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Quantitative light-induced fluorescence: A potential tool for general dental assessment

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Abstract. Current dental diagnostic methods can detect caries but cannot quantify the mineral status of a lesion. Quantitative light-induced fluorescence (QLF) measures the percentage of fluorescence change of demineralized enamel with respect to surrounding sound enamel, and relates it directly to the amount of mineral lost during demineralization. Development of caries-like lesions and subsequent remineralization of the lesions were monitored by QLF. The results showed that the percentage of fluorescence change (ΔQ) increased linearly with the demineralization time and decreased with increased remineralization time. Stained teeth were whitened with a bleaching agent and the change in stain intensity (ΔE) was quantified using QLF. The results showed that ΔE decreased linearly as the tooth regained its natural color. Factors that might affect the use of QLF to detect and quantify caries were also examined. It was concluded that QLF could be used to detect and longitudinally monitor the progression or remineralization of incipient caries, however lesion detection may be limited by the presence of saliva or plaque and enhanced by staining. The change in shade of discolored teeth by whitening agents could be quantitatively measured by QLF. [DOI: 10.1117/1.1427044]

Keywords: quantitative light-induced fluorescence; caries quantification; optical methods; tooth whitening; tooth bleaching; artificial saliva.

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1 Introduction

Repair of the ravages of dental caries is costly in terms of time, resources, and oral health. The prevention of demineralization and the promotion of remineralization of early caries are therefore major goals of preventive dentistry. However, these goals can only be achieved if caries are detected at an incipient stage at which implementation of caries preventive regimes would enable early carious lesions to be remineralized before the need for restorative intervention. Although regular visits to the dental clinic are recommended, diagnostic methods in common use during these visits include visual and tactile examination with a probe,[1] radiography,[2,3] and fiberoptic transillumination.[4] These current clinical methods have limitations. They only have the capability to detect caries at a relatively advanced stage, and cannot quantitatively assess the mineral changes occurring over time in a caries lesion during the application of a therapeutic agent (e.g., a fluoride mouthwash). The current quantitative method, transverse microradiography,[5] involves the destruction of the tooth being studied and as a result could neither be used clinically nor for *in vitro* or *in situ* studies involving longitudinal assessment. Longitudinal monitoring of mineral changes will enable the effect of advice and treatments tailored to inhibit demineralization and promote caries remineralization to be determined. So it seems appropriate to develop a nondestructive method which not only detects the very early lesions that might escape detection by visual examination, but also quantitatively measures the changes in mineral status of the lesions on a longitudinal basis.

Quantitative light-induced fluorescence (QLF) is an optical technique which uses the natural fluorescence of teeth to discriminate between caries and sound enamel[6] based on the fact that the fluorescence radiance of a carious spot viewed with QLF is lower than that of surrounding sound enamel. QLF measures the percentage of change of fluorescence radiance of demineralized enamel with respect to surrounding sound enamel, and relates it directly to the amount of mineral lost during demineralization. In previous studies[7,8] the capability of QLF to detect and quantify caries was determined and compared the technique with other destructive methods and as a result were not capable of longitudinally monitoring the mineral changes over time in a caries lesion in a nondestructive manner. In the present study we determined the capability of QLF to longitudinally monitor the demineralization of teeth to produce caries-like lesions and the subsequent remineralization of the lesions. Certain factors that might influence the reproducibility of QLF imaging and analysis were investigated and possible solutions are discussed.

Improvement of the appearance of discolored teeth using whitening agents is one of the treatment modalities in dentistry, but the major problem associated with this treatment procedure is the difficulty in monitoring the change in color, which occurs gradually and is sometimes unnoticed. Standard

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shade guides, which require subjective visual grading, are used in most cases, but color perception of the human eye is affected by various factors which include ambient lighting, surrounding colors and interpretations of individual assessors.[9] Visual observation, therefore, is not only subjective but cannot detect a very slight change in tooth color. To overcome these difficulties and to provide accurate monitoring of stain removal by whitening agents, a computer-aided method that is not affected by the above factors, and that can quantitatively communicate a change in color would be more reliable. Carious lesions appear dark when viewed with QLF, however, stains on the tooth's surface exhibit the same phenomenon and appear dark, similar to caries, and the darkness increases as the intensity of the stain increases. It was therefore envisaged that QLF might be capable of assessing stain removal with a whitening agent. In the present study therefore it is demonstrated that the use of this device can quantify the gradual change in color of stained teeth following the application of a whitening agent.

