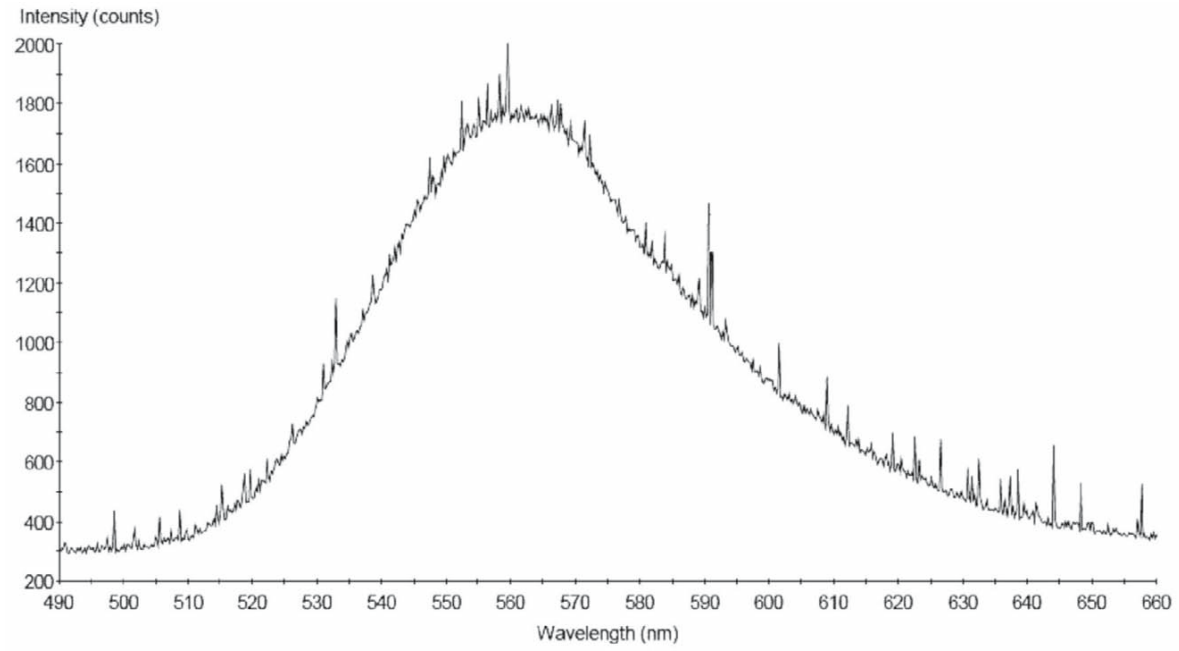
2. Materials and methods

The emission spectrum was recorded in an Ocean Optics HR2000 Series high-resolution fibreoptic spectrometer. The experiments were conducted during early evening to midnight, local time. Before the experiment, the spectrometer was calibrated with the known lines of iron from an arc, and tested against the sodium yellow line. A single firefly was collected just before the experiment from the Gauhati University campus, and kept immobile in a cotton plug with its light organ positioned towards the entrance face of the fibre. *In vivo* emission spectra of fifty specimens, both male and female, of the firefly species were recorded in this way. For recording the continuous glow of the firefly, it was kept in a 1.5 ml capacity microcentrifuge plastic tube 4 cm long. One end of the tube, with an operating diameter of 3 mm, was attached to the entrance face of the optical fibre in the spectrometer. The other end, with a diameter of 1 cm, was filled with cotton dipped in ethyl acetate. It was observed that the rate of flashing of the firefly rapidly decreased. After about 1 min, a constant glow appeared from the last segment of the abdomen of the firefly, which spread to the other light-emitting segment in about 3 min. A black patch in the middle of the upper segment of the lantern finally gave way to the glow in 5–6 min. Ten emission spectra of fireflies emitting this kind of continuous light were recorded. The experiments were performed at laboratory temperatures of 26°C–31°C. Because of the very low intensity of the emitted light, the integration time of the spectrometer had to be increased to 3000 ms, which resulted in the appearance of system noise (figure 2a, b).

