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Quantifying enamel demineralization from teeth with orthodontic brackets—a comparison of two methods. Part 1: repeatability and agreement

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SUMMARY The aim of this investigation was to compare the repeatability of measuring enamel demineralization surrounding an orthodontic bracket using two techniques: computerized image analysis from digitally converted photographic slides and quantitative light-induced fluorescence (QLF). Fifteen human molars were halved and shaped to look like incisors. The teeth were individually numbered and orthodontic brackets bonded to the buccal surface. The crowns were covered with acid resistant varnish, except for windows approximately 1.5 × 3 mm adjacent to the gingival, occlusal, mesial, and distal edges of the bracket. The windows were variously exposed to a demineralizing gel for 0, 3, 7, or 14 days, and the acid resistant varnish was removed. Standardized photographic slides and QLF images of the teeth were taken. These were repeated after 1 week. The slides were converted to grey scale digital format and analysed using Image-Pro Plus 3.0. The QLF images were stored, processed, and analysed using customized software. All images were recoded for blind analysis. The four surfaces of the bracket were inspected and only areas of suspected demineralization were analysed. This was repeated after 1 week.

The limits of agreement and mean difference between repeat readings of the area of demineralization were similar for both techniques (–0.04 ± 0.43 for photographs and –0.10 ± 0.63 for QLF). Mean grey level (photographs) and mean loss of fluorescence from that area (ΔF) (QLF) showed acceptable limits of agreement. The Intra Class Correlation (ICC) was below 0.81 for the measurement of area from QLF, suggesting that random error needs to be reduced. There was evidence of systematic bias for the repeat readings of the grey levels from the photographs ($P < 0.001$). Enamel demineralization surrounding an orthodontic bracket can be measured reproducibly using these two techniques.

Introduction

Demineralization of enamel surrounding orthodontic brackets is a significant clinical problem. Iatrogenic white spot lesions lead to poor aesthetics and, in severe cases, the need for restorative treatment. Strategies to prevent demineralization subsequent to orthodontic treatment must be developed. To test the effectiveness of these preventive regimes, a technique for the recording and measurement of enamel white spots should be used that is both reproducible and valid (Houston, 1983).

The severity of enamel demineralization can be quantified in terms of the area of the tooth surface that it covers and the degree of mineral loss. A recording and measuring technique for demineralization should show good repeatability for both these parameters and it should be clear that the technique is measuring enamel defects that are associated with the orthodontic appliance and not one of the many other causes of white spot lesions (Small and Murray, 1978).

Photographs are commonly used in the clinical environment. They are a convenient and effective means of permanently recording the optical properties of enamel. It has been shown that an area of demineralization on the buccal surface of the tooth can be reproducibly measured using photographic slides that have been converted to digital images and measured with computerized image analysis (Benson *et al*., 2000; Willmot *et al*., 2000).

Various optical methods have also been developed to quantify enamel demineralization. These are well reviewed by Angmar-Månsson *et al.* (1996). They can be categorized into non-fluorescent methods, such as the optical caries monitor (ten Bosch *et al*., 1980), and fluorescent methods. The fluorescent methods have previously involved the use of ultraviolet or laser light, which are potentially dangerous forms of radiation, particularly to the eyes.

Recently, a small portable system with a new light source and filter system has been described for intra-oral use (Al-Khateeb *et al*., 1997). It is called quantitative light-induced fluorescence or QLF. Light from an arc lamp passes through a blue filter, with a peak intensity of 370 nm, along a liquid light guide to a

handpiece that can be directed at the tooth surface. Enamel fluorescence is detected using an intra-oral camera within the handpiece. The reflected light passes through a yellow high-pass filter of 520 nm in front of the camera to exclude light below that frequency. The combination is optimized to minimize reflections. The images are stored, processed, and analysed with customized software (Inspektor Research Systems BV, Amsterdam, The Netherlands). QLF provides quantitative data for area of demineralization and mean loss of fluorescence. The latter has been correlated with mineral loss measured using the destructive techniques of transverse microradiography and chemical analysis (Al-Khateeb *et al.*, 1997).

The aim of this study was to compare the quantification of demineralization surrounding an orthodontic bracket using two methods.

1. Computerized image analysis from a digitally converted photograph.
2. Quantitative light-induced fluorescence.

The experiment is described in two parts. The repeatability and agreement of the two techniques are compared in this article, and in Part 2 the validity is assessed.

Materials and methods

Fifteen extracted human molars were used in this *in vitro* study. It would have been preferable to use incisors, but it was not possible to obtain a large enough sample. The teeth were carefully inspected to ensure the clinical absence of white spot lesions. They were divided in half by cutting mesio-distally down the long axis of the tooth with a diamond wheel (Isomet; Buehler Ltd, Evanston, IL, USA). This produced a buccal and a lingual half for each tooth, which were shaped to look like incisors by using the diamond wheel to contour the mesial, distal, and occlusal surfaces. A unique identifying number was engraved on the cut surface of each half tooth. The cemento-enamel junction of each tooth was grooved with a small round burr and filled with light-cured composite resin dyed with a red vegetable dye to highlight the junction and simulate the gingival margin.

