An in vitro comparison of quantitative light-induced fluorescence-digital and spectrophotometer on monitoring artificial white spot lesions

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Summary
Purpose: The aim of this study was to evaluate the efficacy of quantitative light-induced fluorescence-digital (QLF-D) compared to a spectrophotometer in monitoring progression of enamel lesions.

Methods: To generate artificial caries with various severities of lesion depths, twenty bovine specimens were immersed in demineralizing solution for 40 days. During the production of the lesions, repeat measurements of fluorescence loss (ΔF) and color change (ΔE) were performed in six distinct stages after the demineralization of the specimens: after 3, 5, 10, 20, 30, and 40 days of exposure to the demineralizing solution. Changes in the ΔF values in the lesions were analyzed using the QLF-D, and changes in the ΔE values in lesions were analyzed using a spectrophotometer. The repeated measures ANOVA of ΔF and ΔE values were used to determine whether there are significant differences at different exposure times in the demineralizing solution. Spearman’s rank correlation coefficient was analyzed between ΔF and ΔE.

Results and conclusion: The ΔF values significantly decreased based on the demineralizing period (p < 0.001). Relatively large changes in ΔF values were observed during the first 10 days. There were significant changes in L’, a’, b’, and ΔE values in lesions with increasing demineralizing duration (p < 0.001). A strong correlation was observed between ΔF and ΔE with p = -0.853 (p < 0.001). The results support the efficacy of QLF-D in monitoring color changes. Our findings demonstrate that QLF-D are a more efficient and stable tool for early caries detection.

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Introduction

Early caries lesions are clinically observed as a white opaque spot. Histologically these lesions have a relatively intact surface layer, and the subsurface area (body of the lesion) has low mineral content (10–70 vol%) [1]. Because of these histological features, initial lesions in enamel are more difficult to detect. Currently, consensus in the dentistry emphasizes the importance of accurately detecting the earliest stage of the dental caries process. A new assessment system, the International Caries Detection & Assessment System (ICDAS), was developed to inform decisions regarding the appropriate diagnosis on dental caries. Especially, non-cavitated lesions were divided into two codes, codes 1 and 2, by the ICDAS criteria [2]. White spot lesions of the code 1 type can be visually detected with air-drying, while the lesions of the code 2 type can be visually detected without air-drying [3]. Thus, the lesions of the code 2 type are a more advanced stage of dental caries than the code 1 type [4]. However, these code 2 lesions are difficult to reverse with remineralizing treatment, such as fluoride application because the depth of these lesions is located some place between the inner 1/2 of the enamel and the outer 1/3 of the dentin [4,5]. Therefore, it is essential to detect the code 1 lesions to maximize remineralization of initial lesions [6–9]. Nevertheless, it is difficult to detect and diagnose the code 1 lesions physically with the naked eye [10]. Because of this limitation, a large number of the initial lesions may have been overlooked, which means a period of appropriate remineralization treatment may be missed [11,12]. Therefore, a detection device is required to diagnose the initial lesions at early stage of caries [13–15].

As previously mentioned, a typical characteristic of early caries is the visual change in lesions. This is the reason that demineralization challenges make micro-porosity of the subsurface enamel increases. Recently, it is possible to detect initial lesions as early as possible along with the development of quantitative methods for caries lesion detection, even prior to the non-cavitated stage of lesion formation. Thus we focused on quantitative light-induced fluorescence-digital (QLF-D) system (Inspektor Research systems BV, Amsterdam, The Netherlands) which has been recognized as a useful device to determine not only the occurrence of the initial lesions but also its progression [15,16].

Many previous studies have reported that QLF systems are reasonable devices for detecting and diagnosing early caries lesions [5,17–19]. In recent years, the QLF-D technology, upgraded versions of the QLF systems, have been developed (Fig. 1). These QLF-D technology are an optical instrument set that uses a modified filter set (D007; Inspektor Research Systems BV, Amsterdam, The Netherlands), an increased blue light (405 nm) and white light (general visual light) source, and a digital single-lens reflex (DSLR) camera with high resolution. In taking lesion images, fluorescence and general images were taken at a one shutter. The QLF-D technology can show the teeth in their natural color as well as identify the fluorescence change of the initial lesion. Moreover, the best advantage of the QLF-D technology can help with the continuous management of early caries because it is possible to nondestructively monitor dynamic changes of net de-/remineralization in the lesions. Clinically, the whitish appearance of early caries lesions becomes quite distinct with greater porosity from sound teeth. The clinical sign of the initial lesions is first detected via visual inspection by a dental professional. Thus, it is essential to focus on the color changes in the lesions.

