



## Evaluation of tooth wear by estimating enamel thickness with quantitative light-induced fluorescence technology

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### ABSTRACT

**Background:** Various techniques have been suggested to quantitatively assess tooth wear; most have limited clinical application. The first aim of this *in vitro* study was to estimate the residual enamel thickness of teeth with various degrees of occlusal wear using quantitative light-induced fluorescence (QLF). The second aim was to identify relationships between the fluorescence parameters of QLF and the conventional tooth wear index (TWI) system.

**Methods:** Sixty-nine extracted permanent premolars and molars with initial stages of tooth wear (TWI score 1a-2: enamel wear to dentin exposure) were used. Two blinded and trained examiners participated in evaluation procedures. Occlusal QLF-digital (QLF-D) images were acquired for selecting area of interest (AOI) and calculating fluorescence for occlusal tooth wear ( $\Delta F_{\text{wear}}$ ) of the AOI by the first examiner. Each specimen was cross-sectioned in the buccal-lingual direction. Enamel thickness from images obtained by stereomicroscopy and TWI of each sample was determined by the second examiner. Spearman correlation was used to determine the relationship of  $\Delta F_{\text{wear}}$  with enamel thickness and TWI.  $\Delta F_{\text{wear}}$  values were compared between histological scores with the Mann-Whitney *U* test.

**Results:** Seventy-six AOIs were analyzed. As enamel thickness decreased,  $\Delta F_{\text{wear}}$  values significantly increased and strongly correlated with enamel thickness (Spearman  $\rho = -0.825$ ,  $P < 0.001$ ). There were significant differences in  $\Delta F_{\text{wear}}$  values among TWI scores ( $P < 0.001$ );  $\Delta F_{\text{wear}}$  strongly correlated with TWI (Spearman  $\rho = 0.753$ ,  $P < 0.001$ ).

**Conclusions:**  $\Delta F_{\text{wear}}$  values, which denote fluorescence difference by using QLF, showed a strong correlation with residual enamel thickness and tooth wear severity.

### 1. Introduction

Tooth wear is the irreversible loss of dental hard tissue by physical and chemical factors [1,2]. The main cause of tooth wear is aging, during which the effects of occlusion and mastication processes accumulate. However, abnormal tooth wear due to excessive physical and chemical stimulation is known as pathological tooth wear. Particularly, dentin has a lower hardness than enamel, such that when the dentin is exposed by tooth wear, the subsequent speed of the tooth wear rapidly increases [2–4].

As life span lengthens, the importance of long-term maintenance of

healthy teeth increases. Therefore, problems of pathological tooth wear are increasingly recognized [5,6]. Previous research has shown that tooth wear affects oral health-related quality of life, as well as psychological characteristics [7,8]. Although it is possible to restore lost dental hard tissue with prosthodontic treatment, restored teeth require continuous monitoring and retreatment. Therefore, prosthodontic treatment is not a treatment option to completely replace natural teeth in terms of time and cost [9,10]. Therefore, to ensure long-term preservation of natural teeth, it is essential to detect early stages of pathological wear and provide preventive interventions to inhibit or postpone its progress, primarily by continually monitoring and

