





# Analysis of Cracked Teeth using Quantitative Light-induced Fluorescence Technology and Micro-computed Tomography

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# Analysis of Cracked Teeth using Quantitative Light-induced Fluorescence Technology and Micro-computed Tomography

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## 감사의 글

지난 석사과정 2년과 본 학위논문을 무사히 마치기까지 저에게 도움을 주신 많은 분들께 짧은 지면을 빌려 감사의 말씀을 전하고자 합니다.

본 논문을 작성하며 부족한 저를 관심과 열정으로 지도해주신 신수정 교수 님께 진심으로 감사드립니다. 깊이 존경하는 마음을 이 글을 통해 전하고 싶 습니다. 또한 바쁘신 와중에도 논문 심사를 맡아주시고 귀중한 조언과 통찰을 아끼지 않아 주신 김선일 교수님, 정회인 교수님께도 감사의 말씀을 올립니다.

보존과 수련 기간동안 늘 한결 같은 모습으로 이끌어 주시고 가르쳐 주신 박 정원 교수님, 전미정 교수님께도 감사와 존경의 마음 전합니다. 또한 보존과 수련의로서, 또 대학원생으로서 지식의 넓이와 깊이를 더할 수 있도록 아낌없 는 가르침을 주신 노병덕 교수님, 박성호 교수님, 김의성 교수님, 정일영 교 수님, 신유석 교수님, 김도현 교수님께도 감사의 말씀드립니다.

함께 수련을 받으며 서로에게 힘이 되어주고, 즐거운 수련 생활을 할 수 있 게 해준 동기 양소연 선생님과 의국 선후배님 들에게도 감사의 말을 전합니다.

더불어 이번 연구에 도움을 주신 이형석 선생님, 윤홍철 선생님께도 감사 말 씀 드립니다.

마지막으로 항상 저를 묵묵히 믿어 주시고 든든하게 저를 지탱해주신 부모 님과 동생 그리고 경보오빠에게 말로는 전하지 못했던 감사와 사랑의 마음을 전합니다.



# **Table of Contents**

List of Figuresiii
List of Tables iv
Abstract ······ v
I. Introduction
II. Materials and Methods 4
1. Part I 4
1.1 Specimen preparation 4
1.2 Detection and analysis of crack lines using QLF 4
1.3 Micro-CT evaluation 5
1.4 Statistical analysis 6
2. Part II
2.1 Patient selection ······ 6
2.2 Data collection ······ 7
2.3 QLF image analysis
2.4 Statistical analysis
III. Results ····· 10
1. Part I
1.1 Evaluation of crack detection capabilities by QLF device 10



1.2 The relationship between the depth of crack and QLF parameters $\cdot$ 14
1.3 Association among QLF parameters 14
2. Part II 15
2.1 Distribution and characteristics of cracked teeth 15
2.2 Crack detection using QLF technology 19
2.3 Relationship between clinical signs and symptoms and QLF
parameters 21
2.4 Relationship between the width of crack on QLF images and QLF
parameters, clinical signs and symptoms
IV. Discussion ····· 24
V. Conclusion ····· 29
References
국문요약



# **List of Figures**

Figure 1. Schematic of crack line analysis using 'CrazeLineWizard01 v1.02'
Figure 2. Representative micro-CT images (Sagittal, coronal, axial plane) of extracted
maxillary molar11
Figure 3. Representative QLF analysis of distobuccal and mesiopalatal crack lines on
extracted maxillary molar
Figure 4. Characteristics of the cracked teeth
Figure 5. Classification of restorations
Figure 6. Representative images of crack lines observed in QLF images

# List of Tables

<b>Table 1.</b> Relevance between width of crack on micro-CT and QLF parameters
<b>Table 2.</b> Relevance between depth of crack on micro-CT and QLF parameters
Table 3. Relevance among QLF parameters    15
Table 4. Distribution of cracked teeth    16
Table 5. Clinical signs and symptoms of cracked teeth    19
<b>Table 6.</b> Relevance between QLF parameters with the cold test    21
Table 7. Relationship between the width of crack on QLF images and QLF parameters,
clinical signs and symptoms
<b>Table 8</b> . Relevance between gap on the QLF images and QLF parameters
Table 9. Relevance between gap on the QLF images and clinical signs and symptoms 23



## Abstract

# Analysis of Cracked Teeth using Quantitative Light-induced Fluorescence Technology and Micro-computed Tomography

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A cracked tooth refers to an incomplete fracture of the tooth structure that can progress to affect the pulp and periodontal ligament. By utilizing various diagnostic tools such as magnification, transillumination and dyes, cracked tooth can be detected and diagnosed more accurately. Recently, the Quantitative Light-induced Fluorescence (QLF) technology has been introduced and utilized in clinical environments for diagnosing cracked teeth. The aim of this study was to analyze tooth cracks using QLF technology and micro-CT and to



investigate relationships between QLF parameters and clinical signs and symptoms.

This study consisted of two parts. In Part I, a total of 24 extracted teeth suspected to have cracks were taken with micro-CT images (SkyScan® 1173, Aartselaar, Belgium), and 70 crack lines were identified. The depth and width of each crack line were calculated using Dataviewers software. Subsequently, QLF images were taken using Qraycam Pro (AIOBIO, Seoul, Korea), and fluorescence loss ( $\Delta$ F), maximum fluorescence loss ( $\Delta$ Fmax), red fluorescence ( $\Delta$ R), and maximum red fluorescence ( $\Delta$ Rmax) were analyzed. In Part II, patients diagnosed as cracked teeth were included. The patient' age, sex, and tooth position, the presence of spontaneous pain, biting pain, and the response to the percussion, cold, and bite tests of each tooth were recorded. The QLF image of cracked teeth were taken with Qraypen C (AIOBIO) and the QLF parameters were analyzed.

