

## Caries Diagnosis using a Laser Fluorescence System —Observation of Autofluorescence of Dental Caries—

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The purpose of this study was to evaluate the usefulness of autofluorescence of carious lesions on caries diagnosis. The observation of the micromorphology of caries lesions was conducted using a confocal laser scanning microscope, a fluorescence microscope and a WDX type electron probe X-ray microanalyzer. Observation of autofluorescence under Cy5 and UV fields showed clearly specific images of autofluorescence in the carious lesion. However, observation of autofluorescence under FITC field showed images of autofluorescence with unclear boundaries in the carious lesion. EPMA images showed decreases in Ca and P in the carious areas. As a result of the observation of autofluorescence and the EPMA images in the carious lesion, a correlation was noted between autofluorescence under the Cy5 field as the laser fluorescence apparatus for caries diagnosis and demineralized areas. The usefulness of autofluorescence of carious lesion on caries diagnosis was suggested.

Key words : Laser fluorescence system, Autofluorescence, Dental caries

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### INTRODUCTION

Dental caries were suggested to be a cumulative disease, showing irreversible progression. However, early caries formation without cavitation occurs when the equilibrium between decalcification and recalcification has been lost. New concepts of early caries treatment have been proposed<sup>1-4)</sup>. Cayley *et al.*<sup>2)</sup> reported an assessment of the effects of an audit exercise on the diagnosis of occlusal caries. Zero<sup>4)</sup> reported that the boundaries of caries diagnosis and caries intervention were changing. Dentists currently use visual, tactual and radiographic information to detect relatively advanced changes in the dental hard tissues. The clinical management of dental caries had been primarily directed at the treatment of the consequences of the disease process by placing restorations and not at curing the disease. Therefore, in caries diagnosis, it is important to know not only the presence of caries cavities, but also whether the caries progression is continuing or suspended, and whether

recalcification is observed<sup>5)</sup>.

Penning *et al.*<sup>6)</sup>, Verdonshot *et al.*<sup>7)</sup>, and Lussi<sup>8)</sup> reported that the detection rate of caries cavities by inspection and palpation was low in caries without apparent caries cavity formation. Lussi<sup>9)</sup> and Gray *et al.*<sup>10)</sup> reported that the detection rate of caries cavities became high in caries with apparent caries cavity formation. However, problems of hidden caries have occurred due to the spread of fluoridization. It was reported that since only the surface of caries cavities is recalcified, caries cavities under the surface are not detected by inspection and palpation; therefore, confirmation by X-ray is important<sup>11-13)</sup>. Furthermore, understanding of pathological conditions by X-ray images is difficult in some carious lesions. It was also reported that palpation using explorers destroyed recalcifyable enamel surfaces, and induced caries<sup>14)</sup>. However, there have been no sufficiently appropriate examination methods.

Currently, new caries diagnostic methods are being developed, applying various techniques. Electric conductance measurement (ECM) method<sup>15)</sup>, fibro-optic transillumination (FOTI) method<sup>16)</sup>, laser fluorescence diagnostic method<sup>17)</sup>, and ultrasonic diagnostic method<sup>18)</sup> are now in practical use. Among them, a laser fluorescence system (DIAGNOdent™, KaVo Corp., Biberach, Germany) is a laser apparatus to diagnose as dentinal caries, in which a laser irradiates the tooth matrix, and the strength of specific fluorescent reflections detected in carious areas is indicated numerically. Various clinical studies of this apparatus have been performed, reporting its usefulness<sup>19,20)</sup>. Although clinical application of this apparatus is expected, since fluorescent reflection occurring in carious lesions varies depending on caries conditions, there are many unclarified points. It appears that many factors, such as stains on the tooth structure, thickness of the enamel that covers dentinal carious lesions and proximal surface caries, affect the detection rate of carious lesions.

In addition, carious lesions have a strongly increased autofluorescence compared to their sound counterparts. Foreman<sup>21)</sup> reported that the excitation and emission spectra of fluorescent components on human dentin could be observed. Alfano *et al.*<sup>22)</sup> reported that the spectrum from carious lesions were different from that of noncarious tooth regions. The ultimate cause of this autofluorescence is presently unknown.

The purpose of the present study was to evaluate the usefulness of autofluorescence of carious lesions on caries diagnosis. Using a confocal laser scanning microscope (CLSM) and a fluorescence microscope (FMS), specific autofluorescent reflections in carious lesions were observed, and changes in the components in the carious lesion were investigated using a WDX type of electron probe X-ray microanalyzer (EPMA), to evaluate correlations.