In general, the color changes of a specific sample can be measured with a digital colorimetric device, such as a spectrophotometer [20]. According to previous study, a spectrophotometer based on the CIE L*a*b* color system was recognized as a reliable and an objective tool for quantitative evaluation of the changes in the vital tooth color [21–23]. Above all, it can measure slight color changes between the sound and lesion area in enamel because the spectrophotometer can numerically identify the differences in color that are difficult to physically see.

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Fig. 1  Quantitative light-induced fluorescence-digital (QLF-D) Biluminator™ 2 systems (a), the light sources of the QLF-D technology (b), and the QA2 program for analysis (C).
As previous explanation, typical clinical change of the early caries is the formation of an opaque white spot lesion. We can manage the early caries lesions by continuously monitoring this whiteness change of the initial lesions. The whiteness of the initial lesions is optical phenomena that result from increased porosity within the enamel crystal lattice structure [24, 25]. Thus, it will be possible that color changes of the enamel surface are quantitatively predicted as analyzing mineral loss. Therefore, the aims of this study were to compare a QLF-D analysis, which can assess fluorescence loss, and spectrophotometer measurements, which can assess color changes to monitor mineral changes in the early caries lesions.

Materials and methods

Preparation of enamel specimens

Twenty bovine incisors without cracks and white spots were selected. All specimens were sectioned (8 mm × 4 mm × 3 mm) from the labial surface of the bovine incisors using a low-speed saw with a diamond blade. All specimens were embedded in epoxy resin and ground with 600—4000 grit abrasive disc paper (SiC Sand Paper, R&B Inc., Daejeon, South Korea) on a water-cooled polishing unit to form a flat enamel surface.

Artificial caries lesion creation

Before generating artificial early caries lesions, half of each specimen surface was covered with acid-resistant nail varnish (Mix-nails, Mix&Match, Incheon, South Korea) to preserve the sound tooth surface. Dried specimens were immersed in deionized water for 30 min. To generate artificial caries lesions in enamel, each specimen was immersed in 40 ml of a demineralizing solution for 40 days at 37 °C. After initial lesion formation, the specimens were rinsed with deionized water. The demineralizing solution contained 0.1 M lactic acid gel (pH 4.8) with 1% carbopol (Carbopol® with deionized water. The demineralizing solution contained after initial lesion formation, the specimens were rinsed with deionized water for 30 min. To generate artificial caries lesions in enamel, each specimen was immersed in 40 ml of a demineralizing solution for 40 days at 37 °C. Artificial caries lesion creation

Early caries lesions with various severities of lesion depths were created over a period of 40 days. Repeat measurements on specimens were performed after 3, 5, 10, 20, 30, and 40 days of exposure to the demineralizing solution using the quantitative light-induced fluorescence-digital (QLF-D) technology (QLF-D Biluminator™ 2+, Inspektor Research systems BV, Amsterdam, The Netherlands) and spectrophotometer (CM-3500d, Minolta, Tokyo, Japan).

Measurements of fluorescence loss

The fluorescence loss in enamel was evaluated by ΔF using QLF-D technology. The ΔF is the change of fluorescence between the sound and the demineralized lesion part expressed as a percentage. From this variable, the quantification of mineral changes in initial lesions was determined, that is, using 0 as a reference point for sound enamel, ΔF represents the mineral loss.