2 Materials and Methods

2.1 Assessment of Demineralization and Remineralization

Freshly extracted bovine incisor teeth were collected, cleaned of all debris and soft tissue and examined. Twelve teeth free of caries, cracks or enamel malformations were selected and polished with pumice slurry (Associated Dental Products Ltd., Swindon, UK) to remove organic contaminants from the buccal surface. The teeth were then painted with two coats of a nonfluorescent acid-resistant colorless nail varnish (Max Factor®, Procter and Gamble Int., UK), except for a window of exposed enamel (6 mm in diameter) on the buccal surface of the teeth. Caries-like lesions were then produced on each tooth by demineralization in acidic buffer solutions containing 2.2 mM KH_2PO_4, 50 mM acetic acid, 2.2 mM of 1 M $CaCl_2$ and 0.5 ppm fluoride at a *p*H of 4.5.[10] Prior to demineralization and then every 24 h during demineralization, the fluorescent image of each tooth was captured using the QLF clinical system and stored on the computer (PC) for later analysis. On each occasion the tooth was mounted in the same position on a laboratory bench jack (Harvard Apparatus Ltd., Edenbridge, Kent, England). A standardized hydration condition was maintained by mopping the lesions with cotton wool rolls before the image was captured. This experiment was carried out for 4 days, with a total of five readings.

The QLF system was comprised of a special intraoral camera device connected to a computer fitted with a framegrabber (Comet, Matrox, Electronic Systems Ltd., Quebec, Canada), on which the QLF software (QLF version 2000, Inspektor Research Systems BV, Amsterdam, The Netherlands) was installed (Figure 1). To visualize and to capture the image of the tooth, white light from a special arc lamp (Philips BV, Eindhoven, The Netherlands) based on xenon technology was filtered through a blue-transmitting band pass filter (Philips BV, Eindhoven, The Netherlands) with peak intensity of $\lambda = 370$ nm and bandwidth of 80 nm to provide illumination of the tooth with blue–violet light with intensity of 13 mW/cm^2. A dental mirror provided uniform illumination of the tooth, and with the aid of a color charge coupled device (CCD) sensor (Sony LS-1P, Tokyo, Japan), which had a yellow-transmitting ($\lambda \geqslant 520$ nm) filter (Philips BV, Eindhoven, The Netherlands) positioned in front of it (to filter out all reflected and backscattered light), the fluorescent image of the tooth was recorded and digitized by the framegrabber and was available for quantitative analysis with the QLF software.[11] Once the fluorescent image of the tooth is captured and recorded by the PC, analysis of the lesion can be initiated by a user-defined patch with borders placed on sound enamel surrounding the lesion. The sound fluorescence radiance values inside the patch are reconstructed through two-dimensional linear interpolation of sound enamel values on the patch borders.[12] The decrease in fluorescence was determined by calculating the percentage of difference between the actual and the reconstructed fluorescence surface. Any area with a drop in fluorescence radiance of more than 5% is considered to be a lesion.[6] The QLF software automatically gives the value for the percentage of fluorescence radiance loss, ΔQ (%), and simultaneous data storage.[12,13]

The caries-like lesions produced in the above experiment were subsequently subjected to remineralization in artificial saliva[14] containing (g/L): $MgCL_26H_2O$(0.03), K_2HPO_4 (0.121), KH_2PO_4 (0.049), KCL (0.625), calcium lactate (3.85), fluoride (0.05 ppm), methyl-*p*-hydroxybenzoate (2.0), and sodium carboxymethylcellulose (0.4). The *p*H was adjusted to 7.2 using KOH. Methyl-*p*-hydroxybenzoate served as a preservative, while sodium carboxymethyl cellulose (CMC) was used to increase the viscosity of the artificial saliva, simulating the mucin and protein content of natural saliva. The other constituents provided the inorganic components necessary for the remineralization process at levels comparable to those of natural saliva. The remineralization process was carried out in an incubator (Memmert, Schwabach, Germany) at a temperature of 37 °C. Image capturing, recording and assessment were carried out on a weekly basis for 5 weeks.

