An *in vitro* and Clinical Study of Quantitative Light-induced Fluorescence (QLF) for Validation of Dental Caries Detection and Diagnostic Efficacy in Primary Teeth

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A Dissertation Submitted
to the Department of Dentistry
and the Committee on Graduate School
of Yonsei University in Partial Fulfillment of the
Requirements for the Degree of
Doctor of Philosophy in Dentistry

Cho, Kyung Hyun

June 2025

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감사의 글

무엇보다 지금까지 저의 삶을 인도해주신 하나님과 입학 때부터 본 학위논문을 작성하는 이 순간까지 관심과 사랑으로 지도해주신 연세대학교 치과대학의 모든 교수님께 감사드립니다. 특별히, 소아치과 의사로서의 실력과 인성, 아이들을 대하는 마음까지 부족함 없이 지도하고 알려주신 이제호 교수님, 최형준 교수님, 김성오 교수님, 지도교수님이신 송제선 교수님, 강정민 교수님, 이고은 교수님께 다시 한 번 존경과 감사의 마음을 전해드립니다. 이번 논문의 주제인 정량형광분석 분야를 열어주신 예방치과학교실의 김백일 교수님과 아낌없는 조언과 도움 주신 정회인 교수님께도 감사드립니다. 전공의 시절부터 여전히 함께 웃고 삶을 나누는 동기 김성진, 송민선, 조현민 선생님에게 감사하며 오늘도 그들의 소중함을 느낍니다. 연구와 논문작성에 함께 힘써주신 이태양, 이용혁 선생님께도 감사드립니다.

이 모든 과정이 저의 능력이 아닌 밀어주고 끌어주신 많이 분들의 도움으로 이루어졌음을 고백합니다. 언제나 겸손과 사랑의 마음에 실력을 더한 훌륭한 치과의사로 살아가기 위해 노력하겠습니다. 마지막으로 봄날의 햇살처럼 따뜻하게 다가와 함께 삶을 나누어가는 사랑하는 아내와 언제나 큰 기쁨이 되어주는 귀여운 두 아이들에게 특별한 감사와 사랑의 마음을 전합니다.

감사합니다.

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An *in vitro* and Clinical Study of Quantitative Light-induced Fluorescence (QLF) for Validation of Dental Caries Detection and Diagnostic Efficacy in Primary Teeth

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The Graduate School, Yonsei University

(Directed by Professor Song, Je Seon)

This study evaluated QLF (quantitative light-induced fluorescence) caries detection method under *in vitro* and clinical conditions. The relationships between the cavity volume of carious lesions and QLF analysis results were validated; furthermore, we presented a QLF scoring index (QS-Index) of primary teeth.

For *in vitro* study, total 125 tooth surfaces were investigated with the portable QLF device Qraypen C (AIOBIO, Seoul, Republic of Korea) for detection of dental caries in primary teeth. Micro-CT radiograph was also performed to classify carious lesions and calculate the cavity volume. QLF showed good reliability (sensitivity 0.75-0.94, specificity 0.82-0.95, and AUROC 0.88-0.98) except ΔR average results of proximal surfaces. Statistically significant correlations were found between ΔF average, QS-Index, ΔQ, and the cavity volume ($r = 0.759$-0.832, $p < 0.001$).

In the clinical study, a total of 878 tooth surfaces of 44 children were researched. After visual inspection and radiographic examination, images of dental caries captured with the Qraypen C were classified according to the caries progression and analyzed with special software. ROC analysis was performed on the QLF parameters: fluorescence loss (ΔF) and bacterial activity (ΔR). The reliability of logistic regression model to combine ΔF and ΔR was also evaluated. QLF parameters showed a good sensitivity (0.72–0.91), specificity (0.74–0.96), and AUROC (0.861–0.940). The AUROC of logistic regression model (0.90–0.957) was higher than ΔF or ΔR average alone in all types of carious lesions. Every level of the QS-Index was properly defined to represent the progression of dental caries with corresponding statistical significance.

The reliability of QLF method was similar to or slightly higher than that of the traditional diagnostic methods of visual inspection or radiographic examination in clinical conditions. In conclusion, QLF detection method in primary teeth would be a harmless and reliable way for children to diagnose dental caries without the concern about radiation exposure.

Keywords: Quantitative light-induced fluorescence (QLF) technology, Dental caries, Diagnosis, Caries detection, Primary teeth, Micro-CT, Radiography

An *in vitro* and Clinical Study of Quantitative Light-induced Fluorescence (QLF) for Validation of Dental Caries Detection and Diagnostic Efficacy in Primary Teeth

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I. Introduction

Dental caries is one of the most common oral diseases in patients across all ages; therefore, precise detection and appropriate treatment of dental caries are indispensable aspects of dentistry. Early detection and prompt treatment of dental caries are extremely important in primary dentition, as primary teeth have reduced enamel thickness and easily accumulate dental plaque compared with permanent teeth. These differences render primary teeth weak against dental caries, leading to rapid disease progression (Mo et al., 2004; Wilson and Beynon, 1989). Above all, primary dentition forms the foundation for establishing permanent dentition, making the maintenance of primary teeth health essential for the growth and development of children. Early diagnosis, regular monitoring, and proactive preventive measures for caries in primary teeth can significantly contribute to establishing oral health. This necessitates periodic dental examinations in children to develop patient-specific treatment plans. Early caries detection coupled with active preventive intervention through regular screening and monitoring of dental caries progression helps re-establish healthy oral conditions (Fejerskov et al., 2015).

The most widely used methods for dental caries detection are visual inspection and radiographic examination. Visual inspection is a convenient method for checking the activity of carious lesions (Ekstrand et al., 2007; Nyvad et al., 2003), while radiographic examination offers relatively higher reliability and can detect lesions not visible through direct observation (Newman et al., 2009). Although regarded as highly reliable diagnostic tools, the diagnostic accuracy of visual inspection and radiographic examination is markedly influenced by the varied anatomical morphologies of teeth. Therefore, much of the screening and final diagnosis of dental caries tends to rely on empirical evidence (Lee et al., 2018). Moreover, the early stages of caries tend to develop beneath the tooth surface, making early detection challenging with conventional methods (Stookey, 2005). Quantitative light-induced fluorescence (QLF) has been introduced as a complement to basic dental examinations and aids in providing a precise diagnosis of dental caries. This technology detects quantitative fluorescence changes in the light reflected from the tooth surface when irradiated with visible blue light of 405 nm. It can determine the depth as well as the bacterial activity of dental caries simultaneously like visual inspection (Angmar-Månsson and Ten Bosch, 2001; Van der Veen and de Jong, 2000). QLF also can detect proximal carious lesions that are difficult to identify visually such as radiographic examinations (Ekstrand et al., 2011). It detects fluorescence loss (ΔF) which is representative of the mineral loss of the examined tooth and thus, reveals the lesion depth (Gmür et al., 2006; Jallad et al., 2015; Kim et al., 2013). QLF also detects red fluorescence (ΔR), which corresponds to the porphyrin derivatives of bacterial metabolism (Volgenant et al., 2013). ΔR is usually increased in carious lesions, dental plaque, and dental calculus as these are formed by the aggregation of a plethora of microorganisms (Ando et al., 2017; Lee et al., 2013). Recent studies have proved that ΔR is related with the bacterial activity of dental caries (Felix Gomez et al., 2016; Kim and Kim, 2017; Lennon et al., 2005).

QLF technology has been reported to be sensitive, precise, and reproducible, enabling the monitoring of not only early carious lesions but also the progression of dental caries over time (Stookey, 2004). It has the added benefit of being devoid of detrimental effects of radiation exposure that are associated with traditional radiographic examination and thus, is a better technique for caries screening and detection. Based on the characteristics of the QLF method and results of previous QLF studies, it may be possible to use QLF for the detection of dental caries in primary teeth. We hypothesized that QLF could show a similar caries detection ability as conventional methods such as visual inspection or radiographic examination for primary teeth in children. Previous studies validating QLF's caries detection ability primarily relied on radiographic images or histological specimens to determine lesion depth (Diniz et al., 2019; Jallad et al., 2015; Jung et al., 2018; Ko et al., 2015). However, there is a lack of research examining the direct relationship between the volume of carious lesions and QLF analysis results, especially studies covering both occlusal and proximal surfaces of primary teeth. The aim of this study is to validate the reliability of QLF technology for caries detection using the portable QLF device Qraypen C (AIOBIO, Seoul, Republic of Korea) and analyze the correlation between the actual cavity volume calculated via micro-CT and the QLF analysis results. This study also evaluated the efficacy of QLF method under clinical diagnosis for dental caries in primary teeth and to extend the application of a quantitative light-induced fluorescence scoring index (QS-Index) to primary teeth, which was originally introduced for clinical application on permanent teeth.

II. Materials and Methods

1. *In vitro* Experiment

This *in vitro* experiment involved 60 extracted primary molars collected from the Human Oral Resource Bank at Yonsei University Dental Hospital between September 2019 and March 2020 (IRB No. 2-2019-0065), which were stored at -80℃. Teeth exhibiting severe discoloration, crown fractures, or developmental anomalies were excluded from the study. A total of 125 tooth surfaces, comprising 53 occlusal and 72 proximal surfaces, were selected for analysis. Prior to specimen preparation, all teeth were thoroughly cleaned to remove debris and polished using an ultrasonic scaler.

(1) Specimen preparation

After cleaning and drying the teeth, two adjacent primary molars were fixed in a rectangular block of white utility wax (Atria Inc., Seoul, Republic of Korea) to simulate natural proximal contact (Figure 1). Only the crown portions of the teeth were exposed above the wax block to facilitate QLF and white light imaging.