According to the Part I study, there was no statistically significant difference between crack depth on micro-CT and QLF parameters. However, a positive correlation was observed between the width of cracks on micro-CT and QLF values, indicating that gap in micro-CT images was associated with higher QLF values. In part II study, 155 teeth were included from 142 patients. There was significant relevance between the cold test and maximum fluorescence loss ( $\Delta$ Fmax) (p = 0.02), and  $\Delta$ Fmax was positively associated with the bite test (p = 0.03). The QLF values from crack analysis were higher in the group showing gaps in QLF images. Cracked teeth with obvious gaps in QLF showed significantly higher QLF values compared with those without gaps.



In conclusion, based on the micro-CT analysis, there was no correlation between QLF factors and crack depth. However, micro-CT and clinical analysis demonstrated that cracked teeth with gaps showed higher QLF values than those without gaps. Clinically, QLF factors appear to be related to the cold and bite test. Based on this study, QLF technology could be utilized as an auxiliary tool for diagnosing cracks; if there is a noticeable gap in the crack, it becomes easier to diagnose as cracked teeth.



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## I. INTRODUCTION

According to the American Association of Endodontists, cracked teeth are teeth with a fracture plane of unknown direction that passes through the tooth structure and progresses to communicate with the pulp and the periodontal ligament (Rivera and Walton, 2008). Cracked teeth may cause sharp pain on biting, unexpected sensitivity to cold, and discomfort when pressure is released (Kahler, 2008). As the crack advances into the pulp of the tooth, bacteria could penetrate into the crack lines, leading to symptoms of pulpitis and resulting pulp necrosis (Brännström, 1986).



Diagnosing cracked teeth is not straightforward because crack lines are not always observable on inspection, so detecting crack lines could be challenging. Thus, it may require the use of dyes, transilluminations (Coelho et al., 2016), surgical microscopes (Banerji et al., 2010), cone-beam computed tomography (Gao et al., 2021).

Quantitative light-induced fluorescent (QLF) technology has recently been used for crack diagnosis. The QLF technology utilizes visible blue light at a wavelength of 405nm with a specialized filter to measure different fluorescence reactions in teeth. It has been utilized in clinical practice to diagnose initial caries and assess mineral loss in affected areas (Angmar-Månsson and Ten Bosch, 2001). Contamination of crack lines facilitates the infiltration of metabolites such as porphyrin. These substances appear as red fluorescence QLF images (Kim et al., 2014). Based on detecting fluorescence through light transmission, this method holds promise for clinical use in identifying cracks, which has not yet been established for standardized diagnosis. Previous studies have discussed the clinical efficacy of QLF technology in cracked teeth (Jun et al., 2019), (Jun et al., 2016), (Oh et al., 2021). A previous retrospective study has demonstrated the clinical efficacy of QLF technology to diagnose cracked teeth (Lee et al., 2023).

As Jun et al. (2016) discovered in their histological evaluation of extracted teeth, there exists a significant relevance between the findings of histologic assessments of enamel cracks and the maximum fluorescence loss ( $\Delta$ Fmax) value using QLF on extracted human teeth. This finding opens up possibilities or the QLF device, suggesting its potential in diagnosing cracked teeth and quantifying their depth.



However, verifying the depth of the crack line nondestructively is challenging, and performing a histological assessment after extraction could provide and accurate estimation on its depth. Non-destructive, three-dimensional micro-computed tomographic technique has recently become popular tool for root canal anatomy, as it offers benefits over conventional approaches (Grande et al., 2012). It is an precise, non-invasive method that utilizes geometric magnification to provide detailed comprehensive volumetric data regarding the microstructure, generally without requiring extensive sample damages (Du Plessis et al., 2017). Micro CT could be used as useful method for the qualitatively examining crack lines, offering a high precision (Dumbryte et al., 2021). With these backgrounds, this study was aimed to find out the relevance between QLF parameters and crack depths or widths and evaluate the clinical applicability through clinical studies.

This study consisted of two parts. Part I, an in vitro study aimed to evaluate the efficacy of QLF technology for detecting crack lines detected on micro-CT. It also sought to establish the relevance of the QLF values with the depth and width of cracks detected on micro-CT. Part II, a retrospective clinical study was aimed to evaluate the effectiveness of the QLF technology in detecting crack lines and to investigate the correlation between QLF parameters and clinical symptoms.

The null hypothesis was 1) there is no relationship between QLF parameters and the gap or width of the crack in micro-CT, and 2) there is no relationship between QLF parameters and clinical signs and symptoms.



## **II. MATERIALS AND METHODS**

#### 1. Part I

#### **1.1 Specimen preparation**

Twenty-four extracted teeth consisting of 15 molar teeth and 9 premolar teeth were examined in this in vitro study. The use of the extracted teeth was approved by the Institutional Review Board for Clinical Research in Gangnam Severance Dental Hospital (approval no. IRB 3-2024-0127). The inclusion criteria were as follows : (1) the presence of crack lines on the crown surface, (2) stored in distilled water after extraction. The exclusion criteria as follows : (1) No advanced dental caries and broad restorations, vertical root fracture, endodontically treated, (2) No pre-treatment using chemical agents

The teeth were carefully cleaned with a hand scaler to remove plaque and calculus. All of the evaluations were carried out by a single examiner. On visual inspection with naked eyes, the examiner selected 70 suspected crack lines among twenty-four extracted teeth. To avoid dehydration throughout the experimental procedures, the specimens were kept immersed in distilled water.

