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# Caries detection and quantification around stained pits and fissures in occlusal tooth surfaces with fluorescence

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**Abstract.** Occlusal discoloration due to staining frequently occurs on the pits and fissures of teeth. Noncariogenic discoloration (non-CD) refers to the attachment of staining chromogens to sound surfaces, whereas cariogenic discoloration (CD) represents the discoloration of porous structures due to bacterial metabolites and mineral loss from the enamel surface. This study evaluated whether it is possible to distinguish between non-CD and CD on stained occlusal surfaces with fluorescence assessed by the quantitative light-induced fluorescence (QLF) technology. Sixty-two extracted human permanent teeth with suspected discolorations on the pit and fissure were examined. The maximum values of fluorescence loss ($\Delta F_{max}$) and red fluorescence gain ($\Delta R_{max}$) were calculated using QLF images. Using histology as the gold standard, it was found that 12 teeth were sound (non-CD), while 50 teeth had enamel and dentine caries (CD). The validity tests at the enamel histological caries level, $\Delta R_{max}$ ($\rho = 0.80$) were strongly correlated with the histology ($P < 0.001$). At the optimum threshold (105.0) of $\Delta R_{max}$, it showed high levels of sensitivity and specificity (0.96 and 0.83, respectively). Therefore, QLF can be used to distinguish non-CD from CD on occlusal surfaces using red fluorescence values with high validity.  [DOI: 10.1117/1.JBO.23.9.091402]

Keywords: occlusal caries; tooth discoloration; stained pit and fissure; red fluorescence; quantitative light-induced fluorescence.

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## 1 Introduction

Paradigms in dentistry have been shifting due to the improved understanding of the caries process. The concept of minimal intervention dentistry (MID), namely "prevention for extension," is becoming more important than the old surgical model of "extension for prevention" as suggested by G.V. Black because some caries lesions can now be recovered by preventive treatment. An accurate diagnostic method for dental caries has emerged as a prerequisite of MID.[1,2] Despite efforts to preserve healthy tooth substances via MID, difficulties remain for dental clinicians, especially related to the diagnosis of occlusal caries.

The occlusal surface is highly susceptible to decay due to incomplete removal of dental plaque from structural irregularities such as pits and fissures. The proportion of occlusal caries lesions among all types has increased relatively due to decreasing rates of smooth and approximal caries.[3,4] In addition, the widespread use of fluoride has led to the appearance of various types of occlusal caries lesions (i.e., clinically missed or undetectable subsurface caries lesions).[5,6] From these reasons, a comprehensive diagnosis that considers the enamel roughness, opacity, and discoloration is needed, as opposed to the presence of cavitation previously being the key criterion for a diagnosis of occlusal caries.[7,8]

In terms of discoloration, white or brown opacities are representative features on occlusal surfaces that occur due to the difference in the refractive index between the sound and lesion parts of a tooth. However, color changes in pit and fissure areas are not always due to structural changes of a tooth, instead reflecting the presence of external substances. Staining materials such as foodstuff, beverages, and habitual smoking enter from outside the oral cavity and contain certain chromogens that cause extrinsic discoloration by attaching themselves directly to tooth surfaces. Internal discoloration can also occur when staining chromogens penetrate porous structures on the enamel surface along with opacity from differences in refractive indices during the demineralization process.[9] These various causes of occlusal discoloration can make definitive diagnoses difficult.

Pit and fissure discoloration is one of the factors that can affect decision-making in the diagnosis of occlusal caries, but using such discoloration as a diagnostic criterion is still controversial. A previous study found that the high salivary level of *Streptococci mutans* in pit and fissure areas was associated with brown discoloration in school children.[10] In contrast, some researchers have expressed concern that using occlusal discoloration as a diagnostic criterion could lead to false

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positives because they found that more than half of the teeth regarded as being carious due to their stained pits and fissures were actually sound.[5] Furthermore, most dental clinicians encounter the diagnostic dilemma of deciding whether or not they should remove discolored tissues due to the difficulty of obtaining sufficient evidence about the discoloration of pits and fissures in all clinical situations.[11] Overcoming this diagnostic dilemma requires new diagnostic methods that can provide clear evidence about occlusal caries based on an adequate understanding of discoloration in occlusal pit and fissure areas.

