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Article

Validation of Quantitative Light-Induced Fluorescence Digital Analysis for the Assessment of Transverse Sectional Early Dental Caries Using Micro-Computed Tomography as a Reference Standard: An In Vitro Study

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Abstract: Objectives: The aim of this study was to validate the effectiveness of quantitative light-induced fluorescence digital (QLFD) analysis for assessing mineral loss in transverse sections of early dental caries (EDC) by comparing it with micro-computed tomography (μ CT). **Methods:** Artificial EDC lesions were created by immersing bovine enamel samples in a demineralization solution for periods ranging from 10 to 70 days. The enamel samples were then sectioned transversely and analyzed using QLFD and μ CT to capture and assess the cross-sectional images. Grayscale intensity profiles were generated on a scale of 0 to 100, and thresholds of 90% and 95% grayscale intensity were defined to determine the depth of EDC. The lesion depths obtained from both methods were statistically compared and evaluated using paired t-tests, one-way ANOVA, and linear regression analysis. **Results:** The findings revealed a significant increase in enamel lesion depth over demineralization periods ranging from 10 to 70 days at both 90% and 95% grayscale intensity thresholds ($p < 0.001$). QLFD consistently demonstrated greater enamel lesion depths compared to μ CT across all demineralization stages. A strong positive correlation was observed between QLFD and μ CT ($r = 0.836\text{--}0.962$, $p < 0.001$), with R^2 values of 0.797 and 0.720 for the 90% and 95% grayscale intensity thresholds, respectively, in linear regression models. **Conclusions:** This study confirms that QLFD can reliably quantify the depth and progression of EDC in transverse sections. QLFD has been proven to be a practical and suitable method for replacing μ CT in laboratory settings for the study of EDC.

Keywords: dental caries; quantitative light-induced fluorescence; X-ray microtomography

1. Introduction

Early dental caries (EDC) is a critical stage of tooth decay where mineral loss occurs without structural breakdown. During this stage, remineralization can restore the tooth to its normal state, making it a golden opportunity for intervention [1]. Once EDC progresses to structural collapse and cavity formation, restoration becomes impossible, highlighting the importance of early management and treatment. For this, diverse and in-depth foundational studies on EDC are essential.

The gold standard for in vitro evaluation of mineral loss and lesion depth in EDC is transverse microradiography (TMR) [2,3]. TMR allows for precise analysis of lesion depth and integrated mineral loss (ΔZ , volume % mineral $\times \mu\text{m}$) in transverse sections of EDC. However, TMR is expensive and complex, limiting its widespread use globally. As an alternative, micro-computed tomography (μ CT) has been proposed. Previous study has demonstrated significant correlations between TMR and μ CT in assessing enamel caries lesions, suggesting that μ CT can serve as a viable substitute [4]. Nevertheless, μ CT remains a costly tool that is not readily accessible to individual researchers.

For non-invasive clinical detection, diagnosis, severity assessment, and progression analysis of EDC, several tools have been developed [5–7]. Among them, quantitative light-induced fluorescence (QLF, Inspektor Research Systems BV, Amsterdam, Netherlands) has been widely validated for its diagnostic reliability in EDC [8–10]. EDC involves mineral loss in the tooth structure, which increases porosity and light scattering, leading to reduced fluorescence intensity compared to sound enamel [11]. QLF analysis quantifies this reduction in fluorescence using the delta F (ΔF) value. Numerous studies have demonstrated strong correlations between ΔF and the degree of mineral loss in EDC [8–11]. QLF has been adopted by dental schools, clinics, and research institutions worldwide, with studies on QLF steadily increasing in PubMed listings [12].

While the clinical validity of QLF has been well established, this study aims to evaluate the analytical validity of QLF in experimental research settings. Specifically, we hypothesize that QLF can replace costly methods like TMR or μ CT for analyzing transverse sections of EDC. To test this hypothesis, we analyzed artificially created EDC lesions on transverse sections using QLF and μ CT, comparing the results to validate the reliability of QLF as an analytical tool.