#### 1.2 Detection and analysis of crack lines using QLF



All teeth were dyed with proto-porphyrin IX (PPIX, Aldrich, Milwaukee, USA) solution, immersing for 5 minutes. The solution was prepared with PPIX 0.4 mM dissolved in a 1:1 ratio of water and dimethyl sulfoxide solution. Prior to examination, excess moisture was removed from each specimen by gently blotting with a cotton pellet, allowing the surface to air dry. The QLF images of the specimen were captured vertically from a distance of approximately 8 cm with Qraycam pro (AIOBIO, Seoul, Korea) with the enclosed outer hood to prevent interference of light with the following parameters : ISO setting of 1600, a shutter speed of 1/30, an exposure of 0 and a contrast of 0 with 1920 X 1080 pixel resolution. Subsequently, the crack analysis was performed utilizing the CrazelineWizard01 v1.02 (Inspektor Research Systems B. V., The Netherlands).

#### **1.3 Micro-CT evaluation**

All extracted teeth were scanned with micro-CT (SkyScan® 1173, Aartselaar, Belgium) with the following settings : spatial resolution of 16.07  $\mu$ m, 130 kV, 60  $\mu$ A, and exposure time of 500ms. The images were reconstructed with Dataviewers software (Bruker-microCT, Konitch, Belgium) to quantify the depth and width of crack lines. To determine the depth of the crack line, the specific areas of interest were reconstructed along the longitudinal axis and the linear distance from the occlusal surface to the deepest part on the sagittal plane was quantified. If the maximum width of the crack was wider than 40.00  $\mu$ m, the group was divided into 'a group with a gap'. And if the space was smaller than 40.00  $\mu$ m, the group was regarded as 'a group without a gap'.



#### **1.4 Statistical analysis**

Statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA). According to the result of the normality of data by the Shapiro-Wilk test, the results of the non-parametric tests were used. The Mann-Whitney U test was conducted to examine the association between QLF parameter values and the width of cracks on micro-CT. Additionally, the relevance between the QLF parameters and the depth of crack on micro-CT analysis was assessed using Spearman's rank correlation.

#### 2. Part II

#### 2.1 Patient selection

This study was approved by the Institutional Review Board for Gangnam Severance Dental Hospital (approval no. IRB 3-2024-0105). Those diagnosed as cracked teeth were included among the patients who visited the Department of Conservative Dentistry at the Gangnam Severance Hospital. The patients' QLF images from December 2021 to February 2023 were taken using Qraypen C (AIOBIO). The inclusion criteria for the tooth selection were as follows: (1) Symptomatic (spontaneous pain or biting pain) and suspected visible crack lines. (2) Asymptomatic but suspected visible crack lines. The tooth selection



exclusion criteria were as follows: (1) Symptomatic but no unclear visible crack lines with naked eyes and QLF images. (2) Periodontitis or TMD diseases. (3) Teeth with craze lines.

#### 2.2 Data collection

The patients' age, sex, and tooth position diagnosed as cracked teeth were recorded. The presence of biting pain, spontaneous pain and the response to the percussion, cold, and bite tests were recorded. The bite test was conducted using a roll of rubber, or a cotton roll. The percussion test utilized a mirror handle or a pincette. For the cold test, an ice stick or a cotton swab cooled with a refrigerant spray (Endo-Ice, 1,1,1,2 tetrafluoroethane, Hygenic Corp, Akron, OH) was employed. The directions of crack lines, and the type of previous restorations and the classification of restoration were recorded. The directions of crack lines were divided into 3 groups including mesial-distal, bucco-lingual and multiple (both mesial-distal and bucco-lingual). The previous restorations were divided into 5 subgroups which were no restorations, direct resin, direct amalgam, indirect gold inlay/onlay, indirect resin and ceramic inlay/onlay groups.

Among the captured QLF images, the teeth showing remarkable gaps with red fluorescence in at the QLF image were separately identified as a group with a gap for further analysis.



#### 2.3 QLF image analysis

The CrazeLineWizard01 v1.02 was used (Figure 1) to quantitively analyze crack lines. The examiner manually outlined the crack line using a 72 pixels wide band. This was automatically stretched into a straight line, with the width of the original curved band and a length equal to the flattened curve length. To approximate the original sound surface for each pixel in the image, the program utilized linear interpolation based on the relative colors of the pixels at the far left and right borders of the straightened band, with the crack positioned at the center line of the band. The band border lines, each one pixel thick and as long as the crack, served as the reference field for reconstructing the sound surface within the band. The program could calculate the four parameters:  $\Delta F$  (mean value of the fluorescence change for all points where  $\Delta Fpix < \Delta 3\%$ ),  $\Delta Fmax$  (the maximum value of  $\Delta R$ ). If there are multiple separate crack lines, the crack line was calculated respectively, and the highest was recorded. All images were analyzed by a single clinically experienced post-graduated.



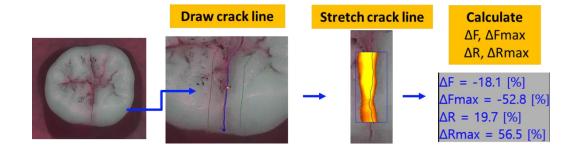


Figure 1. Schematic of crack line analysis using 'CrazeLineWizard01 v1.02'.

 $\Delta F$  = mean value of the fluorescence change for all points where  $\Delta Fpix<-3\%$ ,  $\Delta Fmax$  = the maximum value of the fluorescence loss,  $\Delta R$  = mean value of the fluorescence change for all points where  $\Delta Rpix>5\%$ ,  $\Delta Rmax$  = the maximum value of  $\Delta R$ .