2.2 Assessment of Tooth Whitening

Twenty extracted human premolar teeth were selected and prepared in the manner described in Sec. 2.1. Ten teeth were then painted with two coats of an acid-resistant colorless nail varnish (Max Factor, Procter and Gamble Int., UK), except for a window of exposed enamel (7 mm in diameter) on the buccal surface. The salivary pellicle acquired was formed on all 20 teeth by gentle rotation (10 rpm) of the teeth for 2 h in human whole saliva using a rotary mixer (Sandrest, East Sussex, England). Following pellicle formation, the teeth were stained by 1 h immersion in a 0.2% chlorhexidine gluconate mouthwash (Smith, Kline, Beecham Consumer Healthcare, Brentford, UK) followed by 4 h storage in a standard tea solution. The whole staining process was repeated twice before the teeth were finally stored overnight in tea. Boiling 6 g of tea leaves in 500 mL of water for 2 min produced a tea solution which was then filtered through gauze to remove the leaves and allowed to cool to room temperature. Both pellicle formation and staining were performed at room temperature (approximately 20 °C). Following staining, the nail varnish on the 10 painted teeth was removed with acetone [British Drug House (BDH), Poole, England] leaving only the window of exposed enamel with staining, [Figure 2(a)]. This therefore provided two experiments of 10 teeth each. Experiment A