2. Materials and Methods

2.1. Preparation and Fabrication of Specimens

Sound bovine permanent anterior teeth without any signs of demineralization or cracks were sectioned into 5 mm $\times$ 3 mm specimens using a diamond disc bur (NTI-Kahla GmbH, Germany). The sectioned teeth were embedded in acrylic resin (Curing acrylic denture repair material; Vertex, Soesterberg) molds, with the enamel surface exposed. The specimens were sequentially ground and polished using silicon carbide paper (400p to 2400p; Allied High Tech Products Inc., Rancho Dominguez, CA, USA) mounted on a polishing machine (RB 209 Minipol, R&B Inc., Korea). Transparent nail varnish was applied to a 1 mm $\times$ 3 mm area on one side of the specimen.

The artificial caries solution was prepared by adding tribasic calcium phosphate to 1M lactic acid to create a saturated solution. Subsequently, 2% Carbopol (Carbopol ETD 2050 polymer; The Lubrizol Corporation, Wickliffe, OH, USA) was added, and the final pH was adjusted to 4.8 [13]. Fourteen specimens were assigned to each group. Seven specimens were placed in a 50-ml demineralization solution and incubated at 37°C. Demineralization was performed for 10, 20, 30, 40, 50, 60, and 70 days, with the solution being replaced every 20 days. Using a low-speed saw (Minitome, Struers, Denmark), the specimens were sectioned perpendicular to the enamel surface. To prevent fracturing of the enamel surface, which was expected to be weakened due to mineral loss, the sectioning was performed at 200 rpm. Figure 1 presents the scheme of this study, illustrating the experimental workflow and analysis process.

2.2. Micro-Computed Tomography (μ CT) Analysis

The μ CT images (SkyScan1273, Bruker, Germany) were acquired using X-rays generated at 100 kV and 80 μ A. A 1 mm aluminum filter and a 0.038 mm copper filter were applied with 80% beam hardening correction. To minimize artifacts and noise and enhance data accuracy, ring artifact correction, flat field correction, frame averaging, and random movement were applied. Each tooth specimen was scanned within a rotation range of 206.25°, and the total scan time was 4 minutes and 46 seconds. The distance from the X-ray source to the sample was 53.545 mm, while the distance from the sample to the detector (camera) was 500.742 mm. The data were saved as 1375 TIFF files, with each file having a resolution of 3072 $\times$ 1944 pixels. The reconstructed 3D images had an isotropic voxel size of 7.999 μ m. Cross-sectional surface images of the tooth specimens were obtained using dedicated software (3D.SUITE, Bruker, Germany).

