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Spectroscopic analysis of the autofluorescence from human bronchus using an ultraviolet laser diode

Masakazu Kobayashi
Waseda University
Kagami Memorial Laboratory for Materials Science and Technology
2-8-26 Nishiwaseda Shinjuku
Tokyo 169-0051, Japan

Kiyoshi Shibuya
Hidehisa Hoshino
Takehiko Fujisawa
Chiba University
Graduate School of Medicine
Department of Thoracic Surgery
Inohana 1-8-1
Chuo Chiba 260-8670, Japan

Abstract. A GaN based ultraviolet (UV) laser diode (LD) was used to study the autofluorescence (AF) spectrum of the normal and tumor human bronchial tissues under *ex vivo* conditions. The UV LD generates a coherent short wavelength (around 400 nm) light beam with an intensity of about a few watts. AF spectrum data can be obtained without interference by excitation light. A clear blue peak located at around 483 nm was observed along with a green peak at around 560 nm in the normal tissue. The peak intensities observed were very weak for the tumor tissues. The AF imaging and spectrum analysis were performed along with a histopathological study. The spatial distribution of the elastin in the bronchial tissue affected the intensity of the AF whereas the spectrum shape was not affected. Strong AF was observed from regions that include a high density of the elastin. Biopsy measurements were performed for *ex vivo* samples, and depth profiling of the elastin was studied along with variations of the AF spectrum. AF spectra excited by the UV LD for fluorescence materials including FAD, NADH, and elastin were measured. The spectrum shape of the elastin as well as of NADH was similar to that of normal bronchial tissues. [DOI: 10.1117/1.1506932]

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1 Introduction

The optical properties of human tissues have been extensively studied in recent years. The autofluorescence (AF) properties of various organs using various excitation sources (wavelengths) have provided information associated with age, metabolism as well as various diseases.[1–15] AF observation through a bronchoscope is becoming a standard clinical procedure to detect early stages of lung cancer. The intensity variation of green AF was monitored to diagnose cancerous tissues such as dysplasia; the green AF intensity from the cancerous tissue was much weaker than that of normal tissues. These diagnosis systems visualize AF imaging and the variation of intensity was evaluated to identify the position and the size of the suspicious lesion. Conventional clinical diagnosis systems such as LIFE, SAFE, and D-light systems utilize a multimode He–Cd laser beam (442 nm) or the ultraviolet (UV) region of a Xe lamp for the excitation light source[16–18] to obtain the green AF. One of the disadvantages of these excitation light sources is insufficient separation from the green AF; mixing of the AF and excitation source takes place since they are close to each other in the wavelength region. Optical filters were introduced into those systems to isolate the AF signal from excitation light. The filter cut off the excitation light as well as a certain portion of the AF in the spectrum region. In order to study the precise feature of the AF, conventional excitation light sources should be replaced by a shorter wavelength light with a narrow linewidth so that spectrum analysis can be performed over a wider wavelength region without any ambiguity. Precise analysis of the AF in the spectrum region will be key to understanding details of the characteristics of the AF spectrum.

In this study, a GaN based laser diode was employed as a compact excitation light source for measurement of the AF. The GaN based UV LD can produce coherent light at around 400 nm. Spectra obtained using the UV LD were compared with those obtained using conventional light sources. The spatial distribution of the AF intensity in the human bronchus was measured, and the variation in intensity was studied in correspondence with the position of the elastin. Those spectrum analyses were performed in conjunction with a histopathological study in which elastin staining was employed.

Biopsy was performed for several *ex vivo* samples, and the variation in AF intensity was similarly studied along with the histopathological analysis.

