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

Anurup Gohain Barua,[a,*] Simanta Hazarika,[a] Nayanmoni Saikia[a] and Gauranga Dhar Baruah[b]

[a]Department of Physics, Gauhati University, Guwahati-781014, India

[b]Department of Physics, Dibrugarh University, Dibrugarh-786004, India

[*]Corresponding author; E-mail: agohainbarua@yahoo.com

## Abstract

*In vivo* emission and time-resolved spectra of firefly *Luciola praeusta* Kiesenwetter 1874 (Coleoptera : Lampyridae : Luciolinae) have been recorded. The emission spectrum shows the FWHM value for this particular species to be 55 nm, which is significantly narrower than the *in vivo* 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-catalysed chemiluminescence reaction in the firefly for the emission of light is much faster than is believed to be.


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

## 1 Introduction

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 (Iwasaka and Ueno 1998).

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 (Iwasaka and Ueno 1998, Biggley et al 1967, Bora and Baruah 1991). In this report, we have presented, of firefly *Luciola praeusta*, an *in vivo* emission spectrum, where the full width at half maximum (FWHM) value has come out to be significantly narrow. This value has been consolidated by another spectrum of the firefly emitting continuous light under the influence of ethyl acetate. It is worthwhile to mention here that fireflies of this species emit flashes of light from their abdominal lanterns. Fireflies have a remarkable flash communication system

involving precisely timed, rapid bursts of bioluminescence. 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 been reported (Venel 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 signaling molecule, has been discovered to play a fundamental and novel role in controlling the firefly flash (Trimmer et al 2001), while it has been suggested that the firefly flash could be regulated by calcium (Buck et al 1963). It is worthwhile to mention here that the term *flash* has been used synonymous with the term *pulse* till now. The duration of a single pulse/flash has been reported to very from about 70 milliseconds (Branham and Greenfield 1996) to a few hundred milliseconds (Barry et al 1979, Saikia et al 2001, Carlson 2004). The time-resolved spectrum presented in this communication is clearly in disagreement with these values.

## 2 Materials and Methods

The emission spectrum was 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 from the Gauhati University campus, and kept

immobile in a cotton plug with its light organ positioned towards the entrance face of the fiber. *In vivo* emission spectra of fifty specimens, both male and female, of the firefly species have been recorded in this way. For recording the continuous glow of the firefly, we kept it in a 1.5 ml capacity micro centrifuge plastic tube of length 4 cm. One end, of opening diameter 3 mm, of the tube was attached to the entrance face of the optical fiber in the spectrometer. The other end, of diameter 1 cm, was filled with cotton dipped in ethyl acetate. It was observed that the flashing rate of the firefly rapidly decreased. After about a minute, a constant glow appeared from the last segment in the abdomen of the firefly which spread to the other light emitting segment in about 3 minutes. A black patch in the middle of the upper segment of the lantern finally gave way to the glow in 5 to 6 minutes. Ten emission spectra of fireflies emitting this kind of continuous light were recorded. The experiments were performed at laboratory temperatures $26^0$C - $31^0$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 the system noise (Fig. 1a and Fig. 1b).

The experimental set up for recording time-resolved pulses of the firefly is shown in Fig. 2. 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, 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. Tektronix TDS 520A digital storage oscilloscope was used to record the pulses. Time-resolved spectra of five specimens were recorded in this way. The emission and time-resolved experiments, with arrangements described above, could be easily reproduced, if the flashing fireflies are available.

## 3 Results and Discussion

The peak wavelength and the full width at half maximum (FWHM) in all the emission spectra of the firefly — recorded in trapped as well as ethyl acetate-affected conditions and shown, one each, in Fig. 1a and Fig. 1b respectively — have been found to be remarkably constant. The position of the peak wavelength has been 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 region, with the weak red sector lying outside the halfwidth upto 670 nm. The FWHM value has been measured to be 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 now. From spectroscopic point of view, this small value implies that out of the luciferin molecules excited to the oxyluciferin state of this firefly species, about 50 percent occupy levels in

that state narrower in spacing compared to that of the other species. It has been proposed that different fireflies emit in slightly different spectral regions due to slightly different enzyme structure (Seliger et al 1964); different FWHM values could also be due to this fact. The narrowest half width, by the way, has been 64 nm measured for firefly species *Photinus consimilis* and *Photinus umbratus* (Biggley et al 1967). 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. It could be mentioned here that 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 Fig. 3, exhibits striking similarity with the output of a multimode laser. The spectrum presented here has revealed 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 a *flash*, consisting of a number of microsecond pulses, is of duration 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 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. The event recorded in Fig. 4 shows the 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 advertisement ones emitted by fireflies for courtship and mating; these could be lightly described as SOS signals sent by them!