All specimens were air-dried for at least 15 s at room temperature before QLF-D measurement. QLF-D examinations were conducted in a controlled and dark environment. The distance between the camera lens and surface of the enamel block was kept constant throughout the experiment to facilitate repeat measurements. After taking specimens images using the QLF-D technology, the fluorescence loss (ΔF, %) values in lesions were evaluated in six distinct stages: after 3, 5, 10, 20, 30, and 40 days of exposure to the demineralizing solution. The image taking conditions of the blue light of QLF-D technology were set as follows; shutter speed 1/15 s, aperture value 8.0, ISO speed 1600. Because the lesion window was identical in size for all specimens, only the average ΔF values were recorded at the 5% threshold level between the sound and demineralized enamel using the QA2 program (Inspektor Research systems BV, Amsterdam, the Netherlands) [25].

Measurements of the color changes

A spectrophotometer was used to measure the color changes between the sound and early caries lesion parts. The CIE L*a*b* color system is a three-dimensional uniform color space with axes L’, a’, and b’ where the difference in the distance from the origin measures the color change. The L’ values represent lightness and extend from 0 (black) to 100 (white). Both a’ and b’ values represent the redness to greenness and yellowness to blueness axes, respectively. The color change (ΔE) between the two parts can be automatically calculated within the CIE L*a*b* color system.

All specimens were dried for at least 30 min at room temperature and measured using the same black background in a dark room with controlled lighting settings. Prior to each use, the unit was calibrated using a white calibration plate and a zero calibration box. All specimens were measured on the target mask CM-A121 (3 mm in diameter) to capture the same site of each specimen. L’, a’ and b’ readings were obtained, and the color change (ΔE) between the baseline (sound enamel) and the demineralized part was calculated using the following equation:

\[ \Delta E = \sqrt{\left(\Delta L'\right)^2 + \left(\Delta a'\right)^2 + \left(\Delta b'\right)^2} \]

where ΔL’, Δa’ and Δb’ are the differences between the sound and post-demineralized parts.

Statistical analysis

To analyze changes in early caries lesions based on the exposure times in demineralizing solution, the results were compared by one-way repeated measures ANOVA and Bonferroni’s post hoc analysis with 95% confidence intervals. Spearman’s rank correlation coefficient was found between the ΔF and ΔE. All statistical analyses were conducted using the IBM SPSS package program (IBM SPSS Statistics 20.0 for windows, SPSS Inc., Chicago, USA).
Results

The average ΔF values were monitored by the QLF-D technology for 40 days. According to the result of the repeated measure ANOVA, the ΔF values significantly decreased based on the demineralizing period (Table 1, \( p < 0.001 \)). A relatively drastic decrease in the ΔF values was observed for all specimens within the 10 days of exposure to demineralizing solution. There were no significant changes in the ΔF values from 30 to 40 days.

<table>
<thead>
<tr>
<th>Exposure time (days)</th>
<th>3</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔF</td>
<td>-12.86</td>
<td>-22.86</td>
<td>-34.74</td>
<td>-47.21</td>
<td>-50.62</td>
<td>-51.38</td>
</tr>
<tr>
<td>(SD)</td>
<td>3.90</td>
<td>3.95</td>
<td>4.07</td>
<td>3.04</td>
<td>2.95</td>
<td>4.07</td>
</tr>
<tr>
<td>ΔΔF</td>
<td>-10.00</td>
<td>-11.88</td>
<td>-12.46</td>
<td>-3.41</td>
<td>-0.77</td>
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</tr>
<tr>
<td>(SD)</td>
<td>1.43</td>
<td>2.13</td>
<td>2.51</td>
<td>1.76</td>
<td>2.32</td>
<td></td>
</tr>
<tr>
<td>( p )</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

All values are given as the mean (SD). \( p \)-Values denote statistically significant differences within rows by repeated measures ANOVA and the Bonferroni’s test at \( \alpha = 0.05 \).

