MEASUREMENT OF ENAMEL DEMINERALISATION

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy by Philip Edward Benson

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Measurement of Enamel Demineralisation

Philip E. Benson

Abstract

The overall aim of the investigations in this thesis was to investigate methods of recording and measuring enamel demineralisation applicable to clinical orthodontic research. The methods investigated were firstly, the direct recording and measurement of demineralisation from the tooth surface, using clinical photography and quantitative light-induced fluorescence (QLF). Secondly, the indirect assessment of de/remineralisation utilising the *in situ* caries model adapted for use in the orthodontic patient.

Measuring the area of demineralisation was found to be more reproducible from a photograph (coefficient of repeatability 5.0mm^2), compared with either using a microscope (6.8mm^2) or direct visual examination (7.8mm^2). The position of the masking on the camera flash was found to make a significant difference to reproducibility. Repeat photographs taken from below an angle perpendicular to the buccal surface were found to have lower limits of agreement compared with those taken from above the perpendicular. There was a significant difference between the areas of demineralisation measured from the perpendicular photographs and those taken at 20 and 40-degrees below the perpendicular ($P<0.001$).

The reproducibility of area measurements of demineralisation using QLF on teeth with orthodontic brackets and artificial demineralisation was found to be comparable to computerised image analysis from a photographic image. The mean difference between repeat readings was small for the two techniques (0.08mm^2 and 0.02mm^2) and the coefficient of reliability similar (0.80 and 0.84). QLF might have the advantage of recording and measuring demineralisation at an earlier stage than the photographic technique.

The *in situ* caries model was successfully adapted for use in patients undergoing treatment with fixed orthodontic appliances. There was considerable variability in the de/remineralisation response of an enamel specimen with a preformed carious lesion placed in 14 patients with appliances. Enamel specimens without a small bracket base placed in the orthodontic patients demonstrated significant mineral gain ($P=0.004$) compared with a control that had not been placed in the mouth. The *in situ* caries model is a useful adjunct for investigating the prevention of demineralisation during orthodontic treatment.

The strengths and weaknesses of each technique for recording and measuring demineralisation are discussed and it is concluded that one method would not provide a complete picture of the mechanisms of demineralisation and remineralisation. On the contrary, a combination of direct and indirect techniques is required to study these processes during a clinical trial of a putative preventive agent.

For who would lose,

Though full of pain, this intellectual being,

Those thoughts that wander through eternity,

To perish rather, swallow up and lost

In the wide womb of uncreated night,

Devoid of sense and motion.

John Milton *Paradise Lost*

He was a scholar, and a ripe and good one;

Exceeding wise, fair spoken, and persuading:

Lofty and sour to them that lov'd him not;

But, to those men that sought him, sweet as summer.

William Shakespeare *Henry VIII*

CHAPTER 1

Introduction

Demineralisation of enamel surrounding orthodontic brackets is a significant clinical problem, because, in what is in essence an elective procedure, it represents a major element of risk to the patient risk/benefit treatment equation. Iatrogenic white spot lesions lead to poor aesthetics and in severe cases the need for restorative treatment. The orthodontist must develop strategies to prevent demineralisation consequent to orthodontic treatment.

The effectiveness of agents designed to prevent enamel demineralisation can be established through controlled experimentation in the laboratory and in the mouth. The most clinically definitive method of establishing the effectiveness of agents designed to prevent orthodontic demineralisation is through a clinical trial. The correctness of the results of a clinical trial is dependent upon the accuracy of the technique or techniques for both recording and measuring the relevant outcomes. This can be viewed in two different ways. Firstly, if a technique is able to distinguish the relevant outcome from other non-relevant outcomes (accuracy or validity), but if when a second reading of the same variable is taken, it differs greatly from the first reading (reliability or reproducibility), the results cannot be trusted. Secondly, if repeat readings show little variation, but they include recorded information from non-relevant outcomes, the data are worthless. The technique should demonstrate both validity and reproducibility. The more accurate and reliable

the technique, the greater the power of the study to find a significant result if it is present and fewer participants need to be recruited. If however, there is some variability then the power of the study is reduced and increased numbers are required to provide a significant result.

Aim

The overall aim of the following studies is to investigate a number of methods of measuring enamel demineralisation. These methods are intended to be useful in a clinical orthodontic context, accordingly assessing the validity and reproducibility of such methods is the outcome objective of the studies.

The techniques studied employed two methods:

1. Direct measurement of demineralisation from a subject's tooth using clinical photographs assessed with morphometry and computerised image analysis, as well as a new technique called quantitative light-induced fluorescence.
2. Indirect measurement of the de/remineralising conditions within the mouth, using an *in situ* model assessed with transverse microradiography.

CHAPTER 2

Background and Review of the Literature

2.1 Enamel Demineralisation and Dental Caries – the Pathology

Dental caries is a localised destruction of the dental hard tissues brought about by acid producing bacteria that adhere to the teeth (Thylstrup and Fejerskov, 1986). Normal human enamel consists of tightly packed apatite crystals. The crystals are arranged in an orderly fashion forming rods and inter-rod enamel. The crystals in the areas of the rod peripheries are slightly more loosely packed. Each crystal is surrounded by an intercrystalline space that is filled with water and organic material. These spaces form a potential diffusion pathway in enamel and are referred to as micropores or pores.

Early in the carious dissolution of enamel, there is enlargement of intercrystalline spaces, leading to increased tissue porosity. The changes in tissue porosity can be quantified and used as a sensitive measure of mineral loss. Histologically, the early enamel lesion appears a wedge-shaped defect with the base at the surface. There are four zones in polarised light. A relatively intact surface zone followed by the body of the lesion where porosity is greater than five percent. This may be between 20 and 50μm wide. The body of the lesion is followed by the dark zone with porosity

between 2 and 4 percent and represents an area where demineralisation and remineralisation is occurring. Finally, in the deepest part of the lesion there is a translucent zone that may be between 5 and 100μm wide with a porosity of slightly more than 1 percent. This may be an area where dissolution is occurring along the gaps between rod and inter-rod enamel.

The distribution of plaque and the direction of the enamel prisms determine the spread of the lesion. In some cases, the lesion will reach the dento-enamel junction without cavity formation. In other cases, extensive sub-surface demineralisation and damage to the outer surface will create a cavity.

2.2 Orthodontics and Enamel Demineralisation – The Problem

2.2.1 Orthodontic Appliances and Dental Caries

It has been known for many years that if the orthodontic appliance is not kept clean it will lead to dental caries (Noyes, 1936; Zachrisson and Zachrisson, 1971a). Zachrisson and Zachrisson (1971b) carried out one of the first longitudinal studies into the damaging effects of orthodontic appliances on dental health. They examined the prevalence, severity and distribution of caries in a group of patients who had received orthodontics with fully banded fixed appliances and compared it with an untreated control. They found no difference between the average number of carious lesions in the treated and untreated groups, but they found that banded appliances caused a shift from proximal to smooth surface lesions. They concluded that orthodontic bands

protected completely covered areas of the tooth, whilst making partly covered surfaces more susceptible to demineralisation.

Wisth and Nord (1977) compared the caries experience of a group of untreated controls with orthodontically treated individuals 18 months to two years after the removal of appliances. They found that the Decayed Missing Filled surfaces (DMFS) index was higher in the untreated compared with the treated group. The treated group had significantly more intact surfaces than the untreated group.

Southard *et al* (1986) carried out a retrospective, cross-sectional investigation into the relationship between fixed appliances and caries, on 613 subjects, 308 with previous orthodontic treatment and 305 not. Correlations of treatment time and time since completion of treatment, with filled and decayed surfaces were not statistically significant. It is unclear in the study whether fixed appliances were used and if so, whether they were fully banded or bonded.

Øgaard (1989a) was also concerned with caries rates in orthodontic patients compared with non-orthodontic patients. He carried out a retrospective, cross-sectional, observational study using data collected from patient records, which can be notoriously unreliable. He examined the incidence and location of filled surfaces in the permanent teeth of two groups of 65 individuals, matched for sex, who were born in one particular area of Norway

in the same year and were either treated with bonded fixed orthodontic appliances or not.

Øgaard (1989a) found an increase in filled surfaces from 10 to 18 years. This was not statistically significant between the two groups, neither was the distribution of lesions. He concluded that the identification of high-risk patients at an early stage might be more important in the prevention of caries during orthodontic treatment, than overall protection.

2.2.2 Orthodontic Appliances and White Spot Lesions

The production of a carious cavity following orthodontic treatment is an extreme, but not uncommon end of the spectrum. Much more commonly, there are signs of early demineralisation in the form of white spot lesions following orthodontic treatment. A number of observational studies have looked at the incidence of white spot lesions following orthodontic treatment.

Table 2.1 (page 2.9) summarises the results from four studies that have investigated the prevalence of white spot lesions in individuals that have (orthodontic) and have not (non-orthodontic) undergone orthodontic treatment.

Table 2.2 (page 2.9) provides a description of the individuals that were investigated.

Gorelick *et al* (1982) carried out a retrospective, cross-sectional study to determine the frequency and distribution of white spot formation. There are a number of problems with this study. They compared patients who had just completed orthodontic treatment with patients about to start treatment. A group of individuals who is about to start orthodontic treatment will be significantly younger than a group who has just completed orthodontics. They seemed confident that they could distinguish between clinically "developmental" and "non-developmental" lesions, although there was no explanation as to how this was achieved. No error measurement for either the clinical or photographic techniques was quoted. For the photographic technique, they state that the slides were projected on two separate occasions and scored by two of the authors. They assert that the "observations were completely consistent and reliable", but no figures are given. There is no description of the photographic technique and no mention of it being validated.

Mizrahi (1982) also carried out a retrospective, cross-sectional, observational study to determine the prevalence and severity of enamel opacities in orthodontic and non-orthodontic patients. He used a clinical examination to assess white spots. Mizrahi concluded that there was an overall rise in the prevalence of enamel opacities in the treated group compared with the pre-treatment controls. The severity of the opacities was also greater.

Many of the criticisms of the previous publication (Gorelick *et al*, 1982) are also pertinent here. Mizrahi (1982) did carry out an error assessment re-

examining about 10 percent of the sample within an eight-week period of the initial examination. The average length of treatment and most significantly how long they had been out of appliances were not stated. This was carried out before bonding was commonplace, so the teeth were all banded. In addition, the patients were treated using the Begg technique, which involves the use of more auxiliaries, which make oral hygiene procedures more difficult.

Artun and Brobakken (1986) carried out a retrospective, cross-sectional study to test the effect of fluoride rinses on the prevalence of white spot lesions. In all cases, except the lower first premolar, the trend was for the incidence of carious white spots in the experimental groups to be higher than in the control, however this was only statistically significant in the case of one experimental group. The group that was not statistically significantly different from the controls was, on average longer out of appliances. It has been shown that white spot lesions will become less obvious with time due to microabrasion and remineralisation (Al-Khateeb *et al*, 1998). They stated that the orthodontist for this group also placed greater emphasis on oral hygiene and fluoride mouthrinses, however there had been no attempt to measure the levels of compliance.

Øgaard (1989b) investigated the prevalence of white spot lesions on the vestibular surfaces of 19 year-olds who received and did not receive orthodontic treatment. The study was carried out more than five years after treatment. The author found that the median scores and the incidence of

white spot lesions were significantly higher in the treated group. Øgaard concludes that the effects of enamel demineralisation during orthodontic treatment may still be apparent even five years out of appliances. The assessments were carried out clinically, however it is not stated who performed the examinations and no error assessment appears to have been carried out.

Table 2.3 (page 2.10) summarises the prevalence of white spot lesions on specific teeth. There is considerable agreement between the studies as to which teeth were most severely affected. There are various explanations about the distribution of white spot lesions after orthodontics. Gorelick *et al* (1982) suggest that maxillary molars and premolars had significantly less demineralisation than their mandibular opponents due to the proximity to the salivary duct. The reason the lateral incisors are more susceptible than the maxillary central, is probably due to the size of the tooth surface area between the gingiva and the bracket. This is smaller on the lateral and will retain more plaque and debris. In addition, the use of auxiliaries and closing loops close to the lateral incisors may make cleaning more difficult.

Mizrahi (1982) found no difference in the prevalence of opacities between the sexes, but the males in the experimental group had a significantly higher opacity index number (i.e. worse opacities) than the females.

These studies have shown that there is disagreement about the exact prevalence of white spot lesions following orthodontic treatment, due to

differences in the definition and method of measurement of white spot lesions. However, there is agreement that individuals who have undergone orthodontic treatment have a higher prevalence than non-orthodontic controls. Zachrisson (1977) considers that the change from banded appliances to bonded attachments has reduced the incidence of white spot lesions.

Table 2.1

The results from four studies showing the percentage prevalence of white spot lesions found in orthodontically treated and non-orthodontic individuals. The table is divided into the percentage of individuals and teeth examined that were affected.

Authors	Individuals		Teeth	
	Non-orthodontic	Orthodontic	Non-orthodontic	Orthodontic
Gorelick *et al* (1982)	24%	50%	4%	11%
Mizrahi (1982, 83)*	73%	84%	19%	22%
Artun and Brobakken (1986)	40%	52%	9%	40%
Øgaard (1989b)	85%	96%	7%	24%
Mean	**56%**	**71%**	**10%**	**24%**

* Individuals with banded not bonded appliances

Table 2.2

Numbers and description of participants in the studies into the prevalence of white spot lesions.

Authors	Control Group	N	Experimental Group	N
Gorelick *et al* (1982)	Patients about to start orthodontics	50	Debanded orthodontic patients	121
Mizrahi (1982, 83)	Patients about to start orthodontics	527	Debanded orthodontic patients	269
Artun and Brobakken (1986)	School pupils matched for age	60	2 groups of 60 consecutive patients from 2 practices	120
Øgaard (1989b)	19-year-olds who had not had treatment	47	19-year-olds who had had orthodontics	51

Table 2.3

Prevalence of white spot lesions in specific teeth following orthodontic treatment.

Authors	Upper lateral incisors	Lower canines	Lower first premolars	First molars
Gorelick *et al* (1982)	23%	18%	18%	15%
Mizrahi (1982, 83)[+]	12%	10%	16%	50%
Artun and Brobakken (1986)*	24%	24%	26%	Not scored
Øgaard (1989b)	26%	28%	29%	50%

[+] Exact figures not given therefore estimated from graphs

* Results from the worst experimental group.

2.3 Clinical Trials and the Prevention of Orthodontic Demineralisation

The design of clinical trials is well described elsewhere (Altman, 1991). Altman states that the ideal research method to test the effectiveness of new materials is the prospective, longitudinal, randomised-controlled clinical trial. There must also be the use of valid and reproducible techniques of assessment with adequate blinding and statistical analysis, preferably with the close co-operation of a medical statistician (Altman, 1991).

Table 2.1 to Table 2.4 (pages 2.13 to 2.16) summarise reports of clinical trials, carried out in the last ten years, investigating materials that claim to reduce the prevalence of orthodontic demineralisation.

Table 2.1 (page 2.13) shows that there is a wide range of samples used. No trial reported a sample size calculation to determine whether the study has sufficient power to establish a statistical difference between the groups.

Table 2.2 (page 2.14) shows that there is a trend toward prospective, longitudinal trials, however when the trials are randomised, the method of randomisation is not reported.

Table 2.3 (page 2.15) shows that there are two methods of recording demineralisation in these clinical trials, which are clinical and photographic. There is a wide range of indices for assessment used and more importantly,

the number of assessors and whether the assessment was carried out blind is not reported in the majority.

Table 2.4 (page 2.16) summarises the error assessments carried out. Several studies failed to report an error assessment and those that did used many different statistical techniques to examine agreement. No study carried out the statistical analyses suggested by Houston (1983) or Fleiss (1986) to test for systematic and random error.

Well designed clinical trials to assess materials that prevent enamel demineralisation in the orthodontic patient based on techniques of assessment that have been demonstrated to be both valid and reproducible are indicated.

Table 2.4

Table summarising recent clinical trials testing materials to prevent orthodontic demineralisation - study comparisons, sample size, and sample size calculation.

Authors	Comparisons	Number	Sample Size Calculation
Adriaens *et al* (1990)	Fluoride varnish v no varnish under bands	28 subjects; control = 52 teeth; expt = 52 teeth	No
Geiger *et al* (1992)	Fluoride rinsing	206 subjects from 2 practices; 3133 teeth	No
Mitchell (1992)	Fluoride v non-fluoride composite	24 subjects; control = 62 teeth; expt = 62 teeth	No
Boyd (1993)	3 self-applied topical fluorides	95 subjects; control = 35; 2 expt = 30	No
Turner (1993)	Fluoride v non-fluoride composite	42 subjects; control = 203; expt = 203	No
Banks and Richmond (1994)	2 enamel sealants v non-sealants	2 expt groups of 40 subjects. Group 1 (teeth); control = 282; expt = 289. Group 2 (teeth); control = 305; expt = 306	No
Boyd and Rose (1994)	Electric v manual toothbrush	90 subjects; control= 35; expt1=30; expt2=25	No
Trimpeneers and Dermaut (1996)	Fluoride v non-fluoride composite	50 subjects; control = 379 teeth; expt = 383 teeth	No
Banks *et al* (1997)	Fluoride v non-fluoride composite	50 subjects; control = 371; expt = 366	No
Marcusson *et al* (1997)	Glass ionomer v composite	60 subjects; controls = 111 teeth; expt = 111 teeth	No
Gaworski *et al* (1999)	Glass ionomer v composite	16 subjects; controls = 48 teeth; expt -= 48 teeth	No
Millett *et al* (1999)	Glass ionomer v composite	40 subjects; control = 52 teeth; expt = 52 teeth	No
Wenderoth *et al* (1999)	Fluoride enamel sealant v non-sealant	20 subjects; 113 = teeth; expt = 112 teeth	No

Table 2.5

Table summarising recent clinical trials testing materials for the prevention of orthodontic demineralisation - study design.

Authors	Prospective (P)/ Retrospective (R)	Longitudinal (L)/ Cross-sectional (C)	Interventional (I)/ Observational (O)	Split-mouth	Random/ Non-random
Adriaens *et al* (1990)	P	L	I	Yes	Non; control = upper left and lower left
Geiger *et al* (1992)	R	C	O	No	Non
Mitchell (1992)	P	L	I	Yes	Random; method not reported
Boyd (1993)	P	L	I	No	Non-random
Turner (1993)	P	L	I	Yes	Random; method not reported
Banks and Richmond (1994)	P	L	I	Yes	Non; alternate teeth. Starting point not reported
Boyd and Rose (1994)	P	L	I	No	Non
Trimpeneers and Dermaut (1996)	P	L	I	Yes	Non; control = upper left and lower right
Banks *et al* (1997)	P	L	I	Yes	Random; method not reported
Marcusson *et al* (1997)	P	L	I	Yes	Random; method not reported
Gaworski *et al* (1999)	P	L	I	Yes	Non; alternate teeth
Millett *et al* (1999)	P	L	I	Yes	Non; alternate right or left upper labial segment
Wenderoth *et al* (1999)	P	L	I	Yes	Non; alternate teeth

Table 2.6

Table summarising recent clinical trials testing materials to prevent orthodontic demineralisation – method of assessment, index used, number of judges, whether assessment was blind and statistical tests used.

Authors	Method	Index	Nos of Judges	Blinding	Statistics
Adriaens *et al* (1990)	Photographs	Dichotomous	5	Yes	Chi-sq
Geiger *et al* (1992)	Clinical	Gorelick *et al* (1982)	Not reported	Not reported	Chi-sq
Mitchell (1992)	Photographs	Gorelick *et al* (1982)	1	Yes	Chi-sq; paired *t* test
Boyd (1993)	Clinical	Modified Gorelick *et al* (1982)	2	Yes	ANOVA & ANCOVA
Turner (1993)	Clinical	Modified Gorelick *et al* (1982) & Mizrahi (1982)	4	Not reported	Paired *t* test on frequencies
Banks and Richmond (1994)	Clinical	EDI	Not reported	Not reported	Score per 100 teeth; Wilcoxon
Boyd and Rose (1994)	Clinical	Modified Gorelick *et al* (1982)	2	Yes	One-way ANCOVA
Trimpeneers and Dermaut (1996)	Photographs	Dichotomous	5	Yes	Chi-sq
Banks *et al* (1997)	Clinical	EDI	1	Not reported	Score per 100 teeth; Wilcoxon
Marcusson *et al* (1997)	Photographs	Modified Gorelick (1992)	3	Yes	Wilcoxon & chi-sq
Gaworski *et al* (1999)	Photographs	3 point scale	7	Not reported	McNemar's test
Millett *et al* (1999)	Photographs	DDE	3	Yes	Mean opacity score per patient; paired *t* test
Wenderoth *et al* (1999)	Photographs	3 point score	7	Yes	Wilcoxon

* States that photographs were taken but not clear if these were assessed

Table 2.7

Table summarising recent clinical trials testing materials to prevent orthodontic demineralisation – method of error assessment and statistics used to assess error.

Authors	Error Assessment Reported	Statistics
Adriaens *et al* (1990)	Yes – each photograph scored twice interval of 1 week	Percent success in rating WSL
Geiger *et al* (1992)	No	
Mitchell (1992)	Yes – repeat assessments of photographs of 24 teeth	Paired *t* tests
Boyd (1993)	No – but intra & interexaminer calibrations conducted at start and 6 mthly to maintain 85% reproducibility	
Turner (1993)	Yes – 8 pts reassessed after 1 hour	Paired *t* test for intra-judge; ANOVA for inter-judge
Banks and Richmond (1994)	Yes- 20 pts after 3 months	Kappa
Boyd and Rose (1994)	Yes – see Boyd (1993)	
Trimpeneers and Dermaut (1996)	Yes – each photograph scored twice interval of 1 week	Percent success in rating WSL
Banks *et al* (1997)	No	
Marcusson *et al* (1997)	Yes – interexaminer reproducibility	Kappa
Gaworski *et al* (1999)	No	
Millett *et al* (1999)	Yes – random sample of 21 slides after 1 week	Kappa
Wenderoth *et al* (1999)	Yes –method not reported	Percentage intrajudge reliability

2.4 Techniques for the Qualitative and Quantitative Measurement of Demineralisation in Clinical Studies

Techniques for measurement should fulfil two criteria if their readings are to be considered reliable (Houston, 1983). These are:

I. Validity

II. Reproducibility

Houston (1983) defines validity or accuracy as "the extent to which, in the absence of measurement error, the value obtained represents the object of interest." Therefore, when measuring demineralisation the technique should be able to distinguish between white spots that have occurred because of localised destruction of enamel by plaque acids and the many other causes of white spots, such as developmental defects. Houston (1983) defines reproducibility or precision as "the closeness of successive measurements of the same object."

When undertaking or evaluating the results of research, the validity and reproducibility of the method of measurement need to be examined if the conclusions of the study are to be considered accurate. Clinical studies have employed three fundamental methods for the qualitative and quantitative measurement of demineralisation. These have been based on a clinical or photographic examination or on other optical methods of examination.

2.4.1 Clinical Examination

The majority of studies into demineralisation in the orthodontic literature have used a clinical examination to measure the prevalence of demineralisation. There are advantages and disadvantages to using a clinical examination:

Advantages

1. Simple and inexpensive – no expensive or complex equipment is required.
2. Clinically valid - what the examiner sees and measures is likely to be the patient's perceived problem.

Disadvantages

1. Validity – it is often difficult to clinically distinguish white spots caused by demineralisation and those that are due to other causes, such as developmental hypoplasia or fluorosis. Adequate methods of reducing bias with blinding of examiners are more demanding.
2. Reproducibility – methods of reducing inter-examiner error such as calibration of examiners may be time consuming and inaccurate. Assessment of intra-examiner error requires recalling individuals for re-measuring that may be inconvenient to the patient and may lead to the establishment of a convenience sample for error assessment.

Most studies employing a clinical examination have used an index based on that of Fehr (1961). He compared the anti-cariogenic affect of stannous fluoride, stannic fluoride and sodium fluoride. He induced carious lesions on

the premolars of teeth due to be extracted for orthodontic reasons using a cast gold plate onlay. He designated one half of the tooth to act as the control and covered it with blue inlay wax. The participants then used one of three mouthrinses. The teeth were extracted after three to five weeks and examined under a microscope at 20 times magnification. The scale was as follows:

0° Surface appears intact

1° Limited greyish tinge, with or without accentuated perikymata.

2° Perikymata well accentuated, in some areas confluencing into greyish-white spots.

3° Pronounced white decalcification, having a more or less distinct line of demarcation

Fehr carried out repeat examinations on 43 teeth and stated that the error of the scoring method was $\pm$0.3°, although it was not clear how this was calculated.

This was subsequently amended by Gorelick *et al* (1982), Geiger *et al* (1988) and Gorelick *et al* (1992) who scored the state of the entire buccal surface as follows:

1 No white spot formation

2 Slight white spot formation

3 Excessive white spot formation

4 White formation with cavitation.

An error assessment using this technique was not described in any of these publications.

Mizrahi (1982, 1983) used a scoring system that divided the tooth surface into thirds. Each third of the surface was scored using the following scale:

0 No enamel opacity. An opacity of less than an estimated 1mm in length or diameter was considered absent.

1 An opacity covering up to one-third of the surface area.

2 An opacity covering from one-third to two thirds of the surface area.

3 An opacity covering two-thirds to the full surface area.

He scored the buccal and lingual surfaces of each tooth giving a maximum score of 18 per tooth (two surfaces divided into thirds, each scored to a maximum of 3). One investigator and one chairside assistant carried out all the examinations. He carried out an intra-examiner error assessment by re-examining 75 patients (10% of the total) within eight weeks of the initial examination. To analyse the difference between the two readings he carried out a paired *t* test and Wilcoxon matched pairs signed ranks test. He also calculated a modified percentage reproducibility as described by Shaw and Murray (1975). This takes into account the examiners decision to assess the tooth as caries free as well as when it has caries. He found no statistically significant results with the parametric or non-parametric tests and found 95 percent reproducibility.

Artun and Brobakken (1986) used two scoring systems. They assessed the opacity of the white spot lesion by using the scoring system of Fehr (1961). To assess the size of the opacity they used a scoring system modified from that of Gorelick *et al* (1982) which was as follows:

Score 0 No white spot lesion

Score 1 White spot lesion involves less than one third of the vestibular enamel surface area outside the area covered by the bracket and bonding material during treatment.

Score 2 White spot lesion involves more than one third but less than two thirds of the vestibular enamel surface area in question.

Score 3 White spot lesion involves more than two thirds of the vestibular enamel surface area in question.

A score was given to the four areas surrounding the orthodontic bracket, namely mesial, distal, gingival and incisal. They state that the evaluation was performed jointly by the authors and when there was disagreement, the scores were arrived at after discussion. They tested reproducibility by re-examining 11 patients randomly selected from one of the test groups. The method of randomisation was not described. Neither was it explained if the examiners were blinded as to which group the patient was in, as they had two experimental groups who had received orthodontics and a control group who had not. They calculated a mean score per tooth from each patient and used Pearson's product moment correlation coefficients between the first and second measurements. They found high correlation coefficients, but as Bland

and Altman (1986) point out correlation coefficients measure statistical association and not necessarily agreement.

Øgaard (1989b) used a similar index to Mizrahi (1982,83), however he scored the whole of the buccal surface of the tooth, rather than dividing it into thirds. No mention is made of how many examiners were involved, whether they were calibrated or blinded as to which group the individuals were in. No error assessment was carried out.

Boyd (1993) performed a longitudinal clinical trial to assess two methods of delivering fluoride. He used yet another index. He divided the labial surface of the study teeth into four quadrants and scored each according to the following scale:

Score 0 No visible white spots or surface disruption (no decalcification).

Score 1 Visible white spot without surface disruption (mild disruption).

Score 2 Visible white spot lesion having a roughened surface but not requiring a restoration (moderate decalcification).

Score 3 Visible white spot lesion requiring a restoration (severe decalcification).

Assessments were carried out blind, by two examiners who were calibrated. The author states that intra and inter-examiner calibrations were conducted before the study and at six monthly intervals to maintain 85 percent reproducibility.

Turner (1993) used the qualitative assessment developed by Gorelick *et al* (1982) and the quantitative assessment used by Artun and Brobakken (1986). Four examiners were used, but there was no discussion of calibration before the start of the study. The examiners carried out blind assessments and an error assessment was carried out by the four examiners on eight patients. The study was carried out on patients with fixed appliances and only two of the eight patients used in the error assessment had appliances. An assessment of systematic error was carried out for inter and intra-examiner variability by using paired *t* tests and analysis of variance. No assessment of random error was reported.

Banks and Richmond (1994, 1997) used yet another scoring system, which they have called EDI or Enamel Decalcification Index. This is based on the index of Artun and Brobakken (1986). The buccal surface of the tooth is divided into four areas, gingival, mesial, distal and occlusal. Each area was scored according to the following criteria:

Score 0 no decalcification.

Score 1 mild, but clinically visible decalcification affecting less than 50 percent of the area.

Score 2 moderate to severe decalcification extending over more than 50 percent of the area.

Score 3 decalcification covering the whole area or with obvious surface breakdown or caries.

In the earlier publication (1994) they tested reproducibility by reassessing 20 patients three months after the initial recording and testing agreement with an unweighted kappa. They found an overall good agreement between the first and second reading, but on closer examination, they found differences in agreement between individual areas of the buccal surface. They found excellent agreement for the gingival areas, but only moderate agreement for the mesial areas. Three months would seem to be a long period over which to assess reproducibility as quite rapid remineralisation of enamel lesions can occur (Al-Khateeb *et al*, 1998). It is assumed from the text that there was only one assessor and the kappa statistic was assessing intra-examiner reproducibility. There is no mention of an inter-examiner assessment. In the later publication (Banks and Richmond, 1997) no error assessment is described.

Another problem with these studies is the question of blinding. They were investigating the difference between two techniques to prevent demineralisation. It is not clear from the method whether the assessor was blinded as to which treatment the patient received.

It is clear that the clinical studies examining demineralisation following orthodontic treatment have used many different indices. Some of the studies have been less than rigorous when describing aspects of the investigation such as, calibration and blinding of examiners, as well as methods of error assessment including both systematic and random error (Houston, 1983).

2.4.2 Photographic Examination

Photographic techniques have been extensively used to study the optical properties of the teeth (Hollender and Koch, 1976; Houwink and Wagg, 1979; Dooland and Wylie, 1989; Levine *et al*, 1989; Ishii and Suckling, 1991; Nunn *et al*, 1992; Nunn *et al*, 1993). There are many advantages to the use of photographic records in the assessment of enamel. These have been described well by Ellwood (1993) and include:

1. Photographs are a quick and efficient method of recording enamel defects.
2. They provide a permanent record and can therefore be examined during one diagnosis period and re-examined later if required.
3. Photographs may be examined in random order without reference to any subject details.
4. The method used can be standardised so that clinical variability of diagnostic conditions may be minimised.
5. Photographs taken by several examiners may be scored by one independent expert to remove the effects of intra-examiner variability.

Ellwood (1993) also outlines some potential criticisms of the photographic technique:

1. The camera records details differently to the naked eye.
2. Standardisation of the procedures may be difficult, particularly with respect to film types and processing methods that may change over time.
3. Details that are recorded may be technique sensitive.

A number of studies have used photographs to assess the incidence of enamel opacities in populations (Hollender and Koch, 1976; Levine *et al*, 1989); Nunn *et al*, 1992; Nunn *et al*, 1993). Several techniques have been described (Hill and Geddes, 1975; Callender, 1983; Fleming *et al*, 1989). Many studies have used a photographic technique without validating it first (Houwink and Wagg, 1979; Dooland and Wylie, 1989; Ishii and Suckling, 1991).

Edgar *et al* (1978) carried out a study to assess the reproducibility of a graded index called the Caries Index (CI) devised by Fehr *et al* (1970). Four methods of assessment were carried out. These were a clinical examination, direct examination through a microscope, assessment from black and white and colour photographs. The results of this study indicated the feasibility of using colour photography with controlled lighting and camera position to record changes in the optical properties of enamel. They conclude that recording of the caries index from colour photographs was both valid and reliable. They also point out that the failure of the black and white photographs to reproduce enamel changes is not surprising as the index criteria include colour terms. The authors conclude that use of photographs reduced possible bias inherent in the direct microscopic observations used previously.

Ellwood (1993) carried out an investigation to assess the suitability of using a photographic method to record the developmental defects of enamel (DDE) index for dental epidemiological studies. He endeavoured to develop a

method of recording dental enamel opacities that was suitable to allow comparisons of prevalence and severity over time. To overcome reflection from the tooth surface with flash photography he took two photographs at different angles, one above and one below the occlusal plane.

Comparisons of prevalence of dental enamel defects, using both clinical and photographic methods, demonstrated that significantly more developmental defects of enamel were recorded when using photographs rather than clinically. This agreed with other studies and suggests that this be due to enhancement of the minor forms of defects. Generally, the photographic technique was an extremely powerful method of recording enamel opacity presence. Ellwood points out that the kappa adjusted percentage agreement for comparison of the individual defects was poor at 47 percent. He concludes that although the population prevalence of defects is relatively robust with the photographic method, using this method to monitor individual lesions longitudinally may be difficult, as the agreement is poor when the angle of view is changed.

Mitchell (1992) carried out a longitudinal clinical trial into the effectiveness of a fluoride-releasing composite. Assessment was carried out on black and white prints that were taken of the upper anterior six teeth, before and after treatment. The photographic technique is well described. A special light source (Hill and Geddes, 1975) was used to reduce the amount of reflection caused by a flash. The photographs were examined in sequence by one

operator who did not know which material had been used to bond the bracket.

A qualitative assessment of the presence or absence of demineralisation was carried out using an index similar to that of Gorelick *et al* (1982). A quantitative assessment was made by using a digitizer to trace around the labial surface of the tooth and any opacity. This was repeated three times and the mean value recorded. The proportion of the labial surface of the tooth affected was then calculated. A reproducibility study of the qualitative and quantitative assessments was carried out on 24 teeth, although it was not clear from the text how these were chosen or how long after the original assessment this was carried out.

The results of the error analysis showed that with the qualitative scoring, there was 100 percent agreement when recording whether there was demineralisation (score 0) or not (score 1,2 or 3). There are no details as to the agreement between the different qualitative levels of demineralisation. The quantitative assessment was tested with a paired *t* test and no significant difference was found between the first and second readings. Only five out of the 24 teeth that were re-measured showed signs of demineralisation and only these were included in the error assessment. This is a small number and there would need to be a large systematic difference between the two readings for this to be statistically significant. In addition, random error or the variation in the repeat readings was not explored.

Sonis and Snell (1989) compared demineralisation occurring with a light-activated fluoride-releasing composite with a conventional light-activated composite. The index of Mizrahi (1982) was used to score the opacities, although it was not clear if the buccal surface was divided into quarters. It was also not plain, whether the assessment was carried out clinically or using photographs. The authors state that photographs were taken at the start and at the end of treatment, but the photographic technique is not described. There was no description of the number of examiners and whether they were blinded or not. If the assessments were carried out clinically, then the fact that there was no randomisation of quadrants to experimental or controls groups would be a serious flaw. Finally, no error analysis was carried out.

Adriaens *et al* (1990) carried out a prospective, longitudinal trial to study the effect of a fluoride varnish, as a caries prevention method under orthodontic molar bands. Photographs of the molars were taken before and after treatment. These were projected in a random order and scored by five examiners twice, a week apart. The scoring system was not clear from the description, but was probably a dichotomous recording. Reliability was defined by the percentage of success in identical rating of white spot formation for each observer. There was no attempt to assess the severity or extent of the lesion.

Trimpeneers and Dermaut (1996) carried out a longitudinal, split-mouth crossover design comparing a fluoride-releasing light cured system with chemical no-mix resin. Colour photographs were taken of the labial and

buccal surface of the teeth, by a professional operator before the start of treatment. One week after the removal of appliances the same photographs were taken. The before and after treatment slides were projected in a random order and scored by five different operators. They were asked to score the presence or absence of demineralisation. No qualitative assessment of severity was carried out. The scoring was repeated after one week. Inter and intra-judge reliability was analysed by the number of failures and successes in identical rating on whether a white spot was present or not. They found high intra-judge reliability, with the worst judge changing his or her rating on only 77 out of 836 recordings (91% success rate). They also found an acceptable inter-judge reliability, the worst agreement between two judges was with 84 out of 836 observations (90% success rate).

Millett *et al* (1999) performed a longitudinal, clinical trial to compare demineralisation rates between a glass ionomer cement and a composite resin. Colour photographs were taken of all six upper anterior teeth before bonding the brackets, at debond and at a subsequent review. The photographic technique is described. The three photographic slides for each patient were projected simultaneously and scored independently by three examiners who had been calibrated in the use of the modified DDE index. An error assessment was carried out on 21 slides one week after the initial assessment. The intra and inter-examiner reliability was assessed using the kappa statistic and reliability was moderate-to-high for both the type and extent of enamel opacity recorded. The DDE index is a descriptive index to record developmental defects of enamel, based on the type, number,

demarcation and location of defects. There was some initial concern that the criteria did not clearly state how white spot lesions and demineralised areas should be dealt with (Clarkson and O'Mullane, 1989). It is recommended that defects which are present, but which are not developmental be listed as "Other". Such an index may be too complex and not suitable for research into demineralisation.

Marcusson *et al* (1997) carried out a prospective, longitudinal clinical study to compare white spot formation with a glass ionomer cement and a conventional composite. Colour slides of the upper lateral incisors and lower canines using two projections were taken at the start of treatment. These were repeated at debond and after 1 and 2 years post debond.

All the photographs were magnified and scored using a modified Geiger *et al* (1988) index, by three observers, who were blind to which material had been used. In the case of disagreement the scored agreed by the majority of judges was used. Inter-examiner reproducibility was assessed. A reproducibility assessment was carried out on 186 photographs. Both were acceptable. They found differences between the two materials at debond, but after two years post debond there was no difference. They state that "It may also be that the scoring system was too insensitive to record possible differences in the severity of the lesions, some of which may include microcavities, between the two groups."

2.4.3 Optical Methods

Comprehensive reviews of the various techniques of optical quantification of enamel caries have been provided by Angmar-Månsson and ten Bosch (1987) and Angmar-Månsson *et al* (1996).

2.4.3.1 Non-Fluorescent Methods

Light Scattering

The theory behind the light scattering method of quantifying enamel demineralisation has been well described by Angmar-Månsson and ten Bosch (1987). When a light photon enters sound enamel, it travels an average distance of 0.5mm before being scattered. A large portion of light penetrates the enamel, which is about 1mm thick and is backscattered by dentine. Therefore, the colour of dentine is clinically apparent.

In a carious lesion, the crystallite or prism structure of sound enamel is replaced by carious enamel where the mineral is partly substituted with water. This increase in water ensures that the differences between particles and their environment are much greater than sound enamel and there will be an increased difference in refractive index (or ratio of the velocity of light in two adjacent media) between the scattering particle and the environment. This leads to a light photon travelling a much shorter distance in carious enamel before being backscattered. Thus sound enamel represents low scattering material and caries is high scattering. In carious enamel most photons are scattered within the lesion, fewer are absorbed and the

backscatter is greater, resulting in the clinical appearance of a white spot. When the lesion is dried the water is replaced by air and the average refractive index declines even more. This will increase the chances of scattering occurring and the contrast between lesion and sound enamel will be accentuated.

The scattering results in a sideward displacement of the light. This spreading can be measured and is proportional to the mean free photon path length or the distance between scattering events. This is the basis of the Optical Caries Monitor (OCM) first described by ten Bosch *et al* (1980). They used a 100 watt white light as a light source and measured backscatter with a densitometer. They prepared rectangular blocks of bovine enamel, which were kept in a demineralising solution to produce different sizes of lesions. An early OCM was used to correlate the relationship between backscatter intensity and the length of time in the demineralising solution. A good relationship was found between backscatter and mineral loss, with 20 percent of the visible light absorbed in the enamel and about 75 percent transmitted or backscattered. The intensity of the backscatter levelled off at a depth of about 100μm.

The OCM is described by Borsboom and ten Bosch (1982). It provides two narrow beams of light from a Xenon-arc cylindrical flashbulb transmitted along fibres to illuminate a small circular spot (0.5mm in diameter). The beams of light were supplied from two opposite directions and light emerging from the spot was collected, transmitted along fibres to a photodiodeamplifier

detector. Backscatter data were collected from specimens of bovine enamel (ten Bosch *et al*, 1984) that had previously been demineralised and correlated with chemical and transverse microradiographic (TMR – see section 2.6.1.1, page 2.67) data from the same blocks.

A good correlation was found between the OCM data and the chemical analysis of calcium loss from the enamel specimen. The correlation with the TMR data was poor and the authors suggest that this be due to intra-tooth variation. They also point out that in carious lesions heterogeneities occur that are due to other factors than the mineral content of the sound tissue. These may influence the measurement with the OCM and they must be taken into consideration.

De Josselin de Jong *et al* (1988) followed up the above study with a similar one comparing the non-destructive measurement of mineral changes in human dental enamel. They compared the OCM, with that of chemical analysis and longitudinal microradiography (LMR, see section 2.6.1.2, page 2.72). Because the methods were non-destructive, they could be used to measure mineral change in enamel longitudinally.

Human enamel specimens were prepared in a demineralising buffer solution for time intervals of 24 to 168 hours. It was possible to accurately follow local mineral changes in time of the same tooth sample at the same measuring spots (diameter >0.4mm) on the tooth surface using both LMR and OCM. A wide variation in demineralising behaviour from different spots on the same

enamel specimen was noted. This was the first time that variation in response to the same demineralising challenge had been directly demonstrated. It was proposed that using these two techniques it should be possible to carry out time and position dependent studies with an intra-oral device, which could overcome the difficulties of inter-specimen variation (de Josselin de Jong *et al*, 1988).

Øgaard and ten Bosch (1994) used the OCM to study the remineralisation of enamel lesions that had been induced under specially constructed orthodontic bands (see section 2.5.3, page 2.46). The surfaces of premolars from seven patients, destined to be extracted as part of a course of orthodontic treatment, were assessed with the OCM, then orthodontic bands, which were specifically designed to collected plaque, were placed for four weeks. Following removal of the bands further readings were taken on the day of deband, then at weekly intervals for four weeks. This showed that after four weeks of the band being in place all the teeth had white spot lesions. By the time the teeth were extracted following the four weeks after the band had been removed, almost all-visual signs of the white spot lesions had disappeared. They calculated the half-value time (which they do not explain, but is presumably the average length of time for half the lesion to disappear) as 12.5 days for right-sided teeth and 7.7 days for left-sided teeth. They speculate on this difference between the two sides and give several explanations. The most plausible one is that all the participants in this study were right handed. They were therefore more likely, it was hypothesised, to microabraid the teeth on the left side removing the outer surface of enamel

where the lesion is found. There were only a small number of individuals in this study, which makes the results only indicative. There was a large variation, which was partly due to the machine. However, weekly variations in regression of the lesions were larger than the error of the instrument. It was noted that lesion regression was rapid when the cariogenic challenge was removed, even in the absence of fluoride (Øgaard and ten Bosch, 1994).

In summary, the advantages of the Optical Caries Monitor are; that it enables quantification of enamel demineralisation that is convenient and non-destructive to tooth tissue. It can be applied in the clinical environment and has been correlated with established methods of studying mineral loss. The disadvantage is that it is particularly technique sensitive and results can vary with the degree of wetness or drying of the tooth.

2.4.3.2 Fluorescent Methods

Fluorescent methods have been used to highlight areas of demineralised enamel from the surrounding normal enamel for the purpose of diagnosis and quantification. Angmar-Månsson and ten Bosch (1987) describe the theory of fluorescence in terms of differences in light paths. In carious enamel, the average free photon path or the distance a light photon travels before it is deflected from its path is reduced (see above). The total light path in the material (before it emerges at the surface) will decrease proportionally with the decrease in the average free photon path. If the light is emerging from the surface more rapidly, then the possibility of absorption decreases as well. Fluorescence is a function of light absorption and if less light is absorbed the

intensity of the fluorescence will also decrease. Carious enamel will therefore be shown as a dark area (Angmar-Månsson and ten Bosch, 1987).

There are a number of different techniques for producing fluorescence in enamel.

i) Fluorescent dye uptake

Various dyes fluorescent and non-fluorescent have been used to highlight carious enamel (Rawls and Owen, 1978). These can be viewed under ultraviolet light source. The disadvantage of these dyes is that slight procedural variations can result in widely different degrees of dye uptake. There is also some doubt about whether the dyes are toxicologically safe or whether they can be standardised (Angmar-Månsson and ten Bosch, 1987).

ii) Ultraviolet

Shrestha (1980) used an ultraviolet (UV) light for the early detection of carious lesions on the smooth surfaces of rats. He compared the mean number of smooth surface lesions per rat scored using an ultraviolet light with 366nm filters compared with the same score from murexide staining. He concluded that the UV method was more sensitive that the staining method and showed better delineated lesions.

Angmar-Månsson and ten Bosch (1987) have discussed the disadvantages of an UV system not least of which is that UV radiation is harmful to eyes and special precautions are required to protect the patient, which brings additional ethical difficulties in clinical studies. They also state that it is

difficult to distinguish carious lesions from other causes of disrupted enamel such as developmental defects.

iii) Laser

The dangers of ultraviolet radiation have stimulated the search for a safer form of light to quantify demineralisation by the fluorescent method. Bjelkhagen *et al* (1982) used an argon laser to show differences in luminescence from intact and carious enamel.

Hafström-Björkman *et al* (1992) carried out an experiment to compare laser fluorescence and longitudinal microradiography (see 2.6.1.2, page 2.72) for the quantitative measurement of demineralisation in human enamel specimens. They measured ten enamel slices at set points on the specimen, using the two techniques on days 1, 2, 4, 7, and 9 of a demineralisation cycle. They also measured at the same time, three slices that were not undergoing any demineralisation. They found a good linear correlation between the readings of the two techniques ($r = 0.97$). They discovered that fluorescence decreases with increasing lesion depth, therefore laser fluorescence was most useful in the measurement of shallow initial lesions. They concluded that laser fluorescence had a higher discrimination threshold than longitudinal microradiography, meaning it will detect caries earlier.

De Josselin de Jong *et al* (1995) set out to develop the technique of quantitative laser fluorescence for use *in vivo*. They used a blue-green argon ion laser, with a yellow high-pass filter, which cut off light lower than 520nm. This filter ensures that tooth scattered blue laser light does not reach the

detection apparatus, but fluorescence in the yellow region does. As with all fluorescence techniques the incipient lesions appear as dark areas. The equipment was calibrated to use the decrease in fluorescence to quantify mineral loss and lesion size. The computer programme achieves this by calculating the fluorescence radiance values of the sound enamel surrounding a lesion, then reconstructing the values for the carious lesion. Lesion area is measured by calculating the surface area occupied by points with a difference from the reconstructed sound enamel larger than 10 percent.

The authors evaluated the repeatability of laser fluorescence using several experiments. Firstly, they made 25 clinical measurements, at different times, of one tooth with an arrested, carious lesion. To test the accuracy of the reconstruction method they took measurements from the buccal surfaces of 19 visually sound teeth and compared the reconstructed with the actual values. Finally, they carried out an experiment using an *in vivo* model (see section 2.5.3, page 2.46) performing laser measurement on a premolar destined for extraction, which was bonded with a plaque-attracting bracket. They concluded that the repeatability of laser measurement for the *in vivo* recording of area and fluorescence change from the lesions was acceptable.

The validity of the laser method was tested by Emami *et al* (1996), who compared the results of laser measurements from sections of enamel taken from premolars displaying visible white spot lesions, with measurements from the same sections using longitudinal microradiography (see section 2.6.1.2, page 2.72). They found an acceptable linear correlation (r=0.73) between the

two techniques. They suggest that this correlation was derived from measurements from several teeth and longitudinal measurements of the same lesion from one tooth may produce a better correlation.