Identical standard edgewise twin brackets, with a slot size of 0.018 × 0.025 inch [Ortho-Care (UK) Ltd, Bradford, UK], were bonded to the surface, in the usual position for an orthodontic attachment. The crowns of the teeth were then covered with three coats of acid resistant varnish (Max Factor; Procter and Gamble, Weybridge, UK), except for windows approximately 1.5 × 3 mm of enamel surface on the gingival, occlusal, left, and right aspects of the bracket (Figure 1). The teeth were attached to glass rods and placed in a demineralizing gel (lactic acid, buffered with sodium hydroxide to a pH of 4.5, in hydroxyethylcellulose).

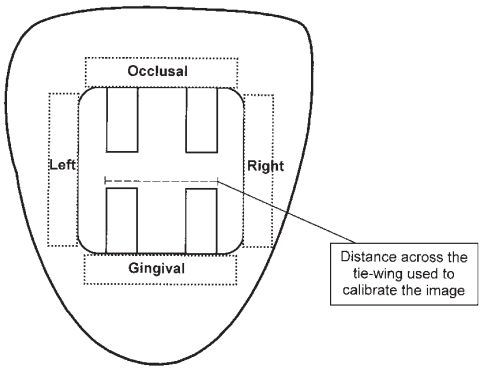

Figure 1 Diagram showing a tooth with an orthodontic bracket and the enamel area on each of the four sides of the bracket that was exposed to the demineralizing gel.

During the time in the gel, the windows on the enamel surface of the teeth were incrementally covered in a systematic arrangement to subject the exposed enamel surface to demineralization for 0, 3, 7, or 14 days (Figure 2). The patterns were chosen to ensure that some teeth had no demineralization challenge, some had severe demineralization, and there was a spread of patterns in between. The occlusal edge of the bracket has been identified as a site without a high prevalence for demineralization (Mizrahi, 1982, 1983), therefore, it

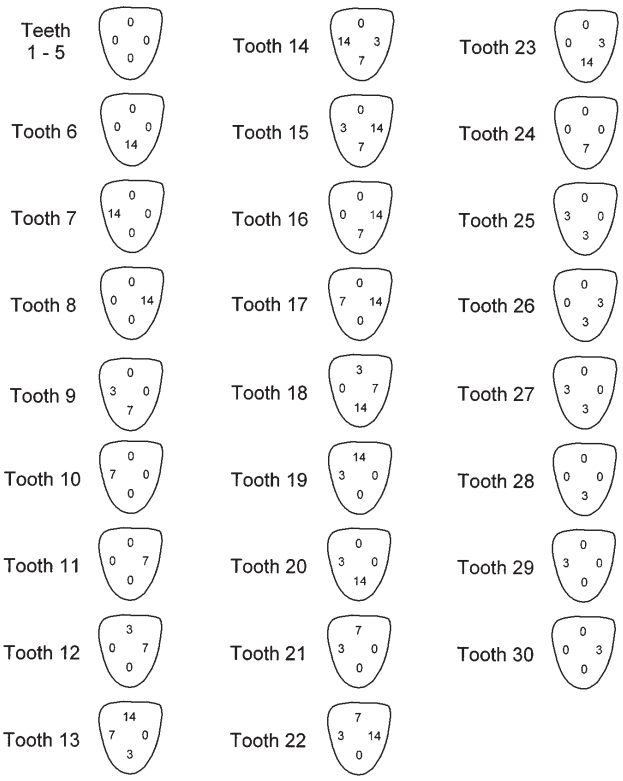

Figure 2 Diagram showing the periods of incremental demineralization in days for the gingival, occlusal, left, and right surfaces of the bracket for the 30 teeth.

was not given priority when arranging the putative patterns of demineralization.

A tooth that was designated to have areas with different periods of demineralization was removed from the gel after the shortest exposure time, washed in distilled water, and three coats of acid resistant varnish applied to cover the relevant window. Once the varnish had dried, the tooth was replaced in the gel. This was repeated until the maximum exposure for that tooth had been completed (between 3 and 14 days). Following exposure to the gel, the teeth were washed in distilled water and the varnish removed with acetone.

Photographic technique and image analysis

Standardized photographs were taken of the teeth, using a technique that has been described previously (Benson *et al*., 1998). The aperture was opened to F16, as a slightly lighter image was found to be beneficial when converting the image to a digital format and performing the computerized image analysis.

Photographs were taken with masking on the ring flash below the lens to reduce reflections (Benson *et al*., 2000; Willmot *et al*., 2000). To improve the replication of the camera positioning, a sighting jig was placed in the bracket slot (Figure 3). The jig consisted of a full sized (0.018×0.025 inch) rectangular stainless steel archwire, with one long and one short arm. The jig was held in the bracket slot with an elastomeric ligature. The camera was lined up at right angles to the bracket using the rectilinear attitude of the jig. The end of the long arm of the jig was identified in the viewfinder of the camera. The camera was then moved toward the tooth until the end of the short arm was in view. When the ends of the long and short arms were adjacent in the horizontal plane, the photograph was taken. After each photograph, the jig was removed. The jig was also constructed with a grey scale consisting of three shades: white, grey, and black, to allow for grey scale calibration of the digital image (Figure 4).