## MATERIALS AND METHODS

In this experiment, nine fresh human decayed teeth with enamel and dentine caries were used, after the removal of soft tissue and dental calculus by a scaler. The teeth were thoroughly washed in distilled water, and preserved in 70% ethanol to be used

as experimental teeth. The history on the teeth is shown in Table 1. Using a laser fluorescence system, measurement of the carious areas in the experimental teeth was performed, the contact tip at right angles to the carious lesion of the enamel and dentin of the specimens. The strength of specific fluorescent reflections detected in carious areas was indicated numerically (from 00 to 99). The measurement was performed five times. Thereafter, after embedding the carious teeth in methyl methacrylate (SpeciFix-20 Kit, Marumoto Struers Corp., Tokyo, Japan), the specimens were cross-sectioned longitudinally through the center of the cavities with a low speed diamond micro-cutter (Micro-cutter 201, Maruto, Tokyo, Japan). The sections were cut 50- $\mu$ m thick using a section device (Roto System, Marumoto Struers Corp., Tokyo, Japan). Using a stereoscopic microscope (SZX 12, OLYMPUS OPTICAL Corp., Tokyo, Japan), carious lesions in the sections were observed. Thereafter, microscopic X-ray (Sofron, Softex Corp., Kanagawa, Japan) was taken under the following conditions, (scanning distance: 55 cm, electric current: 20 mA, voltage: 40 V, scanning time: 30 sec.). Using the CLSM system (FLUOVIEW 500, OLYMPUS OPTICAL Corp., Tokyo, Japan), observation of fluorescence in carious areas at the Cy5 field of the laser fluorescence system was then performed. The CLSM system was mounted on a microscope (PowerBX61, OLYMPUS OPTICAL Corp., Tokyo, Japan) apparatus equipped with an objective (4 $\times$ 0.16 Numerical Aperture (NA) and 10 $\times$ 0.40 NA, OLYMPUS OPTICAL Corp., Tokyo, Japan). A HeNe laser, at 633 nm, was used as the excitation source for the fluorescent probe. A 650 nm/ 667 nm Band Pass (BP) Cy5 filter was used. Using the FMS (Eclipse E800M, Nikon, Tokyo, Japan), autofluorescence was observed at various filters (380-420 nm Exciting filter (EX)/450 nm BA: UV filter, 465-495 nm EX/ 515-555 nm BA: FITC filter, 590-650 nm EX/663-735 nm BA: Cy5 filter). The objective was a 10 $\times$ 0.45 NA (Plan Apo., Nikon, Tokyo, Japan). Furthermore, specimens after being half-cut were viewed with EPMA (EPMA 8705, Shimadzu, Kyoto, Japan) for elemental distribution of calcium (Ca) and phosphorus (P) on the enamel or dentin after critical point drying and carbon coating.

Table 1 The history of the teeth

Sample no.	Age(years)	Gender	A kind of tooth	Carious lesion
1	38	M	upper first premolar	proximal surface
2	25	F	lower first molar	occlusal surface
3	31	F	lower first molar	occlusal surface
4	41	M	upper secound molar	occlusal surface
5	37	M	upper secound molar	proximal surface
6	45	F	lower first premolar	occlusal surface
7	18	F	upper first premolar	occlusal surface
8	19	M	upper first premolar	occlusal surface
9	18	F	upper first premolar	occlusal surface

## RESULTS

The measurements using a laser fluorescence system and carious levels are shown in Table 2. Carious levels were as follows: D<sub>1</sub>: no caries, or histological enamel caries limited to the outer half of the enamel thickness, D<sub>2</sub>: histological caries extending beyond the outer half, but confined to the enamel, D<sub>3</sub>: histological dentinal caries limited to the outer half of the dentin thickness, D<sub>4</sub>: histological dentinal caries extending into the inner half of the dentin thickness. The value became relatively large if the carious lesion was deepened.