Ando *et al* (1997) carried out an *in vitro* investigation to compare the results from quantitative laser fluorescence and a novel dye-enhanced version of laser fluorescence (DELF) with those from transverse microradiography (TMR – see section 2.6.1.1, page 2.67) and confocal microscopy (CLSM- see section 2.6.8, page 2.82). They found that laser measurement was clinically useful for quantifying mineral loss, whereas DELF was not. Laser measurement was able to detect very early lesions (8 hours exposure) and capable of quantifying changes up to the 24 hours of this study.

Quantitative laser fluorescence has been used to study the change in fluorescence with time in teeth exhibiting white spot lesions following orthodontic treatment (Al-Khateeb *et al*, 1998).. Their results showed that radiance levels increased and the area of almost all white spot lesions decreased over time suggesting mineral gain. Remineralisation of the lesions showed an exponential pattern with most mineral gain occurring early, then the rate slowing down. The authors describe the problems with optical monitoring probe employed in a previous study (Øgaard and ten Bosch, 1994) particularly concerning repositioning and sterilisation of the probe. However, they do not make clear how these problems are addressed with the laser equipment.

The technique of quantitative laser fluorescence has also been found useful when used in conjunction with the *in situ* caries model (Al-Khateeb *et al*, 1997a; Hall *et al*, 1997a).

Angmar-Månsson and ten Bosch, (1987) list the advantages and disadvantages of the laser fluorescence method. The advantages are:

1. There is increased contrast between carious and sound enamel, which make earlier detection of caries possible.
2. The depth of lesion can be estimated to a certain extent.
3. Diagnosis of caries can be achieved without a probe.

The disadvantages of this method are:

1. It does not differentiate between active and arrested caries.
2. It does not differentiate between caries and hypomineralised developmental defects
3. It is not able to detect secondary caries next to metal fillings.
4. The equipment is expensive.

iv) Light (Quantitative Light-induced Fluorescence or QLF)

One of the problems with the laser system is the size of the laser equipment used as the light source. A smaller portable system for intraoral use as been developed with a new light source and filter system (Al-Khateeb *et al*, 1997b). This uses an arc lamp with a liquid light guide. The light passes through a blue filter in front of the lamp, with a peak intensity of 370nm. To enable the

enamel fluorescence to be detected there is a yellow high pass filter of 520nm in front of camera to exclude light below that frequency. The combination is optimised so there are no reflections. The images are stored, processed, and analysed with custom software.

Al-Khateeb *et al* (1997b) carried out an *in vitro* investigation to validate the data from the new light with transverse microradiography (TMR) (see section 2.6.1.1, page 2.67) and chemical analysis (see section 2.6.4, page 2.80). The results of this investigation showed that changes in fluorescence correlated with calcium loss (r=0.74) and integrated mineral loss (r=0.64). The correlation between calcium loss and TMR was 0.74. A significant linear correlation was found between the mean fluorescence loss over the lesion (ΔLmean/L) and mineral loss (Δz). The authors point out that a very accurate comparison was not possible as the fluorescence data are derived from the whole of surface of the tooth, whereas the TMR data are from one small slice. They found that lesions up to 500μm could be measured with fluorescence from the regular light source. They also found that the data correlated well (r=0.93) with the previous argon-ion laser light source.

Recently, Lagerweij *et al* (1999) have carried out an *in vitro* study to compare three light-induced fluorescence systems. These were the water-cooled laser-based with ring illuminator; the air-cooled laser based with beam splitter (for detecting occlusal caries); and the arc lamp clinical system with liquid light guide (QLF). Microradiography (see section 2.6.1.1, page 2.67) was used as the reference method. They assessed the precision of each device

by the standard deviation within the specimens and expressed this as a percentage to correct for the size of the lesion. They found that the beam splitter had the lowest variation with a standard deviation of 24 percent. The QLF had a standard deviation of 30 percent and the ring had the largest variation with 32 percent. The highest correlation with TMR was with the beam splitter (r^2=0.70), followed by QLF (r^2=0.63) and finally the ring (r^2= 0.36). They found that the variation with the light sources was generally three or four times that of TMR.

The authors discuss potential sources of error with the fluorescent method. The moisture content on the tooth is important. As discussed previously drier teeth show greater scattering of light. Reflection will also be a factor in the variation in readings. The filters are gradual so there will be overlap between excitation (reflected light) and emission fluorescence from the tooth.

2.5 Experimental Models for Studying Enamel Demineralisation

There are a number of experimental models used to study the process of demineralisation, including:

1. Animal models
2. *In vitro*
3. *In vivo* models
4. *In situ* caries model

White (1992) lists four factors that summarise the value of these models:

a) The cost and timing compared with clinical trials
b) The simulation of caries process
c) The ability of the model to simulate formulation use
d) The correlation/predictiveness of results to clinical findings (validity), which he considers to be the most important.

2.5.1 Animal models

White (1992) considers that only the animal model can study the entire caries process. It can simulate the natural progression of caries under true biological conditions. However, there are concerns regarding the general applicability of animal studies to humans because of differences in the use, application and clearance of preventive agents. There are also differences in tooth morphology and composition, oral flora, saliva, diet and food retention.

Dubroc *et al* (1994) used a rat model to test the effectiveness of a fluoride-releasing composite resin. They bonded stainless steel mesh to the lingual surfaces of the maxillary molars of rats that had been infected with a cariogenic strain of streptococci and fed a cariogenic diet. They found that the teeth bonded with the fluoride-releasing composite had significantly fewer white spot lesions compared with those bonded with a conventional composite. This is contrary to several clinical studies that have compared fluoride-releasing and conventional composites (Mitchell, 1992; Turner, 1993; Trimpeneers and Dermaut, 1996).

2.5.2 *In vitro* models

There have been numerous *in vitro* experiments to test the efficacy of materials to prevent orthodontic demineralisation (Chan *et al*, 1990; Fox, 1990; Chadwick and Gordon, 1995; Basdra *et al*, 1996; Frazier *et al*, 1996; Young *et al*, 1996; Kindelan, 1996).

White (1992, 1995) lists the advantages and disadvantages of *in vitro* models:

Advantages

1. Inexpensive.
2. Not time consuming.
3. Tightly controlled.

Disadvantages

1. Limited relevance to real caries. Neither the microbiology nor the salivary effects can be simulated. It is difficult to simulate the volume and composition of saliva, as well as the usage/clearance factors that will affect the uptake and reactivity of fluoride, which is much lower *in vivo* than *in vitro*. The actual rates of demineralisation and remineralisation are faster than *in vivo*.
2. There is difficulty in matching solid/solution ratios occurring *in vivo*.
3. There may be artefacts associated with substrate choice/reaction conditions.

The *in vitro* methods have the advantage of attempting to standardise many variables present in the mouth, but to represent the clinical reality techniques such as pH cycling, as well as the addition of saliva and plaque will be required (Arends, 1995).

2.5.3 *In vivo* methods

In vivo caries models have involved the banding or bracketing of teeth that are destined for extraction. Following a period in the mouth the teeth are extracted then examined. Mellberg (1992) outlines the disadvantages of the *in vivo* banding techniques. These include the lack of availability of teeth, only patients requiring extractions can participate. There is less control over lesion reproducibility and restrictions regarding lesion location. The patient cannot commence their orthodontic treatment until the tooth is extracted. Consequently, the length of the experiment is limited, otherwise the patient's treatment will be unduly prolonged. The experiment is confined to the initial stages of treatment, usually the first month, whereas orthodontics can take up to two years. This technique is therefore unable to monitor changes in the enamel throughout the duration of the treatment. There may also be a longer time for treatment effects. The advantages of the technique include the fact that the teeth are in their most natural state with original surface pellicle and under natural occlusion and position and function.

Hals and Simonsen (1972) carried out one of the earliest *in vivo* experiments. They used specially constructed plaque attracting orthodontic bands to induce caries on teeth with class V fillings, which were scheduled for

extraction. The bands were left for between 21 to 180 days. Following extraction the teeth were sectioned, ground and studied using polarized light microscopy (see section 2.6.2, page 2.76) and microradiography (see section 2.6.1, page 2.67). They concluded that the pathology was similar to *in vitro* lesions.

Holmen *et al* (1985a&b) carried out a study to examine the progressive stages of enamel caries using an *in vivo* banding technique, polarised light microscopy (see section 2.6.2, page 2.76) and scanning electron microscopy (SEM). They discovered that no teeth showed signs of dissolution after one week, but all showed varying white spot lesions after two, three and four weeks. This study produced interesting histological evidence into the nature of the caries process.

Øgaard *et al* (1988a&b) carried out a study to investigate enamel lesion development during treatment with fixed orthodontic appliances. Five patients, aged 11 to 13 years, with ten premolars destined for orthodontic extraction were fitted with specially designed orthodontic bands, which allowed space for plaque accumulation. They wore the appliances for four weeks during which time no fluoride was used. After four weeks, the teeth were extracted, sectioned and examined using microradiography (see section 2.6.1, page 2.67) and scanning electron microscopy.

Melrose *et al* (1996) also used modified orthodontic bands for four weeks, after which they were removed and the teeth were examined under scanning

electron microscope to investigate the mechanism of formation and determine the nature of enamel damage at an ultrastructural level.

The *in vivo* banding technique has also been used to investigate the anti-cariogenic potential of fluoride and other products. Øgaard *et al* (1986) found that daily fluoride rinsing produced considerable protection from caries. White spot lesions were seen in the non-rinsers, but not seen in rinsers. Lesion depths were reduced by a factor of three in individuals who rinsed with fluoride and mineral loss was reduced by 80 percent. They concluded that fluoride might even be useful in inaccessible areas such as under loose orthodontic bands.

Rezk-Lega *et al* (1991) used the *in vivo* banding technique to assess two glass ionomer cements and a non-fluoride cement. Using transverse microradiography or TMR (section 2.6.1.1, page 2.67) they showed that with respect to lesion depth and mineral loss there was no difference between the two glass ionomer cements, but there was a difference between the glass ionomers and the non-fluoride cement.

The *in vivo* banding technique was used by Ullsfoss *et al* (1994) to examine the caries inhibitory effect of combining twice-daily chlorhexidine mouthrinses with daily fluoride mouthrinses. Four individuals with 14 banded teeth were in the fluoride-rinsing group and five individuals with 15 banded teeth were in the combined chlorhexidine/fluoride mouthrinse group. The teeth were extracted after four weeks and TMR was carried out. Lesion depth and

mineral loss was found to be reduced in the combined chlorhexidine/fluoride mouthrinse group.

O'Reilly and Featherstone (1987) investigated the effect of bracketing on the enamel of teeth destined for extraction. They found measurable demineralisation as assessed with microhardness (see section 2.6.3, page 2.78) as early as one month after placement of brackets.

Øgaard *et al* (1992) studied the cariostatic potential *in vivo* of a visible light-curing composite adhesive compared with a non-fluoridated adhesive. They found that the teeth with the fluoride-releasing resin had lesion depths measured using TMR (section 2.6.1.1, page 2.67) were reduced by an average of 48 percent at the bracket periphery compared with the no-fluoride composite.

Buyukyilmaz *et al* (1994) investigated the anticariogenic activity of a topical application of titanium tetrafluoride. Lesion depths and mineral loss, as measured with TMR (section 2.6.1.1, page 2.67) were both reduced in the experimental group compared with the control and this was statistically significant at the five per cent level (Mann Whitney U). Scanning electron microscopy showed a definite coating with globular deposits of calcium fluoride.

The *in vivo* models developed so far suffer from a number of experimental-design faults. These include small sample sizes, inadequate controls and the

short duration of the experiment. The experimental-model, which involves examining teeth destined for extraction limits the number of orthodontic patients that can be included in the experiment. It also limits the amount of time over which the experiment can be conducted.

2.5.4 The *in situ* Caries Model

Zero (1995) defines the *in situ* model as involving the use of appliances or other devices which create defined conditions in the human mouth that simulate the process of dental caries. The technique entails using a specimen of enamel, placed in a customised holder for investigating the caries process. One *ex vivo* specimen of the same tooth may be kept as a control or an additional specimen measured *in vivo* throughout the duration of the experiment.

The *in situ* caries model has been used in numerous remineralisation studies including the effects of fluoride toothpaste (ten Cate and Rempt, 1986), brushing and not brushing (Dijkman *et al*, 1990), brushing frequency (Vernon *et al*, 1992) and the remineralisation of enamel in close proximity to fluoride-releasing composites (Dijkman *et al*, 1993).

The advantages of the *in situ* appliance have been outlined by Zero (1995):

1. They are performed in the human mouth, unlike *in vitro* or animal studies. The model therefore includes all the elements that contribute to the caries

process, namely a tooth substrate, dental plaque, a carbohydrate challenge and time.

2. They provide adequate controls
3. It is possible to control the experimental variables and provide flexibility of the experimental design to allow crossover studies.
4. They allow the integration of various basic scientific analytical techniques. This will increase the sensitivity and scientific validity of the experiment compared with clinical trials that use cruder and more insensitive means of measurement and recording e.g. probing or visual scoring.
5. They represent a bridge between the laboratory and the clinical trial. They are generally short-term, therefore overcome many ethical and cost problems of long term clinical trials. They mimic the natural caries process of a specimen without causing irreversible damage to the host.

In addition, the *in situ* model has two advantages for the investigation of orthodontic demineralisation:

i. It will not affect the orthodontic treatment.
ii. It can be used at any stage of treatment.

The disadvantages of the *in situ* model are:

1. The technique is very demanding on both clinical and analytical expertise. Due to the large amount of laboratory and analytical work, the number of subjects is limited to between five and 40. This raises the question of whether such a small number is representative of the population.

2. The model depends upon the participant following the instructions of the investigator. Lack of compliance may have a major effect on the study. Wefel (1995) suggests that a means to assess compliance should be included in most *in situ* study protocols.

Zero (1995) reviews the factors that will influence the behaviour of the model.

2.5.4.1 Subjects

The participants should be representative of the population for which the study is intended. However, if the subject panel reflects the breadth of variation in the population then the study may not have sufficient power to detect significant differences. Zero (1995) advocates standardisation according to a number of parameters that include age, gender and race, all of which may influence the model. Also, the subjects should generally be in good health and have not received antibiotics two months before or during the study, as this may influence the composition of the oral flora. They should have a minimum salivary flow level and similar exposure to fluoride.

Stookey (1992) considers that individual panellists are the largest source of variability in their trials. Salivary characteristics such as flow rates, pH, buffering characteristics and concentration of calcium and phosphates are important.

2.5.4.2 Physical Design of the *in situ* Model

The mouth contains many different caries-prone sites each with an individual microenvironment. A number of factors will act on the site and determine if caries will occur. When using the *in situ* model the variability of these factors need to be controlled if useful data is to be collected. The investigator can define a number of variables:

a. *The physical structure of the model*

There are various customised holders. Wefel and Jensen (1992) use gold crowns for holding their sections. Creanor *et al* (1986) use a lower removable appliance, whereas Manning and Edgar (1992) have a method of bonding the structure to a lower molar.

Zero *et al* (1992) developed a method devised by Brudevold *et al* (1984) called the intra-oral enamel demineralization test (IEDT). This involves human subjects wearing palatal appliances holding eight bovine enamel blocks covered by standardised bacterial cell layer prepared by harvesting cultures of *Streptococcus mutans*. Using this technique, they were able to conduct a standardised dietary challenge in a controlled laboratory setting. This method gives data on what happens to enamel during one cycle of plaque pH depression (45 minutes). They found that during the course of one 45-minute test only the outer 15μm of enamel is affected by the acids. They used surface microhardness with a 50g load to assess mineral changes.

Øgaard and Rølla (1992a) reported using slabs from enamel embedded in the acrylic of a removal appliance and covered with orthodontic banding material. The advantage of this technique is that the specimens can be removed, examined and replaced. The disadvantage is that it was dependent on patient co-operation. Whichever method is used the structure of the specimen holder and method of attachment must be standardised, particularly for crossover studies, or more variability will occur.

b. Test site location

Clearance patterns will vary between different sites and the same sites on opposite sides of mouth. This is due to the rate of salivary flow, salivary film thickness, and proximity to salivary ducts. An intra-oral device may actually change these factors, but if this is closely associated with an orthodontic appliance that is also having an influence, then the effect of the intra-oral device should be reduced.

c. Method of plaque accumulation

It is important to control the thickness of plaque covering the specimen, as differences in thickness of 0.5mm can have a profound effect on the *in situ* response (Zero, 1995). Ten Cate (1992) outlines the various methods of plaque accumulation, which include the use of dacron gauze, steel mesh, or placing the specimen in a recess in acrylic. He points out that a steel mesh changes the composition of the flora, whereas a recess leads to differences between periphery and central parts of enamel. Featherstone and Zero (1992) advocate the use of gauze and argue that it will produce a more severe cariogenic challenge and hence more even lesions. The gauze may

inhibit remineralisation. Zero (1995) states that the gauze free model showed consistently greater remineralisation than the gauze covered model. Mellberg *et al* (1992) found that thick plaque lead to approximately twice the mineral loss during use of non-fluoride toothpaste than did thin plaque. They conclude that if a steel mesh is used, care must be taken to minimise thickness of plaque by placing it as close as near specimen as possible.

Øgaard and Rølla (1992b) point out that an orthodontic appliance produces sites of severe cariogenic challenge. In these stagnation areas, the pH of the plaque may fall below 4.5 and at this pH the liquid phase of plaque is undersaturated with respect to hydroxyapatite and fluorapatite. When plaque is undersaturated with respect to fluorapatite no redeposition of lost mineral can occur and the use of additional fluoride in the form of mouthrinses is not effective.

Mellberg (1992) also states that plaque is important for lesion development. The site in which the specimen is placed will be important. Ionic diffusion characteristics of plaque on buccal and lingual surfaces will probably be different from those of plaque in approximal or fissure locations or even around an orthodontic appliance. Approximal lesions will demineralise at about half the rate as those on smooth surfaces, probably due to differences in diffusion. A steel mesh will greatly reduce fluoride diffusion. Even disturbance of the plaque may reduce the demineralisation rate.

2.5.4.3 Type of Hard Tissue Substrate

Enamel for the *in situ* caries model has been used from different animal species and has been prepared in different ways. These can be summarised into three categories:

a. Species of Origin

Human enamel is ideal as an *in situ* model substrate, for reasons of authenticity (Manning and Edgar, 1992). If an experiment is being carried out that is directly related to the anticariogenic properties of an agent in humans, it is relevant to obtain the results with human enamel. However, there are some disadvantages to using human enamel. It is difficult to obtain, it may have defects or caries, and may be of variable age and source, which could lead to an inconsistent response (Mellberg, 1992). If the experiment is not directly related to the clinical situation then the use of non-human enamel might be considered.

Several types of non-human enamel have been used in cariology, but only bovine has been used *in situ*. It is easily obtained and is less variable and therefore more consistent than human enamel. It has a large flat surface, the fluoride concentration of the outer layer is lower and there will be no previous caries. Bovine enamel is more porous than human enamel, leading to more rapid diffusion and hence response (Mellberg, 1992). The rate of lesion formation in bovine teeth is about the same as primary human teeth. Structurally there are differences, with bovine enamel demonstrating thicker crystallites, which Mellberg (1992) considers unimportant. Abraded human

and bovine enamel behave similarly to acid challenges and remineralising conditions, with a faster response from bovine enamel to cariogenic challenges (Øgaard and Rølla, 1992b).

b. Slab or Section

The enamel specimen may be placed in the intra oral holder as either a slab or section. Slabs have the advantage of being easier to handle than thin sections. Additional care needs to be taken to protect the cut surface of a section to prevent it from demineralising. Sections may also demineralise more rapidly than slabs (ten Cate and Exterkate, 1986), although this has not been found to happen when the sections are ground rather than cut (Strang *et al*, 1988). Another complication of sections is that distances away from the specimen are small for thin sections compared with the larger surface of a block. This may lower the concentration of mineral ions adjacent to the surface of a thin section by lateral diffusion, which could alter demineralisation/remineralisation rates (Mellberg, 1992).

A potential disadvantage of slabs is the known variability in the demineralising response between and within the same tooth. Schäfer *et al* (1992) found that enamel taken from the cervical region of a tooth was more susceptible to demineralisation than enamel taken from the coronal region.

Ten Cate (1992) analysed several reported studies for within specimen variation, the intra-side variation, the correlation between contralateral sides and the correlation between studies. They found that the within specimen

variation was small, but there were significant differences between two specimens placed in the same site and between contralateral specimens.

Single sections might overcome the problems of variation in samples, because the same thin section is used as the control before and after treatment, such that longitudinal changes can more accurately be followed (Mellberg, 1992). These sections can also be examined non-destructively several times with polarised light microscopy or longitudinal microradiography. However, the specimens need to be removed for examination, which disrupts the plaque and possibly the lesion. Mellberg (1992) still found inconsistency between specimens using sections rather than slabs. Even so, Stephen *et al* (1992) believe that single section technique increases the sensitivity of the model for measuring small changes in mineral content.

Ten Cate (1992) favours the use of multiple enamel specimens (sandwiches or single sections) placed in sites at risk of caries, for example interproximally, with a sufficient period for natural plaque to form and changes in the specimen to be detectable.

c. Preformed Lesion or Natural

The specimen may be placed in the mouth in the natural state or with a preformed carious lesion. The advantage of using a lesion with a preformed lesion is that remineralisation or further demineralisation may be investigated, unlike sound enamel that only allows the study of demineralisation.

Lesions are usually formed by placing the teeth in a demineralising solution or gel (ten Cate, 1992). Dijkman *et al* (1986) produced lesions *in vivo*, by placing initially sound human enamel in dental prostheses. Subsequently the lesions were remineralised *in vivo* under nearly plaque-free conditions over six weeks or three months respectively and showed a strong participant effect. This study suggested that both the specimen and the participant had an influence on demineralisation/remineralisation. Specimens of one tooth placed in two different participants could remineralise or demineralise. Specimens from two different teeth placed in the same participant would either remineralise or demineralise, but not both.

Zero (1995) challenges the use of specimens with a preformed, subsurface lesion, because clinical dental caries will occur by the net loss or gain of subsurface mineral or interactions with the tooth surface. Most caries research has focussed on the loss or gain of subsurface material, measuring the formation and repair of the preformed lesion. Subsurface lesions are a reversible stage of caries, which do not necessarily precede cavity formation (Zero, 1995). It is possible to go from surface softening to a cavity. He outlines a number of challenges that the use of subsurface lesions presents:

a. The production of a subsurface lesion is difficult to standardise leading to variation.
b. Transverse microradiography is commonly used to analyse mineral loss or gain. It is technically demanding and subject to wide variations in the conditions of analysis.

c. The main interactions between the enamel and the oral environment occur at the surface layer of sample. TMR is mainly of use in the subsurface region and cannot accurately measure in the outer 25μm.
d. Remineralisation of subsurface lesions with intact surface layer occurs only to a limited extent and is more likely to be found with advanced subsurface lesions.

Zero (1995) goes on to support the hypothesis that interactions with the tooth surface are the main factors that determine if clinical dental caries will occur by stating that:

i. Early lesions do not have intact surface layers and the first stage of caries is surface softening.
ii. Outer enamel is tooth material in direct contact with plaque. The fluid phase of plaque is largely responsible for creating conditions that favour either demineralisation or remineralisation.
iii. Fluoride interacts primarily with the outer layer concerning uptake, inhibition of demineralisation and enhancement of remineralisation.
iv. There is evidence that fluoride is much more effective at inhibiting the start of demineralisation than halting the progression.

The natural tooth surface may be important in clinical trials, where the potential of the agent to prevent demineralisation is of more interest than its ability to cause remineralisation (Øgaard and Rølla, 1992b). Fluoride may be more effective in inhibiting demineralisation than increasing remineralisation (Zero, 1995). Surface softened lesions will remineralise fast *in vivo* with or

without fluoride and high concentrations of fluoride may lead to the arrest of the lesion due to precipitation of fluorapatite. A preformed lesion may be misleading because it is unlikely that well-established lesions can fully remineralise *in vivo* owing to the complexity of the reactions (Øgaard and Rølla, 1992b).

The choice of substrate will differ according to whether a study is investigating the factors affecting lesion formation, remineralisation or the effects of treatment on remineralisation (Mellberg, 1992). One way of investigating both preformed and natural tooth surfaces is after Featherstone and Zero (1992), using a sound enamel slab and one with preformed enamel lesion in each subject.

Natural sound surfaces are more useful for studying demineralisation than remineralisation, because there is variation in lesion severity even between areas of the same tooth (Mellberg, 1992). Abrading the tooth with 600-mesh or 120-mesh silicon carbide removes from the surface layer larger crystallites, higher carbonate and fluoride concentrations, which gives a more consistent lesion formation and eliminates curved surfaces before microdensitometric and hardness testing, but the use of the natural surface is lost. Featherstone and Zero (1992) found more reproducible results when they removed the outer 50μm of enamel using 600-grit silicon carbide paper.

Specimens with preformed lesions are usually used to assess remineralisation (Mellberg, 1992). There are various ways of producing

lesions on enamel. Each method may lead to lesions with different characteristics that may not be related to early natural lesions.

Ten Cate (1992) examined three commonly used methods for producing artificial caries *in vitro*. He found that remineralisation differed by a factor of five between lesions with the same uptake capacity but different mineral loss pattern. Thus, there is a cross-comparability difficulty between different centres.

Lesions can be classified as surface-etched, surface-softened or subsurface (Mellberg, 1992). Each may be justified as a stage in the development of natural caries, but will react differently to a remineralising environment. Surface softened lesions may be difficult to measure because of lack of lesion definition and the surface damage during handling. Sub-surface lesions may not remineralise well due to blocking of the surface layer diffusion channels and a good remineralising agent may possibly be overlooked.

The method of demineralisation may also have an effect on the response of the lesion. Lesions formed with acid gel in large blocks may be more severe along the edges. Gel-prepared lesions are not as sensitive to de/remineralisation processes as buffer-prepared lesions (Damato *et al*, 1988; Stephen *et al*, 1992).

Schäfer *et al* (1992) state that lesions with initial mineral loss in the range 3500-600 vol%μm would be suitable for remineralisation studies, whereas less severe lesions might be more applicable to demineralisation studies, because the degree of initial demineralisation of an enamel specimen affects the remineralisation rate. More rapid remineralisation is due to faster diffusion of ions into the more porous extensive lesions (Strang *et al*, 1987).

Manning and Edgar (1992) cast some doubt about the importance of the size of the initial lesion. A shallow lesion would be thought to remineralise faster than a deep lesion because of shorter diffusion distance and smaller amount of mineral required. If the rate of transport of mineral into the lesion is constant then the proportional gain of larger lesions ought to be less.

2.5.4.4 Study Design and Clinical Protocol

In a study design for an *in situ* model the number of subjects and length of the test period are notable (Zero, 1995).

a. Number of subjects

The number of subjects recruited into *in situ* trials may vary from five to 40 (Zero, 1995). One reason for the use of an *in situ* model is to have sufficient statistical power in the clinical study (ten Cate, 1992). The caries rate has dropped, the differences between individuals has increased with only a few people accounting for a large proportion of the lesions. Therefore, the *in situ* study is a means by which inter-individual differences can be reduced. Whilst some individuals consistently remineralised lesions, for others the

demineralisation or remineralisation was a random process, with a coefficient of variation of approximately 0.25 for *in situ* studies. Most studies lack the numbers required for statistical significance, if the expected treatment effects do not differ by more than 10 – 15 percent (ten Cate, 1992).

Stookey *et al* (1992) illustrated the importance of determining a sample size. They investigated 28 subjects who each wore *in situ* enamel blocks. They found that the major sources of variation were between subjects and surprisingly between the enamel specimens. Between them this accounted for 70 and 91 percent of the total variance. They concluded that increasing the number of specimens per subject has a lesser impact upon test efficiency than increasing the number of subjects. They also state that the use of a crossover study is considerably more efficient than a randomised test design. Their view is echoed by Proskin (1992).

b. Length of test period

This has varied between 45 minutes to 6 months. Featherstone and Zero (1992) suggest that a four-week test period is better than two. Ten Cate (1992) found that initially the lesion is highly reactive and will remineralise quickly, it will then be slow. Arends *et al* (1992) examined the rate of enamel demineralisation *in situ* and found a linear relationship between both lesion depth and mineral loss, with demineralisation periods of four and eight weeks

Other factors that may affect the model include the diet. The participants can remain on their normal diet, but subjects need to complete a diet sheet to monitor any changes. Featherstone and Zero (1992) control the

demineralisation challenge by extra-oral immersion of appliances in sucrose for 10 minutes after meals initially then twice daily.

2.5.4.5 *In situ* model validation

Wefel (1995) points out that the *in situ* model assesses the caries process for example mineral loss or gain and not actual caries. The model may also include substrates (bovine enamel, artificially induced caries) and may be covered with materials such as gauze that are different from the actual situation found in locations not associated with the caries process. An important criticism of the technique involves the lack of standardisation of test conditions, analytical techniques and measuring parameters making it impossible to compare results from different centres. There is no agreement on what constitutes a legitimate artificial caries lesion or how to assess this, what is an appropriate technique for assessing means to assess mineral change, creating cariogenic sites and what are key parameters. Stephen (1992) states that a model is only a model and the limitations should be accepted. They may never truly substitute for human clinical trial.

Several authors have pointed out the need to validate the *in situ* model. Mellberg *et al* (1992) suggest that the model is valid because it shows inter and intra subject differences. They have also revealed that fluoride is effective and like some clinical trials, some have shown no significant difference with a placebo. The authors further validated the model by testing a product, which they thought to be compromised in its anticariogenic activity

because it had reduced fluoride release. They found that mineral gain was reduced.

Zero (1995) concludes that *in situ* caries models are the most promising link to "natural" caries short of large scale, long-term clinical trials, but they are only models. The success with the model will be determined by the ability to maintain the clinical relevance while controlling variation. He states that the major source of variation should be biological not experimental by rigorously standardising the major experimental parameters. He suggests that a combination of *in situ* approaches (eg sub-surface, surface and sound enamel) may be necessary to maximise predictive value.

Proskin (1995) provides a statistical test and the criteria associated with it to use with the results from an *in situ* trial to assess if a test agent is an effective anti-caries agent. Raubertas (1995) proposes a stronger definition of validity purpose if a model is to be used as a substitute for a clinical trial.

Ten Cate (1992) concludes that intra-oral models bridge the gap between laboratory and clinical studies. Results can be obtained much quicker than with clinical trials. Increasing the knowledge of the performance of models (reliability and predictive value) will lead to the establishment of a range of designs with different aims and perspectives.

2.6 Techniques for the Quantitative and Qualitative Measurement of Demineralisation with Intra-Oral Models

Arends and ten Bosch (1992) point out that there are over ten different techniques for measuring mineral changes in enamel. The choice of method for evaluation of demineralisation/remineralisation is important. The method should be capable of answering two questions. How much mineral has been lost or gained? Where with respect to the outer surface of the tissue, has the mineral been lost (or gained)?

2.6.1 Microradiography

Microradiography is a technique of using x-rays to examine the mineral content of enamel. There are three techniques:

1. Transverse
2. Longitudinal
3. Wavelength independent

2.6.1.1 Transverse Microradiography (TMR)

Arends and ten Bosch (1992) state that transverse microradiography is the most practical technique for direct and quantitative measurement of mineral content, mineral changes and mineral distributions. The concept was devised by Thewlis (1940), but it was made quantitative by Angmar *et al* (1963). Ten Bosch and Angmar-Månsson (1991) provide a good description of the principles of TMR.

Essentially, TMR involves the measurement of absorption of monochromatic x-rays by a tooth section. The absorption of x-rays is directly reflected in the optical density of a film, which records the x-rays that have past through the specimen and not been absorbed. The optical density of the specimen is compared with the optical density of a simultaneously exposed standard, which is usually an aluminium step wedge. A technique called densitometry is used to calculate values of optical film transmission (OFT). The OFT value of the slice at an area of interest is read. The aluminium thickness that causes the same OFT is applied using the formula of Angmar to calculate the mineral density.

The x-rays are produced from a fine-grain copper anode operated at 20kV and 1-20mA (predominant energy is Cu line of 8.05keV wavelength 0.154nm). A nickel filter suppresses photons with energy of 20keV and less. High-resolution emulsions are used such as Kodak HR 1A on microscope object glass plates. Exposure times vary between 10 and 100 minutes. Slower exposure times are possible, but fine detail is lost.

In the past, the developed film has been analysed using a microscope densitometer with a slit-shaped diaphragm and motor driven cross table which scans the film. Nowadays a charged-couple device (CCD) video camera linked to a computer is used to capture and analyse images of the section. Lagerweij *et al* (1994) compared the results of measuring the mineral content from scans by a densitometer and a video camera. They found that the densitometer produced a slightly sharper profile, but otherwise they

detected no substantial differences between the two techniques. They point out that any differences were smaller than that from biological variation.

Several assumptions must be made. The elemental composition of both mineral and organic material plus water has to be assumed or known. This varies between individuals and between different sites in the same individual (for major constituents roughly $\pm$3% of the average value is common; for trace elements a factor of two can occur). Monochromaticity of x-rays is assumed and the sample must be homogenous over its thickness. Accuracy of TMR is about 5μm for lesion depth (L_d) and 200vol%.μm for mineral loss (Arends and ten Bosch, 1992).

De Josselin de Jong and ten Bosch (1985) summarise the sources of error involved in the measurement of absorbance levels in microradiography. They conclude there are two sources of error:

I. Systematic errors due to beam inhomogeneity; construction and thickness determination of the step wedge and stray light in the densitometer microscope.

II. Random errors due to photographic noise caused by the photographic grain size and random distribution of grains. The area and width of the densitometer window will also have an effect.

They give various criteria to reduce the amount of error and conclude that microradiography is an accurate tool for investigating atomic concentration.

De Josselin de Jong *et al* (1987a) compared the results of measuring the mineral content from 12 human enamel slices using microradiography and chemical analysis. They obtained similar mineral volume percentages with the two techniques, within deviations caused by random error. They point out that to obtain an accurate reading of the calculated mineral volume percentage and mineral loss with TMR care needs to be taken with two variables that are within the control of the operator. Firstly, the thickness of the tooth slices. Samples need to be planoparallel and thickness should be measured as close as possible to the position of the tracing scan. Secondly, the optical film transmission range, which is affected by exposure time, x-ray tube voltage and current adjustment and film development. This should range between 5-50% so the polynomial fit to the calibration stepwedge data is optimal and noise is at a minimum. They conclude that with careful attention to the potential sources of variation, the error in calculating mineral volume percentage is approximately 4 percent of its value with TMR.

Arends and ten Bosch (1992) discuss the disadvantages of TMR. The main disadvantage is that the sample is destroyed and therefore longitudinal measurement of the same specimen cannot be carried out. In addition, phenomena less than 10μm from the anatomical surface are not measured due to finite densitometer slit width and specimen curvature. The presence in the outer layer of ions with a very high absorption coefficient for x-rays, for example tin ions will lead to misinterpretation of the image as remineralisation.

White *et al* (1992) state that radiographic methods (LMR and TMR, WIM) represent the only practical method for direct analysis of demineralisation and remineralisation. Others techniques do not measure these directly. They point out that three things are critical if TMR is to be successful:

i. Specimen preparation procedures - TMR requires planoparallel specimens usually by polishing which can be difficult to achieve. Uneven specimens cannot be analysed. They found 10-20 percent variations in thickness affecting the radiographic precision.
ii. Magnitude of the change – the remineralisation rate *in vivo* is relatively slow with a dependency upon both saliva and substrate. Under high cariogenic stress demineralisation rates may be four times higher. The larger the change the less sensitive the technique has to be to detect a difference.
iii. Protocols of specimen analysis - As intra-oral and *in vitro* methods of assessing demineralisation and remineralisation have become more sophisticated there has been less attention on the details of analysis. Vital information about the techniques used has not been reported making it difficult to assess the accuracy of the results. They suggest that there is a need for standardised profile tests

Ten Cate *et al* (1996) carried out an investigation to assess the variability and reproducibility of TMR between enamel specimens produced and analysed by four different labs. They found that there was general similarity in analysis of lesions produced by a standard method (IML values 2,000 to 3,000) but not for each laboratories preferred method (IML values 1,800-6,300). They

suggest that some of these differences would be due to local factors such as the fluoride content of enamel, others to analytical differences. They also state that the difference in the analysis of the same lesion between different laboratories was of more concern. This was partly explained by differences in the definitions of the lesion parameters. In particular, the defining of the start of the lesion where mineral content is zero had a strong influence on the calculated IML value. They point out that these variations between laboratories would suggest that many of the studies involving TMR would lack the statistical power to produce a significant result. They make several suggestions about the reporting of studies involving TMR so that others may check the accuracy of the results. Damen *et al* (1997) agree that there should be stricter agreement of the definitions of lesion parameters if the full potential of TMR is to be realised.

2.6.1.2 Longitudinal Microradiography (LMR)

Longitudinal microradiography was developed, as a non-destructive alternative to TMR, to determine the x-ray absorption of thicker slabs of tooth material (0.3-0.5mm thick). The increased thickness of material would allow multiple determinations to be carried out on the same slice of tooth tissue. This would provide more accurate longitudinal data on the mineral changes in a section of enamel, for example mineral content could be determined before and after exposure to the intra-oral environment, rather than using a separate control slice. LMR has the added advantage over TMR in that mineral content changes are measured independent of the thickness of the

sample and it can be used on planoparallel samples or natural tooth surfaces.

Unlike TMR where the film is placed perpendicular to the experimental surface, the photographic film for LMR is parallel to the experimental surface. Slab areas of five millimeters by five millimeters are measured. As the slab is thick so must be the step wedge. The same radiation as TMR is used in LMR, but as both slab and wedge act as filters so radiation reaching the film is not monochromatic, therefore film sensitivity for mineral change is much lower. According to ten Bosch and Angmar-Månsson (1991) LMR is subject to systematic errors of up to 20 percent.

De Josselin de Jong *et al* (1987b) used LMR to follow the mineral content variations in five specimens of human enamel ($6mm^2$) that were exposed to an acetic acid buffer solution. In all the specimens, a progressive loss of mineral content was found. They discuss the possible sources of error. They conclude that systematic error due to beam inhomogeneity and the presence of organic material amounts to approximately ± 6.5 percent of the total mineral change value per unit area. Random error due to step wedge calibration and film grain inhomogeneity is small and the main source of random error is inhomogeneous film development and dust particles.

De Josselin de Jong *et al* (1988) investigated the correlation of chemical analysis in enamel with the determination of mineral changes by longitudinal microradiography and scanning optical monitoring. They showed an excellent

linear association (r=99) between the calcium loss due to removal of enamel layer as determined by LMR and chemical analysis. They demonstrated a wide variation in the demineralising behaviour of different human tooth slices. Mineral loss was dependent on the position on the tooth surface. Mineral loss in most samples was greatest in the centre of the exposed portion and lowest at the periphery. They speculate that because the scan area with LMR was smaller than the total exposed portion and was centred on the middle where the mineral loss was greatest, this would lead to an overestimate of the mineral loss. They concluded that both LMR and OM were able to accurately follow local mineral changes in time of the same tooth sample on tooth surface.

2.6.1.3 Wavelength Independent Microradiography (WIM)

Herkströter and ten Bosch (1990) describe the technique of wavelength independent microradiography (WIM). This is another non-destructive technique, which uses polychromatic high-energy x-rays (60kV) rather than monochromatic x-rays. When analysing thick sections, monochromatic x-rays of low energy as used in TMR, are absorbed by the tooth and do not reach the film. Therefore, either monochromatic x-rays of high energy are needed, which are hard to obtain or the method of mineral calculation should not be dependent on monochromatic rays. To ensure this, the stepwedge is made from an alloy with a mass attenuation coefficient that has wavelength-independent ratio to mass attenuation coefficients of enamel and dentine. Samples may have variable thickness between 0.3 to 6mm, with or without natural curved surfaces. Arends and ten Bosch (1992) state that the

accuracy of measuring mineral loss with WIM is about 310vol%.μm and with whole teeth the detection limit is about 1500vol%.μm. Herkströter and ten Bosch (1990) found a correlation coefficient of 0.99 for mineral concentrations in enamel calculated from measurements using LMR and WIM.

Herkströter *et al* (1990) carried out a study to adapt WIM for use on thick curved and whole teeth. They prepared thin specimens with curved surfaces and carried out WIM and LMR before and after demineralisation. They then added dentine sections to thicken the samples and repeated WIM to compare the results with those of the thin sections. They also carried out the procedure with whole teeth. An extra thick stepwedge (11mm) was used, with adapted software to take into account the curve of the tooth.

A linear correlation was found between the WIM measurements of the thin and thick teeth of 0.97 for enamel and 0.90 for dentine. In the discussion, they point out that the graphs of the regression lines do not go through the origin and speculate that this was probably due to errors in stepwedge thickness and beam in homogeneity. The detection level was reduced in the whole teeth due to re-positioning errors of the samples. They conclude that WIM can be used for measurements of minimum concentrations in enamel and dentin from about 0.3 to 6mm with or without natural curve, however when whole teeth are used the reduced detection limit must be taken into account.

2.6.2 Polarised Light Microscopy

When an unpolarised beam of light passes through a crystal, it is split into two rays at right angles to each other. For those rays, the material exhibits different refractive indices. The experimentally measured quantity is the difference in the optical path length of the two rays (retardation). This can be measured with a microscope equipped with two Nicol prisms or Polaroid plates, a polariser, an analyser and a compensator for quantitative measurements. The sample is located between the two prisms and mounted on a rotating stage. The stage is rotated until the detail of interest becomes dark. The compensator reading is used to determine the retardation. When combined with many imbibitions (replacement of pore liquid by aqueous solutions) of the same section and making various assumptions PLM will provide data on mineral content. Lesion depth may be determined using a simpler method with a single imbibition (ten Bosch and Angmar-Månsson, 1991).

Changes in the tissue porosity will lead to changes in the way the light is scattered and therefore the optical properties of enamel. Enamel consists of tightly packed crystals surrounded in healthy tissue by a tiny intercrystalline space. This is usually filled with water and organic material with a similar refractive index to hydroxyapatite. By varying the imbibition media and hence the refractive index in the intercrystalline spaces it is possible to estimate the porosity of the tissue. This is an important method of obtaining information on loss of mineral (Thylstrup and Fejerskov, 1986). PLM can provide quantitative information on pore volume (porosity), but can be difficult to

interpret quantitatively. There are no published reports of correlations between quantifiable techniques such as TMR and PLM (Arends and ten Bosch, 1992).

Polarised light microscopy has been used in several studies of orthodontic demineralisation. Underwood *et al* (1989) carried out an *in vivo* investigation to examine the effectiveness of a fluoride-exchanging resin. The results were not clearly presented. Very few teeth were affected with lesions and those that were observed were in unusual locations such as occlusally. They speculate that certain lesions "were present before the start of the investigation". They quote an attack percentage, but it is not clear what this denotes. A graph suggests that this is a percentage of occurrences, but as they state that very few structural enamel alterations were observed this must be based on a very small number.

Donley *et al* (1995) carried out an *in vitro* study using polarised light microscopy to examine the effects of glass ionomer cement on enamel caries. The results showed that the areas exposed to zinc phosphate had significantly greater pore volume than the varnished controls. The enamel exposed to the glass ionomer cement showed significantly less pore volume than their varnished controls.

Vorhies *et al* (1998) carried out an *in vitro* study of demineralisation surrounding brackets bonded with two glass ionomer cements and a conventional light cured composite. Human premolar teeth were bonded with

the three materials and subjected to an intermittent artificial caries challenge for 30 days. The sections from the teeth were examined by PLM imbibed in water. The results showed that there were significantly larger lesion depths and areas of demineralisation for the conventional composite compared with the glass ionomer cement.

2.6.3 Microhardness

Microhardness techniques involve slowly pressing a diamond onto a test material under a well-defined load for a given amount of time (ten Bosch and Angmar-Månsson, 1991). The size of the indentation is measured by microscope. Two types of diamonds are used, the Vickers which gives a square indentation and the Knoop which is oblong. Typical sizes of indentation range from 10 to 100μm and this is used to calculate the hardness of the material. Quantitative mineral contents can be obtained when calibrated against a quantitative technique like TMR. The Vickers number is directly proportional to microradiography with a correlation coefficient of 0.94 (Arends and ten Bosch, 1992).

Two hardness measurements may be performed. Firstly, when the load is applied perpendicular to surface (Surface microhardness or SMH). Secondly, when the load is applied parallel to anatomical surface (cross-sectional microhardness or CSMH). In the latter technique, several readings are taken from the surface at 25μm to a depth of several 100μm.

Microhardness techniques demonstrate good reproducibility of results in enamel, but not dentine (ten Bosch and Angmar-Månsson, 1991). The disadvantage of surface microhardness is that the test material has to be homogenous over several times the indentation depth and therefore it cannot be used on lesions with a well-mineralised surface layer, although early lesions can be measured.

Other disadvantages of microhardness are that reliable measurements can only be determined on flat surfaces, whereas the tooth surface is not flat. In addition, the outer 25μm cannot be analysed. It only provides a qualitative information only and lesion shape, mineral redistribution as well as protein uptake might influence indentation values. A linear relation between indentation length and lesion depth is valid only in a limited range of lesion depth values (Arends and ten Bosch, 1992).

Wefel and Jensen (1992) point out that hardness testing is not usually practical for longitudinal assessment of enamel sections as indentations are left after each recording.

Microhardness has been used to assess demineralisation surrounding orthodontic brackets. O'Reilly and Featherstone (1987) studied teeth bonded with an orthodontic bracket that had been in the mouth for one month. They found that measurable and significant demineralisation as quantified by microhardness testing occurred around the orthodontic brackets after just one month, even with the use of fluoride toothpaste. They did point out that

tooth enamel variation within a person could be as great or greater than variation between individuals.

The main problem with this study was the lack of a baseline assessment of the mineral content of enamel. They assessed the enamel under the bracket and found three to eight percent mineral loss to a depth of 25μm, which they attributed to acid etching. They concluded that mineral loss exterior to the bracket at depths of 25, 40, 50 and 75μm were not due to acid etching. They also assumed that enamel with a mineral content of less than 85 percent was demineralised and greater than 85 percent was remineralised.

2.6.4 Chemical Analysis

Chemical analysis of enamel may be carried out by dissolving it in acid and analysing the solution for calcium and/or phosphate content (ten Bosch and Angmar-Månsson, 1991). Calcium is determined by atomic absorption spectroscopy and phosphate is determined by the formation of a coloured complex with molybdate. Arends and ten Bosch (1992) state that the disadvantages of this method is that only very large changes are measurable due to biological spreading, calcium and phosphate can only be estimated with an accuracy better than one percent and mineral distributions are not measurable in practice.

2.6.5 Microprobe Analysis

Microprobe analysis is a technique that involves bombarding the tooth sample with a particle beam to induce the release of radiation or particles, which can be analysed (ten Bosch and Angmar-Månsson, 1991). A number of different media may be used for bombarding including electrons, light ions (H^+, He^{2+}) or heavy ions. The energy and/or the flux of the emitted x-rays, secondary electrons or ions released can be measured. A vacuum is required for this process and only concentration ratios are produced. This technique is useful for trace elements.

2.6.6 Iodine absorptiometry

The technique of iodine absorptiometry involves sending a collimated beam of tin-filtered radiation from an ^{125}Iodine source. The difference between the original and the transmitted radiation is measured (ten Bosch and Angmar-Månsson, 1991) using a scintillation counter. The amount of absorbed photon radiation is a measure of the amount of mineral per unit area. Arends and ten Bosch (1992) state that the sensitivity is similar to LMR.

2.6.7 Iodide Permeability

The iodide permeability (Ip) test consists of placing 2M potassium iodide on the enamel surface for three minutes then wiping it off. Water is placed for 40 seconds to permit back-diffusion of iodide. This water is recovered by means of an absorbent disk and the iodide content determined by iodide-specific electrode. This is a measure of the iodide permeability. The relation between

lp changes and amount of mineral lost or gained is not very clear at present (Arends and ten Bosch, 1992). The technique is sensitive to pore blockage and possibly penetration of salivary proteins. lp tests can give sensitive estimates of initial stages of demineralisation and remineralisation, but is probably not very useful.

