euspen's 23rd International Conference & Exhibition, Copenhagen, DK, June 2023
www.euspen.eu

Broadband chromatic light source and inspection system for fluorescence imaging

HyungTae Kim[1], Juhea Kim[1] & Dong-Wook Lee[1]

[1]*Research Institute of Convergence Technology, KITECH, South Korea*

htkim@kitech.re.kr

Abstract
A chromatic light source was constructed to observe the target object using a broadband camera ranging of ultraviolet (UV), visual (Vis) and near infrared rays (NIR) for fluorescence imaging. The chromatic light source generates sufficient light of variable wavelengths and narrow bands. A chromatic light source comprised of a high-power xenon lamp, condensing lenses, a monochromator, and air-cooling u. White light was generated from the high-power xenon arc lamp and concentrated into the inlet slit of the monochromator after passing through the condensing lenses. Light of a specific-band wavelength was achieved after passing through the slits and gratings in the monochromator. The band-width of the output light was adjusted using the slit width, and the centre wavelength was varied by rotating the gratings. The air-cooling system radiated the heavy heat caused by the white light in the xenon lamp and inlet slit. The liquid guide transferred the output light from the outlet slit to the target object with high optical efficiency. Then, the output light of variable wavelengths was illuminated onto the target object to acquire UV-Vis-NIR images. As the camera focus and field of view varied according to the wavelength of the light, a translation unit and motorized zoom lens were applied to the inspection system. The spectral range of the light source was 310-930 nm and the minimum bandwidth was 1.4 nm after measuring the output light using a spectrometer. The proposed system is useful for investigating light-influencing properties; thus, it is applicable for inspecting cells, materials, and art-works.

Image, Inspection, Microscope, Optical

1. Introduction

Fluorescence imaging is widely used for cellular observation, material analysis, and product inspection. In fluorescence imaging, a target object responds to particular wavelengths of incident light, and a high-sensitivity camera captures images of the responses. Thus, a light source for fluorescence imaging generates light of specific wavelengths, and a multiplex light source is commonly preferred because of the various optical responses of the target objects. Arc-based light sources and light-emitting diodes (LEDs) are commonly used for multiplex light source [1]. Arc-based light sources generate a wide spectrum of white light using Mercury, Xenon, or metal halide lamps. Specific wavelengths of light are extracted from white light using optical filters and monochromators [2]. Incident light of specific wavelengths can be obtained from arc-based light sources; however, adjustment of light intensity is difficult. Whereas LEDs are convenient for adjusting light intensity and exhibit high efficiency, the wavelengths in the optical spectrum are fixed, and the number of LED for multiplexing is limited [3]. Thus, this study proposes a light source with variable wavelengths using a high-power xenon lamp and monochromator for fluorescence imaging. The wavelength range of the light source covered ultraviolet, visual and near infrared rays. The wavelength band was adjusted according to the width of the monochromator slits. The wavelength shapes of the output rays were verified using a spectrometer.

2. Principles

The proposed light source is composed of a high-power xenon lamp, condensing lenses, a monochromator, an optical liquid guide, and air-cooling units. A xenon lamp generates white light with wavelengths ranging from UV to NIR. White light is concentrated on the inlet slit of the monochromator using condensing lenses. Light of a specific wavelength is discharged from the outlet of the monochromator. The centre wavelength of the discharged light can be varied by tilting the gratings using a motor. The motor is driven by a PC using an RS-232C. The band-width of the discharged light can be adjusted by changing the slit width. Heavy heat is caused by the white light in the xenon lamp and inlet slit; thus, air pressure is sprayed onto the hot spots for cooling. The light discharged from the outlet slit is transferred to the target object using an optical liquid guide to achieve high transmission efficiency. The transmitted light diffuses onto a target object, and a UV-Vis-NIR camera acquires the spectral responses of the target object. The spectral characteristics of the transmitted light are measured using a spectrometer. Figure 1 and 2 show a conceptual diagram and photo of a broadband chromatic light source.

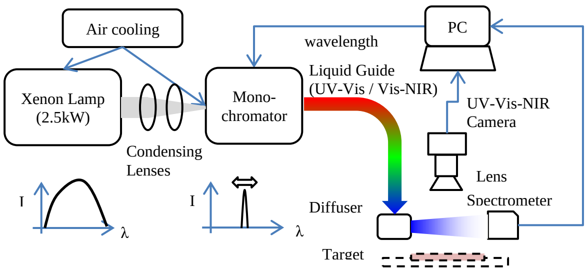

Figure 1. Conceptual diagram of a broadband chromatic light source

Figure 2. Broadband chromatic light source and a spectrometer

3. Experiments and results

The central wavelength of the output light was varied from 200 to 1100 nm using a monochromator after activating the 2.5kW xenon lamp. UV-Vis and Vis-NIR liquid guides were used as alternatives used because of the spectral limits of light transmission. The transmission ranges of the liquid guides were 220-710 nm and 430-2000 nm, respectively. The spectrometer measured the characteristics of the light transmitted from the liquid guides. Because the saturation of the spectrometer, the distance between the liquid guides and the spectrometer was varied according to the slit width.