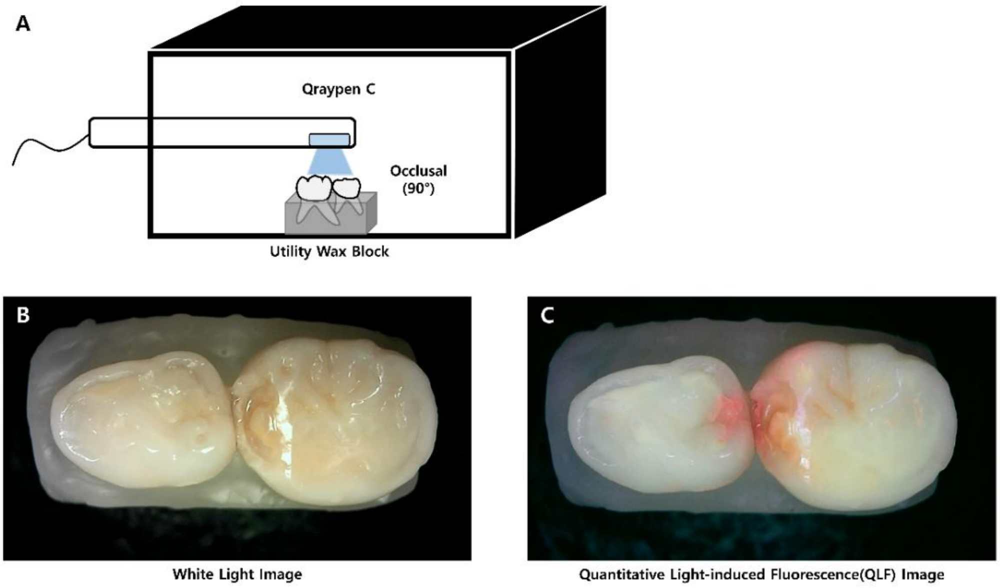


Figure 1. Quantitative light-induced fluorescence (QLF) imaging protocol using extracted primary teeth (A) QLF imaging procedure with Qraypen C (B) White light image of primary teeth (C) QLF image of primary teeth

(2) QLF imaging and analysis

The prepared specimens were photographed using the portable QLF device Qraypen C (AIOBIO, Seoul, Republic of Korea) under white light and QLF modes sequentially. Qraypen C was developed together with the 3rd-generation QLF device (Qraycam Pro; AIOBIO, Seoul, Republic of Korea) and is a device that emphasizes clinical usability. It has the same appearance as a dental curing light and works like an oral camera. When the LED light (405 nm) from the device falls on tooth surfaces, the scattered light is detected through a special double filter to create fluorescence images. White light images and QLF images can be taken consecutively with auto focusing function (1280 × 720 output resolution, 53.05^{o} for the horizontal and 41.14^{o} for the vertical field of view). To enhance QLF image quality, the specimens were placed inside a black box with a light-blocking cloth covering its entrance. Images were taken at a 90-degree angle toward the occlusal surface from approximately 4cm above the teeth to ensure that both teeth of the specimen were visible in one frame (Figure 1). The captured images were analyzed using QA2 software v.1.39 (Inspektor Research Systems BV, Amsterdam, Netherlands). The software provided values for fluorescence loss (ΔF average, ΔF max), red fluorescence (ΔR average, ΔR max), and ΔQ which is the value for fluorescence loss considered size and severity of the carious lesion. Each tooth surface of the QLF images was classified according to the QS-Index for statistical analyses, using the criteria established by Jung EH et al. (Jung et al., 2018) for occlusal surfaces (Table 1) and Kim ES et al. (Kim et al., 2017) for proximal surfaces (Table 2).

Table 1. Quantitative light-induced fluorescence score for occlusal caries (QS-Occlusal)

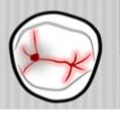
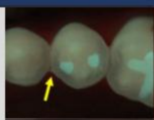
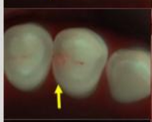
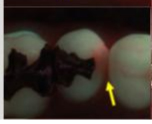
score	Description	Score examples	Fluorescence Images	White-light Images
0 Sound	No fluorescence loss and no red fluorescence increase in pits and/or fissures			
1 Suspected or initial caries	Fluorescence loss and red fluorescence present as a line or spot in pits and/or fissures			
2 Enamel caries	Fluorescence loss and red fluorescence glow extending around pits and fissures			
3 Dentin caries	Red fluorescence glow extending around pits and fissures and a dark shadow from dentin present			

Table 2. Quantitative light-induced fluorescence score for proximal caries (QS-Proximal)

score	Description	Fluorescence Images	White-light Images
0	No dark shadow and no red fluorescence		
1	Irregular dark shadow but no red fluorescence		
2	Faint red fluorescence limited to 1/3 of the buccolingual width		
3	Strong red fluorescence over 1/3 of the buccolingual width		

(3) Evaluation of carious lesions using micro-CT

Micro-CT scanning by Quantum FX (PerkinElmer, MA, USA) was performed on the entire tooth specimens under conditions of 90 kV and 160 μA. The images were reconstructed using Quantum FX μCT control software (PerkinElmer, MA, USA). Carious lesions were identified from sagittal, coronal, and transverse sections (Figure 2). Lesion depth was classified based on the International Caries Classification and Management System (ICCMS) established by Ismail AI et al. (Ismail et al., 2015) (Table 3). We also established the diagnostic level through a simplification procedure from ICCMS to be useful in clinical diagnosis and make obvious statistical significance. It was defined as follows: Level 0: ICCMS stage 0 (sound surfaces), Level 1: ICCMS stages 1-2 (enamel caries), Level 2: ICCMS stages 3-5 (dentin caries). To measure lesion volume of dental caries, we first selected the target cavities one by one. After reconstructing three-dimensional image from the micro-CT data using Bruker CTAn software v.1.18 (Cambridge, UK), the total volume of selected cavities was calculated. For occlusal caries, the volume of all lesions was measured together. For proximal caries, cavities on mesial and distal surfaces were evaluated separately.

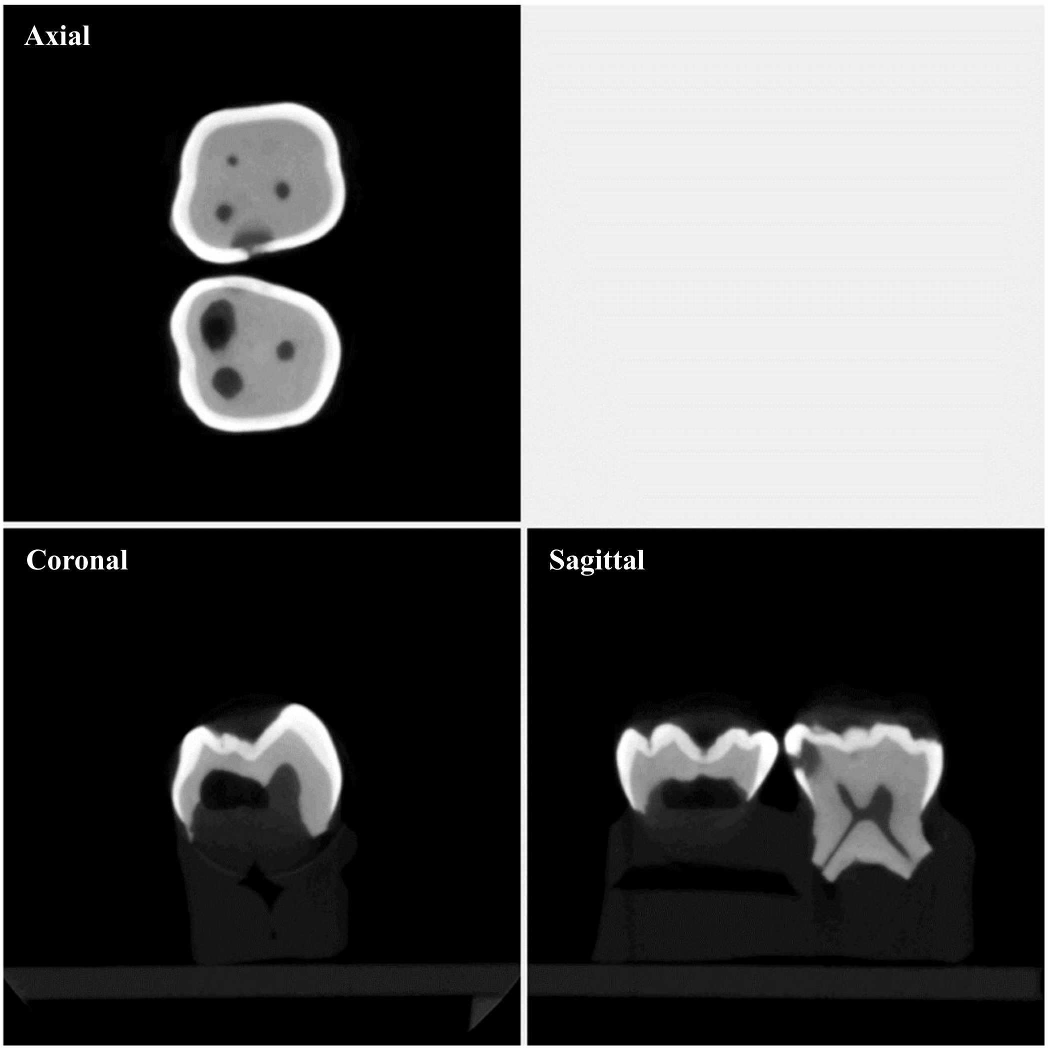


Figure 2. Axial, coronal, and sagittal view of micro-CT radiography for the assessment of dental caries in primary molar

Table 3. The international caries classification and management system (ICCMS)

Level	Description
0	No radiolucency
1	Radiolucency in the outer 1/2 of the enamel
2	Radiolucency in the inner 1/2 of the enamel ± EDJ
3	Radiolucency limited to the outer 1/3 of dentin
4	Radiolucency reaching the middle 1/3 of dentin
5	Radiolucency reaching the inner 1/3 of dentin

EDJ = Enamel-dentin junction

Level 6 represents radiolucency into the pulp is excluded.

(4) Statistical analysis

Statistical analysis and graph plotting were conducted using SPSS Statistics 25.0 (IBM Corporation, NY, USA). ΔF average and ΔR average, which are the most representative QLF analysis values, were used in all analytic procedures. Receiver Operating Characteristic (ROC) analysis was performed to evaluate the detection ability of QLF for occlusal and proximal caries. Sensitivity, specificity, and cut-off values were calculated, with separate analyses conducted for enamel caries and dentin caries. The reliability of the QLF method was assessed by calculating the Area Under the Receiver Operating Characteristic Curve (AUROC).

To compare ΔF average and ΔR average values across diagnostic levels determined by lesion depth, the Kruskal-Wallis test was used, and results were visualized using box-whisker plots. Spearman's rank-order correlation coefficients were calculated to examine correlations between the volume of carious lesions measured via micro-CT and QLF analysis values (ΔF average, QS-Index and ΔQ). To evaluate the reliability of QS-Index and ICCMS classifications for occlusal and proximal caries in primary teeth, results from two independent examiners were compared using Cohen’s kappa coefficient, which demonstrated values exceeding 0.8 ($p < 0.001$), indicating appropriate inter-examiner reliability.

2. Clinical study

This clinical study was granted ethical approval by the Institutional Review Board for clinical research in Yonsei University (IRB No. 2–2019–0022). The data for the study were collected at the Department of Pediatric Dentistry, Yonsei University Dental Hospital, Republic of Korea. Participating patients and their parents received information sheets regarding the procedure and informed consent was obtained prior to the study. Potential patients were recruited from September 2019 to March 2020. Distal surfaces of primary canines and occlusal and proximal surfaces (both mesial and distal) of primary first and second molars were included as eligible tooth surfaces. A total of 1232 tooth surfaces of 44 patients were evaluated in this study (Figure 3). Patients with systemic diseases, tooth malformations, such as enamel hypoplasia or severe periodontitis, and those who were undergoing orthodontic treatment were excluded from the study. Restored tooth surfaces (direct restorations and crowns), extracted teeth, tooth surfaces without matched radiographic images, and low quality QLF images were also excluded. A total of 44 patients (boys = 27, girls = 17, age range: 3–8 years, mean age: 6.02 years) and 1232 primary tooth surfaces were enrolled in this study. In the final analysis, 878 tooth surfaces (occlusal surfaces = 251, proximal surfaces = 627) were selected (Figure 3).