2.6.8 Confocal Laser Scanning Microscopy

Confocal laser scanning microscopy (CLSM) is a new method of obtaining non-destructive microscopic tomographies of the outer subsurface of dental hard tissues. The confocal principle is based on the elimination of stray light from out-of-focus by confocal apertures. Light from a laser is shone through a pinhole onto the object. The same point is imaged on a detector pinhole. The term confocal relates that the laser-illumination pinhole image and the back-projection of the detection pinhole have a common focus in the object (Brakenhoff *et al*, 1989). CLSM allows the study of unsectioned, natural teeth.

Øgaard *et al* (1996) compared TMR and CLSM for evaluation of mineral loss. Specially constructed plaque attracting bands (see section 2.5.3, page 2.46) were cemented for four weeks in seven individuals. Following extraction of the teeth CLSM and TMR were carried out. They found that although the lesion depths varied considerably between individuals, the lesion depths visualised from the TMR measurements corresponded very well with those on the CLSM images.

Ando *et al* (1997) compared CLSM with TMR, QLF and DELF (see section 2.4.3.2, page 2.36) in quantifying *in vitro* artificial carious lesions. Both TMR and CLSM correlated with mineral loss, although lesion depth was lower with CLSM than TMR.

2.6.9 Comparison of Methods

Ten Bosch and Angmar-Månsson (1991) state that TMR is preferable to PLM for sections, as the measurement is more directly related to mineral content. They dispute the contention that imbibition media reaches all the pores. They consider that microhardness of transverse cuts is a reasonable alternative to TMR and is certainly cheaper. They consider that chemical determination of dissolved micro-samples is the only true direct method for measuring mineral content and changes, but spatial resolution is poor.

White *et al* (1992) consider that mineral loss is a primary parameter and can be measured using TMR, WIM or LMR, although CSMH may be used if the others are not available. Lesion depth is also important since it defines the magnitude of penetration and damage by acid. It also provides detail about the nature of the acid destruction, for example surface softening or subsurface. They consider lesion porosity to be a useful secondary measure. They suggest that surface microhardness is best suited as complimentary to direct measures, for example as a calibration measure for sample preparation.

Zero (1995) considers that interactions with the tooth surface are the main factors that determine if clinical caries will occur, therefore evaluating the surface layer may be the best way of assessing the efficacy of agents such as fluoride. He states that techniques such as iodide permeability and WIM evaluate the surface layer. The author's preference is SMH, with a 50g load to increase the sensitivity. Ten Cate (1992) agrees stating that microhardness is more sensitive to changes occurring in the surface layer than microradiography. He goes on to point out that proper alignment of the baseline and final lesion may be difficult and material may be lost due to erosion.

2.7 Summary Of The Review Of The Literature

This review of the relevant literature has shown several deficiencies in our current knowledge that should be explored.

Clinical techniques for recording the development and regression of smooth surface demineralisation associated with orthodontic treatment need to be developed and tested with regard to validity and reproducibility over the length of time of orthodontic treatment and beyond. The advantages of photographs have been outlined in the text. New techniques such as quantitative light-induced fluorescence should also be investigated for use in clinical orthodontic trials of preventive agents. A direct method of studying the mineral loss during orthodontic treatment would be useful in evaluating

preventive agents. The advantages of the *in situ* model have been outlined and need evaluation in the orthodontic context.

Specific aims of this study, as stated in Chapter 1 are:

1. Direct measurement of demineralisation from a subject's tooth using clinical photographs assessed with morphometry and computerised image analysis, as well as a new technique called quantitative light-induced fluorescence.
2. Indirect measurement of the de/remineralising conditions within the mouth, using an *in situ* model assessed with transverse microradiography.

CHAPTER 3

Morphometric Assessment of Enamel Demineralisation from Photographs.

3.1 Introduction and Aim

The advantages of using photographs for recording the appearance of enamel have been discussed previously (section 2.4.2, page 2.25). Briefly, photographs are a swift and efficient method of producing a permanent record. An investigator can examine the data from subjects blindly and in a random order. In addition, photographs taken by several examiners may be scored by an independent viewer during one diagnostic period and re-examined later if required. Several photographic techniques have been described (Hill and Geddes, 1975; Callender, 1983; Fleming *et al*, 1989; Ellwood, 1993). They have been used to assess the incidence of enamel opacities in populations (Levine *et al*, 1989; Nunn *et al*, 1993), the development of enamel demineralisation (Hollender and Koch, 1976; Edgar *et al*, 1978) and the frequency of enamel white spots developing during orthodontic treatment (Gorelick *et al*, 1982; Mitchell, 1992).

Several indices have been devised to assess differences or changes in the optical properties of enamel. Indices that assess the incidence of developmental enamel opacities may not be suitable for the assessment of

enamel demineralisation. The study to be presented in this section uses the technique of morphometry to measure demineralisation.

Morphometry is a microscopic technique for quantification of structures Weibel *et al*, 1966). The morphometric technique uses a test system that is randomly placed on the section. This system consists of "probes" in the form of points, lines or planar areas. The probe may coincide with a particular feature of interest and this is recorded as a positive event. If it does not coincide this is a negative event. The number of positive events can be counted. By using the appropriate mathematical formula various measures can be calculated including volumes, surface areas, surface-to-volume ratios and proportions of constituent components. In this study, a 121-dot array was used to measure the area of demineralisation on the buccal surface of a tooth.

The aim of this study was to investigate the validity and reproducibility of a new method, based on the principle of morphometry, for qualitatively and quantitatively assessing the development of enamel demineralisation.

3.2 Materials and Methods

Twenty two, freshly extracted human teeth (11 molars and 11 premolars) from adolescents and young adults were collected and stored in distilled water. One investigator marked the root of each tooth with a number. The crowns of the teeth were coated with an acid resistant varnish, leaving a

small window on the buccal surface. This was incrementally occluded by the addition of varnish, at intervals, over a 14-day period, during which the teeth were placed in a demineralising gel (see Appendix A section 3.8.1, page 3.29) at pH 4.5 (Figure 3.1, page 3.21). After 14 days the teeth were taken out of the gel and the varnish removed with acetone.

A second investigator, with no knowledge of the details of the protocol used in the first part of the experiment, then mounted the teeth on a wax block, ensuring that the number of the tooth was not visible. Photographs of the teeth were produced using a standardised technique (Fleming *et al*, 1989). The photographs were taken using a Nikon F301 camera body (Nikon UK Ltd, 380 Richmond Rd, Kingston-upon-Thames, Surrey, UK.) with a 90mm Elicar macro lens (Elicar Lenses, Lucfoto Ltd, Unit 3 Grovelands Ave, Winnersh, Berks, UK.) set at a magnification of 1:1. The camera was set to manual with an aperture at f22 and the shutter speed 1/125 of a second. The film used was 50 ASA professional slide film (Agfa-Gevaert N.V. Septestraat 27, B-26640, Mortsel, Belgium). An Elicar ring flash was used masked on the lower half, to reduce the amount of light causing reflections. The teeth were stored in distilled water until they were photographed. They were removed from the water, dried with compressed air for 15 seconds and the photograph taken. They were then replaced in the distilled water.

Each tooth was photographed from approximately 30 degrees above and 30 degrees below a plane arising perpendicular to the buccal surface of the tooth, assessed by eye. A photograph of a grey scale and colour separation

guide was taken with the first frame of every film. These images were examined and compared for consistency of development of the film. The photographs were repeated after one week.

The second investigator, who had no knowledge of the extent or position of demineralisation, then carried out an assessment of demineralisation on the teeth. Three examinations were carried out. One was a direct visual estimation of the area of the buccal surface of the tooth that was affected by demineralisation. The other two examinations were based on the principle of morphometry.

3.2.1 Techniques

3.2.1.1 Direct visual assessment

The teeth were removed from the distilled water, dried with compressed air for 15 seconds and then examined. The length and width of the demineralisation was estimated with a pair of Vernier callipers (Neill Tools Ltd, Napier Street, Sheffield, S11 8HB.) to the nearest 0.1mm. The measurements were repeated after one week.

3.2.1.2 Direct Assessment of the Teeth Microscopically

To ensure accuracy of positioning, the teeth were placed in an acrylic block with the buccal surface uppermost. The teeth were examined at 10 times magnification. A graticule, with a square grid array etched on the surface

(Graticules Ltd, Morley Rd, Tonbridge, Kent, UK.) was placed within the microscope eyepiece. The grid consisted of an array of 11 x 11 dots (Figure 3.2, page 3.22). Each dot was designated a co-ordinate consisting of a letter (A-K) along the y-axis and a number (1-11) along the x-axis. The tooth, which was examined at approximately ten times magnification, was orientated so that the largest dimension filled the grid, either in the x or the y plane. If the widest part was inciso-gingival, the grid was placed on the mesial aspect of the tooth (Figure 3.3, page 3.23). If the widest part of the tooth was mesio-distal, the bottom of the grid was placed on the cemento-enamel junction (Figure 3.4, page 3.24). Therefore, not all the dots fell on the buccal surface of the tooth. Each dot was recorded as positioned on the buccal surface of the tooth or not. The nature of the tooth surface at the site of each dot which was located on the buccal surface was defined and categorised using a modification of the Caries Index of Fehr (Fehr, 1961):

0 - no lesion.

1 - diffuse grey or white opacity or lesion.

2 - white spot with diffuse grey or white surrounding.

3 - pronounced white spot lesion.

Recordings were repeated after one week.

3.2.1.3 Indirect Assessment of Photographs of the Teeth

The slides were projected, in random order, onto a square grid with the 121 dot array. The projected tooth was magnified approximately forty times and

orientated in exactly the same manner as for the microscope readings. If a point fell on a reflection from the flash the letter 'R' was recorded. To prevent assessor fatigue no more than ten slides were scored without a break. The assessments were repeated in a different random order after one week.

Two sets of readings were compared:

1. The first and second readings of the same photographic slide to assess reproducibility of the method of recording from the one slide.
2. The recordings carried out in the same session for the first slide and second slide of the same tooth, to assess the reproducibility of the photographic method.

This method of recording of dots allowed proportions to be calculated, thus:

Area of the buccal surface of the tooth

$$= \frac{\text{Total number of dots given a grade}}{\text{Total number of dots (121)}}$$

Area of buccal surface in grade 0:

$$= \frac{\text{Number of dots given grade 0}}{\text{Total number of dots given a grade}}$$

Proportions are more useful in a clinical study as it is very difficult to measure absolute areas of teeth. However, for the purposes of comparison of techniques in this study an estimate of the actual areas involved was carried out. A stainless steel ruler was placed under the microscope in the same plane as the buccal surface of the tooth. The vertical plane of the grid was

measured to the nearest 0.25mm. This reading was squared to give the surface area of the grid. The proportion of the 121 dots scored on the buccal surface of the tooth was multiplied by the surface area of the grid to provide an estimate of the area of the buccal surface of the tooth. The proportions of dots on the buccal surface placed in each grade were then calculated and these multiplied by the area of the buccal surface to obtain estimates for the areas for each grade. This was undertaken for both microscope and photographic recordings.

3.2.2 Statistics

The following statistics were applied:

3.2.2.1 The kappa statistic

A weighted and unweighted kappa were used to assess agreement between the first and second readings and between the first and second slides. The readings for the premolar teeth were pooled and the readings for the molar teeth were pooled. An overall figure for the pooled results from both sets of teeth was also calculated.

The unweighted kappa was carried out to assess the positioning of the grid. The individual dots on the grid each had a unique co-ordinate. The recordings for the first session were examined and for each point it was noted whether the dot was given a grade or not (i.e. it was assessed as being on the buccal surface of the tooth or not). This was then compared with the

score recorded for the same slide on the second session. Agreement between the two sessions was scored as either reading/reading or blank/blank. Disagreement occurred when on one session, a grade was given and on the next session no grade was given and this was scored as either reading/blank or blank/reading. These frequencies were placed in a two-by-two contingency table and the unweighted kappa statistic was calculated. The same procedure was carried out for the scores recorded on a single session of the two slides of the same tooth taken a week apart.

The weighted kappa was carried out to assess reading reproducibility. The grade given to each point scored on the buccal surface of the tooth on the first recording session was compared with the grade given on the second session. Only those points given grades on both sessions were included and any points scored as a reflection were excluded. The frequencies were placed in a four-by-four contingency table and the weighted kappa statistic calculated. The weighting of Cicchetti (1976) was used. This was also carried out for the repeat slides. Agreement levels were based upon those of Landis and Koch (1977).

3.2.2.2 The Limits of Agreement

To compare the three methods of measuring the area of demineralisation the technique of Bland and Altman (1986) was employed. The mean of the first and second readings for each technique was calculated. For the photographic and microscopic techniques the scores one and above were compared by adding together the results for grade one, two and three.

Bland and Altman (1986) point out that using the mean of repeat readings to compare two techniques will lead to too small an estimate of the standard deviation of the differences unless corrected. This was carried out using the formula $\sqrt{(SD^2 + 1/4S_1^2 + 1/4S_2^2)}$, where SD is the standard deviation of the differences between the means for each method and S_1 and S_2 are the standard deviations of differences between repeat measurement for each method separately. The reading for each tooth using the photographic technique was compared with the reading from the other two techniques. The means and differences between two techniques (photograph and microscope, photograph and Vernier callipers) mean readings were calculated.

3.2.2.3 The Coefficient of Repeatability

Repeatability was examined as described by Bland and Altman (1996). They state that it is expected that 95 percent of the differences between two readings will be less than two standard deviations from the mean difference and that this is the definition of the repeatability coefficient. If it is assumed that the mean difference was zero, this coefficient can be estimated using the following method. The difference between the repeat readings was calculated. The values were squared, added up and divided by n. The square root of this value was found. This was multiplied by two to obtain the coefficient of repeatability.

3.3 Results

3.3.1 Agreement

Table 3.1 (page 3.19) shows the pooled kappa results for the first and second readings of the same slide. The results indicate excellent agreement for grid positioning (kappa>0.81) and substantial agreement for reading reproducibility (kappa 0.61- 0.80). The results for the premolar and molars separately, as well as the above and below occlusal level views demonstrated similar results.

The agreement between readings from the first and second slides are shown in Table 3.2 (page 3.19). Although the kappa statistics were generally lower than for the repeat readings of the same slide, there was still excellent reproducibility for the grid positioning (kappa>0.81) and substantial agreement for reading reproducibility (kappa 0.61 - 0.80). The exception was the molar slide taken from below the occlusal plane that showed moderate agreement (kappa>0.41). It was noted whilst scoring these slides that some views were taken at too steep an angle. The agreement for premolars was marginally better than the scores for molars.

3.3.2 Limits of agreement

Figure 3.5 and Figure 3.6 (pages 3.25 and 3.26) show graphically the limits of agreement for the photographic and microscopic measurements and the photographic and Vernier measurements.

On examining the results for the photographic and microscopic measurements, it was noted that for 18 of the 22 teeth, the mean reading from the photograph was higher than the reading from the microscope and in the other four teeth, the difference was very small. The mean difference between the photographic technique and microscopic technique was 4.3mm^2. Therefore, on average the reading from the photograph measured a greater area of demineralisation than that from the microscope. This accords with previous studies which have shown that higher scores for enamel opacities are scored from photographs than clinically (Ellwood, 1993; Levine *et al*, 1989).

Figure 3.5 (page 3.25) shows a graph of the mean reading derived from the photograph and the microscopic examination techniques plotted against the difference between the two readings. There is no obvious relationship between the difference and the mean. The Normality of the differences was investigated by plotting a histogram. The lower limit of agreement was -9.3mm^2, with a 95 percent confidence limit of -12.9 to -5.7mm^2 and the upper limit was 9.3mm^2 with a 95 percent confidence limit of 5.7 to 12.9mm^2. Bland and Altman (1986) state that 95 percent of differences between the two techniques will lie between these limits. This shows considerable lack of

agreement between the two techniques as the average surface area of the molar was measured at 48.5mm^2 and for the premolars 32.4mm^2. The difference between the two methods is a quarter to a third of the area of the buccal surface of the tooth.

Figure 3.6 (page 3.26) shows a graph of the limits of agreement for the photograph and Vernier readings. The mean difference between these two techniques was less at 1.4mm^2. The photographic technique again tended to score higher, but this was a less consistent feature than with the microscopic technique. The limits of agreement were wide at -9.6mm^2 (95% confidence limits -13.8 to -5.4mm^2) and 12.4mm^2 (95% confidence limits 8.2 to 16.6mm^2).

3.3.3 The coefficient of repeatability

Bland and Altman (1986) state that the repeatability of each method is important, because poor repeatability of one or both methods will affect the limits of agreement. The coefficient of repeatability was calculated for the three techniques and is shown in Table 3.3 (page 3.20). The coefficient for the repeated readings was better for the photographic technique than either of the other two techniques.

The limits of agreement for the repeat readings from the same slide and readings from the two slides taken a week apart are shown graphically in Figure 3.7 and Figure 3.8 (pages 3.27 and 3.28). The limits for the repeated

readings of the same slide were -6.0mm^2 (95% confidence limits -7.9 to 4.1mm^2) and 4.0mm^2 (95% confidence limits 2.1 to 5.9mm^2). The limits for the readings taken on the repeated slide were -5.2mm^2 (95% confidence limits -7.2 to -3.2mm^2) and 5.4mm^2 (95% confidence limits 3.4 to 7.4mm^2). The coefficients of repeatability were very similar between the repeat readings of the same slide and the readings of the repeated slides (Table 3.3, page 3.20). This suggests that the photographic technique was reproducible and much of the error is measurement error.

3.4 Discussion

Photographs are a quick, convenient and effective means of recording the condition of teeth at the start and end of treatment. They have been employed by a number of studies both of enamel demineralisation and opacities. Unfortunately, descriptions of the error assessments carried out in many studies are sparse and comparisons of validity and reproducibility of these indices cannot be made. Some studies are flawed because they used photographs and clinical assessments without calibration of examiners or descriptions of the method errors (Gorelick *et al*, 1982). Many studies have used photographs, but have not discussed whether their technique was validated first (Houwink and Wagg, 1979; Dooland and Wylie, 1989; Ishi and Suckling, 1991).

Ellwood (1993) carried out one of the few studies into the reproducibility of the photographic technique. He investigated the reproducibility of a

photographic technique used to compare the prevalence and severity of enamel opacities over a period of time. He found that colour photography is an extremely powerful method of recording the presence of enamel opacities. The method he used employed the technique of taking two photographs at different angles, one above and one below the occlusal plane, to reduce the amount of reflection from the flash on the image. The reproducibility of the photographic technique was investigated by photographing 50 subjects on two different occasions.

The results of Ellwood's study showed that, using the developmental defects of enamel index to assess enamel opacities, the kappa adjusted percentage, for all the cases, was similar to the present study at more than 70 percent. He found, however, that the kappa adjusted percentage agreement for comparison of individual defects was poor at 47 percent. He concludes that the photographic method was relatively robust in assessing the population prevalence of defects cross-sectionally, but when assessing individual lesions longitudinally, with time, the agreement was poor. This has implications for orthodontic research. It has already been stated that clinical trials should ideally be longitudinal, recording the differences in an individual at the end of treatment compared with the start. Many studies on orthodontic demineralisation have been cross-sectional (Gorelick *et al*, 1982; Mizrahi, 1982, 83; Artun and Brobakken, 1986; Øgaard, 1989b). Cross-sectional studies lack the power of a longitudinal study and therefore more participants need to be recruited.

This study shows that the photographic technique employed was reproducible, as the coefficient of repeatability was the same for the repeat readings of the same slide as for the readings of two slides taken a week apart. It has also shown that measuring demineralisation from photographs is more reproducible than using the same technique with the naked eye or estimating the area of demineralisation using Vernier callipers.

The present study would suggest that there are some potential sources of error in trials investigating enamel defects, particularly when studying individual lesions longitudinally. Measurement from photographs, rather than directly from the patient, may reduce the difficulties for the reasons outlined earlier, but there are still problems that include production of the image, camera angle and subjectivity of the index.

3.4.1 Production of the image

This involves a number of steps, each of which may introduce an element of variation, particularly in a lengthy longitudinal investigation. Alterations in the quality of film, the lighting, development and ageing of the film once processed may lead to misleading changes in the image that could be misinterpreted as optical changes in the enamel surface. With care, it should be possible to minimise the variation due to the production of the image.

3.4.2 Camera angle

Ellwood (1993) speculated that the poorer reproducibility for the repeated slides might be due to variation in the angle of view, when photographing the lesion. It is necessary to vary the camera angle from the horizontal to avoid flash reflections on the buccal surface of the tooth. The size of the angle will change the perspective of the tooth and may affect the area scored as demineralised. The optimum angle required and a technique to reproduce this accurately needs further study. The present study found the same coefficient of repeatability with the repeat readings of the same slide as with the readings of two slides taken a week apart, therefore the variation due to camera technique is probably small.

The agreement for premolars was marginally better than the scores for molars. This is probably due to the shape of the premolar tooth that is square, compared with the rectangular shape of the molar. The grid used was square and adapted better to the shape of the premolar. Generally, the photographs taken below the occlusal plane scored better than those taken above and this may be due to the masking that was placed on the lower half of the flash.

3.4.3 Subjectivity of the four-point index

The results of this study suggest that the subjectivity of the four-point index is a major potential cause of variability. A number of indices have been developed to study the frequency of white spot lesions developing during

orthodontic treatment and a detailed description has been provided in Chapter Two. Many use a four-point scale to measure the presence or absence, as well as the severity of white spots (Gorelick *et al*, 1982; Mizrahi, 1982,83). The Caries Index (CI) described by Fehr (1961) was developed to assess demineralisation. Edgar *et al* (1978) showed that colour photography, used with controlled lighting and camera position, compared favourably with direct visual assessment in recording changes in the optical properties of the enamel using the Caries Index. They concluded that colour was an essential component in the assessment and that black and white photographs were neither valid nor reliable.

Some authors have used indices developed to measure the incidence of enamel opacities to record demineralisation (Millett *et al*, 1999). These are often descriptive and not applicable to measuring demineralisation (Clarkson and O'Mullane, 1989). They frequently do not attempt to measure areas. Crude quantitative assessments have been carried out by dividing the buccal surface into thirds and grading the size of the white spot lesion (Gorelick *et al*, 1982; Mizrahi, 1982,83). Edgar *et al* (1978) attempted to assess the changes in areas of the tooth by recording the Caries Index for individual areas mapped on standard charts, however few details of how this method was applied are given.

Mitchell (1992) used standardised, black and white photographs of patients before and after treatment. The calculation was carried out by tracing around demineralised areas on the labial surface, three times with a computerised

digitiser. The proportion of the labial surface affected was calculated and the values averaged. Although the reproducibility of this technique was satisfactory, it is a time consuming procedure.

The positioning of the morphometric array was highly reproducible as shown by the unweighted kappa results, however the readings themselves show some variation, demonstrating the fickleness of the examiner with a subjective index. The coefficient of repeatability was $5mm^2$. This means that 95 percent of the second readings were within plus or minus $5mm^2$ of the first reading. When it is considered that some indices measure areas in terms of thirds of the tooth surface, this is respectable for a cross-sectional study. Longitudinal studies of individual lesions will require a more objective method of assessment.

3.5 Conclusions

1. The photographic technique used was a reproducible method of measuring artificial enamel demineralisation.
2. Measurement from photographs was more reproducible than direct measurement with the naked eye.
3. Most of the variation in measurement was due to the subjectivity of the index and further investigations into ways of relating the mineral content with the optical properties of enamel need to be undertaken to provide more objective means of assessment.

3.6 Tables

Table 3.1

Table showing the results of the unweighted and weighted kappa statistics for the first and second readings of the same slide when the camera was angled above and below the occlusal plane.

Repeatability test	Kappa	Camera angle	Kappa statistic	Agreement*
grid positioning	unweighted	above	0.87	excellent
grid positioning	unweighted	below	0.92	excellent
reading reproducibility	weighted	above	0.72	substantial
reading reproducibility	weighted	below	0.80	substantial

* according to Landis and Koch (1977)

Table 3.2

Table showing the results of the unweighted and weighted kappa statistics for the readings taken from the first and second slide of the same tooth when the camera was angled above and below the occlusal plane.

Repeatability test	Kappa	Camera angle	Kappa statistic	Agreement
grid positioning	unweighted	above	0.83	excellent
grid positioning	unweighted	below	0.85	excellent
reading reproducibility	weighted	above	0.64	substantial
reading reproducibility	weighted	below	0.63	substantial

* according to Landis and Koch (1977)

Table 3.3

Results of the coefficient of repeatability as calculated from Bland and Altman (1996).

Technique	Coefficient (mm^2)
Photographs 1st v 2nd reading	5.1
Photographs 1st v 2nd slides	5.0
Microscopy	6.8
Vernier callipers	7.8

3.7 Figures

Figure 3.1

Diagram showing the shape and position of the buccal windows with the time intervals (in days) of exposure to the demineralising gel.

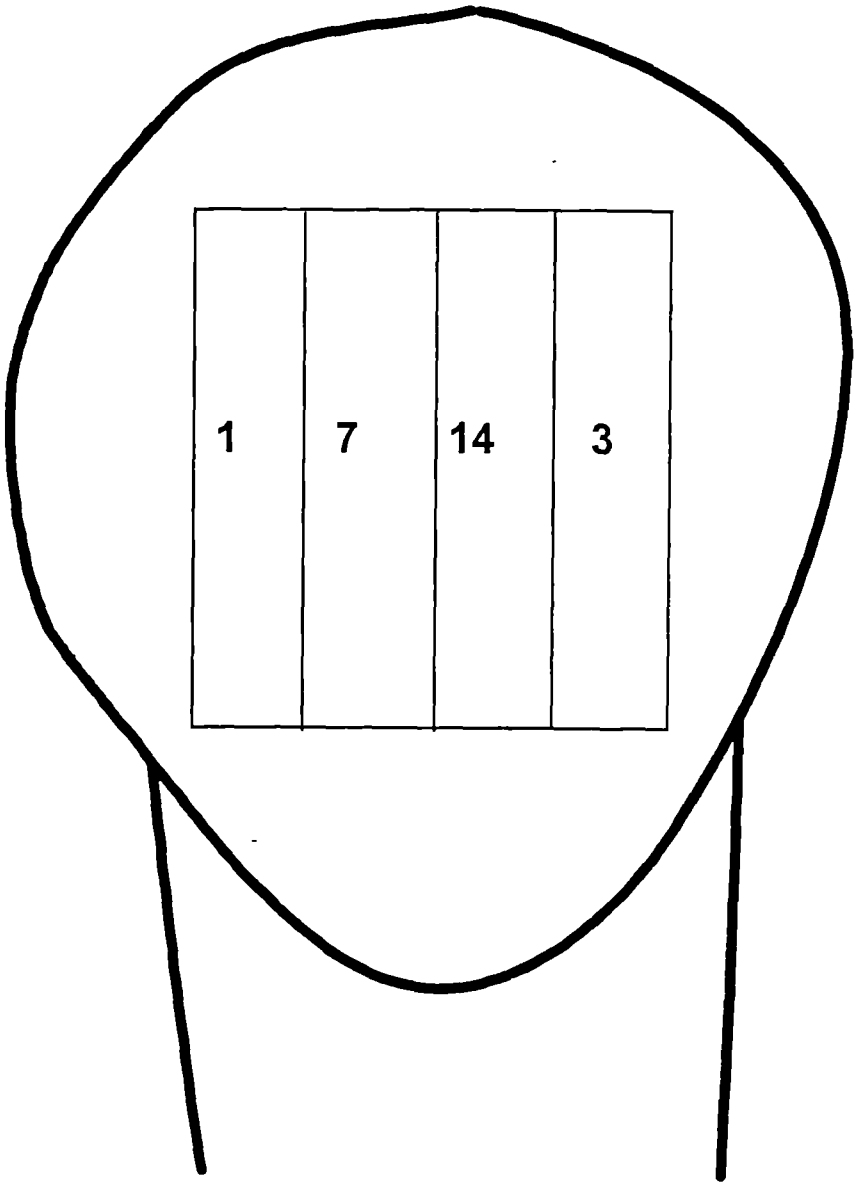


Figure 3.2

Diagram of 121 dot morphometric array.

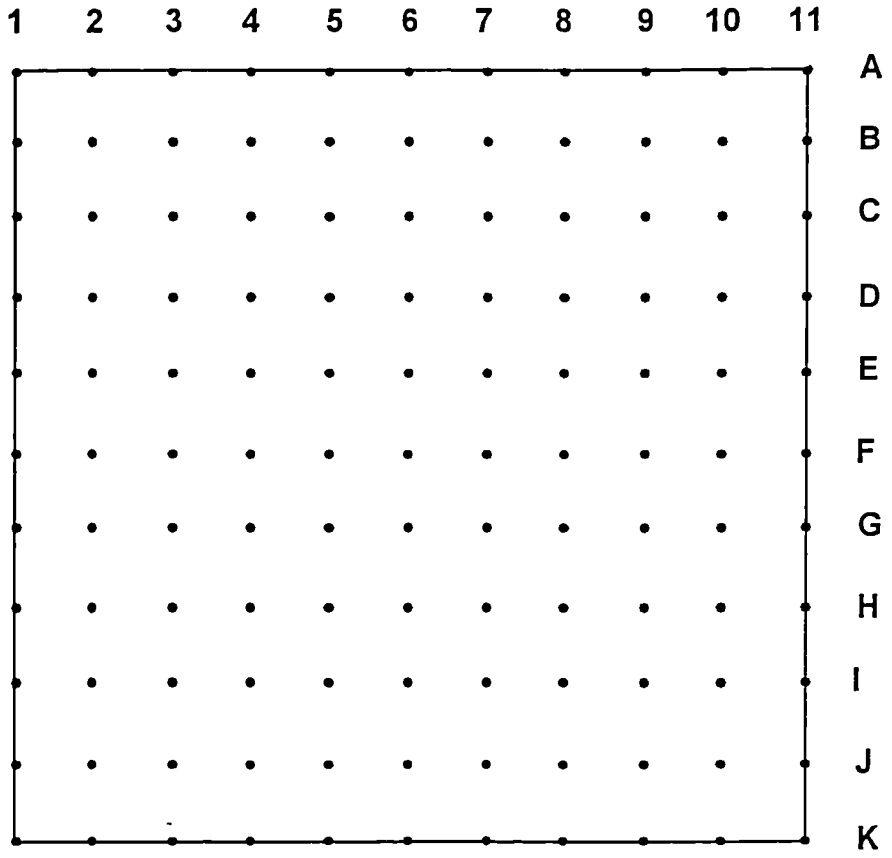


Figure 3.3

Diagram showing premolar aligned to morphometric array.

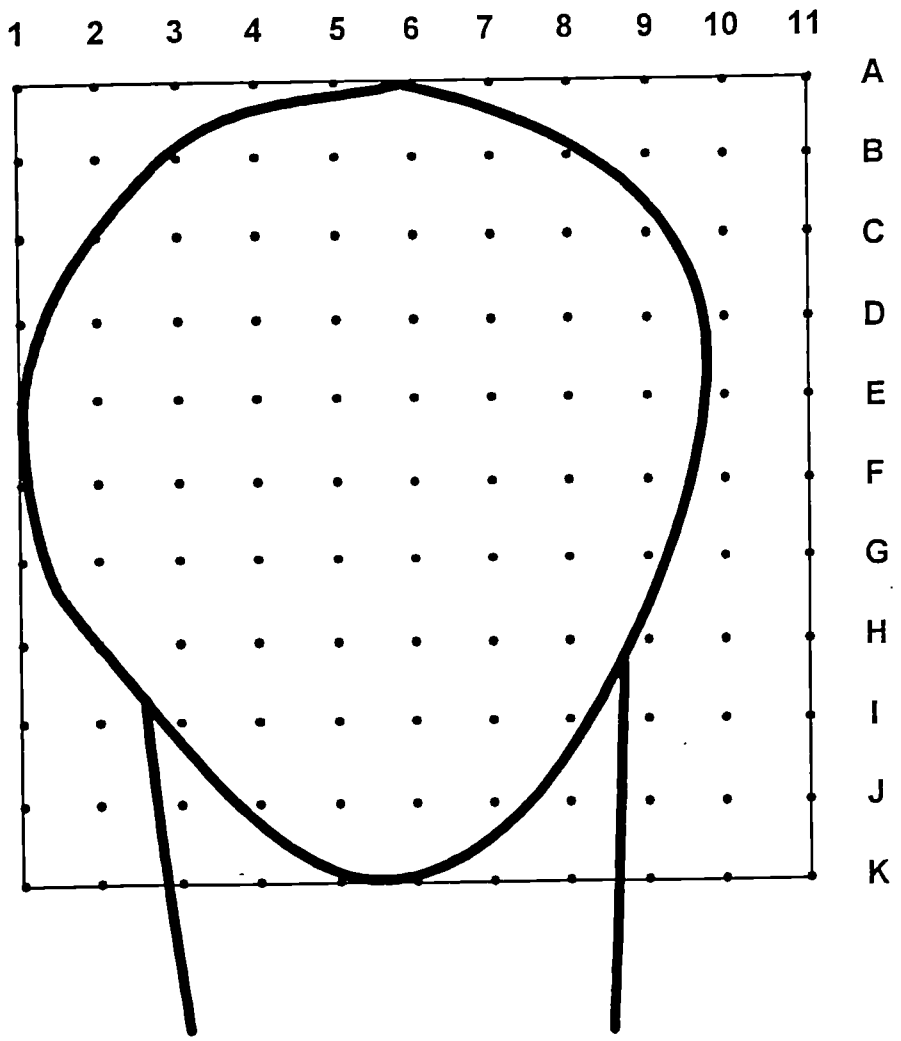


Figure 3.4
Diagram showing molar aligned to morphometric array.

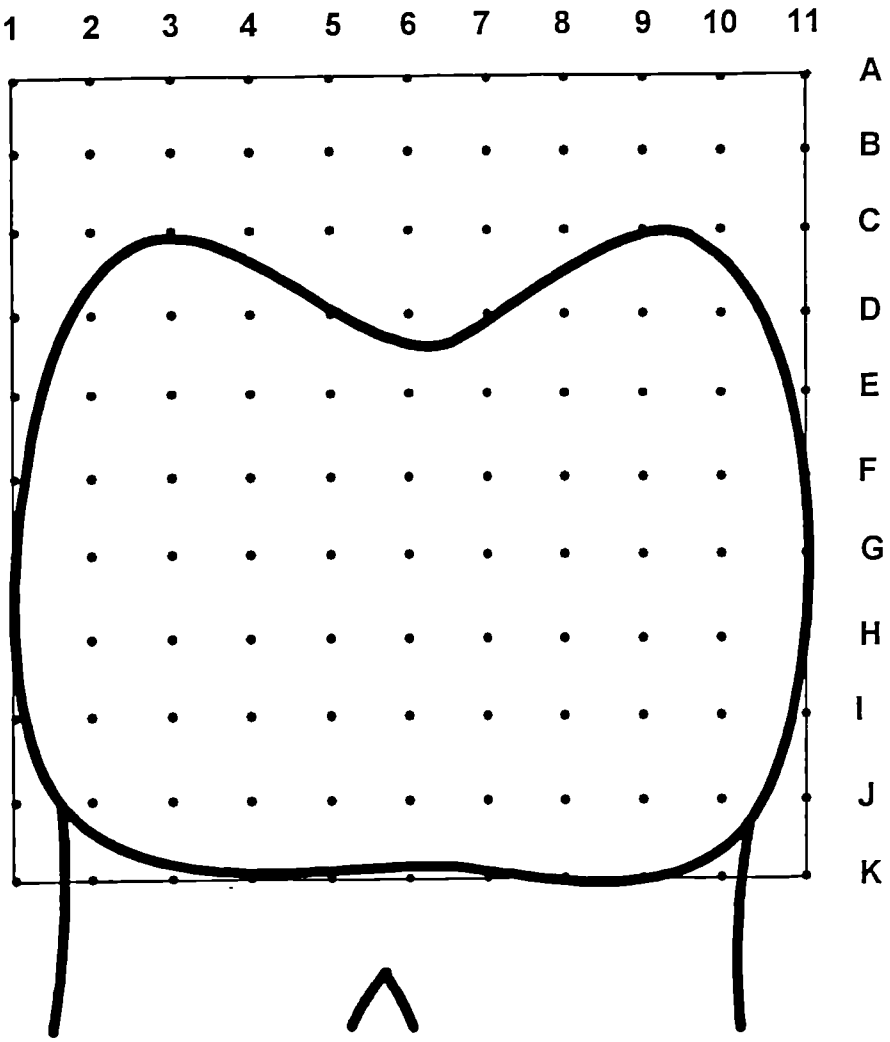


Figure 3.5

Graph showing the mean differences and limits of agreement for the measurements from the photographs and the microscope.

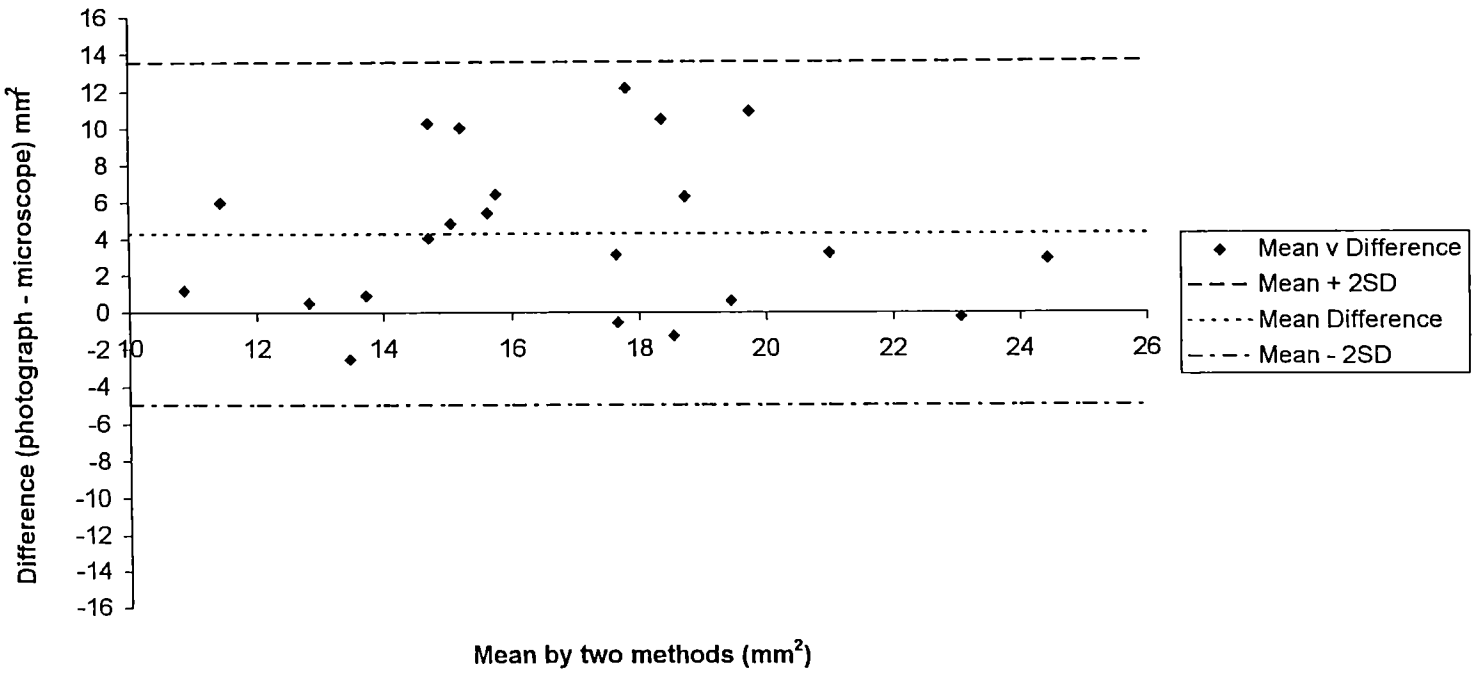


Figure 3.6

Graph showing the mean differences and limits of agreement for the measurements from the photographs and the callipers

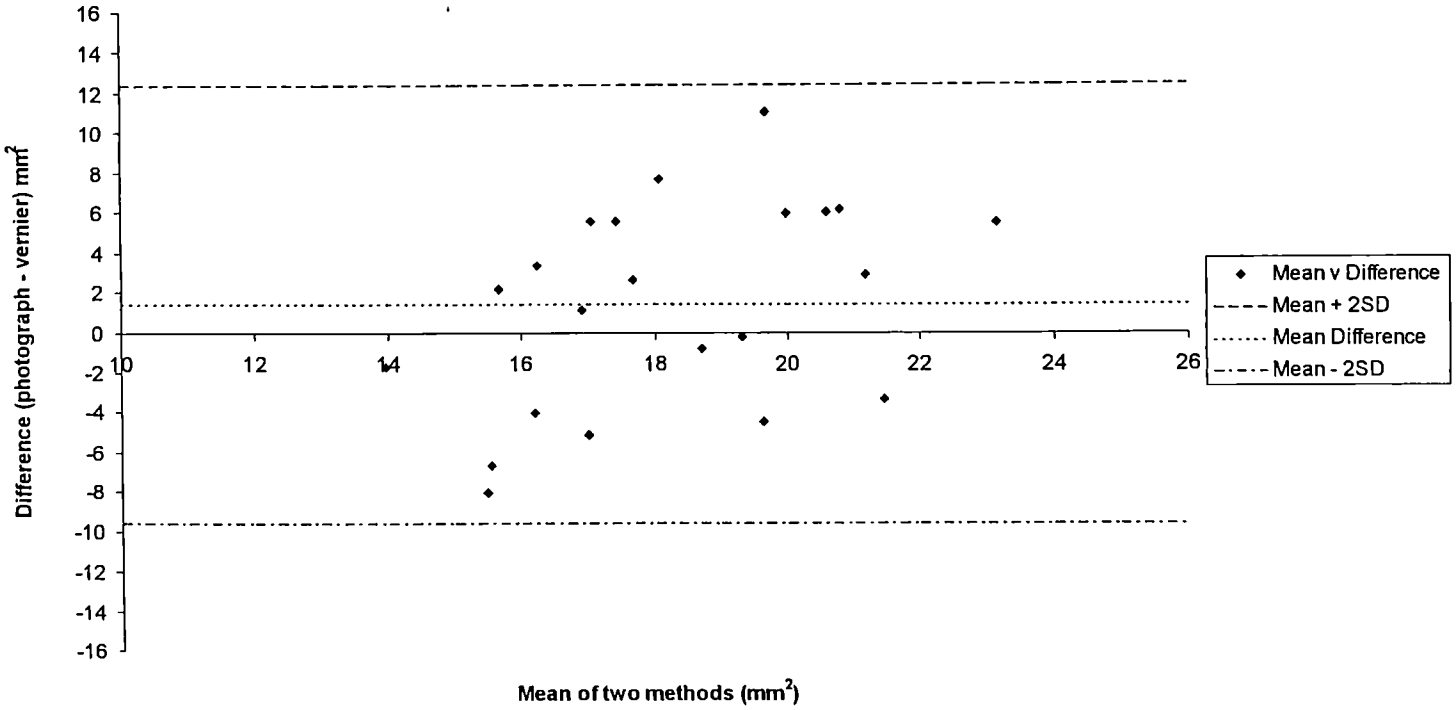


Figure 3.7

Graph showing the mean differences and limits of agreement for the measurements from the repeat readings of the same slide.

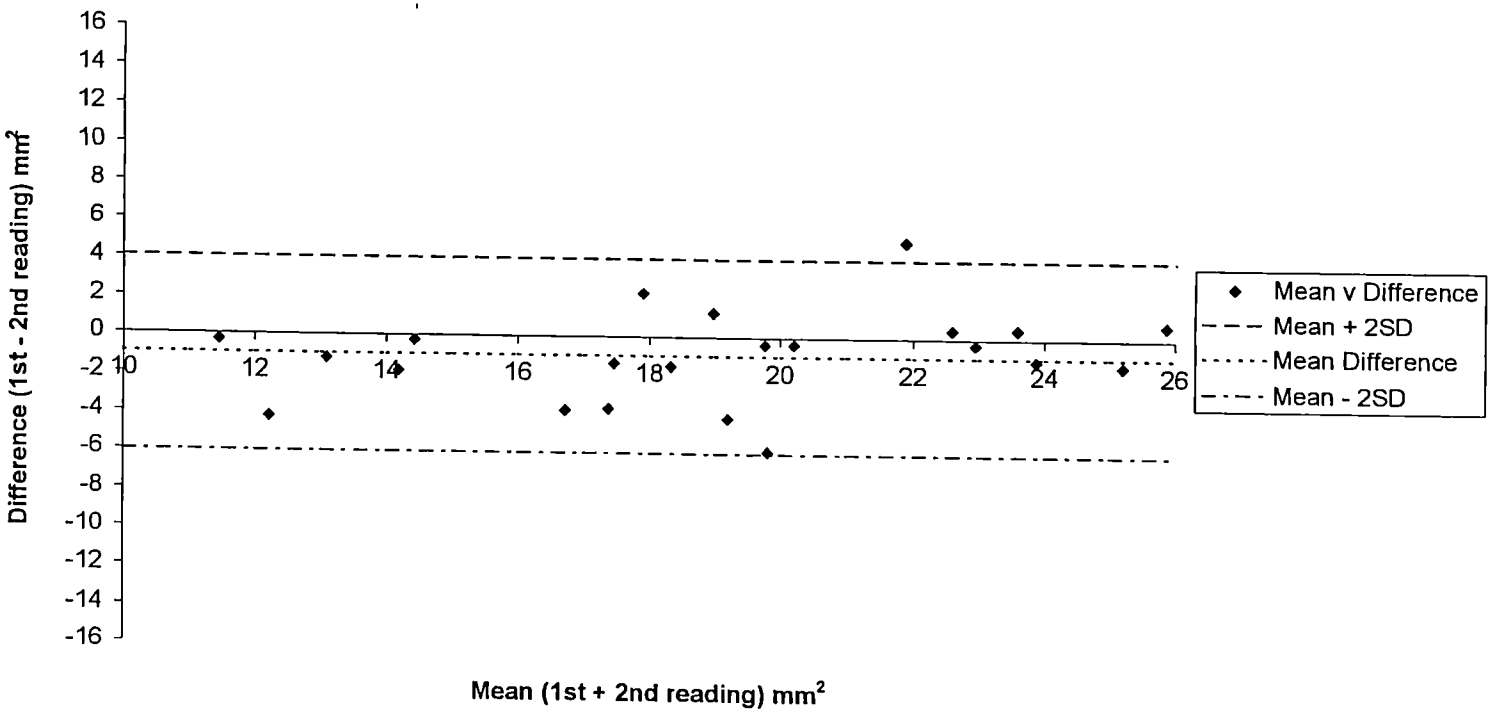
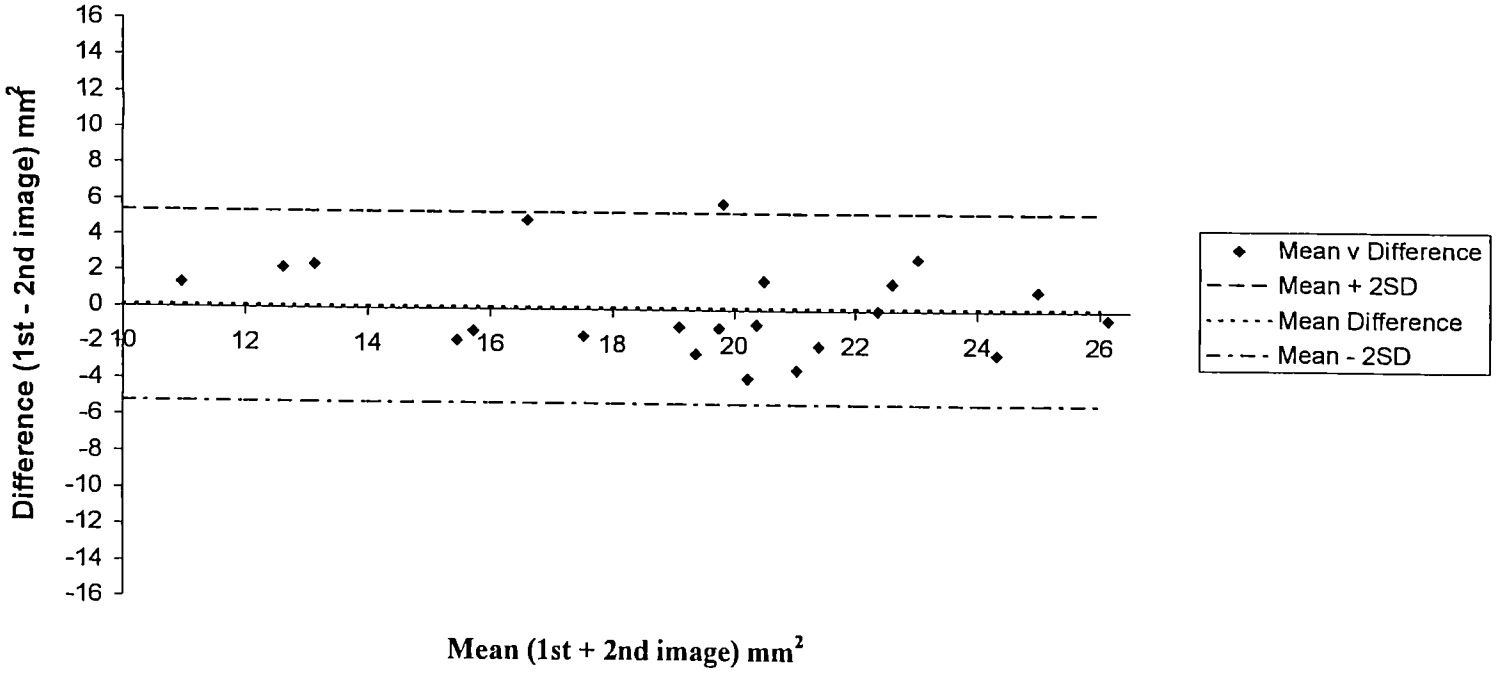


Figure 3.8

Graph showing the mean differences and limits of agreement for the measurements from the repeat slides of the same tooth.