Figure 3 shows the spectrograms of the target wavelength of the monochromator and the spectrum measured by the spectrometer in the case of the UV-Vis liquid guide. The colour of the spectrograms varied from blue to red according to the optical intensity. In the spectrograms, direct-proportion lines were observed between 310 and 705 nm at the target wavelength. Table 1 lists the spectrogram. The mean absolute error (MAE) and linearity were calculated from the target and central wavelengths of the measured spectrum. The mean band-width (MBW) was calculated by averaging the full width at half maximum (FWHMs). The band-width deviations (BWDs) were obtained from the FWHMs. The characteristics increased with the slit width and the linearity was almost perfect.

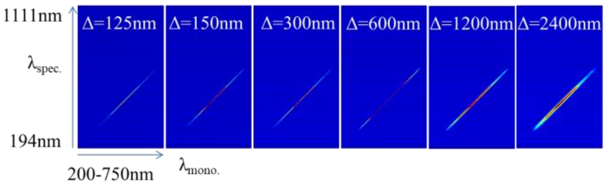

Figure 3. Spectrograms between monochromator and spectrometer using UV-Vis liquid guide and various slits

Table 1 Performances in case of the UV-Vis liquid guide

slit width (nm)	125	150	300	600	1200	2400
distance (mm)	20	20	50	50	100	200
MAE (nm)	1.485	1.513	1.876	1.602	1.920	3.312
MBW (nm)	1.266	1.503	2.308	4.933	10.050	19.865
BWD (nm)	0.297	0.277	0.225	0.864	1.347	1.704
linearity	0.998	0.998	0.999	0.999	1.003	1.008

Figure 4 shows the spectrograms of the target wavelength and the measured spectrum in the case of the Vis-NIR liquid guide. The dominant lines were observed between 430 and 1060 nm at the target wavelength. Additional lines were observed from 900 nm, and the intensity increased above 930 nm. Table 2 lists the spectrogram characteristics. The MAE, MBW, and BWD increased with the slit width. The values of the small slit width were similar to those of the UV-Vis liquid guide. The linearity of the major line was also perfect.

Therefore, the proposed light source could generate light with wavelengths ranging from 310 to 930 nm. The band-width could be adjusted from 1.4 to 19.8 nm according to the slit width. The error between the target and the measured wavelengths was 1.5-3.3 nm according to the slit width. Perfect linearity was achieved; thus, only minor calibration was required when using the light source. The light source ranged from UV to NIR and generated narrow-band light, adjusting the specific wavelength with high accuracy. Optical responses to specific wavelengths, such as fluorescence, can be acquired using a machine vision camera; thus, the light source can be applied to hyperspectral fluorescence imaging. This study also demonstrated that high-power sources can be used for spectroscopic imaging. The proposed system will be useful for inspect cells, materials, art-works as well as products.

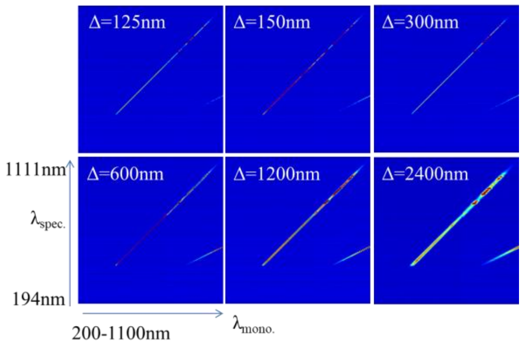

Figure 4. Spectrograms between monochromator and spectrometer using Vis-NIR liquid guide and various slits

Table 2 Performances in case of the Vis-NIR liquid guide

slit width (nm)	125	150	300	600	1200	2400
distance (mm)	20	20	50	50	100	200
MAE (nm)	1.456	1.392	1.711	1.335	1.613	2.166
MBW (nm)	1.359	1.808	2.814	5.767	11.284	30.091
BWD(nm)	0.254	0.430	0.540	1.346	2.348	4.773
linearity	1.001	1.001	1.001	1.001	1.002	1.004

4. Conclusion

A broadband light source with variable wavelengths was proposed for fluorescence imaging. The light source consisted of a high-power xenon lamp, condensing lenses, a monochromator, an optical liquid guide, and air-cooling units. The centre wavelength was varied using a monochromator, and the band-width was adjusted using the slit width. Air-cooling was applied because of the heavy heat generated by white light. The test results showed that the effective wavelength was 310 -930 nm with perfect linearity. The minimum band-width was 1.4 nm and the maximum mean error was 3.3 nm. Therefore, the proposed light source provided high-quality and accuracy of a light for hyperspectral fluorescence imaging. The proposed system is available for bio-engineering, materials science, fine art, and industrial applications

Acknowledgement

We would like to acknowledge the support from the Year 2022 Culture Technology R&D Program of Korea Creative Content Agency (Project Name: Development of the system for digital data acquisition of modern and contemporary fine arts and supporting science-based art credibility analysis, Project Number:R2020060004).

References

[1] Ladouceur A M and Brown C M 2021 Fluorescence Microscopy Light Source Review *Current Protocols* **1** e243

[2] Uhl, R 2012 Arc lamps and monochromators for fluorescence *Cold Spring Harb Protoc.* **9** 931-6

[3] Wessels J T, Pliquett U and Wouters F S 2012 Light-emitting diodes in modern microscopy--from David to Goliath? *Cytometry A* **81** 188-97