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Diagnosis and staging of caries using spectral factors derived from the blue laser-induced autofluorescence spectrum

Ching-Chang Ko^a, Dong-Ho Yi^b, Dong Joon Lee^c, Jane Kwon^d, Franklin Garcia-Godoy^e, and Yong Hoon Kwon^{b,*}

^aDepartment of Orthodontics, School of Dentistry, University of North Carolina, Chapel Hill, NC 27599, USA

^bDepartment of Dental Materials, School of Dentistry, Pusan National University, Yangsan 50612, Korea

^cNC Oral Health Institute, School of Dentistry, University of North Carolina, Chapel Hill, NC 27599, USA

^dDepartment of Biological and Environmental Engineering, College of Engineering, Cornell University, Ithaca NY 14850, USA

^eUniversity of Tennessee Health Science Center, College of Dentistry, Department of Bioscience Research, Memphis, TN 38163, USA

Abstract

Objective—The aim of this study was to identify the factors derived from the 405 nm laser-induced autofluorescence (AF) spectra that could be used to diagnose and stage caries.

Materials and methods—Teeth (20 teeth per stage) were classified as sound, stage II, III, and IV based on a visual and tactile inspection. The specimens were re-examined and reclassified based on micro-CT analysis. From the teeth, the AF was obtained using a 405 nm laser. Three spectral factors (spectral slope at 550–600 nm, area under the curve at 500–590 nm, and two-peak ratio between 625 and 667 nm) were derived from the AF spectra. Using these factors, the diagnosis and staging of caries were tested, and the results were compared with those of DIAGNOdent.

Results—After micro-CT analysis, only 13, 11, and 13 teeth were reclassified as stages II, III, and IV, respectively. The reclassified groups showed less data overlap between the stages, and the spectral slope was 40.1–74.6, 27.5–39.6, 11.1–27.4, and 1.0–9.7 for sound, stage II, III, and IV, respectively. The differentiation of stages III and IV using DIAGNOdent appeared to be difficult due to the considerable data overlap.

*Corresponding author. Tel.: +82-51-510-8230; Fax.: +82-51-510-8228, y0k0916@pusan.ac.kr (Y.H. Kwon).

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Conclusion—Among the factors tested, the spectral slope at 550–600 nm showed the best match with the caries specimens, in which their stage had been identified precisely.

Keywords

Caries diagnosis; Laser-induced autofluorescence; 405 nm laser; Spectral analysis; Spectrum slope; DIAGNOdent

1. Introduction

Caries is one of the most common chronic oral diseases that causes mental and physical distress.^{1,2} In many cases, caries is often neglected until it causes pain. On the other hand, if not properly diagnosed and treated in a timely manner, the affected tooth may need to be extracted due to the extensive damage to the dentin and pulp. Caries is a state of tooth demineralisation caused by the acids produced by oral bacteria, such as *Streptococcus mutans* and *Lactobacillus*, which reside in dental plaque biofilms.^{3–5} These bacteria consume sugars and carbohydrates as their food and produce several acids, such as acetic, lactic, and propionic acid,^{6,7} which can attack the enamel surface and dissolve carbonated hydroxyapatite, leaving a porous structure. During the early stages, the recovery of demineralised enamel can be achieved through the uptake of lost minerals, including calcium and phosphorus, which are dissolved in saliva or present in food.^{8–10} This remineralization process can be compromised when the pH at the tooth surface falls below 4.3,¹¹ and carious lesions can form under such circumstances.