Caries detection methods that have been developed in recent years use physical stimulation methods such as lights and electrical currents to obtain objective information, and they thereby overcome the limitations of conventional diagnostic methods for describing the status of teeth.[12] One representative method is quantitative light-induced fluorescence (QLF), which detects caries lesions by quantifying the autofluorescence emitted from teeth illuminated by light at 405 nm. This technology typically utilizes the light-scattering property that results in the green autofluorescence of early caries lesions becoming darker than that of sound enamel during illumination by narrow-band blue light. Previous studies have demonstrated that QLF could be useful for the detection of early caries lesions due to it varying with only small changes in the mineral content of teeth.[13–15] In addition, numerous studies have been conducted recently to identify various features of teeth, such as remineralization of early caries lesions and the detection of enamel cracks and approximal and occlusal caries.[16–21]

The light used in the QLF technique is well known to be within the optimal wavelength region for eliciting red fluorescence from dental plaque and caries lesions.[14,22] This red fluorescence is reportedly due to the protoporphyrin IX produced by bacterial metabolism.[23] It is therefore expected that the red fluorescence emitted when applying the QLF technique can be helpful for explaining whether bacteria are metabolized along with the existing information of fluorescence changes due to the loss of mineral during the caries process. However, most previous studies have focused on the red fluorescence of dental plaque,[24–26] with there being no previous investigations of the red fluorescence of dental caries.

Based on the above-described situation, this study evaluated whether the QLF technology would be useful for detecting cariogenic discoloration (CD) based on the quantitative analysis of questionable occlusal caries due to stained pit and fissure areas, with the aim of distinguishing CD due to the process of demineralization and bacterial metabolism from noncariogenic discoloration (non-CD).

## 2 Materials and Methods

### 2.1 *Selection of Teeth Samples*

Ethical approval for this study was obtained from the Institutional Review Board for Clinical Research at Yonsei University Dental Hospital (IRB No. 2-2014-0024). Permanent human teeth that had been freshly extracted for orthodontic or periodontal reasons were collected after obtaining informed written consent from all participants older than 20 years. In total, 66 permanent molars and premolars without enamel hypoplasia, fluorosis, or cavities were selected from a pool of extracted human teeth having questionable caries due to the presence of stained pits and fissures. The teeth were placed in distilled water as soon as possible after being extracted, and they were subsequently cleaned of calculus, soft tissues, and other debris using hand scalers and toothbrushes. The cleaned teeth were bottled in a black container to block external light (which can photobleach teeth[27]) and then frozen and stored at −20°C[28] until being analyzed.

### 2.2 *Preparation of Tooth Specimens*

Root areas that were more than 1.5 cm from the top of the cusp of each tooth sample were sectioned using a low-speed saw with a diamond disc (NTI-KAHLA GmbH, Kahla, Germany). Each sectioned tooth was fixed perpendicularly into a 9-mm-diameter hole in an acrylic mold with resin (Ortho-Jet, Lang Dental Manufacturing, Illinois). All tooth specimens were stored at 4°C and 100% humidity to protect them from dehydration throughout the study.

### 2.3 *Quantitative Analysis for Distinguishing between Non-CD and CD Using QLF*

The QLF–digital Biluminator™ 2+ (QLF-D; Inspektor Research Systems BV, Amsterdam, The Netherlands) was used to evaluate the discoloration of the occlusal pit and fissure areas. White-light and fluorescence images were captured from the occlusal aspects of all tooth specimens at a fixed distance from the camera [Fig. 1(a)]. After blocking out the ambient light, the image was acquired using proprietary software (C3 v1.25, Inspektor Research Systems BV) at a shutter speed of 1/20 s and an aperture value of 10.0. For the fluorescence images, an analysis patch was delimited by drawing a border that pointed at sound parts without discolorations from the stained pits and fissures with suspected caries according to manufacturer recommendations using the QLF-D software (QA2 v1.25, Inspektor Research Systems BV) [Fig. 1(b)]. The changes in discoloration and mineral content in the lesion were calculated as the decrease in fluorescence ($\Delta F$) compared with sound enamel, and the level of bacterial metabolites was calculated as the increase in red fluorescence ($\Delta R$), both expressed as percentage values. Considering the feature of occlusal caries, the maximum fluorescence values ($\Delta F_{max}$ and $\Delta R_{max}$) were used to represent the pit and fissure lesions because they show an inverted V-shape with a narrow entrance and progressively wider area to the dentinoenamel junction (DEJ). All analyses were conducted by an experienced examiner.

