Optical detection of secondary caries around amalgam restorations using quantitative light-induced fluorescence (QLF) technology

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Optical detection of secondary caries around amalgam restorations using quantitative light-induced fluorescence (QLF) technology

Directed by Professor Baek Il Kim, D.D.S., M.S.D., Ph.D.

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감사의 글

8년 전, 첫 발을 내딛었던 연구자의 길이 이제 드디어 결실을 맺게 되었습니다. 석사부터 시작한 연세대학교 예방치과학교실에서의 시간은 또 다른 나를 일깨우고 창조해 준 제 인생의 가장 값지고 소중한 시간이었습니다. 그 시간이 없었다면 지금의 저는 있을 수 없었을 것입니다. 지금 이 글을 쓴 이 순간에도 벅차오르는 감정에 눈시울이 붉어지는 듯합니다. 박사 학위를 마친 지금 저는 모든 것에, 모든 분들께 감사한 마음뿐입니다.

먼저 학위를 잘 마칠 수 있도록 끊임없는 관심과 격려로 저를 응원해주신 김백일 교수님께 감사의 마음을 전합니다. 의지와 열정만 가득하였던 저에게 지식과 학문이라는 가치를 끌어내 주시고 지금의 제 모습으로 성장시켜 주신 존경하는 지도교수님, 감사드립니다. 또한 항상 흐트러짐 없는 자세로 교육자, 연구자로서 본보기를 보여주시며 학문을 뛰어 넘어, 인생에 대한 큰 비전과 나아갈 길을 가르쳐 주신 권호근 교수님께도 감사의 인사를 전하고 싶습니다. 지난 8년 동안 동고동락하며 제 연구를 지원해주고 아낌없는 조언으로 최상의 연구 결실을 맺게 도와주신 정회인 교수님, 정말 감사드립니다. 세 분의 교수님이 계시기에 세계에서 으뜸가는 예방치과학교실이 될 것을 믿어 의심치 않고 있습니다. 미력하나마 그 뜻 실현할 수 있도록 함께 그 길을 걷도록 하겠습니다.

또한 박사 연구의 가장 핵심이었던 QLF technology의 창시자이자, 제 연구의 새로운 소프트웨어 프로그램을 만들어 주신 Dr. Elbert 정말 감사합니다.

(Dr. Elbert! Thank you for creating a new software. Your efforts have resulted in a good research). 또한 본 학위 논문의 심사를 맡아 주신 조영식, 정기호 교수님, 부족한 연구가 의미 있는 연구가 될 수 있도록 아낌없는 조언과 도움 주셔서 감사드립니다.

지난 시간동안 저의 조력자로서 아낌없는 격려를 해주었던 강시묵, 이은송 연구교수와 아낌없는 도움과 연구의 멘토가 되어주었던 이형석 선생님 감사합니다. 또한 박석우, 김상겸, 이주영, 김효정, 조무열, 최준혁 예방치과학교실 선생님들께도 감사의 말을 전하고 싶습니다. 이번에 함께 졸업하게 된 나의 사랑하는 동기이자 내 인생 최고의 친구 황혜림 교수님, 교육자의 길을 걸으며 연구의 끈을 놓지 않고 항상 열심인 남상미, 이선영 교수님께도 감사의 말을 전하고 싶습니다.

지금의 제가 있는데 도움을 주신 소중한 분들이 있습니다. 내 안의 숨겨진 나를 깨워주고 지금 이 자리까지 올 수 있도록 격려와 응원을 아끼지 않으셨던 제 인생의 멘토 한양여자대학교의 황윤숙 교수님, 학위를 위한 공부가 아닌 진정한 자기 발전과 학생들을 위한 마음으로 끊임없이 학업에 열중하며 본보기를 보여주고 계시는 동남보건대학의 이선미 교수님, 제가 학위를 잘 마칠 수 있도록 옆에서 배려해주시고 도움을 주셨던 김진수, 심수현, 윤경옥, 장희경, 최부근, 최영숙, 최정연 신성대학교 치위생과 교수님 모두에게 감사의 마음을 전하고 싶습니다. 이 밖에도 저의 주위에서 항상 응원해 주셨던 많은 교수님들과 선생님들께 진심으로 감사의 마음을 전하고 싶습니다.

마지막으로 내 삶의 존재 이유인 나의 사랑하는 가족들에게도 감사의 마음을 전하고 싶습니다. 나의 사랑하는 어머니와 제 인생 최고의 지지자 시어머님, 하늘에서 저를 지켜보고 있을 아버지와 신성대학교 설립자 故 태촌 이병하 박사님, 두 아버님께 부끄럽지 않은 가족이 되도록 더욱 더 열심히 살도록 하겠습니다. 감사합니다, 어머님, 아버님. 또한 내 인생 최고의 조력자, 항상 남의 편이 아닌 나의 편인 사랑하는 남편 이석재 대표께도 감사의 말을 전합니다. 당신이 없었다면 지금의 영광도 저도 없었을 것입니다. 감사하고 사랑합니다. 사랑하는 가족 맹호진, 맹호빈 오빠와 새언니 김민정 감사합니다. 저의 새로운 가족이 되어준 큰 아주버님 가족과 둘째 아주버님, 항상 지켜봐 주시고 응원해 주셔서 감사드립니다. 제가 받은 충만한 사랑, 평생을 걸쳐 베풀도록 하겠습니다.

이 글을 쓰며 몇 번의 눈물을 훔쳤는지 모르겠습니다. 어렵고 힘들었던 마음보다 감사하고 아름다웠던 기억이 제 머릿속에 더 많고 가득하였기에 행복한 눈물을 흘린 것이라 생각합니다. 힘들게 얻은 학위이기에 더욱 더 감사하고 현실에 머무르지 않고 계속 정진할 수 있는 삶을 살도록 노력하겠습니다. 모든 분들께서 저에게 주신 사랑과 도움, 그리고 은혜를 잊지 않고 감사하게 베풀고 살도록 하겠습니다. 감사합니다.

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맹유진 드림

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Abstract

Optical detection of secondary caries around amalgam restorations using quantitative light-induced fluorescence (QLF) technology

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Oral healthcare professionals have been playing an important role in enhancing the oral health-related quality of life of their patients and therefore, the prevalence of dental caries has been decreasing. However, dental caries is still a major oral health problem globally. Secondary caries develops at the tooth-restoration interface, which raises concerns over the optimal tooth restoration. Secondary caries is one of the main reasons for restoration replacement and has been described as a tooth death spiral that continually promotes overtreatment. Thus, it is particularly important to prevent and manage the restored teeth before the development of secondary caries.

Secondary caries lesions start from the tooth-restoration interface and progress along the restoration wall, and therefore, are normally detected only when progressed or cavitated. Visual-tactile examination and bitewing radiography are widely used in dental field; however, confounding factors such as corrosion, discoloration, size of the ditch, microleakage, and radiopacity associated with the use of several amalgam restorations present difficulties in the visualization and evaluation of secondary caries. Quantitative light-induced fluorescence (QLF) is an optical technology that uses a 405 nm wavelength of light for detecting carious lesions using the autofluorescence emitted from teeth. QLF technology not only helps to visualize early carious lesions appearing as fluorescence loss but also measures the level of bacterial involvement as red fluorescence. This study aimed to assess the fluorescent characteristics of secondary carious lesions around amalgam restorations using new QLF evaluation methods.

A total of 85 extracted teeth having amalgam restorations were included in this study after excluding damaged teeth and pulpally exposed teeth. All tooth specimens were randomly numbered and were visually examined using the International Caries Detection and Assessment System – Caries Around Restorations and Sealants (ICDAS-CARS) criteria. QLF-D images were captured from the occlusal aspects of all tooth specimens by blocking the ambient light and the acquired images were quantitatively analyzed using two proprietary software. A new QLF evaluation index using the operational definition was developed based on the common fluorescent characteristics emitted from the surroundings of amalgam restoration following which the severity of secondary caries in all amalgam restorations was histologically examined.

The new SC01 software could significantly distinguish the severity of secondary caries based on the quantitative analysis of QLF images and the associated fluorescence values

($P < 0.01$). The SC01 software showed significant lower fluorescence reduction than the conventional QA2 software owing to its analysis function by excluding the amalgam restoration area ($P < 0.01$). Red fluorescence values (ΔR and ΔR_{max}) showed a strong correlation with histology and helped in significantly distinguishing the carious lesions based on the severity of secondary caries ($P < 0.05$). A new QLF score for secondary caries (QS-SC) index for the evaluation of secondary caries by appropriately defining the fluorescence information was developed based on study findings. QLF technology including both the QS-SC index and QLF quantitative analysis showed excellent diagnostic performance in distinguishing secondary caries in enamel and dentin.

The QLF technology aided in distinguishing secondary caries around amalgam restorations not only by the QLF quantitative analysis method but also by the new evaluation index. Therefore, QLF technology could be a promising tool to evaluate secondary caries quantitatively and intuitively around amalgam restorations on a regular basis.

Keywords: secondary caries, recurrent caries, amalgam restoration, red fluorescence, quantitative light-induced fluorescence (QLF)

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I. INTRODUCTION

Dental restoration is a treatment aimed at restoring the function of teeth by replacing the hard tissue damaged by dental caries or trauma. However, it is not permanent, as carious lesions may recur in case of defects or poorly managed restorations. Lesions that occur at

the periphery of the pre-existing dental restorations are called secondary caries or recurrent caries (Kidd, 2001). Secondary caries are largely divided into two types: the outer lesions and the wall lesions. The outer lesion is a carious lesion caused by the deposition of dental plaque adjacent to the restoration, similar to a primary carious lesion. On the other hand, the inner wall lesion refers to a carious lesion in the gap between the inner wall of the tooth and the restoration, which is caused by a defect in the restoration such as microleakage (Brouwer et al., 2016; Kidd et al., 1992).

In recent years, the prevalence of dental caries has been decreasing with increasing interest in oral care and enhanced quality of life. However, the occurrence of caries is still a heavy burden worldwide. Particularly, secondary caries are of increasing interest, as they occur on teeth that have already been treated. According to previous studies, secondary caries were present in 20% of the population and in 3.6% of all restorations (Nedeljkovic et al., 2020). In addition, secondary caries are reported to be the main reason for restoration replacement. Among the pre-existing restorations, 57.3% were replaced restorations, more than 42.7% placed new restorations, and the rate of re-restoration rose from 56% in 1981–1997 to 58.1% in 1998–2018 (Eltahlah et al., 2018). Secondary caries occur around all types of restorations including composite resin and glass ionomer restorations as well as around amalgam restorations that were being used primarily in the past (Fontana and Gonzalez-Cabezas, 2000; Pink et al., 1994). Various factors shorten the retention and lifespan of a restoration, leading to restoration failures such as restoration defects and dropouts (Eltahlah et al., 2018; Mjor et al., 2000). Thus, secondary carious lesions have a

destructive restorative cycle that continues to expand the scope of restorative therapy and may ultimately result in tooth loss (Nedeljkovic et al., 2020; Nuttall and Elderton, 1983).

Characteristically, secondary caries are discovered only when they reach an advanced stage or when cavitation occurs (Kidd, 2001). This finding can be explained by the following process of secondary caries formation. In the early stages, the outer lesion is formed by demineralization of the dental tissue around the restoration. The lesion then expands into the inner wall as it progresses along the gap or along the enamel rods adjacent to the margins of the restoration. Secondary carious lesions that progress to the inner wall finally result in collapse of the superficial layer of the tooth and formation of a cavity. Only at this stage, secondary carious lesions may be observed around the restoration (Mjor and Toffenetti, 2000). Thus, preventive intervention can be a successful method of managing secondary caries. Early detection of secondary caries and establishment of appropriate preventive strategies are extremely important in maintaining the restoration and in reducing the need for replacement (Dionysopoulos et al., 1990).

Visual-tactile examination and bitewing radiography are typically used in the diagnosis of secondary caries. Visual detection of secondary caries is mainly based on the presence of cavities around the restoration, the translucency of the enamel, and the color of dentin and enamel. These factors are disadvantageous due to subjective judgment of the evaluator (Diniz et al., 2016a). Particularly, gray or blue discoloration at the margins and near the gaps in the restoration and residual caries underneath the restoration increase the false-

positive diagnosis, making the definitive diagnosis more difficult (Kidd, 2001; Rudolphy et al., 1995). Tactile examination with a dental probe is also used to evaluate the hardness of the tissue surrounding the restoration and the continuity of the restoration and dental tissues. However, this method is less accurate and focused probing force may damage the tooth surface when the sharp tip of the explorer lodges at the entrance of the fissure (Ekstrand et al., 1987; Merrett and Elderton, 1984).

Bitewing radiography is commonly used to detect secondary caries below invisible restorations. According to previous studies, sensitivity of this method is lower than its specificity (Espelid et al., 1991; Matteson et al., 1989). Moreover, carious lesions are difficult to detect before they are clearly advanced and thus, small lesions are often missed (Matteson et al., 1989). Dentists generally detect secondary caries through radiolucent sites on radiographs. However, in practice, most of the small secondary carious lesions are radiopaque, which reduces the accuracy of the diagnosis. Tin and zinc ions are released from amalgam restorations and these ions penetrate the carious lesions. Consequently, the lesions appear radiopaque (Rudolphy et al., 1993). The routine two-dimensional radiographic image has reduced accuracy due to the overlap of the restorations on the proximal surfaces (Tarim Ertas et al., 2014). Moreover, there is a risk of unnecessary radiation exposure if routine two-dimensional radiography is used frequently (Wenzel, 2004).

Standardization of diagnostic method for the detection of secondary caries is extremely

important, as existing methods have limitations in diagnosing secondary carious lesions in the initial stage (Mjor and Toffenetti, 2000). Hence, the International Caries Detection and Assessment System (ICDAS) was introduced to evaluate early carious lesions. This method is known for its superior ability to detect carious lesions varying in severity from early to advanced (Gimenez et al., 2015). Particularly, outer lesions are similar to primary caries. Hence, it is possible to additionally apply caries around restorations and sealants (CARS) to existing ICDAS (Diniz et al., 2016b). However, very few studies have evaluated secondary caries using ICDAS-CARS. Reportedly, ICDAS-CARS has limitations in detecting inner wall lesions and secondary caries around amalgam restoration are overestimated, necessitating further assessment (Diniz et al., 2016a; Diniz et al., 2016b; Lenzi et al., 2016).