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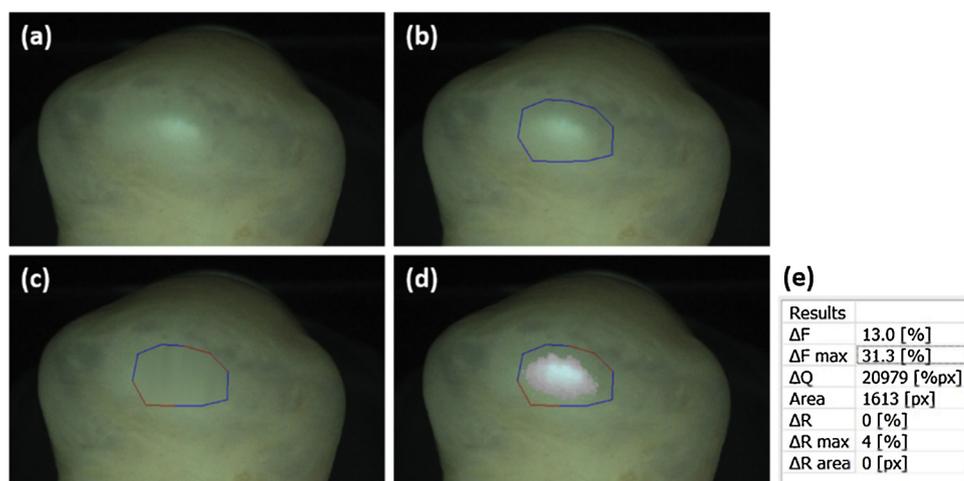
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**Fig. 1.** Quantitative light-induced fluorescence (QLF) image analysis process. (a) A representative fluorescence image of occlusal tooth wear. (b) A designed patch area around the wear. (c) A reconstructed image based on the fluorescence of the sound area. The blue line indicates the sound reference area, whereas the red line indicates the deactivated area. (d) The fluorescence difference between the original and reconstructed images. (e) Results of analysis of the tooth wear area.

managing the associated risk factors [5,10,11].

The primary clinical method for confirming the progress of tooth wear is the tooth wear index (TWI), which categorizes tooth wear based on subjective visual inspection. However, this method may exhibit reduced accuracy or consistency, depending on the subjectivity of the examiner or the individual characteristics of the subject [12,13]. To overcome these limitations, objective tooth wear assessment methods, such as profilometry, computer aided design-computer aided manufacturing, optical coherence tomography, radiography, and ultrasound, have been continuously applied in the research setting. However, most of these methods have not yet been used in a clinical setting; further, they involve disadvantages such as a time-consuming imaging process, difficulties inherent in constant image acquisition and measurement, high cost, and low image resolution [14–20].

Teeth emit fluorescence and phosphorescence when irradiated with ultraviolet light [21–23]. Quantitative light-induced fluorescence (QLF) technology, which exploits this phenomenon, is an optical technology that uses blue wavelength visible light of 405 nm, similar to ultraviolet light. In QLF images, a lack of fluorescence appears as darkened regions that indicate loss of minerals in the dental hard tissue. QLF has been used to noninvasively detect the depth and progression of early caries lesions [24–26]. Additionally, dentin exhibits fluorescence much brighter than enamel under ultraviolet excitation, as dentin contains a relatively large amount of organic components—these serve as fluorophores that induce fluorescence [21]. Kim et al. [27] were the first to apply QLF to assess tooth wear using this phenomenon. In their study, according to the artificial stepwise wear of the enamel layer, fluorescence intensity in occlusal surface fluorescence images showed a strong correlation with degree of wear ( $r = 0.994$ ,  $P < 0.001$ ). However, since the previous study was undertaken to observe changes in fluorescence generated by artificially induced physical wear in a controlled laboratory environment, it is necessary to confirm whether the same phenomenon occurs during multi-factorial tooth wear that occurs in the human oral cavity.

Therefore, the first aim of this *in vitro* study was to predict estimate the residual enamel thickness of teeth with various degrees of wear formed in the oral cavity by using relative fluorescence, measured by QLF. The second aim was to determine relationships between the fluorescence parameters and the conventional TWI [28] that can be used to evaluate the initial (enamel) stage of tooth wear. The null hypothesis of this study was that the relative fluorescence parameters exhibit no relationships with the residual enamel thickness of the occlusal wear or initial tooth wear severity.

## 2. Materials and methods

### 2.1. Sample collection and specimen preparation

This study was approved by the Institutional Review Board of Yonsei University Dental Hospital in the Republic of Korea (Approval No. 2-2015-0032). Power analysis was performed to calculate the sample size needed to compare fluorescence parameters of each TWI score using the Mann-Whitney  $U$  test. Based on data from a pilot study, a minimum sample size of nine samples for each group (36 total samples) was established. Using this sample size, the power was 80% for an alpha level of 0.05.