### 2.4 *Histological Examination*

After completing the image analyses, all teeth were cut perpendicularly into 1-mm-thick specimens (TechCut 4™, Allied High Tech Products, California). The specimens were then ground to a thickness of ~150 $\mu$m with 800-grit silicon carbide paper (SiC Sand Paper, R&B Inc., Daejeon, Korea) and photographed under a polarized-light microscope (PLM; CX31-P, Olympus, Tokyo, Japan) at a magnification of 40×. The PLM images were histologically assessed for the presence and severity of caries lesion as follows: no enamel demineralization or a narrow surface zone of opacity (scored as 0), enamel demineralization limited to the outer 50% of the enamel layer (scored as 1), demineralization involving the inner 50% of enamel up to the DEJ (scored as 2), and demineralization involving the outer 50% of the dentine (scored as 3).

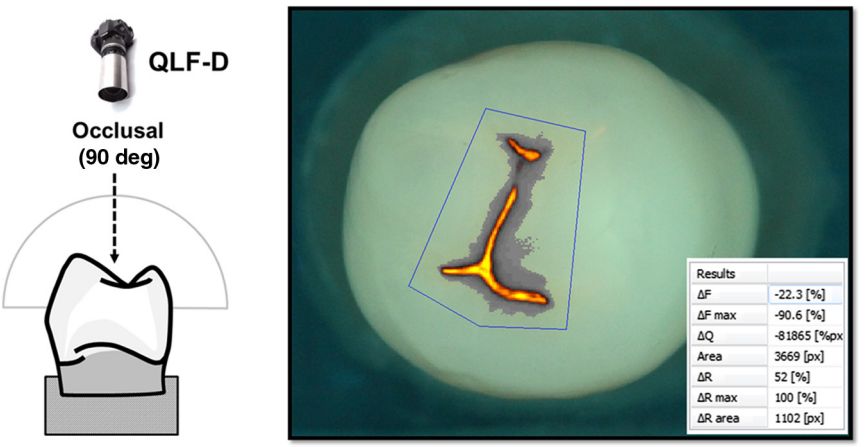


**Fig. 1** (a) Fluorescence image-taking and (b) the analysis process involving manually drawing a patch and automatically calculating QLF parameters.

### 2.5 *Statistical Analyses*

Correlations between the fluorescence parameters and histological results were calculated by Spearman's rank correlation (rho) test. The sensitivity and specificity of each QLF parameter in distinguishing the non-CD and CD were calculated by comparing the specimens with the histology findings. All stained pits and fissures were dichotomized into histological scores of 0 (for non-CD) and >0 (CD at the enamel threshold). The optimum thresholds of $\Delta F_{max}$ and $\Delta R_{max}$ for distinguishing between non-CD and CD were established by the highest combination of sensitivity and specificity in the receiver operating characteristic (ROC) analysis (version 15.8, MedCalc®, MedCalc Software, Ostend, Belgium). The cutoff for significance in all of the statistical analyses was set at $\alpha = 0.05$ using the PASW Statistics software (version 18.0, SPSS, Chicago, Illinois).

## 3 Results

Four teeth were damaged during the sectioning process for the histological examination, so 62 teeth were finally analyzed.

**Table 1** Distribution of QLF parameters of discoloration on occlusal pits and fissures.

| | Histology | *N* | $\lvert\Delta F_{max}\rvert$ (%) | $\Delta R_{max}$ (%) |
|---|---|---|---|---|
| Non-CD | S | 12 | 62.00[a] (55.25, 73.00) | 90.50[a] (68.00, 104.25) |
| CD | E | 43 | 82.00[b] (76.00, 88.00) | 194.00[b] (126.00, 290.00) |
| | D | 7 | 93.00[c] (92.00, 94.00) | 507.00[c] (358.00, 684.50) |
| *P* | | | <0.001 | <0.001 |

Note: Data are median (first, third quartile) values.
Note: Non-CD, noncariogenic discoloration (histological score = 0); CD, cariogenic discoloration (histological score > 0); $\lvert\Delta F_{max}\rvert$ is the absolute value.
Note: S, sound tooth; E, enamel lesion; D, dentine lesion.
Note: Different letters within the same column indicate significant differences between groups by the Kruskal–Wallis test and Mann–Whitney test with Bonferroni *post hoc* correction.