Fig. 1(a) shows a stereoscopic microscope image (sample No.1). The sample is a typical example of caries extending near to the dental pulp. Fig. 1(b) shows a microscopic X-ray image. Apparent decalcified areas colored in brown were observed in the surface layer, and opaque layers along the dentinal tubules were found in the deeper areas, showing an image of smooth surface caries. Measurement using the laser fluorescence system showed a high value ( $99.0 \pm 0.0$ ) in the carious lesion. Fig. 1(c) shows images observed using the CLSM under the Cy5 field as the laser fluorescence system. Autofluorescent images reaching deep areas were noted. Fig. 1(d) shows images observed using the FMS under each field and three-dimensional images of fluorescence intensity. Carious lesion images with clear boundaries were noted using Cy5. Three-dimensional images showed strong fluorescence in the carious lesions. Carious lesion images with unclear boundaries were detected using FITC. Three-dimensional images showed images with unclear boundaries. Carious lesion images with clear boundaries were observed using UV. Three-dimensional images showed decreases in fluorescence strength of the carious lesion. Fig. 1(e) shows component analytical images using the EPMA. Decreases in Ca and P were detected in the areas that showed apparent autofluorescence.

Fig. 2(a) shows a stereoscopic microscope image (sample No.4). This sample is a typical example of caries reaching dentine. Fig. 2(b) shows a microscopic X-ray image. Occlusal caries with apparent decalcified areas colored in brown were present. Measurement using the laser fluorescence system showed a comparatively low value ( $29.0 \pm 3.8$ ) in the carious lesion. Fig. 2(c) shows an image observed using the

Table 2 The measurements using a laser fluorescence system

Sample no.	Measurement value (average, stdev)	Carious level
1	$99.0 \pm 0.0$	D <sub>4</sub>
2	$93.8 \pm 3.4$	D <sub>4</sub>
3	$99.0 \pm 0.0$	D <sub>4</sub>
4	$29.0 \pm 3.8$	D <sub>2</sub>
5	$23.6 \pm 3.0$	D <sub>3</sub>
6	$20.8 \pm 4.2$	D <sub>2</sub>
7	$4.6 \pm 1.1$	D <sub>1</sub>
8	$9.2 \pm 3.0$	D <sub>1</sub>
9	$8.2 \pm 1.9$	D <sub>1</sub>

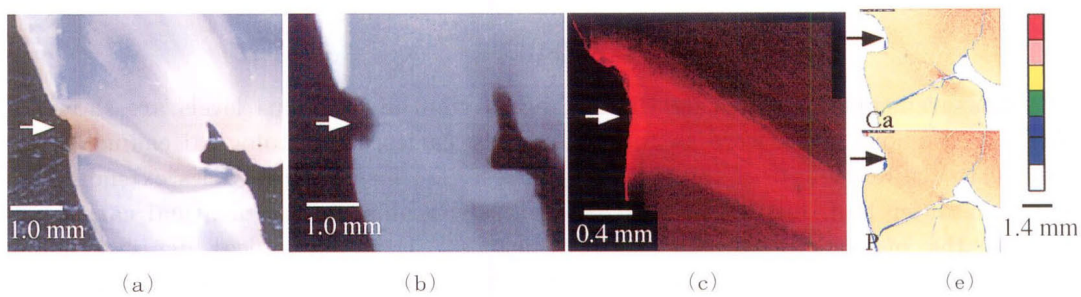


Fig.1(a) A stereoscopic microscope image. (caries extending near to the dental pulp)  
(b) A microscopic X-ray image.  
(c) CLSM image.  
(e) EPMA images.

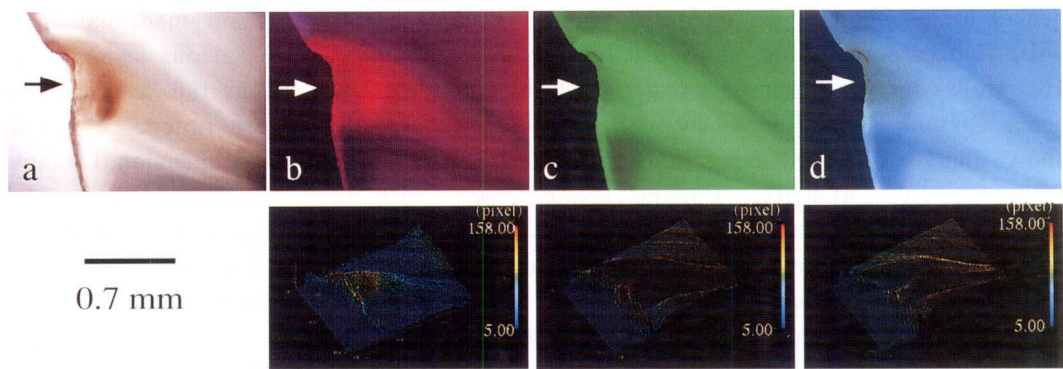


Fig.1(d) FMS images and three-dimensional images.  
a: Transmission image  
b: FMS image under Cy5 field and three-dimensional images of fluorescence intensity  
c: FMS image under FITC field and three-dimensional images of fluorescence intensity  
d: FMS image under UV field and three-dimensional images of fluorescence intensity