2 Materials and Methods

The AF properties of bronchial walls, bronchial bifurcation, and other normal tissues as well as various types of tumor tissues were examined under *ex vivo* conditions. The LIFE system was also used to get reference data for the imaging as well as for the spectroscopic data. Human bronchus samples used for this study (about 50 sections from 13 samples) were obtained within a few hours after lung resection. The sample was rinsed in saline prior to measurement in order to remove excess blood. The *in vivo* AF measurement (including conventional clinical diagnosis methods) would include information associated with blood flow in the tissue (which would help the

Address all correspondence to Masakazu Kobayashi. E-mail: cosmos@mn.waseda.ac.jp

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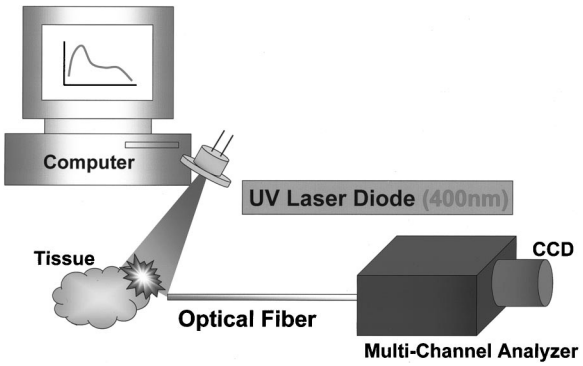

Fig. 1 The experimental setup used for this study.

diagnosis process), but this *ex vivo* measurement would reduce the effect of blood in the AF measurement such that a simplified analysis of the spectrum data can be performed. As shown in Figure 1, samples were directly irradiated from the surface (epithelium) by the GaN based UV LD at an intensity of about a few watts. The AF spectrum was measured using a multichannel analyzer and a charge coupled device (CCD). The AF signal was guided by an optical fiber to the multichannel analyzer, and no optical filters were introduced for the spectroscopic analysis. All the spectrum data acquisition could be performed within a few seconds. Histopathological analysis was performed for most of the samples used for spectroscopic analysis. The relationship between the AF spectrum data and the histopathological feature was also investigated. Hematoxylin and eosin (HE) staining as well as elastica (EL) staining using characteristics of tissues such as the position and density of the elastin, size of the tumor, and the stage of the illness was done.

3 Results

3.1 AF Spectra Obtained using a He–Cd Laser and UV LD

Figure 2 compares an AF image of the normal bronchus excited by the He–Cd laser (through the bronchoscope of the LIFE system) and the UV LD (direct irradiation). The image was obtained using the visualization system of the LIFE system for both excitation light sources through the bronchoscope. Images obtained using the two different excitation sources were very similar. Green bright AF was observed from the normal areas and a reddish color was observed from the tumor areas. This preliminary result could imply that the origin of the AF signal obtained by those sources is probably identical. Figure 3 shows a spectrum of a normal tissue sample excited by the He–Cd laser (442 nm) and the UV LD. The spectrum obtained using the UV LD was essentially the same as that obtained using the He–Cd laser. Much intense green and blue luminescence was observed when the He–Cd laser was used. The spectrum, however, a mix of the excitation beam (the multimode He–Cd laser beam) and the AF signal in the blue region (around 460–480 nm) was difficult to resolve. The intensity of the He–Cd laser of the LIFE system at the end of the bronchoscope was around 20 mW, and the intensity of the UV LD was about a few mW. The difference in excitation source power resulted in a difference in AF

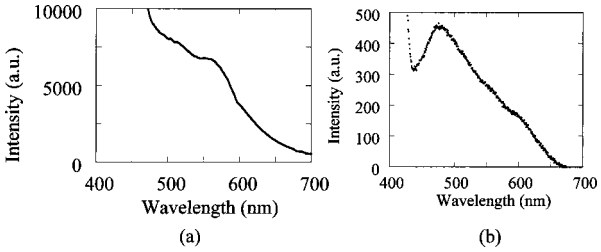

Fig. 3 Autofluorescence spectrum of normal tissue excited by (a) the He–Cd laser and (b) the UV LD.