Appendices

3.8.1 Appendix A

Preparation of the acidified gel system for demineralisation of human premolars

This was described by Edgar (1983). This consists of 0.1M lactic acid and 0.1M sodium hydroxide mixed in proportions to give a pH of 4.5 then add 6 percent (w/v) hydroxyethylcellulose.

0.1M Lactic Acid

The free lactic acid to be used is a liquid with a molecular weight of 90.1, specific gravity of 1.249 and contains 85 percent lactic acid by weight.

Approximate number of ml required in 1l to prepare 1M solution

$$= \frac{\text{m.wt}}{\text{sp.g}} \times \frac{100}{\%\text{ by wgt}}$$

$$= \frac{90.1}{85} \times 100$$

$$= 84.87\text{ml}$$

8.487ml lactic acid in 1l = 0.1M solution

8.487ml lactic acid = 4.244ml in 500ml = 0.1M

0.1M Sodium Hydroxide

Molecular weight = 40g

40g NaOH in 1l = 1M solution

0.4g NaOH in 100ml = 1M solution

NB approximately 500ml (2.0g) 0.1M NaOH is required to increase the pH of the lactic acid (which started around pH 2.5) to pH 4.5.

6% (w/v) hydroxyethylcellulose added to produce a gel.

6g HEC in 100ml = 6%w/v

6 x 5g 30g HEC in 500ml = 6%(w/v)

Add slowly with stirring and when it reaches a consistency of wallpaper paste pour into tubes.

CHAPTER 4

Enamel Demineralisation Assessed by Computerised Image Analysis of Clinical Photographs

4.1 Introduction and Aims

The results outlined in the previous chapter suggested that a number of factors could contribute to variation in measurement of enamel demineralisation from photographic images. One potential source of error was the subjective nature of the index used to record the demineralisation. Objectivity can be given by digital technology, which has been used in the diagnosis of approximal cavities (Schneiderman *et al*, 1997; Firestone, 1998) the monitoring of changes in periapical bone following endodontic treatment (Keruso and Orstavik, 1997) and in measuring tooth wear (Mayhall and Kageyama, 1997). It is relatively simple to convert a photographic image into a digital image, which can then be analysed using a computer. The computer programme is able to objectively distinguish many more shades than the human eye.

A second source of variation suggested was the angle at which the camera is placed relative to the buccal surface of the tooth. Alteration in the angle may change the perspective of the image, which will affect the size of the area of demineralisation measured by the altered perspective.

The aims of this study were:

1. To investigate the use of computerised image analysis to measure the area of demineralisation on the buccal surface of a tooth.
2. To analyse the effect on that measurement of varying the angle at which a photographic image of the buccal surface of a tooth is taken.

4.2 Materials and Methods

4.2.1 Tooth preparation

The preparation of the teeth has been described in detail previously in Chapter Three. In summary, 22 freshly extracted human teeth (11 molars and 11 premolars) were used. The crowns of the teeth were coated with an acid-resistant varnish, except for a rectangular area on the buccal surface. They were placed in a demineralising gel and the exposed area was incrementally occluded over a 14-day period. On the root of each tooth a small ½ round rose head bur was used to place two small holes (Figure 4.1, page 4.14). The horizontal distance between the two holes was measured twice, to the nearest 0.25mm, with a pair of Vernier callipers (Neill Tools Ltd, Napier Street, Sheffield, S11 8HB). The measurements were averaged to produce one reading for each tooth.

4.2.2 Image capture and analysis

The teeth were mounted in acrylic blocks with the exposed buccal surface uppermost. The buccal surface was aligned parallel with the flat surface of the bench on which the photographs were taken. Standardised photographs were taken of the teeth as previously described in Chapter Three. The camera was placed in a camera holder, which was capable of being rotated in the long axis of the tooth. Photographs were taken perpendicular to the buccal surface (0 degrees), at 20 and 40 degrees angulation, above (Cuspal) and below (Gingival) the 0-degree line (Figure 4.2, page 4.15). Masking was placed on the lower aspect of the ring flash to reduce the amount of reflection from the flash. The teeth were stored in distilled water with a few grains of thymol to prevent bacterial contamination, until they were photographed. They were then removed from the water, dried with compressed air for 15 seconds, photographed and replaced in the distilled water. Photographs were repeated on 5 molars and 5 premolars at least two weeks after the first images. One hundred and sixty photographic images were produced and analysed.

The photographic images were developed and mounted as 35mm transparencies. They were recoded by one investigator, to allow for a blind assessment by the principal investigator. The calibration measurement was recorded on the slide, as well as a number code. The slides were converted to high resolution (2720 dpi) grey scale images (8-bit range) using a slide scanner (Canoscan 2700F, Canon Inc., Tokyo 146, Japan) and computer

software (Scancraft FS version 3.1.1. Canon Inc., Tokyo 146, Japan). They were saved as Tagged Image File Format (TIFF).

The images were opened in an image analysis programme (Image-Pro Plus, version 3.0 for Windows 95, Media Cybernetics, Silver Spring, Maryland 20910, USA). Each image was individually calibrated in millimetres, using the distance between the horizontal marks on the root surface and the calibrating measurement recorded on the slide. An Area of Interest (AOI) was drawn to define the buccal surface of the tooth. Each pixel within the AOI records a numeric value corresponding to the brightness of that point on the original image (Russ, 1995). The brightness values for 8-bit range grey scale images fall between 0 (black) and 255 (white). To simplify the analysis the brightness values were categorised into nine ranges (Table 4.1, page 4.11). Figure 4.3 (page 4.16) shows an image of a tooth with the pixels coloured according to their brightness range value.

The brightness range values recorded by the image analysis programme were entered into a spreadsheet (Excel 97, Microsoft Corp., Redmond, WA. USA) and the area in mm^2 for each range were calculated.

A structured sample of 40 slides was randomly selected so that the distribution of angular views within this sub-sample was represented as in the main sample. These were recoded, as previously and blindly reanalysed for an error assessment.

4.2.3 Statistical Analysis

Full details of the statistical analysis are outlined in Appendix C (page 4.22). The intraclass correlation coefficient of reliability (Fleiss, 1986) was calculated for the forty repeated slides, to test the reliability of repeat readings of the same slide. One sample *t* tests were carried out to detect systematic error (Houston, 1983).

To assess agreement between the readings of two different slides of the same tooth, the limits of agreement were plotted according to the technique described by Bland and Altman (1986). The results were plotted for the readings taken from the 20-degree and 40-degree angles of the ten repeated slides (N=20) for the Cuspal and Gingival views.

The difference in the areas between the three different angular views taken of the tooth was assessed with an analysis of variance. The data from the slides of the twenty-two teeth and the repeated slides of ten teeth were analysed (N=32). The data were tested for Normality using Normal Q-Q plots and the Shapiro-Wilk test. The data were Normally distributed therefore parametric statistics were applied. A one-factor within subject analysis of variance was carried out using SPSS (SPSS for Windows V8, SPSS Inc., 444 Michigan Avenue, Chicago, IL. USA). Following this pairwise comparisons were carried out between the different angles using a paired *t* test with a Bonferroni correction to allow for a Type I error (false positive).

4.3 Results

The results of the reproducibility assessment are given in Table 4.2 (page 4.12). There was no evidence of a systematic error between the repeat readings of the same slide as shown by the non-significant one sample *t* test results. The intraclass correlation coefficients of reliability show that the variation of the repeat readings is low, compared with the variation within the sample. Figure 4.4 (page 4.17) shows the limits of agreement for the two slides taken of the same tooth for both the Cuspal and Gingival views. It can be seen that the limits are much narrower for the Gingival views indicating a better agreement between the two slides taken from the gingival.

Table 4.3 (page 4.13) shows the results of the one-factor within subjects analysis of variance comparing the total area of the tooth measured for the three angles, of the Cuspal and Gingival views. There were no significant differences between the areas measured for the Cuspal views ($P=0.587$), but there was a highly significant difference for the Gingival views ($P<0.001$).

Table 4.4 (page 4.13) shows the mean differences and 95 percent confidence intervals of the differences between the areas of the whole buccal surface measured for the three angles of the Gingival views. The results of the paired *t* test are also shown. This reveals a significant difference between the three angles ($P<0.001$). It can be seen that the mean differences between the three angles were not linear. The difference between the 0-degree view and the 20-degree view was $5mm^2$. The difference between the

view and the 40-degree view was 10mm^2. Therefore there was a greater reduction in the area measured when the camera was tipped to 40 degrees from 20 degrees, than when it was tipped to 20 degrees from 0 degrees.

Figure 4.5 (page 4.18) shows two graphs of the mean areas calculated for the nine grey scale categories for the three angles of the Cuspal and Gingival views. It can be seen that there is a progressive shift to the left of the graphs from the 0-degree to the 40-degree angle, suggesting that there is a reduced amount of light reflected back to the camera as the angle to the buccal surface increases. The graphs also show the reduced area measured by the 40-degree angle. The area under the graphs represents the total area measured for each angle. It can be seen, especially with the Gingival views that the area under the graph is reduced for the 40-degree views.

Figure 4.6 (page 4.19) shows graphs outlining the limits of agreement comparing the areas recorded from the first and second readings of the same slide for the calculated areas of the teeth using the computer and morphometric methods. It can be seen that the mean difference between the readings was closer to zero for the computer method than for the morphometric method, suggesting a reduced systematic bias for the former. The Cuspal views have wider limits than the Gingival views for both the computer and morphometric method. The limits are slightly narrower for the computer method.

4.4 Discussion

This study was designed to address two out of the three potential sources of error highlighted in the previous chapter. These were:

1. Alteration in the angle of the camera to the buccal surface of the tooth.
2. The subjectiveness of the indices used to measure demineralisation.

The results of this study suggest that measuring demineralisation by converting 35mm slides to digital TIFF images and using computerised image analysis is a reproducible technique. Repeat measurements of the same 35mm slide showed very good reproducibility. The limits of agreement for the readings of two different slides of the same tooth showed a low mean difference between readings and acceptable limits. However, there were still differences in agreement between two slides of the same tooth and it must be concluded that there are still variations in the processing of the image that can lead to differences in measurement. To overcome the variations in lighting and processing each photographic image could have a calibrating grey scale associated with it. This could be used to manipulate the image digitally so that the errors in the production process can be removed.

Measurement in the present study required the calibration of individual images, as well as the drawing by hand, using the computer mouse, of an Area of Interest around the buccal surface of the tooth. Both these procedures may introduce random error.

The effect of light from the flash reflected back onto the image is important for two reasons. Firstly it may hide an area of demineralisation and secondly it could lead to an over estimate of the area of demineralisation. To reduce the amount of reflected light several authors have suggested tilting the camera (Ellwood, 1993; Fleming *et al*, 1989) and masking the flash.

This study has shown that the position of the masking on a ring flash is an important consideration when taking a photograph of a tooth. Figure 4.4 (page 4.17) shows that the agreement between measurements of two different slides of the same tooth was better for the Gingival views. These views had the lower part of the ring flash, or the area that was closest to the tooth, masked to reduce reflections. This may have been significant in reducing the amount of light from the flash that was reflected from the image. When the masking was placed on the area of the flash furthest from the tooth, as was the case with the Cuspal views, the agreement was poorer. Figure 4.5 (page 4.18) shows that the shapes of the graphs for the Cuspal and Gingival views are different. The graphs for the 20 and 40-degree Cuspal views are similar to the 0-degree graph, which appeared to have more reflected light. They are steeper and the peak is higher than the Gingival view, which shows a broader and flatter shape. This suggests that the masking was in a better position to reduce the reflection from the flash, for the Gingival views.

The investigation into the effect of changing the angle of the camera has shown that changing the angle from perpendicular to the buccal surface (0-

degrees) to 20 degrees will have a slight reduction on the area measured. In addition there is more reflected light from the 0-degree angle which made assessment of demineralisation more difficult. A much larger change occurred when changing from a 20-degree to a 40-degree angle. It would appear that a camera angle of 20 degrees to the perpendicular relative to the buccal surface of the tooth is sufficient to reduce reflection and maintain an adequate perspective of the tooth.

4.5 Conclusions

1. Computerised image analysis on captured 35mm slides is a reproducible technique.
2. The angle of the camera and the positioning of the masking on the ring flash makes a difference to the area measured and the grey scales recorded.
3. Incorporation of a calibrating grey scale with each image may improve the standardisation of photographs for longitudinal studies.

4.6 Tables

Table 4.1

The brightness values for each pixel on the buccal surface of the tooth were divided into nine categories according to the ranges defined in Table 1, where 0 is black and 255 is white.

Category	Range (brightness value)
1	0 – 30
2	31 – 60
3	61 – 90
4	91 – 120
5	121 – 150
6	151 – 180
7	181 – 210
8	211 – 240
9	241 - 255

Table 4.2

Results of reproducibility assessment of the repeat readings from 40 slides.

	Mean Difference (mm^2)	Confidence Intervals (mm^2)	Intraclass Correlation Coefficient of Reliability	One sample *t* test	
				t	p
Cuspal	0.11	-1.23 – 1.46	0.99	0.17	0.864
Gingival	-0.20	-1.16 – 0.77	0.98	-0.44	0.668
All	-0.02	-0.86 – 0.82	0.99	-0.05	0.963

Table 4.3

Results of the one-factor within subjects analysis of variance comparing the total area of the tooth measured from the three angles (0°, 20° and 40°), for the Cuspal and Gingival views (N=32).

	F	P
Cuspal	0.30	0.587
Gingival	101.45	<0.001*

P<0.01

Table 4.4

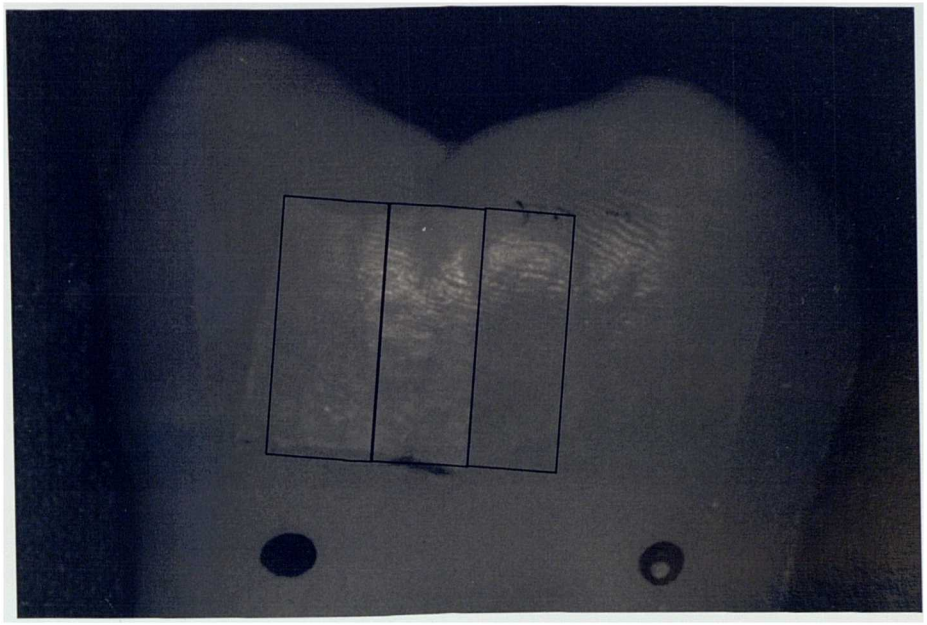
Results of the paired *t* test for pairwise comparison of the three angles for the Gingival slides (N=32).

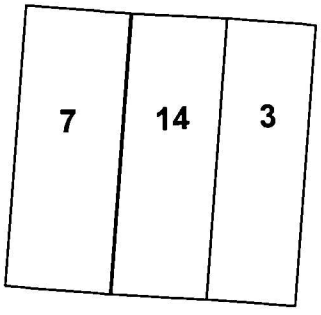
Pairwise comparison	Mean Difference (mm^2)	Confidence Intervals	*t*	P
0° – 20°	5.2	3.4 – 6.9	6.10	<0.001*
20° – 40°	10.5	8.7 – 12.3	11.93	<0.001*
0° – 40°	15.6	12.5 – 18.8	10.07	<0.001*

4.7 Figures

Figure 4.1

Grey scale image of a tooth showing etched areas outlined and the calibrating markers.





Etch areas in days

Figure 4.2

Diagram showing the standardisation of the photographic technique.

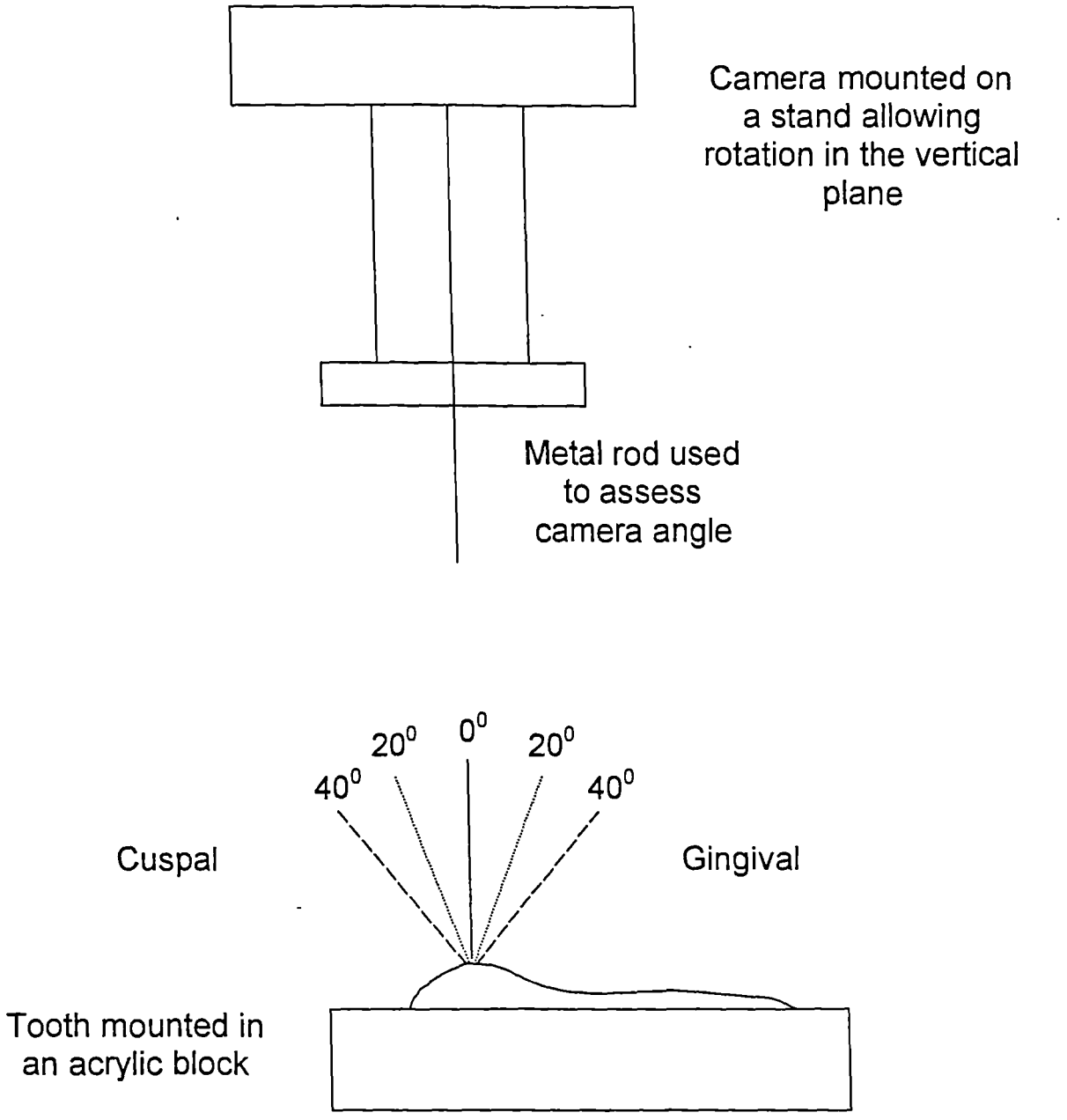
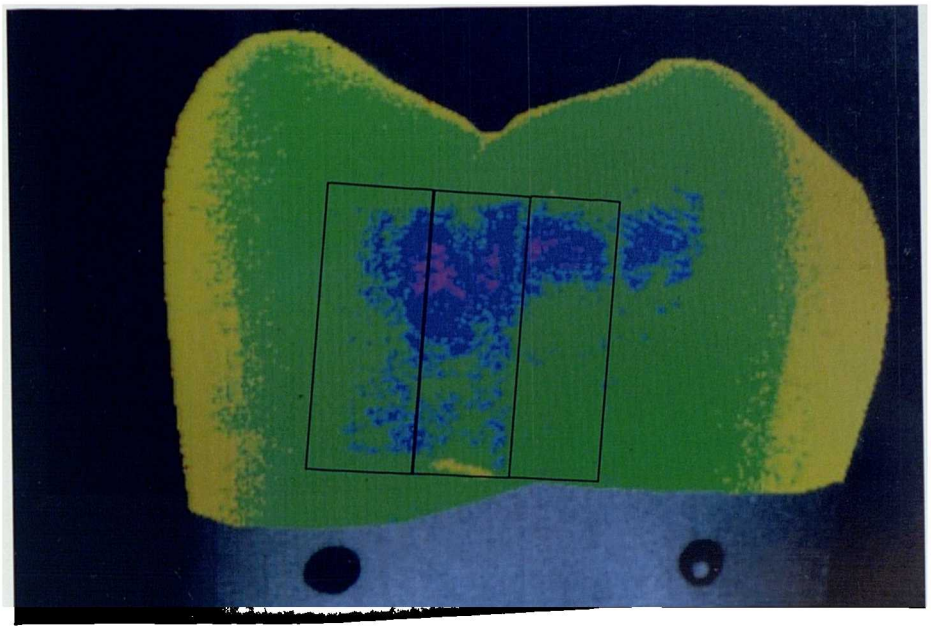
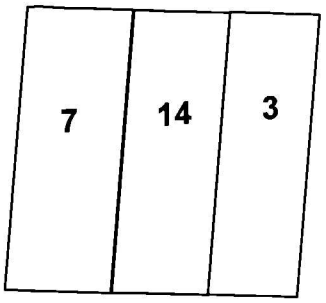


Figure 4.2

Grey scale image with superimposed colours of the image analysis programme showing the intensity ranges in different colours and etch areas (figures in days of exposure) highlighted.



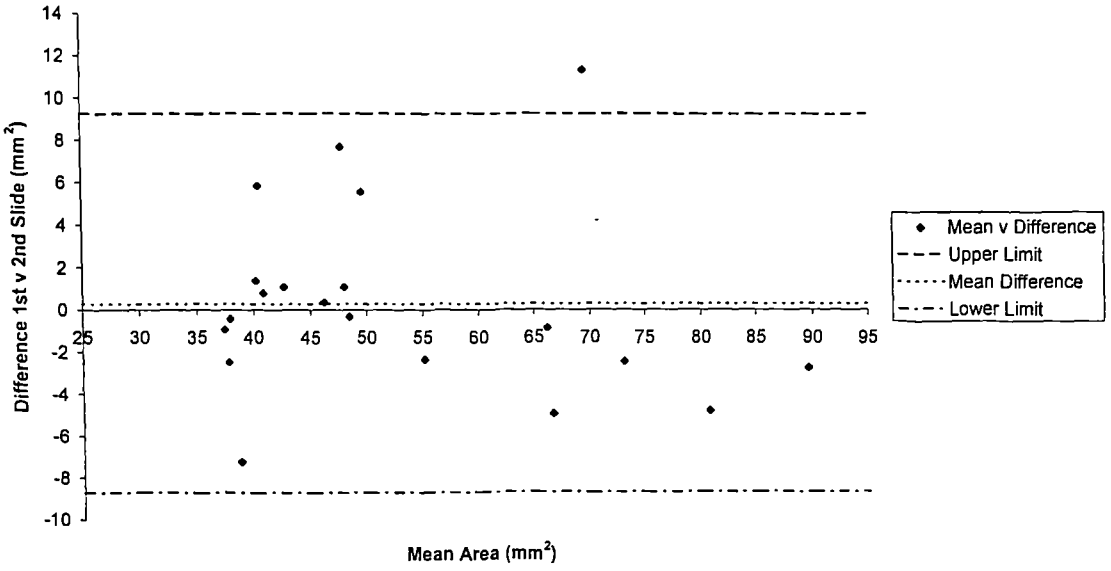


Etch areas in days

Figure 4.4

Limits of agreement for the readings taken from the first and second slides of the same tooth for the 20-degree and 40-degree slides (N=20), Cuspal (a) and Gingival views (b).

a) Cuspal Views



b) Gingival Views

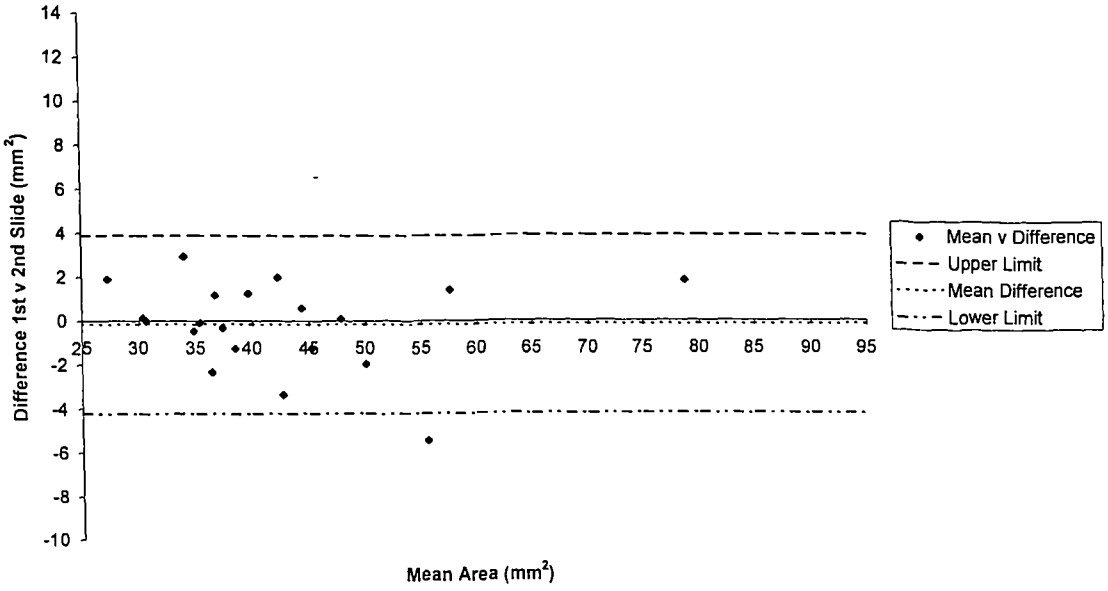


Figure 4.5

Graphs showing the mean areas calculated for each grey scale region for the three angles of slide, Cuspal (a) and Gingival views (b).

Figure 4a - Cuspal Views

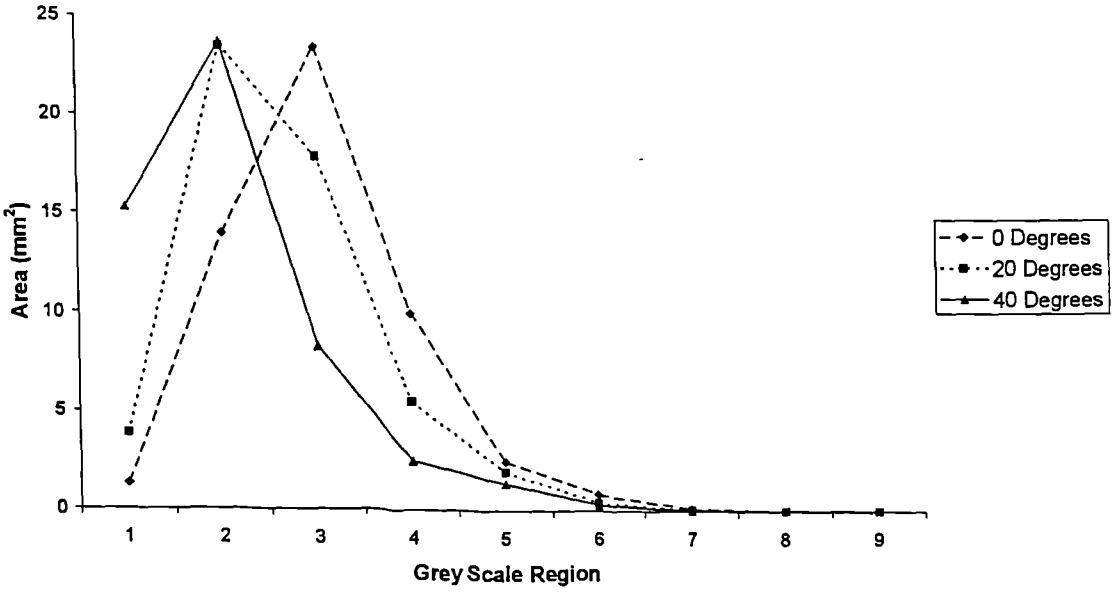


Figure 4b - Gingival Views

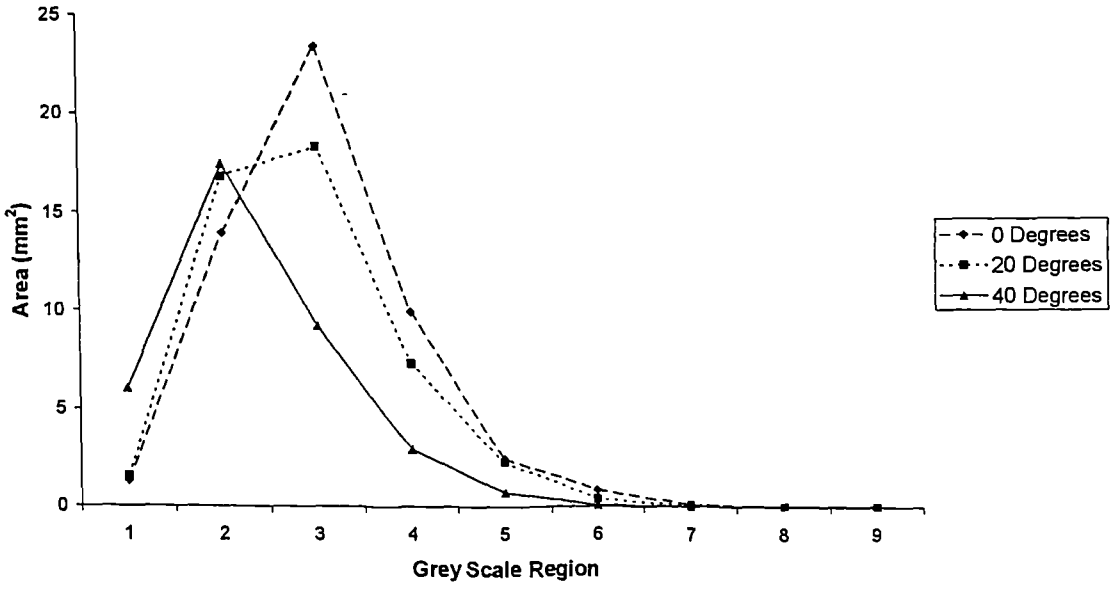
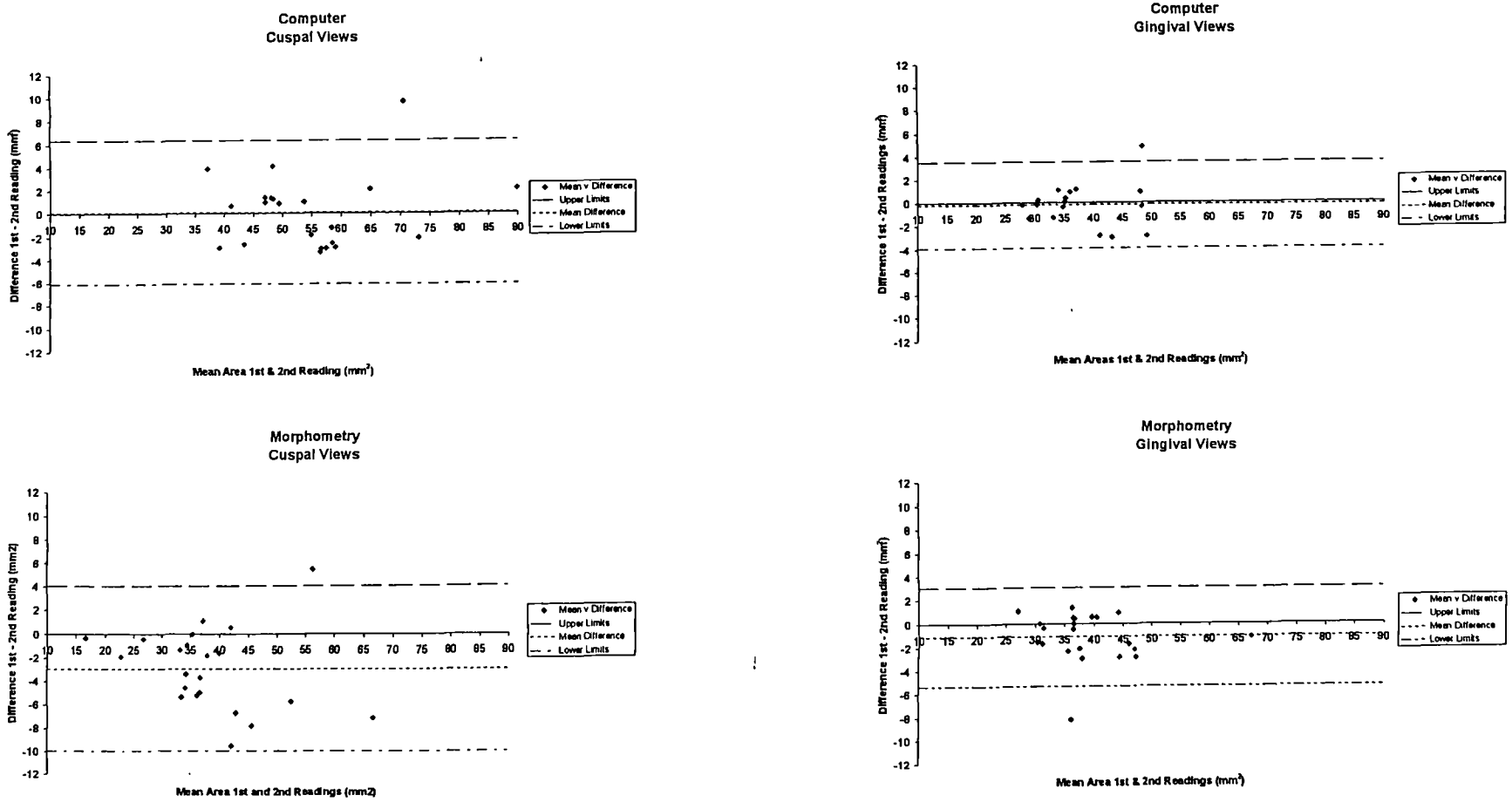


Figure 4.6

Limits of agreement comparing the calculated areas of the teeth using the computer and morphometric methods from the 1st and 2nd readings of the same slide.



4.8 Appendices

4.8.1 Appendix B

Technique for measuring grey scale values using Image-Pro Plus.

1. Open image
2. Measure; Calibration; Spatial. Check the units are mms, then click Image and set calibration bars to horizontal calibration marks. Check the Aspect Ratio is 1 then calibrate using the horizontal reading recorded on the slide.
3. Draw an Area of Interest (AOI) around the outline of the buccal surface.
4. To measure the grey scale levels click Measure; Count/Size. Click on Manual, then Select Ranges and choose PB Range file with the ranges described in Table 4.1 (page 4.11)
5. Make sure the Measure Objects box is ticked and the Apply Ranges and Add Count boxes are not ticked.
6. Click Measure; Select Measurements and make sure 'area' and 'class' are selected.
7. Click Options and make sure Outline style is 'Class-Filled' and Label Style is 'None'.
8. Click Count.
9. Click View and then Range Statistics and Measurement Statistics saving them to separate files.
10. Open the Range Statistics file in Excel and copy this to the Angled spreadsheet.

11. Open the Measurement Statistics file in Excel and calculate the area measurements in SPSS (v8) as follows. Copy and paste the values from Excel into SPSS. Click Statistics; Compare Means; Means. Place Var 0001 in the dependent list and Var 0002 in the independent list. In the options box make sure Sum is transferred. Click Okay. Export the output file. Open the output file in Excel, then copy and paste to the Angled spreadsheet.

4.8.2 Appendix C

Details of Statistical Analysis

4.8.2.1 The intraclass correlation coefficient of reliability (Fleiss, 1986)

Fleiss (1986) explains the intraclass coefficient of reliability.

Let X represent a single observed value or measurement. We know that a single measurement is unreliable, because no matter how the value is obtained, if a second measurement were to be carried out, the second value would be different from the first. If T represents the mean of several measurements or observations of the same phenomenon, a single measurement X will differ from T for a number of reasons, for example random error or imperfect calibration of the measuring device. T should therefore be the closest reading to the true score, as it would reduce the effect of random variation of single readings. If e represents the difference between a single observation X and the mean value T ,then:

$$X = T + e$$

In a population of subjects T will have a variability with a standard deviation and variance (σ^2_T). For a single subject the random error e will vary about a mean of zero. Assuming the distribution of errors is independent of the value of T, e also has variability expressed by a standard deviation and a variance (σ^2_e). There are therefore two parts to the variability among a series of

measurements on different subjects, variability between the subjects and variability of the random error, thus:

$$\sigma^2_X = \sigma^2_T + \sigma^2_e$$

The intraclass correlation coefficient of reliability expresses the relative magnitude of these two components.

$$R = \frac{\sigma^2_T}{\sigma^2_T + \sigma^2_e}$$

As the proportion of random error (σ^2_e/σ^2_T) decreases, reliability increases and R approaches its maximum value of 1. As the proportion of error (σ^2_e/σ^2_T) increases reliability decreases and *R* approaches its minimum value of zero.

In the present example (Table C1):

$$R = \frac{14.88^2}{(14.88^2) + (1.32^2)}$$

$$R = 0.99$$

Table C1
Table showing the readings for the repeated measurements on 40 slides.

Code 1	Reading 1 (mm^2)	Code 2	Reading 2 (mm^2)	Difference	T Mean	e Mean -1
237	42.16	A52	44.76	-2.60	43.46	1.30
239	49.79	A54	48.93	0.86	49.36	-0.43
206	37.77	A57	40.69	-2.92	39.23	1.46
296	41.49	B22	40.83	0.66	41.16	-0.33
269	48.95	B28	47.72	1.23	48.34	-0.62
234	50.37	B32	46.21	4.16	48.29	-2.08
265	39.10	B36	35.18	3.92	37.14	-1.96
390	35.11	A42	35.07	0.04	35.09	-0.02
342	37.54	A43	36.44	1.10	36.99	-0.55
276	39.66	A48	42.51	-2.85	41.09	1.43
230	35.43	A49	35.07	0.36	35.25	-0.18
247	27.77	A56	28.00	-0.23	27.89	0.11
279	50.81	B33	45.98	4.83	48.40	-2.42
270	48.43	B35	47.59	0.84	48.01	-0.42
208	34.62	B37	35.03	-0.41	34.83	0.21
249	30.32	B38	30.53	-0.21	30.43	0.11
280	30.71	B39	30.52	0.19	30.62	-0.09
354	28.40	B40	29.76	-1.36	29.08	0.68
254	34.59	B41	33.56	1.03	34.08	-0.52
211	47.61	A41	46.23	1.38	46.92	-0.69
272	57.29	A45	59.82	-2.53	58.56	1.27
375	56.06	A46	59.02	-2.96	57.54	1.48
227	55.22	A50	58.22	-3.00	56.72	1.50
355	90.92	A51	88.77	2.15	89.85	-1.08
393	57.66	A53	60.56	-2.90	59.11	1.45
274	54.02	A55	55.87	-1.85	54.95	0.92
250	75.38	A58	65.69	9.69	70.54	-4.85
264	71.97	B23	74.04	-2.07	73.01	1.04
244	65.94	B25	63.83	2.11	64.89	-1.06
397	57.80	B26	59.03	-1.23	58.42	0.62
353	54.92	B27	58.23	-3.31	56.58	1.66
291	47.44	B29	46.49	0.95	46.97	-0.47
217	48.64	B30	47.31	1.33	47.98	-0.66
240	54.20	B34	53.22	0.98	53.71	-0.49
373	91.02	B42	92.47	-1.45	91.75	0.73
293	32.51	A44	33.82	-1.31	33.17	0.66
363	48.07	A47	48.46	-0.39	48.27	0.20
221	41.64	B21	44.64	-3.00	43.14	1.50
209	36.46	B24	35.56	0.90	36.01	-0.45
285	47.80	B31	50.70	-2.90	49.25	1.45
			Standard Deviation		14.88	1.32

4.8.2.2 Tests of Normality

Below is an example of the Normality test carried out on the data prior to hypothesis testing with parametric statistical tests. The Normal plot stretches the vertical axis of the cumulative frequency distribution. The horizontal axis shows the numerical value of the observation and the vertical axis gives the relative frequency in terms of number of standard deviations from the mean. If the data are Normal this should be a straight line.

Table C2
Table showing the results from the readings of two different angled views of the same tooth (n=32 as 5 teeth were repeated).

Angle	Tooth	0	20	0-20
Premolar	P1	36.47	37.31	-0.84
	P1	40.34	36.15	4.19
	P2	34.82	34.59	0.23
	P3	39.51	41.01	-1.50
	P3	41.72	44.37	-2.65
	P4	41.49	38.26	3.23
	P5	39.75	37.22	2.53
	P5	39.62	37.54	2.08
	P6	48.72	48.43	0.29
	P7	48.11	44.65	3.46
	P7	49.79	45.90	3.89
	P8	33.15	37.54	-4.39
	P9	48.95	44.71	4.24
	P9	46.03	44.12	1.91
	P10	42.69	41.07	1.62
	P11	55.62	50.81	4.81
Molar	M1	44.64	41.81	2.83
	M2	90.92	79.68	11.24
	M2	89.88	77.85	12.03
	M3	68.02	58.32	9.70
	M3	67.48	56.91	10.57
	M4	56.06	45.87	10.19
	M5	47.61	35.27	12.34
	M5	46.14	37.62	8.52
	M6	61.63	47.90	13.73
	M6	58.96	47.80	11.16
	M7	54.59	49.09	5.50
	M7	54.20	51.05	3.15
	M8	60.76	55.23	5.53
	M9	57.80	48.07	9.73
	M10	64.08	55.22	8.86
	M11	57.64	50.97	6.67

Table C3
Table showing the results of the Shapiro-Wilk test for Normality for the data from Table C2.

Statistic	Df	P
0.963	32	0.426

Figure C1
Figure C1a shows a frequency histogram of the differences between the measurement of the area of demineralisation from the data in Table C2. Figure C1b shows a Normal QQ plot of the same data.

Figure C1a

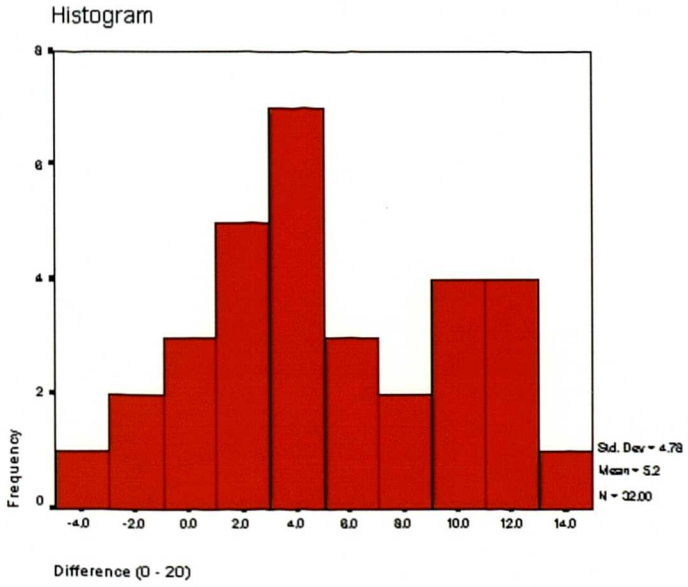
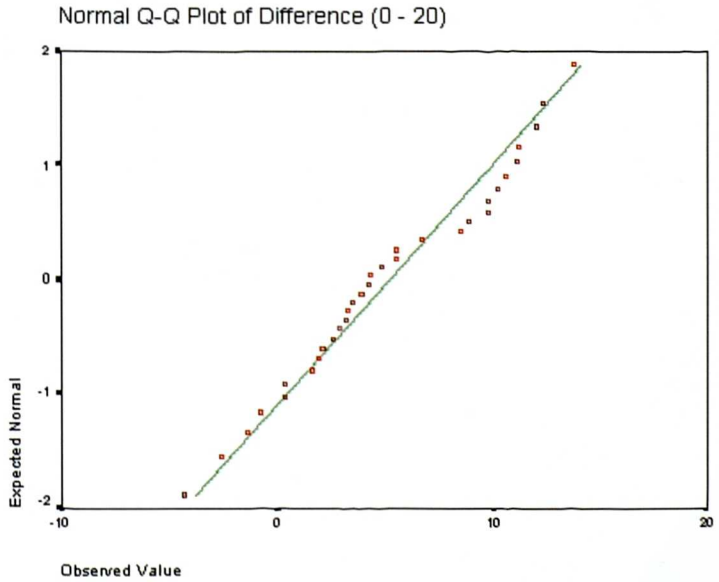


Figure C1b



4.8.2.3 The Bonferroni Correction (from Kinnear and Gray, 1997)

Suppose it is planned to make *c* pairwise comparisons between sets of data. By doing so you increase the chances of a type I error (false positive). It is desirable to keep the *per family* error rate at 0.05. In the Bonferroni method ordinary *t* tests are used for the pairwise comparisons, but the *per family* error rate is divided by the number of planned comparisons. To achieve significance each *t* test must show significance beyond the 0.05 level.