The photographs were repeated after 1 week, to provide two sets of photographs. When all the photographs had been taken and developed using the same machine, the slides were recoded by a second investigator (NP) to allow a blind assessment by the principal investigator (PEB).

The photographs were converted to digital images as previously described (Benson *et al*., 2000). The images were opened using the image analysis software (Image-Pro Plus, version 3.0 for Windows 95; Media Cybernetics, Silver Spring, MD, USA). Each image was individually calibrated in millimetres, using the bracket as the calibrating measure (Figure 1). To determine the calibration measurement, the distance across the outside of the tie-wings of five brackets (Figure 1) was measured on two occasions, 1 week apart, with digital callipers (Mitutoyo Corp., Minato-ku, Tokyo, Japan). The readings were averaged (3.33 ± 0.06 mm) and this figure was used to calibrate each image.

The grey scale images were opened in Image-Pro Plus and calibrated using the bracket tie-wing measurement

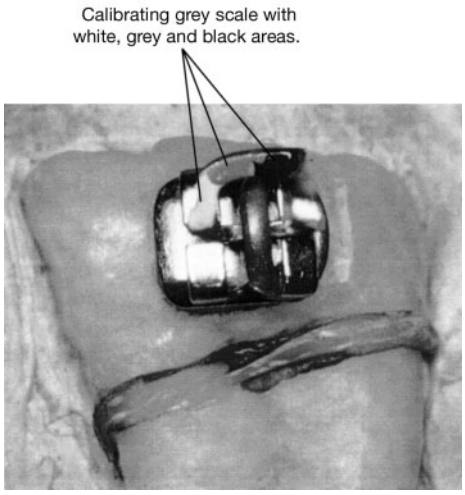

Figure 4 Grey scale image of the bracketed tooth with the positioning jig in place and showing the calibrating grey scale.

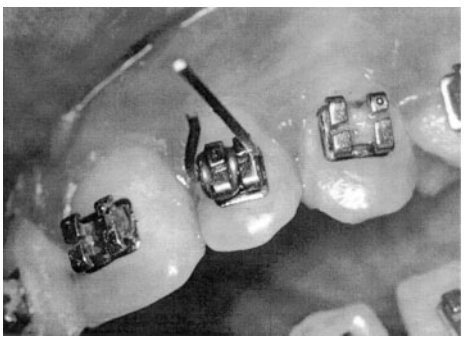

Figure 3 Image of the positioning jig as used in a clinical study. The jig is placed in the orthodontic bracket slot to allow reproducible positioning of the camera.

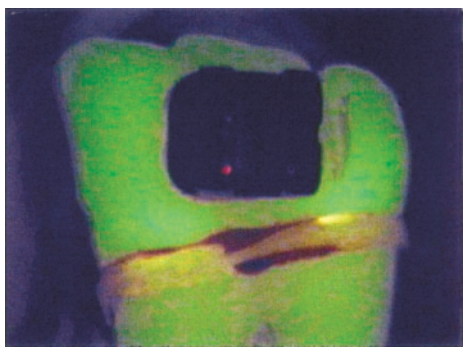

Figure 5 QLF image of same tooth as Figure 4 showing demineralized dark area to the right of the bracket.

(Figure 1). The four edges of the bracket on the grey scale image were individually inspected and, if an area of demineralization was observed, then an area of interest (AOI) was delineated around it. The area and the mean grey scale levels of the AOI were recorded. Only when the observer considered an area of demineralization to be present was a reading taken. Therefore, there were two processes occurring. First, subjective visual assessments to produce a dichotomous estimate of the enamel surface (yes or no to demineralization); and secondly, a measurement of the enamel on those parts of the tooth surface judged to be demineralized.

Quantitative light-induced fluorescence

Images of the 30 teeth were captured using the arc lamp with a liquid light guide system described by Al-Khateeb *et al*. (1997). Two images of each tooth were captured 1 week apart (Figure 5). The images were stored, processed, and analysed with customized software developed by de Josselin de Jong (v 2.00c; Inspektor Research Systems BV). A second investigator (NP) recoded the images, which were stored on the hard drive of a computer. The principal investigator (PEB) then analysed each image, blindly, on two occasions at least 1 week apart.

The images were inspected and only dark areas of suspected demineralization were assessed. The customized software allows the construction of a box around the dark area, including some normal enamel. In order to exclude the bracket from the image, the lesion threshold discriminators of the software were set at an upper limit of 95 per cent and a lower limit of 55 per cent. This was found to exclude the bracket from the calculation. In addition, the edge of the analysis box that included the bracket was 'switched off', so that the computer program excluded this region from the calculation of sound enamel. The outcome measures from QLF are: area of demineralization (mm^2), mean loss of fluorescence of that area, or ΔF (%), and the parameter ΔQ, which is the mean loss of fluorescence integrated over the lesion area (mm^2 %).

