Time Resolved Spectroscopy Using Boxcar Integrator

Bisman Perangin-angin\textsuperscript{1}, Kerista Tarigan\textsuperscript{1,2}, Poltak Sihombing\textsuperscript{2}

\textsuperscript{1} Department of Physics, FMIPA, Universitas Sumatera Utara
\textsuperscript{2} Department of Computer Science, Universitas Sumatera Utara

Email: bipesu@yahoo.com

Abstract. An observation of fluorescence decay signals of condensed-state molecular samples in the time domain has been proved which is very useful for its identification and analysis. To minimize the noise effect in the fluorescence signals, a double-gated boxcar integrator is designed and incorporated into a spectrometer. The design concept and the working principle of the develop spectrometer are presented along with the detailed subsystems description. The performance of the spectrometer is tested with a well-known sample (rhodamine 6G) and the results are dually analyzed. The application of the spectrometer is used to measure the fluorescence spectrum and mean lifetime of an E. Coli bacteria sample. The lifetime of the E.Coli is about 10 ns which is obtained at peak wavelength of 506 nm.

1. Introduction
The first step for analyzing biomedical molecules process especially bacteria and others is material identification\textsuperscript{[1-2]}. There are several method of materials analyze in spectroscopy, one of them is based on the wavelength absorbed by the corresponding molecule. The another method is based on the fluorescence spectrum called as a graph curve between the intensity and wavelength \textsuperscript{[3-4]}. The analysis of biomedical molecule based on decay intensity with the time usually called spectroscopy. The spectroscopy has an advantages of more accurate and faster \textsuperscript{[5-6]}. Most of the biomedical molecules will fluoresce when it is exposed into the light with ultraviolet (UV) region.

The characteristic of the molecule is normally represented by the lifetime of the fluorescence signals. If the molecule lifetime is known, then the molecule can be identified. The fluorescence signals of a molecule can be called as a transient signal which is in addition to being weak \textsuperscript{[7-8]} and lasts very shortly (measure ns). To observe a transient signal, it requires not only a special handler but also a boxcar integrator to capture sensor signal \textsuperscript{[9-10]}. It causes the signal immersed in the sensor must be high speed. The fluorescence signals are repeatedly and stored on high-quality capacitors \textsuperscript{[11-12]}. The tipping points are shifted by setting the delay in mono-stable with a delay determined by the RC time used. The boxcar Integrator is used to support this study to eliminating the noise.

2. Method and Material
The research method is electronically conducted with experimental scheme as shown in the Figure 1. The measurement sample is prepared in the Laboratory of Electronics, FMIPA, Universitas Sumatra Utara (USU). In our spectrometer system, N2 short-pulse lasers in the ultraviolet region are employed as the excitation source. Since the pulse duration of the laser emission is about 5 ns, then the absorption triplet can usually be ignored. Therefore, the correlation between the obtained fluorescence quantum and laser efficiency could become large in the type of pumping \textsuperscript{[13-14]}. Generally, a sample
cell provides sample containment and a uniform illumination surface. These cells are typically constructed of quartz to allow passage of the ultraviolet radiation. The emission-wavelength of the selector system allows the isolation of selected wavelengths from the emission fluorescence. The function of this component is to select the monochromatic or narrow-band radiation for emission. The final component is the detector, which is placed at the excite slit of the emission-wavelength selection system [15-16].

![Figure 1](image1.png)

Figure 1. The block diagram of the Time Resolved Spectroscopy Using Boxcar Integrator.

![Figure 2](image2.png)

Figure 2. Circuit diagram of the Boxcar Integrator.

The detailed circuit diagram of the Boxcar Integrator is shown in the Figure 2 [17-19]. The design (in Figure 2) uses the LF 356 (Q4, Q5) amplifiers which is characterized by the very low input bias current, MOSFET 3 SK 21H (Q1, Q2) bilateral analog switches, and a fast input amplifier of MC 1733 (Q3) type. The use of circuits is to provide the high-quality storage and integrating capacitors.
The switches Q1 and Q2 are under control of monostable Q7 and Q8. The aperture delay is controlled by a monostable Q6 which output pulse could be varied from 35 ns to 15400 ns by choosing appropriate external timing components. The triggering signal is synchronized to the signal repetition rate. Figure 3 shows the timing diagram of the delay and gate pulse.

![Timing Diagram](image)

**Figure 3.** The triggering signal.

### 3. Result and Discussions
The obtained results from the experiments are electronically represented as in the Table 1 to Table 4.

| Table 1. spectrum of rhodamine 6G | Table 2. Decay intensity of rhodamine 6G |
|-----------------------------------|----------------------------------------|
| Wavelength (nm) | Intensity (arb. unit) | Time (ns) | Intensity (arb. unit) |
| 520 | 0.8 | 0 (ref.) | 5.4 |
| 540 | 1 | 4 | 4 |
| 550 | 1.7 | 8 | 3.5 |
| 560 | 2 | 12 | 3 |
| 570 | 3.5 | 16 | 2.3 |
| 580 | 4 | 20 | 1.9 |
| 600 | 4.3 | 24 | 1.5 |
| 610 | 3.8 | 28 | 1.3 |
| 620 | 3 | 32 | 0.5 |
| 630 | 2 | | |
| 640 | 1.5 | | |
| 650 | 1 | | |

| Table 3. spectrum of E.Coli | Table 4. Decay intensity of E.Coli |
|-----------------------------|-----------------------------------|
| Wavelength | Intensity |
| | |
| | |
From the wavelength and intensity in the Table 1 to Table 4 are plotted to indicate the fluorescence spectrum and decay intensities as shown in Figures 3 – 6.

| (nm) | (arb. unit) |
|------|-------------|
| 502  | 0.6         |
| 503  | 1.6         |
| 504  | 4           |
| 505  | 4.5         |
| 505.5| 5.5         |
| 506  | 6           |
| 506.5| 5.8         |
| 507  | 4.8         |
| 508  | 3.8         |
| 509  | 1.8         |
| 600  | 0.5         |

| Time (ns) | Intensity (arb. unit) |
|-----------|-----------------------|
| 0 (ref.)  | 6                     |
| 4         | 4.2                   |
| 8         | 3.4                   |
| 12        | 2.2                   |
| 16        | 1.3                   |
| 20        | 0.8                   |

**Figure 4.** Fluorescence emission of rhodamine 6G.
Figure 5. Fluorescence spectrum of E.Coli.

Figure 6. Decay intensity of E.Coli.

Figure 7. Decay Intensity of rhodamine 6G.
From the fluorescence spectrum curve and from the decay curve, the mean of the peak wavelength and the mean of a lifetime are obtained, respectively. In this work, the least squares method is used to obtain the relations between \( \ln I \) vs \( t \) by which the mean lifetime of the samples is determined. In the case of the rhodamine 6G, the value of around 20 ns at 600 nm wavelength is obtained. This result is consistent with the obtained result with others technique. In the same way with this experiment, the mean of a lifetime of the E.Coli is about 10 ns which is obtained at peak wavelength of 506 nm.

4. Conclusions

Based on the consistent obtained results in the experiment, the measurements with the proposed method is capable of detecting and analyzing the weak transient signals in fluorescence spectroscopy with reasonable accuracy. With further technical improvement, this spectrometer could be used for rapid instrumental detection and analysis of biomedical samples.

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