The mean age of the subjects was  $46 \pm 14$  years (age range: 21–63 years). After obtaining the consent of the subjects, a total of 75 teeth (43 molars and 32 premolars) with enamel wear and suspected dentin exposure, due to wear on occlusal surfaces, were collected. Importantly, teeth with the following conditions were excluded: dental cavities, restorations, tooth cracks, severe dentin wear, and pulp exposure. The reasons for tooth extraction were severe periodontal disease and orthodontic treatment. Calculus and periodontal tissues attached to the root and cervical surfaces were removed with a curette and scaler; whole plaque on the occlusal surfaces was removed with flowing water and a toothbrush. Afterwards, the teeth were stored frozen (at  $-20^\circ\text{C}$ ) until they were evaluated [29]. To maintain an equal distance between the lens and light sources, and the occlusal surfaces of the samples during QLF image acquisition, apical roots of teeth were partially cut so that the apex to crown lengths of all teeth were consistent with each other. Then, all teeth were immersed and fixed in an acrylic mold with acrylic resin (Jet™ Tooth Shade; Lang Dental Manufacturing Co., Inc., Wheeling, IL, USA).

### 2.2. Assignment, training, and calibrating of examiners

Two blinded examiners participated in the evaluation procedures. The first examiner (a dental hygienist) was instructed regarding the basic principles of the QLF analysis algorithm, with reference to Fig. 1; this examiner performed QLF image acquisition and analysis. The second examiner (a dentist) performed evaluations with conventional TWI scoring and enamel thickness measurement. The second examiner was trained regarding the concept of the TWI scoring system [28] and determined the scores of extracted teeth with various types of occlusal wear as a calibration process.

### 2.3. QLF image acquisition

Occlusal surface images were acquired in the vertical direction of the occlusal surface of each specimen by using QLF-D Biluminator™ 2+

system (Inspektor Research Systems BV; Amsterdam, The Netherlands); all specimens were fixed in the acrylic mold for this step. For each specimen, two images were obtained: an image from a general digital camera, obtained under a white light; and a fluorescence image, obtained under a narrowband violet light. The respective image acquisition conditions of the white light and fluorescence images were as follows: aperture value, 11.0 and 9.0; shutter speed, 1/60 s and 1/60 s; ISO speed, 1600 and 1600.

2.4. QLF image analysis

A total of 82 worn areas (cusps) from 75 samples were selected as areas of interest (AOIs), which were then used to quantify changes in fluorescence intensity compared with the sound enamel surface. QLF image analysis software (QA2J version 2.0.0.18, Inspektor Research Systems BV, Netherlands) was used to calculate the relative fluorescence parameters of the worn area, compared with the sound area, in fluorescence images acquired with QLF (Fig. 1).

In this analysis software, an algorithm is available for calculating the positive  $\Delta F$  value, which indicates the rate of increase in fluorescence intensity, through differences in the fluorescence intensity of the worn surfaces that show brighter fluorescence than sound enamel. To compare the calculated parameters with the minimum thickness of the residual enamel of the site, we used the maximum fluorescence parameter ( $\Delta F_{max}$ ) of the relatively brightest fluorescent spot in the worn area in this study; we named this variable " $\Delta F_{wear}$ ". To determine the fluorescence of a sound enamel surface without wear, we excluded the following from the analysis area: anatomical structures, caries lesions, and cracks that showed different color from enamel fluorescence in the periphery of the AOI.