The *per family* type I error rate must be divided by the number of possible pairs (*c*) that can be drawn from an array of k means

$$c = \frac{k!}{2!(k-2)!}$$

Where ! means factorial (eg 4! Is 4 x 3 x 2 x 1 = 24). For example if there are five treatment means, $c = 5! / (2! \times 3!) = (5 \times 4 \times 3 \times 2) / (2 \times 3 \times 2) = 10$, and the test statistic will have to be significant beyond the 0.05/10 = 0.005 level to be significant.

In the present study the $k = 3$, so $c = 3$. The Bonferroni *t* tests will have to show significance beyond the 0.05/3 = 0.02 level approximately.

CHAPTER 5

Quantifying Enamel Demineralisation from Teeth with Orthodontic Brackets

5.1 Introduction and Aim

In Chapter Four it was found that incremental demineralisation could be measured reproducibly using a photographic slide converted to a grey scale image and measured using computerised image analysis. The aim of this study was to examine the reproducibility of measuring demineralisation surrounding an orthodontic bracket. Human teeth, with orthodontic brackets were incrementally demineralised, then two techniques were used to record and measure the demineralisation:

1. Computerised image analysis from colour slides of teeth.
2. Quantitative light-induced fluorescence directly on the teeth.

The validity of each technique was investigated by comparing the measured demineralisation with the known incremental demineralisation period.

5.2 Materials and Methods

5.2.1 Photographic technique

Fifteen extracted human molars were used, because it was not possible to obtain a large enough sample of human incisor teeth. They were carefully inspected to ensure the clinical absence of white spot lesions. The teeth were divided in half by cutting mesio-distally down the long axis of the tooth with a diamond wheel (Isomet, Buehler Ltd, Evanston, Illinois, USA). This produced a buccal and lingual half for each tooth, which were shaped to look like incisors by using the diamond wheel to straighten the mesial, distal and occlusal surfaces. A unique identifying number was engraved on the cut surface of each half tooth.

A previous study (Chapter Four) had found that it was sometimes difficult to detect the cemento-enamel junction (cej) from a photograph and this may have contributed to the error in measuring the area of the buccal surface. Therefore, the cej of the teeth in this study were grooved with a small round bur and filled with light-cured composite resin dyed with a red vegetable dye to highlight it and simulate the gingival margin.

Standard edgewise twin brackets, with a slot size of 0.018 inch x 0.025 inch (Ortho-Care [UK] Ltd. Bradford, W.Yorks, UK), were bonded to the surface, in the usual position for an orthodontic attachment. Standardised photographs were taken of the teeth, using the same camera and settings as previously

(Chapter Four), except for the aperture setting. In the previous study (Chapter Four), the aperture was set at f22 as this was considered to be ideal to allow enough light into the camera for the measurement of demineralisation, without too much reflected light. After the assessment of the photographs in Chapter Four it was recognised that some of the images were too dark for the computerised analysis and opening the aperture to f16 improved the measurement without increasing the amount of reflection noticeably.

Photographs were taken with masking on the ring flash, to reduce reflections. This was placed below the lens, as previous described (Chapter Four) to improve reproducibility. To improve the replication of the camera positioning, a sighting jig was placed in the bracket slot (Figure 5.1, page 5.30). The jig consisted of a full sized (0.018 inch x 0.025 inch), rectangular stainless steel archwire, with one long arm and one short arm (Figure 5.2, page 5.31). The jig was held into 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 from white, grey and black to allow for grey scale calibration of the digital

image (Figure 5.1, page 5.30). The photographs were repeated after one week, to provide two sets of photographs.

Following the initial two sets of photographs, the crowns of the teeth were covered with three coats of acid resistant varnish except for windows approximately 1.5 x 3mm of enamel surface on the gingival, occlusal, left and right aspects of the bracket (Figure 5.3, page 5.32). The teeth were placed in a demineralising gel (see section 3.8.1 Appendix A, page 3.29) and the buccal surface was incrementally covered in a systematic arrangement to expose the four windows to the gel for 0, 3, 7 or 14 days (Figure 5.4, page 5.33). The patterns were chosen so some teeth had no demineralising challenge, some had severe demineralisation 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 demineralisation (Mizrahi, 1982, 1983). Therefore, it was not given priority when arranging the putative patterns of demineralisation.

A tooth that was designated to have areas with different periods of demineralisation, was removed from the gel after the time of the shortest exposure, washed in distilled water and three coats of acid resistant 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 tooth was washed in distilled water and the varnish removed with acetone. Photographs were taken of the enamel surface using the same

photographic technique and jig as described. These views were again repeated after one week.

In summary, there were four images of each tooth taken (Figure 5.5, page 5.34). When all the photographs had been taken and developed using the same machine, the slides were recoded by a second investigator to allow a blind assessment by the principal investigator.

5.2.2 Image Analysis

The images were captured and saved using the same method as Chapter Four. The slides were converted to high resolution (2720 dpi) grey scale images (8-bit range) using a slide scanner (Canoscan 2700F, Canon Inc., Tokyo 146, Japan) and computer software (Scancraft FS version 3.1.1. Canon Inc., Tokyo 146, Japan). They were saved as Tagged Image File Format (TIFF).

The images were opened in an image analysis programme (Image-Pro Plus, version 3.0 for Windows 95, Media Cybernetics, Silver Spring, Maryland 20910, USA). Each image was individually calibrated in millimetres, using the bracket as the calibrating measure (Figure 5.3, page 5.32). To determine the calibration measurement the distance across the outside of the tie-wings of five brackets (Figure 5.3, page 5.32) was measured on two occasions, a week apart, with digital callipers (Mitutoyo Corp, Minato-ku, Tokyo, Japan).

The readings were averaged (3.33 ±0.06mm) and this figure was used to calibrate each image.

Two methods were used to measure the images.

Image Subtraction

The image analysis software allows the subtraction of one image from another. By subtracting the image taken before the experimental demineralisation protocol from the image taken after, the resulting image consists of grey levels that represent the difference between the grey levels of the two images. A large proportion of this new image would be black (grey scale 0), due to there being no difference in the grey levels before and after demineralisation. Any areas of demineralisation would hypothetically show as lighter areas on this new image, because of the increased grey scale levels, as long as the second image (the one being subtracted) was darker than the first image. Hence, the pre-demineralisation process image was subtracted from the post-demineralisation image.

The details of the method are given in Appendix D (page 5.41). Briefly, The images were randomised so that the first image was always slide number 1 (Figure 5.5, page 5.34), which was the baseline pre-demineralisation photograph. The remaining three slides were placed in a random order. Slide number 1 was opened and the grey scale levels altered so that the grey scale levels within the white area of the calibrating grey scale marker recorded a grey scale of 255 and the black area was 0. The image was saved. Areas of Interest (AOI) were drawn around the outline of the buccal

surface of the tooth and the bracket. These were saved to the AOI manager to be used later. The second image was opened and four common points on the two teeth were registered. The software was used to alter the position of the second image to exactly correspond to the first image, allowing for any discrepancies in camera position between the two images. The outline of the buccal surface of the second image was checked with the AOI of the buccal surface of the first image for the accuracy of the registration, which was repeated until they corresponded. This new image (image 3) was calibrated for greyness using the grey scale and for- size using the mean value of bracket calibration measurement. A new AOI was drawn around the bracket and saved. Slide 1 was subtracted from image 3 to produce a fourth image, which represented the difference in grey levels between the two images. The grey levels of this image were measured using the technique described in section 4.8.1, Appendix B, page 4.20), but using a different range for grey scale measurement. This process was repeated by comparing slide 1 with both slide 3 and slide 4.

Outlining areas of demineralised enamel near the bracket

In order to look at repeatability the post-demineralisation slides 3 and 4 (Figure 5.5, page 5.34) of the thirty teeth were measured. Each slide was given a three-digit random number derived from a random number table and assessed blindly in numerical order, on two occasions at least a week apart. The grey scale images were opened in Image-Pro Plus and calibrated using the bracket tie-wing measurement (Figure 5.3, page 5.32). The four edges of the bracket on the grey scale image were closely inspected and if an area of

demineralisation was observed then an 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 demineralisation to be present was a reading taken. Therefore, there were two processes in use. Firstly, a subjective visual assessment to produce a dichotomous estimate of the enamel surface (yes or no to demineralisation). Secondly, a measurement of the enamel on those parts of the tooth surface judged to be demineralised.

Quantitative Light-Induced Fluorescence (QLF)

The teeth in Figure 5.4 (page 5.33) were assessed using QLF. Images of the 30 teeth were captured using the arc lamp with a liquid light guide system described by Al-Khateeb *et al* (1997b). This system uses light from a lamp that passes through a blue filter in front of the lamp with a peak intensity of 370nm. The light reflected from the tooth is captured using a camera with a yellow high pass filter of 520nm to exclude light below that frequency. The images are stored, processed, and analysed with custom software developed by de Josselin de Jong (v 1.22, Inspektor Research Systems BV, Amsterdam, The Netherlands).

Two images of each tooth were captured one week apart. The images, which were stored on the hard disk of a computer, were recoded by a second investigator. The principal investigator then analysed each image on two occasions at least one week apart. A recording was taken from each of the four aspects of the bracket (gingival, occlusal, left and right, see Figure 5.3, page 5.32). If an area of demineralisation was observed then the AOI was

shaped around the area, but including an area of normal enamel surrounding it. If there was no discernible area of demineralisation then a rectangular AOI was drawn. The detailed settings of the QLF machine are described in section 5.8.3, Appendix F, page 5.43.

5.2.3 Statistics

Image Subtraction

A graphical representation of the results of the image subtraction method was prepared. The area readings for the different grey scale ranges were converted into proportions of the total buccal surface of the tooth. Each tooth was given a rank 0, 3, 7 or 14, according to the aspect of the bracket with the maximum exposure to the demineralising solution. The proportions for each rank were averaged and a graph showing the proportion of the area of the buccal surface placed in each grey scale range was constructed for each demineralisation rank.

Outlining areas of demineralised enamel near the bracket

The accuracy of diagnosing demineralisation from a photograph was assessed by recording whether the observer had carried out a reading and comparing this with the actual demineralisation pattern for that tooth. It was assumed that if no reading was recorded then no demineralisation could be detected. This was carried out for the four sites around the bracket of each tooth, which had been photographed twice and recorded twice (n = 480). The

results were placed in a 2 x 2 contingency table and the sensitivity and specificity were calculated. These are defined by Altman (1991):

Sensitivity is the proportion of positives that are correctly identified by the test.

Specificity is the proportion of negatives that are correctly identified by the test.

The probability of a correct diagnosis of demineralisation from the photograph was calculated using the positive and negative predictive values. These are defined by Altman (1991) as:

Positive predictive value is the proportion of patients with positive test results who are correctly diagnosed.

Negative predictive value is the proportion of patients with negative test results who are correctly diagnosed.

A diagnostic test's predictive values will vary widely depending on the proportion of individuals to whom the test is applied, who actually have the condition (Haynes, 1981). If the test is applied to a group of individuals with a high prevalence of the condition, the predictive values are likely to be better than if it is applied to a group with a low prevalence. The effect of the prevalence or proportion of orthodontic patients with demineralisation following treatment has on the accuracy of the test was calculated (Altman, 1991). The data from Table 2.1 (page 2.9) were used to estimate the

proportion of teeth that have demineralisation due to orthodontic treatment. It was estimated that 10 percent teeth that have not been subjected to orthodontic treatment have white spot lesions, as against 24 percent of teeth following orthodontic treatment. Hypothetically, with a baseline of 10 percent of teeth with white spots, it can reasonably be estimated that, the prevalence of white spots due to orthodontic treatment is 14 percent.

The sensitivity, specificity, positive predictive value and negative predictive value were calculated for the QLF data in the same way as for the photographic readings. QLF readings were taken for each site around the bracket, so the calculations were carried out for any area suggesting there was demineralisation. A separate calculation was carried out for areas above $0.5mm^2$. This figure was chosen to represent a clinically significant area of enamel demineralisation. The effect of the prevalence of orthodontic demineralisation was also calculated.

The mean of the two readings from each photographic and QLF image were calculated. The limits of agreement between the two images for each technique and between the photographic and QLF areas were constructed.

The correlation between the mean grey level and the average decrease in fluorescence was investigated with a scattergram and Pearson product moment correlation coefficient.

5.3 Results

Figure 5.6 (page 5.35) shows the graph of the mean proportion of the area of buccal surface in each grey scale range for the image constructed by subtraction of the first photograph from the second photograph. The means for the teeth with different maximum periods of demineralisation are shown in different colours. There was no difference in the mean demineralisation patterns between the teeth with different maximum periods exposed to the demineralising solution.

Table 5.1 (page 5.23) shows the results for the differences between the two readings for both the photographs and QLF. The mean differences for the area of demineralisation measured were similar for both the photographs ($0.02mm^2$) and QLF ($0.08mm^2$), although the one sample *t* test showed evidence of systematic error (P=0.014) for the QLF readings, with the second readings slightly higher than the first. The mean difference was small and the confidence intervals narrow, which suggests that the systematic bias between the two readings was not significant. The intraclass correlation coefficient of reliability was similar for the photographs (0.84) and the QLF (0.80).

The readings for the mean grey levels for the photographs and the mean percentage change in fluorescence did show some differences between the two techniques (Table 5.1, page 5.23). The mean difference in the percentage change in fluorescence was low (0.10) and the confidence intervals narrow. There was no evidence of systematic bias between the two

recordings (P=0.421). The mean difference in grey levels between the first and second recordings was 1.55 greys. There was evidence of systematic bias between the recordings (P=0.003), with the second reading being higher than the first. However, a mean difference of 1.55 greys on a scale from 0 to 255 can be considered small. The intraclass correlation coefficient was higher for the photographs (0.86) compared with QLF (0.75) suggesting a lower random error.

The next series of tables (Table 5.2, page 5.24 to Table 5.8, page 5.27) demonstrate the validity of the photographic and QLF techniques. The tables describe the results of the present experiment, as well as the hypothetical effect of extrapolating the results of the present experiment to the population, taking into account the prevalence of enamel demineralisation following orthodontic treatment.

Table 5.2 (page 5.24) shows the comparison between the results of diagnosing demineralisation from photographs with the actual demineralisation carried out. Table 5.3 (page 5.24) shows the results calculated for the diagnosis for a prevalence of 0.14 derived from the data in Table 3.1. Table 5.4 (page 5.25) shows the calculated sensitivity, specificity, positive predictive value and negative predictive value for the raw data and the data derived to take into account the effect on prevalence.

Table 5.5 (page 5.26) shows the results of detection, using the QLF technique when any area of fluorescence loss was measured compared with the actual demineralisation recorded for the tooth. Table 5.6 (page 5.26)

shows the results when areas below 0.5mm^2 were not considered clinically significant.

Table 5.7 (page 5.27) shows data derived from the figures in Table 5.6 for a prevalence of 0.14. Table 5.8 (page 5.27) shows the calculated results for sensitivity, specificity, positive predictive value and negative predictive value for the data from the previous three tables.

The ability to detect demineralisation when it is present from a photograph was 0.93. The same result for QLF was 0.97 if all areas were included, however this reduced to 0.81 if only areas 0.5mm^2 and over were taken into account (Table 5.8, page 5.27). The ability to correctly identify the absence of demineralisation was 0.84 from the photographs (Table 5.4, page 5.25). The QLF had a much lower ability to identify the absence of demineralisation (specificity of 0.24) if any area with reduced fluorescence was considered to be demineralised, however this increased to 0.88 when areas below 0.5mm^2 were excluded.

The proportion of patients that had been predicted in the correct diagnosis was 0.77 for the photographs (Table 5.4, page 5.25) and 0.44 for the QLF, which increased to 0.80 when areas less than 0.5mm^2 were excluded (Table 5.8, page 5.27). A negative test result correctly predicts the absence of disease in 95% of cases with photographs and 88-92 percent of cases with QLF.

Table 5.9 (page 5.28) and Table 5.10 (page 5.28) show the sensitivity, specificity, positive predictive value and negative predictive values calculated for the four surfaces of the bracket. Similar results are shown for the two techniques, with readings from the left and right sides of the bracket accurately recording demineralisation, however the occlusal and gingival recordings were less reliable.

Table 5.1 (page 5.29) shows the number of correct and incorrect diagnoses for the two techniques for the different times of demineralisation. It can be seen that areas of 7 and 14-day demineralisation were accurately detected from the photographs on every occasion, whereas they were detected 85 and 87 percent of the time using QLF. QLF was also less accurate at detecting the 3-day demineralisation, but it was slightly more accurate at recording no demineralisation.

Figure 5.7 (page 5.36) is a graph of the limits of agreement for the areas of demineralisation recorded from the first and second photograph. There is a small mean difference ($0.01mm^2$) and the limits of agreement are narrow (-0.18 to $0.19mm^2$). Figure 5.8 (page 5.37) is a graph of the limits of agreement for the areas of demineralisation recorded from the first and second QLF images. It can be seen that the scatter of differences increases as the mean area increases. This can be interpreted as there being a relation between the difference and the mean, which will affect the limits of agreement. The limits will be wider apart than necessary for small areas and narrower for large areas (Bland and Altman, 1986). The data were log-

transformed and the results are shown in Figure 5.9 (page 5.38). The mean difference is –0.21 on the log scale, with limits of –0.93 to 0.52. The antilogs of these numbers are 0.39 to 1.68. This is a dimensionless ratio, that shows that for 95% of cases the second reading from the photograph was between 0.39 to 1.68 times the first reading (Bland and Altman, 1986). In other words 60 percent below to 68 percent above. The same relation was found between the difference and the mean for the agreement between the areas recorded from the photograph and QLF, therefore a log-transformation was carried out on this data also. Figure 5.10 (page 5.39) shows a graph of the limits of agreement. The mean difference was –0.52 on the log scale and the limits were –0.2.19 to 1.15. The antilogs of these numbers are 0.11 and 3.1 suggesting that there was not a good agreement between the measurement of demineralisation between the photographic and QLF techniques. The recorded area of demineralisation was higher with the photographic technique compared with QLF.

Figure 5.11 (page 5.40) is a scattergram of the mean change in fluorescence for the QLF readings and the mean grey level from the photographs (n = 62). The correlation coefficient was –0.48 (95% confidence interval –0.38 to –0.57) which was highly significant ($P<0.001$).

5.4 Discussion

The results of subtracting the pre-demineralised photograph from the post-demineralised photograph were disappointing. The technique was unable to differentiate between teeth that had been demineralised and those that had not (Figure 5.6, page 5.35). If the technique had been able to distinguish between the different patterns of demineralisation, the graph would show different peaks of grey levels moving to the right (i.e. the difference in grey levels increasing, indicating whiter areas) as the maximum exposure increased. However, the graph showed the same mean proportion of grey levels within each range for all the groups of teeth, regardless of the maximum period in the demineralising gel.

Despite careful photographic technique to maintain a consistent exposure, calibration of the images using a calibrating grey scale with each photograph and use of a positioning jig to standardised the images, there were inconsistencies between images. It is unlikely that clinical photographs will be as standardised as those produced for this study, therefore there will be more inconsistencies in photographs taken in the clinical situation. It was noted that the differences in grey levels between demineralised areas, particularly the three-day patches and non-demineralised areas were small. The grey scale ranges that are chosen to represent the differences are important in detecting these differences. It may be that the use of a different set of ranges may yield improved results, but the more ranges that are used the more unwieldy the results become and the more difficult to interpret.

The ability to detect demineralisation when it is present (sensitivity) from a photograph using the manual identification was 0.93 (Table 5.4, page 5.25). Studies have shown that more opacities are scored from a photograph than with a clinical examination (Ellwood, 1993) and it is likely that this would be lower for a clinical examination. The same result (sensitivity) for QLF was 0.97 if all areas were included (Table 5.8, page 5.27).

The ability to correctly identify the absence of demineralisation (specificity) was 0.84 from the photographs (Table 5.4, page 5.25). The fact that this was lower than the ability to detect the disease was probably due to reflections from the camera flash being confused with demineralisation. When any area of reduced fluorescence recorded using QLF was considered, this technique had a much lower ability to identify the absence of demineralisation with a specificity of 0.24 (Table 5.8, page 5.27). QLF picked up reduced fluorescence on every tooth. On only six occasions did all four readings from a particular area fail to record an area of reduced fluorescence. This could be interpreted in two ways. Either the QLF is incorrectly diagnosing demineralisation in three-quarters of teeth that had no demineralisation or, there was mineral loss detectable using QLF, that could not be detected by clinical examination. Al-Khateeb *et al* (1997b) carried out an *in vitro* investigation to validate QLF with transverse microradiography that measures mineral loss directly. They showed that changes in fluorescence, using QLF correlated with calcium loss (r=0.74) and integrated mineral loss (r=0.64). It is therefore possible that QLF is measuring mineral loss that is not clinically

detectable and might have a significant advantage, because it would measure carious lesions at an earlier stage than the photographic technique.

Although the study by Al-Khateeb *et al* (1997b) showed good correlation between QLF and TMR results, it may be necessary to be more selective when interpreting the QLF data. By considering only areas of 0.5mm^2 and above as being clinically significant the ability to predict the presence of the experimental demineralisation was 0.81, but the ability to predict the absence of demineralisation improved to 0.88 (Table 5.8, page 5.27).

The proportion of patients that had been predicted in the correct diagnosis was 0.77 for the photographs (Table 5.4, page 5.25) and 0.44 for the QLF, which increased to 0.80 when areas less than 0.5mm^2 were excluded (Table 5.8, page 5.27). A negative test result correctly predicts the absence of disease in 95 percent of cases with photographs and 88-92 percent of cases with QLF.

The effect of estimating the prevalence of demineralisation around a bracket, on the predictive capacity of the two techniques had no effect on the sensitivity and specificity (Table 5.4, page 5.25 and Table 5.8, page 5.27). It had little effect on the accuracy of a negative result either, but it had a profound effect on the predicted accuracy of a positive result. Approximately one half of the positive results from both the photographic and QLF techniques were predicted to be accurate, when the prevalence of the condition was taken into account. Reflections will produce false positive

results for photographs, but this is not a factor when using QLF. It may be necessary to be more discriminating, when using the QLF technique, than measuring each corner of the bracket. QLF will pick up more fluorescence loss than is possible clinically. QLF may be used to measure longitudinal changes in fluorescence.

The values for the different aspects of the bracket showed important differences between the two techniques (Table 5.9, page 5.28 and Table 5.10, page 5.28). The photographic technique showed good negative predictive values for all four corners of the bracket, suggesting that if demineralisation was not recorded it was unlikely to be present. The positive predictive values were poor for the gingival section, where just over one half of positive results were predicted to be accurate and for the occlusal section, where only two thirds were predicted to be accurate. The QLF technique also showed poor positive predictive values for the gingival (0.77) and the occlusal areas (0.59). The occlusal region is an area that has a low prevalence of demineralisation (Mizrahi, 1982, 1983). Unfortunately, the gingival area is an area of the orthodontic bracket with the highest prevalence of demineralisation. Reflections from the flash would account for the low values for the photographic technique. The QLF technique had a low negative predictive value (0.74) and the small area between the bracket and the simulated gingival margin may contribute to the reduced accuracy of the result. The left and right edges of the bracket showed good results for both the photographic and QLF techniques.

In most cases, a negative result suggests that there was no demineralisation present. A positive result was less reliable particularly for gingival, where there is a high prevalence of demineralisation and occlusal readings.

Table 5.11 (page 5.29) shows that the areas of obvious (7 and 14 days) demineralisation were recorded every time using the visual examination of the photograph, however QLF recorded the 7 day demineralisation on 85 percent and the 14 day on 87 percent of occasions. QLF recorded the no demineralisation group slightly more successfully than the visual examination of the photographs (88 percent compared with 84 percent).

The limits of agreement for the recording of the area of demineralisation between the two images for each technique are shown in Figure 5.7 and Figure 5.8 (pages 5.36 and 5.37). The mean difference between the two readings was small for the photographs ($0.02mm^2$) and the limits of agreement acceptably narrow (-1.18 to $1.19mm^2$) suggesting good agreement between the two readings. The QLF technique showed a relationship between the mean and the difference, such that when the mean reading increased the difference between the two readings increased (Figure 5.8, page 5.37). A log-transformation (Figure 5.9, page 5.38) showed that 95 percent of the second readings for QLF were between 0.39 and 1.68 times the level of the first readings (60 percent below to 68 percent above), which is acceptable. The limits of agreement for the two techniques also showed relationship between the mean and the difference, therefore the data were log-transformed (Figure 5.10, page 5.39). The limits of agreement were much

larger for the two techniques (0.11 below to 3.16 above) with the photographs recording a larger area of demineralisation than the QLF.

The correlation between the mean decrease in fluorescence with the QLF and the mean grey level measured from the photograph is shown in Figure 5.11 (page 5.40). The correlation coefficient was –0.48 which was highly significant (<0.001).

5.5 Conclusions

1. Measurement of enamel demineralisation by subtracting a pre-demineralisation image of a tooth with an orthodontic bracket from a post-demineralisation image of the same tooth, was not found to be reliable using the technique described.
2. Computerised image analysis employing manual measurement around areas of demineralisation was found to be reproducible.
3. Quantitative light-induced fluorescence of teeth with orthodontic brackets and artificial demineralisation was found to be comparable to computerised image analysis from a photographic image in terms of validity and reproducibility. This technique might have the advantage of recording and measuring demineralisation at an earlier stage than the photographic technique.

5.6 Tables

Table 5.1

Table showing the mean difference, standard deviation of the differences and 95% confidence intervals (CI) for the mean difference between the repeat readings of the area and mean grey levels for the photographic readings and the area and mean percentage change in fluorescence for the quantitative light-induced fluorescence readings. Also included are the probability (P) that the differences were significant from zero as measured with a one sample *t* test for systematic error and the intraclass correlation coefficient of reliability (*R*) for random error.

		Mean Difference	*sd*	CI	*t*	P	R
Photographs	Area (mm^2) N = 240	-0.02	0.48	-0.08 to 4.1	-0.65	0.519	0.84
	Mean grey level N = 151	-1.55	6.30	-2.56 to -0.54	-3.03	0.003	0.86
QLF	Area (mm^2) N = 240	-0.08	0.49	-0.14 to -1.62	-2.49	0.014	0.80
	Mean percent change in fluorescence N = 240	0.10	1.84	-0.14 to 0.33	0.81	0.421	0.75

Table 5.2

Table showing relation between results of the detection of demineralisation from photographs and whether there was demineralisation present.

Demineralisation Detected	Demineralisation Present		
	Yes	No	Total
Yes	167	49	**216**
No	13	251	**264**
Total	**180**	**300**	**480**

Table 5.3

Table showed predicted effect of the detection of demineralisation from photographs if the prevalence of demineralisation following orthodontic treatment was 0.14, based on data from Table 3.1.

Demineralisation Detected	Demineralisation Present		
	Yes	No	Total
Yes	62	67	**129**
No	5	346	**351**
Total	**67**	**413**	**480**

Table 5.4

Values for sensitivity, specificity, positive predictive value and negative predictive value for the detection of enamel demineralisation from photographs derived from the data in the above tables.

	Current Experiment	Prevalence of 0.14
Sensitivity	0.93	0.93
Specificity	0.84	0.84
Positive predictive value	0.77	0.48
Negative predictive value	0.95	0.99

Table 5.5

Table showing relation between results of the detection of demineralisation from QLF and whether there was demineralisation present.

Demineralisation Detected	Demineralisation Present		
	Yes	No	Total
Yes	178	224	**402**
No	6	72	**78**
Total	**184**	**296**	**480**

Table 5.6

Table showing relation between results of the detection of demineralisation from QLF and whether there was demineralisation present if areas 0.5mm^2 and over are included.

Demineralisation Detected	Demineralisation Present		
	Yes	No	Total
Yes	149	37	**186**
No	35	259	**294**
Total	**184**	**296**	**480**

Table 5.7

Table showed predicted effect of the detection of demineralisation from QLF if the prevalence of demineralisation following orthodontic treatment was 0.14, based on data from Table 3.1.

Demineralisation Detected	Demineralisation Present		
	Yes	No	Total
Yes	54	52	**106**
No	13	361	**374**
Total	**67**	**413**	**480**

Table 5.8

Values for sensitivity, specificity, positive predictive value and negative predictive value for the detection of enamel demineralisation from QLF derived from the data in the above tables.

	All Areas	Areas > 0.4mm^2	Prevalence of 0.14
Sensitivity	0.97	0.81	0.81
Specificity	0.24	0.88	0.87
Positive predictive value	0.44	0.80	0.51
Negative predictive value	0.92	0.88	0.97

Table 5.9

Values for sensitivity, specificity, positive predictive value and negative predictive value for the detection of enamel demineralisation for the different edges of the bracket for the recordings from the photographs.

	Gingival	Left	Occlusal	Right
Sensitivity	0.93	0.98	0.80	0.92
Specificity	0.69	0.84	0.91	0.92
Positive predictive value	0.54	0.85	0.64	0.88
Negative predictive value	0.91	0.98	0.96	0.94

Table 5.10

Values for sensitivity, specificity, positive predictive value and negative predictive value for the detection of enamel demineralisation for the different edges of the bracket for the QLF recordings.

	Gingival	Left	Occlusal	Right
Sensitivity	0.66	0.91	0.71	0.92
Specificity	0.83	0.97	0.80	0.83
Positive predictive value	0.77	0.96	0.59	0.79
Negative predictive value	0.74	0.93	0.92	0.94

Table 5.11

Table showing the number of correct and incorrect diagnoses for the two techniques for the time periods of 0, 3, 7 and 14 day demineralisation periods.

Method	Demin (days)	Correctly Identified	Incorrectly Identified	Total	Percent Correct
Photographs	0	249	47	296	84
	3	65	15	80	81
	7	52	0	52	100
	14	52	0	52	100
	Total	418	62	480	87
QLF	0	260	36	296	88
	3	56	24	80	70
	7	44	8	52	85
	14	45	7	52	87
	Total	405	75	480	84

5.7 Figures

Figure 5.1

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

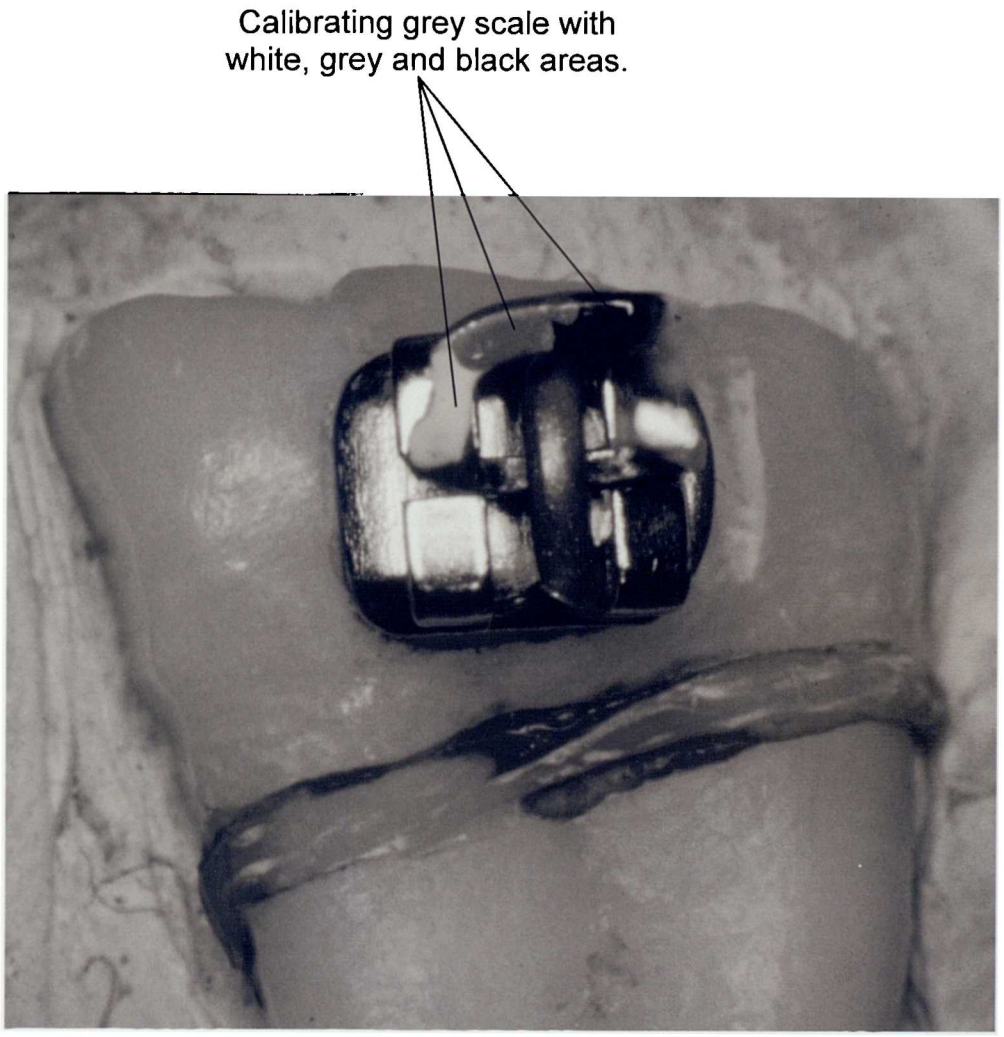


Figure 5.2

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

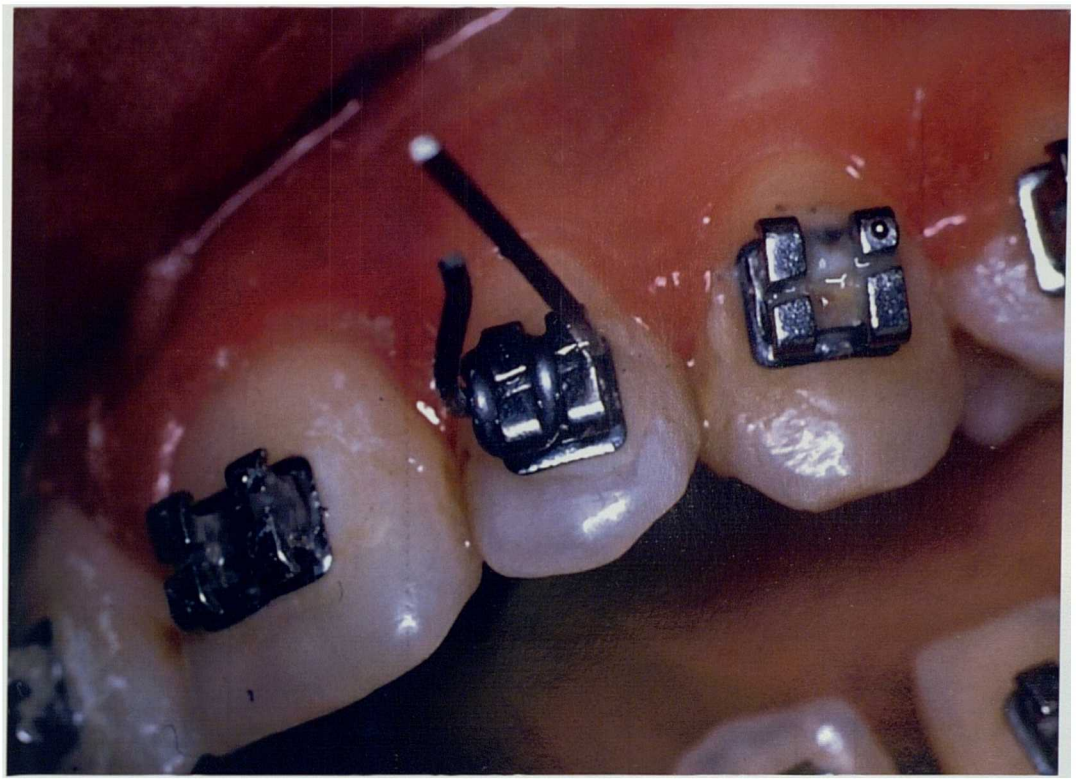


Figure 5.3

Diagram showing a tooth with an orthodontic bracket and the enamel areas on the four sides of the bracket that were exposed to the demineralising gel.

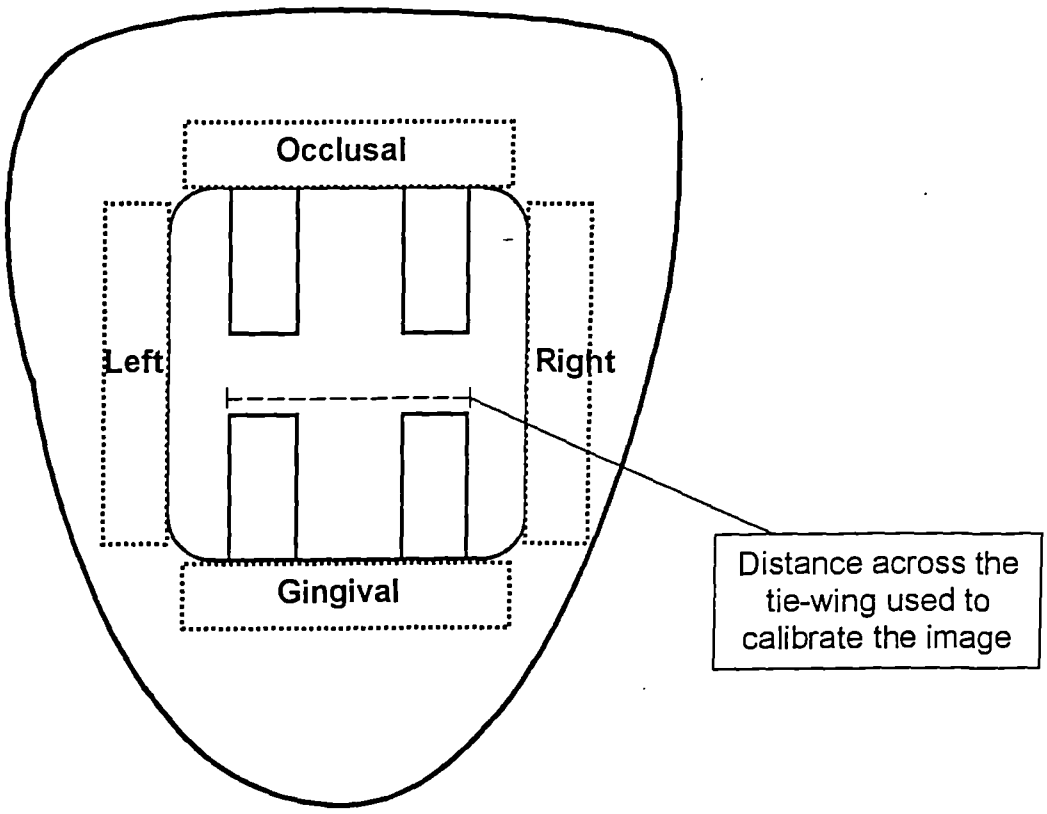


Figure 5.4

Diagram showing the periods of incremental demineralisation in days for the gingival, occlusal, left and right surfaces of the bracket for the thirty teeth.

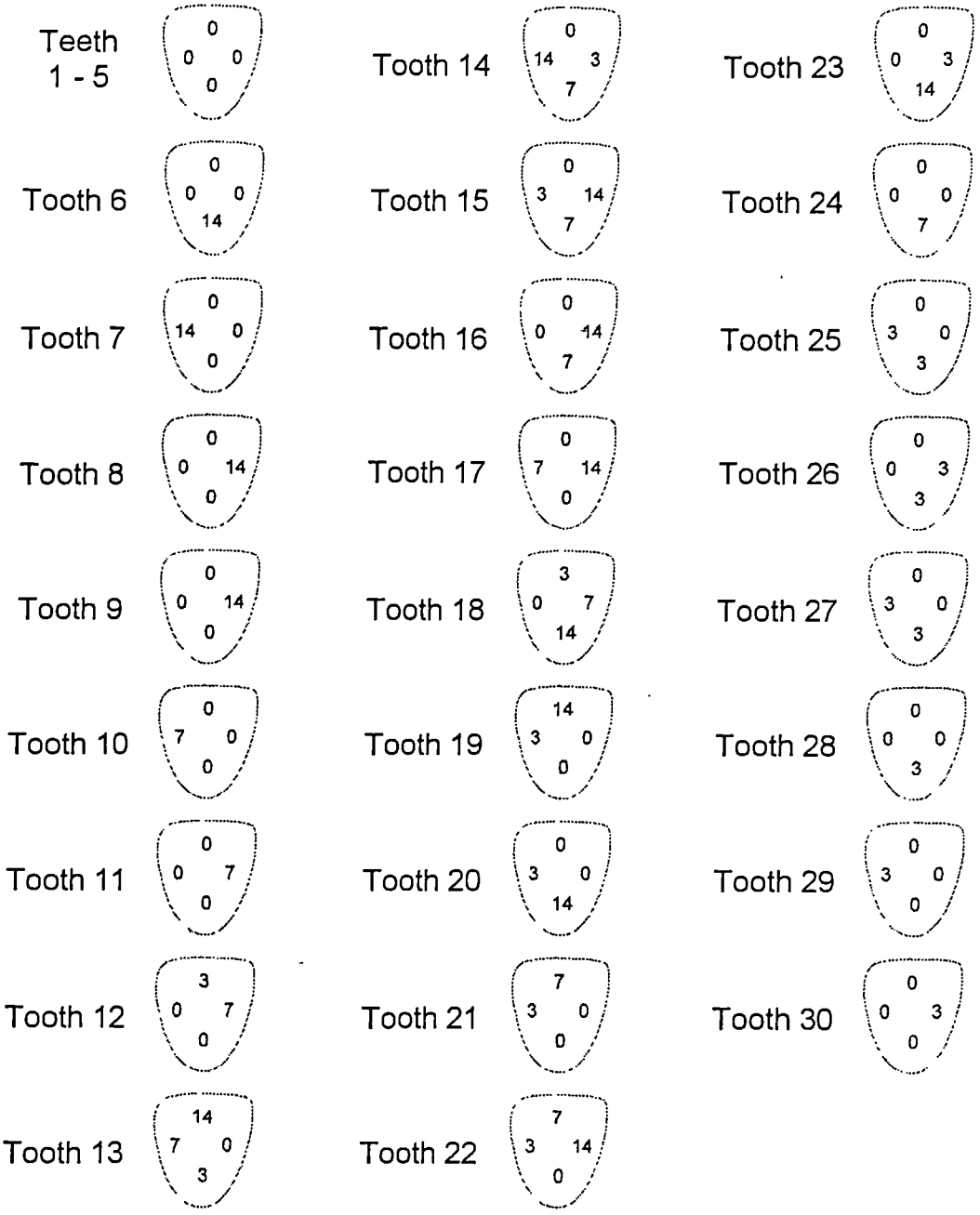


Figure 5.5

Diagram showing the plan of the four images taken of each tooth.

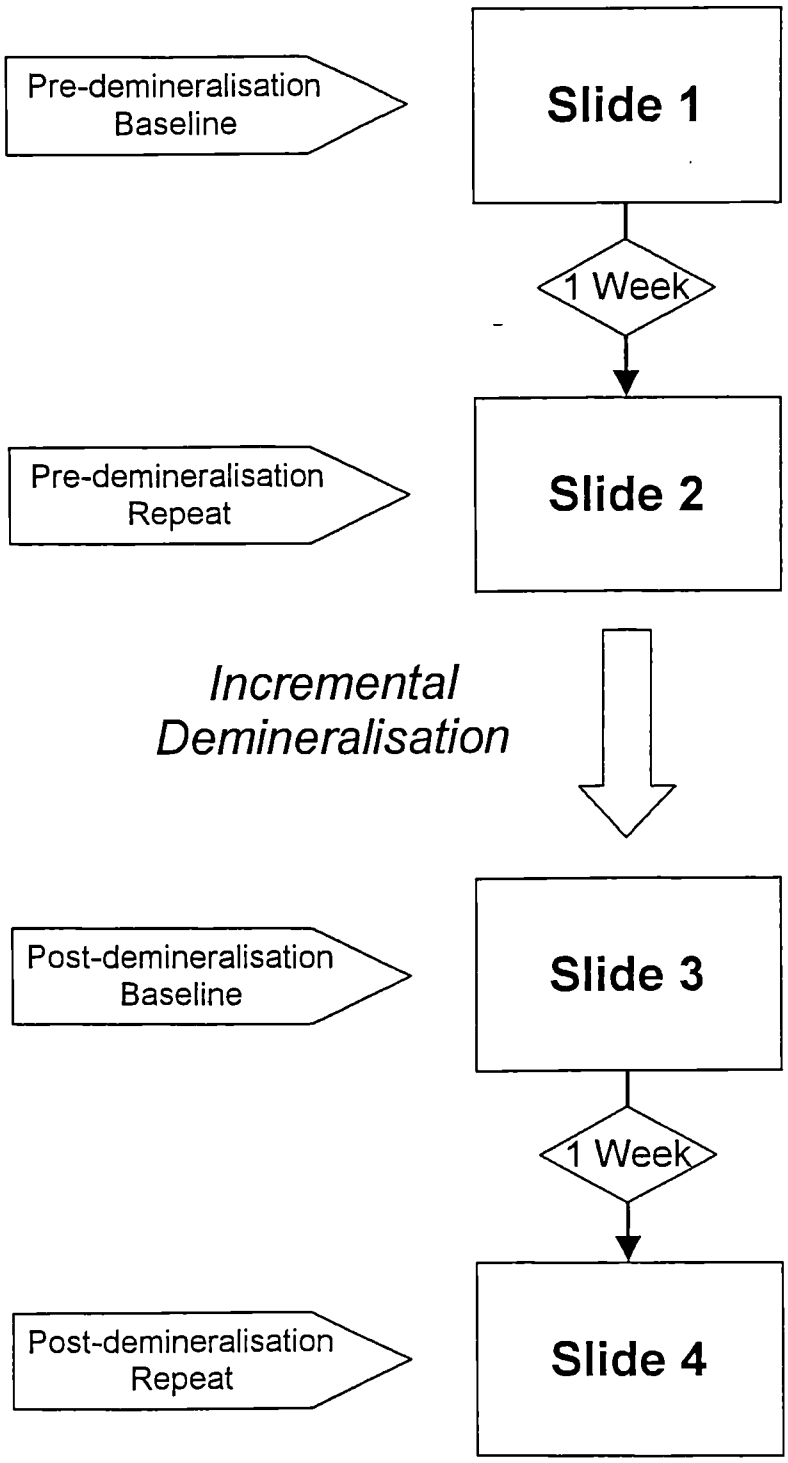


Figure 5.3

Graph showing the poor results from the image subtraction technique. Shown is the mean proportion of the area of buccal surface in each grey scale range for the image constructed by subtraction of the first photograph from the second photograph. The means for the teeth with different cumulative periods of demineralisation are shown in different colours.