#### 2.4 Statistical analysis

Statistical analyses were performed using SAS version 9.4. According to the result of the normality of data by the Shapiro-Wilk test, the results of the non-parametric tests were used. To assess the association between clinical signs and symptoms and QLF parameters, the Mann-Whitney U tests were conducted for the percussion and bite test, while the Kruskal-Wallis test (post hoc analysis: the Mann-Whitney U test) was performed for the cold test.

To investigate the relevance between the existence of a gap in QLF images and the QLF parameters and clinical signs and symptoms, the Mann-Whitney U test was applied for continuous variables, and chi-square tests were conducted for categorical variables.



## **III. RESULTS**

#### 1. Part I

#### 1.1 Evaluation of crack detection capabilities by QLF device

From the micro-CT evaluation of twenty-four extracted teeth, 70 crack lines were identified. Among these, 68 (97.1%) crack lines observed in micro-CT were detected with  $\Delta$ F and  $\Delta$ Fmax values using QLF analysis. In contrast,  $\Delta$ R and  $\Delta$ Rmax values were detected in only 28 (40.0 %) teeth, with no measurements recorded in the other 42 (60.0 %) teeth.

For example, Figure 2 shows a cross-section of a single tooth obtained through micro-CT imaging, confirming a total of 4 crack lines. Figure 3, on the other hand, represents a QLF images of the same tooth taken using Qraycam; two crack lines were analyzed with QLF parameters. While  $\Delta R$  and  $\Delta R$ max were not calculated for the distobuccal crack line, all parameters were analyzed for the mesiopalatal crack, with detecting gaps on the occlusal surface on QLF images.



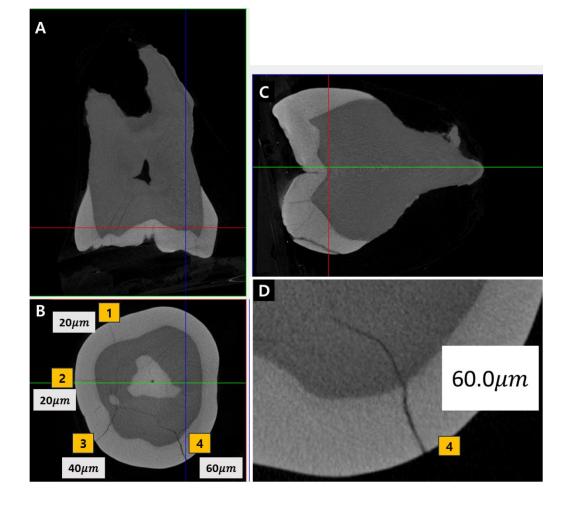


Figure 2. Representative micro-CT images(Sagittal, coronal, axial plane) of extracted maxillary molar. (A-C) The sagittal, coronal, axial images of micro-CT of the extracted maxillary molar respectively. Four prominent crack lines were observed (1. Distobuccal, 2. Distal, 3. Distopalatal, 4.Mesiopalatal). (D) Among these crack lines, only the mesiopalatal crack line (numbering 4) was classified as a "group with gap ( $\geq$ 40 µm)" with a width of approximately 60 µm.



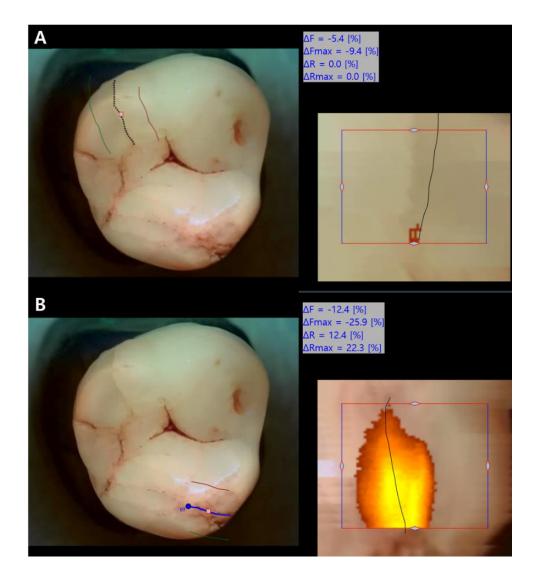


Figure 3. Representative QLF analysis of distobuccal and mesiopalatal crack lines on extracted maxillary molar. (A) The distobuccal crack line. The  $\Delta$ F and  $\Delta$ F max values were detected, but  $\Delta$ R and  $\Delta$  Rmax values were not detected on QLF analysis. (B) The mesiopalatal crack line, all QLF parameters were analyzed.



Of the 70 crack lines, 17 cracks showed a gap ( $\geq$ 40 µm), classified as 'a group with a gap'. And for the group with a gap, all four QLF parameters were analyzed. There was a statistical association between the four QLF parameters and the width of cracks on the micro-CT analysis (Table 1). The group with a gap showed higher QLF parameters than the group without a gap (p<0.01).

Table 1. Relevance between width of crack on micro-CT and QLF parameters.

Variables	Group with gap (n=17)	Group without gap (n=53)	Total	+ 1	
Variables	Median (min-max)	Median (min-max)	Median (min-max)	⁺p-value	
ΔF	-11.2(-18.83.6)	-5.8(-20.9-0.0)	-6.5(-20.9-0.0)	< 0.001*	
ΔF max	-28.6(-52.93.8)	-11.3(-59.2-18.3)	-16.1(-59.2-18.3)	< 0.001*	
ΔR	11.9(0.0-98.0)	6.4(0.0-19.4)	7.2(0.0-98.0)	0.005*	
<b>AR max</b>	25.3(0.0-91.8)	9.2(0.0-55.3)	11.8(0.0-91.8)	0.01*	

Group with gap: showing gap distance of  $\geq$ 40 µm; Group without gap: showing distance of <40 µm; p-value obtained by The Mann-Whitney U test; Asterisk (\*) indicates significant differences at p<.05.