A number of studies have been conducted to objectively evaluate the state of teeth. Among these, optical technology using fluorescence has garnered considerable interest in detecting and quantifying secondary caries around restorations (Ando et al., 2004; Walsh and Shakibaie, 2007). Quantitative light-induced fluorescence (QLF) is a representative technology for quantitative detection of carious lesions through autofluorescence expressed in teeth using light with a wavelength of 405 nm. When the visible blue QLF light is directed toward the teeth, sound enamel expresses green autofluorescence, while early carious lesions appear dark due to scattering of light and loss of fluorescence (Heinrich-Weltzien et al., 2003). In addition, it is known that the wavelength range of QLF is optimal for expressing red fluorescence in dental plaque and carious lesions. This red fluorescence

has been reported to be observed due to protoporphyrin IX produced by metabolism of microorganisms (Lee et al., 2013). Therefore, it is possible to evaluate the initial caries through loss of fluorescence and the involvement of microorganisms in caries progression can be evaluated through red fluorescence. QLF technology can be useful in distinguishing caries from discoloration around the restoration, as it can distinguish cariogenic discoloration through red fluorescence (Lee et al., 2018).

Although studies evaluating various lesions in the oral cavity using QLF technology have been actively conducted, those evaluating secondary caries have been scarce. Previous studies have evaluated secondary carious lesions using information regarding fluorescence loss obtained via QLF technology. Laboratory longitudinal studies evaluating artificial carious lesions induced around sound restorations reported that QLF technology is a useful method for the detection of secondary caries (Gonzalez-Cabezas et al., 2003; Pretty et al., 2003). Previous studies evaluating teeth with secondary carious lesions indicated that QLF technology was effective in detecting initial wall lesions adjacent to resin restorations (Diniz et al., 2016b). However, in case of amalgam restorations, it is difficult to detect secondary caries accurately due to dark fluorescence (Ando et al., 2004; Diniz et al., 2016b; Lenzi et al., 2016). Resin restorations exhibit brighter fluorescence than sound teeth. However, amalgam restorations exhibit dark fluorescence due to the inability of metals to transmit QLF light or due to light being absorbed by the discoloration. Thus, it is difficult to distinguish amalgam restorations from carious lesions that show loss of fluorescence. Therefore, further research is needed regarding secondary caries around amalgam

restorations. However, no studies have evaluated secondary caries using red fluorescence information obtained from QLF technology.

The present study aimed to assess the fluorescence properties of secondary carious lesions around amalgam restorations using new evaluation methods involving QLF technology, which can evaluate carious lesions using red fluorescence as well as the loss of autofluorescence emitted from teeth. We confirmed the diagnostic performance of QLF technology for detecting secondary carious lesions around amalgam restorations.

First, we aimed to explore new quantitative fluorescence analysis methods and fluorescence information to overcome the limitations of the existing methods in analyzing secondary caries around amalgam restorations using QLF technology.

Second, we also tried to develop a new index through operational definition by using the red fluorescence information obtained from objective quantitative analysis. Additionally, we aimed to evaluate the diagnostic ability of the new quantitative fluorescence analysis method and QLF evaluation index in the diagnosis of secondary caries.

II. MATERIALS AND METHODS

2.1. Research materials

2.1.1. Collection of teeth

This study was approved by the Institutional Review Board for Clinical Research of the Yonsei dental hospital (IRB No. 2-2017-0044). Tooth collection was conducted of patients over 18 who visited Yonsei university dental hospital, and the patient received an explanation of the purpose and the method of study before tooth extraction. Among these, teeth extracted from participants who showed voluntary participation and signed an informed consent were collected.

A total of 85 amalgam restored teeth were finally used in this study by selecting only the teeth without damage or pulp exposure among the collected teeth. After extraction, the tooth was immediately placed in a plastic container blocking external light to prevent photobleaching of the fluorescence (Hope et al., 2011), and stored frozen at −20℃ to minimize changes in autofluorescence of tooth (Francescut et al., 2006).

2.1.2. Preparation of tooth samples

Prior to the preparation of specimens, the attachments, exogenous stains, and calculus of the teeth were removed with a manual scaler, and the dental plaque on occlusal surface was removed using the toothbrush. In order to maintain the same height of all specimens during image acquisition, the areas over 1.5 cm from the peak of the occlusal cusps of the cleaned teeth were cut using diamond discs (NTI-Kahla GmbH, Germany) and low-speed handpieces (Lasungmedice, Korea). The cut specimens were then placed on an acrylic block (20 × 12 × 8 mm) with a 9 mm diameter hole and fixed perpendicularly on the ground using resin for orthodontic appliance (Ortho-Jet, Lang Dental Mfg. Co., Inc., USA). Tooth specimens were stored without contact with wet gauze placed underneath the storage container to prevent dehydration and to maintain 100% humidity before use in this experiment (Diniz et al., 2016b).

2.1.3. Acquisition of QLF images

Tooth specimens were dried by removing the moisture on occlusal surface with cotton pellets for image acquisition. Then, white- and fluorescent-light images of all specimens were taken by QLF-D (Biluminator™, Inspektor Research Systems BV, Amsterdam, The Netherlands) with blocking the external light. At this time, the occlusal surface of specimen was positioned to be horizontal to the ground, and then QLF-D was taken to be perpendicular to the occlusal surface (Figure 1).

Table 1. Image acquisitioning condition of QLF-D

	White-light	Fluorescent-light
Shutter speed	1/30 s	1/10 s
Aperture value	16.0	10.0
ISO speed	1600	1600
Pixel size	2592×1728	2592×1728

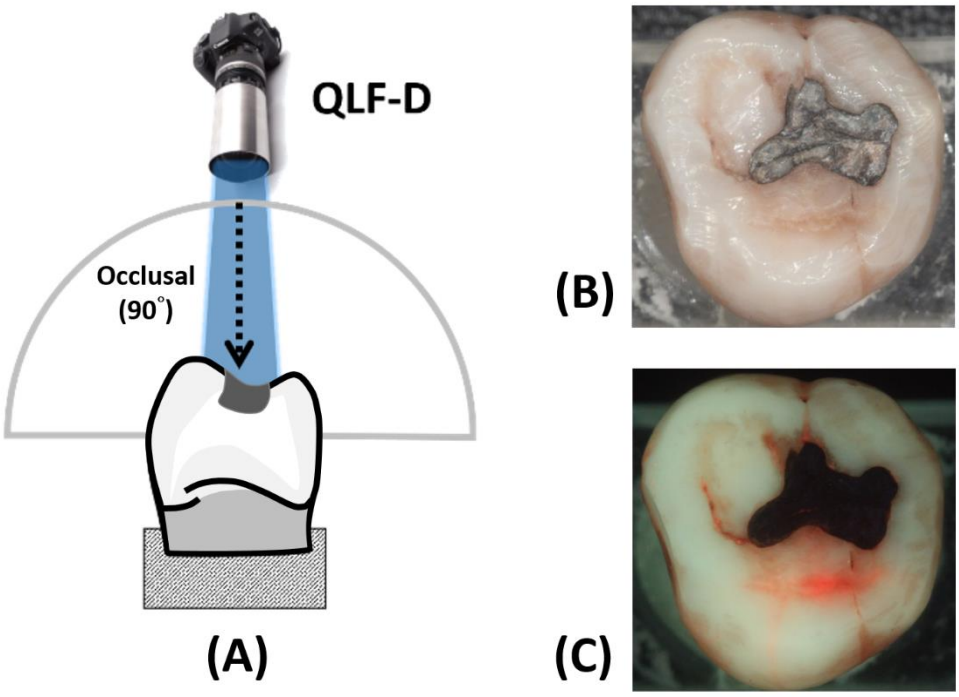


Figure 1. QLF-D image taking position (A) and the obtained images taken under different lights (B: White-light, C: Fluorescent-light).

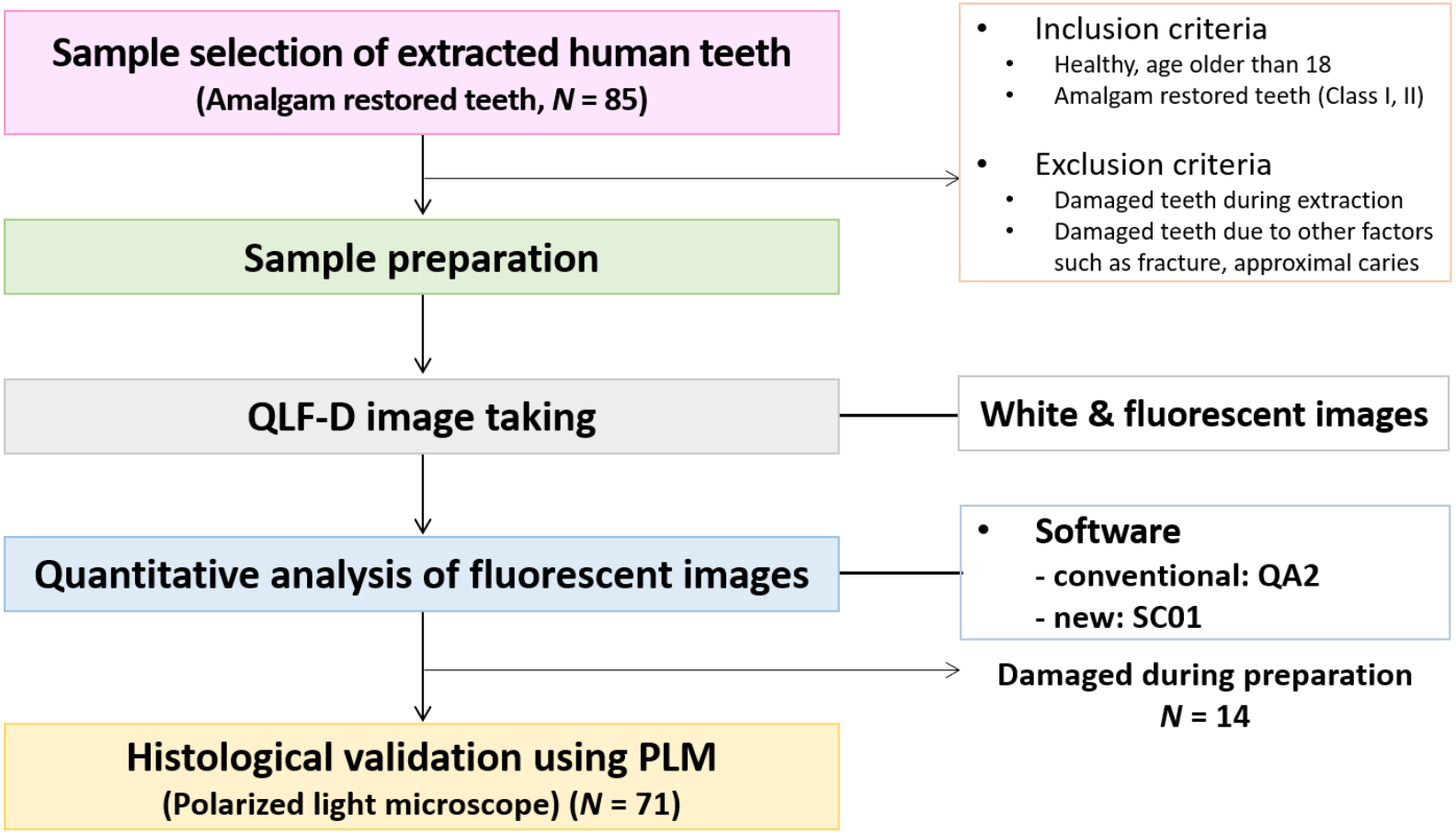


Figure 2. Flow chart of study 1 procedure

2.2. Investigation of a new analysis method for secondary caries

2.2.1. Quantitative analysis of secondary caries

Quantitative fluorescence analysis of all tooth specimens was performed using fluorescence images and analysis software of QLF system. The mineral content of lesions around the restoration was calculated as ΔF [%], which shows reduced fluorescence compared to normal enamel. Discoloration and bacterial metabolites were calculated as ΔR [%], indicating an increase in red fluorescence. Since these two variables (ΔF [%] and ΔR [%]) are average values, the maximum values (ΔF_{max} [%] and ΔR_{max} [%]) reflecting the fluorescence information of the deepest region were also calculated.

2.2.2. Conventional analysis method for quantifying secondary caries

Typically, QA2 software (v1.25, Inspektor Research Systems BV), which is provided with QLF technology, is used to calculate quantitative fluorescence values of teeth. An analysis patch ($Patch_{Lesion}$) was assigned to the area of interest in the fluorescence image using an initial white spot analysis, which is a main function of the software. At this time, the lines of the $Patch_{Lesion}$ were connected along regions where the autofluorescence color of the sound enamel tissue around the restoration was constant (Figure 3). Then, the calculated quantitative variables (ΔF [%], ΔR [%], ΔF_{max} [%] and ΔR_{max} [%]) were used in this study.