<table>
<thead>
<tr>
<th>Exposure time (days)</th>
<th>0</th>
<th>3</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
</tr>
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<tbody>
<tr>
<td>( L^* )</td>
<td>77.74</td>
<td>82.44</td>
<td>81.77</td>
<td>85.07</td>
<td>89.67</td>
<td>91.78</td>
<td>91.41</td>
</tr>
<tr>
<td>(SD)</td>
<td>1.77</td>
<td>1.59</td>
<td>1.49</td>
<td>1.19</td>
<td>1.54</td>
<td>1.20</td>
<td>1.44</td>
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<tr>
<td>( a^* )</td>
<td>-2.22</td>
<td>-2.51</td>
<td>-2.46</td>
<td>-2.47</td>
<td>-1.83</td>
<td>-1.37</td>
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<tr>
<td>(SD)</td>
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<td>0.27</td>
<td>0.30</td>
<td>0.32</td>
<td>0.29</td>
<td>0.25</td>
<td>0.47</td>
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<tr>
<td>( b^* )</td>
<td>1.42</td>
<td>-5.39</td>
<td>-5.07</td>
<td>-5.31</td>
<td>-2.17</td>
<td>-0.80</td>
<td>-1.35</td>
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<tr>
<td>(SD)</td>
<td>1.35</td>
<td>1.36</td>
<td>1.15</td>
<td>0.90</td>
<td>0.96</td>
<td>0.74</td>
<td>0.73</td>
</tr>
<tr>
<td>( \Delta E )</td>
<td>8.40</td>
<td>7.77</td>
<td>10.10</td>
<td>12.61</td>
<td>14.32</td>
<td>14.06</td>
<td></td>
</tr>
<tr>
<td>(SD)</td>
<td>1.40</td>
<td>1.04</td>
<td>1.21</td>
<td>1.15</td>
<td>1.46</td>
<td>1.50</td>
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<tr>
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<td>1.71</td>
<td>0.26</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>0.72</td>
<td>1.13</td>
<td>0.85</td>
<td>1.06</td>
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<tr>
<td>( p )</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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</tbody>
</table>

All values are given as the mean (SD). \( p \)-Values denote statistically significant differences within rows by repeated measures ANOVA and the Bonferroni’s test at \( \alpha = 0.05 \).
### Table 3

<table>
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<th>ΔF</th>
<th>Variables by the spectrophotometer</th>
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<tr>
<td></td>
<td>L⁰</td>
</tr>
<tr>
<td>p</td>
<td>−0.813</td>
</tr>
<tr>
<td>p'</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* p is the Spearman’s rank correlation coefficient.

* p < 0.01.

Fig. 2 Fluorescence and general images in lesions by QLF-D technology were presented according to demineralizing solution exposure duration. Fluorescence changes in lesions were represented by ΔF values and whiteness changes in lesions were represented by ΔE values. Left corresponds to the sound part of specimen and right to the demineralizing part.

The color changes in early caries lesions were also monitored with a spectrophotometer for a period of 40 days. Overall, there were significant changes in each of the L⁰, a°, and b° values in the lesions with an increase in the demineralizing solution exposure duration (Table 2, p < 0.001). In particular, an increase in the demineralizing time resulted in an increase in the L⁰ values, which reflected lightness of the lesion parts. Additionally, the a° values increased as the demineralizing time increased, and the b° values were positive at 0 day and negative 3 days or later. Compared to the sound enamel, the green color of the lesion parts faded, while the blue color intensified. Although the ΔE values changed irregularly between 3 and 5 days, the ΔE values increased continuously after 5 days (Table 2, p < 0.001). There were no statistically significant differences in the ΔE values at 30 days or later.

There was a strong correlation between the ΔF and ΔE values (p = −0.853, p < 0.001, Table 3). Fig. 2 respectively shows the fluorescence loss and color change aspects of specimens under blue and white light according to the demineralization period (Fig. 2). The changes of the fluorescence in the lesion parts were clearly distinguished in the fluorescence images, while it was difficult to differentiate the changes of the whitish appearance in the general images.

### Discussion

In this study, we focus on monitoring lesions of code 1 by the ICDAS, which is especially difficult to detect with the naked eye. With our same demineralizing method, we had confirmed the histological lesion depth values by polarized light microscopy (PLM) according to the demineralizing period. The lesion depths were as follows; 10 days of exposure causes approximately 113 ± 5.70 μm deep lesions, 20 days causes 218 ± 10.79 μm, 30 days causes 268 ± 8.08 μm, and 40 days causes 310 ± 18.38 μm (Data no shown). According to PLM analysis, the histological lesion depth values were significantly correlated with the ΔF values measured with the QLF-D with r = 0.94 (Spearman rank correlation, p < 0.001) [16]. Therefore, we produced artificial early caries lesions with various severities of lesion depths to measure the representative characteristics of early caries lesions, such as fluorescence loss and color changes with the QLF-D and the spectrophotometer.