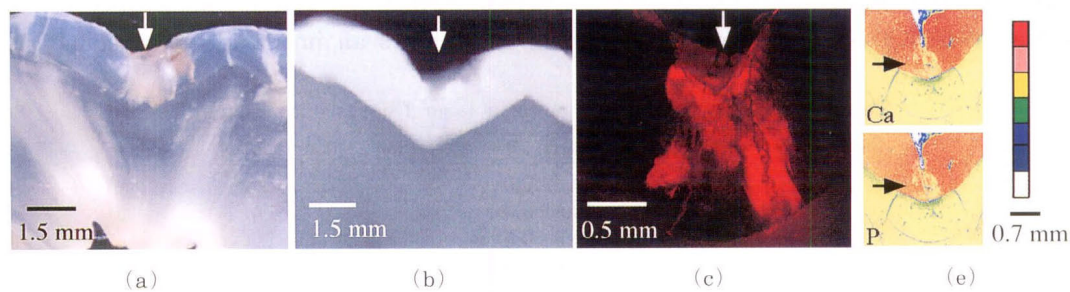


Fig. 2(a) A stereoscopic microscope image. (caries reaching dentine)  
(b) A microscopic X-ray image.  
(c) CLSM image.  
(e) EPMA images.



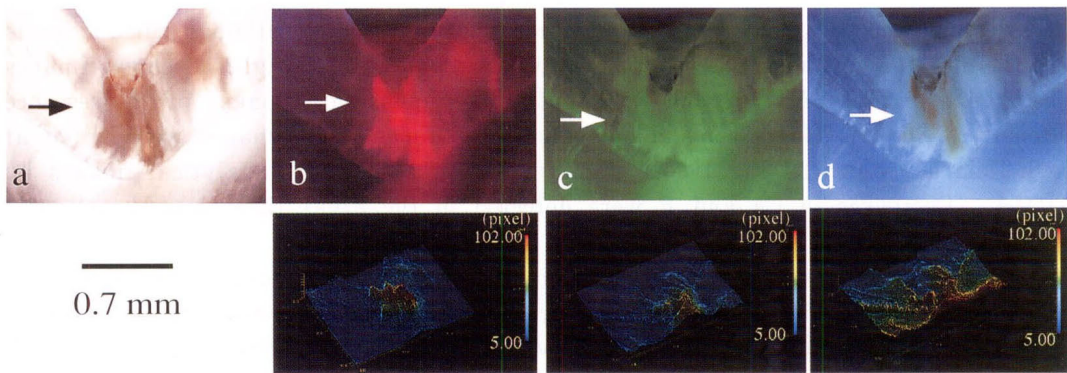


Fig. 2(d) FMS images and three-dimensional images.

a: Transmission image

b: FMS image under Cy5 field and three-dimensional images of fluorescence intensity

c: FMS image under FITC field and three-dimensional images of fluorescence intensity

d: FMS image under UV field and three-dimensional images of fluorescence intensity

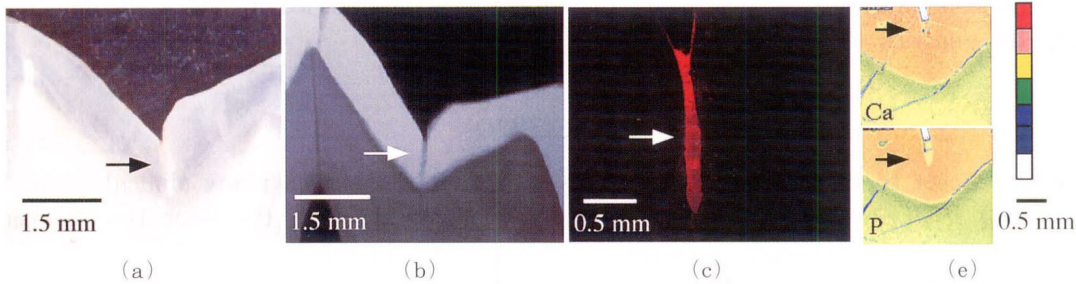


Fig. 3(a) A stereoscopic microscope image. (early caries with pigmentation in the fissure)

(b) A microscopic X-ray image.