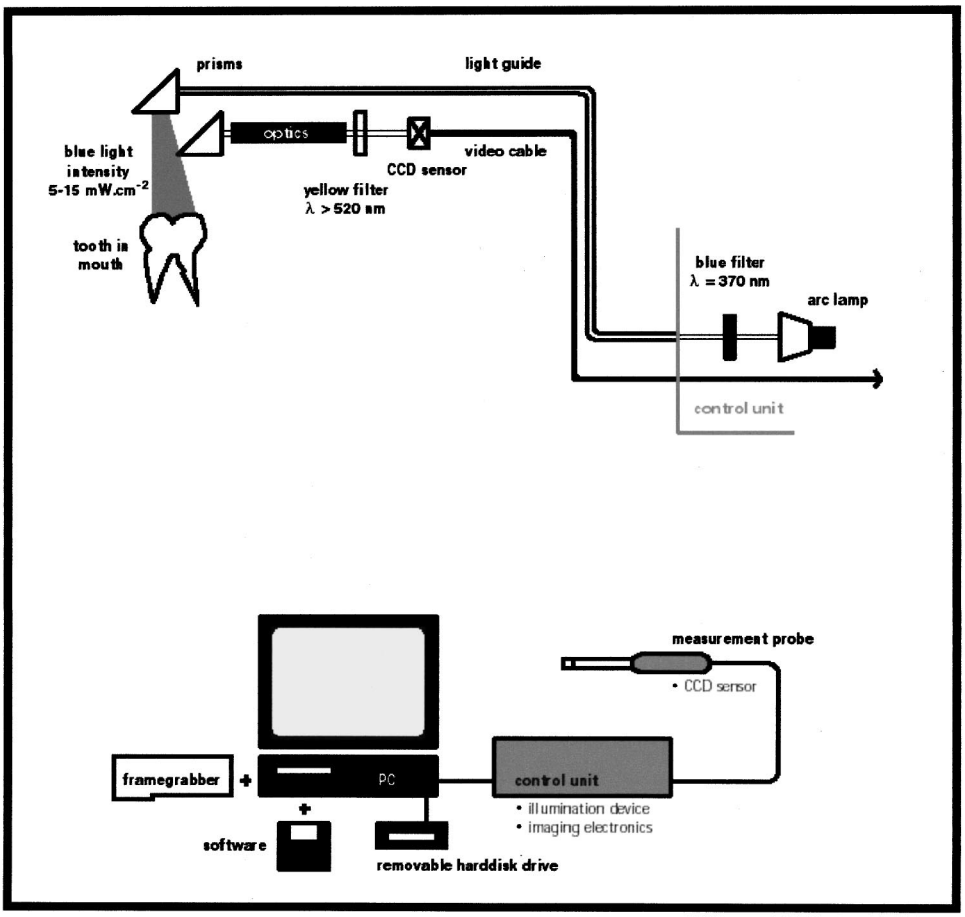

Fig. 1 Schematic illustration of the QLF clinical system. The arc lamp and blue filter are housed in a portable illumination device (control unit). The CCD sensor, yellow filter, prisms and a dental mirror are all contained in a hand-held intraoral camera device (measurement probe). The blue and yellow filter combination is optimized in such a way that the video image is completely free of reflections [see Figures 6(a) and 6(b)]. (Courtesy of Inspektor Research Systems BV, Amsterdam, The Netherlands.)

consisted of 10 teeth with a single spot stain [Figure 2(a)], while experiment B consisted of 10 sporadically stained teeth [Figure 2(b)]. All the teeth were subsequently mounted in a dental periapical radiograph film hanger to facilitate simultaneous immersion into a whitening agent. Prior to whitening, a fluorescent image of each tooth was captured using the QLF clinical system and stored on the computer (PC) for later analysis. This baseline recording was followed by gradual whitening of the teeth by intermittent immersion in 1:10 dilution of sodium hypochlorite (12% w/v available chlorine; BDH, Poole, England) for 60 s on each occasion. Following each immersion, the teeth were dried in air and images were captured with the QLF system and recorded in the PC for later analysis. This procedure was repeated several times until one specimen in each experiment was observed by visual examination to have regained its natural color; there was a total of six and four exposures for experiment A [Figure 2(a)] and experiment B [Figure 2(b)], respectively. Staining of the tooth surface resulted in a reduction in fluorescence radiance on the stained spot, hence the QLF software measured the stain intensity as the percentage of change in fluorescence radiance with respect to the surrounding unstained surface, and this was calculated in the manner described Sec. 2.1. However, the percentage of change of fluorescence on the tooth due to staining was represented as ΔE (in %) in order to distinguish it from the change in fluorescence in the tooth due to demineralization (represented as ΔQ in %).

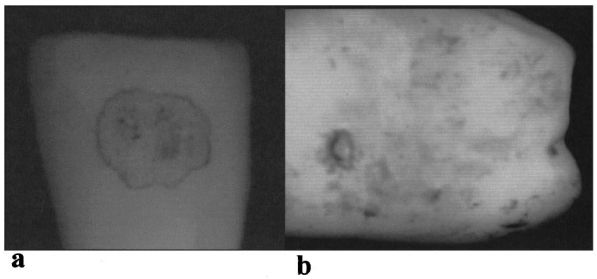

Fig. 2 Fluorescent images of teeth with spot stains (a) and sporadic stains (b) captured by QLF.

2.3 Effect of the Presence of Plaque

Fourteen natural white spot lesions were identified, by visual clinical examination, in three subjects who are members of staff of the dental school. The consent of the subjects was obtained. Following a baseline recording and quantification of

the fluorescence image of the lesions using QLF, the subjects were asked to refrain from oral hygiene to encourage the growth of bacterial plaque. After 3 days of plaque growth, the lesions were again visualized with the QLF clinical system and images of detectable lesions were captured.

2.4 Effect of the Presence of Saliva

Fifteen extracted human molar teeth were selected and prepared again in the manner described in Sec. 2.1. The teeth were then painted with two coats of the same nonfluorescence acid-resistant colorless nail varnish (Max Factor), except for a window of exposed enamel (3×2 mm^2) on the buccal surface of the teeth. Caries-like lesions were then produced on each tooth by demineralization in an acidic buffer solution (see Sec. 2.1). Using QLF, the percentage of change of fluorescence (ΔQ) in the lesion was quantified under three different conditions. (A) The lesions were mopped once with a cotton wool roll, (B) the lesions were wetted with water and (C) the lesions were wetted with a film of natural saliva. On each occasion the tooth was mounted in the same position on the laboratory bench jack.

2.5 Effect of Lesion Staining

Early caries lesions were produced on 15 extracted human premolar teeth, described in Sec. 2.1. The lesions were subsequently captured by QLF and the percentage of change of fluorescence quantified. Following this baseline analysis, the lesions were stained by immersion of the teeth into a standard tea solution, described in Sec. 2.2. The teeth were then washed under running tap water for 10 s. This procedure removed the stain from the surface of the teeth while the lesions remained stained due to imbibition of stain by the porous demineralized lesion. The stained lesions were then recaptured and requantified by QLF.