Initially, dental plaque is a colourless sticky thin layer that can form everywhere on the tooth surface; it then becomes brown or pale yellow when it transforms to calculus. If not controlled or removed properly from its initial stage by careful and periodic tooth brushing, it can progress to caries and various periodontal diseases. To minimise tooth damage and avoid subsequent treatment processes, the monitoring, diagnosis and the accurate staging of caries, if formed, are important for saving the teeth and reducing medical expenses. Conventionally, this is performed by a visual and tactile inspection with the aid of X-ray radiography. According to a wide systemic review and meta-analysis, a visual inspection was concluded to have good accuracy for the detection of caries at various stages on different tooth surfaces, but high study heterogeneity and the risk of bias were unavoidable.^{12,13} In addition to these conventional methods, several adjunctive methods have been introduced,^{14–17} such as, fibre optic transillumination (FOTI), quantitative light-induced fluorescence (QLF), digital image fibre optic transillumination (DIFOTI), and laser fluorescence (DIAGNOdent) using visual or laser light for excitation. Electrical conductance measurements (ECMs) and electrical impedance measurements (EIMs) employ an electrical current. These methods have their unique benefits and limitations in terms of achieving the reliable and accurate detection of caries. Thus far, DIAGNOdent has been adopted widely in clinics. This device uses a laser that emits 655 nm light, detects the fluorescence produced by oral bacteria and bacterial by-products, and produces digital results ranging from 0 to 99, which reflect the severity of caries.^{18–20} Therefore, DIAGNOdent is a cost-effective and nondestructive device that can detect caries from smooth and occlusal surfaces. Despite the claimed sensitivities and specificities of 70–80% regarding early enamel and occlusal dentin

carious lesions, the results of *in vivo* studies conducted on this topic have been contradictory.^{21,22}

Recently, a 405 nm laser was tested for its applicability to the detection of caries.^{23–27} The basic principle of disease detection is the production of a characteristic autofluorescence (AF) spectrum by the disease-specific chromophores. In caries, the 405 nm laser produces a spectrum with peaks near 500 nm and higher than 600 nm, which are related to the organic substances embedded in the inorganic enamel and the by-products of oral bacterial activity, respectively.^{28–30} On the other hand, compared to DIAGNOdent, which responds only to oral bacteria and their endogenous by-products, the 405 nm laser interacts with both inorganic substances and the intrinsic and endogenous organic substances, and might be a more useful diagnostic tool. Thus far, however, there have been limited studies assessing the feasibility of the 405 nm laser for the diagnosis of caries. The purpose of the present study was to evaluate the feasibility of the AF spectra-related factors for the diagnosis and staging of carious lesion on teeth. The factors extracted from the AF spectrum, spectral slope, spectral area, and two-peak ratio, were assessed on the teeth whose state and stage of caries had been identified precisely by micro-CT. The most useful factor of the AF spectrum was determined by a comparison with the DIAGNOdent readings.

2. Materials and methods

2.1. Tooth preparation and classification

The present study was conducted using 325 teeth (molars and premolars) that were cleaned, frozen, and stored at a relative humidity of 100% after extraction. The Institutional Review Board at Pusan National University Dental Hospital, Yangsan, Korea, approved the study and waived informed consent. Two examiners inspected the stored teeth independently through a visual inspection (by eye and under an optical microscope) and by a tactile inspection using an explorer. The examined teeth were categorised as sound, stage II, III, and IV (Table 1), and all teeth were classified identically by the two examiners. Twenty teeth were then selected randomly from each of these classes; these 80 teeth constituted the study groups. The teeth were then re-examined by micro-CT (inspeXio SMX-90CT, Shimadzu, Tokyo, Japan) at 90 kV and 100 μ A. To determine the caries stage, the teeth were scanned longitudinally and transversely. As there is no way to determine the precise caries stage without cutting the specimens, micro-CT was used to determine the caries stage because it can visualise non-invasively the various lesions from the enamel surface to the dentin subsurface.

2.2. Autofluorescence analysis

The laser-induced autofluorescence (AF) spectra of the teeth were obtained using a 405 nm laser (LVI Technology, Seoul, Korea). The laser was operated at an output power of 2 ± 0.1 mW, as measured using a power meter (PM3/FieldMax, Coherent, Portland, OR, USA). To achieve stable and minimally fluctuating output power, the original power (100 mW) was attenuated to 2% using various combinations of filters (Thorlabs Inc., Newton, NJ, USA). For the easy and consistent positioning of the tooth, each tooth was placed on the XYZ-stage of the unit. The laser was focused normally on the center of each lesion for one second using

a convex lens ($f=10$ cm). The spot size on the lesion was less than $10\text{ }\mu\text{m}$. The emission spectra were recorded using a spectrometer (QE65000FL, Ocean Optics Inc., Dunedin, FL, USA) by guiding the emitted light through an optical fibre (QP600-1-UV-VIS; $600\text{ }\mu\text{m}$ diameter silica core). The optical fiber was aligned at a $35\text{--}45^\circ$ angle to the irradiated laser beam. The detection range of the spectrometer was $200\text{--}1100\text{ nm}$ and its optical resolution (grating #: 300; slit size: $200\text{ }\mu\text{m}$) was 6.5 nm according to its slit and grating options. The end of the optical fibre (detector) was positioned 1 cm away from the lesion surface. A 450 nm longpass filter (Thorlabs Inc., Newton, NJ, USA) was placed in front of the optical fibre to attenuate the excitation light.