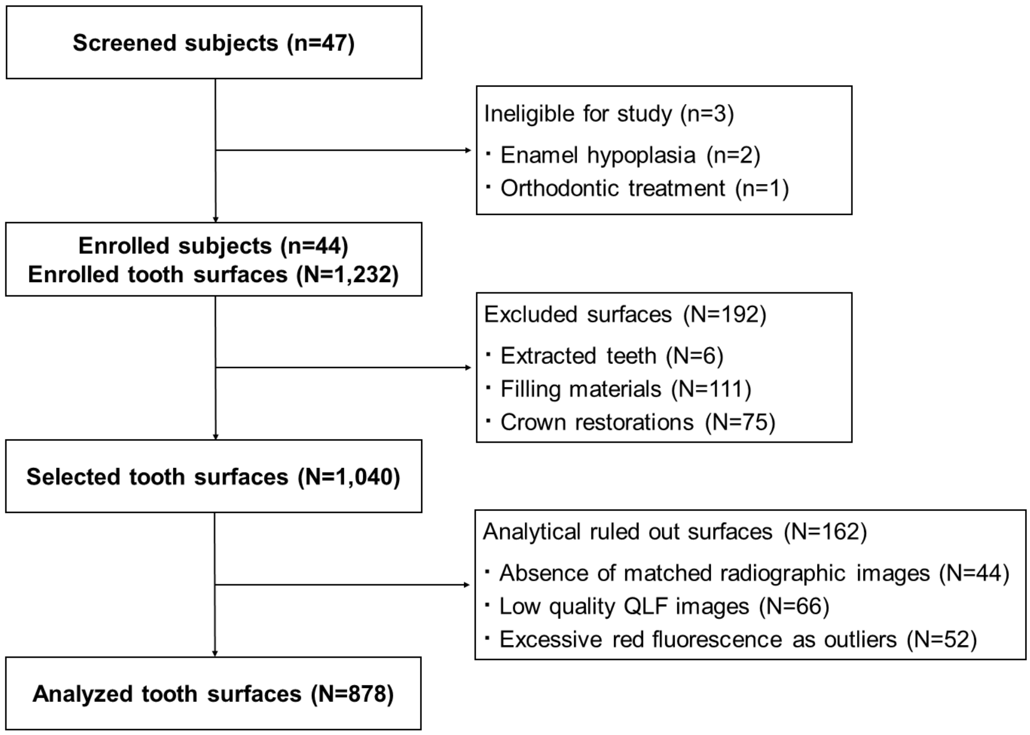


Figure 3. Flow diagram of patient enrollment and inclusion and exclusion process for tooth surfaces in primary teeth (n = number of patients, N = number of tooth surfaces)

(1) Clinical examinations

Two trained dentists in the department of pediatric dentistry conducted the clinical examinations. The included tooth surfaces were examined with a dental mirror, explorer, and air syringe and classified based on the International Caries Detection and Assessment System II (ICDAS II, 0: Sound tooth surface; 1: Visible change in enamel only after prolonged air drying; 2: Distinct visual change in enamel; 3: Localized enamel breakdown because of caries with no visible dentin or underlying shadow; 4: Underlying dark shadow from dentin with or without localized enamel breakdown; 5: Distinct cavity with visible dentin; and 6: Extensive distinct cavity with visible dentin). Inter examiner correlation coefficient was 0.702 ($p < 0.001$).

(2) Radiographic examinations

Digital periapical radiographic images of primary canines, first and second primary molars of every patient were taken by a professional radiologist at Yonsei University Dental Hospital using the dental x-ray machine (Kodak 2200 Intraoral X-ray System; Eastman Kodak Co., Rochester, NY, USA) and extension cone paralleling system. Two trained pediatric dentists scored all periapical radiographs according to the International Caries Classification and Management System (ICCMS, 0: No radiolucency; 1: Radiolucency in the outer 1/2 of the enamel; 2: Radiolucency in the inner 1/2 of enamel to dentino-enamel junction; 3: Radiolucency limited to the outer 1/3 of the dentin; 4:

Radiolucency reaching the middle 1/3 of the dentin; and 5: Radiolucency coming to the inner 1/3 of the dentin). Inter examiner correlation coefficient was 0.819 ($p < 0.001$).

(3) Acquisition of QLF images and assessments

Qraypen C (AIOBIO, Seoul, Republic of Korea), a portable QLF device was used in this clinical study, which is the same device used in the *in vitro* experiments. Two pediatric dentists each captured QLF images of the carious lesions of different patients using the QLF device after cleaning the tooth surfaces with a rubber cup, brush, low speed handpiece, and dental floss to remove plaque or food debris as these could affect the analysis. All images were taken in a darkened room and under the same lighting conditions. Soft tissues and lips were retracted with air blowing by a 3-way syringe to maximize the quality of QLF images. Since Qraypen C can take multiple shots in a short time, the dentist who obtained the QLF images selected the most suitable images of the same carious lesions for accurate analysis. A single examiner (one of the pediatric dentists who examined the patients) classified these QLF images in accordance with the QS-Occlusal (Figure 4) and QS-Proximal (Figure 5) indexes. The classification criteria for these indexes are the same as those of *in vitro* experiments. Intra examiner coefficient was 0.797 ($p < 0.001$).

For the quantification of fluorescence changes, the same single examiner also analyzed QLF images using QA2 software v.1.39 (Inspektor Research Systems BV, Amsterdam, Netherlands). Various types of QLF parameters (ΔF average, ΔF max, ΔR average, ΔR

max, and ΔQ) can be obtained through the QLF analysis process using QA2 software, and the numeric results of each QLF parameter are displayed. QA2 software represents ΔF values indicating a decrease in fluorescence as negative values and ΔR values indicating an increase in red fluorescence as positive values. We used ΔF average and ΔR average for statistical analysis, which are more representative among the QLF parameters.

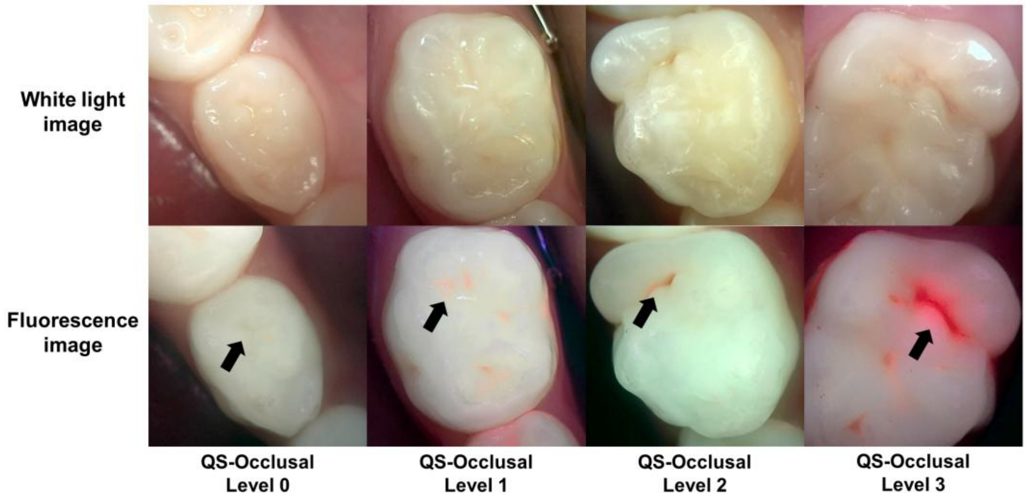


Figure 4. Quantitative light-induced fluorescence score for occlusal caries (QS-Occlusal)

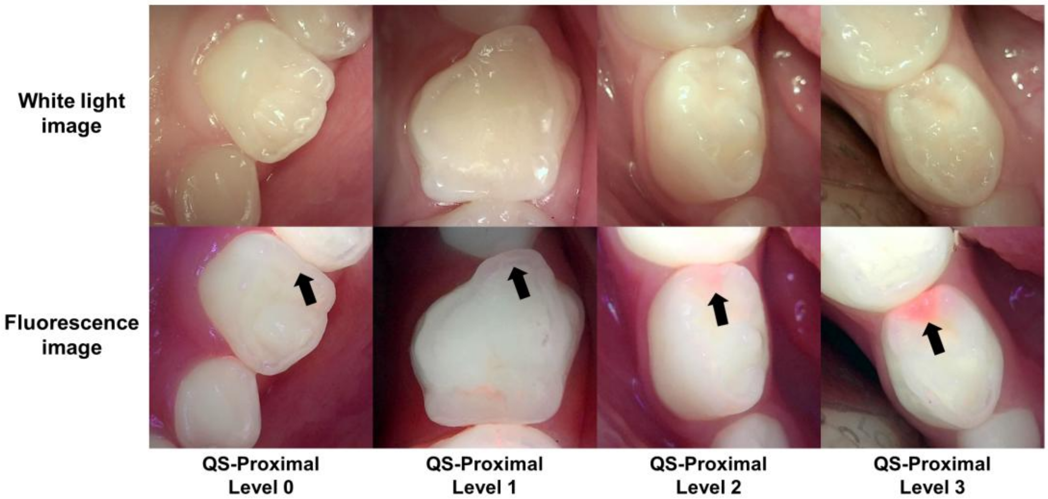


Figure 5. Quantitative light-induced fluorescence score for proximal caries (QS-Proximal)

(4) Statistical analysis

The means and standard deviations of QLF parameters by QS-Index were compared using analysis of variance and Scheffe's post-hoc analysis. Box-whisker plots were made to compare median values of ΔF and ΔR average based on the ICCMS using Kruskal-Wallis test and Mann-Whitney post hoc analysis. For the evaluation of the detection performance of QLF parameters, sensitivity, specificity, and area under the receiver operating characteristic curve (AUROC) were calculated with cut-off values for each type of incipient and moderate caries in primary teeth (95% confidence interval [CI]). The AUROC of the logistic regression model for ΔF average combined with ΔR average were also obtained to compare the caries detection performance of each QLF parameter. In ROC analyses, both visual inspections (ICDAS II) and radiographic examinations (ICCMS) were considered as references to establish the criteria for enamel caries or dentin caries as follows. Level 0 of both ICDAS II and ICCMS was regarded as normal surface. Level 1–2 of ICDAS II or ICCMS was regarded as incipient caries (If one of them was level 0 and the other was level 1, it was regarded as a normal surface considering clinical judgment and the possibility of false positives). Greater than level 3 of ICDAS II or ICCMS were regarded as moderate caries. Cohen's kappa coefficient values were used to confirm the intra-and inter examiner reliability. SPSS Statistics version 25.0 (IBM Corporation, Armonk, NY, USA), R version 4.0.3 (The R Foundation, Vienna, Austria) and R-studio version 1.3.1093 (Rstudio, Boston, MA, USA) were used for all statistical analyses.

III. Results

1. *In vitro* Experiment

(1) Detection of dental caries in primary teeth using QLF analysis

Tables 4 and 5 present the results of detecting dental caries in primary teeth using ΔF average and ΔR average. For the detection of enamel caries, the cut-off values were -7.75 for ΔF average and 20.50 for ΔR average on occlusal surface, and -7.15 for ΔF average and 21.50 for ΔR average on proximal surfaces (Table 4). The sensitivity, specificity, and AUROC values for enamel caries showed generally high accuracy and reliability, except for the ΔR average results on proximal surfaces (sensitivity = 0.75–0.95, specificity = 0.88–0.94, AUROC = 0.88–0.98). Among these, the ΔF results displayed higher values than ΔR for both occlusal and proximal caries.