Designating all enamel and dentine lesions as disease-positive (histological score > 0) resulted in 12 teeth being sound with non-CD and 50 teeth having caries showing CD. Among the 50 lesions with CD, 43 teeth had caries within the enamel and 7 teeth had dentine caries (Table 1).

The $\lvert\Delta F_{max}\rvert$ and $\Delta R_{max}$ values were higher for deeper lesions (Table 1, Fig. 2). Compared with sound parts without discolorations, non-CD teeth exhibited fluorescence reductions of up to 62.00%, with the level of red fluorescence increasing by up to 90.50%. Meanwhile, the $\lvert\Delta F_{max}\rvert$ and $\Delta R_{max}$ values of CD increased continually with the severity of lesions and differing significantly from non-CD ($P < 0.001$). Strong correlations were identified between QLF parameters and histological results, with the correlation coefficient of $\Delta R_{max}$ ($\rho = 0.80$, $P < 0.001$) being higher than that of $\lvert\Delta F_{max}\rvert$ ($\rho = 0.76$, $P < 0.001$). It was also found that red fluorescence existed inside the lesion body by QLF examination of tooth cross sections, and the demineralized area in PLM images were similar with the area of red fluorescence in QLF images within/beyond the DEJ (Fig. 3).

Figure 4 represents the ROC curves of each QLF parameter for distinguishing between non-CD and CD in the occlusal pit and fissure areas. According to histological criteria, the optimum cutoff values of $\lvert\Delta F_{max}\rvert$ and $\Delta R_{max}$ were 75.0 and 105.0, respectively. Comparing the sensitivity and specificity of each QLF parameter, the sensitivity of $\Delta R_{max}$ (0.96) was higher than that of $\lvert\Delta F_{max}\rvert$ (0.80), while the specificity of $\lvert\Delta F_{max}\rvert$ (0.92) was higher than that of $\Delta R_{max}$ (0.83). The AUROC was higher for $\Delta R_{max}$ (0.94) than for $\lvert\Delta F_{max}\rvert$ (0.91).

## 4 Discussion

This study evaluated whether QLF technology can be used to distinguish non-CD from CD surfaces, since the former can be incorrectly diagnosed as indicating caries based on the discoloration of pits and fissures. We have confirmed the potential of QLF technology in evaluating the fluorescence properties of stained pit and fissure areas and in quantitatively distinguishing actual caries from mere occlusal discoloration.

Previous studies have found that visual examinations are not suitable for diagnosing occlusal caries due to the low sensitivity and, especially, the overestimation associated with using discoloration as a criterion.[5] One reason for this problem is the phenomenon known as metamerism that results in dental clinicians

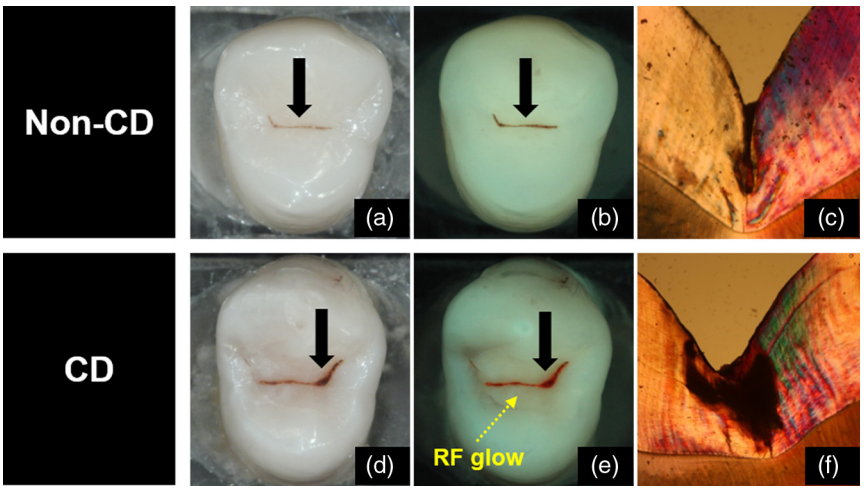


**Fig. 2** Images obtained using the QLF-D under different lighting conditions (a and d, white-light; b and e, fluorescence-light), and the respective polarized-light micrographs (c and f; magnification = 40×). Non-CD, noncariogenic discoloration; CD, cariogenic discoloration. RF glow, red fluorescence (RF) glow around the discolored fissure. Black arrows indicate the point of sectioning.

