# Clinical application of QLF and DIAGNOdent — two new methods for quantification of dental caries

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”Det finns ingen annan jämlikhet än den
och allt annat är inställsamhet och lögn:
det finns ingen människa så avig
så träaktig, så misslyckad, så skev,
att inte världen skulle kunna frambringa
det ögonblick, den unika situation,
som den människan ensam behärskar.”

*Lars Gustafsson*

*Rubriklös dikt ur “Världens tystnad före Bach”, 1982*

***To Mimi with love***

**CONTENTS**

## PREFACE

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:

**I** de Josselin de Jong E, Sundström F, Westerling H, Tranæus S, Angmar-Månsson B, ten Bosch JJ. A new method for *in vivo* quantification of changes in initial enamel caries with laser fluorescence.
*Caries Res, 1995; 29:2-7.*

**II** Tranæus S, Al-Khateeb S, Björkman S, Twetman S, Angmar-Månsson B. Application of Quantitative Light-induced Fluorescence to monitor incipient lesions in caries-active children: comparative study of remineralisation by fluoride varnish and professional cleaning.
*Eur J Oral Sci, 2001;109:71-75*.

**III** Shi X-Q, Tranæus S, Angmar-Månsson B. Comparison of two laser fluorescence methods, QLF and DIAGNOdent for quantification of smooth surface caries.
*Caries Res, 2001;35:21-26.*

**IV** Tranæus S, Shi X-Q, Lindgren L-E, Trollsås K, Angmar-Månsson B. *In vivo* repeatability and reproducibility of Quantitative Light-induced Fluorescence.
*Caries Res, 2001, in press*

**V** Tranæus S, Lindgren L-E, Karlsson L, Angmar-Månsson B. Evaluation of the *in vivo* performance of the DIAGNOdent device.
In manuscript.

## ABSTRACT

The general objective of this thesis was to evaluate the clinical performance of two new methods for quantification of dental caries — the Quantitative Light-induced Fluorescence method (QLF) and KaVo DIAGNOdent.

In Paper I, *in vivo* measurements with QLF on teeth scheduled for extraction, were compared with transverse microradiography (TMR), and tested for intra-operator reliability. In Paper II, the QLF method was applied to monitor active white spot lesions over a period of 6 months in a randomised, controlled study. In Paper IV, the QLF method was tested for intra- and inter-operator reliability *in vivo* for the capturing part as well as the image analysis part of the method. In Paper III, KaVo DIAGNOdent and QLF were validated with TMR and histology for mineral loss and lesion depth. In Paper V, DIAGNOdent readings of occlusal surfaces were compared with visual inspection, and bitewing radiography, before validation by assessment of lesion depth. In a second part of the study, DIAGNOdent was validated with QLF as reference standard for assessment of lesion depth on smooth surfaces. Intra- and inter-operator reliability were tested in both parts of the study.

*Validation*: *In vitro*, both QLF and DIAGNOdent showed good correlation with lesion depth on smooth surfaces, while the QLF method showed higher correlation with mineral loss. Sensitivity and specificity were excellent for QLF and good for DIAGNOdent. *In vivo*, DIAGNOdent showed low sensitivity for deep dentinal caries on occlusal surfaces when balanced to acceptable specificity. Validated with QLF as reference standard, DIAGNOdent readings of smooth surfaces showed a satisfactory correlation.

*Reliability tests* on smooth surfaces showed excellent results for both the QLF method and DIAGNOdent in terms of inter- and intra-operator agreement. For

occlusal surfaces, DIAGNOdent showed a very good intra-operator agreement, and a good inter-operator and inter-device agreement.

*Clinical application*: In Paper II, data obtained by the QLF method showed significant differences over time in the test group (PTR + fluoride varnish) as well as the control group (PTR) regarding lesion area and average change in fluorescence. There was also a significant intergroup difference regarding average change in fluorescence and a tendency towards intergroup difference for lesion area. Despite the fact that the QLF method consists of several steps, both the inter- and intra-operator reliability were excellent. Measurements with the DIAGNOdent (one-step system) are easier to perform, and for operator agreement the results for both methods were excellent. However, because of its closer correlation with mineral content, QLF is the preferred method for scientific purposes such as monitoring de- or remineralisation.

*Conclusions*

1) The Quantitative Light-induced Fluorescence method may be of value for longitudinal monitoring, *e.g.* for assessment of the effects of different caries preventive programmes and the method *per se* may further boost motivation and compliance by the subjects. 2) Under clinical conditions, the DIAGNOdent device showed excellent intra-operator agreement and good inter-operator agreement for measurements of carious lesions on *smooth surfaces* and good intra- and inter-operator agreement for lesions on *occlusal surfaces*. 3) With respect to clinical cut-off thresholds for dentinal caries on *occlusal surfaces,* no definite recommendations for DIAGNOdent could be made on the basis of data obtained in these studies.

# INTRODUCTION

## *Background*

In recent years there has been a pronounced change in the epidemiology and disease pattern of dental caries (Marthaler, 1990; Hugoson *et al*., 2000a, b). However, despite the dramatic decline of caries incidence, particularly in children and young adults, the disease is far from eradicated. The following major changes have occurred in the pattern of the disease. Progression of enamel caries is now slower, and allows preventive intervention before irreversible destruction of tooth substance. There is also a pronounced reduction in lesion development on the smooth surfaces, which are readily accessible to fluoride (Lussi, 1991; Mejàre *et al.*, 1998; Ripa, 1985; Newbrun, 1992; Pereira *et al.*, 1999).