It has been proposed (Saikia et al 2001) that the time-resolved spectrum of the firefly can be considered as the manifestation of oscillating chemical reactions, the so-called BZ reactions (Belousov 1959, Zhabotinsky 1964). 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.

**Acknowledgment**

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. Mukhupadhyay, 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 for the recording of time-resolved spectra.

**References**

Barry J D, Heitman J M and Lane C R 1979 Time-resolved spectrometry of *in vivo* firefly bioluminescence emissions; J. Appl. Phys. 50 7181-7184

Belousov B P 1959 A periodic reaction and its mechanism; Compilation abs. radiation med. 147 145

Biggley W H, Lloyd J E and Seliger H H 1967 The spectral distribution of firefly light II; J. Gen. Physiol. 50 1681-1692

Bora L and Baruah G D 1991 Bioluminescence emission of few species of fireflies; Indian J. Phys. 65B 551-557

Branham M A and Greenfield M D 1996 Flashing males win mate success; Nature 381 745-746

Buck J, Case J F and Hansen F E 1963 Control of flashing in fireflies III. Peripheral excitation; Biol. Bull. 125 251-269

Carlson A D 2004 Is the firefly flash regulated by calcium?; Integr. Comp. Biol. 44 220-224

Coblentz W W 1912 A physical study of the firefly; Bull. Carneg. Instn. 164 1-47

Iwasaka M and Ueno S 1998 Bioluminescence under static magnetic fields; J. Appl. Phys. 83 6456-6458

Saikia J, Changmai R and Baruah G D 2001 Bioluminescence of fireflies and evaluation of firefly pulses in light of oscillatory chemical reactions; Indian J. Pure Appl. Phys. 39 825-828

Seliger H H, Buck J B, Fastie W G and McElroy W D 1964 The spectral distribution of firefly light; J. Gen. Physiol. 48 95-104

Trimmer B A, Aprille J R, Dudzinski D M, Lagace C J, Lewis S M, Michel T, Qazi S and Zayas R M 2001 Nitric oxide and the control of firefly flashing; Science 292 2486-2488

Venel F D and Carlson A D 1998 Proximate mechanisms of sexual selection in the firefly *Photinus pyralis* (Coleoptera: Lampyridae); J. Ins. Behav. 11 191-207

Viviani V, Uchida A, Suenaga N, Ryufuku M and Ohmiya Y 2001 Thr226 is a key residue for bioluminescence spectra determination in beetle luciferases; Biochem. Biophys. Res. Commun. 280 1286-1291

Zhabotinsky A M 1964 Periodic processes of malonic acid oxidation in a liquid phase; Biofizika 9 306-311

## Captions to Figures

**Fig. 1a.** Emission spectrum of firefly *Luciola praeusta*. The peak wavelength appears at 562 nm and the half width is of the value 55 nm.

**Fig. 1b.** Emission spectrum of the firefly *Luciola praeusta* under the influence of ethyl acetate. The peak wavelength and FWHM values are the same as in Fig. 1a.

**Fig. 2.** Experimental arrangement to record time-resolved spectra of the firefly.

**Fig. 3.** Time-resolved pulses of the firefly, showing the relaxation oscillation. The duration of a pulse has come out to be approximately 2 microseconds. 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 have considered the pulses only if they are above the trigger level, i.e. 32 mV, shown by the arrow on the right ordinate in the oscilloscope screen.