The experimental set up for recording the time-resolved pulses of the firefly is shown in figure 3. The outside of the wooden firefly chamber was blackened, while the inside was



(a)



(b)

Figure 2. **(a)** Emission spectrum of firefly *Luciola praeusta*. The peak wavelength appears at 562 nm and the half width has a value of 55 nm. **(b)** Emission spectrum of the firefly *Luciola praeusta* under the influence of ethyl acetate. The peak wavelength and FWHM values are the same as in (**a**).

painted white. We interposed a 'high-pass' (DC-blocking) filter between the anode of the Dumont 6364 photomultiplier tube and the succeeding electronics. Since the time constant, which is the product of resistance (R) and capacitance (C), of the high-pass filter should be higher than the width of the pulse to be recorded, we used different RC values from 500 ms to 150 μs to confirm the result. A Tektronix TDS 520A digital storage oscilloscope was used to record the pulses. Time-resolved spectra of five specimens were recorded in this manner. The emission and time-resolved experiments,

with the arrangements described above, can be easily reproduced if flashing fireflies are available.

The firefly species was identified by Dr L A Ballantyne of Australia and deposited in the Australian National Insect Collection in Canberra.

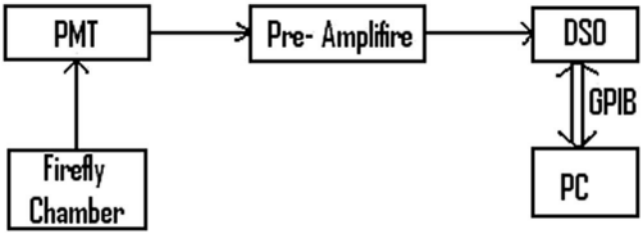


Figure 3. Experimental arrangement to record time-resolved spectra of the firefly. PMT, photomultiplier tube; DSO, digital storage oscilloscope; GPIB, general purpose interface bus; PC, personal computer

3. Results and discussion

The peak wavelength and FWHM in all the emission spectra of the firefly recorded in trapped as well as ethyl acetate-affected conditions are shown in figure 2a, b, respectively (one each). These were found to be remarkably constant. The peak wavelength was observed at 562 nm, that is, in the yellow region. The wavelength spread clearly shows that this particular firefly species emits in the green and yellow regions, with the weak red sector lying outside the half width up to 670 nm. The FWHM value was 55 nm. If we leave aside the outrageously small values reported by Coblentz (1912) (for example, the *Photinus pyralis* FWHM value was reported to be as low as 333 Å!), this is the smallest of all the *in vivo* half-width values of different species of fireflies published till date. From a spectroscopic point of view, this small value implies that about 50% of the luciferin molecules excited to the oxyluciferin state occupy levels narrower in spacing compared with that of other species. It has been proposed that different species of fireflies emit in slightly

