

SCIENCE IMAGING SYSTEMS

Application Note No. 9

Fundamentals of Fluorescence Detection Using Blue LED Illuminator

LAS-1000

Introduction

The Fujifilm LAS-1000 Luminescent Image Analyzer has both chemiluminescence and fluorescence detection capability.

As the excitation light source for fluorescence detection, the LAS-1000 uses a blue light-emitting diode that is superior to the UV illuminators employed in conventional systems in the major points of safety, compactness and cost.

The LAS-1000 is usable with various fluorescence detection methods, including detection of EtBr or SYBR® Green stained DNA/RNA in gel, detection of SYPRO® Orange stained protein, and detection by the enzyme amplified fluorescent method using alkaline phosphatase following Southern or Western blotting using AttoPhos™.

This Application Note covers topics ranging from the emission mechanism of the blue light-emitting diode to specific LAS-1000 applications.

Contents

1. History of Blue Light-emitting Diode Development
2. How the LAS-1000 Excitation Light Source was Chosen
3. Emission Principle of the Blue-SQW-LED
4. Fluorescent Imaging Using the Blue-SQW-LED
5. Features of the LAS-1000 Fluorescence Detection System
6. References

Summary

- The Blue-SQW-LED adopted as the light source for exciting fluorescence is safer, smaller and less expensive than the conventional UV illuminator.
- The service life of the Blue-SQW-LED is 50 to 100 times than that of an UV illuminator.
- The Blue-SQW-LED has a primary wavelength of 470 nm.
- Fluorescent detection capability includes detection of EtBr, SYBR® Green or SYPRO® Orange stained DNA, protein etc. in gel. Detection by the enzyme amplified fluorescent method using AttoPhos™ as substrate is also possible.

1 History of Blue Light-emitting Diode Development

Red and yellow light-emitting diodes (LEDs) with high luminance were developed in the 1960s, but a practical blue LED proved considerably more difficult to realize. The appearance of the much-anticipated large full-color LED display was therefore long delayed for the lack of a bright source of blue light.

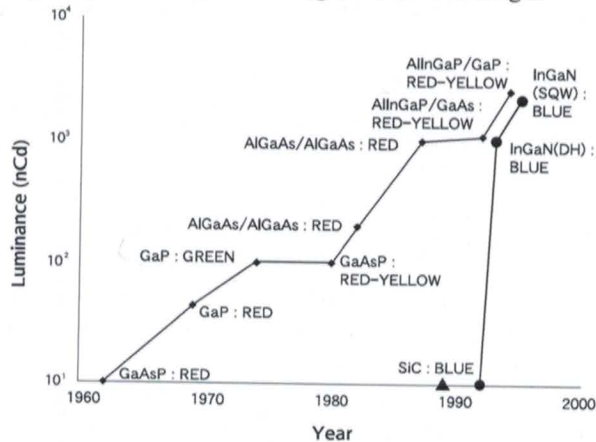


Fig.1-1

Fig.1-1 History of light-emitting diode development

As can be seen from Fig. 1-1, the blue light-emitting diode debuted considerably later than red and yellow LEDs. The first practical blue LED appeared only after production of high-quality films of GaN crystal became possible thanks to breakthroughs in GaN compound semiconductor research between 1985 and 1990. In December 1993, Nichia Chemical Industries, Ltd. announced the development of a high luminance blue LED made of GaN compound semiconductor with a double hetero (DH) structure. With an emission output of 1 mW peaking at 450 nm (blue region), Nichia's blue LED was about 100 times brighter than its SiC predecessor. (Reference 1) Then, in 1994, Nichia developed a blue/green LED with an active layer of InGaP and a single quantum well (SQW). In its blue configuration, the LED achieved an output of 5 mW. (Reference 2) In addition, a high luminance blue LED using a multi-quantum well (MQW) was reported by the Toyoda Gosei Co., Ltd. in 1995. (Reference 3)

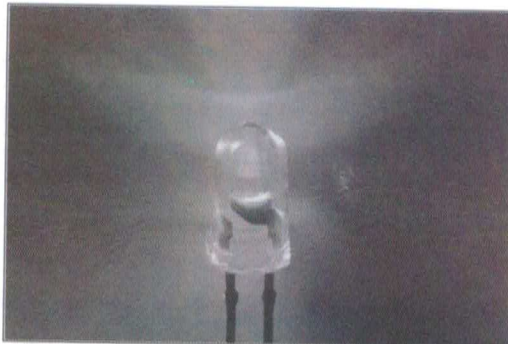


Fig.1-2

Fig.1-2 Blue LED

2 How the LAS-1000 Excitation Light Source was Chosen

Commercially available blue LED materials include GaN, SiC and ZnSe types.