2.5. Conventional evaluation with ordinal grading scales for tooth wear using the TWI system

To compare results between  $\Delta F_{wear}$  and categorized severity of tooth wear, the conventional TWI (an eight-point ordinal scale for (non) occlusal and (non) incisal grading [28]) was calculated. In a previous study, this system successfully evaluated the severity of tooth wear by subdividing initial tooth wear into various enamel phases. In addition, the reliability of occlusal and incisal wear grading was fair-to-good to excellent. Scores were determined as follows: 0 = no (visible) wear; 1a = (within the enamel) minimal wear of cusps or incisal tips; 1b = (within the enamel) facets parallel to the normal planes of contour; 1c = (within the enamel) noticeable flattening of cusps or incisal edges; 2 = wear with dentin exposure and loss of clinical crown height  $\leq 1/3$ ;

3a = wear with dentin exposure and loss of clinical crown height 1/3–1/2; 3b = wear with dentin exposure and loss of clinical crown height 1/2–2/3; 4 = wear with dentin exposure and loss of clinical crown height of  $\geq 2/3$ . The samples used in this study were only from the initial enamel wear stage to the first stage after dentin exposure (score 1a-2 of the list described above).

2.6. Enamel thickness measurement

Cross-sectional lines were established on the basis of AOI. Teeth were then cut with a microtome (TechCut 4™, Allied High Tech Products, Inc., Compton, CA, USA). From samples with multiple AOIs, multiple sectioned specimens were obtained based on the cutting line corresponding to each AOI.

Forty magnification images of the cross-sectioned surfaces were acquired with a stereomicroscope interlaced camera (Leica S9i stereozoom, Leica, St. Gallen, Switzerland) and dedicated image acquisition and analysis software (LAS version 4.12.0, Leica). The minimum enamel thickness of the worn cusp region was measured. Minimum thickness was measured based on the closest distance between the dentinoenamel junction (DEJ) line and the worn enamel surface contour. The light source of the microscope irradiated the upper side of the specimen. When the DEJ contour was not clear, the irradiation direction of the light source of the microscope was adjusted to the posterior and lateral directions of the specimen; then, the DEJ line was determined.

2.7. Statistical analysis

All statistical analyses were conducted with the Statistical Package for the Social Sciences (SPSS) version 23.0 (SPSS Inc.; Chicago, IL, USA), with a significance level of 0.05. Spearman rho analysis was performed between enamel thickness and  $\Delta F_{wear}$  values to determine the relationship between remaining enamel thickness and relative fluorescence intensity in each worn area. In addition, a Mann-Whitney U test was performed on the  $\Delta F_{wear}$  values of each TWI score to determine significant differences in fluorescence intensity based on the TWI. Finally, Spearman rho analysis was performed between TWI and  $\Delta F_{wear}$  values.

3. Results

Of the total 75 teeth, six were excluded in the final analysis because they met exclusion criteria, unclear DEJ contour and fracture of the specimen during the cross-sectioning process; thus, 76 AOIs of 69 teeth