#### 1.2 The relationship between the depth of crack and QLF parameters

There was no statistically significant relevance between the depth of the crack and the QLF parameters (Table 2).

Table 2. Relevance between depth of crack on micro-CT and QLF parameters.

	ΔF		ΔF max		ΔR		ΔR max	
	r(95% CI)	p- value						
Depth of	-0.029		-0.102		0.126		0.094	
crack on micro CT	(-0.262- 0.208)	0.814	(-0.328- 0.137)	0.404	(-0.113- 0.350)	0.299	(-0.145- 0.321)	0.446

CI, confidence interval; p-value obtained by spearman's rank correlation coefficient; Asterisk (\*) indicates significant differences at p<.05.

#### 1.3 Association among QLF parameters

The four QLF parameter values analyzed from the crack lines of the extracted tooth were found to be correlated with each other (Table 3).



	ΔF		ΔF max		ΔR		ΔR max	
	r(95% CI)	p-value						
ΔF								
ΔF max	0.919	<.001*						
ΔR	-0.702	<.001*	-0.621	<.001*				
ΔR max	-0.715	<.001*	-0.637	<.001*	0.989	<.001*		

#### Table 3. Relevance among QLF parameters.

CI, confidence interval; p-value obtained by spearman's rank correlation coefficient; Asterisk (\*) indicates significant differences at p<.05.

#### 2. Part II

#### 2.1 Distribution and characteristics of cracked teeth

One hundred and fifty-five teeth were included. Among 142 patients, 56 were male and 86 were female, with a mean age of 54.65. The mandibular second molars (32.26%) were most frequently cracked, followed by the maxillary second molars (22.58%), the mandibular first molars (21.94%), and the maxillary first molars (16.77%). The occurrence of cracks on premolars was relatively low; the maxillary second premolars showed the highest prevalence (4.52%), followed by the maxillary first premolars (1.30%) and the mandibular second premolar (0.65%). No cases were observed in the mandibular first premolars (Table 4).

Cracked teeth were most commonly found in restored teeth (69.0%), and only accounted

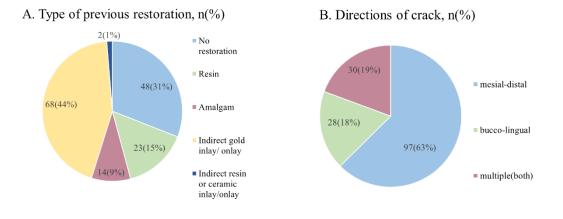


for 31.0% intact teeth. Nonbonded restorations such as gold (43.9%) and amalgam (9.03%) showed a higher incidence of cracks than resin/ceramic restorations (16.1%). Teeth with cracks extending only in the mesial-distal direction accounted for 62.6%, those extending only in the bucco-lingual direction were 18.0%, and teeth with cracks in both directions (multiple or mesial-distal and bucco-lingual) constituted 19.4% (Figure 4). Of the 107 restored teeth, 88 teeth (82.3%) had class I restorations, 17 teeth (15.9%) had class II restorations and two teeth (1.87%) had onlay restorations (Figure 5). Fig 5B, and Fig 5C show the classification of restorations with cracked teeth among non-bonded restorations and bonded restorations respectively.

		Sex			Age			
Tooth position	Male	Female	20-29	30-39	40-49	50-59	60	Total, n(%)
Maxillary								
1 <sup>st</sup> premolars	1	1	0	0	0	1	1	2(1.30)
2 <sup>nd</sup> premolars	1	6	0	0	0	3	4	7(4.52)
1 <sup>st</sup> molars	17	9	2	0	3	11	10	26(16.77)
2 <sup>nd</sup> molars	13	22	1	5	9	8	12	35(22.58)
Total	32	38	3	5	12	23	27	70(45.16)
Mandibular								
1 <sup>st</sup> premolars	0	0	0	0	0	0	0	0(0)
2 <sup>nd</sup> premolars	0	1	0	0	0	1	0	1(0.65)
1 <sup>st</sup> molars	14	20	2	3	3	12	14	34(21.94)
2 <sup>nd</sup> molars	16	34	1	4	21	9	15	50(32.26)
Total	30	55	3	7	24	22	29	85(54.84)

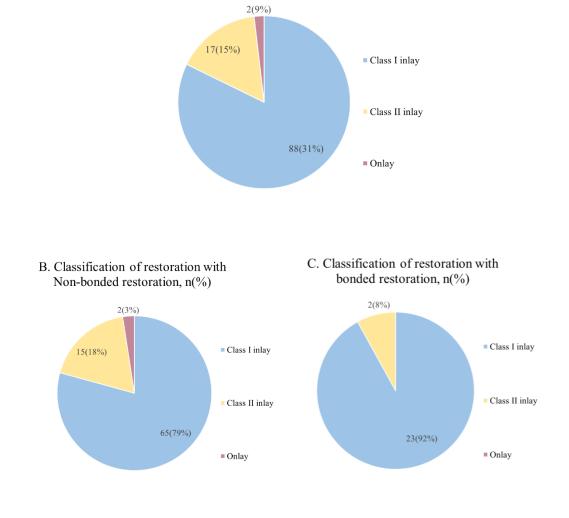
	Table 4.	Distribution	of cracked	teeth.
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**Figure 4. Characteristics of the cracked teeth.** (A: type of previous restoration, B: directions of crack)





#### A. Classification of restored teeth, n(%)

**Figure 5. Classification of restorations**. (A: Total, B: Non-bonded restorations including amalgam, gold restorations, C: Bonded restorations including direct resin filling and ceramic, resin indirect restorations)



Regarding the clinical signs and symptoms of cracked teeth, most teeth with crack lines experienced biting pain (65.8%) and showed a positive response to the bite test (59.4%). Negative responses to the percussion test were recorded in 97 teeth (62.6%) (Table 5).