Since GaN compound semiconductors exhibit the highest external quantum efficiency (see table), they have become the materials of choice for blue LEDs requiring high performance in both color purity and brightness. This led Fuji Photo Film to examine the possibility of using a GaN compound semiconductor LED as the source of blue excitation light in fluorescent detection. Most commercially available blue LEDs made of GaN compound semiconductor fall in one of three structure categories: single quantum well (SQW), multi-quantum well (MQW) and double hetero (DH). Ultimately, the search for an LED excitation light source for use in the LAS-1000 narrowed down to the Blue-SQW-LED made by Nichia. While the LED's acceptably high brightness and long service life were factors, the quality that made it the final choice was its color purity. (Fig. 2-1).

External quantum efficiency

Ratio of emitted luminous energy to injected electrical energy

Color purity

Factors to be considered when using LED light to excite fluorescence dye include primary wavelength, spectrum half-width and impurity-induced emission. The LAS-1000 LED is superior to MQW and DH LEDs on all of these points.

LED Material	Primary wavelength	External quantum efficiency (%)	Emission output (mW)
ZnCdSe	489nm	1.7	0.43
SiC	470nm	0.03	0.036
InGaN	470nm	9.1	3.0

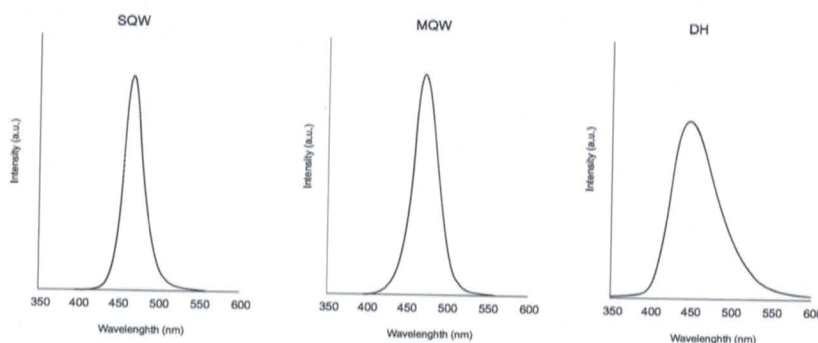


Fig.2-1

Fig.2-1 Emission spectra of differently structured GaN compound LEDs

3 Emission Principle of the Blue-SQW-LED

Basic structure of the Blue-SQW-LED

The diagram in Fig. 3-1 shows the basic configuration of the Blue-SQW-LED.

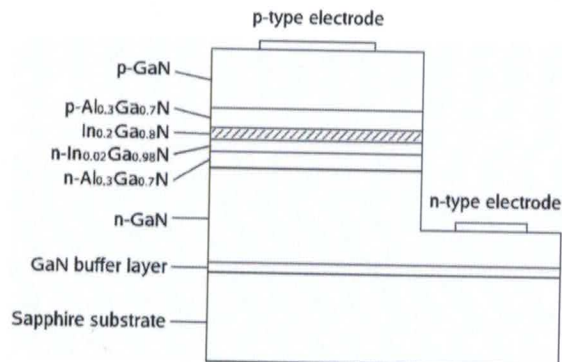


Fig.3-1

The Blue-SQW-LED is fabricated by using metal organic vapor phase epitaxy (MOVPE) to successively grow a GaN buffer layer and layers of n-GaN, n-AlGa_{0.3}N, n-InGa_{0.2}N, In_{0.2}Ga_{0.8}N, p-AlGa_{0.3}N and p-GaN on the C surface of a sapphire crystal substrate. The In_{0.2}Ga_{0.8}N forms the active layer. The wavelength of the light emitted by the LED can be varied by changing the subscript values, between 360 nm (for GaN) and 650 nm (for InN), and is 470 nm when the ratio of In to Ga is 0.2 to 0.8. The single quantum well structure is formed by a combination of the InGa_{0.2}N active layer of this composition and AlGa_{0.3}N.

Emission mechanism

Let us now look at the Blue-SQW-LED emission mechanism.