For the detection of dentin caries, the cut-off values were -11.65 for ΔF average and 27.50 for ΔR average on occlusal surfaces, in addition, -9.40 for ΔF average and 21.50 for ΔR average on proximal surfaces (Table 5). Similar to enamel caries, the sensitivity, specificity, and AUROC values for dentin caries detection showed high accuracy and reliability, except for the ΔR average results on proximal surfaces (sensitivity = 0.89–0.93, specificity = 0.82–0.95, AUROC = 0.94–0.98). Notably, the ΔR results for occlusal dentin caries showed improved values compared to enamel caries on occlusal surfaces. They also were comparable to or higher than ΔF values.

Table 4. Results of the ROC analysis with QLF parameters for the detection of enamel caries in primary teeth

	Occlusal		Proximal	
	ΔF average	ΔR average	ΔF average	ΔR average
Cut-off	-7.75	20.50	-7.15	21.50
Sensitivity	0.94	0.75	0.95	0.19
Specificity	0.89	0.94	0.88	1.00
AUROC (95% CI)	0.97 (0.93–1.00)	0.88 (0.79–0.97)	0.98 (0.96–1.00)	0.62 (0.48–0.75)

CI = Confidence interval

Table 5. Results of the ROC analysis with QLF parameters for the detection of dentin caries in primary teeth

	Occlusal		Proximal	
	ΔF average	ΔR average	ΔF average	ΔR average
Cut-off	-11.65	27.50	-9.40	21.50
Sensitivity	0.93	0.92	0.89	0.36
Specificity	0.85	0.95	0.82	0.98
AUROC (95% CI)	0.98 (0.94–1.00)	0.97 (0.92–1.00)	0.94 (0.88–0.99)	0.69 (0.54–0.84)

CI = Confidence interval

(2) Distribution of QLF analysis results according to carious lesions identified by micro-CT

When comparing the average QLF analysis values (ΔF average and ΔR average) at each diagnostic level classified using micro-CT images, the QLF analysis values increased followed with the diagnostic level (ΔF average showed a negative increase, while ΔR average showed a positive increase). Statistically significant differences were observed between the QLF analysis values at each diagnostic level (Figure 6, $p < 0.001$).

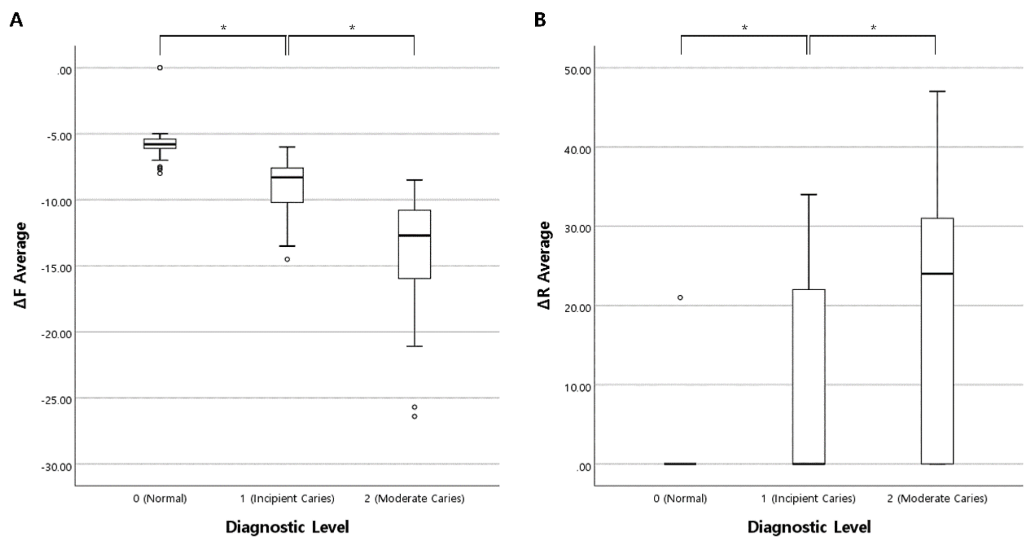


Figure 6. Box-Whisker plots for the comparison between diagnostic level and QLF analysis results (The asterisks indicate statistically significant differences between the groups with post hoc Mann-Whitney U test, $p < 0.001$) (A) Comparison with the values of ΔF (Fluorescence loss) average (B) Comparison with the values of ΔR (Red fluorescence) average

(3) Correlation between carious lesion volume and QLF analysis results

The absolute values of ΔF average, QS-Index, and ΔQ from QLF analysis showed a high correlation with the volume of carious lesions identified by micro-CT (Table 6, $r = 0.76–0.83$, $p < 0.001$).

Table 6. Correlation between results of quantitative light-induced fluorescence analysis with the cavity volume

		∣ΔF Average∣	QS-Index	ΔQ(%mm)
Cavity Volume (mm^3)	Correlation	0.83	0.81	0.76
	p value	< 0.001	< 0.001	< 0.001

p values from Spearman correlation test

QS = Quantitative light-induced fluorescence scoring

2. Clinical study

(1) Distribution of ICCMS, ICDAS II scores and QLF parameters followed by QS-Index

Distribution of ICCMS and ICDAS II scores according to dental caries severity in primary teeth based on QS-Index is shown in Table 7. It shows how the QS-Level for each type of dental caries corresponds with other scoring systems (ICCMS and ICDAS II). Both of the increase in each level of ICCMS and ICDAS II tended to follow the increase in QS-Level. Table 8 presents that mean values with standard deviations of ΔF and ΔR average increased with an increase in the QS-Level. There were significant differences in each level of the QS-Index of all carious lesions ($p < 0.005$).

Table 7. Distributions of ICCMS and ICDAS II scores according to the severity of the dental caries in primary teeth based on QS-Index

QS-Level	N	ICCMS score						ICDAS II score						
		0	1	2	3	4	5	0	1	2	3	4	5	6
0	341	251	83	5	1	1	0	337	4	0	0	0	0	0
1	270	95	123	36	15	0	1	24	227	19	0	0	0	0
2	138	13	74	33	15	3	0	0	3	120	15	0	0	0
3	129	5	19	22	26	37	20	0	2	7	43	31	24	22

Table 8. Means and standard deviation values of QLF parameters in the different lesions of dental caries depending on each level of the QS-Index in primary teeth

	QLF parameters			
	Occlusal		Proximal	
	|ΔF average|	ΔR average	|ΔF average|	ΔR average
QS-Level 0	2.35 ± 3.0[a] (1.58 – 3.13)	0 ± 0[a] (0 – 0)	3.93 ± 3.3[a] (3.54 – 4.30)	0.16 ± 1.9[a] (0.06 – 0.38)
QS-Level 1	7.46 ± 2.2[b] (6.98 – 7.95)	6.75 ± 11.2[b] (4.25 – 9.25)	7.09 ± 2.4[b] (6.75 – 7.43)	4.18 ± 8.7[b] (2.93 – 5.42)
QS-Level 2	9.66 ± 2.9[c] (8.94 – 10.38)	22.62 ± 12.9[c] (19.46 – 25.78)	9.96 ± 3.4[c] (9.16 – 10.76)	19.04 ±11.8[c] (16.28 – 21.81)
QS-Level 3	16.52 ± 7.0[d] (14.43 – 18.62)	39.27 ± 14.9[d] (34.80 – 43.73)	18.78 ± 9.6[d] (16.69 – 20.86)	39.83 ±19.5[d] (35.61 – 44.06)

Data are mean ± SD values.
Different letters within the same column indicate significant differences between groups by Scheffe's post-hoc analysis (cut off α of significant differences is 0.005).
The ranges of numbers in parentheses mean minimum and maximum values for 95% confidence intervals.

(2) Box-whisker plots for QLF parameters based on the ICCMS

ΔF average decreased with the ICCMS score increase (Figure 7a) and ΔR average increased with the ICCMS score increase (Figure 7b). Statistically significant differences appeared at each score ($p < 0.05$) except between 4 and 5, which indicates severe dental caries in both QLF parameters.

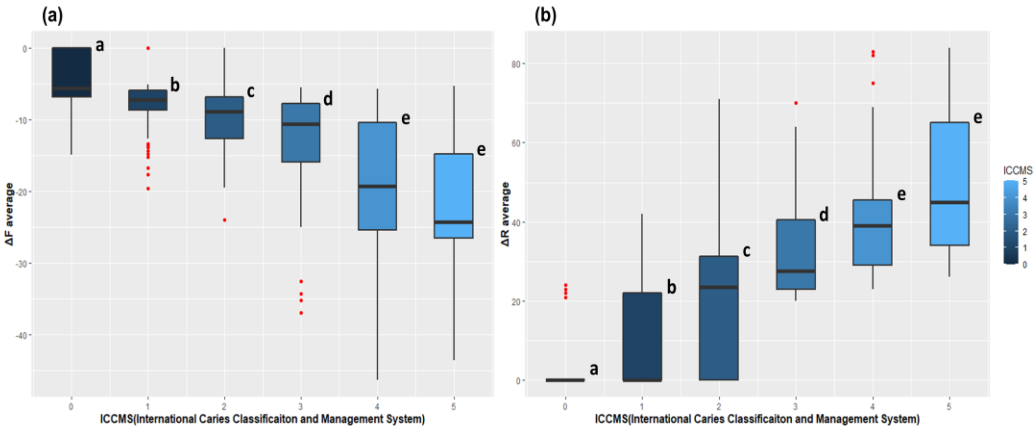


Figure 7. Box-whisker plots of QLF parameters - ΔF average (a) and ΔR average (b) related to the International Caries Classification and Management System (ICCMS)

The boxes mean the upper and lower quartile and horizontal lines show the median values. Different letters within the same graph indicate significant differences between groups (Using Kruskal-Wallis test with Mann-Whitney U test for post hoc, $p < 0.005$).

(3) Evaluation of the detection performance of QLF parameters for each type of incipient and moderate caries in primary teeth

The cut-off values, sensitivity, specificity, and AUROC of QLF parameters (ΔF and ΔR average) to detect incipient caries (Table 9) and moderate caries (Table 10) in primary teeth were calculated. For detection of incipient caries, cut off values were determined for QLF parameters (ΔF average = -7.75, and ΔR average = 20.5). For moderate caries, the cut-off value in case of occlusal surface (ΔF average = -10.85, and ΔR average = 22.5) was slightly higher than that of proximal surface (ΔF average = -9.15, and ΔR average = 21.5). Sensitivity to detect incipient caries was good (0.72–0.88, ΔR average of proximal surface was the lowest value). All results of sensitivity analysis were better for moderate caries (0.81–0.91, ΔF average more than 0.90). Specificity of QLF parameters also demonstrated good results (0.74–0.96, ΔR average in proximal caries more than 0.90), but the results of ΔF average for proximal caries (0.74 for both incipient and moderate caries) and ΔR average for occlusal moderate caries (0.76) were relatively low. The AUROC for detection of incipient caries was reliable for both surfaces (0.861–0.940). In moderate caries, the surfaces showed higher AUROC values (0.912–0.940) than in incipient caries. In both incipient and moderate caries, QLF parameters of occlusal surfaces showed higher AUROC values than proximal surfaces.