Signs and symptoms	n(%)
Spontaneous pain	
Positive	21 (13.6)
Negative	134 (86.5)
Biting pain	
Positive	102 (65.8)
Negative	53 (34.2)
Percussion test	
Positive	58 (37.4)
Negative	97 (62.6)
Cold test	
Two positive	36 (23.2)
Positive	108 (69.7)
Negative	11 (7.1)
Bite test	
Positive	92 (59.4)
Negative	63 (40.6)
Total	155 (100.0)

#### Table 5. Clinical signs and symptoms of cracked teeth.



#### 2.2 Crack detection using QLF technology

All images were obtained as a pair of white light and fluorescent images. The efficacy of the crack detection of QLF technology was analyzed by comparing the white light images as controls. Subsequently, the crack analysis was performed using the same method as before, utilizing the CrazelineWizard01 v1.02 (Inspektor Research Systems B. V). Figure 6 shows representative examples of QLF images.





**Figure 6. Representative images of crack lines observed in QLF images.** Each crack line was indicated by black arrows and analyzed by QLF parameters. (A-C) : The mesial crack line was indicated on mandibular molar restored with gold restoration. (D-F) : The palatal crack line was indicated on maxillary molar restored with gold restoration. (G-H) : The mesial to distal crack line was indicated on maxillary molar restored with resin.

#### 2.3 Relationship between clinical signs and symptoms and QLF parameters

There was a significant relevance between the cold test and the maximum fluorescence loss  $(\Delta Fmax)$  (p=0.02) (Table 6). Group 3 showing no response on cold test showed higher  $\Delta Fmax$  value than Group 1 and Group 2.

Outcome	Group 1 Cold(++) n=36	Group 2 Cold(+) n=108	Group 3 Cold(-) n=11	post-hoc analysis			
	II-30	<b>H</b> =108	11-11	p-value	G1 vs G2	G1 vs G3	G2 vs G3
ΔF	-8.7 (-16.9-3.7)	-9.8 (-27.34.2)	-11.8 (-18.3-7.8)	0.134	1.364	0.223	0.214
ΔF max	-21.5 (-51.8-3.0)	-22.9 (-58.1-6.2)	-32.9 (-61.5-14.7)	0.021*	1.773	0.048	0.021
ΔR	11.0 (6.6-23.6)	12.8 (-9.9-40.2)	13.6 (9.4-22.9)	0.409	1.524	0.537	0.919
ΔR max	24.5 (10.5-66.8)	27.9 (3.0-119.9)	36.5 (17.4-58.2)	0.244	2.537	0.49	0.28

#### Table 6. Relevance between QLF parameters with the cold test.

p-value obtained by Kruskal-Wallis test. Asterisk (\*) indicates significant differences at p<.05.



The  $\Delta$ Fmax was positively associated with the bite test (p=0.03). Teeth with pain upon biting had significantly higher  $\Delta$ Fmax values than teeth without biting pain (p=0.03) (Table 7).

	Negative (n=63)	Positive (n=92)	p-value
	median(min-max)	median(min-max)	
ΔF	-9.2(-27.3-4.6)	-10.4(-27.3-3.7)	0.138
ΔF max	-20.0(-58.1-4.0)	-24.2(-61.5-3.0)	0.031*
ΔR	11.9(-9.9-40.2)	13.0(6.2-39.6)	0.34
ΔR max	24.1(3.0-119.9)	28.9(7.7-100.7)	0.249

Table 7. Relevance between QLF parameters with the bite test.

p-value obtained by Mann-Whitney U test. Asterisk (\*) indicates significant differences at p<.05.

# 2.4 Relationship between the width of crack on QLF images and QLF parameters, clinical signs and symptoms

Among the captured QLF images of 155 teeth, the teeth showing remarkable gaps with red fluorescence occupied on 124 (80.0%); on the other hand, the teeth with ambiguous red fluorescence and gap occupied 31 (20.0%). There was a statistically significant difference between the crack width on QLF images all and four QLF parameters (p<0.001) (Table 8).



Outcome	Total	Group with gap	Group without gap	p-value
	(n=155)	(n=124)	(n=31)	
	Median	Median	Median	
ΔF	-9.8(-27.33.7)	-10.5(-27.33.7)	-6.3(-14.34.2)	<.001*
ΔF max	-23.0(-61.53.0)	-24.7(-61.54.0)	-12.0(-35.53.0)	<.001*
ΔR	12.6(-9.9-40.2)	13.7(-9.9-40.2)	8.8(6.2-16.5)	<.001*
ΔR max	28.2(7.7-119.9)	29.9(9.3-119.9)	14.1(7.7-39.4)	<.001*

 Table 8. Relevance between gap on the QLF images and QLF parameters.

p-value obtained by Mann-Whitney U test. Asterisk (\*) indicates significant differences at p<.05.

There was a statistically significant difference between the width of the crack on QLF images and the percussion (p < 0.001) and the bite test (p=0.02) (Table 9). Teeth with positive responses on the percussion test and the bite test had a higher prevalence in the group with a gap than the group without a gap.