2.6 Effect of Lesion Magnification

Early caries lesions were produced on 15 extracted human premolar teeth, described in Sec. 2.1. Using the QLF clinical system a fluorescent image of each lesion was captured and quantified at six focal distances, of 50, 52, 54, 56, 58 and 60 mm. The focal distance is the distance between the surface of the lens of the QLF video camera and the surface of the lesion. Any alteration of this distance, for example, by adjusting the height of the bench jack on which the tooth was mounted, altered the magnification of the image of the lesion.

2.7 Influence of Tooth Thickness and the Presence of a Dentine Layer

Incipient enamel caries were produced on 15 molar teeth, described in Sec. 2.1. With a step by step reduction of the buccolingual dimension of the teeth using a water-cooled diamond wafering blade (Buehler, Warwick, UK), the change of fluorescence in the lesion was quantified at varying tooth thicknesses of 8, 6, 4.5, 3, 1.5, and 0.7 mm. A reduction in thickness was carried out from the lingual side of the teeth leaving the surface of the lesion intact. Following each reduction, the sample thickness was verified using a Mitutoyo Digimatic micrometer (Mitutoyo Corporation, Japan) before the fluorescence measurement.

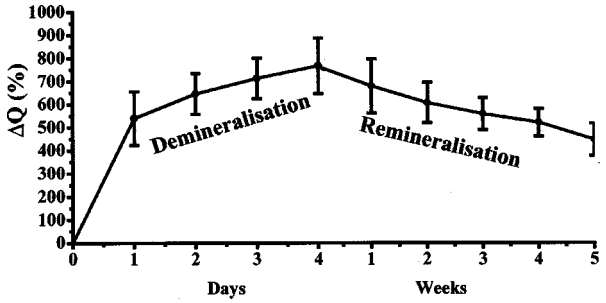

Fig. 3 Graphical illustration of the longitudinal monitoring of caries development and remineralization as quantified by QLF. There were significant differences (ANOVA, $n=12$) between the mean of daily readings for demineralization ($p<0.001$) and the weekly readings for remineralisation ($p<0.01$).

2.8 Detection of Developmental Anomalies

Fifteen teeth with white spot lesions that were confirmed, through their clinical history, to be developmental in origin were identified by visual clinical examination of four subjects. The consent of the subjects was obtained. The lesions were then visualized with the QLF clinical system and an image of the fluorescence of the lesions captured.

2.9 Statistical Analysis

The data obtained were analyzed statistically with a significance level (α) prechosen at 0.05.

3 Results

In assessing the demineralization and remineralization, Figure 3 shows that the percentage of change of fluorescence, ΔQ (%), increased linearly as the tooth loses minerals with an increase in demineralization time. On the other hand, as the caries lesion gained minerals with an increase in remineralization time, the tooth regains its autofluorescence in the lesion with a consequent decrease in ΔQ (Figure 3). There were significant differences (ANOVA, $n=12$) between the mean of daily readings for demineralization ($p<0.001$) and the weekly readings for remineralization ($p<0.01$).

The results of the assessment of tooth whitening are illustrated in Figures 4(a) and 4(b) for experiments A and B, respectively, and they show the mean variation in stain intensity (measured as the percentage of change of fluorescence, ΔE) over time. In both experiments, ΔE decreased linearly as the stain intensity decreased with the whitening time. Analysis of the variance showed statistically significant differences ($p<0.001, n=10$) among the readings in both cases.

After a 3 day abstinence of oral hygiene, 10 of the 14 natural caries lesions were obscured by bacterial plaque and could not be detected by QLF for analysis, while the remaining 4 were faintly visible. Hence, no quantitative analysis was carried out with respect to this investigation.

Figure 5 shows the influence of the hydration condition (A=dry, B=wet with water, C=covered by a film of natural saliva) of the lesion on fluorescence loss in the lesion. There was a significant difference (paired t test, $p<0.001$, $n=15$) in ΔQ (mean$\pm$standard deviation) when A (-35.1 ± 6.0) was compared with B (-10.0 ± 3.0) and C (-11.6 ± 3.8).