From the AF spectra obtained, three factors for determining the caries stage were extracted and assessed: the spectral slope, area under the curve (spectral area), and two-peak ratio. To determine the spectral slope, the AF spectra were analysed at $550\text{--}600\text{ nm}$. This range was determined by checking the linearity (correlation coefficient, R) using a linear fit model. In this range, most specimens (78/80) had an R value of >0.97 . The area under the curve at $500\text{--}590\text{ nm}$ was determined by integrating the curve using ORIGIN (Microcal Software Inc., Southampton, MA, USA). A range of 500 to 590 nm was used because some of the specimens showed an increasing emission profile after 590 nm . The $625/667\text{ nm}$ ratios (i.e., the two-peak ratios, the ratios of the peak emissions at 625 and 667 nm) were calculated to quantify the spectral changes after 600 nm . These wavelengths were determined because some specimens in stages II-IV showed one or two increasing peaks near 625 and 667 nm as caries progressed.

2.3. Examination using DIAGNOdent

The caries in each specimen was assessed using a DIAGNOdent pen (KaVo Dental, Biberach, Germany) according to the manufacturer's instructions. Before the measurement, the device was calibrated against a ceramic standard. The measurements were taken three times on each tooth on the same area that was used to produce the AF spectra. As described by the manufacturer, the peak digital values were classified as follows: $0\text{--}13$, healthy; $14\text{--}20$, beginning of demineralisation; $21\text{--}29$, strong demineralisation; and >30 , dentin caries. Values >30 indicate the consideration of an X-ray test and possible minimally invasive treatment or resin filling. The manufacturer's guidelines do not match the description in Table 1 precisely, but healthy ($0\text{--}13$), beginning demineralisation ($14\text{--}20$), strong demineralisation ($21\text{--}29$), and dentin caries (>30) correspond approximately to sound, stage II, stage III, and stage III/IV, respectively, of the present classification.

2.4. Fourier-transform infrared spectroscopy (FTIR) analysis

To analyse the compositional changes of the carious lesions, a FTIR spectrophotometer (Nicolet 6700/8700, Thermo Fisher Scientific Inc., Waltham, MA, USA) connected to an attenuated total reflection (ATR) accessory was used to obtain the spectra in the range, $7800\text{--}350\text{ cm}^{-1}$. Thirty-two scans were performed per specimen at a resolution of 0.09 cm^{-1} . For each specimen, 2 mg of ground powder that was obtained from a carious lesion using a fissure bur was mixed with 10 mg of potassium bromide (KBr) and made into a thin film for the measurements.

2.5. Statistical analysis

The results (spectral slopes, areas under curves, two-peak ratios, and DIAGNOdent readings) were analysed by one-way ANOVA followed by a Tukey's post-hoc test for multiple comparisons; p values <0.05 were considered significant.

3. Results

Table 2 shows both the number of specimens allocated to sound, stages II, III, and IV after the visual and tactile inspections and after micro-CT analysis, which was performed using the same specimens used for the visual and tactile inspections. Stages II, III, and IV were classified differently; only 13, 11, and 13 out of 20 specimens were re-classified as stages II, III, and IV, respectively, after micro-CT analysis.

Fig. 1 shows optical microscopy and micro-CT images of the carious lesions. The microscopy examinations allowed only superficial observations of the damaged range, colour, and severity of the lesions. On the other hand, the micro-CT images showed both the lesion depth and width and they did not correspond to the original stages determined by the visual and tactile inspections (Table 2).