Statistics

The repeatability was assessed using descriptive statistics for the difference between the first and second readings of the same image. Only data that included two readings from the same site were analysed. When a recording was taken on one occasion but not the other, this was excluded. This was done because it barred from the error calculation the subjective assessment of the image, and only the error of the method was assessed. The Intra Class Correlation (ICC) (Fleiss, 1986) was used to assess random error and the one sample *t*-test for systematic error (Houston, 1983). The differences between the readings of the first and second photographic and QLF images were assessed using the limits of agreement (Bland and Altman, 1986). The agreement between the area measurements of the two techniques was also assessed using the limits of agreement.

Results

The descriptive statistics for repeatability are shown in Table 1. There were 60 images produced for each technique (30 teeth, imaged twice). From these 60 images there were 240 possible sites where demineralization had occurred (four sites or edges to the bracket per image). Of the 240 sites, 92 had actually been exposed to the demineralizing gel and the remaining 148 had not been exposed (Figure 2).

The number of measurements carried out from the photographs was 108 from the first assessment and 108 from the second assessment. Seven readings from the first assessment were not repeated in the second assessment and seven from the second assessment were not carried out in the first assessment. These isolated measurements were excluded from the error assessments for reasons explained in Materials and methods. There were therefore a total of 101 recordings when two measurements were carried out from the repeated readings of the same photograph.

The number of measurements carried out using the QLF technique was 87 from the first reading and 85 from the second reading, of which 83 were performed on the same site from both assessments.

The average area of demineralization measured was 2.1 mm^2 with the photographic technique and 2.5 mm^2 with QLF. Table 1 shows that the mean difference for the area of demineralization measured from the photographs was small (-0.04 ± 0.43 mm^2). The mean difference in grey levels between the first and second recordings was -1.71 ± 4.57 greys. There was no evidence of systematic bias between the recordings for the area ($P = 0.120$). The one sample *t*-test for the mean grey level gave a significant result ($P < 0.001$), suggesting that there was some systematic bias between the repeat readings, with the second reading being higher than the first. However, a mean difference of –1.71 greys on a scale from 0 to 255 can be considered small and would not be clinically significant. The ICC of reliability was slightly better for the measurement of the grey scale level (0.89), compared with the area (0.82), but this was still within acceptable limits.

The differences between the repeat readings from the QLF images are also shown in Table 1. The mean differences for the area of demineralization, mean percentage loss of fluorescence (ΔF), and the integration of fluorescence loss over area (ΔQ) were small. The variances of the area and ΔF were also small; however, the variance for ΔQ was large, leading to a large confidence interval

Table 1 Mean difference, standard deviation (SD) of the differences, and 95 per cent confidence intervals (CI) for the two techniques.

	Photographs (n = 101)		QLF (n = 83)		
	Area (mm^2)	Mean grey level	Area (mm^2)	ΔF (%)	ΔQ (mm^2 %)
Mean difference	–0.04	–1.71	–0.10	–0.10	–0.07
SD	0.43	4.57	0.63	1.91	13.47
CI	–0.12–0.05	–2.61– –0.81	–0.24–0.04	–0.52–0.32	–3.01–2.87
P	0.120	<0.001	0.143	0.639	0.962
R	0.82	0.89	0.79	0.84	0.81

The photographic readings included the area and mean grey levels. The QLF readings included area, mean change in fluorescence (ΔF), and integration of ΔF over area (ΔQ). Also shown are the probability (P) that the differences were significant from zero as measured with a one sample t-test for systematic error, and the ICC of reliability (R) for random error.

for the mean difference. The one sample t-test showed no evidence of systematic error for any of the parameters. The ICC of reliability was similar to the photographic technique, except for the area measurement, which was below 0.81, suggesting that the random error of the technique needs to be reduced.

Figure 6 shows the limits of agreement between the measurement of the areas of demineralization from the first and second photographic images of the same tooth. The limits of agreement were narrow (–0.88–0.82 mm^2) suggesting acceptable agreement between the readings. Figure 7 shows the limits of agreement for the grey scale readings. The limits were –10.85–7.42.

Figure 8 is a graph of the limits of agreement for the areas of demineralization recorded from the first and second recordings of the same QLF image. The limits of agreement were narrow (–1.36–1.16 mm^2). Figure 9 shows graphically the limits of agreement for the mean change in fluorescence (ΔF). The limits were –3.92–3.72. Figure 10 shows the limits of agreement for the product of area and ΔF (ΔQ), which were –26.01–26.87.

The limits of agreement for the recording of the area of demineralization between the two images for each technique are shown in Figure 11.