**Fig. 4.** 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.

**Fig.1a**

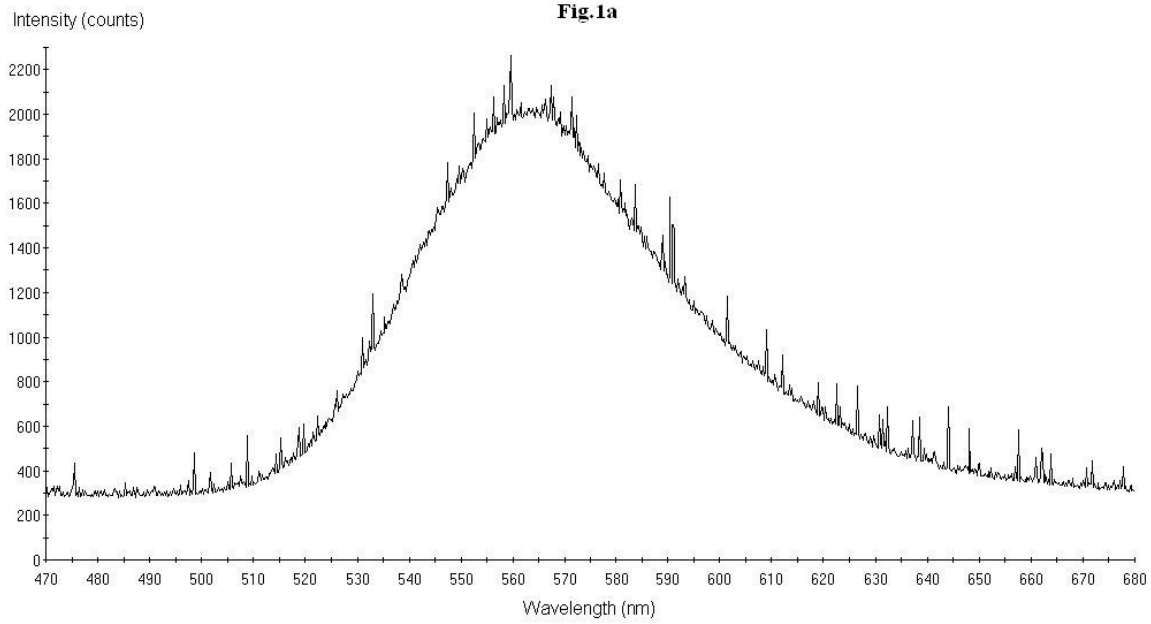

Intensity (counts)
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
470
480
490
500
510
520
530
540
550
560
570
580
590
600
610
620
630
640
650
660
670
680
Wavelength (nm)