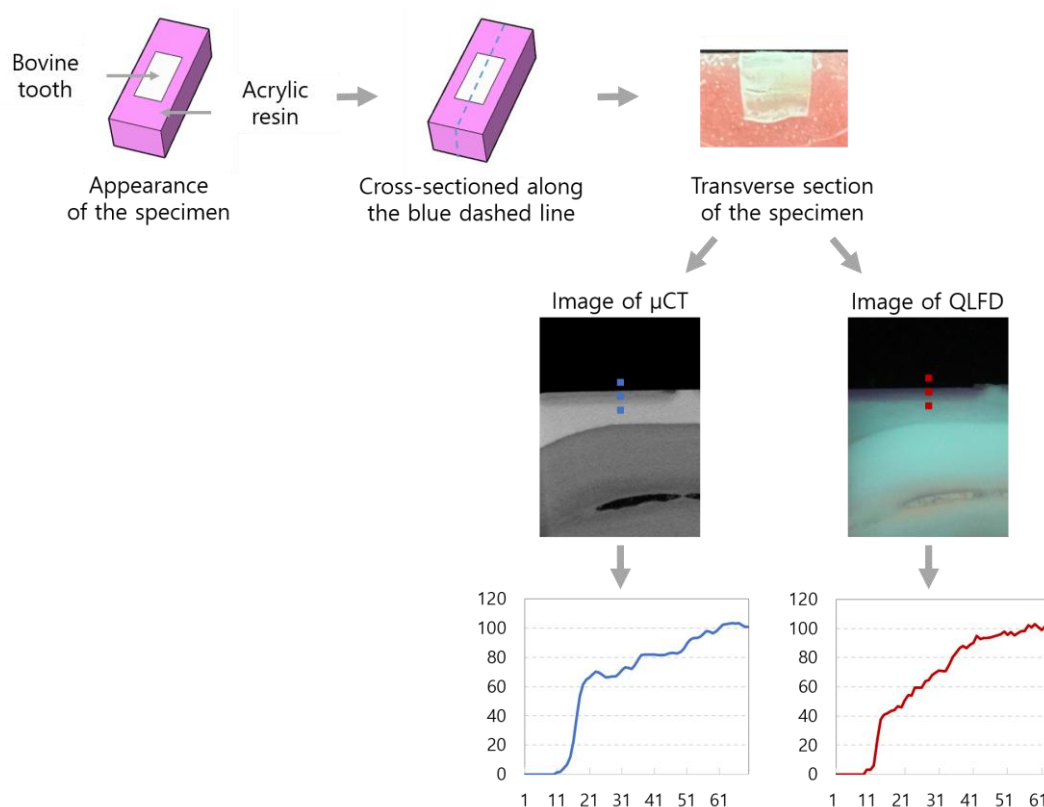


Figure 1. Scheme of this study

2.3. Quantitative Light-Induced Fluorescence Digital (QLFD) Analysis

The cross-sectional surfaces were imaged under QLF-digital (QLFD, 2 + Biluminator™; Inspektor Research Systems BV, Amsterdam, the Netherlands) with all external light blocked and against a black background. The imaging conditions for blue light were set to ISO 1600, a shutter speed of 1/20 s, and an aperture value of 8.0.

2.4. Generation of a Standardized Grayscale Intensity Profile

Image analysis was performed using ImageJ software (version 1.54k, National Institutes of Health, MD, USA). Grayscale intensity profiles across the enamel region were obtained as plot profiles, starting from the black background above the enamel surface, passing through the enamel surface and EDC regions, and extending to areas considered sound enamel. The same regions of interest were selected and analyzed on both μ CT and QLFD images. To standardize the profiles, the grayscale intensity in the black background was set to 0, and the maximum brightness in the sound enamel region was set to 100. These values were used to reconstruct standardized profiles, with the grayscale intensity plotted on the vertical axis and enamel depth plotted on the horizontal axis, using the tooth surface as the baseline. As a result, the enamel depths corresponding to specific grayscale intensity thresholds of 90% and 95% were measured on both μ CT and QLFD images.

2.5. Statistical Analysis

A paired t-test was conducted to compare the mean enamel depths (ED) corresponding to 90% grayscale intensity values obtained from μ CT (90% ED $_{\mu$ CT) and QLFD (90% ED $_{\text{QLFD}}$). Differences in the mean values of 90% ED $_{\mu$ CT and 90% ED $_{\text{QLFD}}$ across demineralization periods were analyzed using one-way ANOVA followed by Tukey's post hoc test. The same analyses were performed for the enamel depths corresponding to 95% grayscale intensity values obtained from μ CT (95% ED $_{\mu$ CT) and QLFD (95% ED $_{\text{QLFD}}$). The correlations among 90% ED $_{\mu$ CT, 90% ED $_{\text{QLFD}}$, 95% ED $_{\mu$ CT, and 95% ED $_{\text{QLFD}}$ were evaluated using Pearson correlation analysis. Simple linear regression analyses were performed

with ED_{QLFD} as the independent variable and ED_{μCT} as the dependent variable. All statistical analyses were conducted using IBM SPSS Statistics version 29.0 (IBM Corp., Armonk, NY, USA), and a p-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Comparison of Enamel Depth at 90% Grayscale Intensity

As the demineralization period increased from 10 to 70 days, both 90% ED_{QLFD} and 90% ED_{μCT} showed a significant increase (p<0.001). Across all demineralization periods, 90% ED_{QLFD} consistently demonstrated greater values than 90% ED_{μCT}. After 10 days of demineralization, 90% ED_{QLFD} was 0.249 ± 0.054 mm, while 90% ED_{μCT} was 0.181 ± 0.054 mm. After 70 days, 90% ED_{QLFD} increased to 0.443 ± 0.087 mm, and 90% ED_{μCT} increased to 0.339 ± 0.069 mm (Table 1).