Discussion

Photographs have been employed in a number of studies investigating the prevalence of both iatrogenic and developmental defects of enamel (Houwink and Wagg, 1979; Gorleick *et al*., 1982; Dooland and Wylie, 1989; Ishii and Suckling, 1991). Frequently, neither the reliability of the recording, nor measurement from photographs has been reported. Ellwood (1996) found

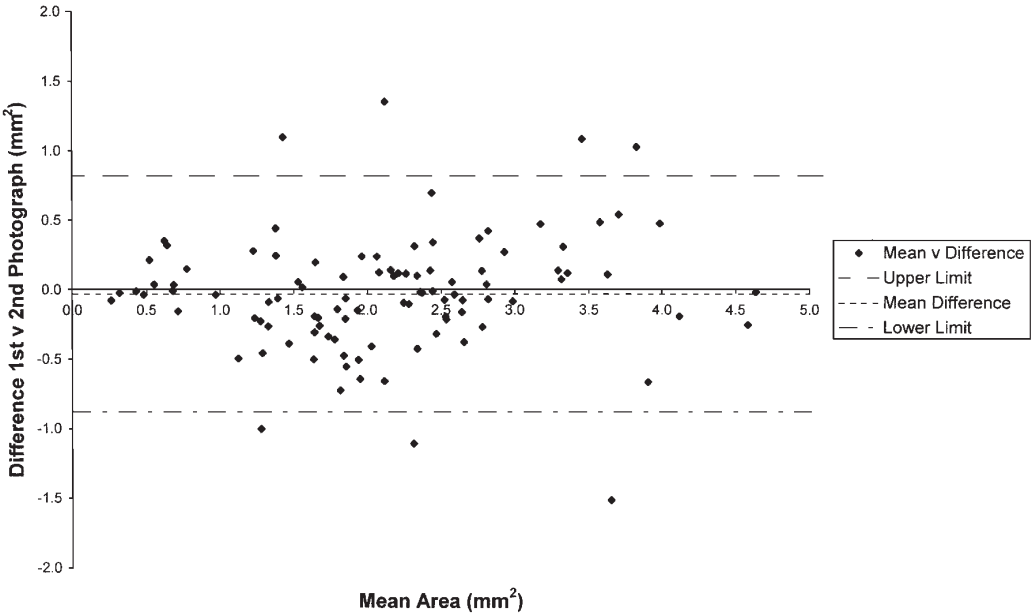

Figure 6 Limits of agreement for the areas of demineralization recorded from the first and second photograph.

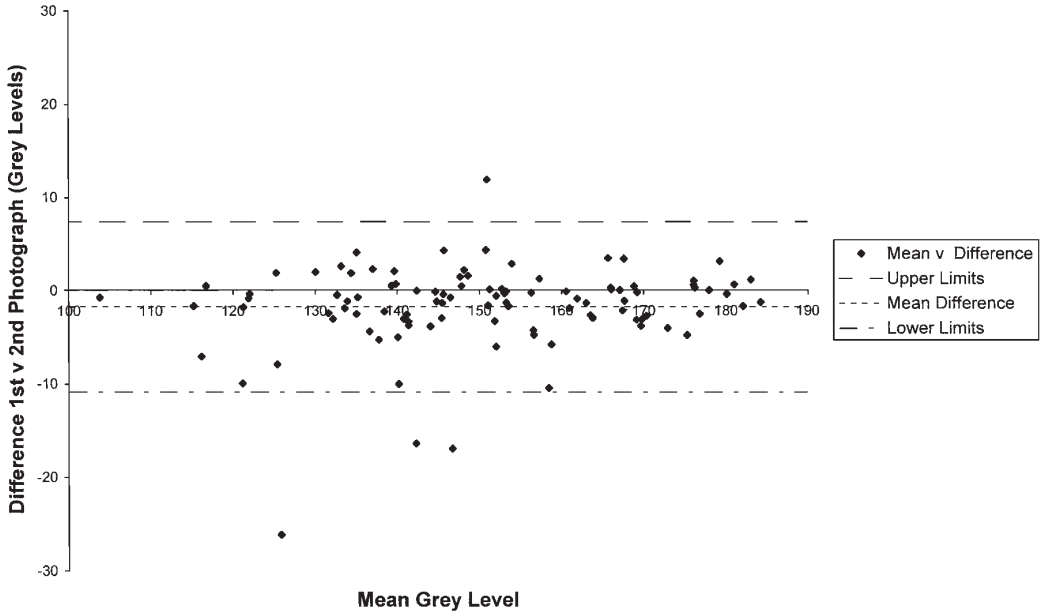

Figure 7 Limits of agreement for the grey scale level recorded from the first and second photograph.