# Table 9. Relevance between gap on the QLF images and clinical signs and symptoms.

Outcome	Total	Group with gap	Group without gap	p-value
	(n=155)	(n=124)	( <b>n=31</b> )	
	Median	Median	Median	
Percussion (+)	97(62.58)	72(58.06)	25(80.65)	0.02*
Percussion (-)	58(37.42)	52(41.94)	6(19.35)	
<b>Bite</b> (+)	97(62.58)	72(58.06)	25(80.65)	0.009*
<b>Bite</b> (-)	58(37.42)	52(41.94)	6(19.35)	

p-value obtained by Mann-Whitney U test. Asterisk (\*) indicates significant differences at p<.05.



### **IV. DISCUSSION**

This study is the first to investigate whether cracks detected by micro-CT imaging in extracted teeth could be analyzed using QLF images and whether there is a correlation between the depth and width of the cracks and the values of QLF parameters.

It was found that certain dental plaques emit red fluorescence if exposed to light at 400-420nm wavelength (Angmar-Månsson and Ten Bosch, 2001). This optical phenomenon is caused by naturally occurring fluorescent porphyrin molecules, like protoporphyrin IX(PPIX) being a major component. These porphyrins are produced by certain microorganisms found in the mouth. These porphyrin molecules exhibit strong red fluorescence when exposed to violet light (Lee et al., 2019). For early smooth surface caries lesions, after staining with PPIX, the laser fluorescence can be efficiently measured using a device called DIAGNOdent (Mendes et al., 2006). Due to the difficulty in observing endogenous porphyrin's influence in teeth extracted long ago, staining was conducted using a protoporphyrin IX (PPIX, Aldrich) solution to analyze crack lines on extracted teeth using QLF. It was expected that the solution could penetrate into the crack lines on the surface of the teeth.

According to Jun et al. (2016), examining histological sections of extracted teeth revealed a relevance between the depth of enamel cracks and the  $\Delta$ Fmax value. However, this study did not significantly correlate with QLF parameters and crack depth (Table 2).



While the study of Jun et al. only focused on enamel cracks and categorized them into groups based on the depth of crack lines, such as reaching the inner half, outer half, or the dentoenamel junction, this study analyzed cracks extended to the dentine layer. In this experiment, the linear distance from the occlusal surface to the deepest point was measured. Although actual cracks do not appear in a straight line, it was difficult to calculate the length of the curved line on micro CT x-ray. The linear distance was measured because the proximity to the pulp was thought to be related to the symptoms in cracked teeth.

It was observed that there was a significant difference in all QLF parameter values depending on the width of the crack; wider cracks showed more pronounced differences (Table 1). Although QLF analysis does not provide information about the depth of the crack lines, it offers insights into the crack width. However, due to the nature of cracks, wider cracks tend to occur more towards the occlusal surface of the tooth and become narrower as they deepen. In this study, the area with the largest gap was used as the reference, and cases exceeding 40  $\mu$ m were classified as 'a group with a gap'. Although the analyzing program was used to measure the width of the cracks, to quantify crack width was difficult, and it varies with the height of the tooth, making it challenging to standardize. The minimum distance of crack that was measure using the program was 20  $\mu$ m, and a distinct gap was observed from 40  $\mu$ m and above. Therefore, the groups was divided into two groups depending on whether their width of the gap exceeds 40  $\mu$ m.



It was thought that the wider the crack, the more PPIX solution could be penetrated, and consequently, higher  $\Delta R$  and  $\Delta R$ max values would be derived. Although the crack patterns in extracted teeth detected in this study may differ from those diagnosed as cracked teeth clinically, it was considered that the cracks with large gaps observed in clinical practice had likely existed for a long time, and would therefore show higher  $\Delta R$  and  $\Delta R$ max values. Based on these results, the part II experiment was designed to investigate whether the width of cracks found clinically is related to QLF values and the signs and symptoms experienced by patients.

The result of the part II study demonstrated that the most teeth with detected crack lines were restored teeth (69.0%). This result differs from studies (Kang et al., 2016; Roh and Lee, 2006), which reported that teeth showing cracks were more frequently found in intact teeth. The inclusion criteria of this study encompassed visible cracks even in the absence of symptoms, which may account for the discrepancy in the study population compared to others. Conversely, similar trends were observed (Seo et al., 2012), where the majority of crack teeth (72%) were restored teeth, with only 28% having received no restorations.

In this study, 155 teeth were included, the teeth showed pain during the bite test demonstrated higher  $\Delta$ Fmax values compared to teeth without pain. This result is similar to the finding of the previous retrospective study (Lee et al., 2023), when 90 teeth were examined, the teeth with positive response on bite test had significantly higher  $\Delta$ F,  $\Delta$ Fmax values. The pain during chewing can be attributed to the changes in the fluid movement



within the dentinal tubules. For cracked teeth, the deeper or more extensive, the higher the maximum fluorescence loss value. This suggests that the movement of fluid within the crack during chewing can explain the pain experienced. However, in this study, there was no statistically correlation between the percussion test and QLF parameters. These findings differ from Lee in 2023 study, which showed that teeth experiencing pain on percussion test had higher  $\Delta F$ ,  $\Delta F$ max, and  $\Delta R$  values. This study included crack lines in asymptomatic teeth, which contributed to the differences in results. Conversely, previous prospective study (Lee et al., 2021) found that pain during percussion test was an important factor of pulp vitality in cracked teeth.

It was observed that the group with a gap showed statistically higher  $\Delta F$ ,  $\Delta Fmax$ ,  $\Delta R$ , and  $\Delta Rmax$  than the group without a gap (p<0.001), resulting in similar trends to the part I in vitro study (Table 8). The width of the crack was also related to the patient's clinical symptoms; teeth with positive responses on the percussion and the bite test showed a higher prevalence in the broader width of cracks. Therefore, the first null hypothesis was partially rejected, and the second null hypothesis was also rejected.