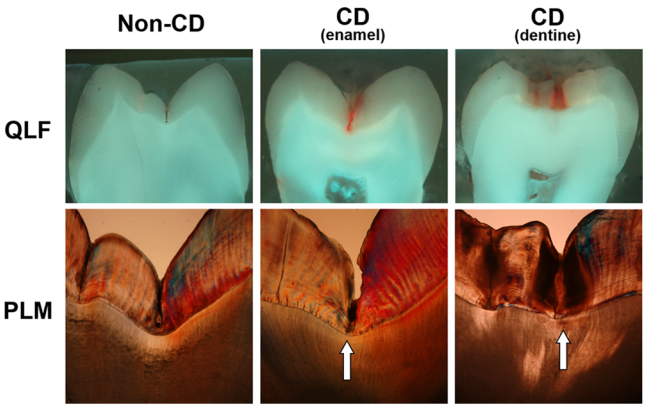


**Fig. 3** QLF images of cross sections of discolored occlusal teeth (upper line), and their respective polarized-light micrographs (PLM, magnification = 10×, bottom line). Non-CD, noncariogenic discoloration; CD, cariogenic discoloration. White arrows indicate the presence of the demineralized lesion.

diagnosing the same tooth differently when using natural light having a large range of wavelengths. Standardizing the lighting conditions can be effective at reducing such metamerism when examining tooth color during caries diagnoses.[9] We therefore used the QLF technology—which involves illuminating a tooth at a specific standardized wavelength and detecting the autofluorescence emitted—to assess stained occlusal pits and fissures.

The present results indicate that the autofluorescence of teeth decreased with the progression of the occlusal caries lesion histologically, with a high correlation coefficient of −0.76 ($P < 0.001$). This trend is in accordance with previous studies that have investigated artificial early caries *in vitro* as well as real approximal and occlusal caries clinically. In contrast to previous studies excluding tooth samples with discoloration, we only evaluated extracted teeth having stained pits and fissures. This resulted in the level of fluorescence loss from tooth specimens used in this study being higher than in previous studies.[16,18,20] This finding is supported by a previous report of the discolored tooth structure appearing darker because an increase in the discoloration intensity corresponded to an increase in the fluorescence loss ($|\Delta F|$).[29] In addition, concerns have been raised that the presence of discoloration could increase the risk of false-positives diagnoses.[30,31] One of these concerns is that the presence of stains can result in fluorescence reduction, similar to that of mineral loss and lesion progression. As the decrease of fluorescence may occur from absorption by stains as well as of light scattering in early white spot lesions, using the fluorescence loss ($\Delta F$) for diagnosis of occlusal discolored surfaces is regarded as not reliable and therefore not effective.

To address this problem, this study also evaluated the red fluorescence of stained pits and fissures—which can reflect bacterial activity—as a new QLF parameter because the red fluorescence emitted at 405 nm is due to porphyrin molecules such as protoporphyrin IX, mesoporphyrin, and coproporphyrin associated with bacterial metabolism.[32] Although it cannot be found in the literature, we believe that these endogenous porphyrins (fluorophores) could be maintained when the teeth samples were frozen at cold temperatures below −20°C (which have a bacteriostatic effect) and stored in a dark condition to block the photobleaching effects.[27,28] We found that the level of red fluorescence around the stained pits and fissures as measured with QLF in this study differed between teeth (Figs. 2 and 4), and it was strongly correlated with the histological lesion severity ($\rho = 0.80$, $P < 0.001$). These findings confirm previous reports of the level of red fluorescence being linearly correlated with the concentration of fluorophore molecules that are easily trapped in the porous structures of caries lesions.[22,30,33,34] The red fluorescence inside the lesion body suggests that porphyrins penetrated inside the lesion, and it might explain why a red fluorescence glow can be detected around discolored pits and fissures on many CD teeth (Fig. 3). The results of the current study also support previous findings of the phenomenon that the red fluorescence in caries-related biofilms increases with their maturation and severity.[24,25] The intensity of red fluorescence of CD was significantly higher than that of non-CD in the present study ($P < 0.001$). Therefore, the red fluorescence