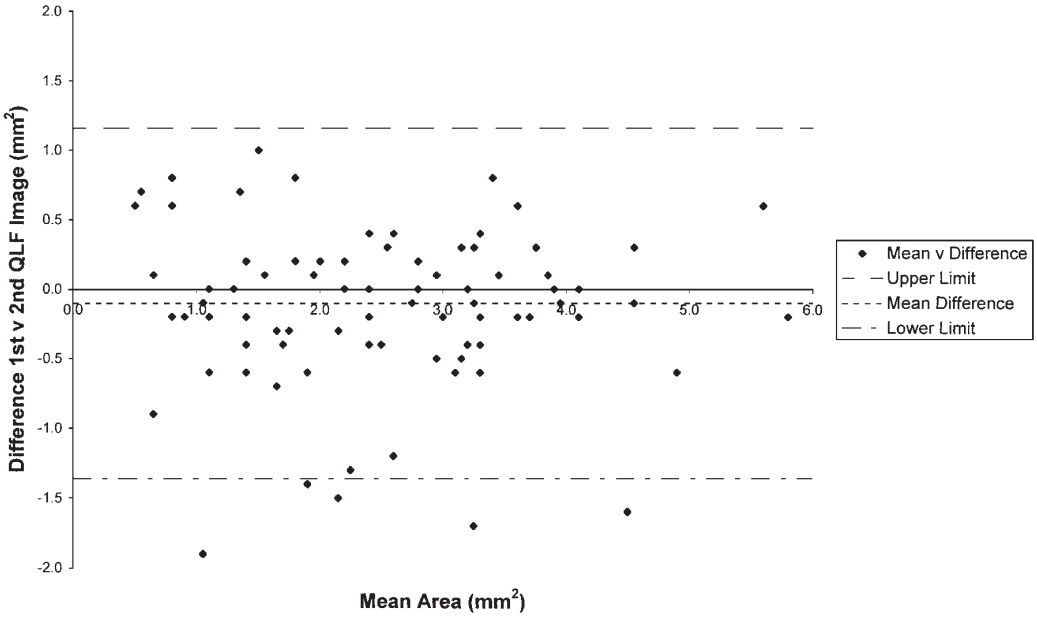

Figure 8 Limits of agreement for the areas of demineralization recorded from the first and second QLF images.

an acceptable reproducibility of recording the prevalence of developmental enamel opacities from photographs; however, he was not concerned with either quantifying the area, or the severity of the mineral loss.

The severity of enamel demineralization can be expressed either in terms of the size of the white spot lesion, or by quantifying the extent of the mineral loss. Recently, it has been shown that the area of a white spot can be reproducibly measured by converting a photograph of the artificially demineralized, buccal surface of a tooth to a digital image and using computerized image analysis (Benson *et al*., 2000; Willmot *et al*., 2000).

This study has also shown that both the area of demineralization and a relative assessment of mineral loss of a white spot lesion surrounding an orthodontic bracket can be recorded and quantified reproducibly using either of the two techniques investigated. The repeatability of using image analysis to measure demineralization

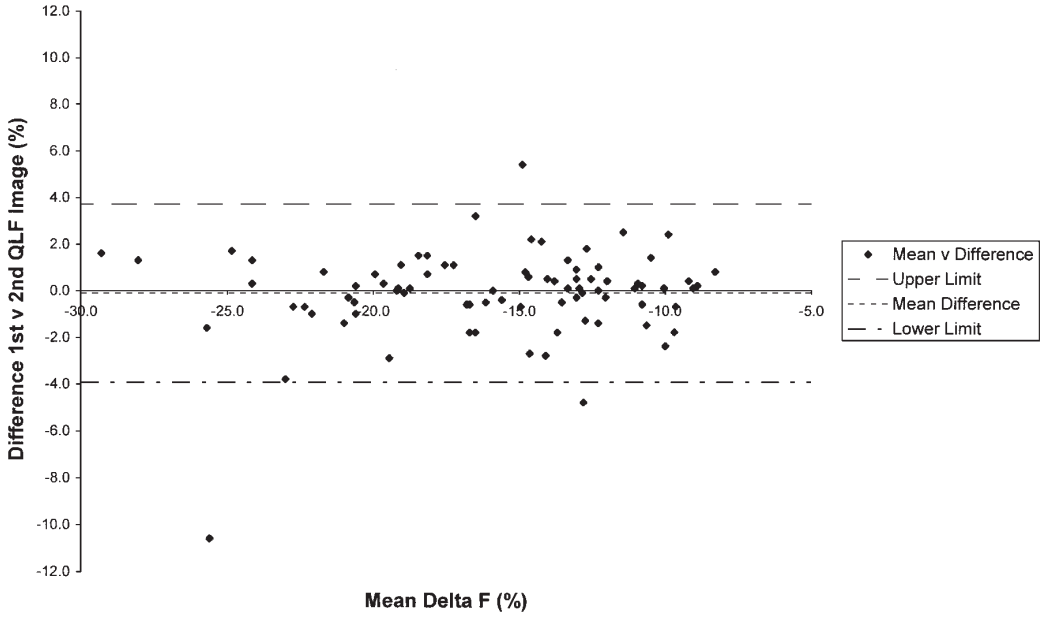

Figure 9 Limits of agreement for ΔF recorded from the first and second QLF images.