In dental practice today, much of the decay for which clinical intervention is required occurs around existing restorations and/or in the occlusal surfaces of the teeth, particularly the complicated fissure systems of the molar teeth. The occlusal fissures of the first permanent molars are generally the first sites in the permanent dentition to develop caries (Kidd *et al.*, 1993).

Dental radiographs are inadequate for detecting decay in the occlusal surfaces until the lesion is well advanced through the enamel and into the dentine (Pitts, 1996). The clinician relies on visual observation of staining, and clinical judgement based upon experience, and on tactile sense, by probing with an explorer. For this purpose, the sensitivity of the explorer is reported to be only about 0.5 – 0.6 (Lussi, 1991), and its use has been questioned by several authors (Ekstrand *et al.*, 1987; van Dorp *et al.*, 1988; Lussi, 1991).

It is hypothesised that the occlusal lesion is initiated on the fissure wall and is therefore obscured by superficial sound tissue (Kidd and Joyston- Bechal, 1987). Additionally, there is evidence that an effect of regular use of fluorides is greater opacity of enamel, which may obscure underlying lesions in dentine, so-called “hidden lesions” (Sawle and Andlaw, 1988). More research is clearly needed on

"hidden caries", especially longitudinal clinical studies in which the long-term fate of these lesions can be documented (Burt, 1997).

For the clinician, a major shortcoming in caries management strategies based on risk assessment is the lack of methods which can reliably establish the extent of the subsurface decay (Pitts, 1996; Featherstone, 1996, 2000; Pine and ten Bosch, 1996; Stookey, 2000; ten Cate and van Amerongen, 1996).

To complement traditional visual assessment by the clinician, there is a role for an objective detection method, to support appropriate clinical decisions about management of the individual lesion: whether invasive therapy or a more conservative, non-invasive approach is indicated [Featherstone, 1999]. For the latter, the aim is to arrest or reverse the disease process. Objective, reliable quantitative data on the outcome of this strategy *i.e.* longitudinal monitoring of lesion response to preventive measures, would allow flexibility in selecting intervention appropriate to the individual patient, before lesion progression to a stage requiring expensive invasive therapy.

Not only in clinical practice, but also in caries research is there a need for sensitive, clinically applicable methods for early detection and quantification of caries lesions (Angmar-Månsson and ten Bosch, 1987, 1993; ten Bosch and Angmar-Månsson, 1991). Traditional methods of caries assessment, which discriminate lesions at the cavitation stage, are no longer clinically appropriate, and are obsolete for research requiring detection of a very early phase of mineral loss. In clinical research and clinical trials of new concepts or products for caries prevention, both the number of subjects and the duration of experimental periods could be dramatically curtailed, saving both time and money.

Carious lesions occur in a variety of anatomic locations and have unique aspects of configuration and rate of spread. These differences make it unlikely that one single diagnostic method will have adequate sensitivity and specificity to detect caries at all sites. Multiple diagnostic tests would increase the overall efficacy and precision of caries diagnosis. Thus there is a need to develop and test a number of different

approaches to lesion detection and quantification and to conduct comparative studies of such methods.

Of recently introduced methods, Quantitative Light-induced Fluorescence is probably the most extensively researched and the most promising method to date. Since its inception 20 years ago (Bjelkhagen and Sundström, 1981; Bjelkhagen *et al.*, 1982) it has undergone a number of modifications and can now be readily applied in the clinical setting. The extensive data published on this method and the careful documentation and re-evaluation of modifications made to the system over time allow its application as a reference standard for other methods.

The method is based on the principle that mineral loss, caused by carious destruction of tooth enamel, can be detected and measured as a change in fluorescence of the tooth substance when exposed to laser light. In the QLF method, fluorescence is induced by blue-green laser light with a wavelength of 488 nm.

Recently, research by Hibst and Gall (1998), showed that fluorescence induced by red light (638 nm, 655 nm) could differentiate between sound and carious tooth tissue. This work led to the development of a new laser-based instrument for detection and quantification of dental caries on smooth and occlusal surfaces: KaVo DIAGNOdent (KaVo, Biberach, Germany).

The following section presents a brief description of current theories about fluorescence in the dental hard tissues and an historical overview of research and development of the QLF and DIAGNOdent methods.

### *Light and dental hard tissues*

Light interacts with the dental hard tissues in different ways. It can be reflected, scattered, transmitted or absorbed. Fig. 1 shows a) a photon that is reflected by the material, b) a photon that is scattered several times in the medium, c) a photon that is transmitted right through the material, and d) a photon that is absorbed and then transformed into heat. The different phenomena can occur alone or in combination.

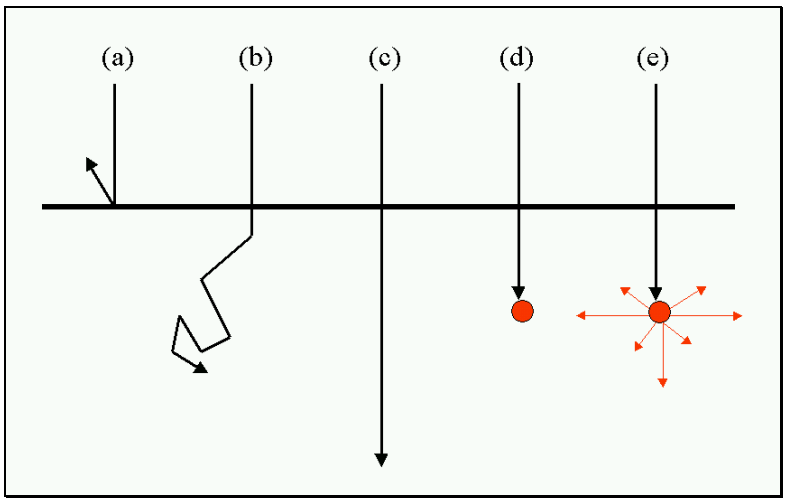


Fig. 1 a) Reflection; b) Scattering; c) Transmission; d) Absorption; and e) Absorption with fluorescence.

