Purkinje effect and bioluminescence of fireflies

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Observations on numerous specimens of fireflies have established the fact that the emitted light is not what is actually perceived by the naked eye. We have analysed the spectra of fireflies, and observed that those belonging to the species *Luciola praeusta* Kiesenwetter 1874 (Coleoptera: Lampyridae: Luciolinae) emit a substantial part of the radiation in the red sector of the spectrum. Here we show that the red sector is not visible to the naked eye because of the Purkinje effect, which is related to the biochemistry of the rods and cones. Interference patterns produced by the light of the firefly have also been presented.

Keywords: Bioluminescence, fireflies, red sector, Purkinje effect.

BIOLUMINESCENCE is the process by which living organisms like fireflies convert chemical energy into light. The enzyme luciferase catalyses the bioluminescence reaction, which uses luciferin, O_2, ATP and Mg^{2+} to yield an electronically excited oxyluciferin species. Visible light is emitted as the oxyluciferin decays to the ground state. The emission of light by natural means is of considerable interest to biophysicists and biochemists due to the complicated reactions involved. Electro-optical physicists have observed an analogy between the *in vivo* emission of fireflies and laser light[1]. A recent study has shown micropulses hidden inside a flash of fireflies belonging to a particular species[2]. The light of the firefly has also been of interest in biomagnetics, due to the effect of magnetic fields on enzymatic activities[3]. The spectral distribution of bioluminescence has been the subject of numerous investigations, and existence of distinct groups of bands in a few species of fireflies has been reported[3–5]. Fireflies have a remarkable flash communication system involving precisely timed rapid bursts of bioluminescence. A comprehensive synthesis of work over the past two decades on firefly signal evolution, mate choice and predation is provided in a review by Lewis and Cratsley[6]. Nitric oxide, a ubiquitous signalling molecule, has been found to play a fundamental and novel role in controlling the firefly flash[7].

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Fireflies emit a substantial percentage of colours in the longer and shorter wavelength sides, which are not observable to the naked eye under usual conditions. In the present work, we report the spectrum of the firefly in colour and show that fireflies do emit a substantial part of the radiation in the red sector of the spectrum. The reason why we do not observe the red colour is explained on the basis of the Purkinje effect. Discovered in 1819 by Jan Evangelista Purkyne[8,9], the effect introduces a difference in colour contrast under different levels of illumination. For instance, in bright sunlight geranium flowers appear bright red against the dull green of their leaves, but in the same scene viewed at dusk, the contrast is reversed with the red petals appearing dark red or black, and the leaves and blue petals appearing relatively bright. The sensitivity to light in scotopic (night) vision varies with wavelength, though the perception is essentially black and white. The Purkinje shift is the relation between the absorption maximum of rhodopsin, reaching a maximum at about 500 nm, and that of the opsins in the long-wavelength and medium-wavelength cones that dominate in photopic (bright light) vision, about 555 nm. In visual astronomy, the Purkinje shift can affect visual estimates of variable stars when using comparison stars of different colours, especially if one of the stars is red. This Purkinje shift has an interesting psychophysical correlate. It may be observed, as evening draws on, that the luminosities of different colours of flowers in a garden change: the red becomes much darker, while the blue becomes much brighter. This implies that in this range of luminosities, called mesopic (dusk), both rods and cones are responding, and, as the rod responses become more pronounced, i.e. as darkness increases, the rod luminosity scale prevails over that of the cones. Thus the effect occurs because the colour-sensitive cones in the retina are more sensitive to yellow light, whereas the rods, which are more light-sensitive (and thus more important in low light) but which do not distinguish colours, respond best to green–blue light[10].

The bioluminescence spectrum was recorded in colour on an ASCO glass spectrograph using KODAK colour film of speed 400 ASA. The primary objective to photograph the spectrum in colour film was to visualize directly the colour emitted by the firefly species *Luciola praeusta* Kiesenwetter 1874 (Coleoptera: Lampyridae: Luciolinae). The experiments were carried out during early evening to midnight hours local time, using fireflies collected just prior to the experiment. A single firefly was held immobile inside a cotton plug with its light organ positioned towards the slit. An exposure time of 10–20 min was sufficient to record the spectra. The average temperature in the laboratory during the experiments was 32°C. The intensity distributions of spectra were worked out with the help of the open-source software, ImageJ. For this purpose the spectrograms were scanned with the help of a scanner and the software installed in the computer was