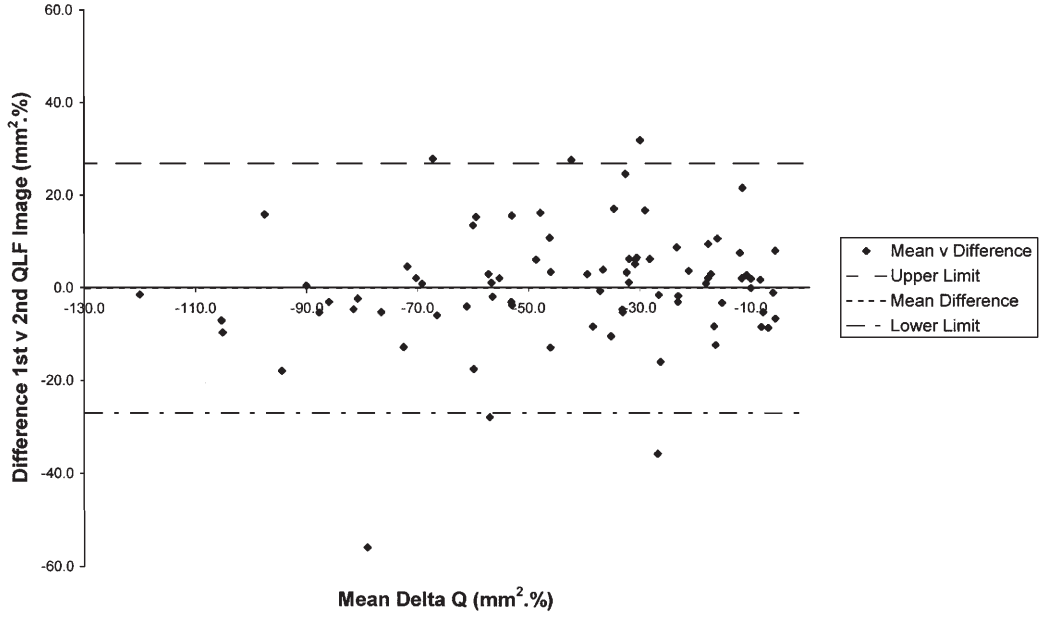

Figure 10 Limits of agreement for ΔQ for the first and second QLF readings.

from a photographic image is very similar to the technique of analysing a fluorescent image of the tooth using the customized software with QLF. The advantage of the QLF technique over the photographic technique is that it estimates mineral loss by extrapolating the change in fluorescence of the lesion compared with the fluorescence of the surrounding sound enamel. The photographic technique produces a figure for the grey level, which in itself is relatively meaningless, as the absolute figure will be dependent upon lighting conditions, changes in processing, and even film type. This will be discussed more in Part 2.

The mean differences between repeat readings of the same sites on the photographic images were small and of the same magnitude as in a previous study (Benson *et al*., 2000). There was some evidence of systematic bias in the grey scale readings, but the error was low compared with the size of the scale.

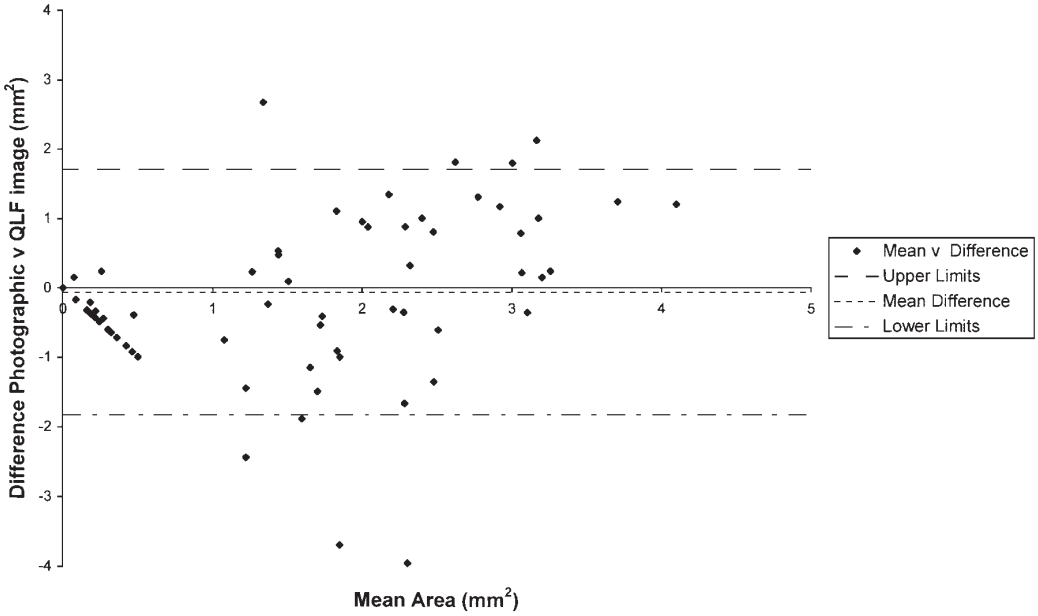

Figure 11 Limits of agreement of area measured from the photograph and QLF.