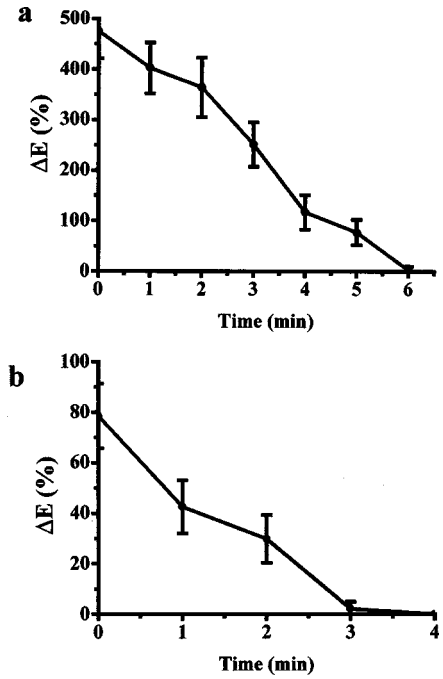

Fig. 4 Graphical illustration of the change in stain intensity (ΔE) with the whitening time measured by the QLF clinical system. (a) Experiment A with single-spot stained teeth [see Figure 2(a)], (b) experiment B with sporadically stained teeth [see Figure 2(b)]. Analysis of the variance showed statistically significant differences ($p<0.001, n=10$) among the reading groups in both cases.

Staining of the caries lesions with tea enhanced the loss of fluorescence observed when the lesions were viewed by QLF, with a consequent significant difference (paired t test, $p<0.001$) in the mean percentage of change of fluorescence, ΔQ (mean±standard deviation) under the two conditions, stained (-318.7 ± 103.3) and nonstained (-147.7 ± 53.1).

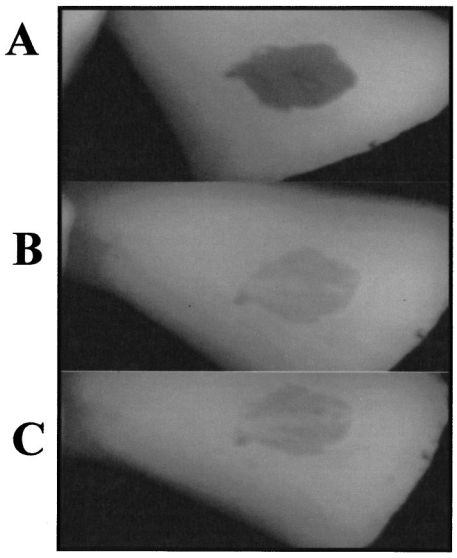

Fig. 5 Changes in the fluorescence image of an incipient enamel lesion under three different conditions as visualised by QLF. A=dry, B=wetted with water, C=covered by a film of saliva.

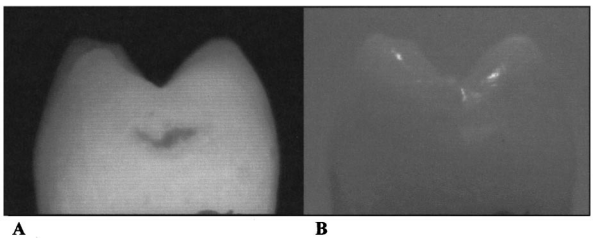

Fig. 6 Comparison of the fluorescent image of a carious tooth viewed by QLF (a) and white light (b). Note the reflections on the white light image that obscure the caries. The QLF image is completely free of reflections making caries detection easier than with white light. The yellow-transmitting filter ($\lambda \geqslant 520$ nm) positioned in front of the CCD camera filters out all reflected and backscattered light and when combined with the blue band pass filter the video image is completely free of reflections.

When the focal distance was adjusted from 50 through 60 mm, there was no statistical significant difference (ANOVA) observed in ΔQ between the focal distances investigated. Furthermore, at the point of sharpest image, which varied with each tooth, a distance of ±2 mm did not change the numerical value of ΔQ.