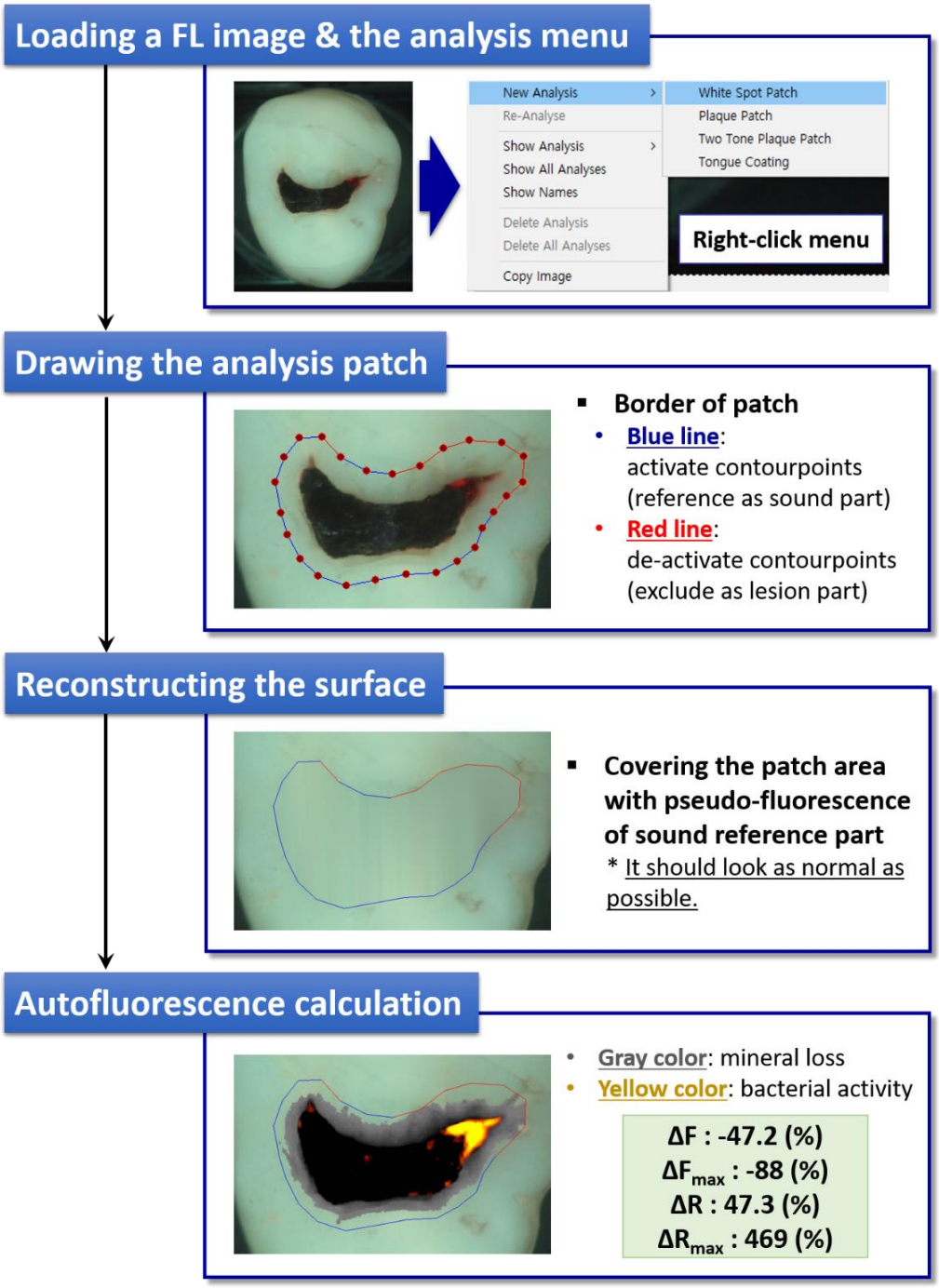


Figure 3. Conventional QLF software (QA2 v1.25) analyzing process

2.2.3 New analysis method for quantifying secondary caries

Existing QLF software calculates fluorescence parameters by including the restoration within the region of interest. This software has a disadvantage that the metal restoration included in the analysis patch affects the level of quantitative values. This is because metal restorations that do not express fluorescence, such as amalgam, appear dark and discoloration by the metal material is also relatively dark compared to sound teeth. To overcome this limitation, a manufacturer has developed the software (SC01 v1.0.0.1, Inspektor Research Systems BV) to evaluate secondary caries by excluding metal restorations. First, the restoration patch ($Patch_{Amalgam}$) was drawn and stored along the margin of the amalgam restoration seen in the magnified white-light image in order to exclude the restoration part and was then copied to the fluorescence image. After that, the patch of the lesion ($Patch_{Lesion}$) drawn in the existing software was applied to the fluorescence image and then analyzed (Figure 4). The calculated quantitative variables (ΔF [%], ΔR [%], ΔF_{max} [%] and ΔR_{max} [%]) reflected the information excluding the fluorescence value of the restoration patch ($Patch_{Amalgam}$) from the entire analysis patch ($Patch_{Lesion}$). The differences between the two analytic software used in this study are presented in Figure 5.

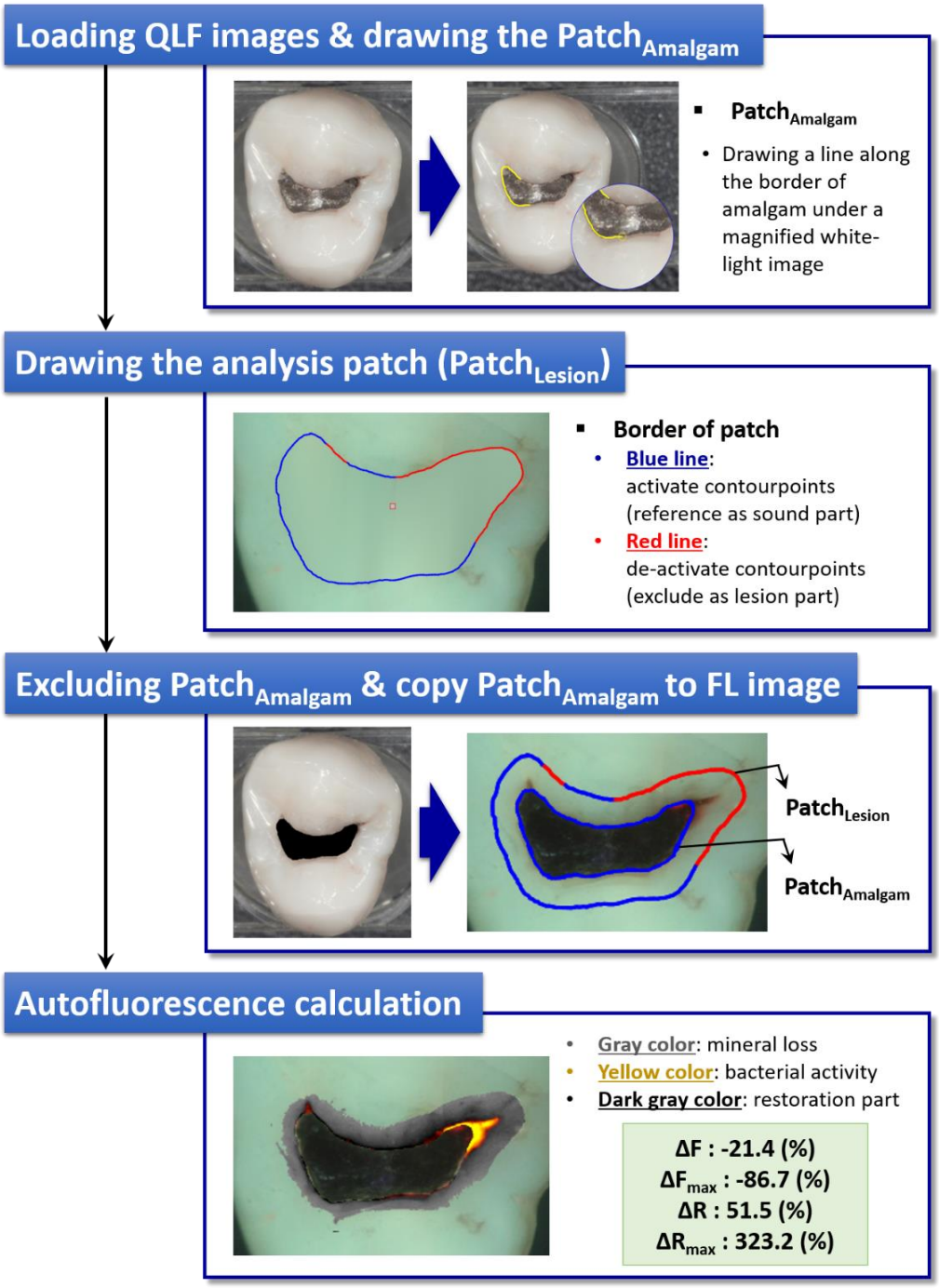


Figure 4. New QLF software (SC01 v1.0.0.1) analyzing process

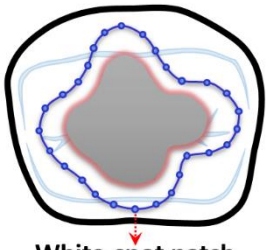
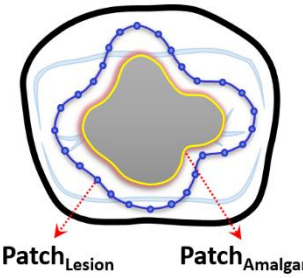
	Conventional QA2 software	New SC01 software
Patch image		
Type of patch	1 White-spot patch (analyzing caries lesions)	1 Patch$_{Lesion}$ (sound reference) 1 Patch$_{Amalgam}$ (restoration)
Common characteristic	Can detect both fluorescence loss and red fluorescence	
Weakness	**Overestimation due to dark restoration and stain**	Time-consuming
Strength	-	**Can exclude restoration part on the image analysis**

Figure 5. Different characteristics between conventional (QA2) and new (SC01) QLF analysis software

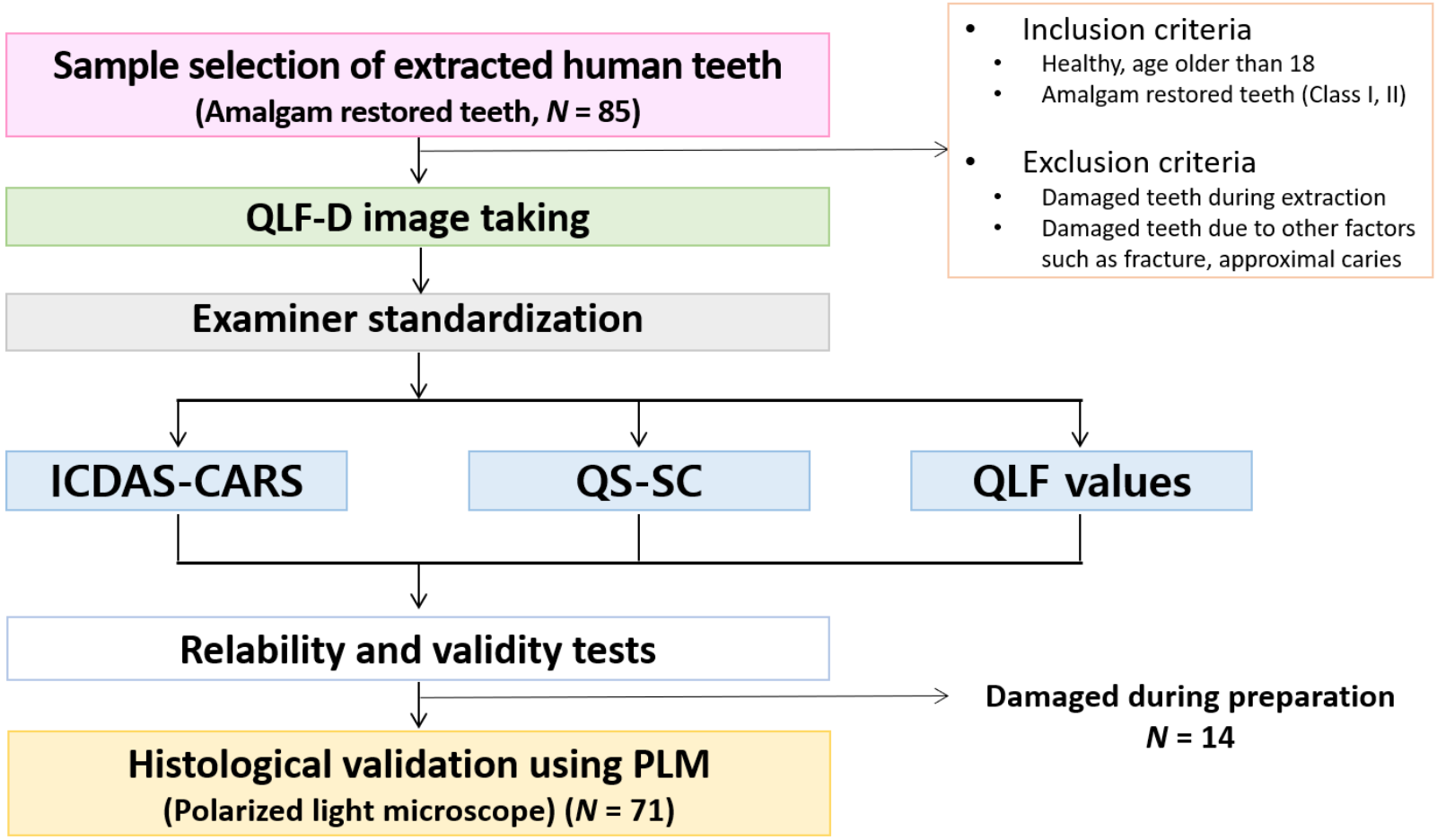
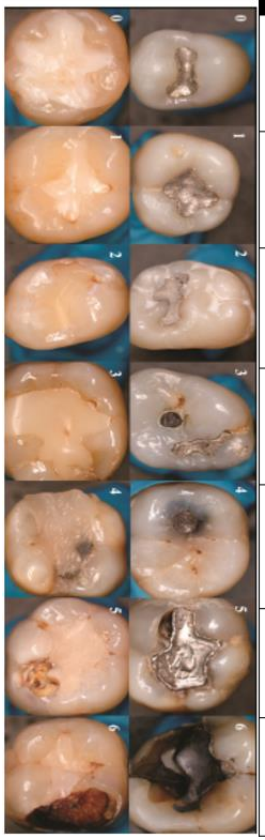


Figure 6. Flow chart of study 2 procedure

2.3. Investigation of a new evaluation method for secondary caries

2.3.1. Visual inspection using ICDAS-CARS

Prior to the evaluation, a random identification number was assigned to a total of 85 prepared tooth specimens, and the examiner performed a visual examination by randomly selecting the specimens. In the visual examination, the CARS (Caries around Restorations and Sealants) criteria based on ICDAS (International Caries Detection and Assessment System) which can distinguish early caries lesions was used (Ismail et al., 2007). Prior to the main evaluation, the examiner was trained through the e-learning program provided on the ICDAS web site (www.icdas.org), fully acquired the details through the examples in standard manual and the diagnostic decision flow chart (Figure 7). In case of ambiguous tooth specimens, two researchers observed teeth together and assigned the final score after discussion.