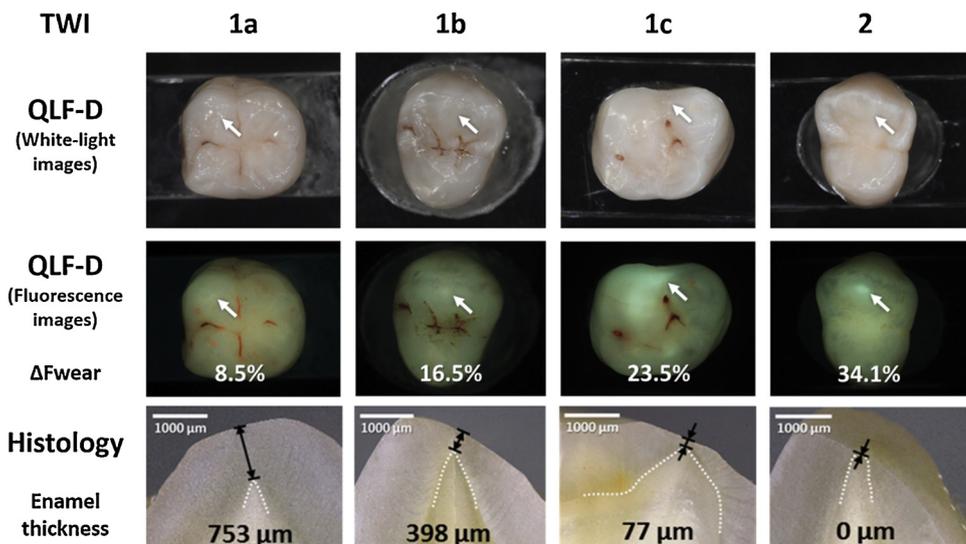


Fig. 2. Representative quantitative light-induced fluorescence-digital (QLF-D) images (white-light and fluorescence images) and stereomicroscope images of specimens, according to tooth wear index.  $\Delta F_{wear}$  values were calculated from fluorescence images. Enamel thickness was measured from stereomicroscope images. The white arrows indicate area of interest. White dotted lines indicate the dentinoenamel junction.

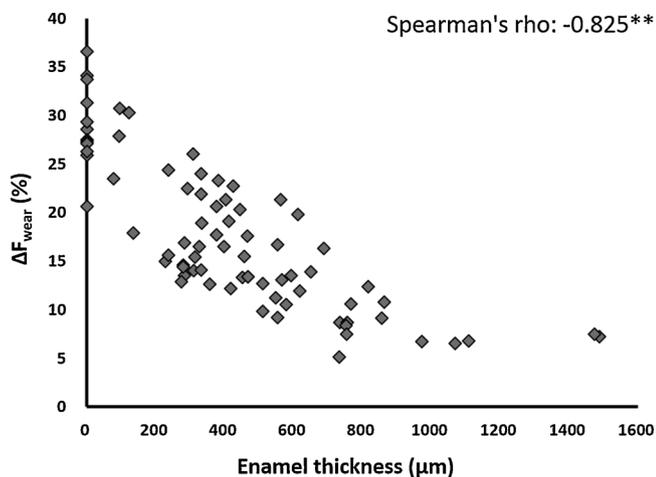


Fig. 3. A scatter plot of ΔF<sub>wear</sub> values for the residual enamel thickness of each sample. The dotted line indicates an exponential trend. \*\*P < 0.001.

(42 molars and 27 premolars) were included in the final analysis. Relatively higher ΔF<sub>wear</sub> values were measured in samples with thinner residual enamel or higher TWI score. Fig. 2 shows the representative QLF-D and stereomicroscope images after the initial ordinal grading scale for tooth wear (TWI score 1a-2).

ΔF<sub>wear</sub> values and corresponding enamel thicknesses of all samples included in the analysis are shown in Fig. 3. ΔF<sub>wear</sub> values and enamel thickness showed a strong negative correlation (Spearman rho = -0.825, P < 0.001). The samples used in this study were only from the initial enamel wear stage to the first stage after the dentin exposure (score 1a-2). Thus, there were no samples corresponding to a sound stage (score 0). The numbers of specimens for TWI scores 1a, 1b, 1c, and 2 were 10, 20, 35, and 11. Median values of ΔF<sub>wear</sub> were 8.0%, 13.4%, 17.7%, and 28.6%, respectively; there were statistically significant differences between the groups according to the TWI score (P < 0.001) (Fig. 4). In addition, ΔF<sub>wear</sub> values and TWI scores were strongly positively correlated (Spearman rho = 0.753, P < 0.001).