used to measure the intensity. The emitting organ was photographed using a video camera (Model Nikon Coolpix, 10 megapixels). For the purpose of scanning, the photograph was placed on a scanner (Model Canon Scan Lide 25) connected to the computer. The software for measuring the intensity in two dimensions, and also in three dimensions, was installed in the computer. Figure 1 shows a photograph of the light-emitting organ. Figure 2 shows its intensity distribution pattern in two dimensions in three different directions. Figure 3 shows the spectra of the bioluminescence emission and Figure 4 shows the corresponding intensity distribution patterns. To study the nature of the interference pattern, a double slit with separation of 0.05 cm was conveniently prepared by drawing two vertical lines on a glass slide covered with lamp black. When this double slit was held in front of the luminous organ of the firefly, interference fringes were observed, as shown in Figure 5 *a*. When a transmission grating (with 2000 LPI) was used, we observed a pattern as shown in Figure 5 *b*. Both these patterns were recorded in the Nikon Coolpix (10 megapixels) video camera. The intensity distribution patterns demonstrate the nature of the fringe patterns in each case.

As may be inferred from Figure 1, the elliptical pattern of the photograph of the light-emitting organ exhibits prominent yellow colour in the periphery followed by

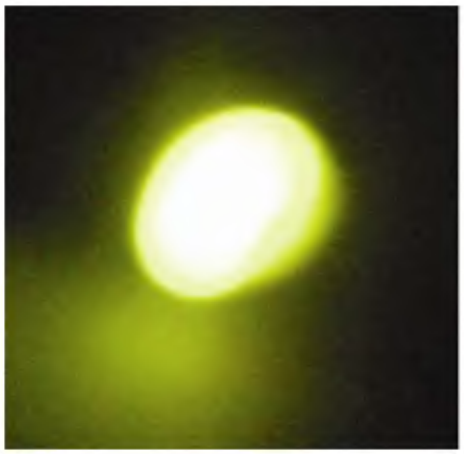

Figure 1. Photograph of the light-emitting organ of the firefly *Luciola praeusta* Kiesenwetter 1874 (Coleoptera: Lampyridae: Luciolinae).

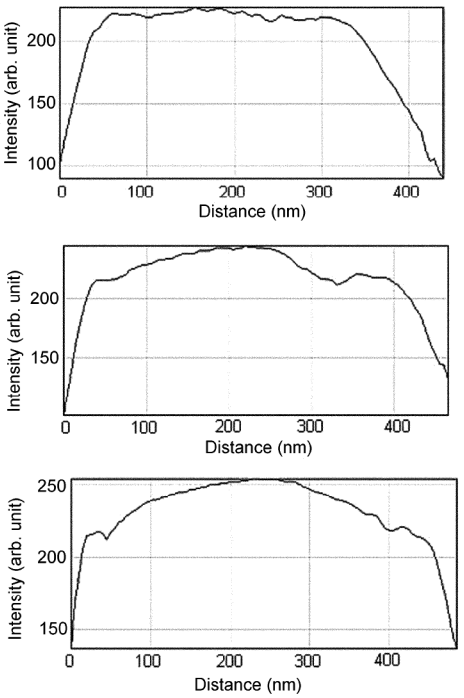

Figure 2. Intensity distribution pattern of the light-emitting organ using the open-source software, ImageJ.

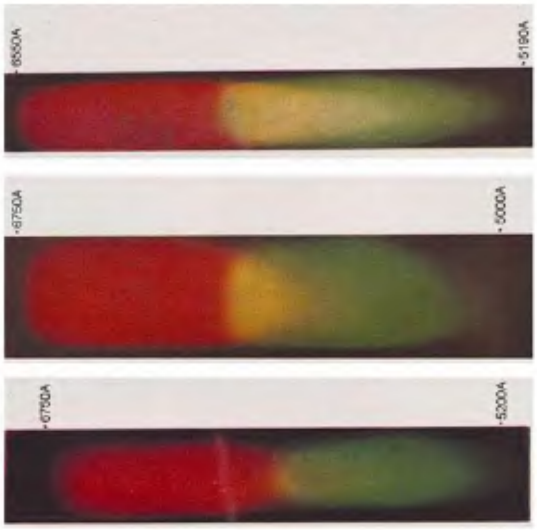

Figure 3. Emission spectra of three different specimens of the firefly species in colour.

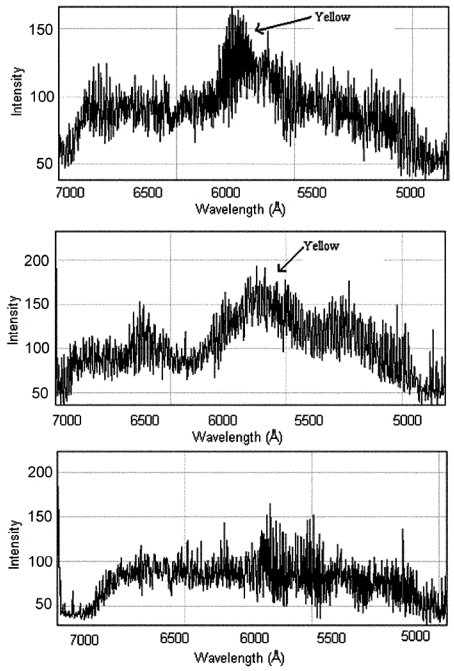

Figure 4. Intensity profiles of the emission spectra displayed in Figure 3 worked out using ImageJ.