SCORE	DESCRIPTION
0	Sound tooth surface
1	First visual change in enamel
2	Distinct visual change in enamel/dentin adjacent to a restoration margin
3	Carious defect <0.5 mm with the signs of score 2
4	Marginal caries in enamel /dentin/cementum adjacent to a restoration with underlying dark shadow from dentin
5	Distinct cavity adjacent to a restoration
6	Extensive distinct cavity with visible dentin

Figure 7. ICDAS-CARS (International Caries Detection and Assessment System for Caries Associated with Restorations and Sealants) criteria on occlusal aspect

2.3.2. Development of a new QLF classification system

The new QLF index was developed in this study to reduce the time required for quantitative analysis in the actual clinical field and to evaluate secondary caries intuitively. Firstly, QLF experts classified all QLF images according to the histological scores to make groups of the severity of secondary caries. Subsequently, fluorescence characteristics (fluorescence reduction and red fluorescence) and color changes (white/brown spot and brown/blue discoloration), which were seen in images of each histological group, were recorded. The QLF index was categorized into four stages (score 0-3) according to the operational definition made on a basis of the common characteristics expressed in each histological group. Finally, this evaluation index was named QS-SC (QLF score for secondary caries), and a representative examiner evaluated all tooth specimens in an independent environment (Figure 8).

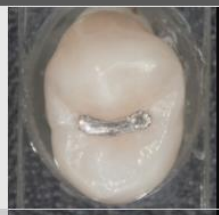
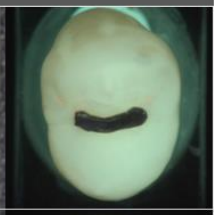
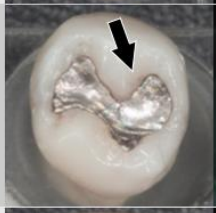
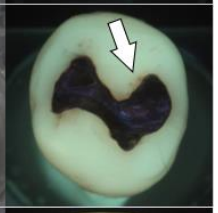
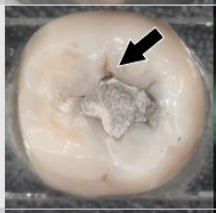
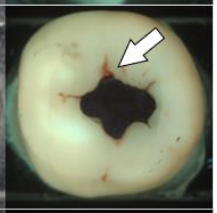
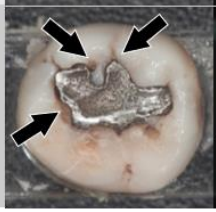
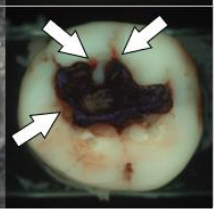
SCORE	Description		Representative images	
	White-light image	Fluorescence image	WLI	FLI
0 Sound	**No evidence of caries**	No fluorescence loss and no red fluorescence around restoration		
1 Non-cariogenic discoloration	**Brown** or **dark discolored margins**	Fluorescence loss and no red fluorescence around restoration at stained margins		
2 Enamel SC lesion	**Distinct white/brown spots discoloration** and **defective margin**	Fluorescence loss and red fluorescence around restoration at margins		
3 Dentin SC lesion	**Distinct dark shadow** around margins and, **defective margin** or **distinct cavity**	Vivid red fluorescence around restoration at margins		

Figure 8. QS-SC (QLF score for secondary caries) criteria on occlusal aspects

2.3.3. Reliability and reproducibility of all evaluation methods

In evaluating secondary caries, reproducibility tests for newly developed fluorescence evaluation methods were additionally performed. The same examiner visually reevaluated all secondary caries specimens using ICDAS-CARS of study 1 and QS-SC of study 2, and quantitatively recalculated fluorescence values using SC01 software of study 1. At this time, the examiner performed the reevaluation in the same environment as the first test. It is generally recommended that the wash-out period for retests be performed 2-4 weeks after the first test. Therefore, in this study, reevaluation was performed 2 weeks after the first test point.

2.4. Histological evaluation using polarized-light microscope (PLM)

After completing all evaluations, cutting sites were selected for the histological evaluation of cross sections of the tooth specimens. Two researchers discussed and designated the site of the most severe secondary caries lesion as a cutting site through visual inspection and fluorescence images of each tooth specimens. Before cutting the teeth, the crown of all specimens was buried with resin (Ortho-Jet, Lang Dental Mfg. Co., Inc., USA) to prevent the amalgam restoration from falling out during the cutting process. The area of interest was then vertically cut to a thickness of 200-250 μm using low speed fine diamond cutter (TechCut 4™, Allied High Tech Products, Inc., California, USA). Thin sections were photographed using QLF-D (Biluminator™, Inspektor Research systems BV, Amsterdam, The Netherlands) for further fluorescence observation. The cut surface was then polished to a thickness of about 150 μm with 800 grit abrasive paper (SiC Sand Paper, R&B Inc., Daejeon, Korea) and placed on a slide glass. Lastly, the slides were photographed under a polarized-light microscope (PLM, CX31-P, Olympus Co., Japan) at a magnification of 40X. The PLM images were observed histologically (Figure 9), and scored according to the severity of caries lesion as Downer's criteria (Downer, 1975) (Table 2).

Table 2. The criteria of histology according to the lesion severity (Downer's criteria)

Score	Description
S	No caries (sound)
D1	Caries lesion limited to the outer half (50%) of the enamel layer
D2	Caries extending into the inner half of the enamel
D3	Caries limited to the outer half of the dentin
D4	Caries involving the inner half of the dentin

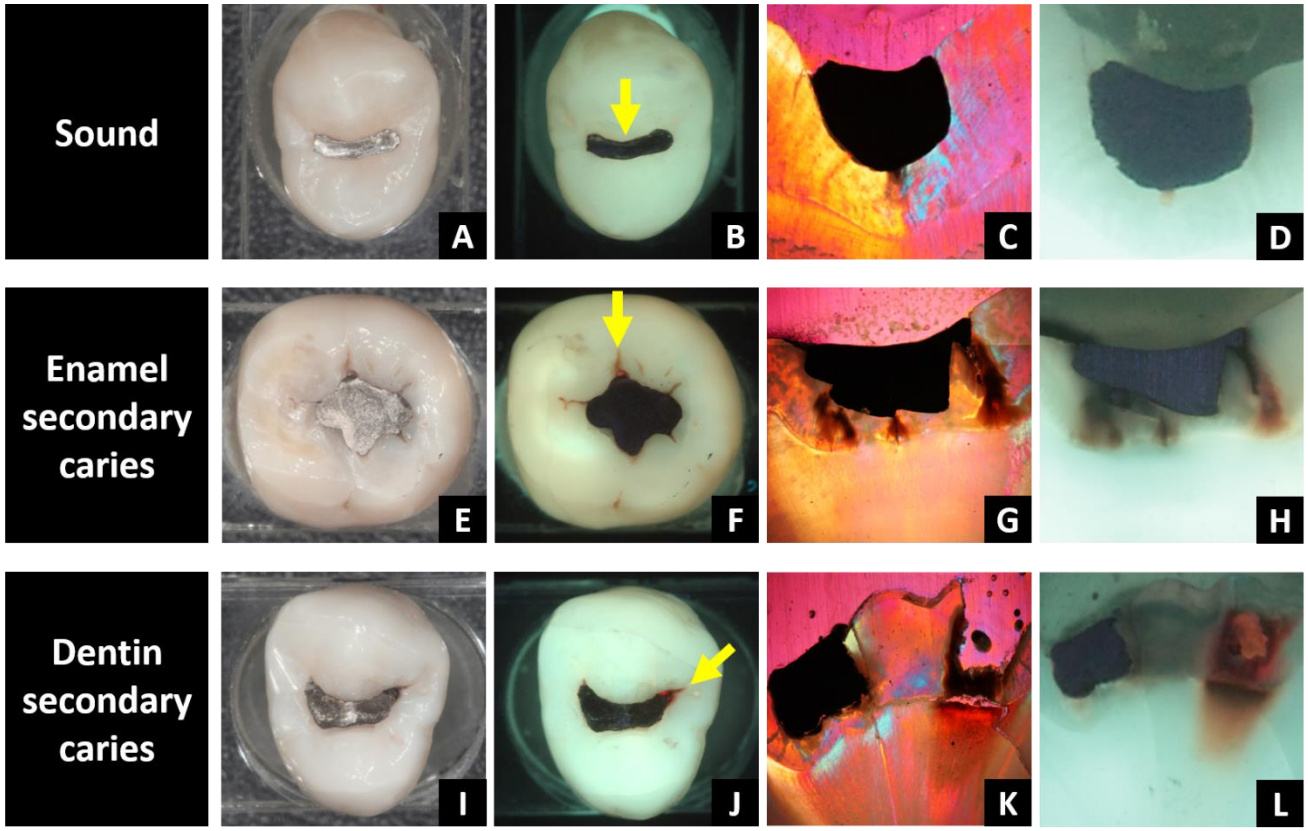


Figure 9. Representative images of secondary caries under white-light of QLF-D (A, E, I), blue-light illumination (B, F, J), the respective Polarized Light Micrographs (PLM, magnification = 40X) (C, G, K), and the respective hemisectioning fluorescent images (D, H, L). Yellow arrows indicate the direction of section.

2.5. Statistical analysis

All the statistical analyses were performed using the statistical package for the social science (SPSS Statistics ver. 23.0, Chicago, IL, USA) at a significance level of 0.05.

The histological results were set to the gold standard, and then statistical analysis was performed.

2.5.1. Study 1: comparison of fluorescence values obtained from conventional and new QLF analysis software

Statistical analysis was performed on four variables, ΔF [%], ΔR [%], ΔF_{max} [%], and ΔR_{max} [%], calculated by the quantitative analysis software of secondary caries. Kruskal-Wallis tests were performed to confirm the significant differences in quantitative fluorescence variables for each severity of secondary caries according to histological criteria. The median values of fluorescence intensity between each caries groups were compared using the Mann-Whitney post hoc test. In addition, the differences between the two quantitative analysis software methods used in this study were confirmed by independent t-test.

2.5.2. Study 2: evaluation of reliability and diagnostic performance of QLF methods for secondary caries

Intra-examiner reproducibility of all secondary caries evaluation methods in this study was assessed using the intra-class correlation coefficient (ICC) and compared. In addition, the correlations between each fluorescence variables and evaluation methods were analyzed using Spearman's rank correlation test. From this, optimal quantitative fluorescence variables available for quantitative analysis of secondary caries were determined. Significant differences in quantitative fluorescence variables for each severity of secondary caries according to QS-SC index were assessed using the Kruskal-Wallis test and Mann-Whitney test.

The sensitivity and specificity of all evaluation methods were calculated at the cut-offs of enamel (D1) and dentin (D3) histological level. At the D1 threshold, both enamel and dentin secondary caries were considered to be disease positive, and at the D3 threshold, dentin secondary caries was established as a disease, and sound teeth and enamel secondary caries were set as disease-free (disease negative). In ICDAS-CARS, the cutoff point of D1 was between 0 and 1, that of D3 was between 2 and 3, and the cutoff point of D1 in QS-SC was between 1 and 2, and that of D3 was between 2 and 3. Since ΔF and ΔR of the QLF quantitative fluorescence analysis do not have an available scale for classifying secondary caries, the cutoff value of the point where sum of sensitivity and specificity is the maximum was calculated (MedCalc v19.2, Ostend, Belgium).

Finally, receiver operating characteristics (ROC) statistics were performed to evaluate the degree of agreement between each evaluation method and histological results, and the area under the ROC curve (AUROC) was calculated.

III. RESULTS

A total of 71 teeth (23 premolars and 48 molars) among the 85 teeth were used for the final analysis. The 14 excluded teeth could not be evaluated because dental tissue was damaged due to severe caries or the amalgam restoration was dropped together with the dental tissue during the preparation process for histological evaluation.

3.1. Severity distribution of secondary caries lesions

The histological distribution of tooth specimens used in this study showed that 14 of the total 71 teeth were sound. 36 teeth had enamel secondary caries, and the remaining 21 teeth were dentin secondary caries teeth.

3.2. Distribution of fluorescence values calculated by conventional and new quantitative analysis methods

3.2.1. The decrease in fluorescence of secondary caries lesions

When the secondary caries lesions were classified by severity according to histological criteria, the degree of demineralization by secondary caries was evaluated as the absolute values of the average fluorescence loss (ΔF) and the maximum fluorescence loss (ΔF_{max}). On a histological evaluation of the cross sections of the tooth, the deeper the lesion depth, the higher all fluorescence loss values. As the depth of the lesion was deeper according to the histological evaluation, the absolute values of the fluorescence loss variables increased except for the |ΔF| value of the QA2 software (Figure 10). As the severity of secondary caries increased, |ΔF| and $|\Delta F_{max}|$ calculated by SC01 software were increased with significant differences for each severity group ($P < 0.05$). On the other hand, |ΔF| and $|\Delta F_{max}|$ of QA2 software showed no significant differences according to the severity of secondary caries ($P = 0.871$ and $P = 0.117$, respectively). When comparing the differences between the software, there was no significant difference in $|\Delta F_{max}|$ value in dentin secondary caries($P = 0.082$), but statistically significant differences in other |ΔF| and $|\Delta F_{max}|$ values ($P < 0.01$, Table 3). Figure 11 shows the fluorescence values of the representative images generated by both software.