One possible consequence of absorption is fluorescence (Fig. 1e), in which electrons in a lower status are moved to a higher status, and when they fall back to the original situation, energy is emitted in the form of light, called fluorescence. In other words, fluorescence occurs as a result of the interaction of electromagnetic radiation with molecules in the tissue.

The cause of enamel fluorescence is still unclear. Most of the fluorescence is induced by organic components, proteinic chromophores, but some is probably attributable to the apatite (Spitzer and ten Bosch, 1976). It has been proposed that fluorescence in dentine is caused by inorganic complexes, as well as some organic components (Armstrong, 1963). In sound enamel, the path lengths are long, with a high probability that the photons will hit a chromophore. Thus, fluorescence is relatively intense.

Demineralisation of dental hard tissue, enamel or dentine, results in loss of autofluorescence, the natural fluorescence. Several factors may contribute to the decreased fluorescence of incipient caries lesions. Four possible mechanisms have been proposed (Angmar-Månsson and ten Bosch, 2001):

1) The light scattering in the lesion causes the light path to be much shorter than in sound enamel: the light absorption per volume is much smaller in the lesion and the fluorescence is weaker.
2) The light scattering in the lesion acts as a barrier for excitation light to reach the underlying fluorescing dentine, and a barrier for fluorescent light from dentine to reach the surface.
3) Fluorescence is quenched by a change in molecular environment of the chromophores.
4) Proteinic chromophores are removed by the caries process.

***The QLF method — yellow/orange fluorescence***

In the QLF method, yellow fluorescence is induced by irradiating the tooth by visible light in the blue-green region. Early last century this phenomenon had already been proposed as a useful tool for diagnosing dental caries (Benedict, 1929). More recently, laser light was used to induce fluorescence of enamel in a sensitive, non-destructive method for detection of enamel demineralisation and dental caries (Alfano and Yao, 1981; Bjelkhagen and Sundström, 1981; Bjelkhagen *et al.*, 1982).

The tooth was illuminated with a broad beam of blue-green light from an $argon^{+}$ ion laser, producing diffuse monochromatic light with a wavelength ($\lambda$) of 488 nm. The fluorescence of the enamel occurring in the yellow region was observed through a yellow high-pass filter ($\lambda \geq 540$ nm), which filters out all reflected and back-scattered light.

Demineralised areas appear as dark spots. The fluorescent radiance of a carious lesion viewed by QLF is lower than that of sound enamel. A relationship between mineral loss and fluorescent radiance (decreasing fluorescence with increasing mineral loss) was found for artificial and natural carious lesions with correlation coefficients of r = 0.73-0.86 (Hafström-Björkman *et al.*, 1992; Emami *et al.*, 1996, Al Khateeb *et al.*, 1997b).

The laser fluorescence method was developed further for *in vivo* quantification of mineral loss in natural enamel lesions, using a colour micro-video CCD camera and

computed image analysis (de Josselin de Jong *et al.*, 1995, this thesis). The principles of the method are illustrated in Fig 2.