**Fig. 1b**

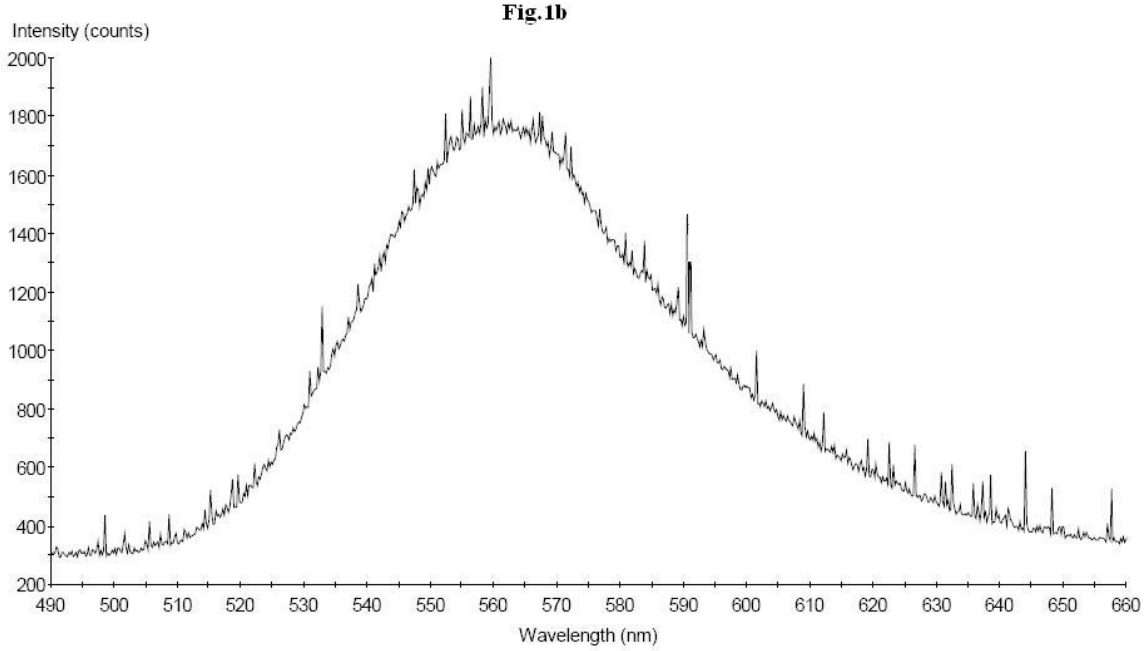

Intensity (counts)
2000
1800
1600
1400
1200
1000
800
600
400
200
490
500
510
520
530
540
550
560
570
580
590
600
610
620
630
640
650
660
Wavelength (nm)