4. Discussion

In this study, the actual residual enamel thickness and initial

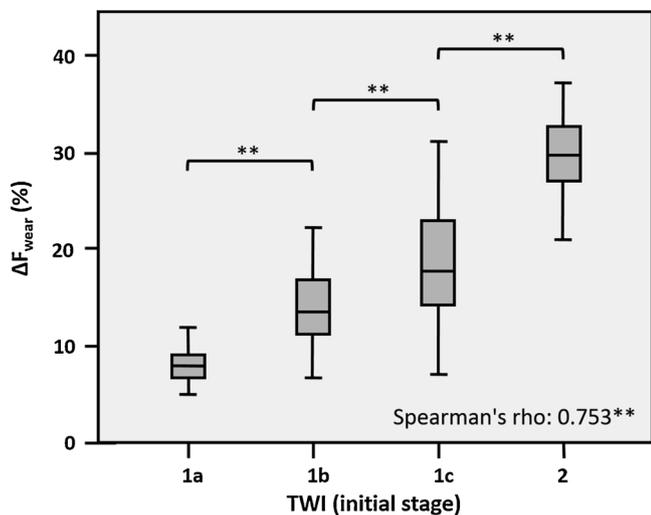


Fig. 4. Box-and-whisker plots of ΔF<sub>wear</sub> related to tooth wear index (initial stage). The boxes show the lower quartile, median (horizontal line), and upper quartile. The asterisks indicate statistically significant differences between each set of scores (Kruskal-Wallis test with post hoc Mann-Whitney U tests). \*\*P < 0.001.

occlusal wear can be evaluated objectively and noninvasively using the QLF technology, based on the autofluorescence properties of teeth. This indicates the applicability of QLF for assessing the severity of tooth wear and monitoring its changes in clinical situations. In addition, the results of this study will serve as important preliminary data for future clinical tooth wear evaluation and monitoring using autofluorescence of the teeth.

To the best of our knowledge, this is the first study to use QLF to evaluate the initial stage of tooth wear occurring in the actual oral environment of various subjects, based on the thickness of the residual enamel. Traditionally, in dentistry, indices have been used to subjectively determine and quantify the shape or severity of wear, based on the anatomical outlines of teeth and/or casts. The index system can be used easily at the chairside, but there is a limit to the prediction of residual enamel thickness. In addition, optical techniques for predicting enamel thickness and observing wear patterns in clinical situations are limited [11,14,30,31]. In this study, there was a slightly higher correlation between ΔF<sub>wear</sub> and residual enamel thickness than between ΔF<sub>wear</sub> and TWI. This suggests that ΔF<sub>wear</sub> more accurately reflects residual enamel thickness than tooth wear severity. Samples belonging to TWI score 1c showed a relatively higher variance of ΔF<sub>wear</sub>, compared with other scores (Fig. 4), because score 1c includes all samples with a flat facet due to wear, regardless of the actual thickness of the enamel. Accordingly, ΔF<sub>wear</sub> values would have been calculated by varying the enamel thickness of the samples.

Previous studies regarding the fluorescence of teeth on ultraviolet light excitation have identified a number of factors that contribute to the diversity of natural tooth fluorescence. The most important factor is age. It is known that, with increasing age, the formation and increase of secondary dentin, reduction in the volume of the pulp chamber, and extrinsic staining change the overall dental fluorescence [32–35]. Similarly, fluorescence intensity and color of the sound enamel areas of the collected samples varied. To reflect this phenomenon, the relative fluorescence intensity of the worn enamel or exposed dentin surfaces was calculated based on the fluorescence intensity and color of sound enamel for each tooth. This calculation method is similar to the algorithm for calculating the ΔF value, which has been widely used in the previous studies with QLF. The demineralization (early caries lesion) area, which is relatively dark due to mineral loss, is expressed as ΔF negative value, indicating relative fluorescence loss [25,36]. In contrast, areas where the fluorescence appears bright, due to decreased enamel thickness, are expressed as positive values; thus, the relative increase in the rate of fluorescence can be measured. In previous studies of artificial and physical tooth wear, the maximum fluorescence intensity of the occlusal surface was expressed by simple absolute values [27]. However, absolute values may have limitations in clinical situations where teeth with varying fluorescence properties must be evaluated. In addition, since the teeth collected in this study were samples with tooth wear that had been caused by various factors in the human oral cavity, they could more accurately reflect real-life clinical situations.