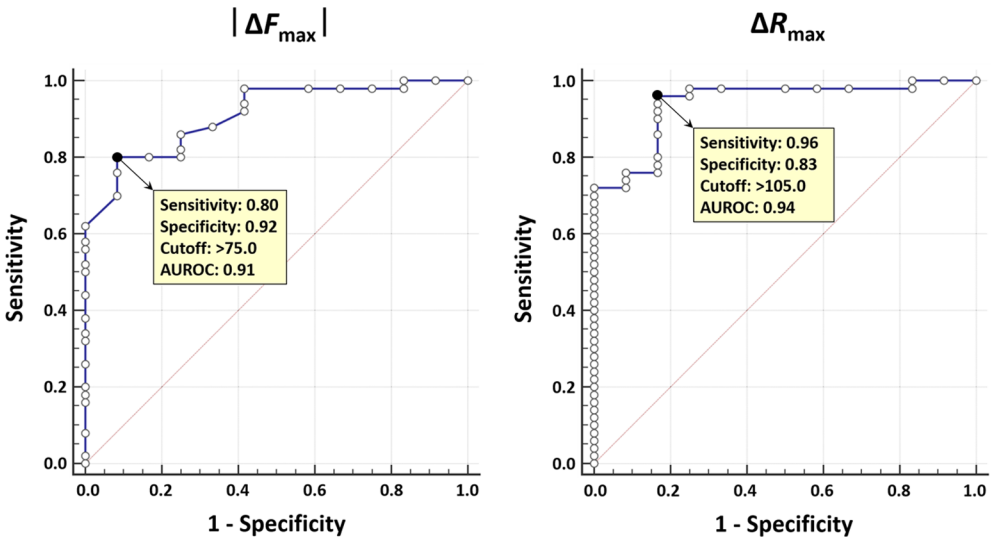


**Fig. 4** ROC curves of QLF parameters ($|\Delta F_{max}|$ and $\Delta R_{max}$) at the enamel histological caries level to distinguish between non-CD (histological score = 0) and CD (histological score > 0).

parameter can be used as an indicator for distinguishing caries lesions from simple discolorations on the occlusal surfaces.

To our knowledge, this is the first study to apply the red fluorescence properties in QLF technology to determine the validity and optimum cutoff values for detecting caries in discolored occlusal teeth. The $\Delta R_{max}$ value in the present study showed a high sensitivity of 0.96 but a lower specificity of 0.83. Despite the possibility of false-positive results, the ROC analysis indicated that the new red fluorescence parameter could be used to distinguish between non-CD and CD with a high AUROC for $\Delta R_{max}$ (0.94). The $|\Delta F_{max}|$ value, reflecting the maximum level of mineral losses from tooth surfaces, had a sensitivity of 0.80 and specificity of 0.92, and its AUROC was 0.91. However, note that the AUROCs of QLF parameters in this study might have been artificially high due to it being conducted *in vitro* and hence not including external factors that could be present in clinical situations. In addition, the possibility of errors in the sensitivity and specificity may exist because those values are dependent on the histology results, which were used in this study as the gold standard.

The QLF technique may be most superior with respect to evaluating occlusal caries as compared with other available