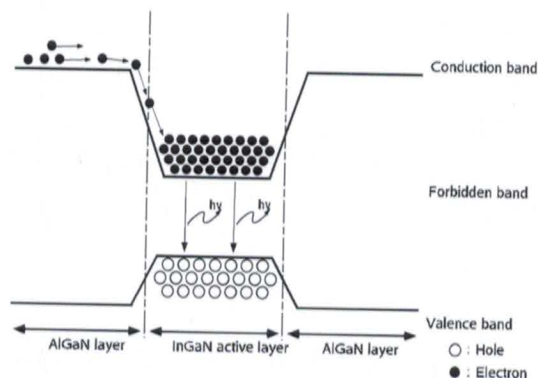


Fig.3-2

Fig. 3-2 is a diagram showing the Blue-SQW-LED bands. The depression holding electrons is the "well" that gave the structure the name "quantum well." When the structure has only one layer, it is called a "single quantum well." An electron caught in the InGa_{0.2}N active layer by this quantum well recombines with a hole. This produces a photon (light particle) whose wavelength of about 470 nm corresponds to the bandgap (forbidden band width) of the active layer.

Fig.3-1 Basic structure of Blue-SQW-LED

Although the wavelength spectrum can theoretically be varied between 360 nm (GaN) to 650 nm (InN) by selecting the ratio between In and Ga, the longest wavelength realized in a marketed product is 530 nm (green). Longer wavelengths are hard to achieve because a high molar fraction of In degrades the crystallinity of the layer and makes it unusable for light emission.

Metal organic vapor phase epitaxy

A method of growing a single crystal by using the gas of an organometallic compound to grow successive single crystal films on a crystal substrate.

Fig.2-2 Blue-SQW-LED band diagram

The band structure is called a "quantum well" because it catches electrons in a well-like indentation. A "single quantum well" has only one such layer.

Conduction band

Energy band that contributes to conduction.

Forbidden band

Energy band where electrons cannot exist.

Valence band

The highest energy band, completely filled with electrons.

Hole

In an insulating material or semiconductor material that is not completely filled with electrons, a vacancy caused by the absence of an electron where one might exist. It has been found convenient to treat holes as particles.

4 Fluorescent Imaging Using Blue-SQW-LEDs

The LAS-1000 is equipped with a pair of Blue-SQW-LEDs arranged to illuminate the sample uniformly. The fluorescence detection system using the Blue-SQW-LEDs is illustrated in Fig. 4-1. The filters located in front of the LEDs are for passing only the blue light needed for selective excitation of fluorescence dye. The filter in front of CCD sensor is for cutting reflected excitation light and passing only the desired fluorescence from the sample.

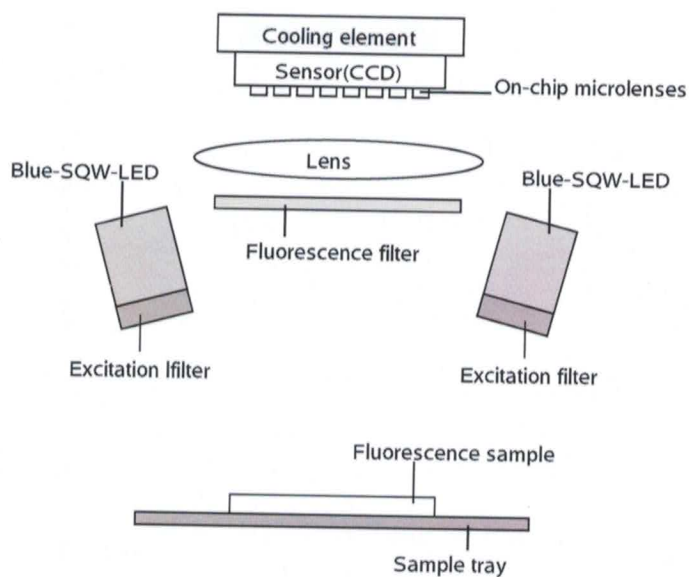


Fig.4-1

Fig.4-1 Conceptual diagram of
fluorescent detection

5 Features of the LAS-1000 Fluorescence Detection System

■ Ideal excitation light source for high-sensitivity fluorescence reagents

Recent years, use of SYBR® Green and other newly-developed high-performance fluorescence reagents which has higher sensitivity than the long-popular EtBr is increasing. (References 5-9)

The excitation light of the LAS-1000 has a primary wavelength of 470 nm, which is very close to the optimum excitation wavelength of these reagents.

