Bioluminescence emissions of the firefly

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We have recorded *in vivo* emission and time-resolved spectra of the firefly species *Pyrophorus noctilucus*. The emission spectrum shows the FWHM value for this particular species to be 55 nm, which is significantly smaller than the half widths reported till now. The time-resolved spectrum reveals that a flash, of duration about a hundred milliseconds, is in fact composed of a number of microsecond pulses. This result suggests that the speed of the enzyme-catalyzed chemiluminescence reaction in the firefly for the emission of light is much greater than is believed to be.

Bioluminescence is an enchanting process in which living organisms convert chemical energy into light. Fireflies are common organisms exhibiting this process. The enzyme luciferase catalyzes 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. 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 been of interest in biomagnetics, even, due to the effect of magnetic fields on enzymatic activities[1].

The spectral distribution of bioluminescence has been the subject of numerous investigations. Existence of distinct groups of bands in a few species of fireflies has also been reported[1-3]. We have recorded *in vivo* emission spectra of fifty specimens of the firefly species *Pyrophorus noctilucus*; one of these is shown in Fig. 1. Males of this species emit flashes of light from their abdominal lanterns. We have found the peak wavelength as well as the full width at half maximum (FWHM) to be remarkably constant. The position of the peak wavelength has been observed at 564 nm, that is, in the yellow region. The wavelength spread clearly shows that this particular firefly species emits in the green and yellow region, with a bit of red thrown in. The FWHM width has been

measured to be 55 nm. If we leave aside the outrageously small values reported by Coblentz[4] in 1912 (for example, the *Photinus pyralis* FWHM width was reported to be as low as 333 Å!), this is the smallest of all the half width values of different species of fireflies published till now. The smallest half width, by the way, has been 64 nm measured for firefly species *Photinus consimilis* and *Photinus umbratus*[2]. The asymmetric 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, while the same for the upper half is 30 nm. No discrete bands are observed in the spectrum.

Fireflies have a remarkable flash communication system involving precisely timed, rapid bursts of bioluminescence. Females of a firefly species were shown[5] to discriminate between males on the basis of variation in the flash rate of male patterns. It has been reported[6] that female fireflies prefer flashes of greater intensity and precedence — suggesting that flash 'synchronisation' is a competitive display. Nitric oxide (NO), a ubiquitous signaling molecule, has been discovered[7] to play a fundamental and novel role in controlling the firefly flash, while it has been suggested[8] that the firefly flash could be regulated by calcium. Our experimental arrangement to record time-resolved spectra of the firefly is shown in Fig. 2. The time-resolved spectrum, shown in Fig. 3, exhibits striking similarity with the output of a multimode laser. The duration of a single pulse has

been reported to be a few hundred milliseconds[6-7,9-10], but the spectrum presented here reveals that the duration of a pulse is a couple of microseconds! A survey of literature indicates that this is the first report of a bioluminescence system emitting microsecond pulses. On a bigger scale (Fig. 4) it is evident that the duration of a *flash*, consisting of a number of microsecond pulses, is about 100 ms, and from studies of similar spectra of five such specimens it can be concluded that the flashes are separated from each other by a few hundred ms. We have found that the flashes, on an average, are repeated after 800 ms, and have noted the minimum separation between two flashes as 150 ms.

It has been proposed[11] that the time-resolved spectrum of the firefly can be considered as the manifestation of oscillating chemical reactions, the so-called BZ reactions. The oscillatory nature of the time-resolved spectrum in our work (Fig. 3) also points towards 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 time-resolved spectra in a natural environment for finding out (a) whether the wavelength spread remains the same as in the 'trapped' condition in the laboratory, and (b) by what amount the flash duration and flash repetition rate vary from specimen to specimen.

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Figure 1 Emission spectrum of the firefly *Pyrophorus noctilucus* recorded in an Ocean Optics HR2000 Series High-Resolution Fiber Optic Spectrometer. The experiments were conducted during early evening to midnight hours, local time. Prior to 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 and kept immobile in a cotton plug with its light organ positioned towards the entrance face of the fiber. 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 the system noise.

Figure 2 Experimental set up for recording time-resolved pulses of the firefly. The outside of the wooden firefly chamber was blackened, while the inside was 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 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. Tektronix TDS 520A digital storage oscilloscope was used to record the pulses.

Figure 3 Time-resolved pulses of the firefly, showing the relaxation oscillation. The duration of a pulse has come out to be approximately 2 microseconds.

Figure 4 Time-resolved pulses of the firefly, on a larger scale, showing the *flash*. Flash duration as well as flash separation could be easily found out from this spectrum.