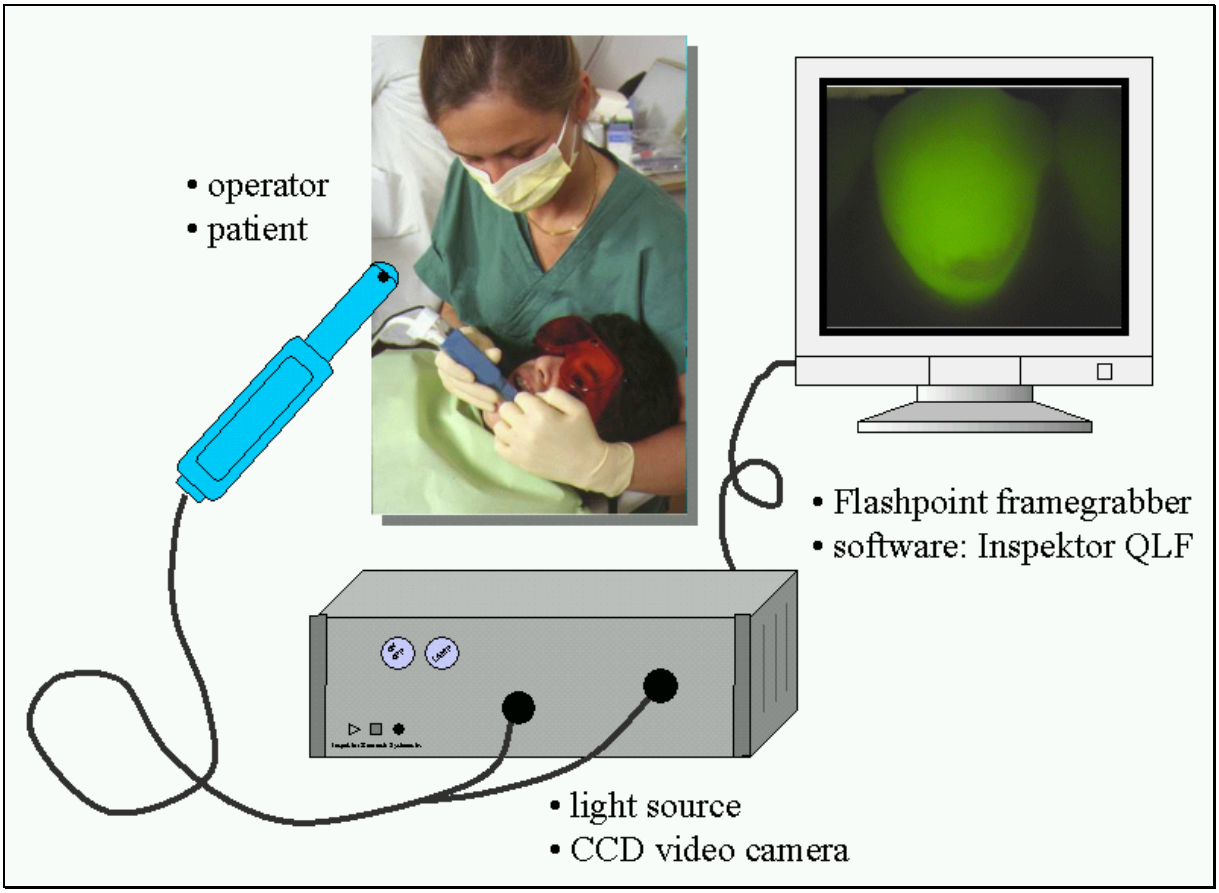


Fig. 2 QLF principle

To enable calculation of loss of fluorescence in the carious lesion, the fluorescent radiance of sound tissue at the lesion site is reconstructed by interpolation from the radiance of the sound tissue surrounding the lesion. The difference between the measured values and the reconstructed values gives the resulting fluorescence loss in the lesion. Originally, three quantities were obtained: mean fluorescence loss over the lesion (in %), maximum fluorescence loss in the lesion (in %), and area of the lesion (in $mm^2$). In the later versions of the QLF software, maximum fluorescence loss has been excluded. Lesion area ($mm^2$), $\Delta F$ (average change in fluorescence, in %), and $\Delta Q$ (area $\cdot$ $\Delta F$) were introduced as parameters.

To facilitate clinical studies at different locations, a small portable system for intraoral use was developed with a regular, *i.e*. noncoherent, light source and filter system to replace the laser source (Al-Khateeb *et al.*, 1997a). The illumination system consists of a 50 W Xenon microdischarge arc lamp equipped with an optical bandpass filter with a peak intensity of 370 nm (full-width half-measure of 80 nm) in order to produce blue light. The light illuminating the tooth is transported through a liquid filled light guide. The fluorescent filtered images (high pass filter, $\lambda \geq 520$ nm) are captured using a colour CCD video camera and a frame-grabber. Data are collected, stored and analysed by custom-made software (Inspektor Research Systems BV, The Netherlands).

The portable QLF device was validated against chemical analysis and microradiography for assessment of mineral changes in enamel and compared with results from measurements with the laser equipment (Al-Khateeb *et al.*, 1997a). It was concluded that QLF was a sensitive, reproducible method for quantification of enamel lesions, to a depth of about 400 nm.

In one of the first clinical studies using QLF, the subjects were orthodontic patients in their early teens (Al-Khateeb *et al.*, 1998a). Removal of their orthodontic brackets and bands had disclosed active carious lesions, and caries preventive measures were continued. The lesions were monitored with the QLF method: immediately after removal of the orthodontic brackets and once a month thereafter. During a one-year follow-up period, the areas of the lesions decreased, and the lost enamel fluorescence was partly regained, indicating remineralisation. It was concluded that QLF is appropriate for *in vivo* monitoring of mineral changes in incipient enamel lesions and is useful for the evaluation of preventive measures in caries-susceptible individuals, such as orthodontic patients (Al-Khateeb *et al.*, 1998a).

Ferreira Zandoná *et al.*, (2000) conducted a 1-year pilot clinical trial on children, 9-12 yr old, to compare caries detection by traditional clinical examination and by QLF. Based on preliminary data from the study, the authors concluded that QLF was

able to monitor changes in the lesions, even small changes over time, and these changes were in agreement with the clinical findings.

To validate the ability of QLF to accurately detect lesion area, exfoliated, deciduous teeth were collected from study subjects participating in the clinical trial and examined in a multi-site laboratory study (ten Cate *et al.*, 2000). Sections of the teeth were examined by transverse microradiography, histology and polarised light microscopy. The authors concluded that the QLF analysis under clinical conditions, for the bucco-lingual specimens, showed a sensitivity of 79% and a specificity of 75%.

Compared to other detection methods, the values for sensitivity are higher but - consequently - lower for specificity, e.g. FOTI (Pine, 1996). This can be understood from the physical principle of the method which enables very early, non-destructive detection of caries under clinical conditions. Judging from the data this gave rise to a number of false positive scorings (ten Cate *et al.*, 2000).

### *The DIAGNOdent device — near infrared fluorescence*

In 1985, Sundström and co-workers conducted a comparative study on the fluorescence of sound tooth substance for various excitation wavelengths and reported no fluorescence within the visible range for illumination by red (633 nm) light. As mentioned earlier, recent research by Hibst and Gall (1998) showed that red light (638 nm, 655 nm) induced fluorescence could differentiate between sound and carious tooth tissue.