Table 9. The cut-off value, sensitivity, specificity, and AUROC of QLF parameters to detect incipient caries in primary teeth

	QLF parameters (Incipient dental caries)			
	Occlusal		Proximal	
	ΔF average	ΔR average	ΔF average	ΔR average
Cut-off value	-7.75	20.5	-7.75	20.5
Sensitivity	0.88	0.83	0.83	0.72
Specificity	0.82	0.93	0.74	0.96
AUROC (95% CI)	0.940 (0.913–0.967)	0.911 (0.872–0.950)	0.866 (0.835–0.897)	0.861 (0.824–0.898)

CI = confidence interval.

Table 10. The cut-off value, sensitivity, specificity, and AUROC of QLF parameters to detect moderate caries in primary teeth

	QLF parameters (Moderate dental caries)			
	Occlusal		Proximal	
	ΔF average	ΔR average	ΔF average	ΔR average
Cut-off value	-10.85	22.5	-9.15	21.5
Sensitivity	0.90	0.89	0.91	0.81
Specificity	0.83	0.76	0.74	0.91
AUROC (95% CI)	0.940 (0.908–0.973)	0.920 (0.872–0.968)	0.912 (0.884–0.941)	0.921 (0.887–0.955)

CI = confidence interval.

(4) ROC analysis of logistic regression model with combined ΔF and ΔR

We also examined caries detection ability through a receiver operating characteristic (ROC) analysis of logistic regression model with combined ΔF and ΔR. When values of ΔR average were added to ΔF average, the AUROC was increased significantly for occlusal moderate (Figure 8b, 0.943, $p < 0.001$), proximal incipient (Figure 8c, 0.902, $p < 0.001$), and proximal moderate caries (Figure 8d, 0.940, $p < 0.001$). In occlusal incipient caries, the AUROC was increased, but the increase was not statistically significant (Figure 8a, 0.957, $p = 0.388$). There were more improvements in the AUROC with the logistic regression model of QLF parameters in case of proximal caries than occlusal caries.

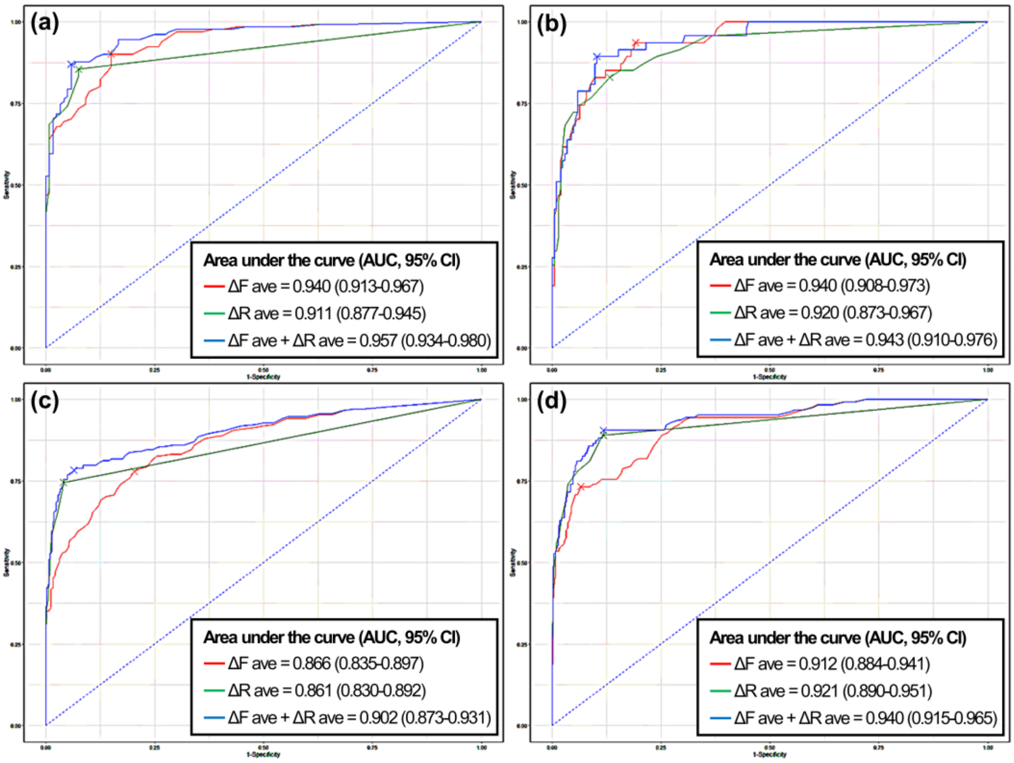


Figure 8. ROC curves and corresponding areas under the curve (AUCs) of QLF parameters in dental caries of primary teeth following locations and depth of caries lesions – occlusal incipient caries (a), occlusal moderate caries (b), proximal incipient caries (c), and proximal moderate caries (d). ΔF average + ΔR average show AUCs of logistic regression models for ΔF average together with additional predictors ΔR average. CI = confidence interval.

IV. Discussion

Initially, we compared the results of *in vitro* and clinical study with previous researches. QLF method showed excellent caries detection ability in both *in vitro* (sensitivity 0.75-0.95, specificity 0.82-0.95, and AUROC 0.88-0.98) and clinical study (sensitivity 0.72–0.91, specificity 0.74–0.96, and AUROC 0.861–0.940) except the results of ΔR average in proximal caries under *in vitro* condition. Sensitivity was prioritized when determining cut-off values, sensitivity, and specificity in the ROC analysis, because high sensitivity could be advantageous to detect dental caries in actual clinical conditions with QLF method.

Although there may be some differences in the execution, these results can be compared with those of other previous studies. Park SW et al. reported that, *in vitro* studies on occlusal caries detection in permanent teeth, QLF exhibited ΔF sensitivity of 0.92–1.00, specificity of 0.69–1.00, and AUROC of 0.90–0.97, while ΔR sensitivity was 0.85–1.00, specificity was 0.72–0.93, and AUROC was 0.84–0.91 (Park et al., 2019). Ko HY et al. investigated the use of ΔF in proximal caries detection for permanent teeth, reporting sensitivity of 0.64–0.75, specificity of 0.84–0.88, and AUROC of 0.76–0.80 (Ko et al., 2015). A clinical study on adult patients reported that ΔF / ΔR had sensitivity of 0.825 / 0.842, specificity of 0.816 / 0.879, and AUROC of 0.860 / 0.902 when detecting dentin caries on proximal surfaces (Kim et al., 2017). Another clinical study with permanent teeth to detect occlusal and proximal dental caries with QLF method showed the results of ΔF average. They used both of QLF devices (Qraycam pro and

Qraypen C), however, results using Qraypen C would be better to compare with the results of our study. For occlusal caries, QLF method got higher values (sensitivity 0.89-1.00, specificity 0.75-0.96, AUROC 0.92-0.99) than proximal caries (sensitivity 0.00-0.62, specificity 0.62-0.79, AUROC 0.60-0.67) (Oh et al., 2022).

Recently, an *in vitro* study was reported to compare the utility of various diagnostic methods, including Quantitative Light-induced Fluorescence (QLF), for detecting occlusal caries in primary teeth. In the study by Diniz MB et al. (Diniz et al., 2019), the sensitivity, specificity, and accuracy (calculated using the McNemar test) of ΔF for enamel caries in primary teeth were reported as 0.68, 0.80, and 0.71, respectively. While for dentin caries, these values were 0.93, 0.87, and 0.88, respectively. Cho HJ et al. also reported about proximal caries detection ability for primary teeth in clinical conditions with Qraypen C device. The results of ΔF showed 0.677-0.734 of sensitivity, 0.678-0.751 of specificity, 0.702-0.794 of AUROC; and ΔR showed 0.273-0.519 of sensitivity, 0.981-0.989 of specificity, 0.631-0.750 of AUROC (Cho et al., 2021). It was found that results about caries detection ability of QLF in this study were similar or even higher than those obtained in the other previous studies employing QLF for caries detection (Figure 9).

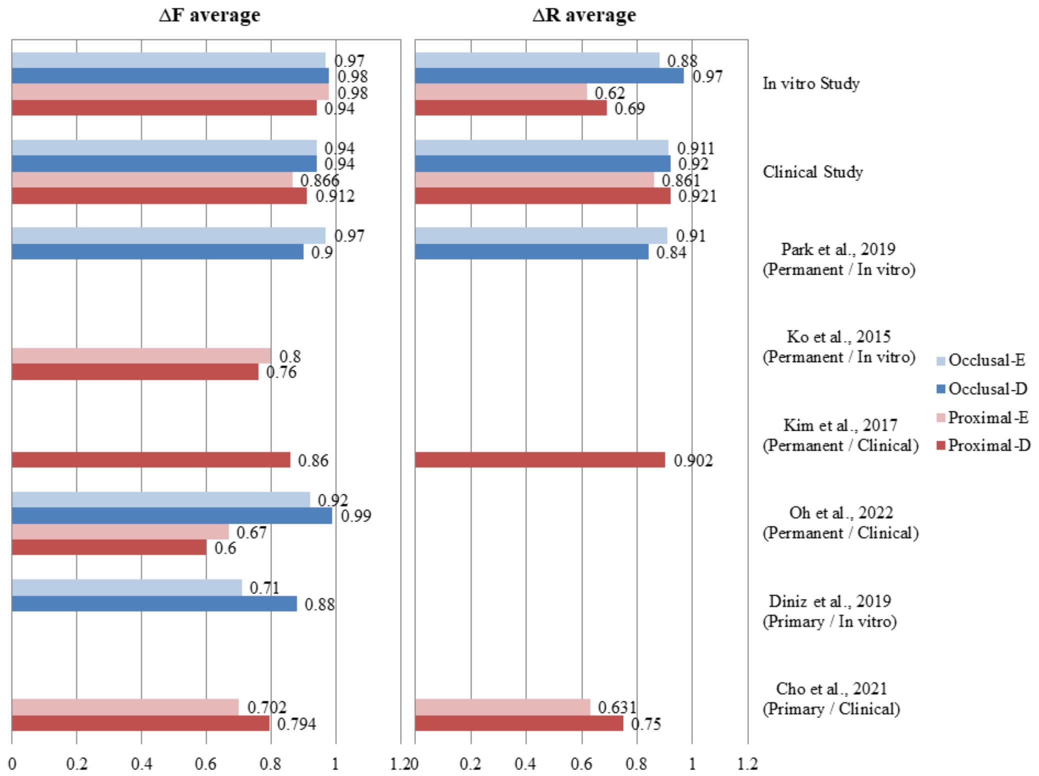


Figure 9. Comparison the results of reliability for QLF caries detection in previous studies with those of *in vitro* and clinical studies – Occlusal-E: enamel caries in occlusal surface, Occlusal-D: dentin caries in occlusal surface, Proximal-E: enamel caries in proximal surface, Proximal-D: dentin caries in proximal surface; numeric values for reliability of each study cited the results of AUROC or accuracy test.