Tooth wear continuously progresses throughout the lifetime. Therefore, long-term monitoring and management is important to provide effective preventive intervention and to determine the appropriate starting point for restoration treatment [6,28,37]. QLF technology can acquire fluorescence images immediately using non-invasive visible light, which can be advantageous for long-term monitoring and frequent examinations [27]. In addition, extended use of the relative calculation method in the monitoring process may facilitate consistent analysis results, even if there are differences between images due to changes in the image acquisition environment during longitudinal monitoring. These advantages have been proven in previous studies, which demonstrated excellent reproducibility for *in vivo* longitudinal monitoring of early caries lesions with QLF [24–26,38].

In the clinical setting, fluorescence images of the entire occlusal surface can be obtained with QLF. This approach can be used to easily

capture and continuously monitor the appearance of tooth wear due to pathological factors (e.g., traumatic occlusion, parafunctional oral habits). Fluorescence images of the cervical area may be helpful to confirm the wear of the tooth by cervical abfraction, compared with the occlusal image, and to recognize the subject. Previous studies have shown that QLF can be used to obtain information regarding occlusal and proximal caries, as well as tooth wear, even within the same occlusal fluorescence images [39–41]. In addition, when applied to large-scale epidemiological surveys, it is possible to screen with fluorescence images, including those of mutable regions, thereby increasing economic and time efficiency. Furthermore, as a part of teledentistry, a remote evaluation system through image storage and transmission may also be available.

Because the QLF-D used in this study is optimized for laboratory research, control of the ambient light source was not needed when capturing a sample images. However, in order to obtain ideal clinical images over an extended period of time using QLF-D, the ambient lighting conditions of the clinical environment should be kept as constant as possible [27]. In addition, the use of QLF-D in the clinical setting may be limited in terms of cost and convenience (e.g., weight and mobility). Recently, new QLF devices have been developed that can be conveniently used in clinics; these are cost-effective and are actively used in clinical situations. For instance, Qraycam (AIOBIO, Seoul, Republic of Korea) is capable of imaging the entire occlusal and labial/buccal surfaces, in a manner similar to that of QLF-D. Qraypen (AIOBIO) is a form of intra-oral camera that can acquire more precise images of specific teeth and sites [42,43]. Subsequent *in vivo* studies using these devices will be performed to evaluate tooth wear in real clinical situations, as well as to determine the optimal image capture conditions for assessment of tooth wear using fluorescence. In this study, the results were retrospectively acquired by autofluorescence analysis of previously worn extracted teeth. Therefore, they were limited in the ability to predict the main cause of wear, the original shape of the tooth, and the speed of wear. Further studies should be conducted to confirm the applicability of autofluorescence for differential diagnosis of various types of tooth wear, which are caused by a variety of factors. Particularly, future *in vivo* studies are needed for substantive clinical applications. For example, a cross-sectional study could be conducted comparing fluorescence parameters between control and patient groups with pathological abuse by erosion, malocclusion, or parafunctional oral habits such as bruxism or clenching. In addition, a longitudinal study should be performed for comparison of wear speed between the groups by fluorescence parameter monitoring. Further studies are needed to determine the relationships between properties of autofluorescence, with regard to dentin, and symptoms of dentinal hypersensitivity. In conclusion,  $\Delta F_{\text{wear}}$ , calculated from changes in autofluorescence that occurred with occlusal wear, showed a strong correlation with residual enamel thickness and TWI. Therefore, QLF may enable early-stage evaluation and monitoring of tooth wear.

### Conflict of interest

Inspektor Research Systems BV provided the salary for author EdJdJ, but did not have any additional role in the study design, data collection, analysis, decision to publish, or preparation of the manuscript. EdJdJ's involvement in this research was under the auspices of his status as adjunct professor at Yonsei University College of Dentistry supported by BK21 PLUS Project. The specific role of EdJdJ was to provide his expertise regarding the fluorescence technology. This does not alter the author's adherence to the policies of the Journal of Biomedical Optics on sharing data and materials. EdJdJ holds several patents with respect to QLF technology. The remaining authors declared no conflicts of interest.

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