intensity. On the other hand, a well resolved peak at around 483 nm (blue) was clearly observed without the necessity of using any optical filters when the UV LD was used. The excitation light of the UV LD influenced only the region whose wavelength was shorter than 420 nm. Both samples showed a side peak at around 560 nm. The spectrum obtained from the tumor tissue by UV LD excitation (Figure 4) was also similar to the spectrum of tumor cells reported using other conventional methods.[18–20] The intensity of the green (blue) fluorescence mostly disappeared when excited by the either He–Cd laser or the UV LD. The AF intensity was affected by the excitation source intensity, but the peak position of the green AF spectrum was not affected by the wavelength or the intensity of the excitation source used for this study.

3.2 AF Signal Intensity versus Histopathological Findings

The AF signal intensity was measured for various regions of the human bronchus. Figure 5 shows an AF image of the bronchial bifurcation region. As is shown the wide area of the sample was irradiated by the UV LD beam. A green band pass filter was used to obtain the green AF image. An AF image obtained using the blue band pass filter was also taken and the image was similar to that obtained using the green band pass filter. As can be seen from Figure 5, the intensity of the green fluorescence showed spatial distributions; bright fluorescence was obtained from the region of bronchial bifurcation as well as from part of the bronchial wall along the airway where the elastin fiber bundle would exist. The signal intensity variation for various areas of the normal bronchial wall was measured

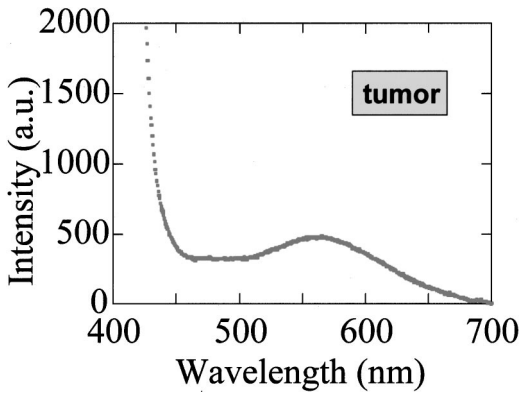

Fig. 4 Autofluorescence spectrum of tumor tissue excited by the UV LD.

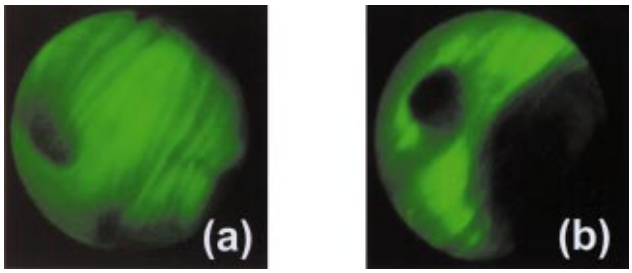

Fig. 2 Autofluorescence image of the human bronchus using (a) the He–Cd laser and (b) the UV LD.

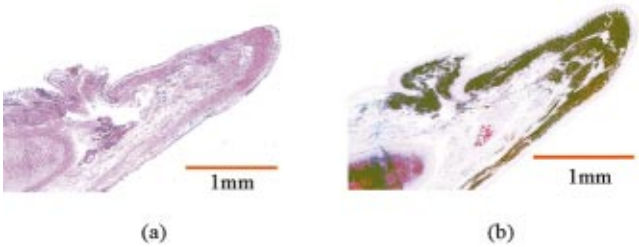

Fig. 7 Histopathological findings of normal bronchial bifurcation. Cross-sectional views of (a) HE staining, and (b) EL staining.