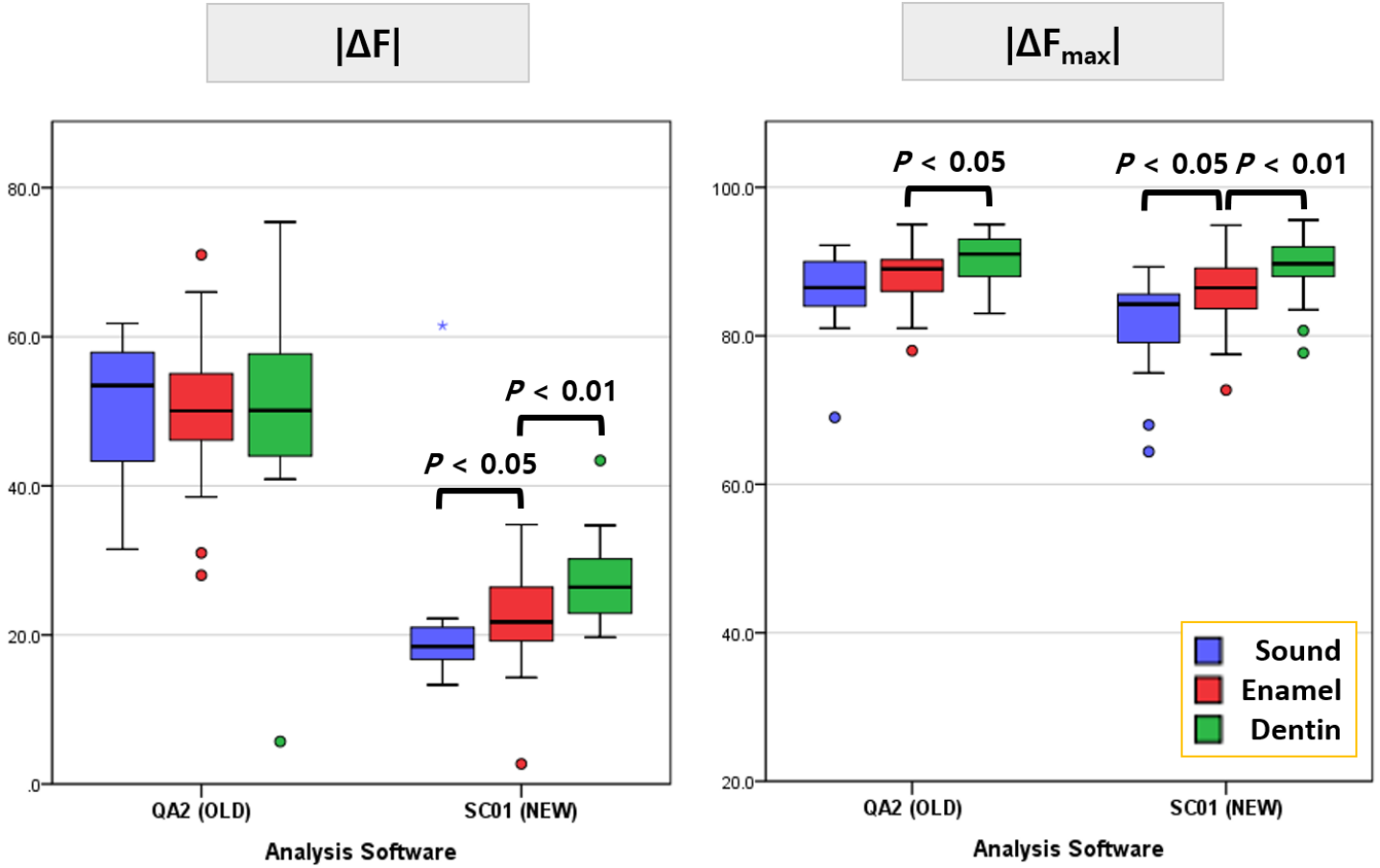


Figure 10. Comparison of |ΔF| and $|\Delta F_{max}|$ values from conventional (QA2) and new (SC01) QLF analysis software according to the histological results of secondary caries. Significant differences between groups by the Kruskal-Wallis test and Mann-Whitney test with Bonferroni *post hoc* correction.

Table 3. Distribution of ΔF values (fluorescence loss) of secondary caries calculated by conventional and new analysis software according to the histology

Histology		|ΔF| (%)		*P*	$\|\Delta F_{max}\|$ (%)		*P*
	N	QA2	SC01		QA2	SC01	
Sound	14	53.45[a] (42.88, 58.58)	18.45[a] (16.23, 21.23)	0.001	86.50[a] (83.50, 90.00)	84.25[a] (78.08, 85.73)	0.002
Enamel	36	50.05[a] (45.73, 55.23)	21.75[b] (19.15, 26.35)	<0.001	89.000[a] (86.00, 90.38)	86.45[b] (82.98, 89.10)	<0.001
Dentin	21	50.10[a] (44.55, 59.20)	26.40[c] (23.55, 30.40)	<0.001	91.000[b] (87.50, 93.00)	89.70[c] (87.50, 92.20)	0.082
P		0.871	<0.001		0.007	<0.001	

Data are median (first, third quartile) values.
QA2, conventional QLF analysis software; SC01, new QLF analysis software only for evaluation of secondary caries.
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.

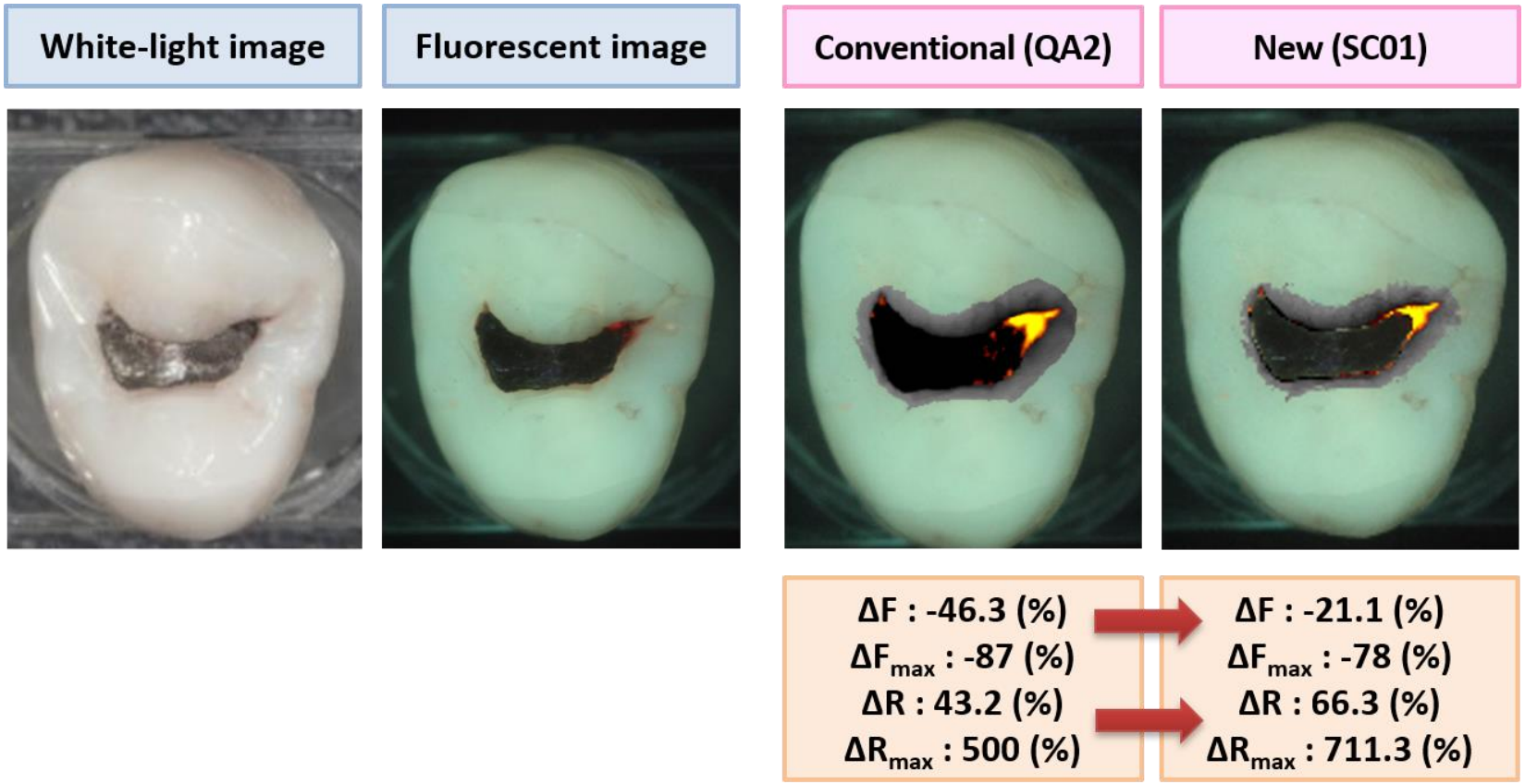


Figure 11. Representative image of different QLF values obtained from conventional (QA2) and new (SC01) QLF software

3.2.2. The increase in red fluorescence of secondary caries lesions

The degree of microbial intervention according to the histological severity of secondary caries was evaluated by the average increased red fluorescence (ΔR) and maximum increased red fluorescence (ΔR_{max}). ΔR and ΔR_{max} values of all software increased as the lesion severity according to the histological evaluation of the cross sections with significant differences, so that all variables were able to distinguish each severity of secondary caries ($P < 0.01$, Figure 12). When comparing the differences between software at all secondary caries severity group, the red fluorescence intensity of enamel and dentin secondary caries between two software was significantly higher in SC01 than QA2 software ($P < 0.01$). There was no difference in red fluorescence intensity between two software in the sound teeth group (Table 4). Figure 11 shows the fluorescence values of the representative images generated by both software.

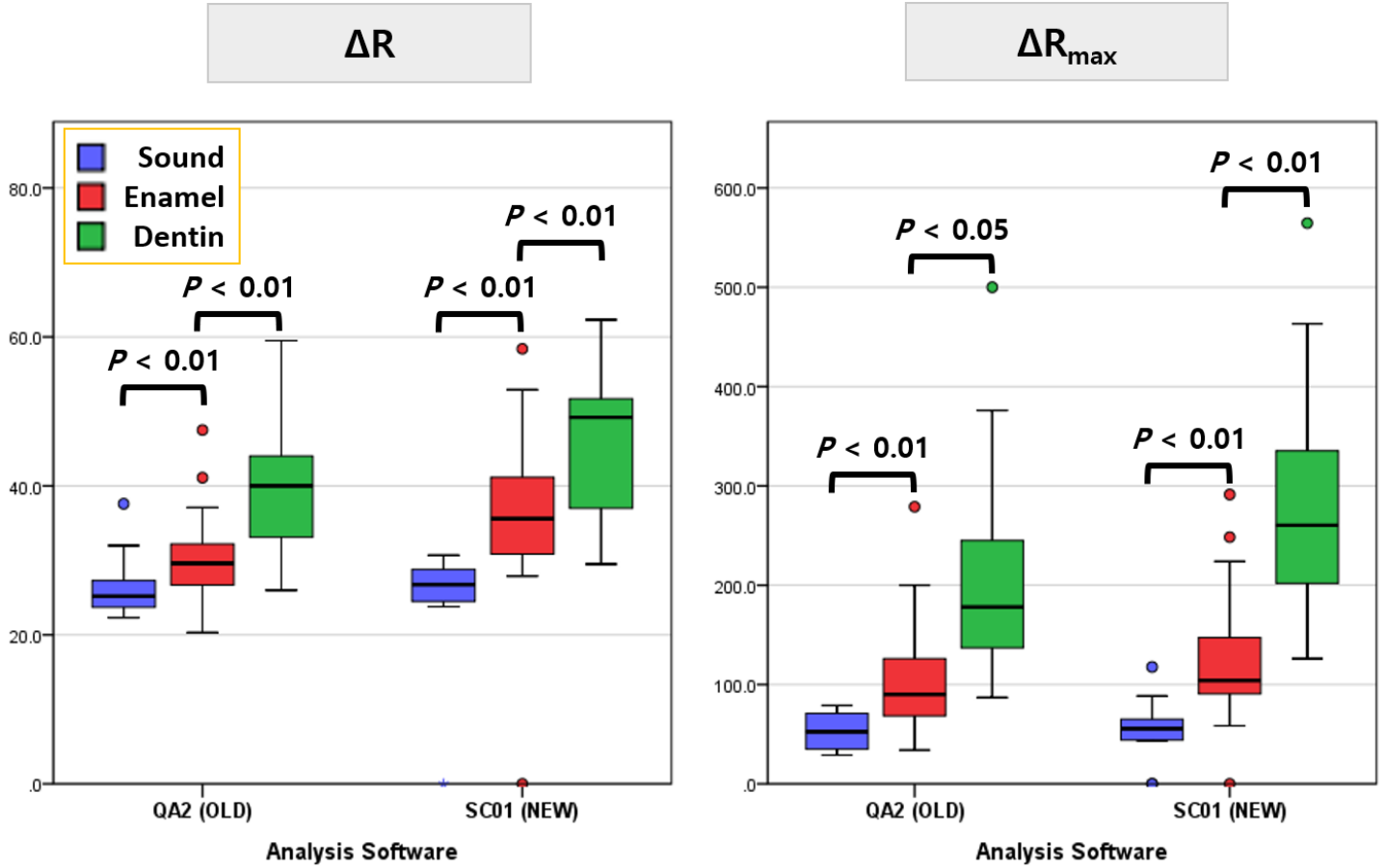


Figure 12. Comparison of ΔR and ΔR_{max} values from conventional (QA2) and new (SC01) QLF analysis software according to the histological results of secondary caries. Significant differences between groups by the Kruskal-Wallis test and Mann-Whitney test with Bonferroni *post hoc* correction.