**Fig.2**

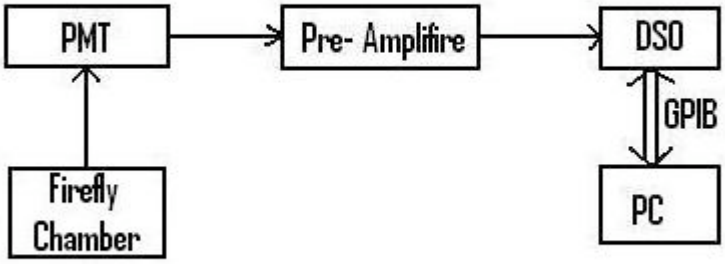


**Fig.3**

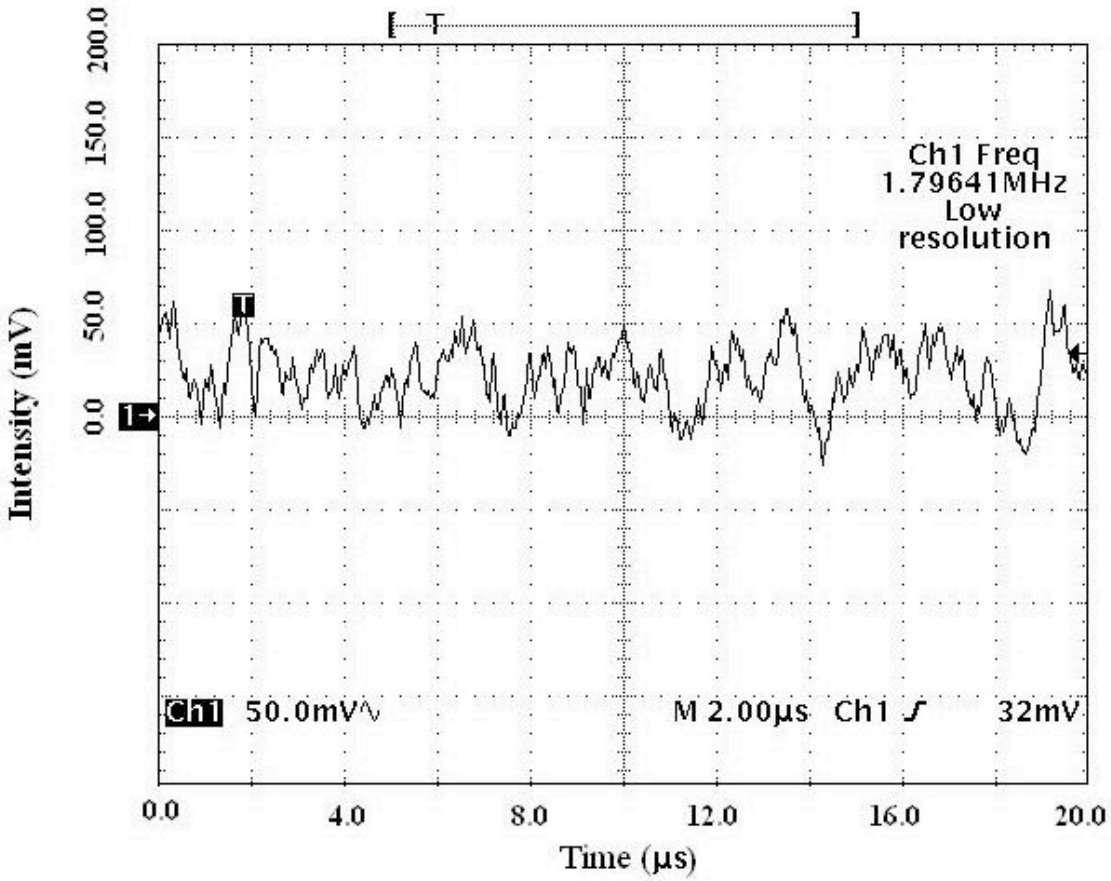
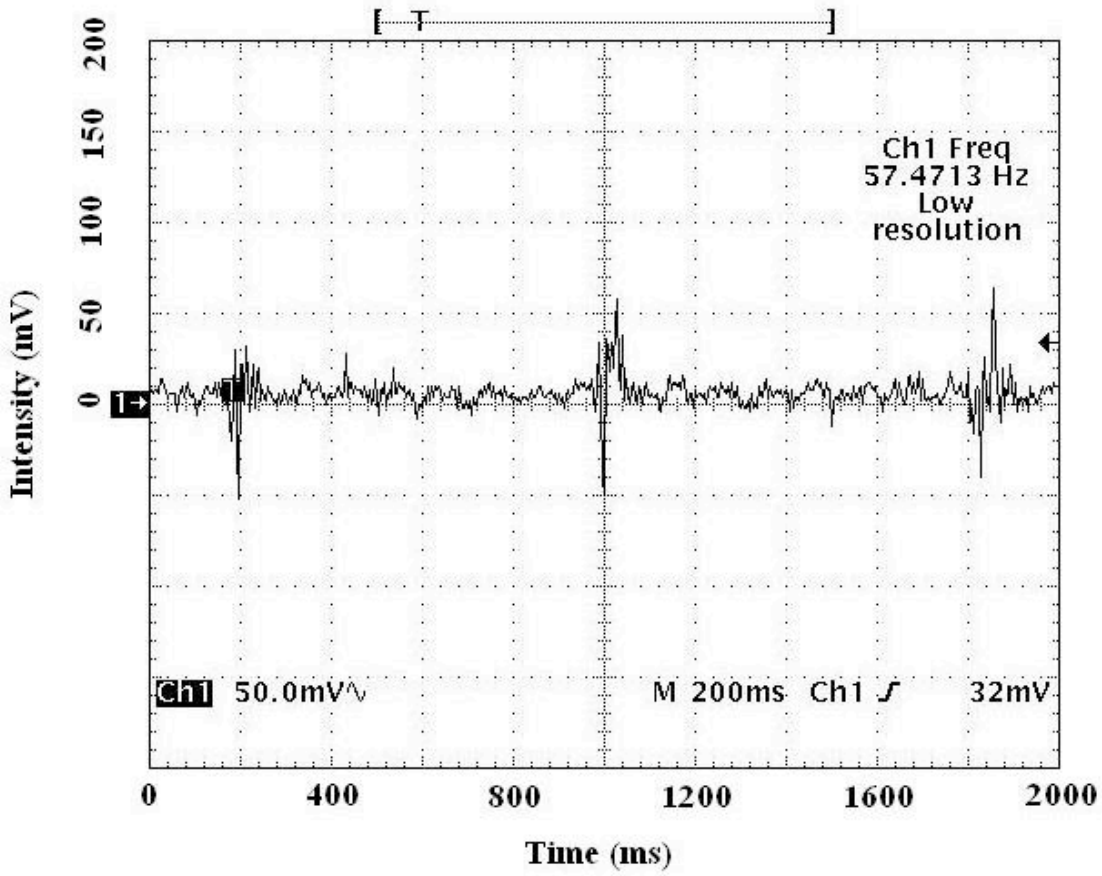

Intensity (mV)
200.0
150.0
100.0
50.0
0.0
Ch1 Freq
1.79641MHz
Low
resolution
Ch1 50.0mV
M 2.00µs Ch1 32mV
0.0
4.0
8.0
12.0
16.0
20.0
Time (µs)

**Fig.4**


Intensity (mV)
200
150
100
50
0
Ch1 Freq
57.4713 Hz
Low
resolution
Ch1 50.0mV
M 200ms Ch1 32mV
0
400
800
1200
1600
2000
Time (ms)