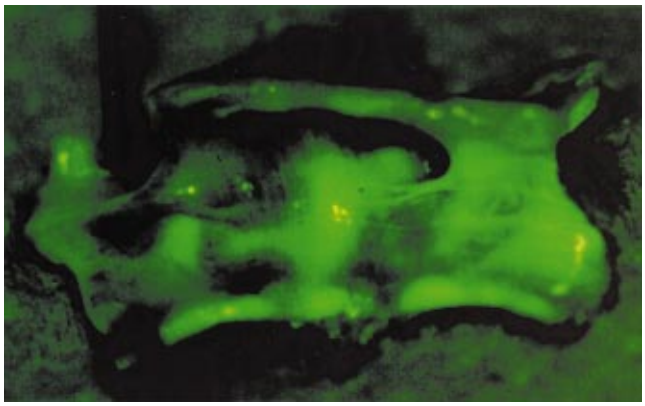

Fig. 5 AF image of the bronchus bifurcation.

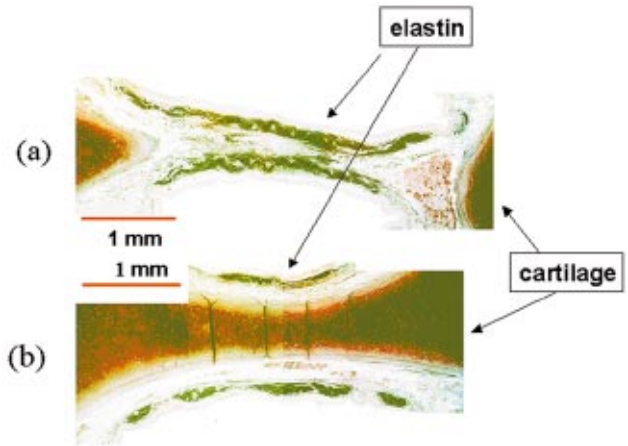

Fig. 8 EL staining of the bronchus bifurcation. The sample was sliced along the contour. (a) Near the saddle point of the bifurcation and (b) far from the saddle point.

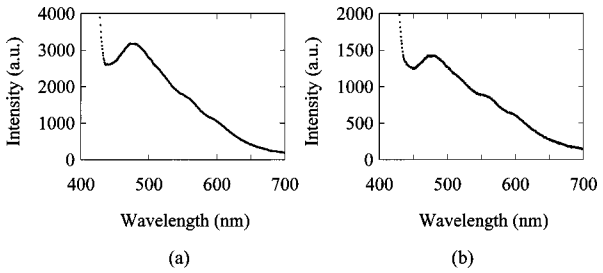

Fig. 6 Variation in signal intensity of the AF for various regions: (a) the region with a high density of elastic fiber bundles and (b) the region with less elastic fiber bundles.

and is given in Figure 6. As is shown, the AF intensity was affected by the density of the elastin fiber bundle, but the AF spectrum shape was not affected; a strong AF signal was observed in the sample which included a high density of elastin fiber bundles whereas the AF signal intensity was weak for areas where the bundle density was less.

Histopathological analysis was carried out along with spectrum analysis. Figure 7 shows histopathological findings of a saddle point region for the bronchial bifurcation. An adjacent region of the sample was sliced and stained by HE and EL, respectively. As can be seen from the cross-sectional view of the bifurcation, the existence of dense elastin in the saddle point region below the basement emblem was confirmed. Elastin was not clearly confirmed in the epithelium region. Figure 8 shows EL staining of the bifurcation for two different regions. The sample was sliced along with two different contours. The density of the elastin was high near the saddle point of the bifurcation, and the density became weak as the region became farther from the saddle point. The variation of the elastin density observed from these histopathological findings agreed well with the variation in intensity of the green AF shown in Figure 5; the region that has a high density of elastin seemed to show strong AF compared to regions that showed less density of elastin. There are several articles which describe the AF characteristics of elastin.[5,11,15] The reported spectrum data associated with elastin were similar to the data obtained using the UV LD, but the peak position of the AF reported was slightly different, probably because of variation of the excitation sources.

The depth profile of the elastin was characterized, and the EL staining along with the HE staining (shown in Figures 7 and 8) confirmed that elastin was observed in the submucosal area of the bronchial bifurcation, and was hardly observed in the epithelium region. This observation was consistent with previous reports that AF could be observed in the upper region of the submucosa, but not in the epithelium.[21] The tumor sample which did not include elastin (evaluated by EL staining) showed a very weak AF; blue and green AF peaks were barely observed from spectrum analysis.