Notably, ΔF values exhibited higher reliability compared to results of previous studies in both occlusal and proximal caries detection, which may be attributed to the use of earlier generation QLF device and analysis software in the previous study. Over time, continuous advancements have been made in both the QLF equipment and analytical programs. Regarding the development of QLF devices, Park SW et al. (Park et al., 2019) described that since the introduction of QLF technology in the 1980s, it has progressed to the third generation. These advancements include differences in the type of light source (e.g., the use of LEDs), variations in the wavelength range of emitted light, changes in the background color of captured images, and modifications to fluorescence detection filters. Qraypen C, which was used in our study, can be considered as a third-generation portable QLF device. Additionally, QLF image analysis software has undergone continuous version upgrades, enhancing usability and pixel-based analytical functions. These improvements have now made it possible to detect enamel caries, corresponding to early carious lesions.

Comparing the cut-off values obtained in this study with those reported for recent researches (Figure 10), enamel caries cut-off values for permanent teeth were reported as ΔF = -10.3, ΔR = 20, and dentin caries cut-off values as ΔF = -13.1, ΔR = 29.5 for occlusal lesions. Proximal caries cut-off values were ΔF = -13.8 for enamel caries and ΔF = -28.3 for dentin caries (Ko et al., 2015; Park et al., 2019). One of other studies with permanent teeth showed cut-off values for proximal dentin caries as ΔF = -12.4, ΔR = 23.3 (Kim et al., 2017). Another clinical study for permanent teeth reported cut-off values of ΔF in both occlusal caries (-12.9 for enamel caries, -21.4 for dentin caries) and proximal caries (-10.4 for enamel caries, -14.3 for dentin caries) (Oh et al., 2022). Although direct comparisons are difficult, a clinical study on treatment decision making with regard to dental caries in permanent teeth showed that a ΔF cut-off values of -12 for incipient caries needed preventive resin restoration only whereas a value of -23 for moderate caries needed operative treatments (Alammari et al., 2013). On the other hand, cut-off values from the previous researches with primary teeth were similar to results of our *in vitro* and clinical studies. In the study by Diniz MB et al., the cut-off values for enamel and dentin caries were determined to be -7.4 and -13.8, respectively (Diniz et al., 2019). Another clinical study of QLF method to detect proximal caries in primary teeth reported cut-off values as -5.35 for enamel caries and -6.15 for dentin caries (Cho et al., 2021).

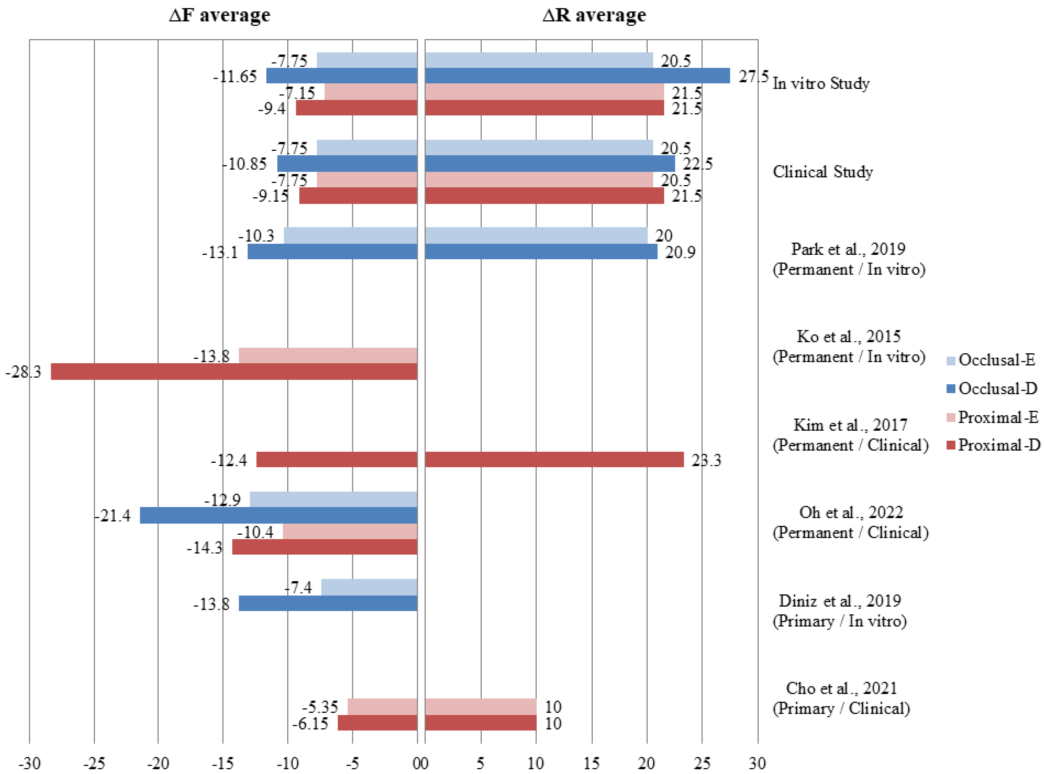


Figure 10. Comparison the results of cut-off values for QLF caries detection in previous studies with those of *in vitro* and clinical studies – Occlusal-E: enamel caries in occlusal surface, Occlusal-D: dentin caries in occlusal surface, Proximal-E: enamel caries in proximal surface, Proximal-D: dentin caries in proximal surface

The relatively lower cut-off values observed in primary teeth in our study would be caused by the histological difference between primary and permanent teeth. The histological features of primary teeth such as thin enamel layer or translucency due to less mineralization that may allow the QLF device to detect fluorescence changes better when compared with permanent teeth (De Menezes Oliveira et al., 2010; Wilson and Beynon, 1989). This may be because light entering enamel easily reaches the level of DEJ (Dento-enamel junction) and dentin where the chance of light absorption by fluorophores which remit the fluorescence is a magnitude higher (Van der Veen and de Jong, 2000). If QLF can catch smaller changes of fluorescence of the carious lesion in primary teeth than in permanent teeth, it could be more efficient to use QLF as a caries detection method for children. Moreover, the results of QLF for the caries detection were comparable with the results of visual inspections or radiographic examinations. Especially, sensitivity of QLF method showed higher results than these conventional caries detection methods (Gimenez et al., 2015; Gomez et al., 2013). It is encouraging that QLF not only showed similar caries detection ability to the conventional methods, but also could contribute to early detection of dental caries. .

Meanwhile, the results of ΔR average showed relatively low values especially in proximal surface at *in vitro* experiment, and the reason for this is that *in vitro* experiment used extracted teeth. As mentioned earlier, red fluorescence expressed as ΔR is a value that indicates the porphyrin, a metabolites of bacteria on the teeth. Therefore, new porphyrin synthesis by bacterial metabolism could not be expected in extracted teeth. The length of time the extracted teeth were stored may also affect the results; although we used teeth with a short storage period of less than 6 months in our *in vitro* experiment, some of the porphyrin may had been washed out during storage. These are the reasons why we should make an exception for the results of ΔR average in proximal caries under *in vitro* condition.

A detailed analysis of the ROC results in this study reveals that, in most cases, the values associated with ΔF were higher than those of ΔR. This suggests that detecting dental caries based on fluorescence loss may be a more reliable approach than red fluorescence detection. The difference in results between ΔF and ΔR was particularly pronounced for early-stage enamel caries compared to dentin caries. This can be attributed to the lower presence of bacteria emitting red fluorescence in early-stage lesions than in deeper carious lesions, as demonstrated in previous research (Lennon et al., 2005). Therefore, fluorescence loss analysis could appear to be a more accurate diagnostic method in the early stages of caries progression.

However, red fluorescence could have an important role in the QLF caries detection method. An in vitro experiment to monitor the degree of maturation of dental biofilms by observing the red fluorescence emitted from the biofilms that were grown on bovine enamel discs, reported that red fluorescence increased according to biofilm maturation and was significantly associated with the cariogenicity of the biofilm (Kim et al., 2014). Another clinical study published in 2022 reported the correlation between dental caries activity and QLF parameters with identified bacteria collected from dentin caries of patients. The ΔR results showed a statistically significant difference between the inactive and active lesion. Furthermore, Lactobacillus, a representative acidogenic bacterium, was found to be significantly higher in the active carious lesions (Kim et al., 2022). This is a result that shows the ΔR value and bacterial composition are followed by the activity of the caries lesion, not just the difference between early and advanced caries. This may also explain why ΔR values are lower in early stage of dental caries with low activity.

Some of the lower results of ΔR can be attributed to its poor detection results of proximal caries. In the same way, the overall accuracy of QLF in detecting occlusal caries was superior to that in detecting proximal caries, and this result follows a trend consistent with findings from previous studies on both permanent and primary teeth. The lesions on occlusal surfaces can be confirmed directly; however, in case of proximal surfaces, fluorescence from the carious lesions can only be seen through the marginal ridge. QLF devices detect fluorescence loss less effectively in the presence of thick mineralized enamel layers, making it difficult to identify minor changes of fluorescence from the carious lesions (Ando et al., 2003). A previous study pointed out that 75% of proximal carious lesions exist at the proximal contact area and 25% are below the contact point (Arnold et al., 1998). In proximal caries, unless the carious lesion is large or severely cavitated, a thick marginal ridge typically overlies the carious lesion. This enamel structure makes a challenge for detecting both fluorescence loss and red fluorescence. The closer carious lesions are to the marginal ridge, the better detection of proximal caries with QLF in the occlusal direction (Ko et al., 2015). The current approach of detecting fluorescence changes by QLF devices from the occlusal direction may therefore be a limiting factor in caries detection. To enhance the detection ability of proximal caries using QLF, considering the location, size, and width of the carious lesion, QLF light sources should be applied not only from the occlusal direction but also from the buccal or lingual aspects to improve detection sensitivity.

In case of occlusal moderate caries, AUROC results of ΔF revealed higher values than those of ΔR in our study, especially in our clinical study. The relatively low AUROC of ΔR in occlusal caries may be due to red fluorescence from the plaques in pits and fissures, which can lead to false positive results in the QLF analysis. Red fluorescence released from the remaining plaque can give rise to a false perception of increased cariogenic potential of a lesion. Although we cleaned patient's teeth thoroughly with professional instruments before commencement of the study to minimize the effect of debris and plaque, it is impossible to completely remove bacteria and their metabolites in pits and fissures (Manton and Messer, 1995). Despite of these variations, QLF parameters showed excellent AUROC values in all types of carious lesions.

For *in vitro* study, radiographic examination, which is considered the optimal standard for caries detection, was used to classify the stages of dental caries (Gomez et al., 2013). Among radiographic methods, micro-CT, which allows for three-dimensional image reconstruction, was utilized to minimize errors due to the overlapping of structures observed in conventional radiographs. The use of micro-CT, which had not been employed in previous QLF studies, is particularly significant as it enabled direct comparison between QLF analysis results and actual caries lesion volumes.