KaVo DIAGNOdent (KaVo, Biberach, Germany), based on the above research by Hibst and Gall, is a recently introduced laser-based instrument, developed for detection and quantification of dental caries on smooth and occlusal surfaces. It operates with light from a diode laser with a wavelength of $\lambda$=655 nm and 1 mW peak power (Hibst and Gall, 1998). The light is transmitted through a descendent optic fibre to a hand held probe with a bevelled tip with a fibre optic eye.

Both organic and inorganic molecules in the tooth substance absorb the light, and fluorescence within the infrared spectra occurs. The emitted fluorescence, as well as backscattered ambient light, is collected through the tip, and passed in ascending fibres

to a photo diode detector. The backscattered excitation and short wavelength ambient light is absorbed by a band pass filter in front of the photo diode detector. To discriminate the fluorescence from the ambient light, the laser diode is modulated. By amplifying only the modulated portion of the signal, the ambient light is suppressed (Hibst *et al.,* 2001). The signal is finally processed and presented on the display as an integer between 0-99. The principles of the method are presented in Fig 3.

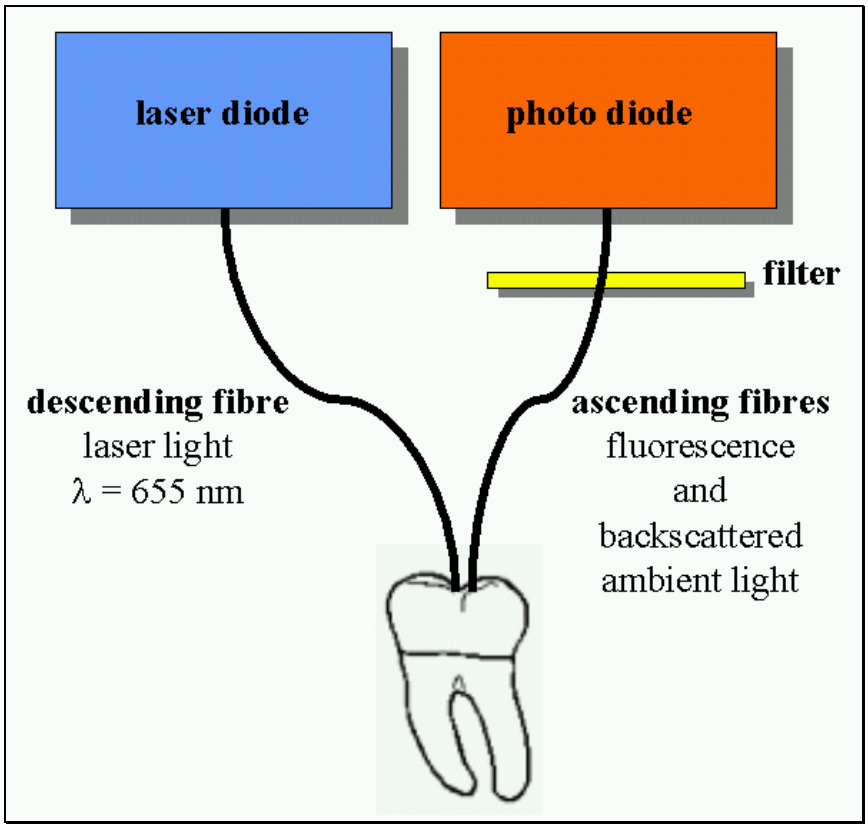


Fig. 3 DIAGNOdent principle

In order to collect fluorescence from the maximum extension of carious lesions on occlusal surfaces, the instrument has to be tilted around the measuring site. This ensures that the tip picks up fluorescence from the slopes of the fissure walls where the carious process is believed to originate. Variations in the laser diode output power should regularly be compensated by calibration of the instrument against a given fluorescence standard, in accordance with the manufacturer's instructions.

In the presence of carious tooth substance, fluorescence increases. The origin of the fluorescence is still the subject of debate, but proto-porphyrins and meso-porphyrins, bacterial metabolites, probably play a major role (Hibst and Paulus, 1999, 2000).

The DIAGNOdent device has been evaluated in several *in vitro* studies (Lussi *et al.*, 1999; Longbottom *et al.*, 1998, 1999; Shi *et al.*, 2000, 2001). To date there is only one published clinical study comparing traditional examination and treatment and the concurrent use of the DIAGNOdent device (Lussi *et al.*, 2001). Good to excellent sensitivity and excellent reproducibility were reported.

# AIMS

## *General aim*

The general objective of this thesis was to evaluate the clinical performance (validation, repeatability- and reproducibility tests, and clinical applicability) of two new methods for quantification of dental caries — QLF and DIAGNOdent.

## *Specific aims*

- The aim of *Paper I* was to develop the laser fluorescence method to a method for *in vivo* assessment of changes in initial enamel carious lesions.

- The aims of *Paper II* were twofold: firstly, to test quantitative light-induced fluorescence (QLF) methodology in a short-term, randomised, controlled clinical study; and secondly, to compare the response of white spot lesions in caries active adolescents to fluoride varnish or professional toothcleaning, using QLF to monitor changes in lesion fluorescence during the experimental period.

- The aim of *Paper III* was to correlate *in vitro* readings by DIAGNOdent and QLF with lesion depth and mineral loss, and also compare the diagnostic performance of the two methods, in terms of sensitivity and specificity.