3.3 AF Spectra from Biopsied Samples

Several human bronchus samples after resection were analyzed in conjunction with standard biopsy procedures in order to obtain a depth distribution profile of the fluorescent materials. The histopathological analysis indicated that the tissues used for this experiment were normal. As illustrated in Figure

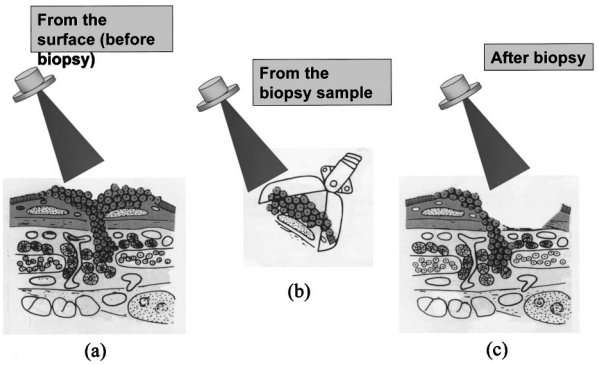

Fig. 9 Schematic of the *ex vivo* biopsy procedure and the AF analysis: (a) prior to the biopsy, (b) the biopsied sample, and (c) after the biopsy.

9, the spectroscopic analysis was performed for samples prior to biopsy, immediately after the biopsy, as well as for the biopsied sample. An *ex vivo* biopsy was performed with standard biopsy tools used for routine diagnosis in hospitals. The thickness of the biopsied sample was about 0.4 mm. Figure 10 compares spectra of the three samples. A spectrum obtained from the sample prior to the biopsy was a typical spectrum of normal tissue. The biopsied sample showed a fairly weak but dominant broad peak at around 560 nm. There is an additional feature in the shorter wavelength region. A certain intensity of the AF was observed in the wavelength region between 450 and 500 nm; the peak position was not clearly identified. EL staining of the biopsied sample showed that the sample included the epithelium and a very thin layer of submucosa which included a very small amount of elastin. The spectrum shape obtained from the sample after biopsy was similar to that of the sample prior to biopsy. The AF spectrum shape and intensity were quite comparable. These phenomena would suggest that the AF peak observed at around 483 nm in the normal tissue probably mainly originated from tissue beneath the epithelium, and that the AF material whose spectrum peak was around 560 nm would be located in a much wider region in the depth direction.

A typical AF spectrum of dysplasia samples which were biopsied and classified through pathological analysis is shown in Figure 11. The spectrum shape was similar to that of the

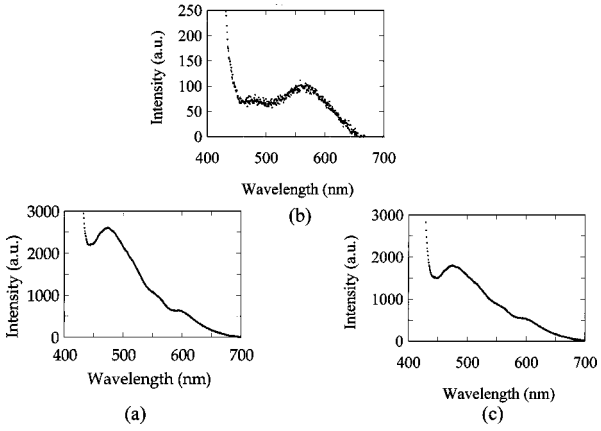

Fig. 10 AF spectrum of the *ex vivo* biopsy samples: (a) prior to the biopsy, (b) the biopsied sample, and (c) after the biopsy.