Table 4. Distribution of ΔR values (red fluorescence) of secondary caries calculated by conventional and new analysis software according to the histology

Histology		ΔR (%)		*P*	ΔR_{max} (%)		*P*
	N	QA2	SC01		QA2	SC01	
Sound	14	25.20[a] (23.50, 27.85)	26.75[a] (24.33, 28.93)	0.802	52.50[a] (34.75, 71.25)	55.60[a] (43.88, 66.98)	0.594
Enamel	36	29.60[b] (26.85, 32.25)	35.25[b] (30.78, 41.18)	<0.001	90.00[b] (71.25, 130.00)	104.00[b] (89.43, 144.68)	<0.001
Dentin	21	40.00[c] (32.25, 44.40)	49.20[c] (38.35, 53.45)	0.001	178.00[c] (128.00, 246.00)	260.30[c] (203.55, 344.25)	<0.001
P		<0.001	<0.001		<0.001	<0.001	

Data are median (first, third quartile) values.
QA2, conventional QLF analysis software; SC01, new QLF analysis software only for evaluation of secondary caries.
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.

3.3 Reproducibility of evaluation methods for secondary caries

3.3.1. Distribution of secondary caries according to ICDAS-CARS

Distribution of tooth specimens according to ICDAS-CARS criteria showed that 5 of 71 teeth were sound, 41 of the enamel secondary caries, and 25 of the dentin secondary caries. Among the enamel secondary caries teeth, 14 were code 1 and 27 were code 2 teeth. Whereas, in dentin secondary caries teeth, 20 were code 3 and 5 were code 4 teeth (Table 5). Compared with histological results, ICDAS-CARS was found to be able to distinguish the severity of secondary caries with a moderate correlation coefficient ($\rho = 0.545$, $P < 0.001$).

3.3.2. Distribution of secondary caries according to QS-SC

According to the distribution of tooth specimens based on QS-SC index, which is the new QLF secondary caries evaluation criteria, 27 of 71 teeth were enamel secondary caries, 24 of dentin secondary caries, and 20 of sound teeth. The number of code 0 teeth with no color and fluorescence changes in the tissue surrounding the restoration were 8, and code 1 restored teeth with noncariogenic discoloration were 12 (Table 5). Each code of QS-SC was found to be able to distinguish the severity of secondary caries with excellent correlation ($\rho = 0.849$, $P < 0.001$). Table 5 is a cross-tabulation showing the distribution of ICDAS-CARS and QS-SC according to histological results.

Table 5. Cross-tabulation for the secondary caries classification methods with the corresponding histology scores

Histological score	ICDAS-CARS					QS-SC				Total
	0	1	2	3	4	0	1	2	3	
S	4	6	4			7	6	1		14
D1	1	3	4	3			6	4	1	11
D2		4	11	9	1	1		19	5	25
D3		1	8	8	4			3	18	21
Total	5	14	27	20	5	8	12	27	24	71

S, sound; D1, demineralization in outer enamel; D2, demineralization in inner enamel; D3, demineralization in outer dentin.
ICDAS-CARS, international caries detection and assessment system for caries around restorations and sealants; QS-SC, QLF score for secondary caries.

3.3.3. Reproducibility of the methods in evaluating secondary caries

Table 6 shows the reproducibility of the examiner in the evaluation of secondary caries performed using various methods. The new QS-SC index and the QLF software analysis method using QLF technology showed ICC values of 0.934 and 0.969, respectively, and indicated almost perfect repeatability ($P < 0.0001$). ICDAS-CARS also showed an excellent ICC of 0.850, but lower than the QLF methods. The reproducibility of all methods was excellent, and it was confirmed that the examiner of this study performed the evaluation with excellent reliability.

Table 6. Intraclass correlation coefficients of intra-examiner reproducibility obtained for the methods in evaluating secondary caries

	ICDAS-CARS	QS-SC	QLF software analysis
ICC	0.850***	0.934***	0.969***
95% CI	0.770-0.904	0.896-0.958	0.961-0.976

ICC, intraclass correlation coefficient; CI, confidence interval.
***P<0.0001

3.4 Validity of all methods for evaluation of secondary caries

3.4.1. Correlations of fluorescence values compared with other evaluation methods

To assess the correlations of quantitative variables obtained from fluorescence analysis, comparison tests between QLF values, ICDAS-CARS, QS-SC index, and histological results were conducted. The $|\Delta F|$ and $|\Delta F_{max}|$ values indicating the absolute values of fluorescence reduction showed moderate positive correlations in all methods ($P < 0.01$). The ΔR and ΔR_{max} values representing the red fluorescence intensity showed strong positive correlations with the histological results and QS-SC index, and the correlation coefficient between ΔR_{max} and QS-SC was 0.806 and the highest ($P < 0.01$, Table 7).

Table 7. Correlation coefficients of QLF values for secondary caries compared with ICDAS-CARS, QS-SC and histology

		ICDAS-CARS	QS-SC	Histology
$\|\Delta F\|$	Spearman's correlation	0.450**	0.548**	0.499**
$\|\Delta F_{max}\|$	Spearman's correlation	0.533**	0.433**	0.458**
ΔR	Spearman's correlation	0.695**	0.800**	0.715**
ΔR_{max}	Spearman's correlation	0.646**	0.806**	0.804**

*$P < 0.05$
**$P < 0.01$

3.4.2. Distribution of ΔR values according to QS-SC criteria

The distribution of average increased red fluorescence (ΔR) and maximum increased red fluorescence (ΔR_{max}) according to the QS-SC index is presented in Figure 13. Both ΔR and ΔR_{max} values were increased with significant differences as the lesion severity according to QS-SC index increased, and it was possible to distinguish each severity of secondary caries ($P < 0.001$, Table 8). The sound group of the QS-SC index means code 0 and code 1, and the teeth of code 0 and code 1 did not show significant differences in both ΔR and ΔR_{max} values ($P = 0.305$ and $P = 0.208$, respectively).

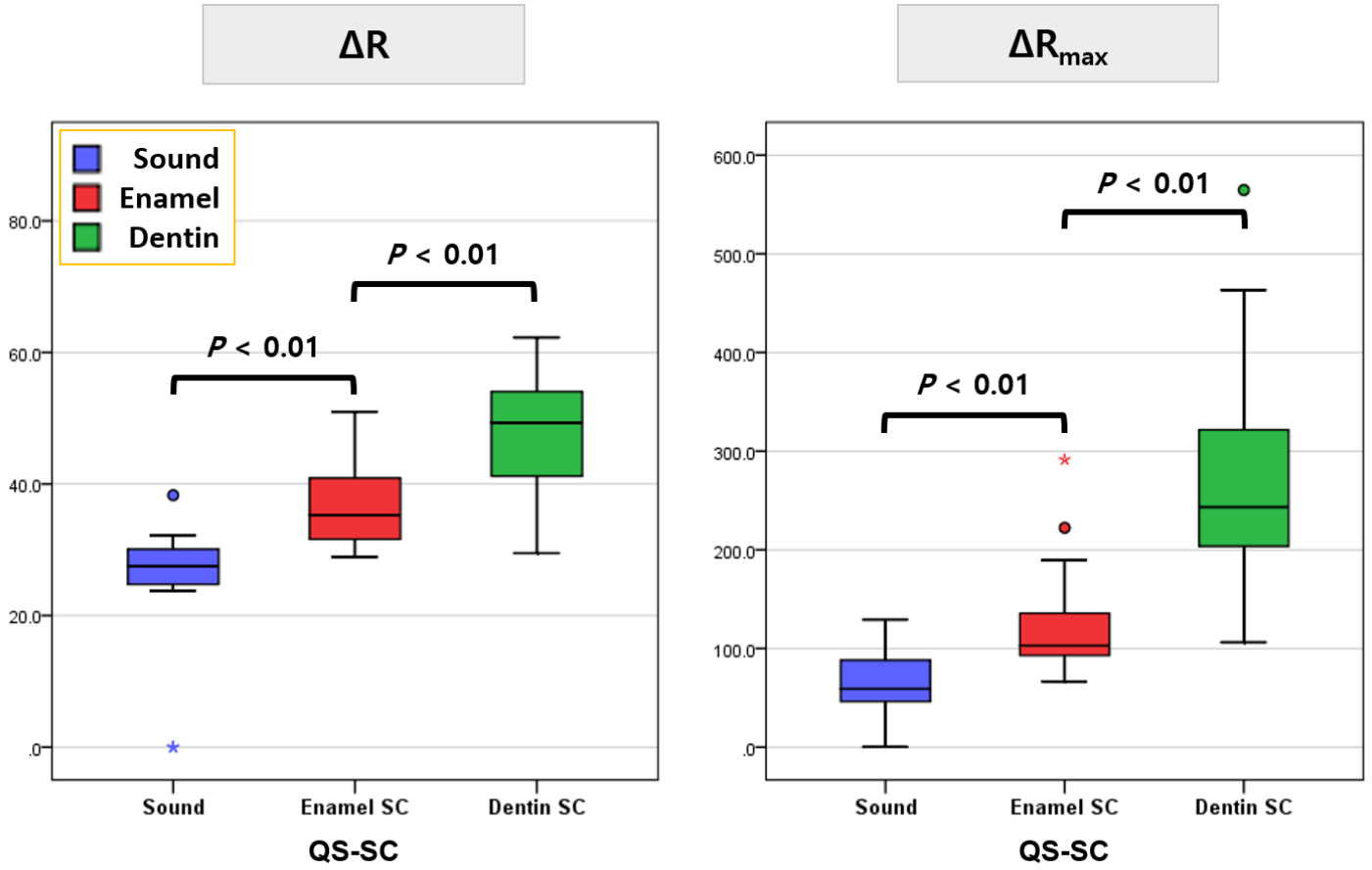


Figure 13. Distribution of ΔR and ΔR_{max} values from new (SC01) QLF analysis software according to the lesion severity of QS-SC (QLF score for secondary caries) criteria. Significant differences between groups by the Kruskal-Wallis test and Mann-Whitney test with Bonferroni *post hoc* correction.

Table 8. Distribution of ΔR values (red fluorescence) of secondary caries calculated by new analysis software according to the lesion severity of QS-SC criteria

QS-SC	*N*	ΔR (%)	ΔR_{max} (%)
Sound (Score 0 and 1)	20	27.55[a] (24.63, 30.40)	59.15[a] (45.13, 88.25)
Enamel SC lesion (Score 2)	27	35.30[b] (31.00, 41.10)	102.90[b] (92.80, 145.30)
Dentin SC lesion (Score 3)	24	49.35[c] (40.45, 54.63)	243.35[c] (202.73, 328.50)
P		<0.001	<0.001

Data are median (first, third quartile) values.
SC, secondary caries; QS-SC, QLF score for secondary caries.
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.

3.4.3. Determination of cut-offs for QLF fluorescence values

Before calculating the sensitivity and specificity of the evaluation methods, the optimal cut-off limits of the quantitative fluorescence method were obtained to distinguish secondary caries lesions according to histological criteria (Table 9). Among the QLF quantitative variables, red fluorescence values (ΔR and ΔR_{max}), which significantly distinguished secondary carious lesions in study 1, were selected. The optimal cut-offs of the ΔR과 ΔR_{max} values were calculated based on histological enamel and dentin lesions (Figure 14 and Figure 15).

Table 9. Optimal cut-off limits of the QLF red fluorescence values for evaluation of secondary caries according to the histology

Histology	ΔR	ΔR_{max}
Sound	≤ 29.4	≤ 72.6
Enamel SC	$29.4 < \Delta R \leq 35.9$	$72.6 < \Delta R_{max} \leq 122.2$
Dentin SC	> 35.9	> 122.2

SC, secondary caries.

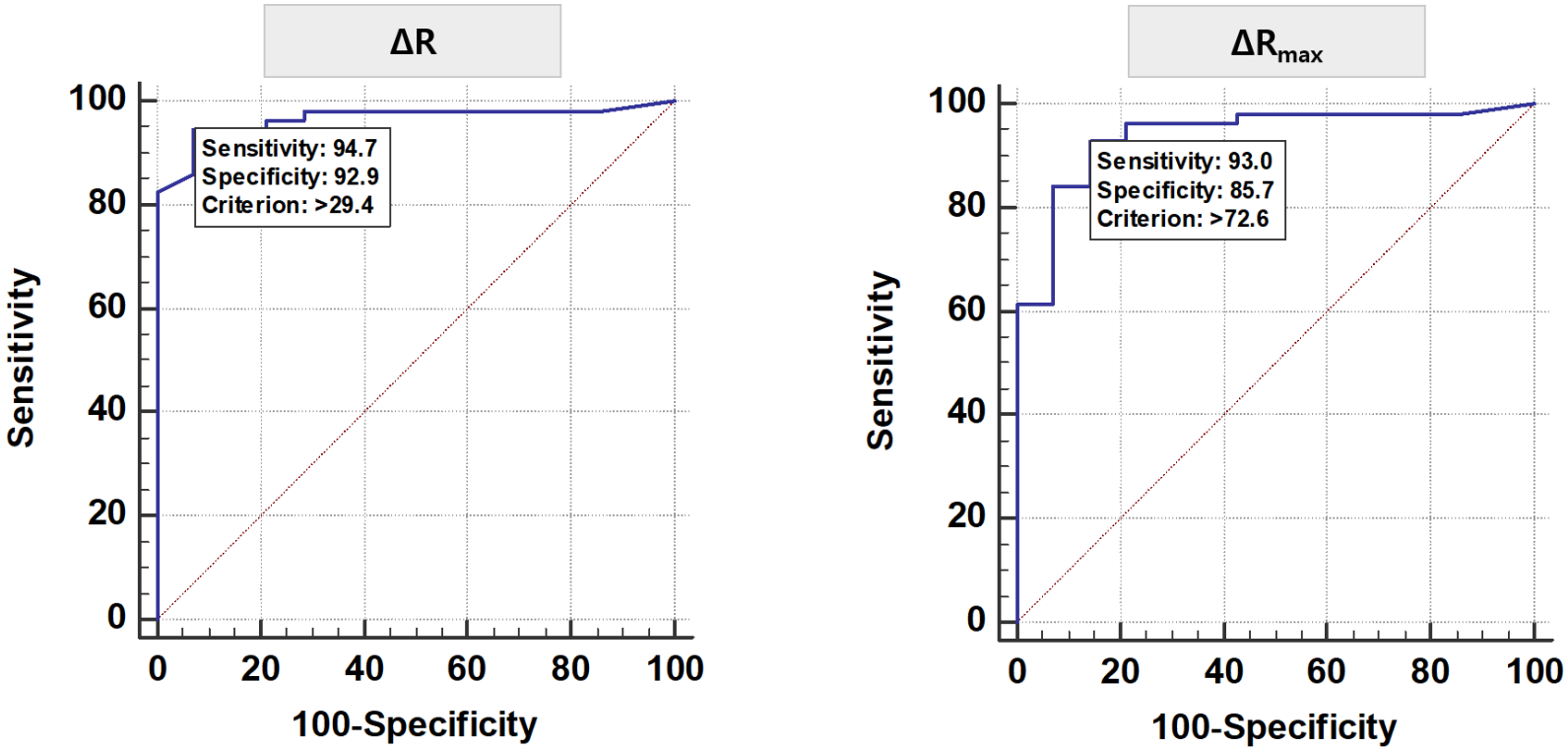


Figure 14. Selected ROC curves of ΔR and ΔR_{max} values at histological enamel caries level