Fig. 2 shows the emission AF spectra of the specimens at different stages. As the tooth changed from sound to stage IV, the emission peak near 500 nm decreased gradually to the baseline. Some specimens, however, showed two peaks after 600 nm. For stage III-b, the peak at 625 nm was higher than that of 667 nm. For stage IV-b, however, the peak at 667 nm was much higher than that of 625 nm.

Tables 3 and 4 show the numerical results (mean, standard deviation, minimum, and maximum values) of the three derived factors (spectral slope, area under the curve, and two-peak ratio) and DIAGNOdent readings. The estimated mean values of the three factors in these two tables were similar. On the other hand, the standard deviations (STD) of the three factors were narrower in table 4 than in table 3. In table 4, for the DIAGNOdent readings, stages III (83.2 ± 20.6) and IV (87.3 ± 16.7) were similar, but significantly different from those of the sound (13.7 ± 7.1) and stage II (44.0 ± 23.9) teeth. With the exception of the DIAGNOdent readings, the three factors values were all significantly different for the different stages ($p < 0.001$).

Fig. 3 shows the compositional changes on the specimens at the different stages. The intensities of the characteristic bands for CO_3^{2-} (at 1550, 1460–1415, and 870 cm^{-1}), PO_4^{3-} (at 1090–1032 and 960 cm^{-1}), C-H (at 2930 and 2860 cm^{-1}), and water/ OH^- (at 3700–3400 cm^{-1}) decreased gradually as caries progressed.

Fig. 4 presents images of a brown spot on a tooth surface. Optical microscopy indicated a stage III carious lesion (a), but micro-CT indicated a caries-free sound tooth and did not provide any evidence of subsurface damage (b).

4. Discussion

Caries is one of the most common oral diseases in the oral cavity that can be recovered by a remineralisation process from the ingredients dissolved in saliva and food or through a simple restoration at a dental clinic using amalgam or composite resin. If ignored, however, it can progress to serious tooth destruction ultimately requiring extraction. To maintain a healthy, controllable tooth condition, routine teeth monitoring by regular check-ups is required, and if caries is suspected, a precise diagnosis and treatment are important.

The diagnosis of caries by a visual and tactile inspection is a straightforward, routine process that is performed in dental clinics during regular check-ups.^{12–14} Despite its simplicity, a diagnosis of caries requires experience and despite the presence of guidelines for classification, it can sometimes be subjective because examiners cannot see inside the teeth, as is the case with X-rays, and the states of all lesions are not identified clearly and simply. For this reason, an X-ray test can be added, but identifying early or mild caries is still difficult because at this stage, the size, affected boundary, and severity of caries is not clearly defined. In other words, they are not discerned easily by X-ray due to insensitivity of the intraoral X-ray test at this stage. Indeed, in the present study, except for ‘sound’ teeth, the diagnosis of caries, regardless of the stage, by a visual and tactile inspection was inaccurate compared to the results of micro-CT analysis. Only 13, 11, and 13 out of 20 stage II, III, and IV teeth, respectively, matched their initial designations. In some specimens, caries was underestimated (2, 5, and 7 teeth for stages II, III, and IV, respectively) and in others overestimated (5 and 4 teeth for stages II and III, respectively). In many cases, chalky white and/or brown spots on the enamel surfaces (Fig. 4) caused misreadings.^{31–33} This type of misreading can be worsened when a carious lesion is located between two adjacent teeth or premolars and molars because the viewing angles are suboptimal and the surface geometries are unfavourable. The wide STD distributions in the present study would be the result of an inaccurate diagnosis of the caries stage. Therefore, for reliable results, a comparison with accurately classified caries is essential.

The 405 nm laser-induced AF spectrum shows a characteristic spectral pattern from 450 to 800 nm.^{23–30} From the spectrum of sound to carious teeth, the changes in the spectral slope at 550–600 nm due to a change in the peak intensity, area under the spectrum at 500–590 nm, and the appearance and ratio between the two peaks at 625 and 667 nm were apparent. Generally, the spectral slope increases to 500 nm and then decreases to 800 nm, but the slopes at this spectral range differ according to the stage. Demineralisation results in decreases in the peak intensity near 500 nm and in the spectral slope before and after 500 nm with increasing stage (Fig. 3). Demineralisation due to the oral acids, produced by oral bacteria after their consumption of dietary sugars and carbohydrates, leaves a porous structure by removing the inorganic substances. During the early stages of caries, the tooth structure is intact, but cavitation and disintegration occur as caries worsens (Fig. 1). As a result, carious lesions show structural and colour changes from the surface to subsurface. Sound teeth have a shiny bright colour with an intact smooth surface. On the other hand, carious teeth have pores with a rough surface or cavity into the subsurface that become pale to dark brown. As the tooth becomes porous due to demineralisation, the fluorescence intensity from the minerals will be reduced due to the lower mineral concentration. In