(c) CLSM image.

(d) EPMA images.

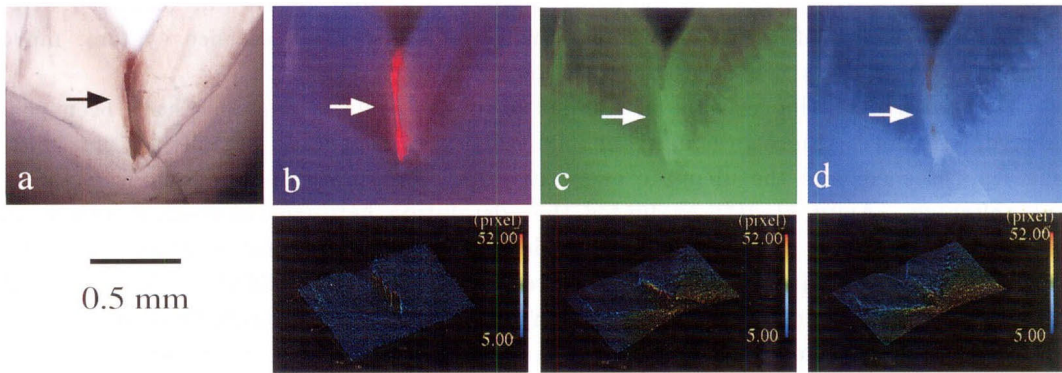


Fig. 3(d) FMS images and three-dimensional images.

a: Transmission image

b: FMS image under Cy5 field and three-dimensional images of fluorescence intensity

c: FMS image under FITC field and three-dimensional images of fluorescence intensity

d: FMS image under UV field and three-dimensional images of fluorescence intensity

CLSM under the Cy5 field as the laser fluorescence system. Although carious areas were within enamel, localized strong fluorescence was noted. Fig. 2(d) shows images observed using the FMS at each field and three-dimensional images of fluorescence intensity. Specific fluorescent images and three-dimensional images were noted at each field as shown in Fig. 1(d). Fig. 2(e) shows component analytical images by EPMA. Decreases in Ca and P were detected in the areas that showed apparent autofluorescence as shown in Fig. 1(e).

Fig. 3(a) shows a stereoscopic microscope image (sample No.7). The sample is a typical example of early caries with pigmentation in the fissure. Fig. 3(b) shows a microscopic X-ray image. Early caries with pigmentation in the fissure was noted. Measurement using the laser fluorescence system showed a low value ( $4.6 \pm 1.1$ ). Fig. 3(c) shows images observed using the CLSM under the Cy5 field as the laser fluorescence system. Localized markedly strong fluorescence was found. Fig. 3(d) shows images observed using the FMS at each wavelength and three-dimensional images of fluorescence intensity. Specific fluorescence images and three-dimensional images were observed at each wavelength as shown in Figs. 1(d) and 2(d). Fig. 3(e) shows component analytical images using the EPMA. No decalcified images were detected by the analysis using the EPMA.

## DISCUSSION

With respect to the laser fluorescence system, there have been many clinical studies. Shi XQ *et al.*<sup>19)</sup> reported that the diagnostic performance of the laser fluorescence system method was superior to that of radiography in this *in vitro* study of detection of occlusal caries. In addition, Ross<sup>20)</sup> reported that the laser fluorescence system has shown itself to be very accurate in the diagnosis of pit and fissure lesions. Furthermore, Senda *et al.*<sup>23)</sup> reported that comparing the measurement using a laser fluorescence system with carious levels, 20 was the boundary line with treatment needs and non-needs. However, they reported that it was necessary to consider other evidence and the symptoms. In the present study, a similar result was observed. Regarding the results of the measurements using the laser fluorescence system, more detailed examinations are necessary in future studies.