- In *Paper IV*, the aim of the study was to test *in vivo* repeatability and reproducibility of the QLF method for quantification of enamel carious lesions.

- In *Paper V*, the aim of the study was to perform clinical evaluation of the KaVo DIAGNOdent device to determine whether the previously reported laboratory values are comparable to those found in a clinical study.

## MATERIAL AND METHODS

### Paper I

The study was approved by the Ethics Committee at Huddinge University Hospital, Huddinge, Sweden (123/91).

*Subjects*

Twelve patients, scheduled for orthodontic treatment, were selected and included in the study.

*Repeatability test*

The repeatability of clinical image capturing was tested by capturing images of one arrested initial lesion on the buccal surface of a premolar tooth, 25 times.

*Test of the reconstruction*

To test the reconstruction, the buccal surfaces of 19 visually sound teeth were examined *in vivo*. The sound surface fluorescence values inside the patch were reconstructed from the values at the patch border, *i.e.* sound enamel fluorescence values. The difference between the actual and the reconstructed values were then averaged in terms of the number of pixels covered, the area in $mm^2$, and the mean change in fluorescence.

*Clinical application*

Two intact maxillary premolars, scheduled for extraction on orthodontic indications, were selected in each participant The participants were provided with a non-fluoridated dentifrice and instructed to use it throughout the study, until extraction of the premolars. Images of the buccal surfaces of all teeth included in the study were captured at baseline. The imaging equipment comprised an $argon^+$ ion laser ($\lambda$=488 nm, 10 - 20 mW $cm^{-2}$) as a light source and a micro-CCD-video camera (Panasonic WV - KS 152, length 50 mm, diameter 17 mm) equipped with an orange high-pass

filter (Hoya O-54, λ›540 nm) to exclude scattered light. Individually formed plastic brackets (1.5 x 3 mm) were fabricated. Plastic slabs were fixed to the brackets and placed at a distance of 1 mm from the tooth surface, partially covering the buccal enamel surface. In the space between the plastic slab and the enamel surface, plaque formation could proceed undisturbed by mechanical forces, thereby creating an environment conducive to initiation of carious lesions. The brackets remained in place for a period of 4-6 weeks, and were then removed. The underlying plaque was removed with a rotating rubber cup, and QLF images of the experimental surface were captured. Images were captured again after 3 weeks and after 5 weeks, whereafter the teeth were finally extracted.

**Paper II**

The study was approved by the Ethics Committee at Huddinge University Hospital, Huddinge, Sweden (224/96) and conducted according to ICH/GCP regulations (Harmonised Tripartite Guideline for Good Clinical Practice, 1996).

*Subjects*

The subjects comprised 31 healthy subjects aged 13 to 15 yr. The inclusion criteria were that the subjects should be aged between 13 and 15 yr, and each have two or more white spot lesions, on the buccal surfaces of the premolars or permanent molars. After stratification by gender, the subjects were randomly assigned to one of two groups.

*Study design*

The study was conducted as a single-blind study. At all visits, lesion fluorescence was first measured by QLF at the Clinical Research Unit. The subject then proceeded to the Department of Paediatric Dentistry for preventive treatment. In the fluoride varnish group, professional tooth-cleaning was followed by an application of fluoride varnish (Fluor protector®): at study start (baseline), the following week and then four times, six weeks apart, for 6 months (visit 1 – visit 4). The group comprised 13

patients with a total number of 32 lesions. Before application of the fluoride varnish, the teeth were thoroughly dried with compressed air. Fluoride varnish was applied with an applicator tip, in accordance with the manufacturer's instructions. The other group underwent professional tooth-cleaning at study start (baseline), and then four times, six weeks apart, for 6 months (visit 1 – visit 4). In this group, 18 subjects, with a total number of 30 lesions, completed the study. All subjects received similar oral hygiene instructions and counselling on dietary habits (meal frequency, the importance of sugar-free carbonated drinks, confectionery, chewing gum, etc). All subjects were issued with two new extra soft toothbrushes (Pepsodent®) and instructed to use the "Bass technique" and brush for 2 min. They were also supplied with a fluoride dentifrice (Pepsodent®, 1450 ppm F) to be used twice a day. The subjects were instructed to refrain from any other kind of supplementary fluorides, such as F-lozenges, F-rinses or F-gels during the study period. The teeth were professionally cleaned with a rotating rubber cup and the same toothpaste as supplied to the subjects. If necessary, calculus was removed with standard scaling instruments. At the first visit, samples of paraffin-stimulated saliva were obtained for standardised chair-side Mutans streptococci tests (Dentocult Strip Mutans®), Lactobacillus (Dentocult LB®), and buffer capacity (Dentobuff®) tests from Orion Diagnostica, Helsinki, Finland.

*Longitudinal measurements of fluorescence changes*

QLF images of the lesions were subsequently captured. The equipment comprised an argon$^{+}$ ion laser ($\lambda$=488 nm, 10-20 mW cm$^{-2}$) as a light source, and a micro-CCD-video camera (Panasonic WV - KS 152, length 50 mm, diameter 17 mm), equipped with an orange high-pass filter (Hoya O-54, $\lambda$›540 nm) to exclude scattered light. Enamel fluorescence was measured at the beginning of the study (baseline) and at each six-weekly visit (visit 1 – visit 4) over the following 6 months. Two parameters were measured: lesion area, and the average change in fluorescence over the lesion. Data analyses, using the QLF-program (Inspektor QLF 1.97, Inspektor Research Systems, Amsterdam, The Netherlands), were performed blindly, in random order, after all panellists had reached end point.