addition, light transmission into the subsurface can be reduced because more light is absorbed by the dark area than a bright colour, but the scattering of transmitted light will be increased by the inhomogeneous porous structure. A decrease in peak intensity near 500 nm is probably due to these combined effects.³⁴ The two peaks after 600 nm in the AF spectrum (Fig. 2) of stage III and IV were assigned to oral bacteria and endogenous porphyrins, such as protoporphyrin IX, which were synthesised by the oral bacteria in the carious lesions.^{24,28–30} The species complexity of oral bacteria, their activities, and ability to accumulate on the tooth surfaces may be responsible for the inconsistent appearance of the peak(s) in the AF spectrum and the high spread of two-peak ratio data. Regarding the two peaks at wavelengths longer than 600 nm, dental plaque can be a possible entity in absence of caries.²⁴ The production and detection of two fluorescence peaks can be possible because dental plaque resides everywhere on the tooth surface as a thin biofilm or mass of bacteria. According to the stage description (Table 1), however, all carious lesions (from stage II to IV) accompany visible morphology modifications from the surface to the deep subsurface of the tooth; peaks after 600 nm without surface modification can be excluded as evidence of a carious lesion. In Table 2, two and three teeth, which were initially classified as stage II and III, respectively, would be such cases. According to micro-CT, their surfaces were clean and showed no evidence of caries formation.

Among the factors, the spectral slope at 550–600 nm showed the highest correspondence to the stages classified by micro-CT. According to Table 4, the spectral slope for sound, stage II, III, and IV carious teeth was in the range of 40.1–74.6, 27.5–39.6, 11.1–27.4, and 1.0–9.7, respectively. These values showed the least overlap among the data for each stage and the results suggest that this method has high potential and reliability for the diagnosis and staging of caries compared to the DIAGNOdent readings. Although these results were obtained only from the limited specimens under optimal in vitro measuring conditions, the feasibility of the staging of carious lesions can be improved further by taking the in vivo conditions that reflect the clinical situations with a much greater specimen size. Interpretations of the diverse AF spectra that have been obtained under different detection angles for the incident light and distances from the lesion to the detector head would be challenging for future clinical applications.

A tooth is a rigid composite of various inorganic and organic substances and water. In a complex situation within the oral cavity and on the tooth surface, these constituents may have different dissolution rates depending on the pH and interaction time. Inorganic minerals are quite soluble in acidic solutions and their dissolution will be high because of their high content in the tooth. Organic substances, such as collagen and proteins, are insoluble in acidic solutions, but can be decomposed by saliva or other solvents in food stuffs or beverages. During the demineralisation process, dehydration or decomposition of water can occur. As a result, FTIR spectral changes in all tooth-composing molecular bonds are inevitable.^{35,36} Therefore, a decrease in the peak intensities of the carbonate (CO_3^{2-}) and phosphate (PO_4^{3-}) bands is apparent. The weak peak intensity at the C-H bands can be attributed to the low content of organic substances in the tooth. The low peak intensities at the water/ OH^- band at both sound and carious lesions are probably due to dehydration while preparing a specimen. The heat produced while grinding lesions with a fissure bur and exposure to air of the airborne state of the ground powder may enhance dehydration.