With regard to fluorescence in carious lesion, various evaluations have been performed. However, there are many unclarified points regarding carious lesions emitting fluorescence, and the ultimate cause of autofluorescence on the carious lesion is not unknown. Avijit *et al.*<sup>24)</sup> evaluated the correlation between fluorescent images in dentinal carious and mineral content. Measurement of mineral content was performed using Backscattered scanning electron microscopy (BSE-SEM). They reported that no correspondence was noted between BSE images after decalcification by acid and fluorescent images. However, they added that this could be because accurate fluorescence observation was impossible due to the influence of tissue opacity. The assumption that the chromophores responsible may be organic in nature appears logical<sup>25)</sup>: if it were the opposite, *i.e.* purely inorganic, one would expect to see

healthy dentine (especially hypermineralised areas and enamel) fluorescing brightly and carious demineralised dentine to exhibit significantly or markedly reduced fluorescence. The major bacterial products associated with dentinal decay are lactic acid and proteolytic enzymes. Van der Veen *et al.*<sup>26)</sup> reported the pH was reduced in lesions, but pH changes did not affect the autofluorescence signal. There was no evidence for autofluorescence of the exogenous proteases. A good candidate for the cause of autofluorescence might be exogenous fluorescent molecules imported during the carious process, and support for this was seen in the progressive enhancement of autofluorescence with the progress of lesions and the degree of perfection of destruction of the original tissue. Candidates might possibly include certain plasma proteins, gaining access to the tissues via the circulatory system. Linde<sup>27,28)</sup> reported these ubiquitous molecules were present in small amounts in healthy dentin and their concentrations might increase once the degradative process had started. Lormée *et al.*<sup>29)</sup>, Adriaens *et al.*<sup>30)</sup> and Gonzáles-Cabezas *et al.*<sup>31)</sup> reported bacteria could penetrate dentinal tubules. In addition, König *et al.*<sup>32)</sup> reported some bacteria species had the potential to autofluorescence, *Actinomyces odontolyticus*, *Bacteroides intermedius* and *Pseudomonas aeruginosa*. The bacteria (*B. intermedius* and the Gram-positive *A. odontolyticus*) have the ability to synthesise endogenous protoporphyrins. And König *et al.*<sup>32)</sup> reported these heme-us spelling-related molecules had been shown to have the same fluorescence characteristics as those found in carious tissue. Although the excitation maximum was found to be at 407 nm, the peak emission wavelength at around 650-700 nm would suggest that there would still be sufficient excitation at longer wavelengths. However, these bacteria are in the minority and those most commonly associated with dentine caries (*Streptococcus mutans* and various lactobacilli) have not been shown to have significant autofluorescence due to excitation at 488 nm.

Concerning the fluorescent reflection in carious areas, studies have been performed at various wavelengths. Foreman<sup>21)</sup> reported that the excitation and emission spectra of fluorescent components were observed on human dentin (285 nm EX/ 355 nm BA). Alfano *et al.*<sup>22)</sup> reported that the spectrum from carious lesions was different from that of noncarious tooth regions (350 nm EX/ 427 nm BA). In the present study, observation of CLSM was performed using the Cy5. Furthermore, using the FMS, observations and comparisons were performed at various wavelengths. As a result of fluorescent reflection observations at various wavelengths (Cy5, FITC and UV), carious lesions showed stronger fluorescence than healthy areas by using the Cy5 filter. Furthermore, carious lesions showed weaker fluorescent reflection than healthy areas using the UV filter. The ultimate cause of this autofluorescence is presently unknown.

In the present study, fluorescence observation of typical specimens of 3 types of different caries progression was performed. In every specimen, markedly strong fluorescent reflection localized in carious areas was observed at infrared wavelengths. With regard to Figs. 1 and 2, degeneration of the tooth matrix itself, and cariogenic microorganisms entering the tooth matrix and their production substance, was



suggested to be involved. Furthermore, localized markedly strong fluorescent reflection was noted in the Cy5 image on Fig.3. It was suggested that this was because external factors entering the pits and fissures were highly involved, rather than the degeneration of the tooth matrix itself.

EPMA images showed decreases in Ca and P in the carious areas in Figs.1 and 2. Strong fluorescent reflection was noted in the Cy5 image in Fig.3. However, no micromorphological change was recognized in the area of this fluorescent reflection. Demineralized layers, *i.e.*, layers with reduced calcium and phosphorus levels, were seen in the carious lesion. Since autofluorescent images were observed even in the pit and fissure areas showing no apparent decalcification, further evaluation is necessary regarding the progression of carious lesions and autofluorescent emission. It was suggested that autofluorescence of carious lesions was useful for caries diagnosis.

### CONCLUSIONS

As a result of the observation of autofluorescence and the EPMA images in carious lesions, a correlation was noted between autofluorescence under the same wavelength as the laser fluorescence apparatus for caries diagnosis and demineralized areas. The usefulness of autofluorescence of carious lesions on caries diagnosis was suggested.

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