In correlation analysis, the absolute value of ΔF average, QS-Index, ΔQ and carious lesion volume showed a higher than 0.76 correlation coefficient. The fluorescence loss represented by ΔF directly reflects mineral loss in the tooth (Gmür et al., 2006; Jallad et al., 2015), demonstrating a strong association with lesion depth, which explains its high correlation with volume measurements obtained via micro-CT. Similarly, QS-Index exhibited a strong correlation with micro-CT volume results. This index was developed as a scoring system for assessing caries progression using only the QLF device, without requiring analysis software, making it a cost-effective and time-efficient method with high reproducibility (0.86–0.94) (Jung et al., 2018). Since QS-Index considers both fluorescence loss and increased red fluorescence, it demonstrated a high correlation with the volumetric results of carious lesions obtained via radiographic examination and three-dimensional reconstruction. More importantly, given its relative simplicity, QS-Index can be expected clinical applications. ΔQ is a value that integrates both fluorescence loss and lesion size, thus, it could be an indicator of mineral loss with relation to size and severity of the carious lesions (Pretty et al., 2002). ΔQ was expected to have the highest correlation with the actual carious volume, but conversely, the results were the lowest. This might be due to errors of the QLF analysis program. Some of QLF images taken by Qraypen C in *in vitro* experiment showed certain areas where shadows were created due to the structure of the primary teeth, and it seems that the QLF analytic program sometimes recognized these areas as caries lesions. More accurate results could be obtained through improvements in the protocol or software program of QLF analysis.

When classifying QLF analysis results according to diagnostic levels, both the mean values of ΔF and ΔR increased progressively followed with each stage, and statistically significant differences were observed at each level. However, for ΔR, there was a relatively wide interquartile range overlap. In particular, between stage 0 and 1, the median values were same, which suggests that in early carious lesions where red fluorescence changes are not sufficient, the QLF analysis software may not numerically express these differences effectively. It is anticipated that comparative analysis with a larger sample size could yield statistically more reliable results. Thus, future studies would better to focus on a larger number of teeth samples for analysis with further developed of QLF equipment and analysis software.

Consequently, a limitation of this *in vitro* study is the relatively small number of sample teeth and tooth surfaces analyzed. When detecting dental caries using QLF technology, it is essential to distinguish between occlusal and proximal surfaces to yield meaningful results. Therefore, securing an adequate number of teeth for analysis is crucial. Additionally, excessively low or high values of fluorescence results during the QLF analysis process sometimes needed to be excluded from statistical processing due to software limitations. Furthermore, the distribution and relationship between QLF parameters (ΔF and ΔR average) and radiographic examination results were not assessed based on ICCMS stages, but on diagnostic levels. Although these diagnostic levels were used for clinical utility, employing a more detailed ICCMS classification could have allowed for a more precise evaluation of caries progression. However, the distribution of QLF analysis values across ICCMS stages did not show statistically significant differences. A larger number of tooth surfaces might have led to different distributions of QLF results according to ICCMS classification. Therefore, future studies should prioritize securing an adequate sample size.

During the QLF imaging process of this *in vitro* study, the direction of light exposure, the fixed position of primary molars, and the type of tooth could introduce subtle artifacts, such as shadowing or overly bright areas, which also may influence analysis results. Although QLF images were captured at a consistent 90-degree overhead angle relative to the specimens, variations in tooth morphology and positioning could cause minor deviations in the irradiation of light from a QLF device. Especially, proximal surfaces may appear darker due to shadowing, while some areas of the occlusal surface may appear excessively bright due to intense light exposure. Given that the QLF analysis software compares fluorescence differences between QLF images and white-light images, these factors could influence the results. Therefore, continued advancements in imaging resolution of QLF devices and analysis software, with standardized imaging protocols, are necessary.

Despite of these limitations, the results of this *in vitro* study for QLF technology to detect dental caries in primary molars and compared with micro-CT, which is a highly reliable reference, may provide meaningful implications in the process of finding ways to complement traditional caries detection methods. It could also suggests that QLF detection methods may have sufficient reliability and accuracy in detecting dental caries in primary teeth and can be safely used clinically in pediatric dentistry along with visual inspection and radiographic examination.

Although significant results were obtained in this *in vitro* study, the caries detection ability and reliability of QLF methods of primary teeth in clinical conditions are not yet clearly revealed. Hence, we also focused on evaluating the efficacy of QLF technology in clinical diagnosis of dental caries in primary teeth. In clinical study, QLF analysis can provide information regarding quantitative changes taking place during dental caries progression. Among the QLF parameters obtained through our analysis, ΔF average and ΔR average were mainly used for our clinical study because they are considered to be more representative.

At first, when ΔF average and ΔR average were compared to the ICCMS radiographic scores, they showed the same tendency. QLF results increased with the severity of dental caries according to the ICCMS level and were appropriately distributed to each level with statistically significant differences. Thus, it could be said that QLF can indicate the states of carious lesions of primary teeth in clinical conditions similar to the radiographic examination that is still considered as the gold standard for caries detection. In addition, it was thought that these analytic results of QLF can distinguish the progression of dental caries at dentin level better than others because the difference between the mean values corresponding to level 3 and 4 of ICCMS was the largest.

Secondly, QS-Index is a scoring process of QLF images to infer progression of dental caries easily without the use of any specific software. It can indicate the severity of carious lesion and related bacterial activity together. Recent studies on permanent teeth have reported that QS-Index is cost-effective, timesaving, and highly reproducible. In addition, the sensitivity was 0.895–0.912, specificity was 0.563–0.839, and AUROC was 0.807– 0.929 for detecting occlusal caries (Jung et al., 2018). For proximal caries, the sensitivity was 0.702–0.894, specificity was 0.835–0.951, and AUROC was 0.826–0.864 (Kim et al., 2017). As per the results of this clinical study, QLF analysis results were evenly distributed according to the QS-Index levels. On confirming the relation between the QS-Index and other scoring systems (ICCMS and ICDAS II), the QS-Index showed a tendency to increase with increasing ICCMS and ICDAS scores. This indicates that the QS-Index can represent dental caries severity clinically in primary teeth similar to other detection methods. However, a weakness of the QS-Index is that each level of that was oversimplified, especially for dental caries with greater severity than moderate caries. The number of tooth surfaces for QS-Level 3 corresponded to several levels of other scoring systems representing deep carious lesions (levels 3–5 of ICCMS or 4–6 of ICDAS II). Nevertheless, we hope that QS-Index of primary teeth, which was introduced in this clinical study at first, can be helpful in the clinical judgement about dental caries in children.

As mentioned above along with the results of in vitro experiment, ROC analysis reported that QLF showed high reliability even in clinical conditions; however, we also used logistic regression analysis to obtain further improved accuracy of the QLF method. In all types of dental caries, the AUROC of the logistic regression model were higher than the results obtained with ΔF or ΔR alone in the clinical study. These differences were more significant for proximal caries. Thus, it can be said that it is efficient and accurate to check the bacterial activity expressed by red fluorescence combined with mineral loss. Since there were the differences in detection ability of ΔF or ΔR between enamel and dentin caries, the importance of each parameter should be set differently according to the depth of the carious lesion when considering two QLF parameters together (For example, ΔF values would be more important in enamel caries, both ΔF and ΔR should be considered equally for dentin caries). Through subsequent studies, if a method is developed which can appropriately combine ΔF and ΔR to show comprehensive figures according to the types of dental caries, we believe that it will markedly improve the caries detection ability of QLF.

The QLF technology also has its limitations in the use of QLF devices under clinical conditions and analysis procedures. Above all, obtaining high-quality QLF images can be challenging depending on patient cooperation, which may lower the reliability of analysis results. This is particularly relevant in pediatric dentistry, children and adults with severe gag reflexes may experience discomfort during imaging procedure with QLF devices. Additionally, QLF imaging procedure requires the assistance of additional personnel, and using retractors such as W-block (AIOBIO, Seoul, Republic of Korea) or mouth props that can help patients maintain a stable mouth-opening position for taking QLF images comfortably. Moisture in the oral cavity, saliva, and mouth-breathing habits may cause blurring in QLF images. Therefore, appropriate air blowing and suction are necessary to ensure clear imaging, along with maintaining proper focal distance and preventing image blurring caused by shaking devices during capture. It is also difficult to be free from the effects of the accumulated dental plaque on the final results. Excessive tooth cleaning or flossing for better quality of analysis induced bleeding which is again disadvantageous. We tried to establish the same conditions for every patient included in the clinical study while using QLF detection, but problems arose because of variability in level of cooperation of every child and contrasts of light or shadow in QLF images. These factors could have led to skewed results from the actual state of dental caries.

Another weakness of this clinical study was the relatively low values of inter examiner coefficients of ICDAS II or ICCMS because these scoring systems served as the basis for many analysis processes. In the ROC analysis, for example, the diagnosis of enamel caries or dentin caries was determined using ICCMS (radiographic examinations) and ICDAS II (visual inspections) together for greater reliability. Both visual inspections and radiographic examinations (available as gold standards for caries detection) were considered in the analysis procedure, but if repeated tests show different results of these scoring systems for the same carious lesion, the reliability of the diagnostic criteria may decrease. Establishing more reliable standards such as 3D radiographic data using CT radiography as our *in vitro* study or histological analysis data will be needed in subsequent studies.

Visual inspection and radiographic examination are considered basic caries detection methods and will remain an essential part of the dental examination process in clinical conditions. However, the guideline of the American Dental Association (ADA) does not recommend radiography for dental caries screening (Affairs, 2006). As such, the QLF method may be a reliable method for screening and detecting dental caries in children without such restrictions. This method can complement visual inspection and may serve as a good alternative to radiographic examination, which has known detrimental health effects due to associated radiation exposure. Therefore, it is essential to consider that visual inspection and radiographic examination may be required in conjunction with findings of QLF method to enhance diagnostic accuracy for dental caries.

V. Conclusion

This study evaluated the accuracy and reliability of QLF technology using a portable device (Qraypen C) in detecting dental caries in primary teeth under both *in vitro* and clinical conditions. QLF caries detection method demonstrated sufficient accuracy and reliability for detecting enamel caries and dentin caries in primary teeth except the *in vitro* results of ΔR average in proximal caries. The reliability of the QLF method was found to be similar or slightly higher than those of previous QLF studies. The results of QLF analysis (i.e., ΔF or ΔR values) and QS-Index, which was presented in this study for primary teeth, can satisfactorily represent the progression of dental caries. These were confirmed by comparison with the radiographic examination results such as the diagnostic levels or ICCMS. The volume of carious lesions measured by micro-CT showed a strong correlation with ΔF average, QS-Index, and ΔQ, thus, these parameters could be sufficiently used to determine the severity of dental caries. Although it can be difficult sometimes to obtain good quality images in children, detection with the Qraypen C is a useful and harmless way for caries screening. Thus, QLF used together with traditional caries detection methods can make the caries detection process more efficient and precise.

VI. References

Affairs ADACoS (2006). The use of dental radiographs: update and recommendations. *The Journal of the American Dental Association* 137(9): 1304-1312.

Alammari M, Smith P, De Jong EDJ, Higham S (2013). Quantitative light-induced fluorescence (QLF): a tool for early occlusal dental caries detection and supporting decision making in vivo. *Journal of dentistry* 41(2): 127-132.