Unlike the AF spectra obtained using a 405 nm laser, which exhibited two characteristic peaks near 500 nm and after 600 nm, the DIAGNOdent (a 655 nm laser device) responds only to oral bacteria and their endogenous by-products in the lesions. This means that DIAGNOdent does not respond to demineralisation if organic substances are not involved. Hence, DIAGNOdent is not as sensitive in the diagnosis and staging of caries as the AF spectra obtained using the 405 nm laser. In a preliminary test, DIAGNOdent did not respond to tooth demineralised artificially with acidic solutions regardless of the demineralisation time. In the present study, eight out of eighty specimens had DIAGNOdent readings equivalent to the initial stage or strong demineralisation state (>14) according to the manufacturer's instructions, but were classified as sound by micro-CT. In addition, nine out of eighty specimens showed readings equivalent to the dentin caries state (>30), but were classified as stage II (cariou lesions remain in less or around 1/3 of enamel) by micro-CT. Such overestimations by DIAGNOdent were also observed easily in the specimens of stages III and IV. For these overestimation readings, brown spots or dark stains are a possible reason. Regarding these spots or stains, several causes are probable; the caries produced by demineralisation/remineralisation can produce a brown spot on the tooth.^{31–33} In addition, unremoved tartar over the lesion or exposed dentin by enamel wear after demineralisation is also likely. In any case, accompanied morphological changes on the affected lesion can be observed. On the other hand, dental plaque, tartar, or discoloration by food stuffs, beverages, or tobacco can be formed on the sound tooth surface without a change in morphology. In this case, although they are not related to caries, DIAGNOdent can identify it as a carious lesion by overestimating it.^{32,37}

Three spectral factors tested in the present study were extracted from the AF spectrum of a precisely classified carious lesion using micro-CT. After the comparisons, the ranges of the spectral slope for the different carious lesions were informative enough to be applicable directly to the diagnosis and staging of unknown teeth. Because the data were obtained under optimal in vitro experimental conditions, further studies under more simulated oral and optical conditions and with larger sample sizes will be needed before the spectral slope can be applied both to the diagnosis and staging of carious lesions in addition to DIAGNOdent and to obtain a more agreeable conclusion regarding the feasibility under more complex and unfavourable clinical situations.

5. Conclusion

Of the specimens classified initially as stage II, III, and IV by two examiners through visual and tactile inspections, only 13, 11, and 13 out of 20 specimens matched stages II, III, and IV classifications, respectively, made by micro-CT inspections. The data obtained from the micro-CT-reclassified specimens showed less overlap between the stages than those initially classified. Among the three factors identified, the spectral slope at 550–600 nm showed the least overlap between the stages and the apparently differentiable ranges of the slope for each stage. Regardless of the classification method, the DIAGNOdent readings showed significant overlap between the stages. In addition, regardless of the caries stage, most DIAGNOdent readings were >30 , indicating the presence of dentin caries, which means an overestimation of the carious state than that of the real state. Within the limitations of the

present study, an analysis of the spectral slope from the 405 nm laser-induced AF spectra appears to provide a useful means for diagnosing and staging caries.

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Clinical significance

The 405 nm laser-induced AF spectra can be applied to the diagnosis and staging of caries alone or in conjunction with conventional methods, such as visual, tactile, and X-ray inspection.

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Fig. 1. Light microscope and micro-CT images of specimens with different stages. Tooth a (a1 and a2) is stage II, b (b1 and b2) is stage III, and c (c1 and c2) is stage IV. The dark region in the micro-CT image from the enamel subsurface to enamel-dentin junction or dentin subsurface is a carious lesion.

Fig. 2.

Autofluorescence (AF) spectrum of specimens with different stages. As caries progressed, the slope after peak intensity gradually decreased to baseline. However, some specimens of stage III and IV showed two peaks after 600 nm with different intensities.

Fig. 3. FTIR spectra obtained from specimens with caries of different stages (sound, stage III, stage IV). As caries progressed from sound to stage IV, water and mineral (calcium and phosphorus) contents in carious lesions gradually decreased due to demineralization.

Fig. 4. Images of brown spots on tooth surfaces. Brown spots were clearly visible on enamel surfaces under a light microscope (a1, a2) and teeth were initially allocated to stage III by visual and tactile inspection. However, they show no marks of carious lesions on the micro-CT images (b), and thus, should have been classified as sound.

Table 1

Description of caries stages defined in this study.