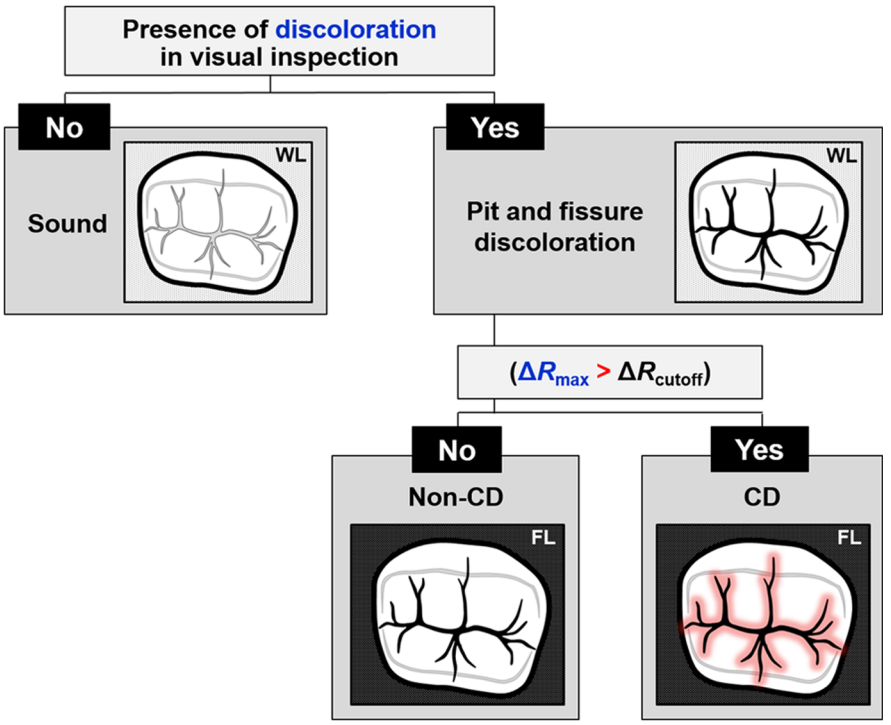


**Fig. 5** Decision flow chart for quantitatively distinguishing between non-CD and CD during diagnosing occlusal caries. WL, white-light image; FL, fluorescence image; non-CD, noncariogenic discoloration; CD, cariogenic discoloration.

optical methods, which include fluorescence-aided caries excavation (FACE), DIAGNOdent, and optical coherence tomography (OCT). The rationale for using FACE, DIAGNOdent, and QLF is that carious tissue emits more intense red fluorescence than sound tissue. QLF, FACE, and DIAGNOdent are used to detect caries by detecting porphyrins, which emit red fluorescence in the caries lesion (excitation wavelengths are 405, 405, and 655 nm, respectively).[24,30,35] FACE and DIAGNOdent were considered to be not appropriate for evaluation in this study because these methods cannot quantitatively assess changes of the mineral content in caries in time.[30,35] With OCT, cross-sectional images of the internal tooth can be made noninvasively. OCT uses the different scattering properties of enamel and dentine (excitation wavelength, 1310 nm), so it can detect changes in mineral loss using backscattered signal of a caries lesion. However, it is difficult to assess occlusal surfaces due to the high variation of the optical penetration and surface reflectivity.[36] Furthermore, with OCT, red fluorescence from bacterial metabolism cannot be detected. In view of these aforementioned limitations, it was concluded that QLF in this study may surpass other optical methods because it could quantitatively assess not only bacterially induced red fluorescence but also mineral changes at the occlusal surfaces at a time as well.

The concept of MID means that when diagnosing occlusal caries it is very important to consider the etiology of the discoloration that frequently occurs on tooth pits and fissures. Due to the absence of clear evidence about the mechanism of chromogenic microorganisms, understanding the etiology of discolored occlusal surfaces is necessary for developing appropriate treatment plans.[9] This requires a comprehensive evaluation of changes in color and mineral contents, and whether bacterial metabolism is present on the occlusal surfaces. Considering all of the results obtained in the present study, we suggest that information about red fluorescence increases and fluorescence decreases should be utilized together to diagnose occlusal caries with QLF technology. Using the optimum cutoff of $\Delta R_{max}$ (105.0), a decision flow chart to quantitatively distinguish non-CD from CD could be established; see Fig. 5. Future longitudinal clinical studies should attempt to validate the potential of QLF technology in distinguishing between non-CD and CD of occlusal tooth surfaces.

## 5 Conclusions

This study found significant differences in the red fluorescence parameters of non-CD and CD, with QLF being demonstrably useful for distinguishing non-CD from CD surfaces in teeth with high validity in relation to occlusal caries.

It can be concluded that QLF can be a useful tool for the differential diagnosis of discolored occlusal tooth surfaces. Future clinical validations may reveal that QLF technology can provide dental clinicians with meaningful information for diagnosing occlusal caries.

### Disclosures

Inspektor Research Systems BV provided the salary for author EdJdJ, but it did not have any role in the study design, data collection, analysis, decision to publish, or preparation of the manuscript. EdJdJ's involvement in this research was under the auspices of his status as adjunct professor at Yonsei University College of Dentistry supported by Brain Pool Program and BK21 PLUS Project. The specific role of EdJdJ was to provide his expertise regarding the fluorescence technology. This does not alter the author's adherence to the *Journal of Biomedical Optics* policies on sharing data and materials. EdJdJ holds several patents with respect to QLF technology. The remaining authors declare no conflict of interest.

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