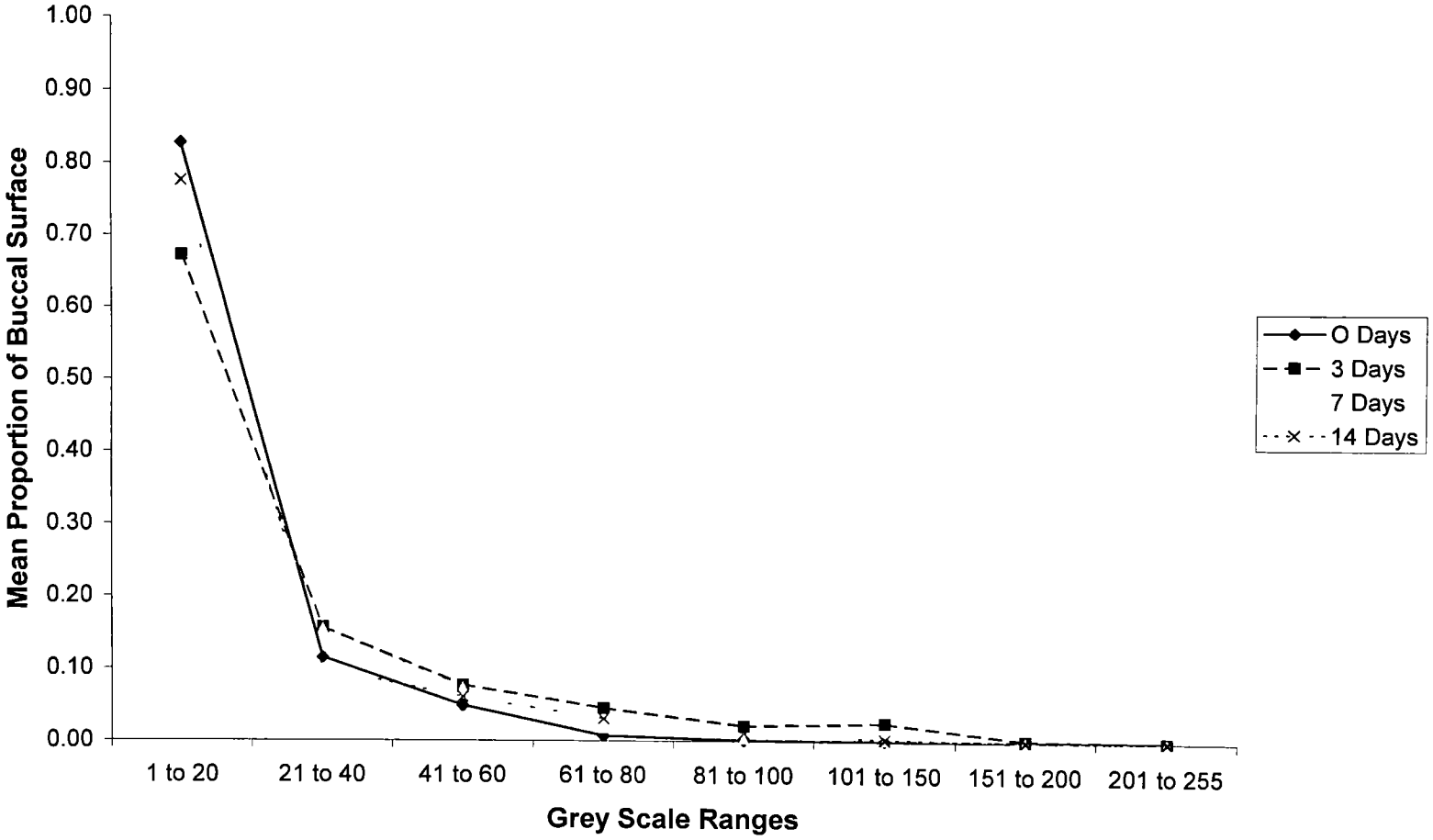


Figure 5.7

Limits of agreement for the areas of demineralisation recorded from the 1st and 2nd photograph.

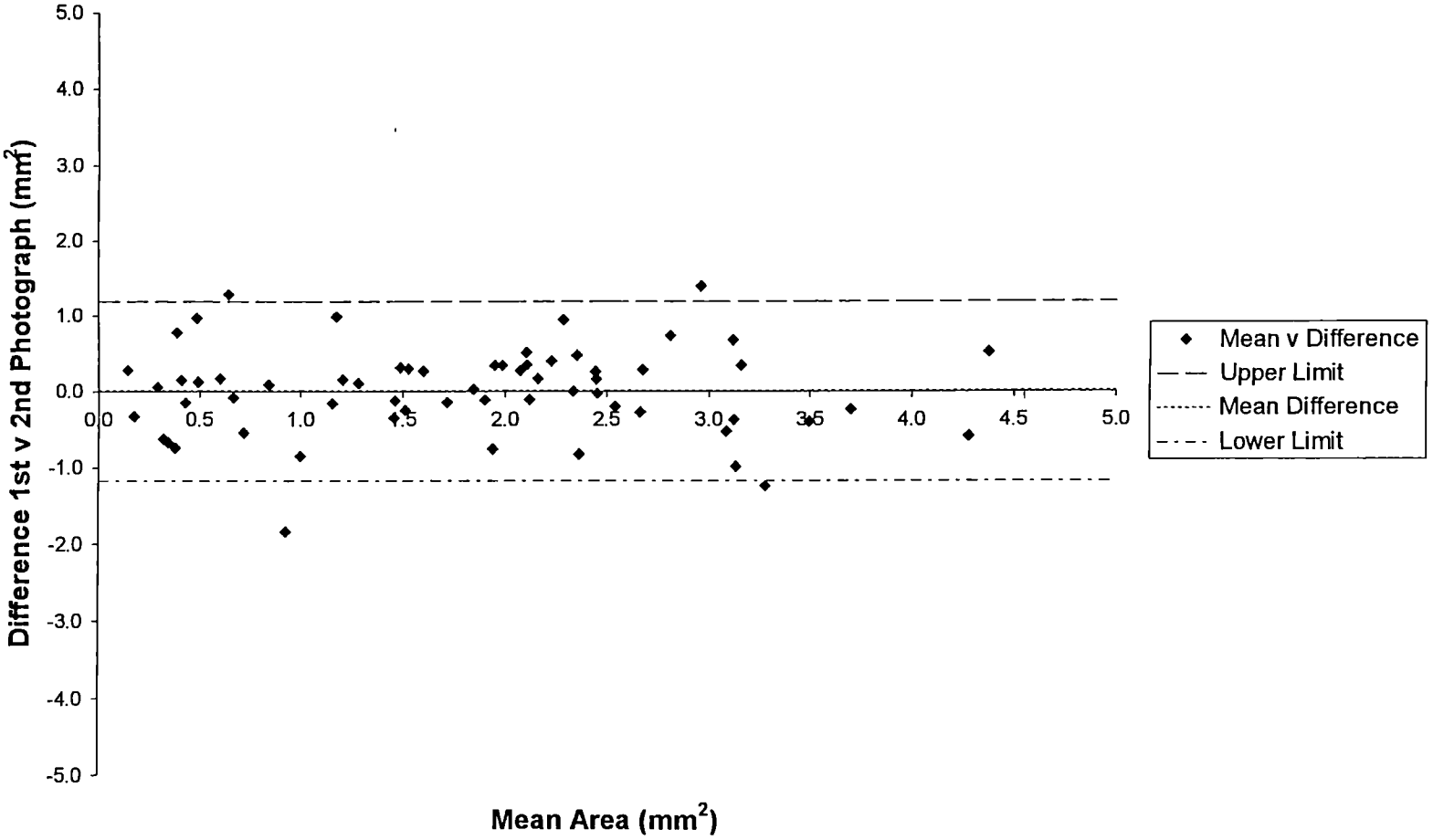


Figure 5.8

Limits of agreement for the areas of demineralisation recorded from the 1st and 2nd QLF images.

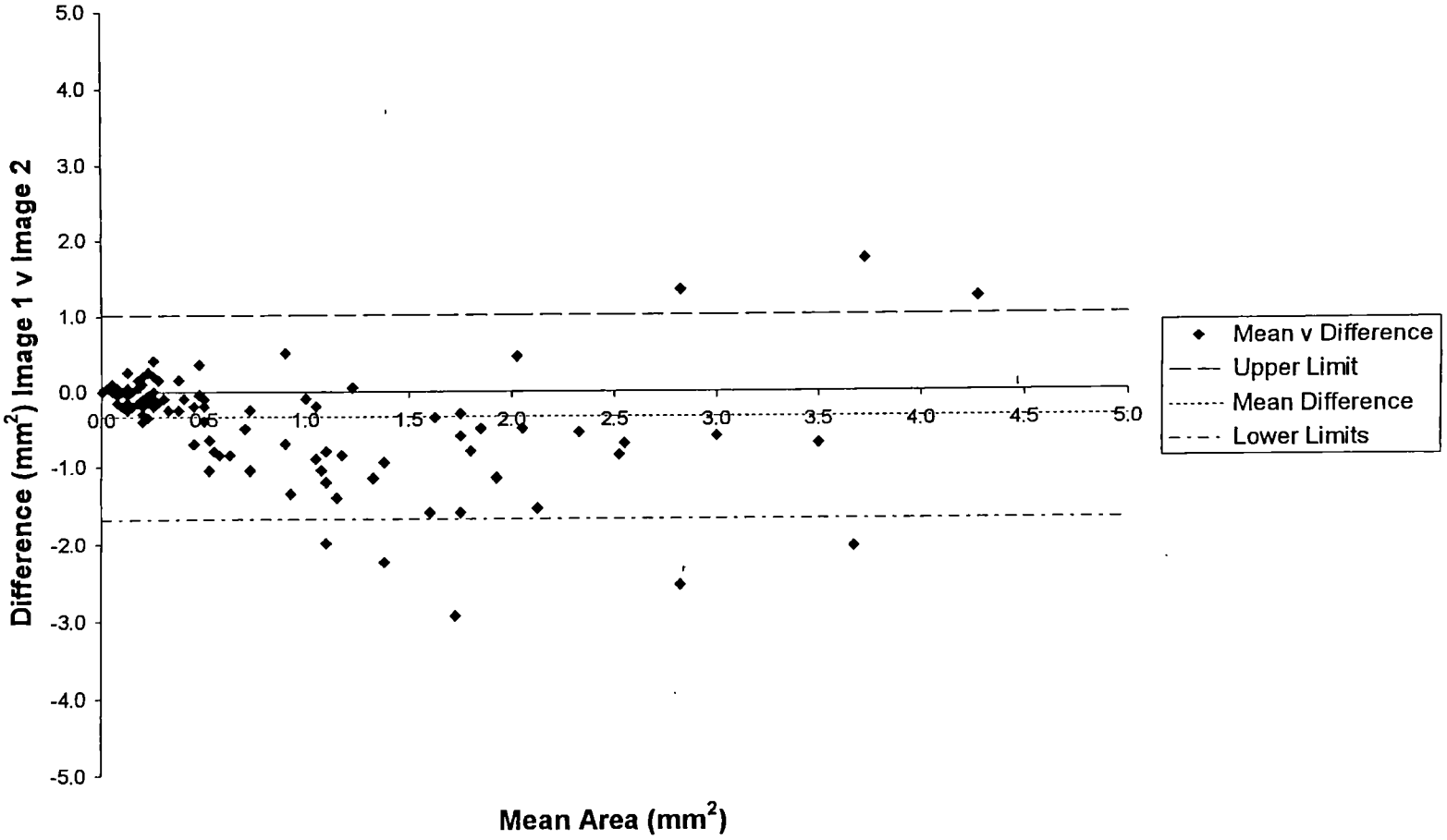


Figure 5.9

Limits of agreement using the logarithm$_e$ of the areas of demineralisation recorded from the 1st and 2nd QLF images.

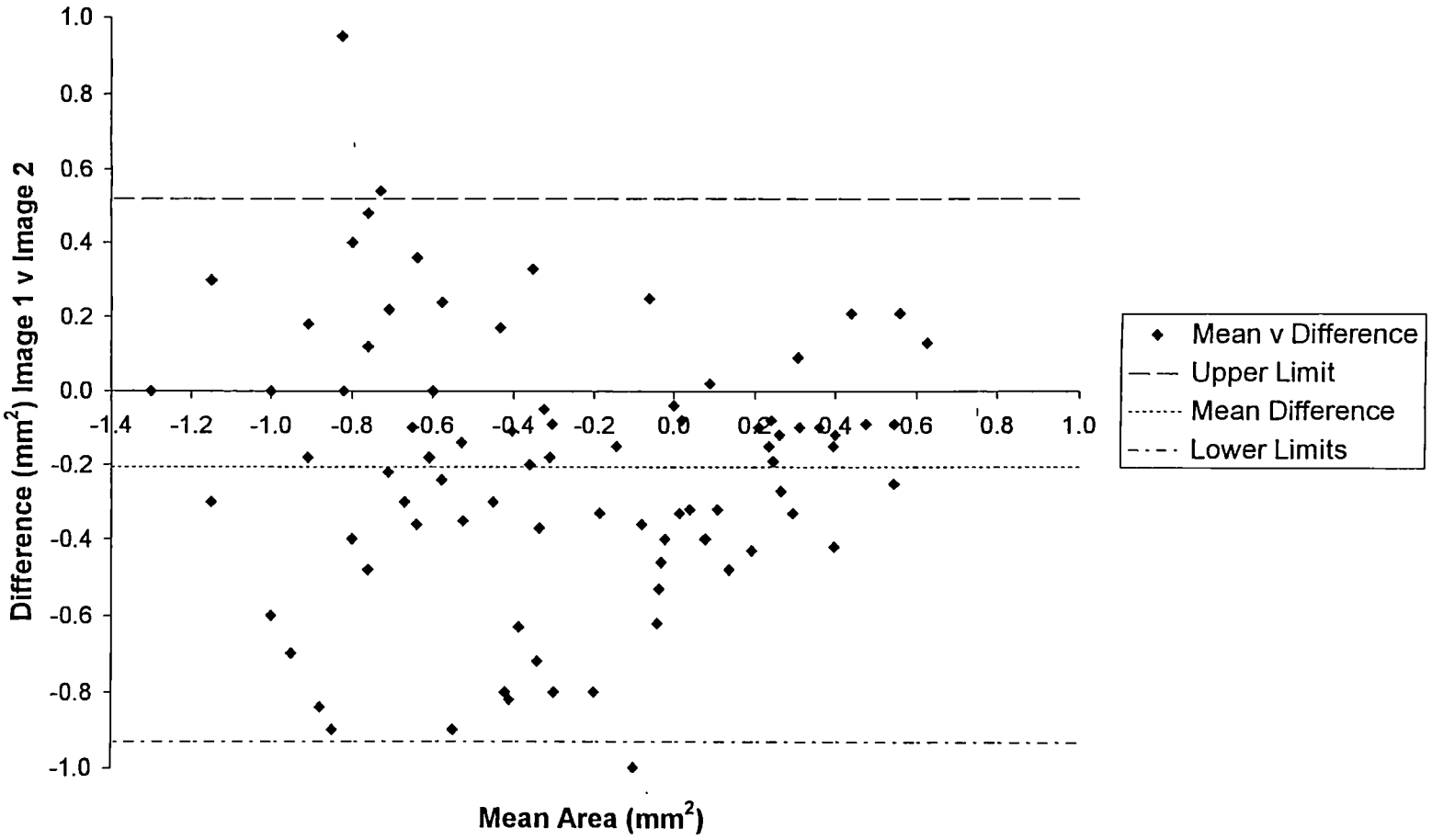


Figure 5.10

Limits of agreement using the logarithm$_e$ for the areas of demineralisation recorded from the photographic and QLF readings.

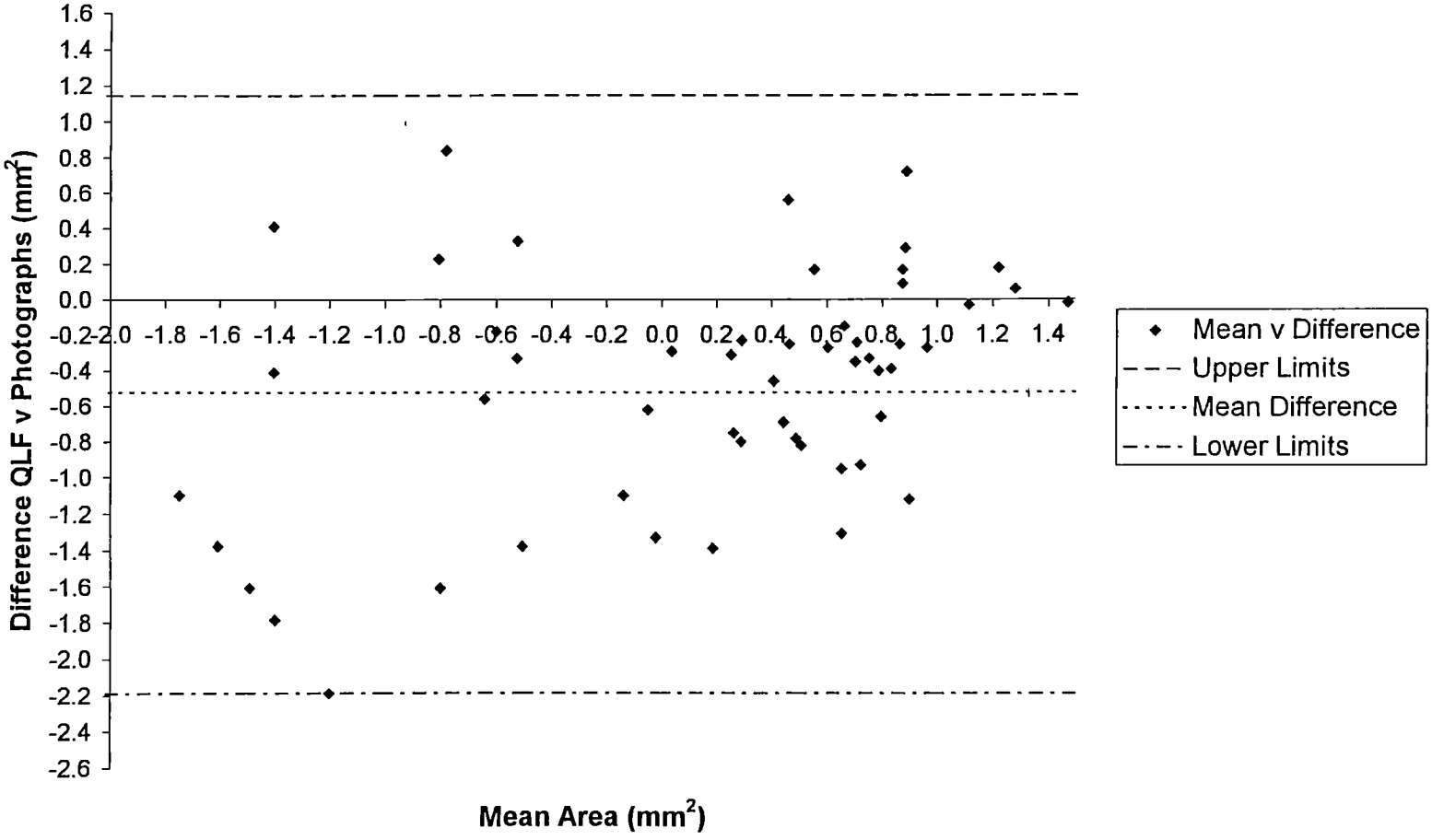
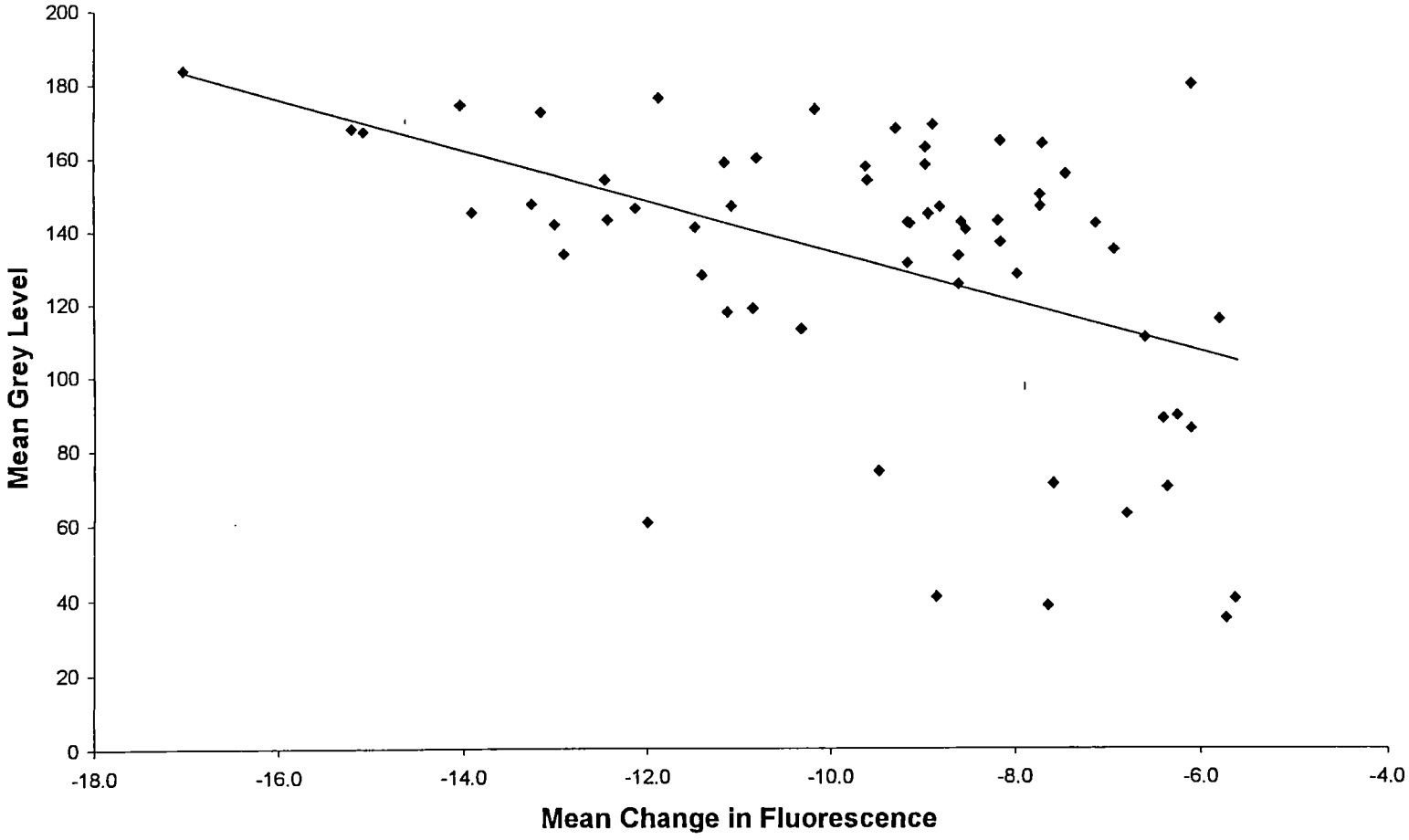


Figure 5.11

Scattergram of mean change in fluorescence (QLF) v mean grey level (photographs), including a regression line (N=62)



5.8 Appendices

5.8.1 Appendix D

Details of the Technique used in Image-Pro Plus to superimpose one image on another, subtract it and measure the difference in grey levels.

1. Open coded Image 1(Im1). Zoom in using right mouse button.
2. Draw an Area of Interest (AOI) around the buccal surface of the tooth and save it to the AOI manager as AOI Im1.
3. Draw an AOI around the bracket and save it to the AOI manager as AOI Im1b.
4. Adjust contrast of Im1; Process; Contrast Enhancement; click on Brightness symbol and use the cursor to increase the white area of the calibration grey scale to 255 and the black area to 0 using the readings in the before and after boxes (Curve type – Highlight and Shadow = linear).
5. Apply contrast and save image as Im1a.
6. Open coded Image 2 (Im2). And zoom in.
7. Adjust Im2 to same position as Im1a by: Process; Registration. Move the 4 points onto the 4 corners of the tooth positioned on Im1b as on Im2.
8. Transform Im2 onto Im1a.
9. Set AOI Im1 onto transformed Im2. If there is a good fit then save as Im2a. If not repeat (make sure AOI is off when image is saved otherwise the image will be cropped).
10. Close Im2.

11. Calibrate Im2a: Measure; Calibration; Spatial> Use the mesial and distal tie-wings of the bracket and calibrate to 3.35mm.
12. Draw an AOI around the bracket of Im2; add to AOI manager as AOI IM2b.
13. Alter the contrast of Im2 as previously using Process; Contrast Enhancement.
14. Set AOI Im1 onto both Im1a and Im2a.
15. Subtract Im1a from Im2a by using the following: Process; Operations; Subtract.
16. On the new image fill in the areas covered by the brackets by setting AOI Im1b and AOI Im2b then filling them as black (Edit; Fill).
17. To measure the grey scale levels click Measure; Count/Size. Click on Manual, then Select Ranges and choose PB Range file with the ranges described in Appendix E (page 5.43).
18. Make sure the Measure Objects box is ticked and the Apply Ranges and Add Count boxes are not ticked.
19. Click Measure; Select Measurements and make sure 'area' and 'class' are selected.
20. Click Options and make sure Outline style is 'Class-Filled' and Label Style is 'None'.
21. Click Count.
22. Click View and then Range Statistics and Measurement Statistics saving them to separate files.
23. Open the Range Statistics file in Excel and copy this to the spreadsheet.

24. Open the Measurement Statistics file in Excel and calculate the area measurements in SPSS (v8) as follows. Copy and paste the values from Excel into SPSS. Click Statistics; Compare Means; Means. Place Var 0001 in the dependent list and Var 0002 in the independent list. In the options box make sure Sum is transferred. Click Okay. Export the output file. Open the output file in Excel, then copy and paste to the spreadsheet.

5.8.2 Appendix E

Table E1

Range PB Bracket range used to measure grey levels in Image Pro Plus.

Area	Grey Scale Range
Area 1	1 to 20
Area 2	21 to 40
Area 3	41 to 60
Area 4	61 to 80
Area 5	81 to 100
Area 6	101 to 150
Area 7	151 to 200
Area 8	201 to 255

5.8.3 Appendix F

Settings for the Quantitative Light-Induced Fluorescence.

Lesion threshold discriminators

Level 1 95% Decrease

Level 2 55% Increase

Reconstruction borders all ticked

Inner patch box not ticked

Contour box ticked

Iso line Nos 2

Paint lesion in blue

Show result after reconstruction box ticked.

CHAPTER 6

An *In situ* Caries Model to Study Demineralisation During Fixed Orthodontics

6.1 Introduction and Aim

The use of the *in situ* caries model for the study of enamel demineralisation and remineralisation has been reviewed in section 2.5.4 (page 2.50). In summary, the method uses a standardised, pre-prepared enamel sample to test demineralisation and remineralisation within the oral environment. It has a number of advantages over other *in vivo* models.

The sample of enamel that is placed in the mouth is taken from a control with an artificial carious lesion. Following a suitable test period, the sample is removed from the mouth. Any change in the parameters of the lesion, either further demineralisation or remineralisation, can be measured and compared with the control, that has not been in the mouth.

The technique has the additional advantage in a study on orthodontic patients, that the experimental procedures may be carried out at routine appliance adjustment appointments and so do not affect the patient's treatment. The *in situ* model may test conditions in the mouth at various stages of orthodontic treatment. Finally, because the patient's treatment is not delayed or affected by having the enamel sample, a crossover design of

trial can be employed, whereby several preventive regimes can be tested in the same individual at different times. They therefore act as their own control, increasing the power of the experiment.

In situ enamel samples have been placed in removable appliances (Øgaard and Rølla, 1992a), but as far as the author is aware to date, they have not been used to investigate fixed orthodontic appliances.

The aim of this study was to develop and test the use of the *in situ* caries model in the study of de- and remineralisation of dental enamel during orthodontic treatment with fixed appliances.

6.2 Materials and Methods

6.2.1 Preformed Enamel lesions

The preformed enamel lesions were prepared using a technique described by Leach *et al* (1989). Premolar teeth extracted for orthodontic purposes were collected and stored in distilled water containing a few grains of thymol. The teeth were carefully examined and those with signs of caries or damage to the enamel were excluded. Selected teeth were lightly abraded with fine abrasive paper to remove the outermost enamel and remnants of the pellicle from the buccal surface. The teeth were varnished with an acid resistant nail varnish except for a window approximately 12 x 2mm on the buccal surface. They were mounted on glass rods using inlay wax and immersed into an

acidified gel described in Chapter Three (section 3.8.1, Appendix A, page 3.29) (0.1M lactic acid, 0.1M sodium hydroxide and 6% hydroxyethylcellulose, pH 4.5) for seven days.

After withdrawal from the gel, the varnish was removed and the block of enamel containing the lesion was cut from the crown of the tooth, together with a margin of sound enamel, which had been under the varnish, above and below the lesion. The lesions were divided to give three sections of approximately 4mm x 2mm in size. One of the sections was retained as the baseline control and the remaining two sections were prepared as if they were to be placed in the mouth. The experimental and control lesions were sterilised by gamma irradiation with a dose of 4080 Grays over three days under a $Cobalt^{60}$ source. Amaechi *et al* (1999b) has established that this dose sterilises an enamel sample of bacteria, without causing discoloration or change in mineral loss values. A recent *in vivo* study (Kielbassa *et al*, 2000) has confirmed that irradiation does not have a significant effect on the de/remineralisation potential of an *in situ* sample, although this was a much lower dose of radiation than that advocated by Amaechi *et al* (1999b).

Following sterilisation the control samples were cut perpendicular to the surface and polished to give planoparallel specimens of approximately 100μm thickness. The sections were examined under polarised light microscopy for a subsurface lesion of even quality. Samples with evidence of surface lesions or lesions of poor quality were rejected. The control sections from each acceptable lesion were placed together with an aluminium stepwedge with

25μm steps, on high-resolution radiographic film (Kodak, Rochester, NY, USA). They were radiographed in a Phillips X-ray set with a copper target and nickel filter. The exposure time was 18 minutes at 25kV and 10mA. The anode film distance was 30cm.

The microradiograph images were developed and measurement of the lesion parameters carried on a computerised image analysis system (TMRW program version 1.22) using an algorithm developed by de Josselin de Jong *et al* (1987a). The mineral content of the sections were expressed as mineral loss (ΔZ), lesion depth (l_d), lesion width (l_w) and ratio ($\Delta Z/l_d$) (Figure 6.1, page 6.30).

6.2.2 Development of the Customised Enamel Specimen Holder

6.2.2.1 Method 1

Initially it was thought that the ideal position for the enamel specimen would be on the lower first molar as advocated by Manning and Edgar (1992). During orthodontic treatment bands, rather than brackets are placed on the first molars therefore the design had to be incorporated onto a band (Figure 6.2, page 6.31). The enamel was bonded to the mesh base of a gauze pad using composite resin (Concise®, 3M Dental Products, St Paul, MN, USA).). This was welded to a small piece of band tape that was be used to attach the gauze pad to the molar band. A ring of two layers of 0.8mm hard stainless steel wire was welded to the mesh of the base to protect the enamel specimen from toothbrush abrasion and other trauma.

The incorporation of Dacron gauze over the enamel specimen has been advocated (Manning and Edgar, 1992). This has two functions, firstly it prevents the enamel from being lost if it becomes detached from the metal base and secondly it attracts plaque over the enamel specimen. It was decided not to incorporate the gauze into the orthodontic model for two reasons. Firstly, the enamel would be in position for longer than the three or four weeks usually employed, therefore they would be held in place by a strong composite bond, unlike the standard technique that uses varnish. Secondly, it was an aim to try to ensure that the enamel was in the normal orthodontic environment and the encouragement of further plaque might alter this.

The customised holder, containing the enamel specimen bracket was sterilised by irradiation using the protocol advocated by Amaechi *et al* (1999b).

Four individuals, who were already undergoing orthodontic treatment, were chosen and consent obtained to test the method. Details are given in Table 6.1 (page 6.20). Alternate left and right bands were chosen. A lower first molar band was chosen and tried on the respective tooth. Before cementation with a glass ionomer cement the enamel specimen and bracket base were welded to the bracket of the molar band. Adjustments were made to the orthodontic appliance and the patient given an appointment for six weeks time. No special instructions were given to the patient regarding the specimen to simulate the normal orthodontic environment. At six weeks the

patient was seen and questioned as to the comfort of the specimen. The band was removed and a new band cemented. If the specimen had been lost, a note was made of the site of failure.

6.2.2.2 Results of Method 1

The patient details and fate of the enamel are given in the Table 6.1 (page 6.20). The recovery rate was poor. It was noticed that the site of failure was at the enamel/bracket interface. There may have been a number of reasons for the failure of this method:

1. The specimen was very prominent being placed on top of the bracket. This led in one case to extreme discomfort and possibly contributed to the loss of other specimens. It also made the enamel susceptible to possible toothbrush abrasion.
2. Irradiating the band after bonding may have weakened the composite bond.
3. Some specimens had a residue of dentine on their bonding surface. The composite used would not have bonded to this and this would have led to a weaker bond.

The results for the recovered specimens are given in Table 6.2 (page 6.21). It is noted that specimen PA13 (patient 1), which was the removed after approximately two weeks showed some remineralisation. Specimen PA14b (patient 4) lasted the full six weeks and no change was observed.

6.2.2.3 Method 2

After the unsatisfactory results of the first pilot study the design of the bracket was radically altered (Figure 6.3, page 6.32). The bracket was designed to be placed on the archwire rather than the molar band (for full details see Appendix G, page 6.35). This has a number of advantages:

1. This reflects more accurately the position of the bracket in the arch
2. The specimen is protected from trauma and toothbrush abrasion
3. This was a more comfortable design for the patient
4. It is more easily placed and removed

Problems

Firstly, design of the customised bracket holder was more complex and construction more time-consuming. Secondly, the ballhooks used are rectangular with internal dimensions of 0.021 inch x 0.025 inch. They fitted neatly on the second levelling archwire, which is generally a 0.018 inch x 0.025 inch rectangular wire. However, there was particular interest in the initial stages of orthodontic treatment, when a round archwire of diameter 0.014 inch is used. The bracket rotates and slides freely on this wire which is uncomfortable for the patient. Initially it was thought that this could be overcome by crimping the hook to the archwire. The disadvantage of crimping the bracket means that it can only be used for one, six-week visit.

The second way of tackling the problem of the rotating holder was to design the holder with a loop for attaching an elastic chain or stainless steel ligature to the adjacent bracket.

Following the first pilot study, which established that the bond between the enamel and the bracket was found to be weak, the enamel specimens were fully prepared for the mouth before irradiation. All three sections including the control were irradiated. The control specimen was then ground to 80 μm and examined under polarised light microscopy to assess the suitability of the lesion. Satisfactory specimens were bonded to the bracket with a material that bonds to dentine and enamel (Scotchbond Multi-purpose®, 3M Dental Products, St Paul, MN, USA).

A second pilot study was carried out using the new design of bracket. The details of the patients and sites are given in Table 6.3 (page 6.22). This time the harvest rate was much more satisfactory. The specimen that was lost was due to a failure of a solder joint between the ballhook and the gauze pad and more care was taken in preparing this joint.

The results of the second pilot study are given in Table 6.4 (page 6.23). Patient three, who had two enamel specimens, showed some remineralisation of the lesions. After questioning, it was apparent that he regularly used a fluoride mouthwash, as well as a fluoridated toothpaste. Patient one showed further demineralisation. She was also patient 4 in the first pilot study when the lesion showed no change in mineral content.

6.2.3 Experimental Procedures

Following the second pilot study it was decided to test the use of the *in situ* caries model using the successful customised enamel specimen holder. The principal outcome of interest was the difference in the parameters of an artificial enamel lesion among three samples. The first sample was bonded with a small bracket base; the second was without the bracket, but placed in the mouth of the same individual at the same time. The third was a control that had not been placed in the mouth.

Secondary outcomes of interest were changes in these parameters between the dominant (toothbrushing hand) side and non-dominant side and the change in the parameters with length of time the sample was left *in situ*.

The design of the experiment was such that each subject required four samples. Each prepared tooth yielded three sections (two experimental and one control). The subjects therefore required samples from two prepared teeth. The controls of the two samples in each patient were carefully matched according to their mineral loss, as it has been shown that the baseline lesion mineral loss may affect the demineralisation properties of the sample (Strang *et al*, 1987).

The experimental enamel lesions were mounted onto customised holders (Figure 6.4, page 6.33) using a dentine and enamel primer with a light cured composite resin (Prime and Bond/Prismafil, Dentsply De-Trey-Strasse 1, D78467, Konstanz, Germany). The small bracket base, of approximate size

1.5mm x 1.5mm, was constructed from a larger orthodontic molar bracket base (American Orthodontics, 1714 Cambridge Avenue, Sheboygan, WI 53081, USA). It was bonded to the enamel sample with an orthodontic composite resin (Concise®, 3M Dental Products, St Paul, MN, USA) according to the manufacturer's instructions. Excess of material was removed from around the bracket edges with a sharp probe. A small bracket base was used to simulate the enamel environment around an orthodontic bracket, but without the bulk.

6.2.3.1 Subjects

Ethical approval for the study was obtained from the Local Ethics Committee. The subjects for the trial were selected from those individuals about to undergo fixed orthodontic treatment in the Orthodontic Department of Liverpool Dental Hospital and who required orthodontic extractions as part of their treatment. The latter requirement was to ensure there was enough space to place the customised holders with the enamel sample (Figure 6.4, page 6.33). Written consent of all patients and parents agreeing to participate was obtained.

Statistical advice concerning sample size was obtained. Data from a previous experiment suggested a clinically relevant mineral loss of 300 vol%.μm with a standard deviation of 200 vol%.μm would give us sufficient power using 10 patients assuming a paired *t* test with $\alpha = 0.05$. It was decided to recruit fifteen individuals to allow for loss of samples or withdrawals from the study. The fifteen individuals consisted of nine females and six males. The median age was 13.5 years (range 12.3 years to 38.8 years).

The study was designed so that each patient acted as his or her own control. Each patient received four enamel sections. Two sections were placed at the start of treatment when the first archwire was placed. These were removed at the first adjustment visit (mean 52 ±15 days) and two new enamel sections were placed. These remained in the mouth for two adjustment appointments (mean 90 ±19 days).

The samples were placed in pairs. On the surface of one sample in each pair was bonded a small bracket base to mimic the environment of the conventional orthodontic bracket. The other sample had no bracket. The customised holder was placed on the archwire of the lower orthodontic appliance in the extraction site (Figure 6.4, page 6.33). It was secured with a stainless steel ligature, to prevent rotation. One holder was placed in the left extraction site and one in the right.

The side containing the sample with the small bracket base was randomly allocated by a block randomisation technique to either be placed on the dominant (or toothbrushing hand) side or on the non-dominant side. All patients were instructed in the use of a fluoride toothpaste and fluoride mouthrinses, so that standardisation could be achieved.

6.2.3.2 Measurement of de- and remineralisation

Following removal from the mouth, the samples were taken off the customised holders with an orthodontic debonding instrument. The small bracket base was carefully removed from the bracketed samples. The

enamel was stored in distilled water, before preparing for microradiography. During the preparation the samples were cut perpendicular to the surface and polished to give between two to four plano-parallel sections of approximately 100μm thickness. After preparation, the samples were recoded by a second investigator to allow blind assessment by the principal investigator. Each patient had six samples (four experimental and two controls). All six samples were microradiographed on the same film, together with the calibrating stepwedge, to minimise random error due to problems with exposure and developing. The microradiographs were quantified by computerised image analysis. The parameters of the lesions, expressed as mineral loss (ΔZ), lesion depth (l_d), lesion width (l_w) and ratio ($\Delta Z/l_d$) were compared by statistical analysis (see below).

To investigate the reproducibility of the technique, five radiographs containing 30 samples were re-coded for a second blind assessment by the principal investigator two weeks after the first assessment.

6.2.3.3 Statistical Analysis

Each sample was cut into between two to four sections depending on the size of the original sample. All the sections were examined and a total of between three and five readings were taken to obtain a representative reading for that sample. The mean of these readings was then chosen for statistical analysis. All statistics were carried out using SPSS for Windows version 8 (SPSS Inc., 444 Michigan Avenue, Chicago, Il. USA).

Reproducibility

The index of reliability was calculated and a one sample *t* test carried out on the repeat readings to assess random and systematic error (Houston, 1983).

Hypothesis testing

The null hypothesis was that there is no difference in the changes to the lesion parameters amongst the three samples. The data was examined graphically and tested with a Shapiro-Wilk statistic to assess whether it had a Normal distribution. On two occasions the data was found to be skewed and was transformed to a Normal distribution. Hypothesis testing was carried out with a one-factor repeated analysis of variance. Multiple comparisons were carried out with a paired *t* test correcting for type I error by using the Bonferroni *t* (Maxwell, 1980).

To test the change in the parameters with length of time the sample was left *in situ,* the percentage change in the respective variables was calculated (Strang *et al*, 1987). This was carried out by dividing the sample value by the control value and multiplying by 100 (thus a value of 100 would signify no change, less than 100 would signify remineralisation and more than 100 would signify further demineralisation). Scatter plots were prepared of the change in the variable against the number of days the sample was in the mouth to examine for any association. The Pearson's product moment correlation coefficient was calculated to assess for any linear association.

6.3 Results

Fifteen patients were recruited for this investigation. One patient withdrew in the early stages, as he was unable to tolerate the intra-oral carrying device. Of the 56 samples placed in the mouth four samples, from two patients, were lost due to fracture of the archwires. Two samples, from one patient, were lost in processing. No samples were lost due to debonding of the sample from the bracket. Thus a total of 50 samples (25 with the simulated bracket and 25 without the simulated bracket) from 14 patients were analysed. Fifteen of the bracketed samples and ten of the non-bracketed samples were placed on the same side as the dominant toothbrushing hand. Conversely, ten of the bracketed samples were placed on the non-dominant side and fifteen of the non-bracketed samples.

The results of the reproducibility assessment are given in Table 6.5 (page 6.24) The index of reliability provides an indication of the proportion of the total error that is due to random error (Houston, 1983). If the random error is a large proportion of the total variability, a result that would have been significant without error may become non-significant (a Type II error). In this study the proportion of random error did not exceed 10 percent of the total variability.

The *t* tests for systematic error (Table 6.5, page 6.24) showed that for the lesion width (P=0.108) and ratio (P=0.178) there was no evidence of a systematic error between the first and second reading. However, for the

mineral loss (P=0.020) and lesion depth (P=0.035) there was evidence of systematic error at the five percent level. In both readings, the second recording was lower than the first. The variability of these readings was such as to suggest that this was unlikely to be responsible for a Type II error.

Table 6.6 and Table 6.7 (pages 6.25 and 6.26) show the means, standard deviations, confidence intervals of the means and the ranges of the four parameters. Table 6.6 refers to the control, bracketed and non-bracketed samples. Table 6.7 refers to the control, dominant, and non-dominant samples.

These descriptive statistics indicate that there was a trend toward reduction in mineral loss and ratio values in both the bracketed and non-bracketed samples, but this reduction was greater in the non-bracketed sample. The depth and width of the lesions did not show a similar reduction. The results were similar to the dominant and non-dominant statistics, the dominant samples showing the greater reduction. There was however a large variation both between and within individuals.

The analysis of variance between control, bracketed and non-bracketed samples (Table 6.8, page 6.27) showed a statistically significant difference (P=0.006) between the ratio values. The pairwise comparisons corrected for a type I error showed (Table 6.9, page 6.28) a significant difference between the control and the non-bracketed sample.

The analysis of variance between control, dominant and non-dominant samples (Table 6.8, page 6.27) also showed a statistically significant difference (P=0.013) between the ratios of the three groups. The pairwise comparisons (Table 6.9, page 6.28) showed a significant difference between the control and the dominant sample and the control and the non-dominant sample, but no difference between the dominant and non-dominant samples.

Figure 6.5 (page 6.34) shows a typical scattergram of the results of percentage change in mineral loss against the time the sample was in the mouth. No association is apparent. Table 6.10 (page 6.29) shows the Pearson's product moment correlation coefficients. There were no linear associations between change in any of the parameters with time.

6.4 Discussion

The aim of the present study was to investigate whether, within the oral environment of an orthodontic patient, a sample of demineralised enamel containing a simulated orthodontic bracket was at risk of further demineralisation, compared with a sample without a simulated bracket. Examination of the results on an individual basis demonstrated great variability both between and within patients. The overall trend was for remineralisation of both bracketed and non-bracketed samples; however only the non-bracketed sample showed a statistically significant reduction in the ratio compared with the control.

The ratio is calculated by dividing the estimated mineral loss by the lesion depth (Figure 6.1, page 6.30). Arends *et al* (1987) consider the ratio to be an important parameter. They state that the ratio corresponds to the average amount of mineral that is absent or has been lost in a section, therefore it also represents the average amount of mineral loss from an average enamel prism. The caries process starts with diffusion of mineral from the prism periphery (Haikel *et al*, 1983). Small ratio values suggest loss of interprismatic mineral, whereas large ratio values suggest loss from the prism surfaces, breakdown of prism structure and cavitation.

The results of this study show that the average mineral loss was significantly lower for the non-bracketed sample than for the control. This suggests that there was significant remineralisation for the non-bracketed sample. There was no difference between the average mineral lost between the control and the bracketed sample, although there was notable individual variation and in a few cases there was significant further demineralisation. The largest average mineral loss ratio for a bracketed sample was 24 vol%. This is well below the figure of 36 vol% which Arends *et al* (1987) suggest is the point at which there may be collapse of the prism structure and cavitation.

In the present study, the interest was in examining the conditions that were present in a representative group of patients with fixed appliances. All patients were instructed in the use of a fluoride toothpaste and mouthwash, however neither was provided and no attempt was made to measure compliance. The reduction of demineralisation during orthodontic treatment by the use of fluoride

has been demonstrated (Boyd 1993, 1994). The advantage of the *in situ* caries model is that both samples were tested, at the same time, under the same conditions. If the patient did not comply with the mouthwash instructions then both samples would be equally affected. It was the difference between the two samples that was of interest, as well as the difference between these samples and a control sample that had not been placed in the mouth. This study found that although there was considerable individual differences there were no statistically significant differences between the bracketed sample and the control.

Investigation into the affect of dominant versus non-dominant placement of the samples showed no difference between the two sides. There was a significant reduction in average mineral loss whether the sample was placed on the dominant toothbrushing side or the non-dominant side.

Examination of the effect of length of time the sample was left *in situ* with any of the parameters measuring de/remineralisation showed no relationship. This is contrary to the findings of Øgaard *et al* (1988c) and Arends *et al* (1992). Øgaard *et al* (1988c) used the orthodontic banding model to look at demineralisation when the band was left for four, six or eight weeks. They found an approximately linear relationship between enamel demineralisation and the time the band was left *in situ*. The orthodontic banding model has been discussed elsewhere. This is an excellent model for the study of demineralisation under a loose or poorly fitting orthodontic band. It will not accurately represent the environment of a bracket, which may be subjected to

intermittent cleaning. Arends *et al* (1992) examined the rate of enamel demineralisation *in situ* and found a linear relationship between both lesion depth and mineral loss, with demineralisation periods of four and eight weeks. The results of this study would suggest that enamel might be at risk of demineralisation any time during orthodontic treatment. The clinician must therefore be vigilant throughout treatment in monitoring the patient for signs of demineralisation.

6.5 Conclusions

1. The *in situ* caries technique can be used as a model to investigate demineralisation with fixed orthodontic appliances. The advantages over other *in vivo* techniques are discussed
2. When orthodontic patients were given instructions in the use of a fluoridated toothpaste and mouthwash, there was no increase in the demineralisation of an artificial enamel lesion with a simulated orthodontic bracket, compared with a control.
3. A bracketed sample showed reduced remineralisation in the oral environment compared with a similar enamel sample without a simulated bracket and in some cases further demineralisation was seen.
4. There was no relationship between the side the sample was placed and demineralisation.
5. There was no relationship between the length of time the sample was in the mouth and the extent of demineralisation.

6.6 Tables

Table 6.1

Details of patients and fate of specimens from pilot study method 1.

Patient Nos	Sex	Age (yrs)	Position of Band	Fate of Specimen
1	M	18	Lower left first molar	Patient in a lot of discomfort specimen removed by GDP
2	M	16	Lower left first molar	Specimen became loose, patient lost it
3	F	14	Lower right first molar	Specimen lost without the patient's knowledge
4	F	29	Lower right first molar	Specimen recovered

Table 6.2

Results for pilot study 1 for control (c) and test (t) specimens from 2 patients with calculated parameters of mineral loss (ΔZ, vol%.μm), lesion depth (L_d, μm), lesion width (L_w, μm) and ratio (vol%).