Code	Description	Relevant ICDAS II
Sound	Sound and glossy surface	Sound tooth surface (0)
Stage II	Demineralization less 1/3 enamel, visible white spot, no glossy surface, remineralization can occur, dentist warns finding of incipient caries	First/Distinct visual change in enamel (1,2)
Stage III	Demineralization over 1/3 enamel and possibly damaged to dentin, more clearly visible white spot than stage II, sometimes light to dark brown spot is visible, no cavitation yet, immediate treatment depend on internal demineralization	Enamel breakdown, no dentin visible (3)
Stage IV	Cavitation is visible and progressed into dentin, white and/or brown discolored lesion is frequently visible, immediate treatment is required	Underlying dentinal shadow (not cavitated into dentin) – in our case, this case was grouped to III if there is no loss of surface integrity (4) Distinct cavity with visible dentin (5) Extensive distinct cavity with visible dentin (6)

Table 2

The number of the allocated specimens to each group after visual and tactile inspections and the number of reclassified specimens based on the micro-CT analysis using the same specimens used for visual and tactile inspections.

After visual and tactile inspection		# of specimens Micro-CT analysis of the visually and tactilely inspected specimens
Sound	20	Sound: 20
Stage II	20	Sound: 2, Stage II: 13, Stage III: 5
Stage III	20	Sound: 3, Stage II: 2, Stage III: 11, Stage IV: 4
Stage IV	20	Sound: 0, Stage II: 1, Stage III: 6, Stage IV: 13

Table 3

The estimated values from the AF spectrum and DIAGNOdent readings for specimens of different stages which were classified by visual and tactile inspections.

	Sound ^a	Stage II ^b	Stage III ^c	Stage IV ^d	P-value
550–600 nm slope	-56.0 ± 10.1 (40.1–74.6)	-31.2 ± 8.2 (17.3–52.6)	-20.4 ± 12.3 (5.4–46.2)	-9.9 ± 8.6 (1.0–35.8)	< 0.001
	Sound ^a	Stage II ^b	Stage III ^b	Stage IV ^c	
Area under curve at 500–590 nm	712.9 ± 81.4 (562–865)	520.0 ± 74.0 (389–699)	459.0 ± 111.8 (307–702)	360.0 ± 100.7 (255–586)	< 0.001
	Sound ^a	Stage II ^b	Stage III ^c	Stage IV ^d	
625/667 nm ratio	1.26 ± 0.04 (1.19–1.33)	1.19 ± 0.04 (1.12–1.26)	1.14 ± 0.06 (1.01–1.24)	1.09 ± 0.07 (0.99–1.27)	< 0.001
	Sound ^a	Stage II ^b	Stage III ^c	Stage Ib ^d	
DIAGNOdent reading	11.1 ± 3.0 (6–17)	41.8 ± 16.3 (14–78)	72.9 ± 27.5 (13–99)	90.2 ± 11.3 (65–99)	< 0.001

* Statistically significant difference on carious stage is shown by superscript letters^{a, b, ...}. Same letters in the same row are not significantly different ($p > 0.05$).

The estimated values from the AF spectrum and DIAGNOdent readings for specimens of different stages which were reclassified based on the micro-CT analysis.

Table 4

	Sound ^a	Stage II ^b	Stage III ^c	Stage IV ^d	P-value
550–600 nm slope	−53.7 ± 10.3 (40.1–74.6)	−33.0 ± 3.0 (27.5–39.6)	−17.4 ± 4.5 (11.1–27.4)	−5.7 ± 2.6 (1.0–9.7)	< 0.001
	Sound ^a	Stage II ^b	Stage III ^c	Stage IV ^d	
Area under curve at 500–590 nm	701.8 ± 78.6 (562–865)	534.8 ± 43.5 (476–654)	440.7 ± 53.5 (357–585)	307.8 ± 36.0 (255–385)	< 0.001
	Sound ^a	Stage II ^b	Stage III ^c	Stage IV ^d	
625/667 nm ratio	1.25 ± 0.05 (1.15–1.33)	1.20 ± 0.03 (1.14–1.24)	1.15 ± 0.05 (1.04–1.27)	1.06 ± 0.04 (0.99–1.12)	< 0.001
	Sound ^a	Stage II ^b	Stage III ^c	Stage Ib ^c	
DIAGNOdent reading	13.7 ± 7.1 (6–35)	44.0 ± 23.9 (14–99)	83.2 ± 20.6 (32–99)	87.3 ± 16.7 (46–99)	< 0.001