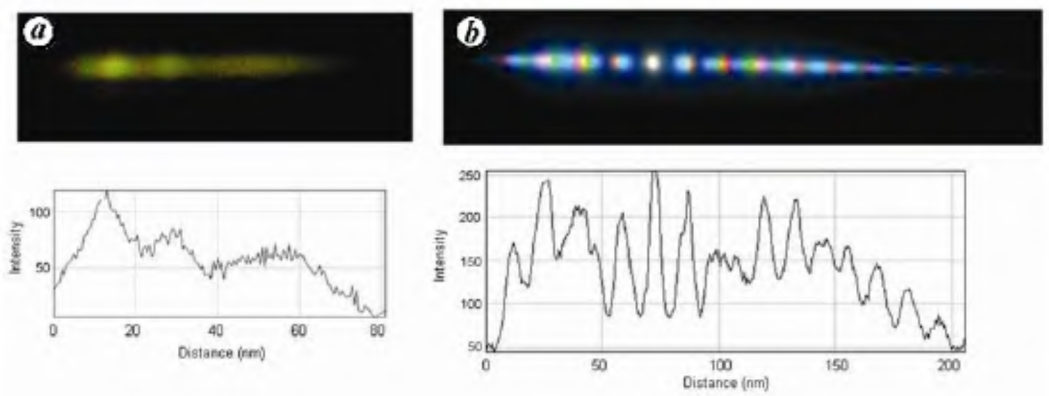

Figure 5. ***a***, Double-slit interference pattern (above), and the intensity distribution of the pattern (below). ***b***, Diffraction pattern (above) and the corresponding intensity distribution (below) when a transmission grating is used. It may be noted that the central fringe is yellow, whereas for white light the central fringe is bright and colourless.

weaker rings of colour. The central part is white. The intensity distribution pattern strikingly demonstrates the presence of alternate bright and dark rings. A careful examination indicates the presence of red colour outside the ring. It is reasonable to believe that these patterns are manifestations of diffraction and interference. However, this statement needs to be qualified with further studies. The primary objective of the present work is the manifestation of the Purkinje effect in bioluminescence of fireflies. The spectra in colour exhibit a remarkable effect. It is a matter of common experience that there is an immense disparity between the colour of the firefly light perceived by naked eyes and the colour recorded from the sources on films with the help of a spectrograph. The recorded bioluminescence spectra show three prominent colours: red, yellow and green. The colours of yellow and green are mixed up with no sharp line of demarcation separating them. In contrast, there is a line of demarcation which separates the red sector of the spectrum from the rest. This may be considered as significant, keeping in view the fact that in the usual VIBGYOR for white light recorded on similar films the line of demarcation separating the colours does not exist. Visual observations on the firefly light show that it is greenish-yellow with no indication of any colour in the red or orange sector of the spectrum. We refer here to one of the most striking phenomenon of vision known as the Purkinje effect related to the dark adaptation of the eye. It is generally understood that the colour depends on intensity. There are a number of phenomena that one can appreciate and which are observed because of transfer of the function from the rods and cones located in the retina. In bright light the rods are at a very low level of sensitivity, but in the dark they pick up their ability to see light. So the red sector of the spectrum is black so far as the rods are concerned. This rod–cone duplicity and the reversibility of the sensation of brightness of colours are best understood and illustrated by the Purkinje effect. The colours emitted by fireflies and those actually perceived are a manifestation of this effect.

Regarding the interference patterns illustrated in Figure 5 *a*, we infer that the yellow sector of the spectrum predominates. Similarly, from Figure 5 *b* we observe that the central maximum is yellow and the accompanying orders exhibit spectral colours similar to that shown in Figure 3. The salient feature in Figure 5 *b* is that the central fringe is bright yellow in colour, whereas for white light the central fringe is always white. It is observed that the red colour, prominent in Figure 3, is hardly seen in these two figures (Figure 5 *a* and *b*). This is easily understandable, as the emitted light is mainly greenish-yellow, with the red sector falling outside the full width at half maximum[2]. From the patterns, it is difficult to comment about the coherence of the firefly emission, but from the predominance of the intensity in the yellow sector within a narrow range of wavelength (~30 nm), it is reasonable to infer that the firefly emission has a tendency for spectral narrowing within the narrow yellow sector of the spectrum.

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