Ando M, Ferreira-Zandoná AG, Eckert GJ, Zero DT, Stookey GK (2017). Pilot clinical study to assess caries lesion activity using quantitative light-induced fluorescence during dehydration. *Journal of Biomedical Optics* 22(3): 035005-035005.

Ando M, Schemehorn B, Eckert G, Zero D, Stookey G (2003). Influence of enamel thickness on quantification of mineral loss in enamel using laser-induced fluorescence. *Caries Research* 37(1): 24-28.

Angmar-Månsson B, Ten Bosch J (2001). Quantitative light-induced fluorescence (QLF): a method for assessment of incipient caries lesions. *Dentomaxillofacial Radiology* 30(6): 298-307.

Arnold W, Gaengler P, Kalkutschke L (1998). Three-dimensional reconstruction of approximal subsurface caries lesions in deciduous molars. *Clinical Oral Investigations* 2: 174-179.

Cho H, Kim H, Song J-S, Shin TJ, Kim J-W, Jang K-T, et al. (2021). Detecting of Proximal Caries in Primary Molars using Pen-type QLF Device. *Journal of the korean academy of Pediatric Dentistry* 48(4): 405-413.

De Menezes Oliveira MAH, Torres CP, Gomes-Silva JM, Chinelatti MA, De Menezes FCH, Palma-Dibb RG, et al. (2010). Microstructure and mineral composition of dental enamel of permanent and deciduous teeth. *Microscopy research and technique* 73(5): 572-577.

Diniz MB, Campos PH, Wilde S, Cordeiro RdCL, Zandona AG (2019). Performance of light-emitting diode device in detecting occlusal caries in the primary molars. *Lasers in medical science* 34: 1235-1241.

Ekstrand K, Luna L, Promisiero L, Cortes A, Cuevas S, Reyes J, et al. (2011). The reliability and accuracy of two methods for proximal caries detection and depth on directly visible proximal surfaces: an in vitro study. *Caries Research* 45(2): 93-99.

Ekstrand KR, Martignon S, Ricketts DJN, Qvist V (2007). Detection and activity assessment of primary coronal caries lesions: a methodologic study. *Operative dentistry* 32(3): 225-235.

Fejerskov O, Nyvad B, Kidd E (2015). Dental caries: the disease and its clinical management. John Wiley & Sons.

Felix Gomez G, Eckert GJ, Ferreira Zandona A (2016). Orange/red fluorescence of active caries by retrospective quantitative light-induced fluorescence image analysis. *Caries Research* 50(3): 295-302.

Gimenez T, Piovesan C, Braga MM, Raggio DP, Deery C, Ricketts DN, et al. (2015). Visual inspection for caries detection: a systematic review and meta-analysis. *Journal of dental research* 94(7): 895-904.

Gmür R, Giertsen E, van der Veen MH, de Josselin de Jong E, ten Cate JM, Guggenheim B (2006). In vitro quantitative light-induced fluorescence to measure changes in enamel mineralization. *Clinical Oral Investigations* 10: 187-195.

Gomez J, Tellez M, Pretty I, Ellwood R, Ismail A (2013). Non-cavitated carious lesions detection methods: a systematic review. *Community Dentistry and Oral Epidemiology* 41(1): 55-66.

Ismail AI, Pitts NB, Tellez M, Classification AotIC, System M The International Caries Classification and Management System (ICCMS™) an example of a caries management pathway. Proceedings of the BMC oral health; 2015. p. 1-13.

Jallad M, Zero D, Eckert G, Ferreira Zandona A (2015). In vitro detection of occlusal caries on permanent teeth by a visual, light-induced fluorescence and photothermal radiometry and modulated luminescence methods. *Caries Research* 49(5): 523-530.

Jung E-H, Lee E-S, Jung H-I, Kang S-M, de Jong EdJ, Kim B-I (2018). Development of a fluorescence-image scoring system for assessing noncavitated occlusal caries. *Photodiagnosis and Photodynamic Therapy* 21: 36-42.

Kim CH, Bae K, Lee TY, Song JS, Kim S-O, Kang C-M (2022). Assessment of dental caries lesion activity status using quantitative parameters obtained from the quantitative light-induced fluorescence method and difference of microbial distribution in primary molars. *Photodiagnosis and Photodynamic Therapy* 39: 102942.

Kim E-S, Lee E-S, Kang S-M, Jung E-H, de Jong EdJ, Jung H-I, et al. (2017). A new screening method to detect proximal dental caries using fluorescence imaging. *Photodiagnosis and Photodynamic Therapy* 20: 257-262.

Kim H-E, Kim B-I (2017). Analysis of orange/red fluorescence for bacterial activity in initial carious lesions may provide accurate lesion activity assessment for caries progression. *Journal of evidence based dental practice* 17(2): 125-128.

Kim HE, Kwon HK, Kim BI (2013). Recovery percentage of remineralization according to severity of early caries. *Am J Dent* 26(3): 132-136.

Kim Y-S, Lee E-S, Kwon H-K, Kim B-I (2014). Monitoring the maturation process of a dental microcosm biofilm using the Quantitative Light-induced Fluorescence-Digital (QLF-D). *Journal of dentistry* 42(6): 691-696.

Ko H-Y, Kang S-M, Kim HE, Kwon H-K, Kim B-I (2015). Validation of quantitative light-induced fluorescence-digital (QLF-D) for the detection of approximal caries in vitro. *Journal of dentistry* 43(5): 568-575.

Lee E-S, Kang S-M, Ko H-Y, Kwon H-K, Kim B-I (2013). Association between the cariogenicity of a dental microcosm biofilm and its red fluorescence detected by Quantitative Light-induced Fluorescence-Digital (QLF-D). *Journal of dentistry* 41(12): 1264-1270.

Lee J-H, Kim D-H, Jeong S-N, Choi S-H (2018). Detection and diagnosis of dental caries using a deep learning-based convolutional neural network algorithm. *Journal of dentistry* 77: 106-111.

Lennon AM, Buchalla W, Brune L, Zimmermann O, Gross U, Attin T (2005). The ability of selected oral microorganisms to emit red fluorescence. *Caries Research* 40(1): 2-5.

Manton DJ, Messer LB (1995). Pit and fissure sealants: another major cornerstone in preventive dentistry. *Australian dental journal* 40(1): 22-29.

Mo K-H, Yoon J-H, Kim S-G, Lee S-H (2004). Detection of proximal caries using laser fluorescence. *Journal of the korean academy of Pediatric Dentistry* 31(2): 323-330.

Newman B, Seow W, Kazoullis S, Ford D, Holcombe T (2009). Clinical detection of caries in the primary dentition with and without bitewing radiography. *Australian dental journal* 54(1): 23-30.

Nyvad B, Machiulskiene V, Baelum V (2003). Construct and predictive validity of clinical caries diagnostic criteria assessing lesion activity. *Journal of dental research* 82(2): 117-122.

Oh SH, Choi J-Y, Kim S-H (2022). Evaluation of dental caries detection with quantitative light-induced fluorescence in comparison to different field of view devices. *Scientific Reports*

12(1): 6139.

Park S-W, Kim S-K, Lee H-S, Lee E-S, de Jong EdJ, Kim B-I (2019). Comparison of fluorescence parameters between three generations of QLF devices for detecting enamel caries in vitro and on smooth surfaces. *Photodiagnosis and Photodynamic Therapy* 25: 142-147.

Pretty I, Hall A, Smith P, Edgar W, Higham S (2002). The intra-and inter-examiner reliability of quantitative light-induced fluorescence (QLF) analyses. *British dental journal* 193(2): 105-109.

Stookey G (2004). Optical methods—quantitative light fluorescence. *Journal of dental research* 83(1_suppl): 84-88.

Stookey GK (2005). Quantitative light fluorescence: a technology for early monitoring of the caries process. *Dental Clinics* 49(4): 753-770.

Van der Veen M, de Jong EdJ (2000). Application of quantitative light-induced fluorescence for assessing early caries lesions. *Monogr Oral Sci* 17: 144-162.

Volgenant CM, van der Veen MH, de Soet JJ, ten Cate JM (2013). Effect of metalloporphyrins on red autofluorescence from oral bacteria. *European Journal of Oral Sciences* 121(3pt1): 156-161.

Wilson PR, Beynon AD (1989). Mineralization differences between human deciduous and permanent enamel measured by quantitative microradiography. *Arch Oral Biol* 34(2): 85-88.

Abstract in Korean (국문요약)

정량형광분석(QLF)을 이용한 유치의 치아우식증 탐지와 진단 효능 확인을 위한 *in vitro* 및 임상연구

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조 경 현

지도교수: 송 제 선

이 연구의 목적은 *in vitro* 및 임상적 환경에서 정량형광분석(QLF) 방식의 치아우식증 탐지를 평가하는 것이다. 나아가 우식병소의 부피와 QLF 분석결과 사이의 상관관계를 확인하고, 유치에 해당하는 QLF 분석지수인 QS-Index를 제시하였다.

In vitro 연구에서는 총 125개 유치 치면의 치아우식증을 휴대용 QLF 장비로 확인하였다. 또한 치아우식을 분류하고 우식병소 부피의 계산을 위해 micro-CT 방사선검사를 시행하였다. 인접면 우식의 ΔR 결과를 제외하면, QLF 분석결과는 유치의 치아우식증 진단에 충분한 수준의 신뢰도를 보였다(민감도 0.75-0.94, 특이도 0.82-0.95, AUROC 0.88-0.98). ΔF average, QS-Index 및 ΔQ와 우식병소 부피는 통계적으로 유의한 상관성을 확인할 수 있었다($r = 0.76-0.83$, $p < 0.001$).

임상연구에서는 44명 소아에서 총 878개의 유치 치면을 확인하였다. 시진 및 방사선 검사 후, 휴대용 QLF 장비로 치아우식증 사진을 촬영하였고, 우식진행 단계에 따라 분류 후 특정 소프트웨어로 분석하였다. 분석과정에서 형광소실(ΔF)과 세균활성(ΔR)을 의미하는 QLF 지표에 대해 ROC분석을 시행하고, ΔF와 ΔR 수치를 혼합한 회귀분석모델의 신뢰도를 확인하였다. QLF 지표는 준수한 결과 (민감도 0.72-0.91, 특이도 0.74-0.96, AUROC 0.861-0.940)를 보였으며, 회귀분석모델의 AUROC(0.90-0.957)는 모든 종류의 우식병소에서 ΔF와 ΔR 각각을 이용해 분석한 수치보다 더 높게 나타났다. 함께 제시한 QS-Index는 모든 단계에서 통계적으로 유의미하게 치아우식증의 진행을 적절히 표현하는 것으로 확인되었다.

QLF의 신뢰도는 임상적인 환경에서도 시진 또는 방사선 검사로 대표되는 기존 진단방식의 신뢰도와 비슷하거나 다소 높은 수치로 확인되었다. 결론적으로 QLF를 이용한 유치의 치아우식증 탐지는 소아에서 방사선 노출의 걱정 없이 치아우식증을 진단할 수 있는 무해하고 신뢰할만한 방법이 될 수 있을 것이다.

핵심되는 말: 정량형광분석(QLF), 치아우식증, 진단, 우식증 탐지, Micro-CT, 방사선촬영