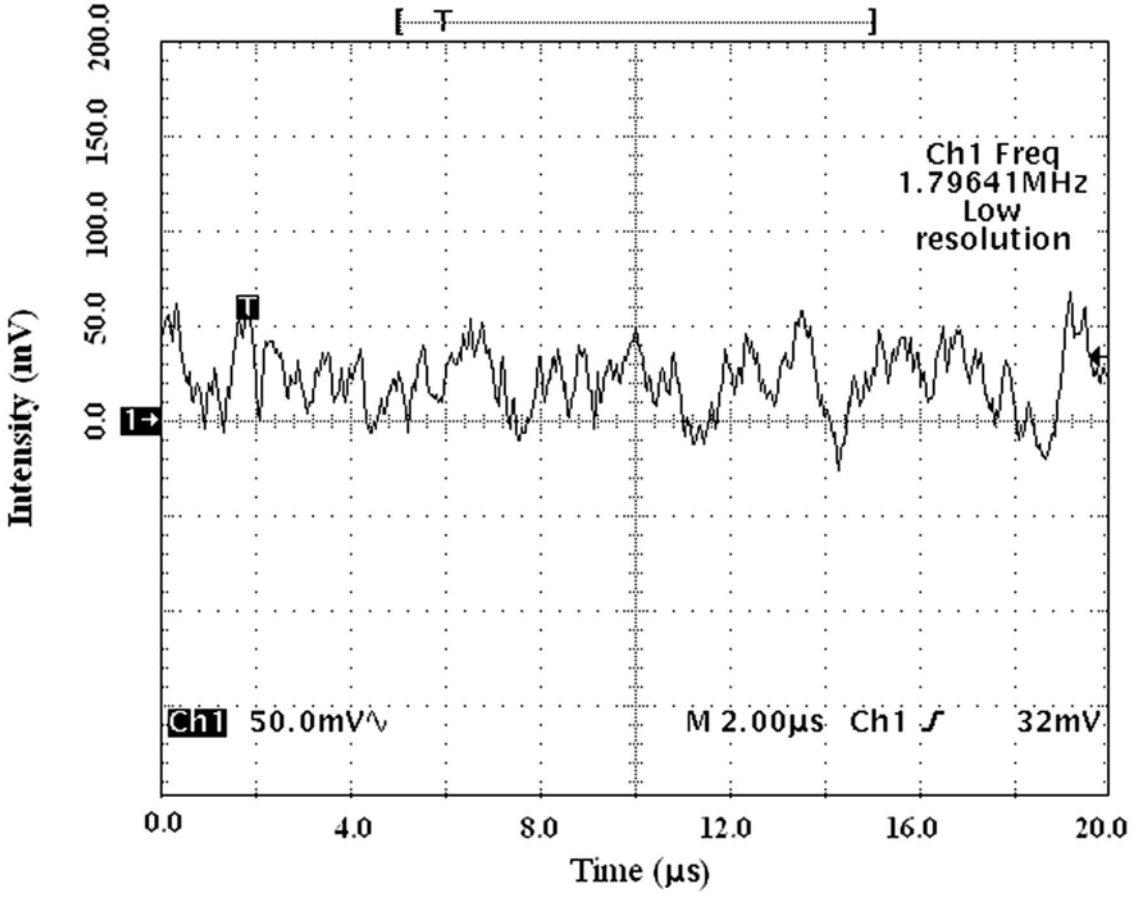


Figure 4. Time-resolved pulses of the firefly showing relaxation oscillation. The duration of a pulse is approximately 2 μs. Before the application of the signal, the noise level was approximately 15 mV, which got amplified to about 20 mV after the firefly began flashing in the chamber. To be absolutely on the safe side, we considered pulses only if they were above the trigger level, i.e. 32 mV, shown by the arrow on the right ordinate in the oscilloscope screen.