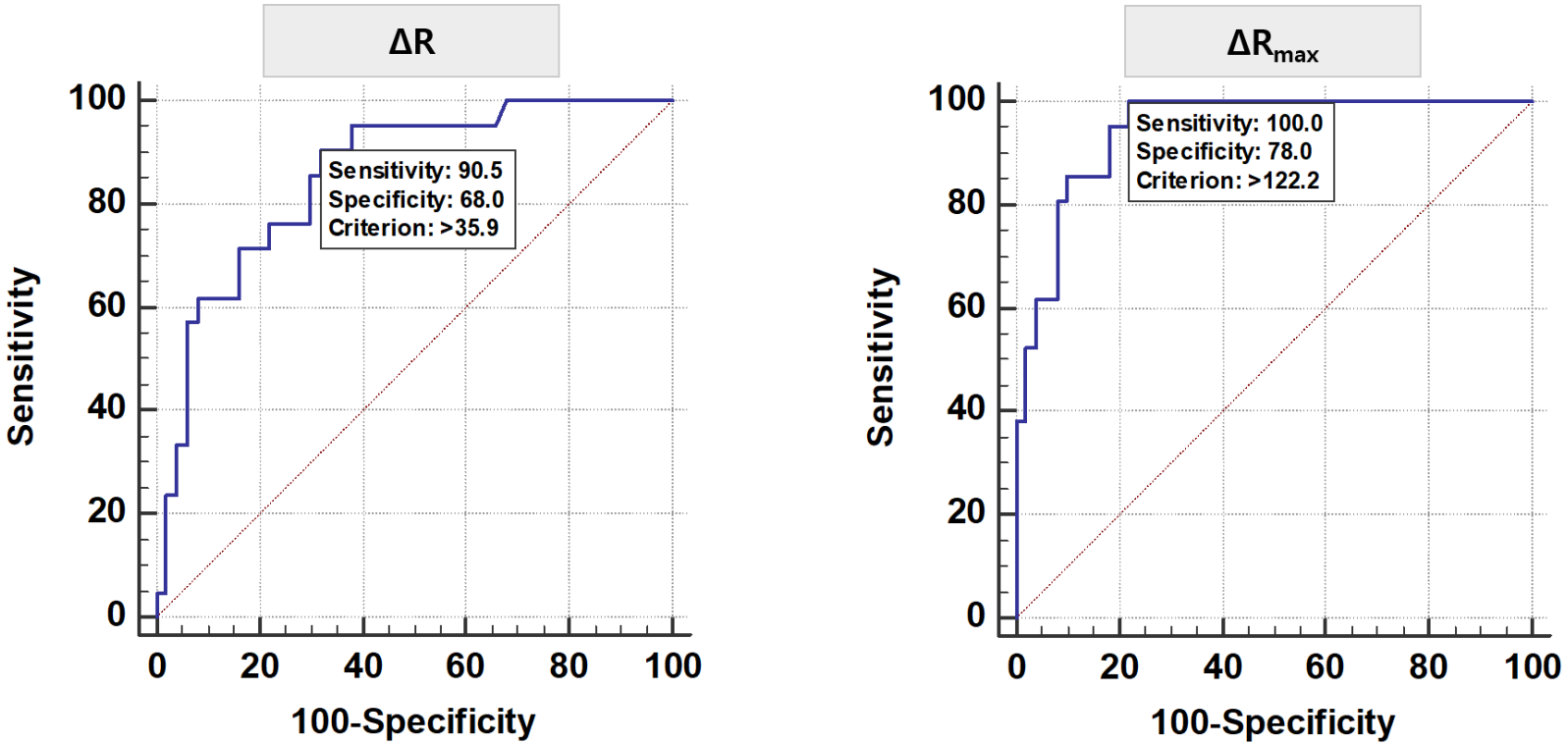


Figure 15. Selected ROC curves of ΔR and ΔR_{max} values at histological dentin caries level

3.4.4. AUC analysis for all evaluation methods

Sensitivity, specificity, and the area under the ROC curve (AUROC) of all evaluation methods at enamel (D1) and dentin (D3) histological caries levels are presented in Table 10 and Table 11. ICDAS-CARS was the most sensitive (0.98) method at the enamel secondary caries threshold, but the specificity (0.29) was extremely low. In contrast, QLF ΔR showed the best diagnostic performance due to the highest AUROC (0.94) among the evaluation methods, and both sensitivity (0.95) and specificity (0.93) were over 0.9 (Figure 16).

At the dentin secondary caries threshold, the sensitivity of ΔR_{max} was 1.00, so that all dentin lesions could be identified, but the specificity (0.78) was relatively lower than sensitivity. The remaining QLF evaluation methods showed sensitivities of 0.90, of which QS-SC showed a specificity of 0.90 and the highest AUROC (0.90). While QLF methods showed excellent diagnostic performances, ICDAS-CARS had the lowest AUROC (0.66) due to low sensitivity (0.57) (Figure 17).

Table 10. Sensitivity, specificity, and area under receiver operating characteristics (AUROC) curve of secondary caries evaluation methods at enamel histological threshold

	Cut-off points	Sensitivity	Specificity	AUROC (SE)
ICDAS-CARS	0/1	0.98	0.29	0.63 (0.06)
QLF ΔR	ΔR>29.4	0.95	0.93	0.94 (0.04)
QLF ΔR_{max}	ΔR_{max}>72.6	0.93	0.86	0.89 (0.05)
QS-SC	1/2	0.88	0.93	0.90 (0.04)

ICDAS-CARS, international caries detection and assessment system for caries around restorations and sealants; QS-SC, QLF score for secondary caries.
SE, standard error.

Table 11. Sensitivity, specificity, and area under receiver operating characteristics (AUROC) curve of secondary caries evaluation methods at dentin histological threshold

	Cut-off points	Sensitivity	Specificity	AUROC (SE)
ICDAS-CARS	2/3	0.57	0.74	0.66 (0.06)
QLF ΔR	ΔR>35.9	0.90	0.68	0.79 (0.05)
QLF ΔR_{max}	ΔR_{max}>122.2	1.00	0.78	0.89 (0.03)
QS-SC	2/3	0.90	0.90	0.90 (0.04)

ICDAS-CARS, international caries detection and assessment system for caries around restorations and sealants; QS-SC, QLF score for secondary caries.
SE, standard error.

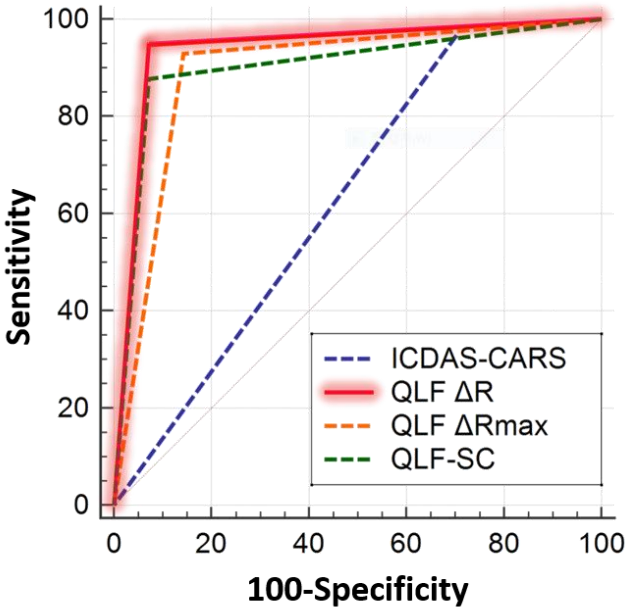


Figure 16. ROC curves of the evaluation methods for secondary caries at enamel histological threshold

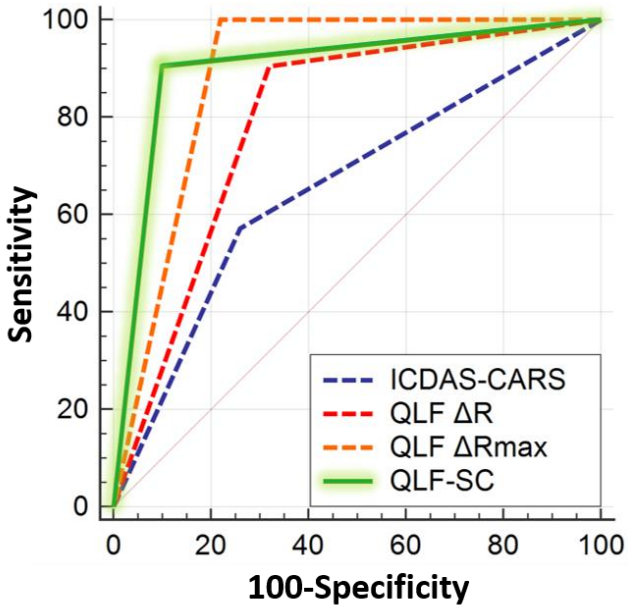


Figure 17. ROC curves of the evaluation methods for secondary caries at dentin histological threshold

IV. DISCUSSION

Amalgam restorations are still considered one of the main types of restorations due to their economic benefits despite a reduction in the rate of their use (average annual decreased by 9.8% in Korea). According to the 2012 Korean National Oral Health Survey, placement rate of amalgam restorations was 44.6% in the primary teeth and 27.1% in the permanent teeth (Park et al., 2016). Amalgam restorations are associated with many confounding factors that make it difficult to diagnose secondary caries. Thus, objective and accurate assessment and diagnosis are necessary to minimize unnecessary replacement. Therefore, the present study evaluated the diagnostic performance of QLF technology in evaluating secondary carious lesions around amalgam restorations. We confirmed that the new fluorescence evaluation method using red fluorescence has an excellent ability to determine the severity of secondary caries around amalgam restorations.

In the first part of this study, secondary carious lesions around amalgam restorations were quantitatively evaluated using the fluorescence loss values. Quantitative evaluation of the severity of the carious lesions based on QLF technology is typically performed using a computer analysis software (de Josselin de Jong et al., 1995). The present study quantitatively calculated the red fluorescence variables due to microbial intervention and fluorescence loss due to demineralization using the QA2 software according to a number of previous studies (Ku et al., 2019; Lee et al., 2018). Initially, the fluorescence loss values were calculated to quantitatively evaluate the severity of secondary caries around amalgam restorations. Many previous studies have reported that the fluorescence loss variables (ΔF and ΔF_{max} values) are useful for quantitative evaluation of the degree of demineralization in dental carious lesions (Gomez et al., 2013; Jong et al., 2009; Stookey, 2004). However,

the |ΔF| value calculated by the QA2 software could not distinguish the severity of secondary caries around amalgam restorations in the present study ($P = 0.871$). Secondary caries in dentin could be significantly distinguished from other lesions using the $|\Delta F_{max}|$ value ($P < 0.05$). However, there was no significant difference in the $|\Delta F_{max}|$ values between sound teeth and secondary caries in enamel ($P = 0.117$). At the wavelength used in QLF, amalgam restorations express reduced fluorescence (Pretty et al., 2003). It is believed that fluorescence of amalgam restorations influences the |ΔF| and $|\Delta F_{max}|$ values, as the analytical patch of the QA2 software includes the restoration part. Previous studies have reported that QLF showed false-positive results in diagnosing secondary caries around amalgam restorations (Diniz et al., 2016b).

To overcome this limitation of the existing QA2 software, we needed a new method of quantitative analysis. While evaluating the secondary caries around the restorations, we suggested to the QLF manufacturer that the restoration part from the analytical patch of the QA2 software should be removed. Thus, the manufacturer developed the new SC01 software for the evaluation of secondary carious lesions. In the analysis of tooth specimens using this software after excluding the amalgam restoration part, it was confirmed that the |ΔF| and $|\Delta F_{max}|$ values could significantly distinguish the severity of all secondary caries ($P < 0.05$). The |ΔF| and $|\Delta F_{max}|$ values from the SC01 software were significantly lower than those from the QA2 software ($P < 0.01$). We confirmed that the fluorescence of amalgam restorations was excluded from the analytical patch of the SC01 software.

In the present study, red fluorescence as well as fluorescence loss values were calculated to evaluate secondary caries around amalgam restorations. In the progression of dental caries, the intensity of red fluorescence increases with an increase in the degree of microbial

metabolism. The ΔR and ΔR_{max} values calculated by the QA2 and SC01 softwares increased significantly with an increase in the severity of the secondary caries. The softwares were also able to differentiate the severity of secondary carious lesions ($P < 0.01$). It is believed that evaluating secondary caries around amalgam restorations using red fluorescence, there will be no effect of the analysis software on red fluorescence values. Nevertheless, the ΔR and ΔR_{max} values from the SC01 were significantly higher than those from the QA2 in secondary caries involving enamel and dentin, but not significantly different in sound teeth ($P < 0.01$). Thus, amalgam restoration is considered to affect the red fluorescence variables.