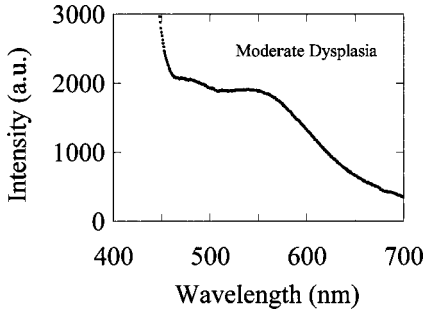

Fig. 11 AF spectrum of the biopsied sample. The sample was classified as dysplasic by pathological analysis.

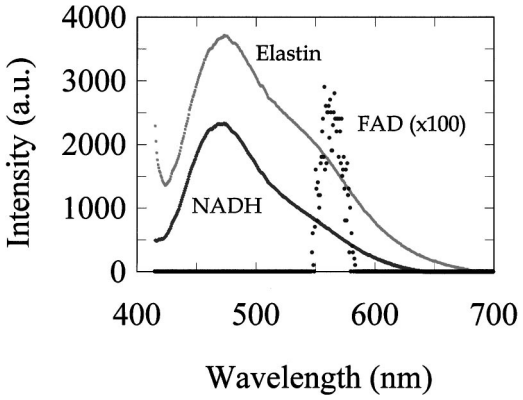

Fig. 12 AF spectra of various fluorescent materials.

biopsied normal tissue, and a relatively weak signal was observed in the blue region (around 480 nm) as well as a clear peak in the green region (around 560 nm). Further study is required to understand the change in AF intensity associated with the development of precancerous tissues since those changes would take place in epithelium tissues, not in submucosa tissues where elastin is located.

AF spectra of elastin (soluble, of bovine origin), NADH, and FAD solved in saline were also measured and compared with those from bronchial tissues. Measurement of the liquid was performed using the same setup as that for measuring the bronchial tissues. The liquid solution was excited directly by the UV LD. As shown in Figure 12, the AF spectrum obtained from the elastin solution was similar to that of the normal tissues obtained from the human bronchus, and an intense peak was observed at around 483 nm with a weaker peak at around 560 nm. The spectrum obtained from NADH is similar to that of elastin, but the spectrum from the FAD was quite different. These facts imply that the AF peak located at around 483 nm through UV LD excitation should be related to elastin and/or NADH. Monitoring and precise analysis of the condition of elastin (which could easily be performed using the UV LD whose excitation wavelength is about 400 nm) would provide further information for detecting the early stage of lung cancer and of other diseases associated with the biochemical condition of elastin.[22] Monitoring of AF associated with elastin would also provide useful information for laser welding techniques of tissues.[15]

4 Conclusions

Autofluorescence spectra of the human bronchus were measured using the UV LD under the *ex vivo* conditions. The AF spectrum could be obtained over a wider wavelength dynamic range because the AF peak could be substantially isolated from the excitation source. The AF spectrum in the blue region was hard to measure by conventional methods because the separation between the AF and the excitation source was insufficient. The spectroscopic analysis revealed that blue fluorescence peaking at 483 nm (blue) was clearly observed in normal tissues along with the green peak at around 560 nm. On the other hand, the AF from tumor tissues exhibited very weak AF spectra. Histopathological analysis was performed for the sample whose AF spectrum was measured. The AF feature varied in several regions of the bronchus tissues, and the intensity was strong where elastin existed. The density of elastin seems to affect the intensity of the AF, but the spectrum shape remains unchanged.

The *ex vivo* biopsy and the spectroscopic analysis indicated that the AF peak at 483 nm probably originated from tissue beneath the epithelium. The comparison with the AF spectra of the fluorescent materials suggested that the AF peaking at 483 nm would have originated from elastin and/or NADH. The condition of elastin could be characterized by using the UV LD, and it could provide additional information for detecting the early stage of lung cancer as well as of other diseases related to elastin. Further study is required to resolve the precise mechanism of change in the AF due to the development of the illness.

Acknowledgment

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