Upon examination of the influence of tooth thickness and the dentine layer, there was no significant difference (ANOVA, $n=15$) in ΔQ (mean±standard deviation) among the thickness groups, 8 mm (-20.0 ± 5.2), 6 mm (-21.7 ± 4.0), 4.5 mm (-21.5 ± 4.1), 3 mm (-21.3 ± 4.2), 1.5 mm (-20.7 ± 4.7) and 0.7 mm (-21.0 ± 4.0), investigated. However, at a thickness of 0.7 mm no dentine layer was left below the enamel, and the fluorescence image of the entire tooth sample appeared dark [under QLF, only lesions appear dark surrounded by bright fluorescent sound enamel, Figure 6(a)] and cannot be quantified, but when the enamel sample was replaced on a dentine block the lesion alone appeared dark and was quantified.

The developmental anomalies ($n=15$) viewed by QLF gave a similar appearance (dark surrounded by bright fluorescent sound enamel) as caries lesions [Figure 6(a)].

4 Discussion

In the present study we have demonstrated the capability of QLF to detect incipient caries as early as 24 h after development. The observation, in the present study, of the capability of QLF to longitudinally monitor enamel demineralization and remineralization over time shows that it would not only be a suitable tool to use in a dental clinic to monitor *in vivo* the mineral changes over time in a caries lesion upon application of a reparative agent, but would also be applicable in *in vivo*, *in situ* or *in vitro* testing of the efficacy of products formulated to inhibit demineralization and/or to promote remineralization. Production of caries-like lesions by the demineralization solution used in the present study has been reported,[10] while the artificial saliva used has demonstrated significant remineralization of caries in previous studies.[14] The validity, reproducibility and sensitivity of the QLF technique for detection and quantification of caries lesions in enamel have been evaluated with previously used caries quan-

tification methods such as chemical analysis, transverse microradiography and laser-induced fluorescence as well as longitudinal microradiography.[6,7,15,16]

QLF uses the natural fluorescence of the teeth, which is determined by the light absorption and scattering properties of teeth, to discriminate between caries and surrounding sound enamel.[6] The dark appearance of a caries lesion viewed by QLF is based on the principle that a demineralized enamel surface limits the penetration of light, resulting in more scattering of photons entering the carious surface, with the consequent limitation to the chance of a photon being absorbed and fluorescence being remitted from the demineralized surface than from the surrounding sound surface. Hence the lesion is observed as a dark spot surrounded by highly luminescent sound enamel [Figure 6(a)]. The blue and yellow filter combination in QLF is optimized in such a way that the fluorescence image is completely free of reflections [Figures 6(a) and 6(b)], thereby making caries detection easier and quicker. QLF detected 9.5 times more demineralized surfaces than visual clinical examination.[8] It can be seen from Figure 6(b) that the reflections on the white light image of the tooth have obscured the caries, whereas the QLF image is completely free of reflections, making the caries very evident [Figure 6(a)]. Furthermore, instantaneous provision of quantitative data gives QLF an advantage for use in dental clinics over other existing methods of caries diagnosis.[1–4]

Apart from demineralization, the fluorescence radiance of dental enamel can be affected by some other factors such as hydration of the tooth, as demonstrated in the present study (Figure 5), possibly due to a change in the optical properties of the enamel resulting from differences in the refractive indices of water and enamel. However, although in the present study we have shown that the detection of a caries lesion by QLF may be limited by the presence of bacterial plaque or saliva, the application of prophylactic cleaning prior to QLF imaging would facilitate the efficiency of the device while the use of a standardized moisture control technique would ensure the reproducibility of QLF analysis in a longitudinal study. The application of a cotton wool roll used in the present study offered a reproducible hydration state. The levels of moisture control by other methods such as drying in air, either by a time delay or using a dental three-in-one syringe, cannot be controlled since the hydration state obtained within a specific period of time would depend on the ambient temperature while the level of dryness obtained with a three-in-one syringe depends on the amount of pressure applied by the operator.