Table 1. Enamel depth at 90% grayscale intensity

Demineralization period	N	Mean±S.D.		p-value ²
		90% ED _{QLFD} ¹	90% ED _{μCT} ¹	
10days	28	0.249±0.054 ^a	0.181±0.054 ^a	<0.001
20days	28	0.304±0.076 ^b	0.232±0.066 ^{ab}	<0.001
30days	28	0.361±0.073 ^{bc}	0.278±0.069 ^{bc}	<0.001
40days	28	0.362±0.077 ^c	0.281±0.073 ^{bcd}	<0.001
50days	28	0.396±0.052 ^{cd}	0.292±0.046 ^{cdf}	<0.001
60days	28	0.425±0.079 ^d	0.334±0.081 ^{df}	<0.001
70days	28	0.443±0.087 ^d	0.339±0.069 ^f	<0.001
p-value ³		<0.001	<0.001	

¹ Different letters (a–f) indicate statistically significant differences as determined by Tukey's post hoc test. ² Statistical significance was determined using paired t-tests (p < 0.05). ³ Statistical significance was determined using one-way ANOVA (p < 0.05).

3.2. Comparison of Enamel Depth at 95% Grayscale Intensity

Both 95% ED_{QLFD} and 95% ED_{μCT} significantly increased as the demineralization period lengthened (p<0.001). Across all demineralization periods, 95% ED_{QLFD} consistently showed higher values than 95% ED_{μCT}. For instance, after 10 days of demineralization, 95% ED_{QLFD} was 0.284 ± 0.062 mm, while 95% ED_{μCT} was 0.208 ± 0.054 mm. After 70 days, 95% ED_{QLFD} increased to 0.473 ± 0.086 mm, and 95% ED_{μCT} increased to 0.382 ± 0.074 mm (Table 2).

Table 2. Enamel depth at 95% grayscale intensity

Demineralization period	N	Mean±S.D.		p-value ²
		95% ED _{QLFD} ¹	95% ED _{μCT} ¹	
10days	28	0.284±0.062 ^a	0.208±0.054 ^a	<0.001
20days	28	0.334±0.078 ^a	0.257±0.064 ^{ab}	<0.001
30days	28	0.396±0.086 ^b	0.309±0.077 ^{bc}	<0.001
40days	28	0.410±0.092 ^{bc}	0.319±0.073 ^{cd}	<0.001
50days	28	0.431±0.051 ^{bcd}	0.331±0.049 ^{cdf}	<0.001
60days	28	0.466±0.083 ^{cd}	0.367±0.077 ^{df}	<0.001
70days	28	0.473±0.086 ^d	0.382±0.074 ^f	<0.001
p-value ³		<0.001	<0.001	

¹ Different letters (a–f) indicate statistically significant differences as determined by Tukey's post hoc test.² Statistical significance was determined using paired t-tests (p < 0.05). ³ Statistical significance was determined using one-way ANOVA (p < 0.05).

3.3. Correlation Between $ED_{\mu CT}$ and ED_{QLFD}

Strong positive correlations were observed between $ED_{\mu CT}$ and ED_{QLFD} at both 90% and 95% grayscale intensity values ($r=0.836-0.962$, $p<0.001$). The correlation was slightly higher between 90% $ED_{\mu CT}$ and 90% ED_{QLFD} ($r=0.903$) compared to 95% $ED_{\mu CT}$ and 95% ED_{QLFD} ($r=0.849$, Table 3).

Table 3. Correlation between QLF-D and μCT

	90% $ED_{\mu CT}$	90% ED_{QLFD}	95% $ED_{\mu CT}$
90% ED_{QLFD}	0.893 ($p<0.001$)		
95% $ED_{\mu CT}$	0.962 ($p<0.001$)	0.898 ($p<0.001$)	
95% ED_{QLFD}	0.836 ($p<0.001$)	0.944 ($p<0.001$)	0.849 ($p<0.001$)

The Pearson correlation coefficients and p-values were obtained using a two-tailed test.

3.4. Linear Regression Analysis

At the 90% grayscale intensity threshold, the regression model showed an R^2 value of 0.797, indicating that 90% ED_{QLFD} had a strong influence on 90% $ED_{\mu CT}$. The regression equation was $Y = 0.782X - 0.007$ (where Y represents 90% $ED_{\mu CT}$ and X represents 90% ED_{QLFD}). The Durbin-Watson value was 1.900, indicating no autocorrelation and independence of residuals. The model's goodness of fit was significant, with an F-value of 761.098 and a p-value < 0.001 , demonstrating that the regression equation was statistically significant. Similar results were observed at the 95% grayscale intensity threshold. The R^2 value was 0.720, and the regression equation was calculated as $Y = 0.741X + 0.015$. Both models yielded statistically significant results ($p<0.001$). The Durbin-Watson value was 1.826, and the model's goodness of fit was significant, with an F-value of 499.361 and a p-value < 0.001 , confirming the statistical significance of the regression equation (Table 4).