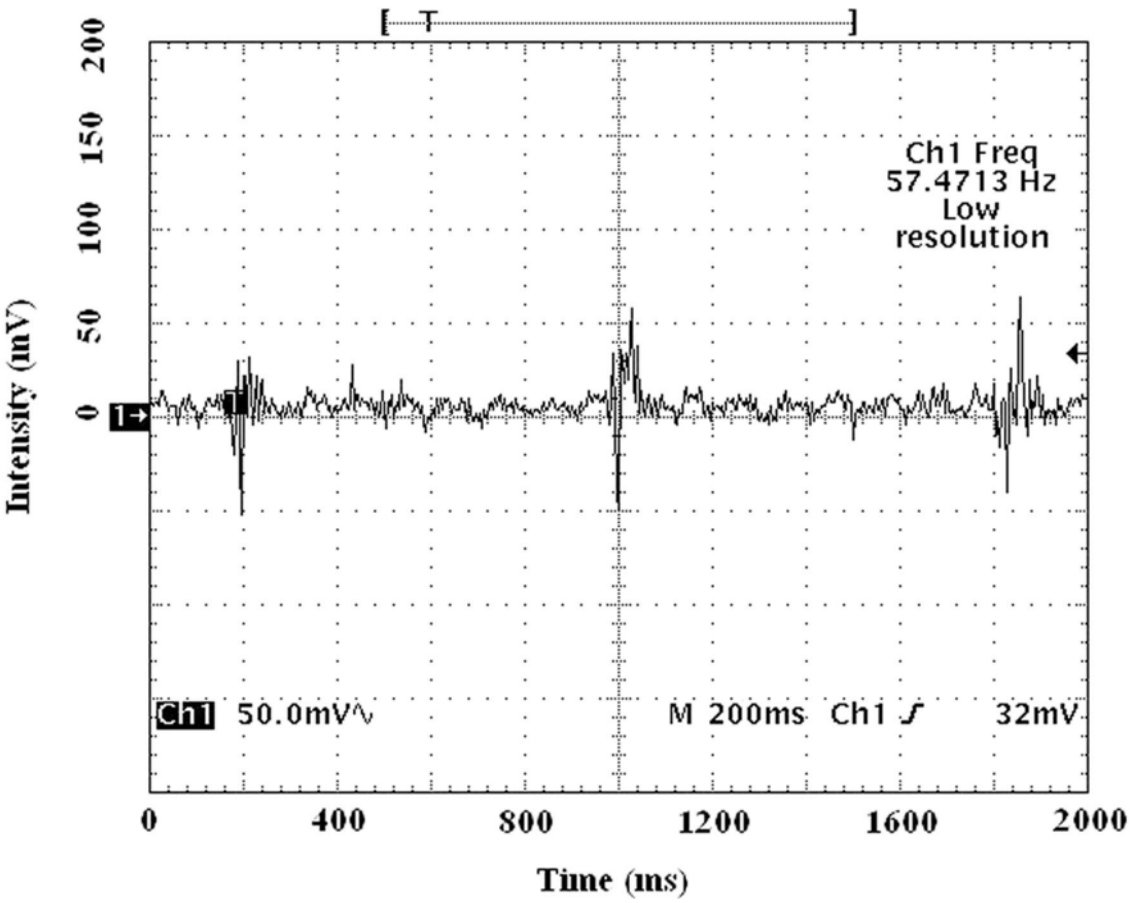


Figure 5. Time-resolved pulses of the firefly, on a larger scale, showing the *flash*. In the figure, the duration of a flash appears to be about 100 ms, while the three flashes are separated from one another by approximately 800 ms.

different spectral regions due to slight differences in enzyme structure (Seliger *et al.* 1964); the different FWHM values could also be due to this fact. The narrowest half width of 64 nm was measured for firefly species *Photinus consimilis* and *Photinus umbratus* (Biggley *et al.* 1967). The asymmetrical nature of the intensity profile is in agreement with earlier investigations. The half width at half maximum (HWHM) value for the lower half is 25 nm and for the upper half it is 30 nm. No discrete bands are observed in the spectrum. Approximately equal *in vitro* FWHM values have been reported, for example, in the green-emitting luciferase of the Japanese firefly *Pyrocoelia miyako* (Viviani *et al.* 2001).

The time-resolved spectrum of the firefly, shown in figure 4, exhibits a striking similarity with the output of a multimode laser. The spectrum presented here reveals that the duration of a pulse is a couple of microseconds! A survey of the literature indicates that this is probably the first report of a bioluminescence system emitting microsecond pulses. On a larger scale (figure 5), it is evident that a *flash*, consisting of a number of microsecond pulses, is for a duration of about 100 ms, and consists of about 30 000 pulses. From studies of similar spectra of five such specimens, it can be concluded that the flashes are separated from one another by a few hundred milliseconds. We found that the flashes, on an average, are repeated after 800 ms, and noted that the minimum separation between two flashes is 150 ms. The event recorded in figure 5 shows an average separation of 800 ms between flashes. The time-resolved spectra appear to be noisy because of the very low energy of the signal. The signal-to-noise ratio is approximately 1.8. Of course, the signals recorded were not the ones emitted by fireflies to advertise courtship and mating; these could be lightly described as SOS signals sent by them!

In vivo bioluminescence emission spectra recorded for 55 species of North American (temperate zone) fireflies (Lall *et al.* 1980) showed that 23 of 32 dark-active species emit green light ($\lambda_{max} \leq 558$ nm) and 21 of 23 dusk-active species emit yellow light ($\lambda_{max} \geq 560$ nm). The particular species under investigation falls in the group of 'early-starting' species, and also emits light whose peak wavelength is in the yellow region. It has been proposed (Saikia *et al.* 2001) that the time-resolved spectrum of the firefly can be considered as a manifestation of oscillating chemical reactions, the so-called BZ reactions (Belousov 1959; Zhabotinsky 1964).

The oscillatory nature of the time-resolved spectrum in our study (figure 4) also points in this direction. The characteristics of the pulses suggest that the speed of the chemiluminescence reaction must be remarkably high. The challenge at the moment is to record both the emission as well as the time-resolved spectra in a natural environment for finding out (i) whether the wavelength spread remains the same as in the 'trapped' condition in the laboratory, and (ii) by what amount the flash duration and flash repetition rates vary from specimen to specimen.

Acknowledgments

This work was supported by University Grants Commission, India, Major Research Project F.31-15/2005 (SR). We thank Dr L Ballantyne, School of Agricultural and Wine Sciences, Charles Sturt University, Australia, and Dr P Mukhopadhyay, Coleoptera section, Zoological Survey of India, Kolkata, for identifying the firefly species of the specimens collected. We are grateful to Professors K Boruah and P K Boruah, Departments of Physics and Instrumentation, respectively, Gauhati University, for allowing us to use the facilities in the UHE Cosmic Ray Research Laboratory to record the time-resolved spectra.

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MS received 5 November 2008; accepted 13 April 2009

*e*Publication: 23 May 2009

Corresponding editor: VIDYANAND NANJUNDIAH