Example of sample detection using Blue-SQW-LED

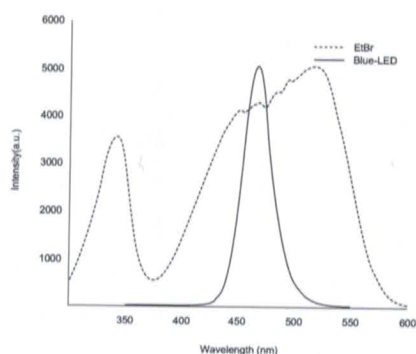


Fig.5-1

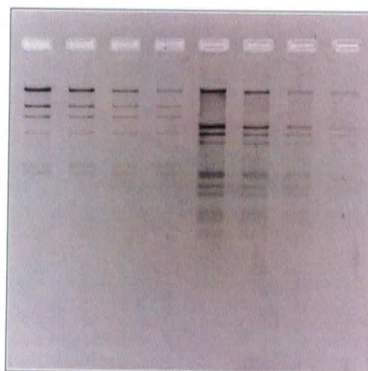


Fig.5-1 Detection of EtBr-stained λ DNA / Hind III
Left : EtBr excitation / Blue-LED emission spectra
Right : Example of detected image

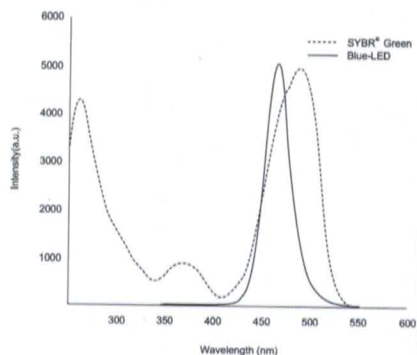


Fig.5-2



Fig.5-2 Detection of SYBR® Green-stained λ DNA / Hind III
Left : SYBR® Green excitation / Blue-LED emission spectra
Right : Example of detected image

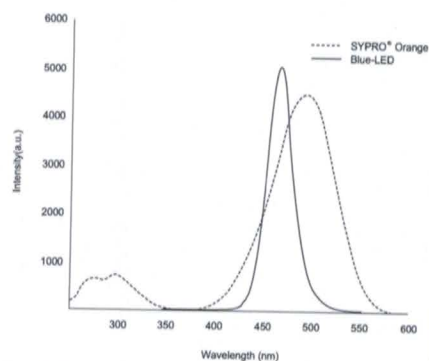


Fig.5-3

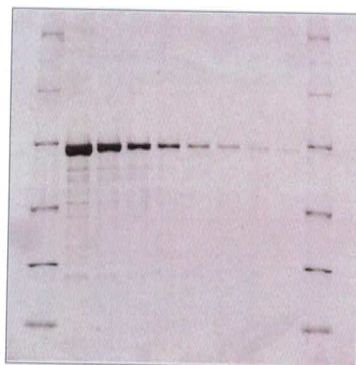


Fig.5-3 Detection of SYPRO® Orange-stained bovine serum albumin

Left : SYPRO® Orange excitation / Blue-LED emission spectra
Right : Example of detected image

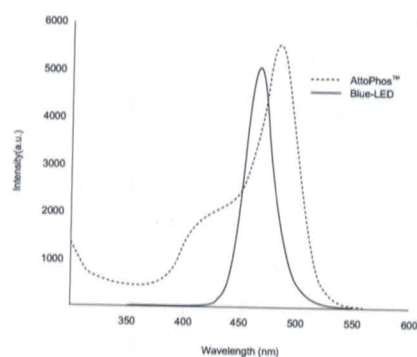


Fig.5-4

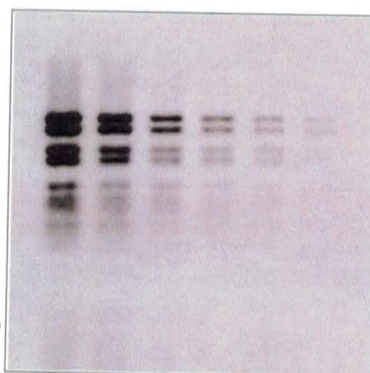


Fig.5-4 Detection of AttoPhos™-labeled pBR328/*Bgl* I + *Hinf* I

Left : AttoPhos™ excitation / Blue-LED emission spectra
Right : Example of detected image

■ Damage safety

The conventional method of measuring electrophoresed EtBr-stained gels and the like using an UV transilluminator as the excitation light source (Reference 10) is problematic since ultraviolet rays are known to damage fluorescence dyes, nucleic acid and protein. Operator safety is also a concern.

The excitation light source of the LAS-1000 emits visible light with a primary wavelength of 470 nm. Light of this wavelength causes no damage to the sample and is perfectly safe for the operator.

■ Durable, inexpensive and safe

The Blue-SQW-LED installed in the LAS-1000 has a longer service life and is more compact than an UV transilluminator.

Uniform illumination is relatively easy to achieve by arraying LEDs two-dimensionally.

6 References

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