Random error, as measured using the ICC, was higher than in the previous study (Benson *et al*., 2000). This might have been due to the different techniques used in the two investigations. In the earlier study (Benson *et al*., 2000) the whole of the buccal surface of the tooth was automatically placed in nine different ranges of grey scale by the computer. This reduced the random error because the computer was making the assessment, but it made the results difficult to interpret, as it was not clear which was sound and which was demineralized enamel.

In the present study, the technique employed by Willmot *et al*. (2000) was used. A visual assessment of the enamel was carried out. If an area of demineralization was located then an AOI was manually drawn around it. Whilst random error could be introduced when outlining the AOI, other investigators (Mitchell, 1992; Linton, 1996) have found that this error is a small proportion of the total error. Another potential source of random error occurs when calibrating the image, but the technique for calibrating the images was common to both investigations.

The ICC was of the same order as the photographs taken above the occlusal plane (Willmot *et al*., 2000) and worse for those taken below the occlusal plane. Those authors speculated that the difference between these two views was due to the position of the masking used on the flash to reduce the amount of reflection. In the present study the masking was placed on the lower part of the ringflash as indicated by the improved results. However, the camera was orientated with the jig that was placed in the bracket slot. This was generally perpendicular to the buccal surface of the tooth and not below the occlusal plane. This could explain the poorer random error from this technique. The ideal position of the masking for photographs of teeth with orthodontic brackets may require further investigation.

The ICC for the area measurement using QLF was below 0.81, which is considered to be acceptable for random error (Fleiss, 1986). The random error for QLF needs to be reduced, possibly with improvements in the camera resolution and capture facilities.

The limits of agreement for the area measurements were small (Figure 6). This indicates good agreement in measuring the area of demineralization between the two slides taken 1 week apart. The limits are smaller than that of the previous study (Benson *et al*., 2000), because in that investigation the limits were measured on the total area of the buccal surface of the tooth (mean 42.0 mm^2). In the present study the area measured was only that believed to be demineralized, which was much smaller (mean 2.1 mm^2). Any discrepancy in the outlining of the AOI in the earlier study would magnify the error, because the area involved was greater.

In the previous study (Benson *et al*., 2000), the camera was placed in a holder that allowed rotation of the camera body. Photographs of the teeth were taken at a reproducible angle. In this study, a jig was used to allow reproducible positioning of the camera. The good agreement between the two slides taken 1 week apart indicates that the jig is a satisfactory method of producing reproducible photographs.

The limits of agreement for the measurement of mean grey levels from the two slides taken 1 week apart show similar agreement to the results of Willmot *et al.* (2000).

Variations in the lighting and processing of the image might lead to differences in the images that affect the grey scale measurement. The photographs were taken and developed using a standardized technique under standardized conditions, which exceeded that possible in the clinic. It is likely that there will be more inconsistencies in photographs taken in the clinical situation. To overcome these variations, a calibrating grey scale was incorporated into the jig (Figure 4). The use of the calibrating grey scale to manipulate the image digitally so the grey scales of the two images are closely matched is an area that requires further investigation.

The agreement of the QLF technique as measured with the limits of agreement (Figures 8 and 9) was very similar to the photographic technique for both the area and ΔF measurements. The limits of agreement for the area measurement were slightly wider than for the photographic technique, but were still acceptable. A similar precaution applies to the QLF technique as to the photographic technique. These images were taken in the laboratory under ideal conditions. In the clinical situation, in the presence of saliva the images will not be so good. In addition, although QLF is optimized to reduce reflections, precautions need to be taken to exclude all fluorescent lighting, which can also adversely affect the images.

Delta Q has been advocated as a summary measurement of the area and the mean change in fluorescence (van der Veen and de Josselin de Jong, 1999). However, the ΔQ measurement in this study showed a large variation and wide confidence limits. The limits of agreement were also wider for ΔQ (Figure 10) indicating poorer agreement between the two recordings. It is suggested that when measuring demineralization around orthodontic brackets the area and ΔF are stated separately.

Delta F is a more valuable figure than the grey level from a photograph. It represents a quantitative measurement of demineralization for that particular tooth surface. On the other hand, the grey level is a generic number that represents relative change only if subtracted from an area that is considered sound. Differences in lighting and developing may denote that a certain grey level on one image that represents demineralized enamel may represent sound enamel on another image. Therefore further subjective assessments and calculations are required to make the grey level a practical figure for analysis.

There was reasonable agreement between the photographic and QLF techniques for the measurement of area (Figure 11). The limits of agreement were –1.8–1.8 mm^2. The interesting cluster of results running in a straight line with a mean area of 0.5 mm^2 were data that were recorded from the photograph, but not using QLF, many of which were false positives. Elimination of these data did not affect the limits of agreement.

Part 2 of this investigation describes the validity of each technique.

Conclusions

This *in vitro* study carried out under ideal conditions has shown that:

1. Demineralization surrounding orthodontic brackets can be quantified reproducibly using the two techniques of computerized image analysis from photographic slides converted to digital images and QLF.
2. The two techniques show good agreement with respect to quantifying the area of demineralization.

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