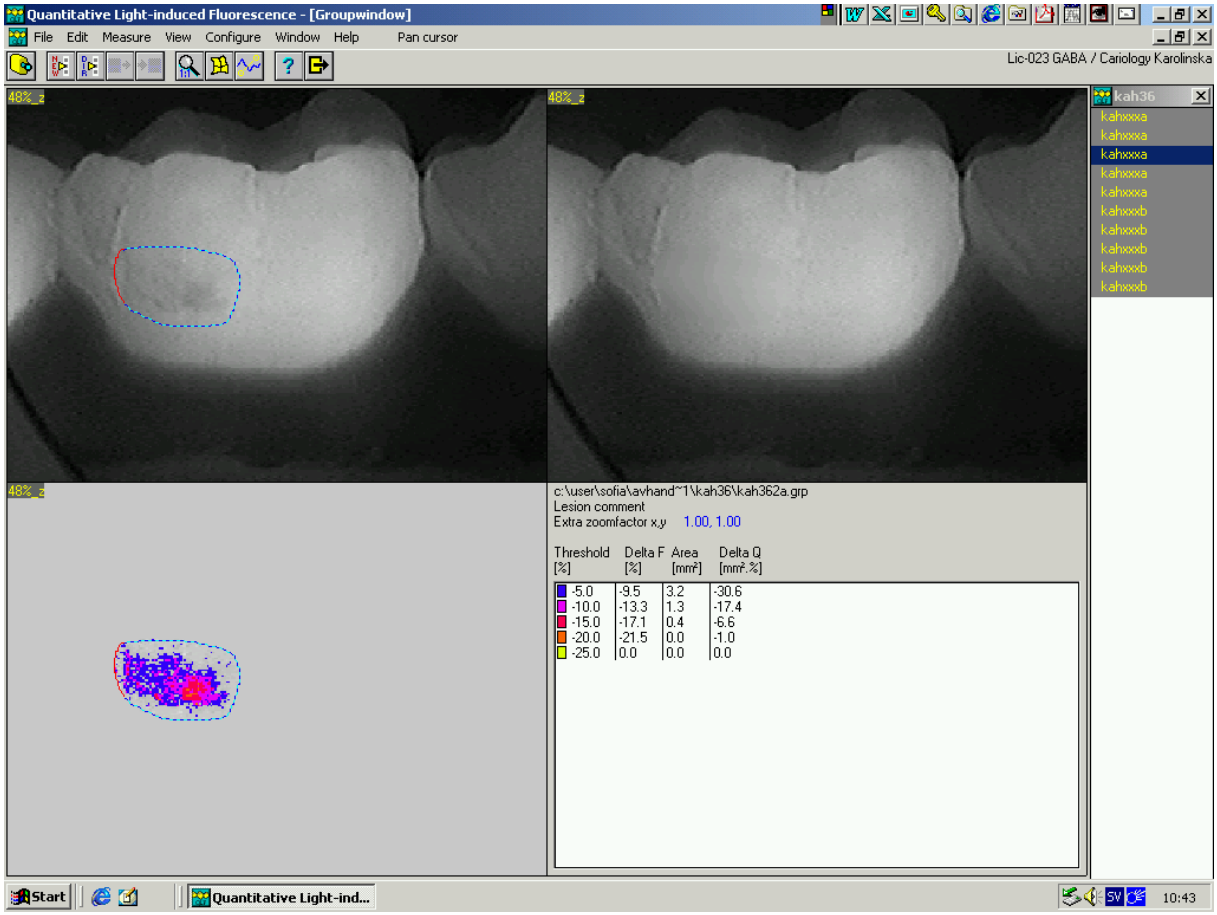


Fig. 4 An example of QLF analysis (screen catch) of an incipient lesion on 36 in a patient enrolled in the clinical trial presented in paper II.

## Paper III

The study was approved by the Ethics Committee at Huddinge University Hospital, Huddinge, Sweden (001/02).

*Teeth*

The material comprised 40 premolar teeth, extracted from young adolescents on orthodontic indications, and stored in 10 % neutral buffered formalin solution. The test sites in this study were the approximal surfaces. Visual inspection of the 80 approximal surfaces revealed that 11 were sound, 5 had cavities, and the remaining 64

had caries without cavitation. The teeth were thoroughly rinsed under tap water and then cleaned with a toothbrush. Each of the approximal surfaces was photographed by a digital camera (Kodak DC210), to facilitate subsequent application of the laser fluorescence methods and orientation for producing sections through the lesion sites.

*Caries quantification with DIAGNOdent and QLF*

The teeth were retrieved from the formalin solution, gently wiped with tissue, and dried with compressed air for about 8 sec. The areas of caries predilection on the approximal surfaces were measured by DIAGNOdent, using the flat tip. The highest values were registered and these sites were marked on the hard copy of the photographs of the teeth. After the measurements, the teeth were returned to the storage solution. Two QLF devices were used: both consisted of a camera (Panasonic WV – KS 152), one with an argon$^{+}$ ion laser as the light source (de Josselin de Jong *et al.*, 1995, this thesis), and the other with non-coherent light from a Xenon lamp as the light source, combined with a filter system (Al-Khateeb *et al.,* 1997a). The teeth were retrieved from the storage medium and wiped dry with tissue. Images of the 80 approximal surfaces were captured independently and recorded. The images were stored, processed, and analysed with the QLF-program (Inspektor QLF 1.97, Inspektor Research Systems, Amsterdam, The Netherlands). Two parameters were measured: average fluorescence loss (%) and maximum fluorescence loss in the lesion (%).