Patient	ΔZ	L_d	L_w	Ratio
1c	2110.4	55.8	36.0	37.8
1c	1445.6	29.1	21.6	49.8
1c	1611.9	38.1	29.9	42.3
1t	831.4	43.4	30.8	19.1
1t	507.0	30.1	18.5	16.9
1t	851.4	44.8	32.4	19.0
4c	896.6	38.3	31.9	23.4
4c	1052.0	47.5	34.9	22.1
4c	1118.6	40.4	32.9	27.7
4t	1078.0	38.3	24.7	28.1
4t	661.6	32.1	22.6	20.6
4t	999.8	34.0	20.8	29.4

Table 6.3

Details of patients and fate of specimens using method 2

Patient Nos	Sex	Age (yrs)	Position of Band	Fate of Specimen
1	F	29	Lower left side between 4 & 5	Specimen recovered
2	F	18	Lower right side between 4 & 5	Specimen became loose, patient lost it
3	M	16	Lower right and left sides between 3 & 5	Specimens recovered

Table 6.4

Results for pilot study 2 for control (c) and test (t) specimens from 2 patients with calculated parameters of mineral loss (ΔZ, vol%.μm), lesion depth (L_d, μm), lesion width (L_w, μm) and ratio (vol%).

Patient	ΔZ	L_d	L_w	Ratio
3c	1539.4	44.4	30.4	34.7
3c	1582.2	59.9	43.2	26.4
3t	1098.2	49.6	36.0	22.1
3t	964.5	50.6	37.0	19.0
3t	1209.9	53.7	39.1	22.5
1 & 3c	1320.0	57.0	34.2	23.1
1 & 3c	1143.6	53.1	35.7	21.5
1 & 3c	1155.3	54.7	37.4	21.1
1 & 3c	1478.3	53.9	31.5	27.4
3t	1507.1	63.0	50.4	23.9
3t	1371.1	53.7	40.1	25.5
3t	1204.6	50.6	38.0	23.8
1t	2149.3	63.0	44.8	34.1
1t	3168.8	46.5	34.9	68.1
1t	3251.5	58.9	49.3	55.2

Table 6.5

Descriptive and reproducibility statistics for the repeat measurement of mineral loss (ΔZ, vol%.μm), lesion depth (L_d, μm), lesion width (L_w, μm) and ratio (vol%).in 30 samples, where the index of reliability test for random error and P is the significance of a one sample *t* test for systematic error (n = 30).

	Mean Difference	*sd*	95% Confidence Interval	Index of Reliability	P
ΔZ (vol%.μm)	51.8	113.3	8.6 – 94.8	93.4	0.020
L_d (μm)	2.0	4.8	0.2 – 3.8	91.1	0.035
L_w (μm)	1.3	4.3	-0.3 – 2.9	92.4	0.108
Ratio (vol%)	0.37	1.4	-0.2 – 0.9	93.2	0.178

Table 6.6

Means, standard deviations, confidence intervals (CI) and ranges for control, bracketed and non-bracketed samples parameters of mineral loss (vol%.μm), lesion width (μm), lesion depth (μm) and percentage mineral loss (vol%).

Parameter	Statistic	Control (n=25)	Bracket (n=25)	Non-bracket (n=25)
ΔZ (vol%.μm)	Mean	803.3	807.7	750.4
	sd	296.1	445.8	400.2
	95% CI	681.1 – 925.5	623.7 – 991.7	585.2 – 915.6
	Max	1392.3	2006.7	1706.9
	Min	440.7	367.9	181.5
L_d (μm)	Mean	50.1	53.5	52.5
	Sd	8.1	13.4	14.5
	95% CI	46.7 – 53.4	48.0 – 59.1	46.6 – 58.5
	Max	67.1	86.4	79.4
	Min	34.0	35.1	23.4
L_w (μm)	Mean	39.1	39.6	39.3
	Sd	8.9	14.1	13.5
	95% CI	35.4 – 42.8	33.8 – 45.4	33.7 – 44.8
	Max	57.6	73.7	68.1
	Min	20.1	23.2	14.4
Ratio (vol%)	Mean	15.8	14.2	13.5
	Sd	4.4	4.5	4.6
	95% CI	14.0 – 17.6	12.4 – 16.1	11.6 – 15.4
	Max	23.9	24.8	24.6
	Min	9.8	8.5	6.4

Table 6.7

Means, standard deviations, confidence intervals (CI) and ranges for control, dominant and non-dominant samples parameters of mineral loss (vol%.μm), lesion width (μm), lesion depth (μm) and percentage mineral loss (vol%).

Parameter	Statistic	Control (n=25)	Dominant (n=25)	Non-dominant (n=25)
ΔZ (vol%.μm)	Mean	803.3	784.1	774.1
	sd	296.1	421.3	427.9
	95% CI	681.1 – 925.5	610.2 – 958.0	597.4 – 950.7
	Max	1392.3	2006.7	1706.9
	Min	440.7	181.5	217.2
L_d (μm)	Mean	50.1	53.1	52.9
	sd	8.1	13.5	14.3
	95% CI	46.7 – 53.4	47.5 – 58.7	47.0 – 58.8
	Max	67.1	86.4	80.8
	Min	34.0	23.4	27.8
L_w (μm)	Mean	39.1	38.9	40.0
	sd	8.9	13.1	14.4
	95% CI	35.4 – 42.8	33.5 – 44.3	34.0 – 45.9
	Max	57.6	73.7	72.4
	Min	20.1	14.4	19.3
Ratio (vol%)	Mean	15.8	13.9	13.8
	sd	4.4	4.3	4.8
	95% CI	14.0 – 17.6	12.1 – 15.7	11.8 – 15.8
	Max	23.9	24.8	24.6
	Min	9.8	7.7	6.4

Table 6.8

Results of one factor repeated measures analysis of variance for mineral loss (vol%.μm), lesion width (μm), lesion depth (μm) and percentage mineral loss (vol%) between control, bracketed and non-bracketed samples and control, dominant and non-dominant samples (n=25).

Samples	Parameter	F	P
Bracketed v Non-bracketed	ΔZ	0.48	0.622
	L_d	0.91	0.409
	L_w	0.02	0.976
	Ratio	5.75	0.006*
Dominant v Non-dominant	ΔZ	0.10	0.903
	L_d	0.84	0.438
	L_w	0.13	0.882
	Ratio	5.05	0.010*

* $P<0.010$

Table 6.9

Results of paired *t* tests and Bonferroni *t* between control, bracketed and non-bracketed samples (n=25) and control, dominant and non-dominant samples (n=25) for the ratio parameter.

Samples	*t*	Critical *t*	P
control v bracket	2.32	2.57	0.029
control v nonbracket	3.17	2.57	0.004*
bracket v nonbracket	1.10	2.57	0.282
control v dominant	2.69	2.57	0.013*
control v nondominant	2.80	2.57	0.010*
dominant v nondominant	0.14	2.57	0.891

* significant Bonferroni *t* .

Table 6.10

Pearson's product moment correlation coefficients (r) to assess linear correlation of percentage change in parameter with time for mineral loss (vol%.μm), lesion width (μm), lesion depth (μm) and percentage mineral loss (vol%)

	Parameter	Correlation Coefficient (r)	P
bracketed v time	ΔZ	0.293	0.156
	L_d	0.186	0.373
	L_w	0.125	0.553
	Ratio	0.181	0.386
non-bracket v time	ΔZ	0.022	0.915
	L_d	-0.086	0.683
	L_w	-0.176	0.401
	Ratio	0.126	0.550
dominant v time	ΔZ	0.120	0.567
	L_d	0.038	0.856
	L_w	-0.096	0.647
	Ratio	0.178	0.393
non-dominant v time	ΔZ	0.182	0.383
	L_d	0.024	0.911
	L_w	0.007	0.972
	Ratio	0.136	0.517

6.7 Figures

Figure 6.1

Diagram of a transverse microradiography plot showing the measurements used to calculate the parameters of mineral loss (ΔZ), lesion depth (l_d) and lesion width (l_w).

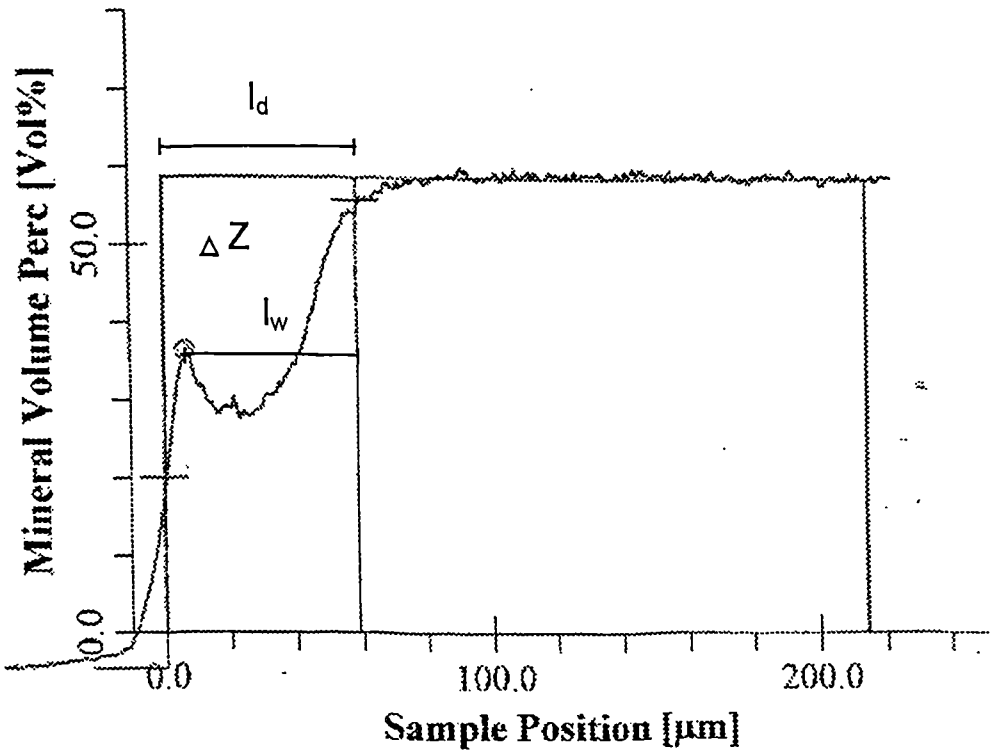


Figure 6.2

Diagram of the first enamel specimen holder designed for the pilot study 1.

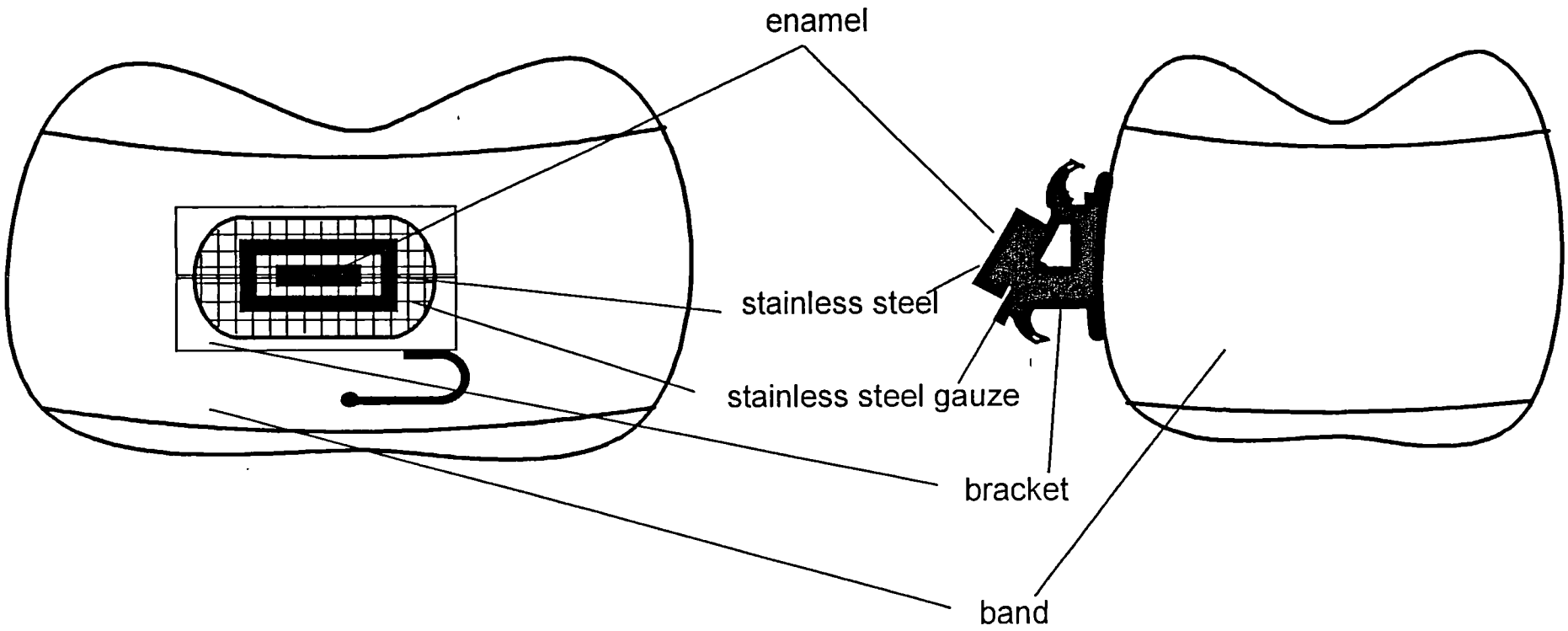


Figure 6.3

Diagram of the enamel specimen holder design for pilot study 2.

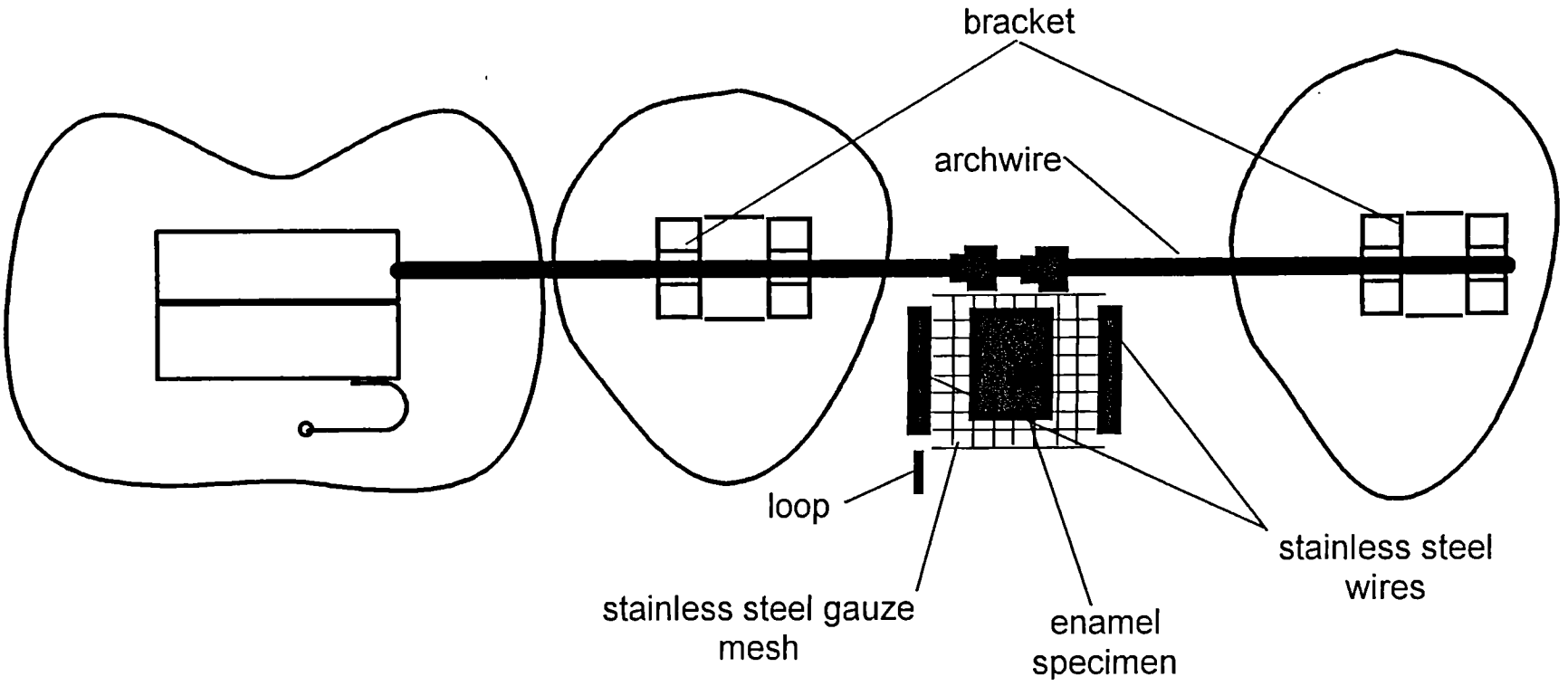


Figure 6.4

Image of customised *in situ* enamel specimen holder with enamel specimen and orthodontic bracket base.

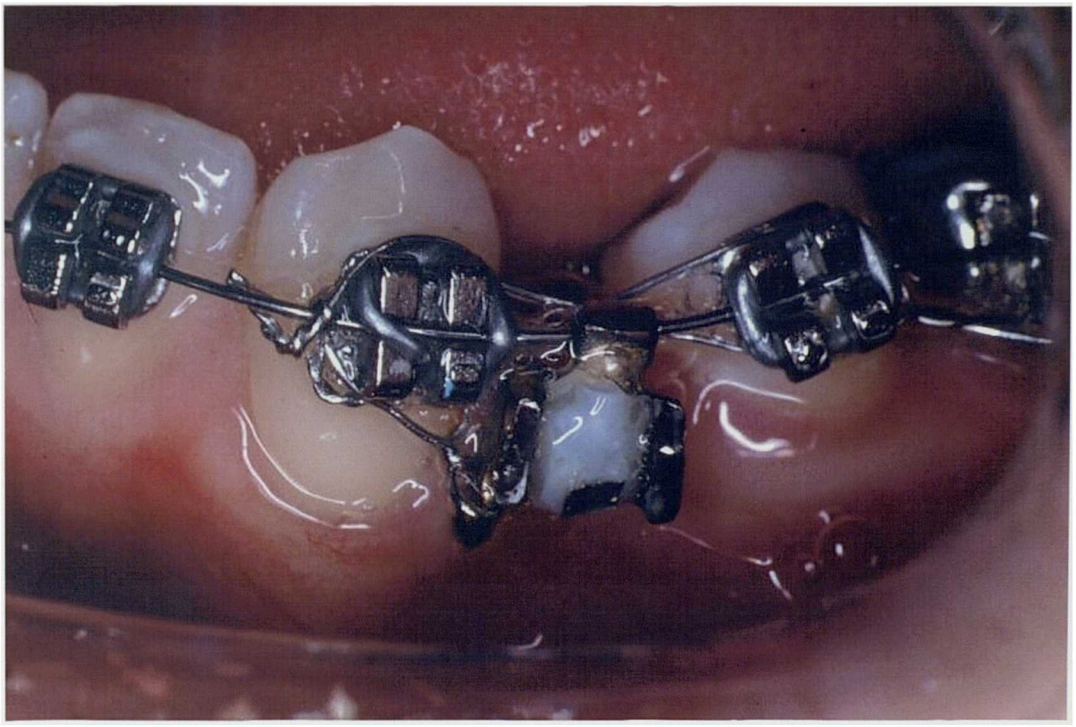
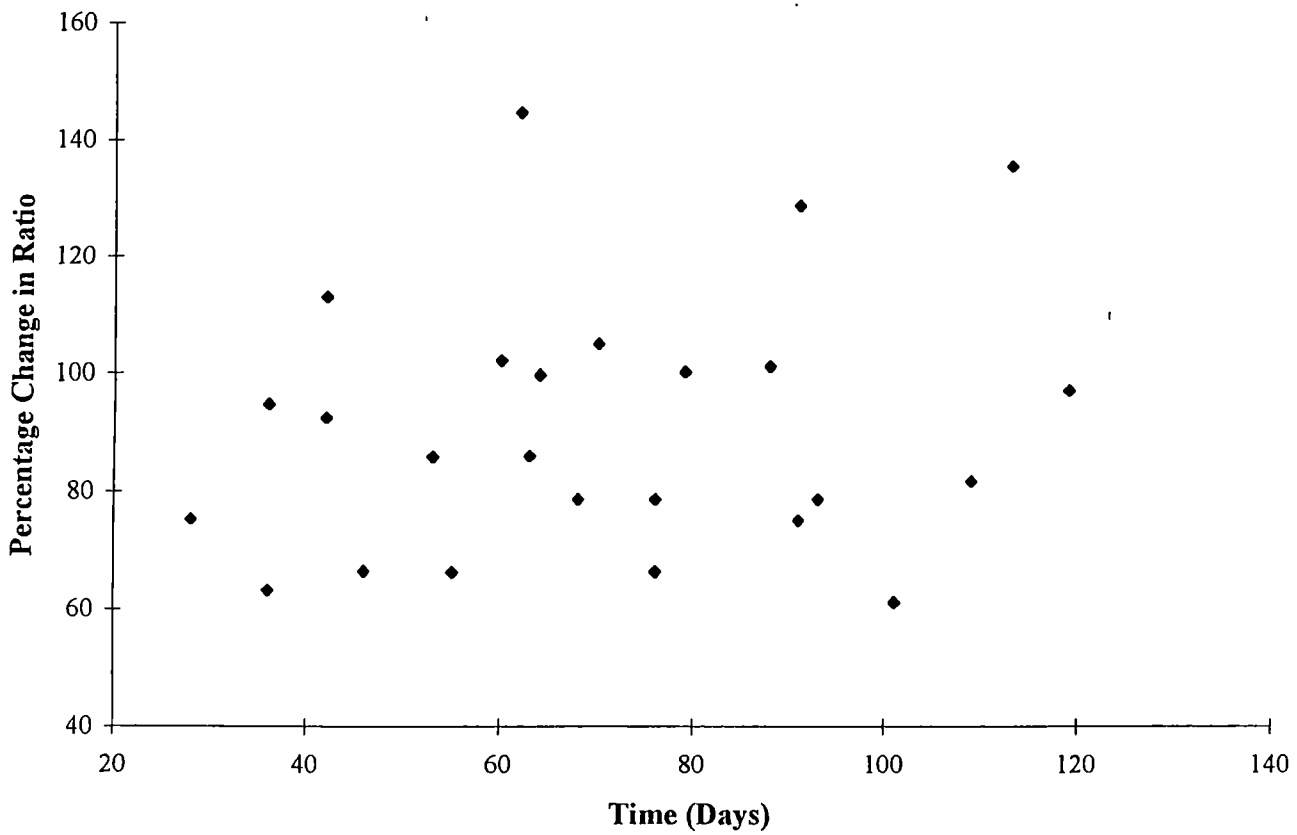


Figure 6.5

Scattergram of change in ratio with time for bracketed specimens (r=0.181).



6.8 Appendices

6.8.1 Appendix G

Manufacture of the *in situ* Enamel Holder

1. Use a 2mm cast slide-on surgical hook with ball hook (American Orthodontics, 1714 Cambridge Avenue, Sheboygan, WI 53081, USA) with internal dimensions 0.021" x 0.025".

2. Place a full size 0.021" x 0.025" stainless steel rectangular archwire through the slide on hook. This makes handling easier and ensures that solder does not flow into the box.

3. Spot-weld the hook to the back of a stainless steel molar bracket base (American Orthodontics, 1714 Cambridge Avenue, Sheboygan, WI 53081, USA), trying to avoid damage to the mesh. It should be placed approximately in the middle, with the box running parallel with the flat surface of the mesh and sufficiently clear to allow free running of the archwire.

4. Solder the hook to the bracket base, ensuring that solder does not run over the mesh and the wire is still running freely in the box. Discard if this is not the case.

5. Spot-weld two pieces of 0.8mm diameter hard stainless steel orthodontic wire to each end of the bracket base, running parallel to the long arm of

the hook. Solder these to the bracket base, again ensuring that no solder flows onto the mesh and discard if it does. (This was initially done to protect the enamel sample, but no enamel samples were lost during the experiment, so this step may not be necessary).

6. Grind with a green stone and polish with a rubber to produce an acceptable appearance, then place in an ultrasonic cleaner bath to remove any residual debris.

7. Enamel specimens were bonded to the bracket with dentine and enamel primer with a light cured composite (Prime and Bond /Prismafil, Dentsply, Konstanz, Germany).

8. The specimens were carefully removed by sqeezing gently at the composite/bracket interface with a narrow debonding instrument such as the Ormco straight debonding plier. These have the advantage of including an adjustable screw so that the beaks cannot be approximated too rapidly. Only gentle force was applied and if there was any resistance, the bracket base was bent to break the enamel composite bond.

6.8.2 Appendix H

Mean or Median

It could be argued that taking the mean of three readings from one section to provide an overall reading that is representative of that section, is not a legitimate statistical technique, as this is not a discrete reading but a manufactured one. If this were the case the median would be more statistically sound, as it is an actual recorded figure. Statistics were carried out on both the mean readings and the median readings. The results mineral loss and ratio for the bracketed and non-bracketed samples are shown in Tables G1 and G2. The results were the same

Table G1

Table comparing the results from taking a mean of the three recordings for each section compared with the median reading. Means, standard deviations, confidence intervals (CI) and ranges are quoted for the control, bracketed and non-bracketed samples parameters of mineral loss (vol%.μm) and percentage mineral loss (vol%).

		Means				Medians		
Parameter	**Statistic**	**Control (n=25)**	**Bracket (n=25)**	**Non-bracket (n=25)**	**Statistic**	**Control (n=25)**	**Bracket (n=25)**	**Non-bracket (n=25)**
ΔZ (vol%.μm)	Mean	803.3	807.7	750.4	Mean	792.4	764.8	734.7
	sd	296.1	445.8	400.2	*sd*	302.9	392.6	414.4
	95% CI	681.1– 925.5	623.7– 991.7	585.2– 915.6	95% CI	667.7 - 917.4	602.7 - 926.9	563.7 - 905.8
	Max	1392.3	2006.7	1706.9	Max	1400.3	1676.5	1809.3
	Min	440.7	367.9	181.5	Min	397.5	362.4	176.75
Ratio (vol%)	Mean	15.8	14.2	13.5	Mean	15.7	14.0	13.4
	sd	4.4	4.5	4.6	*sd*	4.4	4.3	4.7
	95% CI	14.0 – 17.6	12.4 – 16.1	11.6 – 15.4	95% CI	13.9 - 17.5	12.2 - 15.8	11.4 - 15.3
	Max	23.9	24.8	24.6	Max	23.8	23.8	24.2
	Min	9.8	8.5	6.4	Min	9.9	8.3	6.4

Table G2

Table comparing the results from taking a mean of the three recordings for each section compared with the median reading. Results of one factor repeated measures analysis of variance for mineral loss (vol%.μm) and percentage mineral loss (vol%) between control, bracketed and non-bracketed samples and control.(n=25).

		Means		Medians	
Samples	**Parameter**	**F-statistic**	**P**	**F-statistic**	**P**
Bracketed v Non-bracketed	ΔZ	0.48	0.622	1.39	0.258
	Ratio	5.75	0.006**	5.74	0.006**

CHAPTER 7

Validation of the Acid-Etch Technique for Use in the *in situ* Caries Model

7.1 Introduction and Aim

The use of the *in situ* model to study the process of demineralisation around orthodontic attachments was investigated in the previous chapter. If the model is to have a clinical application, then acid etching of the enamel specimen and bonding of an attachment is required to produce an experimental model similar to the orthodontic environment. One criticism of using the acid-etch technique on the experimental specimen and not the control is that it could lead to mineral loss, which may invalidate the experimental sample in comparison with an non-etched control. The design of an experimental protocol that included an additional etched control would address this problem. However, if every *in situ* experiment needed two controls for each experimental sample, the scope of the experiments undertaken would be limited.

The aim of this study was to investigate whether mineral loss from enamel after acid etching can be detected using the technique of microradiography. The null hypothesis was that there would be no difference in mineral loss as measured by microradiography, between an area of enamel that had been subjected to the acid-etch technique and an area that had not. Because the

enamel used in the *in situ* model can be either intact or incorporate a lesion, the effect of acid etching on enamel both with and without a pre-existing enamel lesion was investigated as both have been advocated for use with the *in situ* model (Featherstone and Zero, 1992). The purpose of the pre-existing enamel lesion in the *in situ* model system is to allow study of both putative remineralisation and demineralisation with the same experimental conditions.

In the present experiment bovine enamel was used. The main advantage of bovine enamel is that the composition is less variable than human enamel and therefore, hypothetically a more consistent response would be expected (Mellberg, 1992). Bovine enamel also has the advantage that it has a large, relatively flat surface. Bovine enamel is more porous than human enamel leading to more rapid diffusion and lesion formation, therefore bovine enamel should show a significant level of mineral loss more readily than human enamel. For example, Edmunds *et al* (1988) found that with the same cariogenic challenge the depth of the bovine lesion was approximately twice that of the human enamel. Although the artificial carious lesions produced by acid gel in bovine and human teeth examined with a scanning electron microscope showed many similarities.

7.2 Materials and Methods

7.2.1 Specimen Preparation

Forty bovine incisor teeth were used in the experiment. They were extracted from the jaws of freshly culled cattle and stored in water with a few grains of thymol to prevent bacterial contamination. The teeth were assigned to two groups, each of 20 teeth. The allocation of each group is shown in Figure 7.1 (page 7.17).

The procedures carried out on the two groups of teeth are shown diagrammatically in Figure 7.1(page 7.17). The crowns of the teeth were covered with three layers of an acid resistant varnish, except for a rectangular area on the buccal surface. The teeth in Group I (GI) were not exposed to an initial period of demineralisation to produce a pre-existing enamel lesion. The teeth in Group II (GII) were prepared with a pre-existing enamel lesion. They were attached to glass rods and placed individually in 10ml of a 40mmol/L acetic acid/potassium hydroxide buffer (pH 4.5) containing 2.2mmol.L^{-1}. of calcium chloride and potassium dihydrogen phosphate and 0.026μmol/L of sodium fluoride. The solution was stirred at room temperature for 72 hours, when the teeth were removed. The teeth were thoroughly washed in distilled water, dried and a fresh coat of varnish was applied.

Following the production of the pre-existing carious lesion for GII, both groups of teeth were treated in the same way. A rectangular window on the

buccal surface of the tooth was surrounded by acid resistant varnish (Figure 7.1, page 7.17). One third of the exposed window on the buccal surface of the teeth was covered with three further layers of acid resistant varnish. The whole of the remaining exposed area was etched with 37% phosphoric acid for 30 seconds, then thoroughly washed for 15 seconds. The area was dried with compressed air for a further 15 seconds. To simulate the normal orthodontic environment a rectangular stainless steel bracket base incorporating a mesh for bonding purposes (American Orthodontics, 1714 Cambridge Avenue, Sheboygan, WI 53081, USA), was bonded to the left edge of the exposed area of enamel, in the centre of the original rectangular window. The base was contoured to the shape of the tooth surface and a no mix composite resin (Right-on®, TP Orthodontics, Inc., La Porte, Indiana, USA) was used. This positioned the base in the centre of the original exposed enamel window. Any excess composite was removed with a Ward's wax carver. The right portion of the exposed box was left uncovered and exposed.

The teeth were attached individually to glass rods and placed in a fresh preparation of the demineralising solution. The teeth were stored in the solution, which was stirred at room temperature, and subsequently removed at periods of 24, 48, 72 and 96 hours. The different time periods were chosen to represent varying increments of demineralising challenge.

After removal from the solution, the teeth were washed in distilled water. The acid resistant varnish was removed with acetone. The bonded bracket base

was carefully separated from the enamel with a sharp excavator, leaving the composite in position.

The crown of each tooth was sectioned from the root with an Isomet saw (Buehler Ltd, Evanston, Illinois, USA). The saw was then used to cut the crown longitudinally on the non-exposed side of the bracket base area leaving a margin for analysis (Figure 7.1, page 7.17). This was carried out so the sample could be orientated with the regions, in a set order, once it had been ground. The crown was then cut transversely with the Isomet saw into several sections. Each section was ground to 100μm, during which they were turned once to obtain a plano-parallel section. Three sections from each tooth were prepared, making a total of 15 sections for each sub-group and total of 120 sections for the two groups (Figure 7.1, page 7.17).

The sections were placed, in a known but random order, on a specimen holder that also contained an aluminium stepwedge, with 25μm steps. Each section was orientated with the flat edge, produced by the orientating saw cut, to the left. A diagram of the plate and the individual sections was produced. A high-resolution radiographic film (Kodak, Rochester, NY, USA) was placed in the specimen holder in a photographic dark room with a photographic safe red light. The specimens were radiographed in a Phillips X-ray set with a copper target and nickel filter. The exposure time was 18 minutes at 25kV and 10mA. The anode film distance was 30cm.

The microradiograph films were developed using a standard method. Both the film and diagram were re-coded by one investigator to allow for blind analysis by the principal investigator, who carried out all the assessments. The measurement of mineral loss (ΔZ) from each section, was carried out on a computerised image analysis system (TMRW program version 1.22) using an algorithm developed by de Josselin de Jong *et al* (1987a) and expressed as volume%.μm.

Three regions were measured for each section. The three regions were designated as follows:

C Control - the area next to the orientating flat surface, which was the area under the acid resistant varnish during the second stage of the experiment. This had not been exposed to the acid-etch technique (Figure 7.1, page 7.17; Region C).

E1 Experimental Area 1 - the area under the orthodontic bracket base (Figure 7.1, page 7.17; Region E1).

E2 Experimental Area 2 - the area that remained exposed throughout the experiment (Figure 7.1, page 7.17; Region E2).

Samples were rejected if the composite could not be visualised. Three readings of each area were taken and these were averaged to obtain a mean reading, which was taken to be representative of the whole area. The readings were made at a site distant from the edge of the region to avoid possible crossover of effects between treatments given to each region.

Three films, containing a total of 29 sections were randomly chosen for an error analysis. They were re-coded by one investigator, to allow a second blind assessment by the principal investigator, at a time interval of at least two weeks after the first measurement.

7.2.2 Statistical Analysis

Statistical analysis was carried out using SPSS for Windows version 8 (SPSS Inc., 444 Michigan Avenue, Chicago, Il. USA). The data was checked for Normality using frequency histograms of the differences between the groups, Normal Q-Q plots and the Shapiro-Wilk test (see section 4.8.2.2 Tests of Normality, page 4.25). The data were considered to be Normally distributed and therefore parametric statistics were applied.

The design of the experiment was a mixed design with both within sample and between sample factors. The within sample factors included the regions C, E1 and E2 that had been subjected to different conditions. The between sample factors included whether there was a pre-existing lesion or no pre-existing lesion and the length of time the exposed area was subjected to the demineralising solution. A multivariate analysis of variance was therefore carried out. The main independent variable of interest was the within sample factor. The dependent variable was mineral loss. A two-way analysis of variance was performed to assess the within sample factors. Paired *t* tests, with the Bonferroni correction (see section 4.8.2, Appendix C, page 4.22), were carried out to make pairwise comparisons between the regions.

To assess reproducibility a one sample *t* test was used to monitor any systematic error and the intraclass correlation coefficient of reliability between replicates was calculated to assess random error (Fleiss, 1988).

7.3 Results

The reproducibility results showed no systematic error and a low random error (Table 7.1, page 7.15).

Many of the specimens subjected to the 96 hour exposure to the demineralising solution showed evidence of cavitation making measurement of mineral loss using TMR difficult. Because of this cavitation only the results from the 24, 48 and 72-hour exposures are analysed. Several of the specimens were lost during processing or were rejected because the composite could not be visualised. The number of specimens analysed for each sub-group is shown in Table 7.2 (page 7.15). The means and 95 percent confidence intervals for the two groups are shown graphically in Figure 7.2 and Figure 7.3 (pages 7.18 and 7.19). Mean mineral loss was greater for GII, because they had undergone two periods of demineralisation. There was also greater variability in mineral loss in GII.

The multivariate analysis of variance was highly significant for both between ($P=0.002$) and within ($P<0.001$) factors. The results of the two-way analysis of variance are given in Table 7.2 (page 7.15). Only specimens with readings from all three regions were analysed. They show significant results for the within-group factors (between the regions) for the 48 ($P<0.001$) and 72 hour

(P=0.001) exposure regions in GI and the 24 (P=0.042) and 48 hour (P=0.009) exposure regions in GII.

The results of the pairwise comparisons are shown in Table 7.3 (page 7.16). This showed that for both the 48 and the 72-hour exposures in GI, there was a highly significant difference between the area under the acid resistant varnish (C) and the exposed area (E2) and between the area under the bracket base (E1) and the exposed area (E2). There was no significant difference between the area under the acid resistant varnish (C) and the area under the bracket base (E1). The GII results showed a reduced probability of a significant difference between regions. There were significant differences at the 5% probability level between C and E2 for the 24 hour exposure (P=0.047), C and E2 (P=0.035) and E1 and E2 for the 48 hour exposure (P=0.018) and between C and E1 (P=0.044) for the 72 hour exposure. However, if the Bonferroni correction were used to rule out a Type I error (see section 4.8.2.3, page 4.27), none of these results for GII were shown to be statistically significant.

7.4 Discussion

The present study detected no difference in mineral loss between the area of bovine enamel that had been under the acid resistant varnish (Figure 7.1, page 7.17; region C) and the area under the orthodontic bracket base (Figure 7.1, page 7.17; region E1). The difference in the treatment of these two zones was that region C had been protected from the acid-etch technique, whereas region E1 had been subjected to it. This suggests that it was not

possible to detect a significant amount of mineral loss from bovine enamel following etching with 37% phosphoric acid for 30 seconds using the technique of transverse microradiography.

In Chapter Six the use of the *in situ* technique to study demineralisation surrounding bonded orthodontic attachments was investigated. A difference was found between human enamel specimen placed in a patients' mouth, that had no orthodontic attachment and a specimen that had an orthodontic attachment bonded using the acid-etch technique. One interpretation of the result of this study is that the acid etching led to mineral loss in the experimental specimen. The difference in mineral loss between the two specimens during the experiment could therefore have been due to a difference at the start of the *in vivo* stage, rather than differences in the rate of remineralisation. The present study has shown that using transverse microradiography a significant amount of mineral loss was not detected as a result of the acid-etch technique, which in turn suggests that the difference in mineral loss between the two specimens in the *in situ* study may have been due to the remineralisation rate.

The results of this study tend to agree with that of Hall *et al* (1997b). They used transverse microradiography to detect acid erosion on human enamel specimens. They prepared sections of enamel and dentine 100-150μm thick. These were mounted on lead foil and covered with acid resistant varnish except for a small strip 0.4mm wide down the centre of the section, which was covered with adhesive tape. Once the varnish had dried, the strip was

removed. The specimens were exposed to 37 percent *ortho*-phosphoric acid at pH 3.0 for 0, 15 minutes, 30 minutes and 1, 2, 5, 12 and 24 hours. They then performed TMR on the specimens and carried out a microdensitometric scan. They were not able to detect significant mineral loss with an exposure time of 15 minutes in enamel, but they did in dentine. They were able to detect mineral loss in enamel specimens exposed for 30 minutes or greater.

Amaechi *et al* (1998) also employed TMR to detect the loss of mineral in bovine enamel following exposure to orange juice. They used a two-step image analysis approach as follows. An image of the erosive lesion together with the sound enamel was captured. An analysis box was used over the sound enamel to reconstruct the sound enamel surface. The box was then moved over the erosive lesion to measure mineral loss and lesion depth. There was considerable mineral loss when they immersed the teeth in orange juice for five minutes, six times a day for 24 days (total exposure of 12 hours). Mineral loss was worse when the teeth were stored in distilled water following the exposure, rather than artificial saliva. This two-step method would not have been appropriate for a study of etching because the edge effect may not have been representative of the area as a whole.

Figure 7.2 (page 7.18) shows a graph of the mean mineral loss and 95% confidence intervals for the teeth with no prepared lesion. The graphs for the C and E1 regions were very similar for all three exposure times, suggesting there was no difference in mineral loss between the area that had been acid etched (E1) and the area that had not (C). The mean mineral loss increased

for the exposed area (E2), although not in a linear way. There was an increased mineral loss between the 48 and 72-hour exposure specimens, compared with difference between the 24 and 48-hour exposures. It was noted that two of the 72-hour specimens could not be measured due to cavitation and several others approached it.

Figure 7.3 (page 7.19) shows a graph of the mean mineral loss and 95 percent confidence intervals for the teeth with a prepared carious lesion. It can be seen that there is an increase in the mean mineral loss after the initial exposure to the demineralising solution that produced a pre-existing enamel lesion. The confidence limits are wider reflecting the variability in mineral loss between the teeth exposed to the same demineralising environment. The graphs for the region C and E1 are similar for both the 24 and the 48-hour exposure times. The mean mineral loss for region C and E1 72-hour exposure is higher and the confidence limits wider even though these regions had the same exposure as C and E1 for the 24 and 48-hour regions. On closer inspection of the figures, it was noted that three specimens from one tooth displayed much greater mineral loss than the other specimens. When these were excluded the graphs for region C and E1 were similar to the graphs for the 24 and 48-hour exposure times.

The increased confidence limits for the mean mineral loss from the specimens with the pre-existing enamel lesion has an important effect on their sensitivity to further mineral loss. Table 7.3 (page 7.16) shows that when there is no pre-existing enamel lesion (Group I) there is a highly

significant difference between the regions that were exposed to the demineralising solution for 48 hours or more (E2) and those that were not (C and E1). In the teeth with a pre-existing lesion (Group II) the difference in mineral loss between the regions was not statistically significant when a correction was carried out to allow for a Type I error. In other words, the increased confidence limits for the mean mineral loss from the specimens with the pre-existing enamel lesion ensures that these specimens showed reduced sensitivity to further mineral loss after being placed in the demineralising solution.

These results agree with Mellberg (1992), who considers that the choice of whether to use a sample with or without a pre-existing enamel lesion will differ according to whether the study is investigating the factors affecting lesion formation or is investigating the effects of treatment on remineralisation. He states that the natural sound surfaces are useful for studying demineralisation, but not remineralisation, as variation in lesion severity even between areas of the same tooth is too great. He suggests ways of producing more consistent lesion formation and eliminating the problem of curved surfaces. These include abrading the tooth with 600-mesh or 120-mesh silicon carbide, which will remove the surface layer with larger crystallites and higher carbonate and fluoride concentrations. This may help for microdensitometric and hardness testing, but any advantage of using the natural surface is lost.

7.5 Conclusions

1. No significant detectable mineral loss was found when bovine enamel was exposed to the acid-etch technique.
2. An *in situ* human enamel sample with a simulated orthodontic bracket bonded to the surface using the acid-etch technique can reasonably be compared with a control sample that has not undergone this procedure.

7.6 Tables

Table 7.1

Tables showing the mean difference (vol%.μm), standard deviation and confidence intervals for the difference between the repeat readings of the specimens (N=29). Also shown is a one sample *t* test to assess systematic error and the intraclass correlation coefficient of reliability to assess random error.

Mean Difference (vol%.μm)	-14.0
sd	199.0
Confidence Intervals (vol%.μm)	-57.6 – 29.6
One sample *t* test (t)	-0.6
P value for *t* test	0.531
Intraclass correlation coefficient of reliability	0.911

Table 7.2

Table showing the results of the two-way analysis of variance to assess the differences between within sample factors (the regions) for Group I (GI) and Group II (GII) and for the different exposure times to the demineralising solution, where N is the number of specimens with readings from all three regions.

Exposure Times (hrs)	GI			GII		
	N	F	P	N	F	P
24	15	3.2	0.056	8	4.0	0.042
48	15	17.8	<0.001	14	5.7	0.009
72	13	18.4	0.001[+]	13	1.2	0.335

[+] more conservative statistic applied as heterogeneity of covariance detected.

Table 7.3

Results of the pairwise comparisons between the groups of teeth, which showed a significant difference in mineral loss (vol%.μm) for the within sample factors (where C = control under the acid resistant varnish, E1 = under the orthodontic bracket base and E2 = exposed throughout the experiment). The critical P with the Bonferroni correction in this experiment is 0.02.

Group (see Figure 7.1)	Regions Compared	Mean Difference	*sd*	Confidence Intervals	P
	C– E1	0.9	134.7	-73.7 – 75.5	0.979
GI24	C– E2	-176.2	384.0	-388.9 – 36.5	0.097
	E1 – E2	-177.1	355.9	-374.2 – 20.0	0.074
	C– E1	60.3	169.8	-33.7 – 154.3	0.191
GI48	C– E2	-323.5	306.4	-493.2 – -153.8	0.001
	E1 – E2	-383.8	304.1	-552.2 – -215.3	<0.001
	C – E1	-30.7	97.8	-87.2 – 25.8	0.261
GI72	C – E2	-1422.5	1176.5	-2133.5 – -711.6	0.001
	E1 – E2	-1412.1	1201.5	-2138.2 – -686.1	0.001
	C – E1	90.9	303.4	-101.9 – 283.6	0.322
GII24	C – E2	-290.6	340.6	-575.4 – -5.9	0.047
	E1 – E2	-174.5	716.1	-686.7 – 337.8	0.461
	C – E1	52.7	184.4	-49.4 – 154.8	0.287
GII48	C – E2	-203.7	323.9	-390.7 – -16.6	0.035
	E1 – E2	-254.2	351.9	-457.4 – -51.0	0.018
	C – E1	181.5	291.2	5.6 - 357.5	0.044
GII72	C – E2	54.9	584.3	-298.2 – 408.0	0.741
	E1 – E2	-126.6	404.3	-370.9 – 117.7	0.281

7.7 Figures

Figure 7.1

Flow diagram showing the design of the experiment with the two main groups, Group I (GI) without a pre-existing caries lesion and Group II (GII) with a pre-existing caries lesion. There are four subgroups with exposure times to the demineralising solution of 24, 48, 72 and 96 hrs. A total of 120 sections were produced. The crown was covered with acid resistant varnish (▨) except for a rectangular window on the buccal surface. One experimental region (E1=▨) was covered with an orthodontic bracket base. A second experimental region (E2=□) was left exposed. A control region (C=▩) was coated with acid resistant varnish either after an initial period of demineralisation (GII) or no demineralisation (GI).

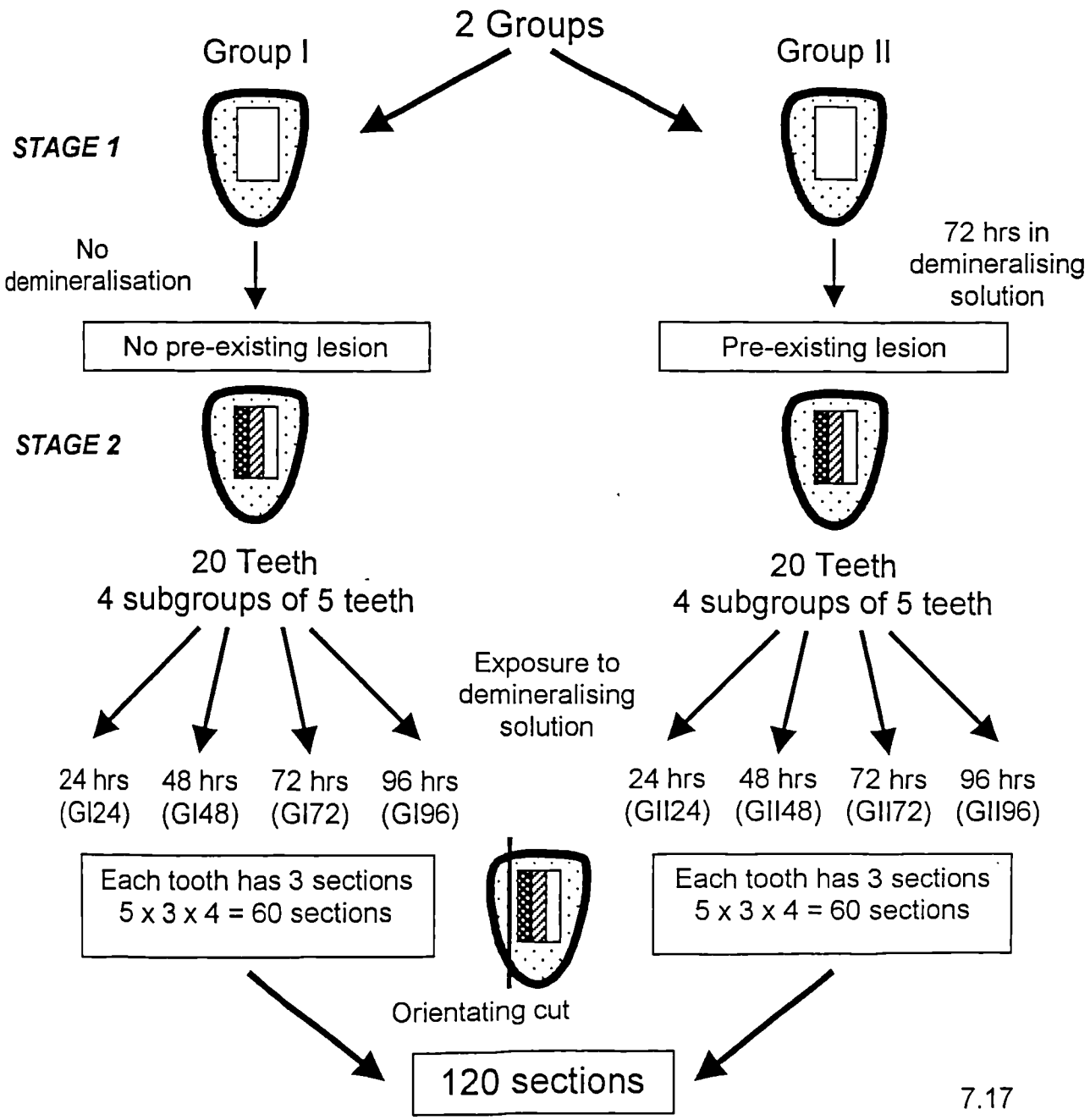


Figure 7.2

Mean mineral loss (vol%.μm) and 95% confidence intervals for the mean mineral loss in the samples without a pre-existing enamel lesion (Group I).