The presence of a dentine layer beneath the enamel influences the light scattering and absorption properties of the tooth.[6] Furthermore, in an *in situ* study, enamel blocks of varying thicknesses bearing artificially produced caries lesion are bonded onto the surface of a natural tooth in the mouth.[17] Hence the effect of the thickness of these enamel blocks and of the presence or absence of a dentine layer on the reproducibility of QLF was investigated in the present study, and it showed that, providing dentine is present, neither the thickness of the enamel block used in an *in situ* trial nor the buccolingual dimension of the tooth *in vivo* would influence the fluorescence radiance of the tooth. Although in the present study we demonstrated that staining of a caries lesion by chromogens present in dietary foods would enhance the loss of fluorescence, the capability of the QLF clinical system to longitudinally monitor lesion activity is the most important clinical requirement. Furthermore, although white spots resulting from enamel malformations gave a similar appearance to incipient caries when viewed by QLF, the capability of this device to monitor lesion activity over time makes it the most suitable technique with which to distinguish between a caries lesion and a white spot due to developmental hypomineralization, since caries are a dynamic process whereas a developmental anomaly such as fluorosis is an inactive white spot which does not exhibit any change in its mineral status over time. Another important observation from the present study is the fact that, providing the image is sharp before it is captured and recorded by QLF, slight variations in image magnification which may occur through intra- and interobserver imaging do not appear to influence the reproducibility of QLF analysis.

The use of QLF to monitor and quantitatively communicate the gradual change of the shade of discolored teeth upon application of a whitening agent, which was successfully demonstrated in the present study [Figures 4(a) and 4(b)], is a major development in the field of clinical dentistry. Apart from the fact that improvement of the appearance of discolored teeth using whitening agents is one of the treatment modalities in dentistry, and has been facing the major problem of difficulty in monitoring the change in color, there are many commercially available tooth-whitening products (e.g., toothpaste, mouthwash) which dentists and researchers in oral care are eager to examine and confirm as to their tooth-whitening potential. QLF proved to be the most suitable measure of shade and monitoring device for use by clinicians because of its capability to instantaneously provide quantitative data, which gives it an advantage over other existing methods of color assessment.[18,19] The use of these currently existing methods by clinicians is limited by the fact that a series of calculations with standard equations is required before final quantitative data can be obtained. Furthermore, these devices measure the light reflected from the tooth surface being examined so as to obtain accurate and reproducible data, and a color reading can only be taken on a flat surface of the tooth so that the aperture can be positioned to prevent loss of reflected light and interference from external light sources. QLF uses the same principle as that which depicts early caries as a dark spot on the fluorescence image of the tooth to show stain on a tooth surface of similar appearance. As a result, a stained spot on the tooth surface viewed by QLF will appear dark, similar to a caries lesion [Figures 2(a) and 2(b)]. It was surprising to observe in the present study that QLF was capable of analyzing sporadically stained teeth [Figures 2(b) and 4(b)], possibly by making use of intervening sound enamel surfaces. However, the system is currently being developed to make it capable of using a patch from a distant sound enamel surface for analysis of a homogeneously stained tooth.

Although the discoloration of teeth from extrinsic sources has been ascribed to a wide variety of substances,[20] the stain formation technique used in the present study has been used for many years in dental research.[21–23] The stain developed is almost entirely organic and represents discoloration that would develop initially *in vivo*. There has been controversy over the mechanism of stain formation by dietary substances. The more conclusive evidence to date tends to favor the precipitation of chromogenic dietary compounds onto locally ad-

sorbed antiseptic cations.[24] However, an ionic exchange type of mechanism has been reported to predominate, in which ions on the pellicle surface are simply exchanged for those contained in foods or beverages,[19] with chromophore retention occurring as a result of electrostatic attraction[25] and is easily removed by surface-active agents.[26] The whitening agent, sodium hypochlorite (NaOCL), used in the present study is an active bleaching agent that acts by direct oxidation of the stain through its ability to release nascent oxygen.[27]

5 Conclusions

The QLF clinical system was shown to be able to longitudinally monitor mineral changes over time in an early caries lesion, and to quantitatively monitor the gradual change in shade of discolored teeth by a whitening product. QLF, therefore, has the potential for wide application in clinical dentistry and dental research. It would not only be a suitable tool to use in a dental clinic to monitor the activity of early diagnosed caries upon application of a reparative agent, but would also be applicable in *in vivo*, *in situ*, or *in vitro* testing of the efficacy of products formulated to inhibit demineralization and/or to promote remineralization.

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