The limitation of this study is that the cracks observed in the extracted teeth mainly occurred the storage process and may differ from the crack patterns caused by actual biting force. It may be difficult to collect a tooth extracted due to cracks without any dental procedures. The observed cracks in this micro-CT study may not fully represent the real crack patterns caused by chewing situations. Therefore, if it is experimentally possible to



create cracks in the extracted teeth, it would be more desirable to get more accuracy and reliability.

Secondly, using an artificial porphyrin solution staining on extracted teeth in order to drive  $\Delta R$  values may not have perfectly replicated the natural porphyrin-induced bacterial activity. This limitation could have interfered with the accurate measurement of the  $\Delta R$  values. The natural porphyrin produced by bacteria likely has different properties and effects than the artificial porphyrin used. Among extracted crack teeth with QLF analysis, 97.1% of crack lines were detected with  $\Delta F$  and  $\Delta F$ max, however  $\Delta R$  and  $\Delta R$ max were detected in only 40.0% of teeth. Thus, measuring the fluorescence loss ( $\Delta F$ ) would be more sensitive to finding crack lines than the red fluorescence ( $\Delta R$ ).

Furthermore, in a clinical practice, it is not always possible to take QLF images at a constant distance and angle. Variations in the angle can affect the area and maximum loss of fluorescence. Therefore, it is important to optimize the image capture conditions and standardize the analysis process.



## **V. CONCLUSION**

1. Based on the micro-CT analysis, there was no relevance between the crack depth and QLF values.

2. Micro-CT and clinical analysis demonstrated that cracked teeth with gaps showed higher QLF values than those without gaps.

3. Clinically, QLF factors appear to be related to the cold and bite test.

4. The QLF technology could be utilized as an auxiliary diagnostic aid for the crack detection, and in particular, when a gap is present in the crack, it becomes easier to identify.



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### **Abstract (IN KOREAN)**

# 정량광형광기술 및 마이크로 전산화 단층 촬영을

## 이용한 균열 치아의 분석

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(지도교수 신 수 정)

균열 치아는 치수와 치주인대에 영향을 미칠 수 있는 치아 구조의 불완전한 파절로 정의될 수 있다. 확대, 투광, 그리고 착색제를 이용하여 균열치아를 감지하고 더욱 정확하게 진단할 수 있다. 최근에는, 정량광형광기술이 소개되어 균열치아의 진단을 위해 임상상황에서 이용되고 있다. 본 연구의 목적은 정량광형광기술 및 마이크로 전산화 단층 촬영을 이용해 치아의 균열을



분석하고 정량광형광기술의 수치와 임상적 징후 및 증상 간의 관계를 조사하고자 하였다.

본 연구는 두 부분으로 구성되었다. 제1부에서는 균열이 의심되는 총 24개의 발거치에서 마이크로 전산화 단층 촬영(SkyScan® 1173, Aartselaar, Belgium)을 시행하였고 70개의 균열선을 확인하였다. 각 균열의 깊이와 폭은 Dataviewer software 를 이용하여 계산하였다. 이어서 Qraycam Pro(AIOBIO, Seoul. Korea)를 이용해 정량광유도 형광 사진을 촬영하였으며 CrazelineWizard 소프트웨어(Inspektor Research Systems B. V., The Netherlands)를 이용하여 형광소실도(△F), 최대 형광소실도(△Fmax), 적색형광도(△R), 최대 적색형광도(△Rmax)를 분석하였다. 제 2 부에서는 임상적으로 균열치아로 진단받은 환자들이 포함되었다. 환자의 나이, 성별, 치아 위치, 자발통, 저작통의 여부 및 타진, 냉, 저작검사의 결과가 기록되었다. 균열치아의 QLF 사진은 Qraypen C 로 촬영되었고 정량광형광 수치가 분석되었다. 제 1부 연구에 따르면, 마이크로 전산화 단층상의 균열 깊이와 정량광형광 수치에는 통계적으로 유의미한 상관관계가 확인되지 않았다. 그러나, 마이크로 전산화 단층상의 균열 폭과 정량광형광 수치에는 양의 상관관계를 확인하였고 마이크로 전산화 단층상에서 균열에 틈이 있을수록 더 높은 정량광형광 수치를

갖는 다는 것을 의미한다. 제2부에서는 142명의 환자 중 155개의 치아가

34



포함되었다. 냉자극 검사와 최대형광소실도(△Fmax) 사이에 유의미한 차이를 확인할 수 있었으며(p=0.02), 최대 형광소실도는 타진 검사와 양의 상관관계를 보였다. 정량광형광 사진에서 틈을 보이는 집단은 균열 분석 시 더 높은 정량광형광 수치를 보였다. 정량광형광사진에서 명확한 틈이 있는 균열치아그룹은 틈이 없는 집단에 비하여 유의미하게 더 높은 정량광형광 수치를 보였다.

결론적으로, 마이크로 전산화 단층 분석에 기반하면 정량광형광수치와 균열의 깊이 사이에는 관련성이 없었다. 그러나, 마이크로 전산화 단층 및 임상 분석에 따르면 틈이 있는 균열치아는 틈이 없는 균열치아에 비하여 더 높은 정량광형광수치를 보인 것을 확인할 수 있었다. 본 연구의 결과, 정량광형광수치와 냉 자극 검사 및 저작검사와는 관련성이 있는 것으로 보여졌다. 본 연구의 결과에 따르면, 정량광형광 기술은 균열의 진단에 보조도구로 이용될 수 있고 균열치아에 틈이 있는 경우 더욱 진단에 용이할 것이다.

핵심되는 말: 균열 치아, 정량광형광기술, 마이크로 전산화 단층 촬영, 진단