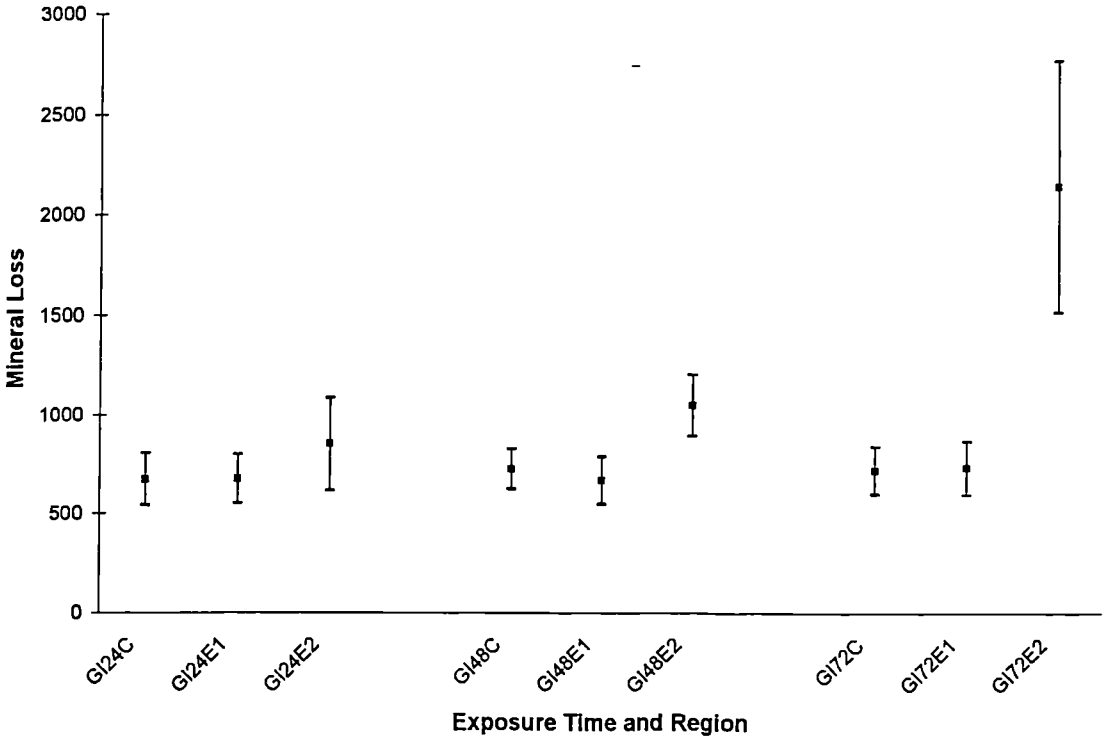
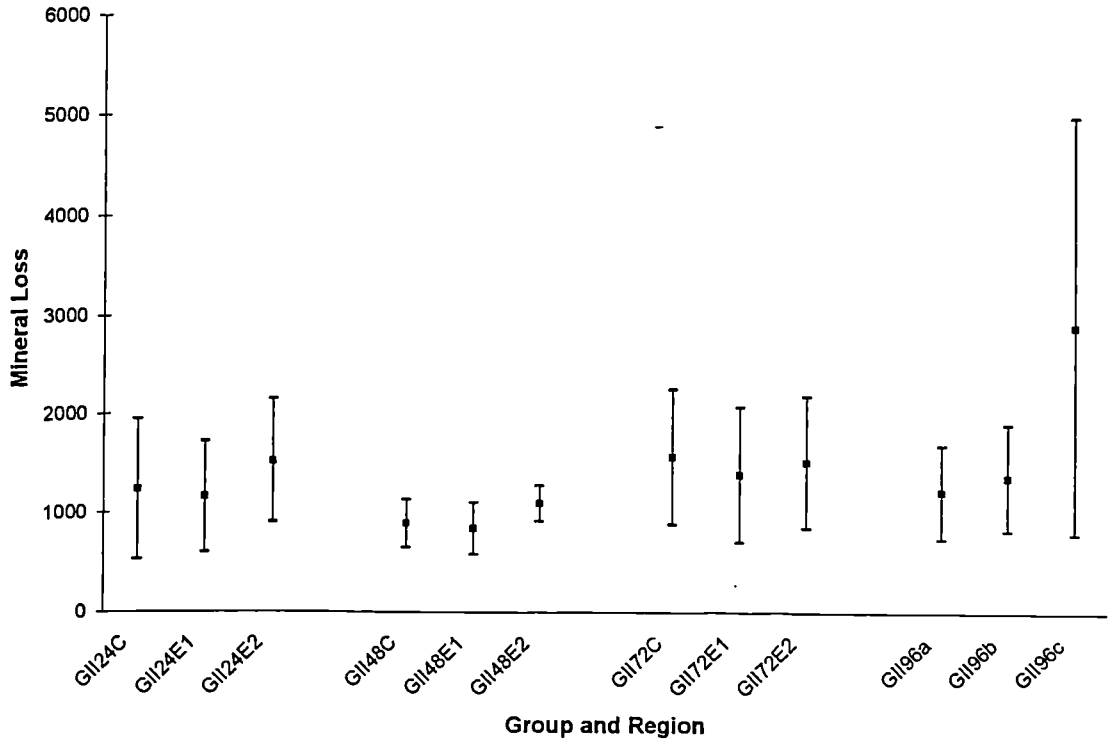


Figure 7.3

Mean mineral loss (vol%.μm) and 95% confidence intervals for the mean mineral loss in the samples with a pre-existing enamel lesion (Group II).



7.8 Appendices

7.8.1 Appendix I

Preparation of Demineralising Solution

Prepare 200ml containing the following:

0.0598g KH_2PO_4 (=2.2mM)

0.44ml of 1M $CaCl_2$ AVS grade (=2.2mM)

0.57ml of glacial HAC A.R. grade (=50mM)

Add 50ml of distilled deionised water

Adjust pH to 4.5 with conc KOH solution

Pour into a 200ml volumetric flask

Add 0.1ml of 22.1mg NaF per 100ml distilled water

Make up tp 200ml with distilled water (1ml contains 0.5 μgF)

Use 10ml per tooth with a magnetic stirrer.

CHAPTER 8

Discussion

The overall aim of the investigations in this thesis was to investigate methods of assessing enamel demineralisation, applicable to clinical orthodontic research, which are both valid and reproducible. The most clinically valid method of establishing the effectiveness of agents designed to prevent orthodontic demineralisation is through clinical trials. The ideal design of a clinical trial is prospective, longitudinal and properly randomised (Altman, 1991). The use of a crossover design, which permits the study of all the agents (including placebo or control) in one participant, increases the power of the study, by reducing inter-participant variability (Altman, 1991).

The results of a clinical trial will be dependent upon the accuracy of the technique or techniques for both recording and measuring the relevant outcomes. The more accurate and reliable the technique, the greater the power of the study and fewer participants need to be recruited. If however, there is some variability then the power of the study is reduced and increased numbers are required to test the result to a significant level of determination.

The prevalence of the condition will also be a factor in the accuracy of the results. If a condition has a high prevalence and the measuring technique is moderately reliable, a few participants with the condition will be missed, but because more participants suffer from it, sufficient will be recorded to

produce a meaningful result. If there is a low prevalence and the method of recording or measuring is missing the condition, then larger numbers of participants will be required to obtain a statistically valid result. For many conditions it is not possible to predict who will suffer the condition and target those for special investigation, so larger numbers need to be studied.

A major obstacle in researching orthodontic demineralisation is the length of orthodontic treatment, which frequently extends over two years. In order to start orthodontic treatment oral hygiene must be good, however plaque levels, which may be associated with demineralisation, frequently rise in the early stages (Pender, 1986). Thus, it is likely to be informative when the first few months after placement of the appliance are studied. On the other hand, some studies have shown that the longer the treatment the higher the prevalence of demineralisation (Marcusson *et al*, 1997). Therefore, demineralisation studied only in the early stages of treatment may fail to detect some important aetiological factors, because the long term prevalence of demineralisation may be an effect of short-term inadequacies in plaque control. The ideal technique should be capable of measuring demineralisation over the whole length of orthodontic treatment.

8.1 Evaluation of Techniques for Measurement of Enamel Demineralisation

The investigations reported in this thesis have concentrated on two aspects that would be relevant for a putative clinical trial into the prevention of orthodontic demineralisation. Namely, the direct assessment of enamel demineralisation using photographs and quantitative light-induced fluorescence and the indirect assessment of demineralisation/ remineralisation conditions in the oral environment, using the *in situ* caries model during stages of orthodontic treatment.

The reasons for choosing these techniques have been explained in detail in Chapter Two. In summary, they were studied because of their simplicity allowing clinical applicability avoiding disruption of orthodontic treatment, which is an important ethical consideration. In addition, the *in situ* model lends itself to controlled experimentation and crossover studies, which tend to increase the statistical power of studies.

8.1.1 Direct Measurement of Enamel Demineralisation

There are two aspects to the direct measurement of demineralisation, firstly the recording of the information and secondly the measurement. Two direct methods of recording demineralisation have been studied:

1. Photographs
2. Quantitative light-induced fluorescence

The methods of measurement employed were an ordinal index recorded by visual examination and computer analysis of images.

8.1.1.1 Photographs

Photographs are commonly used in the clinical environment, therefore they are a convenient and effective means of permanently recording the optical properties of enamel. They have been employed in a number of studies, but frequently neither the reliability of the recording, nor measurement from photographs have been reported (Gorelick *et al*, 1982; Houwink and Wagg, 1979; Dooland and Wylie, 1989; Ishi and Suckling, 1991). Ellwood (1993) found an acceptable reproducibility of recording the prevalence of developmental enamel opacities from photographs.

The first study in this thesis (Chapter Three) compared the reproducibility of measuring demineralisation with an incremental pattern, on the buccal surface of a tooth. Three techniques were used; the naked eye, estimating the area of demineralisation using Vernier callipers or measuring from photographs. In addition, the reproducibility of repeat readings from the same slide of the teeth, with repeated slides of the same tooth was investigated.

The aim of that study was to determine if recording and measuring demineralisation from a photograph was at least as good as recording and

measuring using the naked eye. The results were surprising as it was found that the photographic technique was more reproducible than recording and measuring using the naked eye. This was shown by the coefficient of repeatability, which was lower for both the repeat readings of the same slide and the two readings from different slides of the same tooth, compared with the Vernier or microscope techniques. The coefficient of repeatability represents twice the standard deviation of the differences between the repeated measurements, therefore the smaller this value, the lower the variability between repeat readings.

There was poor agreement between the measurement from the photographs and both the Vernier and microscope readings, as shown by the wide limits of agreement. The question concerning which technique was measuring genuine demineralisation more accurately is important. At the beginning of the experiment, it was considered that measuring with the naked eye through a microscope would produce definitive results on the area of demineralisation that the photographic results would have to match up to. The results showed that the readings from the naked eye were more variable compared with those from photographs. This suggests that it was easier to make a decision about whether demineralisation was present or not from the photographs rather than with the naked eye. Even the assessments from two different photographs of the same tooth were more comparable than two different naked eye assessments.

It could be argued that photographic images record detail differently to the naked eye (Ellwood, 1993). I confirm the view of Ellwood (1993) that more defects of enamel are recorded when the teeth are assessed from photographs, rather than with the naked eye. This was shown by the mean difference between the two techniques, which showed that the photographs on average scored 4.3mm^2 more demineralisation than the naked eye. If this is the case can the measurement from a photograph be considered valid? I would maintain that the measurement from a photograph is valid because it is the difference between the start and finish-levels of demineralisation that is important. A major advantage of a photograph is that it will record the condition of the tooth at the start of treatment and the condition at the end. The relevant reading will not be the actual recordings from the two photographs, but the difference between the two, which will represent the changes that have occurred to the tooth during treatment. Direct assessment by one observer over time would probably be subject to drift.

The application of the morphometric technique to recording demineralisation was original, but was unsatisfactory. The grid did not fit the surface of the tooth accurately, therefore the algorithmn to describe the proportion of the surface affected, upon which the technique is based, may not be correct. The positioning of the grid was highly reproducible, as long as strict criteria were observed, however the technique is unlikely to be useful for measuring demineralisation following orthodontic treatment, because this tends to be patchy and irregular. Morphometry would be unlikely to produce an accurate representation of this demineralisation because of the 'hit-or-miss' nature of

the recording. For this reason, morphometry would be more useful for generalised conditions of enamel, such as fluorosis.

Three potential sources of error in recording and measuring enamel demineralisation, particularly when studying individual lesions longitudinally, were highlighted by this study:

1. The production of the image.
2. The angle at which the camera is placed to take the photograph.
3. The subjectivity of the index.

I believe that by careful attention to detail the production of the image, namely variations in the lighting, quality, development and ageing of the film can be minimised. The effect of camera angle and the subjectiveness of the index used to assess demineralisation required further investigation and this was carried out in Chapter Four.

The study in Chapter Four examined the same teeth as were used in the initial study, however this time the camera was placed in a holder that could be rotated in the vertical plane. Photographs were taken perpendicular to the buccal surface of the tooth and at angles of 20 and 40-degrees above and below the perpendicular. In addition, to remove the subjectiveness of the human eye assessing demineralisation, the grey levels of the image were measured using a computer.

I considered that by converting the photograph to a digital image, then using computerised image analysis, the sophistication of both the qualitative assessment of change in the optical properties of the enamel and the quantitative measurement of the area of demineralisation would be increased.

Concerning the change in the optical properties of the enamel the element of subjectivity could be reduced. The index used in the first study was chosen to represent the indices that were used in various clinical studies. It recognised four grades from normal enamel, through early demineralisation, to obvious demineralisation and finally, severely demineralised enamel. It was found that the eye could interpret normal enamel and obviously demineralised enamel with some accuracy, but the grades between were recognised with less precision. The computer provided more exactitude, as it can recognise 255 different grey levels. In fact, this proliferation of information produced by using the computer created as many problems as it solved.

The main problem was how to deal with information represented by 255 different grey levels. This was overcome by placing the grey levels into groups or ranges of approximately 30 grey levels. Although it was thought to be expedient at the time, further investigation showed this to be somewhat artificial, as only a relatively small range of grey scales are represented on the buccal surface of the tooth. So for the critical values of grey levels present on the image of the buccal surface the range was too large. For the grey levels outside these critical values the range was too small. In addition,

the difference in grey level between sound and demineralised enamel was much smaller than expected and well within the range of differences produced by different lighting conditions. This will be discussed further later in this chapter.

Concerning the quantitative measurement of the area of demineralisation, the computer method could be equated with the morphometric method. Each pixel that makes up the digital image could be considered a point or probe on a morphometric grid. Instead of 122 dots, there would be literally thousands, depending on the resolution of the digital image. The measurements, instead of representing a 'snapshot' of the buccal surface, would be a highly accurate picture of the optical properties of the enamel, as represented by the grey levels. With adequate calibration of the image, the area of the buccal surface could be determined with greater accuracy than with the morphometric method. The area of each range of grey levels could be calculated.

The results of subsequent investigations were to show that my optimism with regard to using the computer to quantitatively measure demineralisation was well founded, but an accurate qualitative assessment of demineralisation was to prove more elusive.

The results of the study using digital images and computerised image analysis showed that the technique was reproducible. The repeat measurements of the same image showed very good reproducibility. The

limits of agreement for the readings of two different slides of the same tooth showed a low mean difference between readings and acceptable limits.

Changing the angle of the camera to the perpendicular of the tooth did have an effect on the measurement of the area of demineralisation, as speculated by Ellwood (1993). He advocated tilting the camera to reduce the amount of reflected light on the image. It was noted that the perpendicular or 0-degree view had more reflected light than the tilted views and this could confuse the measurement of demineralisation by either hiding or mimicking white spots, making assessment more difficult and increasing the random error. Because of this (and for reasons discussed later in this chapter) it was not possible to directly compare the areas of demineralisation measured by the three angles. Instead, the areas of the whole buccal surface measured for the three angled views of each tooth, were compared by adding together the area measurements for all the grey scale ranges. This revealed that the effects of changing the angle from 0 to 20-degrees and changing from 20 to 40 degrees were not linear. The mean area of the buccal surface was 5mm^2 less for the 20-degree views compared with the 0-degree views. The mean area of buccal surface was 52mm^2, therefore this represents a reduction of 10 percent. The difference increased to 10mm^2 between the 20 and 40-degree views, which represents a reduction of 30 percent from the mean area of the 0-degree views. In fact, it was apparent that the 40-degree photograph was taken at too steep an angle and the investigator would recognise this. The next question is how precise can the investigator be when determining the angle of the camera to the buccal surface? The answer

is not very, unless there is a way of aligning the camera to the buccal surface. This is investigated in Chapter Five. It would be interesting to examine the differences between 10-degree and 30-degree views, as this would probably represent a range within which the error of the measurement of the buccal surface will probably fall. Some of the differences might be due to the random error of drawing the area of interest around the buccal surface of the tooth, however the magnitude of this error will be the same for the three angled views.

A surprising result was the difference in reproducibility between the images that were taken above the perpendicular (towards the Cuspal) and those taken below the perpendicular (toward the Gingival). The results showed the repeat readings had better agreement for the Gingival views compared with the Cuspal views. The major difference between these views was the positioning of the masking on the ringflash. The masking is placed in order to reduce the amount of reflected light from the flash (Ellwood, 1993; Fleming, 1989). The positioning of the masking has not previously been considered important, but these results suggest that the masking had an important influence on the reproducibility of the results. When the masking was placed on the lower part of the flash, which was closest to the tooth, the limits of agreement were narrower than when the masking was placed on the upper part of the flash, furthest from the tooth. I suggest that the masking is in the best position to reduce the reflection from the flash when placed close to the tooth.

I believed, following the investigation in Chapter Three, that the error due to the production of the image could be minimised. I was therefore disappointed, during the subsequent investigation in Chapter Four, to find that despite the fact that the photographs were taken and developed using a standardised technique under standardised conditions (which exceeded that possible in the clinic), there remained differences in agreement between two slides of the same tooth. Random error could have been introduced at the calibration and outlining of the Area of Interest, but other studies have shown this is small proportion of the total (Mitchell, 1992; Linton, 1996). Hence, variations in the processing of the image were still leading to differences in the images that were affecting the measurement. However, the results from the study using computerised image analysis did indicate to me that digital technology could be used to improve the reliability of the method in two ways.

Firstly, to overcome the variations in lighting and processing. The variations in the production of the image may be compensated for if a calibrating grey scale was photographed along with the relevant tooth to be measured. The grey scales of two images could be matched more closely if the images were manipulated digitally so the grey scales on the two calibrating markers coincided.

Secondly, digital technology could be used to more accurately measure the differences in grey levels between two images. I mentioned previously that a photograph records more demineralisation than the naked eye. This would not matter if this bias were systematic, for example, if the increase in

demineralisation on a post-treatment photograph, were the same as the increase on the pre-treatment photograph. The difference between the two images is of interest, because this represents the change in the enamel that has occurred during treatment. It is possible, using digital technology, to create an image showing the differences between the grey levels of two images, by placing one image on top of another and subtracting the grey levels of one from the other. Thus the picture of a tooth before treatment could be subtracted from the picture of the same tooth after treatment and the resulting image would represent the change in the optical properties of the enamel during treatment.

These techniques were the subject of the next investigation (Chapter Five). This study assessed the digital manipulation of pre- and post-demineralisation images of teeth with orthodontic brackets, using a calibrating grey scale and a subtraction method. The results of both the subtraction method and the use of the calibrating grey scale were disappointing.

The result of subtracting the two images was disappointing. Areas of demineralisation that could be detected by eye were not registered on the subtracted image. It was clear that the visual assessment was looking at the buccal surface as a whole and subjective comparisons were being made about areas that were considered lighter (demineralised) than other areas. I found that with practice I was able to discriminate areas that were lighter due to flash reflection rather than demineralisation. The computer is unable to

make that subjective comparison. It will provide a grey scale number for individual pixels down to the smallest detail, but because it is not possible to state which grey level represents normal enamel and which is demineralised enamel, the number in itself is meaningless, without that subjective assessment. To quote a well-known analogy, the computer is unable to "detect the wood from the trees".

A second problem is that the grey levels for normal enamel and demineralised enamel will vary for different teeth with different lighting levels. Because of this variation, it is not possible to state a threshold grey level above which it can be said that demineralisation is present. Indeed, the difference in grey levels between areas of normal and demineralised enamel was smaller than expected. Therefore, a grey level that would represent demineralised enamel on one image could be in the range of normal enamel on another image, because the image itself was generally brighter.

To overcome some of the problems with variation in brightness of different images a calibrating grey scale was incorporated into each image. Unfortunately calibration of the images using the standard grey scale was found to be difficult for two reasons. Firstly, it was found that differences in the grey levels between the two images were not general across the whole image, but localised. It was not possible to calibrate the two images using the grey scales and find that the differences in the grey levels of the two grey scales was a reflection of the differences between the grey levels of the two

images over the whole tooth. This is probably due to the complex, curved nature of the tooth surface, reflecting light in different ways.

Secondly, the grey scale calibration marker may have been part of the problem. The calibration of a radiograph for TMR is performed by assessing the radiation that passes through an aluminium strip of varying thickness. The grey scale calibration marker worked by reflecting back from the surface to the film in the camera. This might be a more variable quantity than transmitted light, due to variation in the angle of the camera or scatter of the photons. Ideally, the calibrating grey scale should consist of a black area that absorbs all light and a white area that reflects all light.

In summary, it was found that photographs are an excellent medium for recording the optical properties of enamel. Some of the problems concerning measurement of enamel demineralisation from photographs have been addressed, but there are still areas of investigation, which are explored further in section 8.2 (Further Research, page 8.33).

8.1.1.2 Quantitative Light-induced Fluorescence (QLF)

I have to confess to being excited when I first heard about QLF. A major drawback of photographs is the lack of contrast in the image. There is the small difference in grey levels between normal and demineralised areas. The images produced using the fluorescence techniques (section 2.4.3.2, Fluorescent Methods, page 2.36) show a high contrast between normal and

demineralised enamel, which makes area measurement of demineralisation easier. I thought it would be interesting to compare the manual measurements taken from the captured, digital photographic images with a new quantitative light-induced fluorescence (QLF) technique.

The method of investigation differed slightly for the two techniques. The QLF measurements were carried out around all four edges of the bracket of the demineralised teeth, regardless of whether it was considered demineralisation was present or not. The photographic technique recordings were taken only if, after visual examination of the image by the investigator, it was considered that demineralisation was present. I was interested to see if the investigator could rely solely on the software for QLF to determine if demineralisation were present or if some subjective assessment with the eye was necessary first. It was not possible to remove this subjective element from the photographic technique for the reasons mentioned above, namely the inability to determine a threshold grey level above which enamel demineralisation is defined to be present.

The photographic and QLF techniques were both found to be reproducible, when repeated measurements were examined. The accuracy of diagnosing demineralisation from a photograph and using QLF was assessed by recording the sensitivity, specificity, positive predictive and negative predictive values, as defined in Chapter Five. In most cases, it was found that a negative result suggested that there was no demineralisation present. A positive result was less reliable particularly for gingival and occlusal readings,

where it was speculated that the size of the area between the bracket and the gingival margin and the presence of reflections from the ringflash, would reduce the reliability of these readings. Importantly, the gingival region is an area of high prevalence of orthodontic demineralisation.

QLF was found to have poor specificity and positive predictive value when all areas of demineralisation were included in the results. There are two possible explanations for this. Either the technique was falsely finding demineralisation when none was present, or it was diagnosing demineralisation that could not be seen either clinically or from a photograph and therefore was a more sensitive technique. Al-Khateeb *et al* (1997b) found reasonable correlations between QLF and calcium loss, measured by chemical analysis and mineral loss, measured using TMR. Without definitive measurements of mineral loss from the teeth used in this experiment, it is impossible to tell which is the case. It could be argued that if the demineralisation cannot be detected by eye or on a photograph then is it clinically important? By defining the rather arbitrary level of $5mm^2$ or greater as a clinically significant area of demineralisation, the specificity and positive predictive value were improved. On the other hand, if QLF were detecting demineralisation at a much earlier stage than could be detected with the eye, this would be a very sensitive technique, which would be very useful when conducting a clinical trial. It would allow small differences in the effectiveness of preventive agents to be measured, which, as discussed earlier, would increase the power of a study. At present, QLF is predominantly a research tool to define and test appropriate regimes. It is unlikely to be used routinely

the clinic for the foreseeable future, although from a clinical perspective, the earlier demineralisation is found the sooner additional preventive measures can be taken.

I am not yet convinced that QLF is a significant advance on the subjective assessment and measurement of photographic images using computerised image analysis, although my experience with the technique is limited. The equipment is more unwieldy than a camera and sometimes the teeth can be difficult to visualise and capture from a computer screen. QLF is useful when monitoring the change in a lesion that is already apparent, rather than detecting demineralisation occurring in intact enamel. At present, I believe the two techniques of computerised image analysis from captured photographic images and QLF should be regarded as complimentary to each other, for the direct recording and measurement of enamel demineralisation.

8.1.2 Indirect Measurement of Demineralisation

Photographs and QLF (perhaps more sensitively) provide a direct record of what is occurring to the enamel of a tooth in a person's mouth. From that record, it may be inferred that demineralisation (or remineralisation) has happened by the change in the optical properties of the enamel (section 2.4.3, Optical Methods, page 2.32). It is not possible to directly measure mineral loss from a live volunteer, as the techniques frequently require destruction of the tooth material being examined (section 2.6, Techniques for the Quantitative and Qualitative Measurement of Demineralisation with Intra-

Oral Models, page 2.67). If the effect of preventive agents on the direct loss or gain of mineral from enamel is to be studied within the mouth, then a model has to be used.

The various models used for the *in vivo* study of de/remineralisation have been reviewed in section 2.5 (Experimental Models for Studying Enamel Demineralisation, page 2.43). For the reasons outlined previously, the *in situ* model has many advantages over other *in vivo* techniques. Primarily, the *in situ* method has a control with an artificial-carious lesion with measurable parameters. The sample parameters can be directly compared with the control and de/remineralisation can be measured directly. Investigators using other *in vivo* models have assumed that the mineral content of the enamel was 85 volume percent mineral (O'Reilly and Featherstone, 1987). They postulated that any decrease in the mineral content below this level was due to demineralisation and any increase due to remineralisation.

The aim of the investigation in Chapter Six was to adapt the *in situ* caries model to study de/remineralisation in the orthodontic patient with a fixed appliance. The model is a well-established technique for investigating the therapeutic effects of preventive agents. The first problem when adapting the model to the orthodontic environment was to develop a customised holder for the enamel specimen. The usual location for the specimen in the laboratory I was working in was the buccal of the lower molar (Manning and Edgar, 1992). There were a number of problems with this position. Firstly, the only place the enamel specimen could be secured, without interfering with the

appliance, was the buccal attachment of the molar band, which is already quite prominent. The specimen was therefore even more conspicuous, which caused a considerable amount of irritation to the patient. It was also subjected to extra trauma that led to several specimens being lost.

After further thought and discussion it became apparent that placing the specimen on the archwire would have a number of advantages. This position would have a minimal disruption to treatment, as the archwire is routinely removed and replaced during adjustment appointments. The specimen would be in line with the rest of the appliance and was unlikely to cause more irritation than the appliance itself. The specimen would also be in a position (buccal on the lower premolar) that is susceptible to demineralisation.

There were some initial problems with the design of the holder. The first specimen holders were large and cumbersome to manufacture and position in the mouth. They could only be placed in the space provided by an extraction. After several attempts and with more experience of making the holder, I was able to reduce the bulk. Following the investigation in Chapter Six, which demonstrated that the enamel specimen could be successfully attached to the holder, the size of the holder might be reduced further and could be placed in non-extraction cases.

A major problem with the specimen holder was that when placed on round archwires it rotated, which the patient found annoying. Initially I considered crimping the holder to the archwire. The difficulty with this was that if the

archwire were changed the specimen holder would have to be changed too. The problem with rotation was overcome by manufacturing a hook on the holder that a stainless steel ligature could be attached to. The ligature was then tied around the nearest bracket anchoring the holder and preventing rotation.

The advantage of the removable specimen holder was the flexibility it introduced into the experimental design. If an archwire had to be changed, the specimen holder was simply removed from the old archwire and placed on the new one. There were no constraints on the length of time the specimen could be left in the mouth. Another advantage that had not been envisaged when the removable specimen holder had been designed, was that once the enamel specimen had been carefully removed, the specimen holder could be sent for recycling. This removed any residual composite from the gauze base and sterilised it, so that the holder could be reused.

The removal of the enamel specimen was a worrying phase of the experiment, as I foresaw the possibility of the enamel shattering. I used some fine bracket-removing pliers to fracture the composite at the base of the specimen. If this was unsuccessful I was prepared to destroy the enamel holder rather than lose the specimen. Gentle flexing of the bracket base was sufficient to free the enamel specimen. Throughout the experiment no enamel specimens were lost during removal from the customised holder.

The design of specimen holder used in Chapter Six proved to be successful. Only one patient was unable to tolerate it from the start. One of the reasons that patients did not complain could have been that the holder was placed when the brackets were located, therefore any extra irritation from the holder was indistinguishable from the general irritation caused by the appliance itself. The other reason the holder was successful was that no enamel specimens were lost from it (although several of the holders and specimens were lost). I believe that this was due to sterilising the enamel before attaching it to the holder with a dentine-bonding agent.

Five enamel specimens were lost during the experiment (a sixth was rendered useless, as the contralateral side was lost). Two were lost due to carelessness. They were thrown away by overzealous clearing up, after being removed from the mouth at the end of a clinical session. Despite, several hours searching through the clinical waste the next day they were not recovered. Three holders were lost when both the archwire and ligature securing the holder fractured. It is difficult to see how these mishaps could be avoided in a clinical trial.

The second consideration, when attempting to adapt the *in situ* model to study demineralisation occurring during orthodontic treatment, was how to mimic the area of enamel in an orthodontic patient that is susceptible to demineralisation, specifically that surrounding the orthodontic bracket. It could be argued that because the enamel specimen is placed within the holder, which is essentially a customised bracket, that this was sufficient to

reproduce the orthodontic environment. However, I did not believe that this was sufficiently authentic. Firstly, the specimen would lie within the bracket, which would surround it. This is different to enamel in the orthodontic environment, in which the attachment lies on top and the enamel surrounds the bracket. Secondly, prior to attachment of orthodontic brackets the enamel is subjected to an acid, which produces an etch pattern that allows bonding of composite resin to the tooth. Acid etching of the enamel may alter the properties, which may affect the de/remineralisation rate. I therefore attached a small piece of bracket base to the enamel sample, using an acid-etch technique.

There may be a number of criticisms regarding the attachment of the bracket base. Firstly, as previously stated the acid etching may alter the properties of the enamel, rendering the intra-oral specimen different to the control. This criticism is addressed further in Chapter Seven.

The second criticism would be the positioning of the bracket base. Demineralisation occurs at the edge of the bracket. Underneath the bracket there will be no demineralisation, as the composite resin protects this area. The further one moves from the edge of the bracket the lower the mineral loss becomes (O'Reilly and Featherstone, 1987). The difficulty with the *in situ* model is measuring the precise area at the edge of the bracket and not the area under the bracket or areas further away from the bracket, which would lead to an underestimate of the extent of demineralisation. I was saved from this dilemma by the fact that the composite was radiopaque on the

microradiographs. As long as the composite remained on the enamel specimen I was able to determine where the edge of the bracket was. This however, produced another dilemma. Following removal and grinding of the specimens for microradiography, the sections were recoded to allow for blind assessment. Therefore, I was unable to distinguish between the control, bracket and unbracketed specimens. Except that the bracketed samples had composite on the surface. The composite on some of the sections did not survive the sectioning and grinding of the specimens and I considered removing the material that did. The problem with this was that removing it might lead to mineral loss that would be incorrectly attributed to demineralisation. I argued that in a clinical trial using this technique, the specimens for both the placebo and the active ingredient would have the small bracket base and would be indistinguishable. I was testing this technique for that situation and therefore decided to leave the material in place.

The overall conclusion from the study in Chapter Six was that the *in situ* model could be used successfully to measure de/remineralisation within an orthodontic patient. This is an important adaptation of a technique that is widely used in the study of the effectiveness of preventive agents. It can now be used to study products designed to prevent orthodontic demineralisation.

No significant difference in mineral loss or gain was found between an enamel sample that had an orthodontic bracket base bonded to the surface and was placed in the mouth of an orthodontic patient and a control sample

that had not been in the mouth. However, a significant reduction in mineral gain was found between an enamel sample that had the bracket base and one that had not. This suggests that although the bracket does not necessarily shift the environment of enamel in the orthodontic patient toward demineralisation, it does shift it against remineralisation.

A number of problems with the *in situ* caries model were highlighted by this study. The laboratory work for the *in situ* caries model is extremely time-consuming and there is a large amount of wasted effort. A high proportion of the preformed lesions created on the extracted teeth was unsuitable for the experiment, because they did not have an intact surface layer. The amount of mineral loss also varied between teeth. Considering the amount of time it takes to create the lesions I would advocate spending extra time to ensure that more of the preformed lesions could be used in the experiment. It has also been shown that the method of formation of the lesion also has an affect on the response to demineralising and remineralising conditions (Damato *et al*, 1988). A pH cycling technique (ten Cate and Duijsters, 1982), may produce a lesion with a more consistent size and response rate, in a similar way to the production of artificial erosive lesions (Amaechi *et al*, 1999a).

A major disappointment of the *in situ* caries model was the large variation in mineral loss. If this variability had been confined to between patients, it could be overcome by carrying out a crossover trial. However, the variability within patients was almost as great as the variability between patients. Patients lost mineral at different rates. Some lost mineral with the first sample and gained

it with the second and visa versa. There was no pattern to the mineral loss. The problem with this variability is that it will reduce the power when undertaking a clinical study. To overcome this variability within individuals and demonstrate a statistical difference between a therapeutic agent and a placebo, larger numbers of volunteers will need to be recruited than originally envisaged if a study is to have sufficient power.

It is possible to perform a power calculation using the data from this experiment. Sample size and power calculations are based on a quantity known as the standardized difference (Altman, 1991). The standardized difference for continuous paired data is:

$$2\delta/s_d$$

Where δ is a clinically relevant difference and s_d is the standard deviation of the changes. The standard deviation of the differences for mineral loss between the control and the bracketed samples in the *in situ* experiment was 337.2 vol%.μm. A value for δ needs to be estimated. Figure 6.5 (page 6.34) shows a graph of the changes in mineral loss in the enamel specimens with time. The 100 percent line represents no change in the specimen, above the line represents further loss of mineral and below the line represents remineralisation. A reasonable estimation of the mineral loss during the experiment was a 40 percent change (above or below the 100 percent line). The mean mineral loss of the control specimen shown in Table 6.6 (page 6.25) is 803 vol%.μm. A 40 percent change in this value (δ) is:

$$803 \times 0.4 = 321$$

The standardized difference is therefore:

$$\frac{2 \times 321}{337}$$

$$= 1.9$$

Using the nomogram (Altman, 1991), a standardized difference of 1.9, with a sample size (N) of 14 and a significance level of 0.01 gives a power of 0.85. This means that the experiment had an 85 percent probability of detecting a difference in mineral loss at the 0.01 percent level. This represents an acceptable level of power.

One cause of variability with the *in situ* model is the initial size of the mineral loss from the preformed enamel lesion, as this has been shown to affect the de/remineralisation response (Strang *et al*, 1987; Schäfer *et al*, 1992). This is unlikely to have made a significant contribution in this experiment as every effort was made to standardise the size of the lesion for each individual. The two specimens placed in the mouth at the same time were from an identical preformed lesion. The two batches of specimens placed in each patient were matched for size of mineral loss.

Another cause of variability could be due to differences in mineral loss between the control and experimental enamel at the start of the experiment. It is known that the rate of mineral loss is variable not only between teeth, but also between different sections of the same tooth (Mellberg, 1992). Variability could be reduced by measuring the same area of enamel before and after the experiment. This could be achieved by using thin sections (ten Cate,

1992) or by using a method of measuring mineral loss that is non-destructive (section 2.6, Techniques for the Quantitative and Qualitative Measurement of Demineralisation with Intra-Oral Models, page 2.67).

A further cause of variability in mineral loss may have been due to differences in the diet between the two stages of the experiments. I asked the volunteers to record everything they ate and drank over a three-day period, of which one was at the weekend. No obvious changes in diet were revealed.

The length of time the enamel sample was left in the mouth was a matter of some debate when the design of the experiment was first discussed. The initial protocol was for one specimen to stay in the mouth for the entire length of orthodontic treatment. This was changed for two reasons. This first experiment with the *in situ* model was really an extended pilot study to test that the model could be successfully applied to the orthodontic patient. The length of experiments with the *in situ* model is usually no longer than six weeks. I was not certain that the customised holder and enamel specimen would survive extended periods in the orthodontic patient. The maximum length of time one specimen was in the mouth was 119 days or exactly 17 weeks and I see no reason why a specimen should not last for the entire length of orthodontic treatment.

The second reason for not leaving a specimen in the mouth for the entire length of orthodontic treatment was that I was interested in using this pilot study to test the enamel specimen under two differing conditions (bracketed and non-

bracketed). This required a paired study with removal of the two specimens at the same time. I was not convinced that an observational study alone would provide enough information to develop the technique for use in a clinical trial.

Examination of the effect of length of time the sample was left *in situ* with any of the parameters measuring de/remineralisation showed no relationship. This is contrary to a number of other studies (Øgaard *et al*, 1988c; Arends *et al*, 1992; Marcusson *et al*, 1997). The results of this study would suggest that enamel be at risk of demineralisation any time during orthodontic treatment. The clinician must therefore be vigilant throughout treatment in monitoring the patient for signs of demineralisation.

The study carried out by O'Reilly and Featherstone (1987) found a mineral content below 85 volume/percent mineral in the surface enamel beneath the bracket. They postulated that this was due to mineral loss from the acid etching. If this were the case, the bracketed enamel sample used in Chapter Six may have had a greater mineral loss than the control when it was placed in the mouth and therefore could not be considered a valid control for the bracketed sample. It was of interest to compare the effects of a sample of enamel that had undergone all the procedures involved in placing an orthodontic bracket with a sample of enamel that had not. Acid etching is routinely performed in orthodontics and the comparison would not be clinically valid without it.

This problem was investigated in the study in Chapter Seven, which examined whether the acid-etch technique led to a significant amount of mineral loss, when measured by transverse microradiography. No difference in mineral loss could be detected between etched or unetched bovine enamel, either with or without a preformed enamel lesion. The results did show that when there was no pre-existing enamel lesion there was a highly significant difference between the regions unexposed to the demineralising solution and those that were exposed for 48 hours or more. In the teeth with a pre-existing lesion, the difference in mineral loss between the regions was generally not statistically significant. In other words, the increased confidence limits for the mean mineral loss from the specimens with the pre-existing enamel lesion ensures that these specimen show reduced sensitivity to further mineral loss after being placed in the demineralising solution.

The results of this study agree with the observations of Mellberg (1992), who considers that if the process of demineralisation is being studied, an enamel sample with no pre-existing lesion is used. Whereas, if remineralisation is of interest, then an enamel lesion with a pre-existing lesion would be appropriate.

Zero (1995) also questions the use of specimens with a preformed, subsurface lesion, because clinical dental caries is not necessarily preceded by a subsurface lesion.

In section 2.5.4 (The *in situ* Caries Model; page 2.50) it was noted that several authors have challenged the use of subsurface lesions in specimens prepared for the *in situ* caries model. Strang *et al* (1987) demonstrated a linear relationship between the size of the pre-existing lesion and the rate of remineralisation. It could be argued that by introducing a pre-existing lesion the investigator might be altering the enamel environment to encourage remineralisation, which may not reflect the true orthodontic environment. Conversely, the *in vivo* orthodontic condition with no pre-existing lesion or minimal demineralisation might not produce an oral environment capable of affecting an enamel slab with no pre-existing lesion.

Another important consideration is to determine which therapeutic effect of preventive agents is being investigated by the study, namely the prevention of demineralisation or the promotion of remineralisation (Zero, 1995). In a clinical trial of patients undergoing orthodontic treatment surely the important effect we wish to demonstrate is that a therapeutic agent will prevent demineralisation from occurring. In such a trial an enamel specimen with no preformed enamel lesion could be used.

On the other hand if we wish to investigate an agent that is designed to be used in patients who have demineralisation that we wish to arrest or reverse (either pre or post debond), then the important effect we wish to investigate is remineralisation. In such a trial we could use an enamel lesion that has a preformed carious lesion.

One way of investigating both preformed and natural tooth surfaces is to follow the method of Featherstone and Zero (1992). The subjects in their trials each carry a sound enamel slab and one with preformed enamel lesion. It should be possible to carry this out by placing slabs bilaterally in the lower extraction sites. The investigation in Chapter Six showed that there was no difference in mineral loss between the two sides.

Alternatively, ten Cate (1992) advocated using sections of enamel rather than slabs. This has the advantage of giving the investigator the option of placing sections both with and without preformed enamel lesions in the same position in the mouth. The use of thin sections also allows the measurement of demineralisation from the same area of enamel before and after the experimental period. This will reduce the variability due to possible differences in mineral loss between the experimental and control sections. The use of thin sections has been criticised because they may demineralise more rapidly than slabs (ten Cate and Exterkate, 1986). Strang *et al* (1988) showed that the demineralisation rate is the same with sections if they ground to 100μm rather than cut.

A disadvantage of using sections is that it will be difficult to reproduce the environment of the orthodontic bracket, by placing a small bracket base. Another disadvantage of using thin sections is that there are technical difficulties in protecting the cut surfaces from demineralisation that may affect the response of the specimen.

8.2 Further Research

One common source of error that has been highlighted by the studies in this thesis has been the presence of reflections from the flash. This needs to be addressed if improvement in reliability of measuring demineralisation from clinical photographs is to be made. The author carried out some preliminary pilot studies with a polarising filter on the camera lens, but this proved unsatisfactory. Other investigators have examined the use of polarising filters on both the lens and the flash (Willmot, personal communication) and this may be a source of further investigation.

The position of the masking placed on the ringflash to reduce the amount of reflected light needs further investigation. It has been shown that placing the masking on the area of the flash closest to the tooth has improved the reproducibility. The effect of the position of the masking on measuring demineralisation surrounding an orthodontic bracket needs to be assessed.

It would be beneficial to have a reproducible method of calibrating the grey scale of a photographic image, so errors in the lighting of the subject and processing the image can be reduced. A method similar to the calibrating stepwedge for transverse microradiography would be desirable. However, there are some important differences between the calibrating aluminium stepwedge used in TMR and a calibrating grey scale for photographs. The most important difference is that the aluminium stepwedge involves the calibration of electromagnetic radiation that passes through the material,

whereas the grey scale would involve calibrating the amount of light reflected from the surface. Hence, the grey scale may form part of the problem, if the camera is angled slightly differently the amount of reflected light may alter. Ways of producing a calibrating scale that contains a medium that absorbs all light for the black end of the scale and one that reflects all light for the white end needs to be investigated.

This investigation used photographs to measure demineralisation in order to employ tools that are standard in a modern clinical setting, without resorting to complex and expensive equipment. The process of taking clinical slides and converting them to digital images is potentially time-consuming. The direct use of a digital camera, though more expensive, would reduce this problem, however the quality of images from these cameras is insufficient at present.

The *in situ* caries model requires further refinement. I would recommend investigating different methods of producing the preformed enamel lesions in order to reduce the number of teeth that need to be discarded because the lesions are not suitable. A more consistent amount of mineral loss for the preformed lesions would also be helpful, as it has been shown that the size of the lesion may affect the response (Strang *et al*, 1987; Schäfer *et al*, 1992).

The change in mineral loss was found to be variable between and within patients was found to be high. Ways of reducing this variability require further

investigation. This might include the longitudinal measurement of mineral loss from the same area of enamel either by the use of thin sections rather than slabs, or by using a non-destructive method of measurement. The disadvantages of thin sections have been outlined previously. The advantage of using a non-destructive method of measuring mineral loss is that several recordings may be obtained from one slab or section. This would considerably reduce the quantity of laboratory work required with the *in situ* caries model. The variability due to changes in the diet of individuals taking part needs further investigation. It is not clear how much this contributes to the variability of the model.

Further investigation into the use of specimens with and without preformed enamel lesions is required. This may involve the use of thin sections or slabs. This would allow the study of both the demineralisation and remineralisation processes to be carried out.

Summary

This thesis has examined three methods of recording and measuring demineralisation of enamel during orthodontic treatment. It has highlighted strengths and weaknesses in each technique. It is unlikely that one method will provide a complete picture of the mechanisms of demineralisation and remineralisation. On the contrary, a combination of direct and indirect techniques is required to study these processes during a clinical trial of a putative preventive agent.

CHAPTER 9

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Appendix J

Publications Arising Directly from the Work in this Thesis to Date

Benson PE, Pender N, Higham SM, Edgar WM (1996) Morphometric assessment enamel white spot lesions from photographs. J Dent Res 75: 1134 (abs 39).

Benson PE, Higham SM, Pender N, Edgar WM (1996) Morphometric assessment of orthodontic demineralisation from photographs. Europ J Orthod 18: 509-510 (abs 11).

Benson PE, Higham SM, Pender N, Edgar WM (1997) An *in vitro* investigation of a simulated orthodontic bracket. J Dent Res 76: 1046 (abs 221).

Benson PE, Pender N, Higham SM, Edgar WM (1998) An *in situ* model to study demineralisation during fixed orthodontics. J Dent Res 77 Spec Iss B 633 (Abs 11).

Benson PE, Pender N, Higham SM, Edgar WM (1998) Morphometric assessment of enamel demineralisation from photographs. J Dent 26: 669-677.

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Benson P E, Pender N, Higham S M (2000) Reproducibility of measuring enamel demineralisation from teeth with orthodontic brackets. J Dent Res (Abs in press).

Publications Arising from Work Associated with this Thesis

Willmot DR, Benson PE (1998) Quantitative measurement by image analysis of white enamel demineralization. J Dent Res 77 Spec Iss B 766 (Abs 1073).

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Doherty U, Benson PE, Higham SE (2000) Fluoride-releasing elastomerics assessed with the in situ caries model. Europ J Orthod (Abs in press).

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