Most of the previous studies related to the diagnosis of secondary caries were performed based on visual observations such as color changes and gaps in the restorations. However, there is no established standard for the diagnosis of caries around restorations controversies still exist regarding this issue (Kidd et al., 1995; Kidd et al., 1992). Evaluation of color change is less accurate due to the subjectivity of examiners and the discoloration around the amalgam restorations makes the evaluation much more difficult due to many confounding factors such as corrosion, metal translucency, and exogenous pigments (Gonzalez-Cabezas et al., 2003; Kidd et al., 1992). In addition, residual caries left by the dentist during the restorative treatment process may also be misdiagnosed as secondary caries (Kidd et al., 1995). Ditching of the marginal sites is also used frequently for the evaluation, but previous studies have reported that there is no correlation between the gap in this area and secondary caries. This finding may be due to the fact that the occlusal surface is easy to manage and evaluate compared to the gingival margin, which has a high risk of secondary caries (Mjor, 2005; Moncada et al., 2008). Therefore, we evaluated secondary caries around amalgam restorations using the ICDAS-CARS, which was

suggested as a visual examination method in some of previous studies. The ICDAS-CARS results showed that 66 out of the 71 teeth used in the present study had secondary carious lesions. The number of teeth with secondary caries according to the ICDAS-CARS was higher than that according to the histological results (66 vs. 57 teeth, $\rho = 0.545$, $P < 0.001$), which is considered the gold standard. This result is consistent with the result from a previous study, which reported that ICDAS-CARS overestimates secondary caries around amalgam restorations (Ando et al., 2004).

Considering the aforementioned limitations, the second part of this study aimed to find useful fluorescence information about the secondary carious lesions, to develop a new evaluation index through operational definition for these lesions, and to compare it with other methods. Since secondary carious lesions are derived from metabolism of cariogenic biofilms at the margins of the restorations (Magalhaes et al., 2009), it is important to check whether microbial intervention is present. Therefore, we tried to confirm the evidence of microbial intervention at the margin of the restoration using QLF. We developed a new secondary caries fluorescence evaluation index, namely the QS-SC (QLF score for secondary caries) using common fluorescence characteristics of secondary caries observed on white-light and fluorescence images. Evaluation of secondary caries using the QS-SC showed a strong positive correlation with histological classification ($\rho = 0.849$, $P < 0.001$). Particularly, ΔR and ΔR_{max} (the red fluorescence variables) were strongly correlated with the QS-SC ($\rho = 0.800$ and $\rho = 0.806$, respectively) and the histological results ($\rho = 0.715$ and $\rho = 0.804$, respectively) ($P < 0.001$). It was confirmed that the red fluorescence information obtained from the QLF images can be useful in detection of secondary carious lesions. Results of a previous study confirmed the leakage around all-ceramic crowns using red fluorescence. Hence, it can be speculated that the red fluorescence detected at the border

of the amalgam restorations in this study resulted from bacteria that penetrated the tooth-restoration interface through the marginal gap. Consequently, biofilm formation and cariogenicity increased with increasing biofilm maturity, leading to subsequent carious lesions (Maeng et al., 2020). Previous studies that intuitively evaluated carious lesions using QLF technology developed the QS-occlusal index for detection of occlusal caries and the QS-proximal index for the detection of proximal caries (area under the receiver operating characteristic curve [AUROC]: 0.807–0.976 and 0.826–0.864, respectively), which showed excellent diagnostic validity (Jung et al., 2018; Kim et al., 2017). Thus, the red fluorescence information along with fluorescence loss observed using QLF technology can be useful for the development of a scoring system through operational definition. It is believed that various carious lesions can be evaluated with QLF-based scoring system that has intuitiveness and high diagnostic performance.

The ΔR and ΔR_{max} values calculated by the SC01 software increased significantly in each severity group of secondary carious lesions classified using the QS-SC index ($P < 0.01$). In the QS-SC index, code 0 denotes sound teeth with no evidence of caries (no change) and code 1 denotes noncariogenic discoloration of teeth (Lee et al., 2018). Statistical analysis revealed that ΔR and ΔR_{max} values of teeth with codes 0 and 1 were not significantly different ($P = 0.305$ and $P = 0.208$, respectively). Nevertheless, the present study divided sound teeth according to the QS-SC index into codes 0 and 1, since the absence of discoloration is a good indicator for distinguishing sound teeth from caries and management of sound teeth should be different from the management of teeth with discoloration (Rudolphy et al., 1995).

In the present study, the cut-off values of ΔR and ΔR_{max} at each histological threshold

were calculated to help distinguish the secondary carious lesions around amalgam restorations on the occlusal surface. The ΔR and ΔR_{max} cut-off limits for distinguishing secondary carious lesions in enamel were 29.4 and 72.6, respectively. The ΔR and ΔR_{max} cut-off values for distinguishing secondary carious lesions in dentin were 35.9 and 122.2, respectively. According to the ROC analysis, methods that used QLF technology showed excellent diagnostic performance for detection of secondary caries. At the enamel histological threshold, the ΔR value showed the highest AUROC of 0.94 and sensitivity and specificity were also high. The QS-SC showed an excellent AUROC of 0.90 in all secondary carious lesions. At the dentin threshold, the AUROC of ΔR_{max} was 0.89 and specificity was relatively low, while sensitivity was high. This finding may be attributed to the limitations of QLF technology in the detection of red fluorescence that has spread to the bottom of the restoration. This indicates that different surface conditions can easily produce considerable changes in the variables. Previous studies that evaluated the secondary caries around resin restorations using ΔF reported high AUROC values. Moreover, there was no limiting effect on ΔF values of resin restorations brighter than the autofluorescence of teeth (Diniz et al., 2016b; Lenzi et al., 2016). However, since the brightness of fluorescence varies among resin restorations (Kim et al., 2016), the procedure may have limitations in evaluating secondary caries around resin restorations with darker fluorescence than teeth. Hence, it is believed that red fluorescence information is more effective in detecting secondary caries than information regarding loss of fluorescence.

In the histological evaluation using polarized light microscopy, it was difficult to distinguish secondary caries from residual caries under the amalgam restorations. Due to the lack of defined guidelines and scarce previous literature regarding this distinction, history of the restoration, patients' caries risk, and the activity of secondary caries should

be further evaluated (Kidd et al., 1995). In this study, the teeth were sectioned and the sites with the highest severity of secondary carious lesions were evaluated via discussion by two examiners. However, since the wall lesion of secondary caries progresses along the restoration wall, it is possible that the starting point of the outer lesion and the deepest area below the restoration are different (Mjor, 2005). There is still no clear standard for histological evaluation of secondary caries around the restorations and further investigations are needed (Hintze and Wenzel, 2003). In addition, since the present study used extracted teeth with amalgam restorations, studies regarding red fluorescence expressed in teeth with secondary caries around restorations in actual clinical situations should be performed.

To the best of our knowledge, this is the first study that used the red fluorescence properties of QLF technology to detect secondary caries around amalgam restorations. The present study objectively evaluated secondary carious lesions around amalgam restorations associated with the possibility of over-treatment. The results of this evaluation suggest that QLF technology can reduce restorative death spiral, which is harmful to teeth (Eltahlah et al., 2018). The paradigm of restorative treatment, which is moving toward minimally invasive treatment, recommends repair of the restoration or long-term observation of the lesion rather than replacement due to secondary carious lesions (Nedeljkovic et al., 2020). QLF is an optimal technology for minimally invasive dentistry, as it is not only harmless to the human body, but also provides real-time images of the condition of the oral cavity in a noninvasive and quick manner. It can easily explain the oral condition by evaluating and showing the long-term cumulative images of patients. Therefore, QLF could be a promising tool to evaluate secondary caries around amalgam restorations quantitatively and intuitively on a regular basis.

V. CONCLUSION

The purpose of this study was to evaluate the autofluorescence characteristics of secondary caries lesions around amalgam restoration of the extracted tooth using QLF technology. And it was also aimed to determine whether the new quantitative analysis method and evaluation index using QLF technology are useful for distinguishing the severity of amalgam secondary caries. The specific conclusions of this study are as follows:

1. As a result of quantitative analysis of fluorescence images, the new SC01 software was able to evaluate secondary caries by excluding the amalgam restoration part and showed different fluorescence values from existing QA2 software. All fluorescence variables of SC01 software significantly differentiated the severity of secondary caries around amalgam restorations ($P < 0.05$).

2. The red fluorescence values (ΔR and ΔR_{max}) of QLF technology showed excellent correlation with histology ($\rho = 0.715$ and $\rho = 0.804$, respectively, $P < 0.001$), which was found to be useful information for detection of secondary caries. Based on this information, the newly developed quantitative QS-SC index significantly distinguished secondary caries with excellent correlation compared with the histology ($\rho = 0.849$, $P < 0.001$).

3. Compared to the ICDAS-CARS using visual examination, the new quantitative analysis and QS-SC index showed excellent diagnostic performances for detection of amalgam secondary caries in each histological severity.

Red fluorescence of QLF technology had excellent diagnostic ability in quantitative analysis and intuitive evaluation of amalgam secondary caries, and thus can be used as an objective and effective tool for evaluation of secondary caries in actual clinical practice.

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ABSTRACT (in Korean)

정량 광형광 (QLF) 기술을 이용한 아말감 수복물 주위 이차 우식의 광학적 탐지

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맹 유 진

구강 관리에 대한 관심과 삶의 질이 향상됨에 따라 치아우식증 유병률은 수년간 감소하였지만, 여전히 우식 발생은 전세계적으로 큰 부담이다. 특히 이차 우식증은 우식 경험이 있어 수복 처치가 된 치아에 다시 발생한 병소라는 측면에서 관심을 가지고 볼 필요가 있다. 왜냐하면 이차 우식 병소는 수복물 교체의 주 원인 중 하나이고, 수복 치료의 범위를 계속 확장시키는 수복의 치아 파괴 주기(restorative death spiral)를 갖기 때문이다. 따라서 이차 우식 병소가 발생하기 이전에 예방하고, 조기 진단을 통해 관리하려는 노력이 필요하다.

이차 우식은 치아-수복물 경계부에서 시작되어 수복물의 벽을 따라 진행되

는 특성이 있어 병소가 진전된 단계이거나 와동이 형성되었을 때 주로 발견된다. 치의학 영역에서 널리 사용된 방법은 시각-촉진 검사와 bitewing 방사선 방법이다. 아말감 수복물은 corrosion, 변색, 틈의 크기, 누출, 방사선 불투과성 등의 여러 혼란 변수들을 가지고 있어 기존의 방법들을 이용하여 이차 우식을 평가하는데 어려움이 있다. Quantitative light-induced fluorescence (QLF)는 405 nm 파장의 빛을 이용하여 치아에서 발현되는 자가형광(autofluorescence)을 통해 우식 병소를 정량적으로 탐지하는 기술이다. QLF 기술은 형광 소실로 보여지는 초기 우식뿐만 아니라 붉은 형광을 통해 미생물의 개입 정도를 평가할 수 있다. 따라서 본 연구는 QLF 기술의 새로운 분석 방법을 이용하여 치아의 아말감 이차 우식 병소에서 나타나는 형광학적 특성을 평가해 보고자 한다.

본 연구는 아말감 수목물이 존재하고, 조직의 손상 및 치수의 노출이 없는 총 85개의 치아를 사용하였다. 먼저 치아 시편에 임의로 번호를 부여한 후 각각의 치아에 대해 ICDAS-CARS 기준을 이용하여 시진 평가를 수행하였다. 이후 QLF 형광 검사를 위해 모든 시편에 대해 치아 교합면이 지면과 수평이 되도록 위치시킨 후 QLF-D를 이용하여 촬영하였다. 촬영된 모든 사진은 QLF 제조사에서 제공하는 두 가지 분석 소프트웨어를 이용하여 정량적으로 분석하였다. 이 후 아말감 수복물 주변에서 발현되는 공통의 형광 특성을 기반으로 하여 조작적 정의를 통한 새로운 QLF 평가 지수(index)를 개발하고 평가하였다. 최종적으로 모든 아말감 수복물의 이차 우식 병소의 심도는 조직학적 검사를 통하여 확인하였다.

본 연구의 QLF 형광 사진을 정량적으로 분석한 결과, 새로운 SC01 소프트

웨어의 모든 형광 변수들은 유의한 차이를 보이며 이차 우식 병소의 심도를 구분하였다(P<0.01). 기존의 QA2 소프트웨어와 비교하였을 때, SC01 소프트웨어는 분석 시 아말감 수복물을 제외할 수 있어 유의하게 낮은 형광 소실량을 보였다(P<0.01). 조직학적 검사 결과와 우수한 상관성을 보인 붉은 형광 변수 (ΔR and ΔR_{max})는 이차 우식의 심도를 유의하게 구별하였고(P<0.05), 이를 통해 형광 정보를 조작적으로 정의함으로써 새로운 이차 우식 평가 지수인 QS-SC를 개발하였다. QS-SC 지수는 QLF 정량 분석 방법과 함께 법랑질 및 상아질 이차 우식 모두를 구별하는데 우수한 진단 능력을 보였다.

결론적으로, QLF 기술은 기존의 방법으로 진단이 어려운 아말감 수복물의 이차 우식을 정량적인 분석 방법뿐만 아니라 새로운 평가 지수를 이용하여 구분할 수 있었다. 따라서 QLF 기술은 아말감 수복물의 이차 우식을 정량적이고 직관적으로 평가하는데 유용하였다.

핵심되는 말 : 이차 우식, 재발성 우식, 아말감 수복물, 붉은 형광, quantitative light-induced fluorescence (QLF)