Table 4. Linear regression analysis results

Grayscale intensity threshold	Constant (Intercept)	Coefficient (B)	R^2 value	Standardized beta coefficient	p-value
0.90	- 0.007	0.782	0.797	0.903	<0.001
0.95	0.015	0.741	0.720	0.849	<0.001

The regression analysis was conducted to evaluate the relationship between enamel depth measured by QLFD and μCT .

4. Discussion

This study aimed to evaluate mineral loss in transverse sections of EDC using QLFD and to validate the analytical reliability of QLFD by comparing it with μCT . The analytical approach used in this study is distinct from previous research, as it focuses on transverse sections of teeth.

To assess the maximum depth of EDC, artificially formed on the enamel and extending to areas considered sound enamel, 90% and 95% thresholds were established, as referenced in previous studies [14,15]. Significant correlations were observed between QLFD and μCT at both thresholds ($r = 0.836-0.962$, $p<0.001$). Furthermore, a significant regression equation was identified between 90% ED_{QLFD} and 90% $ED_{\mu CT}$, indicating that for every unit increase in 90% ED_{QLFD} , 90% $ED_{\mu CT}$ decreased by 0.782. Similarly, for 95% ED_{QLFD} , a unit increase corresponded to a decrease of 0.741 in 95% $ED_{\mu CT}$. In both cases, the standardized beta coefficients were 0.903 and 0.849, respectively, indicating a strong influence of the independent variable, QLFD, on μCT measurements. These findings validate the effectiveness of QLFD in assessing mineral loss in transverse sections of EDC.

Most previous studies on QLFD have focused on validating its utility as a clinical research tool for surface-level analysis of EDC [5,8–11]. In earlier research utilizing QLF, the mineral loss in early caries lesions was assessed, and both artificial and natural lesions were reported to exhibit a linear

relationship between fluorescence loss and mineral loss [11]. The slope of this relationship indicated that a 10% fluorescence loss corresponded to a mineral loss of 0.15 kg·m⁻² [11]. Other studies evaluated the sensitivity and specificity of QLFD for caries detection, demonstrating that fluorescence loss data accurately reflect lesion depth and mineral status [10]. Additionally, numerous studies have reported that QLFD analysis of the tooth surface reflects the depth of EDC [9,16–18]. Although prior QLFD studies primarily focused on surface-level analysis, the linear relationship identified in this study between mineral loss measured by μ CT and ED_{QLFD} values in transverse sections is consistent with previous findings.

μ CT is a high-resolution imaging tool capable of precisely evaluating the depth of early caries lesions in three dimensions and is considered a viable alternative to TMR. However, its high cost and time-intensive analysis process limit its practical applicability in clinical settings. In contrast, QLF-D offers simplicity and cost-effectiveness. This study aimed to validate the quantitative assessment of lesion progression and depth in transverse sections of EDC. The strong correlation observed between ED_{QLFD} and ED _{μ CT} underscores the potential of QLFD as a practical tool for quantitative evaluation of EDC lesions in experimental environments.

This study was conducted on artificially induced EDC lesions. Therefore, future research should assess the validity of QLF for naturally occurring EDC lesions in extracted human teeth, encompassing various tooth types and lesion locations.

5. Conclusions

This study demonstrated that QLFD is a suitable and practical alternative to μ CT for assessing EDC in laboratory settings, providing a reliable method for quantitative evaluation of lesion depth and progression.

Data Availability Statement: <https://doi.org/10.5281/zenodo.14634776>

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Conflicts of Interest: The author declares no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

EDC	Early dental caries
TMR	Transverse microradiography
QLF	Quantitative light-induced fluorescence
QLFD	Quantitative light-induced fluorescence-digital
μ CT	Micro-computed tomography
ED	Enamel depth
90% ED _{μCT}	Enamel depth at 90% grayscale intensity values obtained from μ CT
90% ED _{QLFD}	Enamel depth at 90% grayscale intensity values obtained from QLFD
95% ED _{μCT}	Enamel depth at 95% grayscale intensity values obtained from μ CT
95% ED _{QLFD}	Enamel depth at 95% grayscale intensity values obtained from QLFD

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