In general, clinical signs of the early caries lesions in enamel are assessed as color changes in lesions by visual inspection or roughness of surface on lesions by tactile examination with probe. However, in a previous study, although visual and tactile examinations were used together, the sensitivity of the dental caries diagnosis is reduced.
Monitoring of enamel caries by QLF-D and spectrophotometer

According to the observed lesion with the spectrophotometer, the L’ values increased as the demineralization period increased (Table 2). Lesions tended to be brighter as the severity of the lesion increased. This result agrees with that of a previous study which reported that the caries progression could be assessed based on lightness [30]. Additionally, the ∆E values also increased proportionally to the demineralization period (Table 2). In this case, because the ∆E values were greater than seven units, it was possible to distinguish color changes between the sound and lesion parts. This is because a casual viewer can distinguish the color difference when the ∆E values between two colors are 5–6 units [29]. Nevertheless, the observation of early caries lesions with a spectrophotometer has the following limitation. First, all ∆E values in initial lesions were under 3 units. It means that the color changes during lesion progression were not physically visible. This is because a trained eye can distinguish color differences when ∆E values are 3 to 4 units [28]. Secondly, the ∆E values were irregularly changed between 3 and 5 days, which is the earliest stage in progression of the caries lesions (Table 2, p < 0.001). This could be because the enamel surface roughness increased drastically at the earliest stage of early caries. Thus, there is the limitation that stable detection for code 1 lesions through only color change analysis of lesion surface cannot perform. Finally, the spectrophotometer has limitations in correctly measuring color changes of teeth with a curved surface. The reason is that this device can measure the only flat surface of a solid, but not liquid, sample because it is very sensitive in light intervention.

Because of the aforementioned limitations for color changes in the lesion area, we focus on assessing early caries lesions using the QLF-D technology. Based on our results, mineral loss in lesions increased as the demineralizing solution exposure duration increased (Table 1). The ∆F values sharply decreased for all specimens in the first 10 days of exposure. However, there were relatively small changes in the ∆∆F values (below 5%) 20 days and later. Trend of these results was similar to those of previous studies, which analyzed mineral loss using TMR after demineralization [31–33]. The dynamic equilibrium of mineral ions was probably reached in the interface between the enamel surface and demineralizing solution because the demineralizing solution was never replenished. Nevertheless, we found that infinitesimal mineral changes in a shallow lesion in the outer half of enamel can be correctly monitored with the use of the QLF-D technology (Fig. 1). This result was opposing to the result of the spectrophotometer analysis. In other words, the clinical changes in the initial stage with progression of caries lesions are more stably analyzed with QLF-D technology than with a spectrophotometer. Additionally, the accuracy and reliability of QLF have already been demonstrated in many previous studies [5–7,13,17,34,35]. Therefore, the QLF-D technology, upgrading the version of QLF could also help with longitudinally quantifying early mineral losses or gains in the initial lesions, and this technique can be used to capture high resolution images of arrested initial and subclinical lesions, which will critically facilitate clinical assessments [36]. Therefore, the changes in the fluorescence images according to the lesion depth that are not observed in the general images by white light can be confirmed with blue light such as in Fig. 2. Additionally, patients can easily understand their oral condition because the pathological changes of the initial lesions can be quantitatively confirmed through a chair-side computer system.

We found that the ∆F values can be used more stable than the ∆E values in the lesion area. Additionally, the QLF-D technology can simultaneously provide visual image information and quantitative analysis of lesion changes at during incremental oral health care. Therefore, we suggest that preventive management of early caries lesion will become more useful with the QLF-D technology. However, further studies are required conducting experimental setups that mimic an oral situation, such as using in situ model.

Conflicts of interest
The authors declare that they have no conflicts of interest.

Acknowledgment
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