*Histopathological and microradiographic analyses*

The teeth were then sectioned for histopathological examination and transverse microradiography. Tooth slices, 300-µm-thick, were sawn perpendicular to the enamel surface at the test sites. The tooth slices were subsequently examined under a microscope at 16x magnification. The following five-point scale was used to stratify the sites according to the histopathological evidence of penetration by caries of the dental hard tissues:

0 = sound, 1 = enamel caries limited to the outer half of enamel, 2 = caries extending into the inner half of the enamel, but not to the dentinoenamel junction (DEJ), 3 = caries penetrating the DEJ, but limited to the outer half of the dentine, and 4 = caries involving the inner half of the dentine.

The tooth slices were then manually ground to 80-90 μm thickness. The microradiographic image of the tooth slice, together with an aluminium step wedge for calibration, was recorded on holographic film (Kodak SO-253) exposed to Ni-filtered CuKα radiation at 20 kV and 55 mA. The focus-to-film distance was 315 mm, and the exposure time was 20 s. Mineral content-depth profiles were measured with a microscope-densitometer-computer set-up equipped with software for transverse microradiography, TMR (Inspektor Research Systems BV, Amsterdam, The Netherlands). Densitometric values were used to calculate the mineral content according to the formula of Angmar *et al*. (1963). The tracings were expressed as integrated mineral loss (ΔZ), and lesion depth (μm), using the established parameter definition (Arends and ten Bosch, 1992). The integrated mineral loss, ΔZ, was obtained only for enamel caries because of the technical complexity of measuring both enamel and dentinal caries at the same time.

**Paper IV**

The study was approved by the Ethics Committee at Huddinge University Hospital, Huddinge, Sweden (224/96 and 430/97) and conducted according to ICH/GCP regulations.

*General set-up*

Tooth surfaces with caries lesions were illuminated by either diffuse blue-green light (λ=488 nm, 10-20 mW $cm^{-2}$) from an $argon^{+}$ ion laser, or blue light from a Xenon arc lamp with a blue filter (peak intensity of λ=370 nm with a full width half measure of 80 nm). A micro-CCD-video camera, equipped with a high-pass filter (orange, λ›540 nm for the laser light, and yellow, λ›520 nm for the lamp light) to exclude scattered light, was used to film the lesions. A computer program, Inspektor QLF 1.99

(Inspektor Research Systems BV, Amsterdam, The Netherlands) was used to display, store, browse and analyse the images. All images were captured under identical physical conditions, in a dental surgery. A roller blind in the window excluded direct daylight, which might cause reflection at the enamel surface and thereby disturb the image. It is known that dehydration of lesions results in greater loss of fluorescence (Al-Khateeb *et al*., 1998b, 2002). To standardise the level of hydration, the patient rinsed with water between the capturing of each image. The actual area was then wiped once with a cotton roll, and the image was captured. Capturing of each image took approximately 5 s, an interval brief enough to avoid dehydration. Corrections in distance deviations were made by the use of contour points, a software facility that automatically compensates for changes in distance between the camera and the lesion during imaging. The material used in this study was a combination of tooth material from two parallel clinical trials, one with the $argon^{+}$ ion laser system, and the other with the Xenon lamp system.

*Image capturing*

To test the repeatability of the capturing procedures, three analysts captured 15 images each of one natural incipient smooth surface lesion. Analysts 1 and 2 were highly experienced with the QLF method, while analyst 3 was less experienced, and would therefore represent a new user of this method. The Xenon arc lamp with a blue filter was used as the light source for both the repeatability and the reproducibility tests. After completion of image capturing, one analyst analysed all images. To test the reproducibility of the image capturing procedures, three analysts captured one image each of 15 different natural smooth surface lesions on 15 teenagers. To standardise tooth position, only mandibular left first molars, in 15 patients, were included. The lesions were active, well-defined (lesion border visible), non-stained, incipient, buccal lesions. Analyst 1 captured the first image, and analysts 2 and 3 then repeated the process. The primary image, captured by the first analyst, served as a guide for repositioning of the other two images. After the session, all images were analysed by one analyst.

*Image analysis*

To test the repeatability and reproducibility of the analytical stages of the QLF method, images of 15 different caries lesions were analysed by three analysts. As before, these lesions were active, well-defined (lesion border visible), non-stained, incipient, buccal lesions in 15 teenagers, randomly selected from a total of 60 lesions, evenly distributed with respect to both size and depth and typical of lesions encountered clinically in this age group. The $argon^{+}$ ion laser was used as the light source. The images were presented to the analysts in three set-ups with copies of the 15 lesions, in which the lesions were placed in random order. The images were analysed independently by the analysts, three times each. Individual patches were created in all images in the first set-up, and analyses were performed. A fourth independent person, who in addition placed the patches so that the analysts had to perform necessary adjustments before the analyses, transported these patches to the second set-up of images. In the third set-up of the images, new patches were created, and analyses were subsequently performed.

*Application of different patch sizes*

Four images, randomly selected from the 15, were examined in order to determine how different patch sizes would affect the result. One analyst performed analyses for three patch sizes. The range of sizes was dependent on the subjective judgement of the analyst, and only a successful reconstruction pattern was accepted (Fig. 5). All images throughout the study were analysed blindly, without reference to the lesion data. When a good reconstruction was obtained, the lesion data were saved for later statistical analysis.

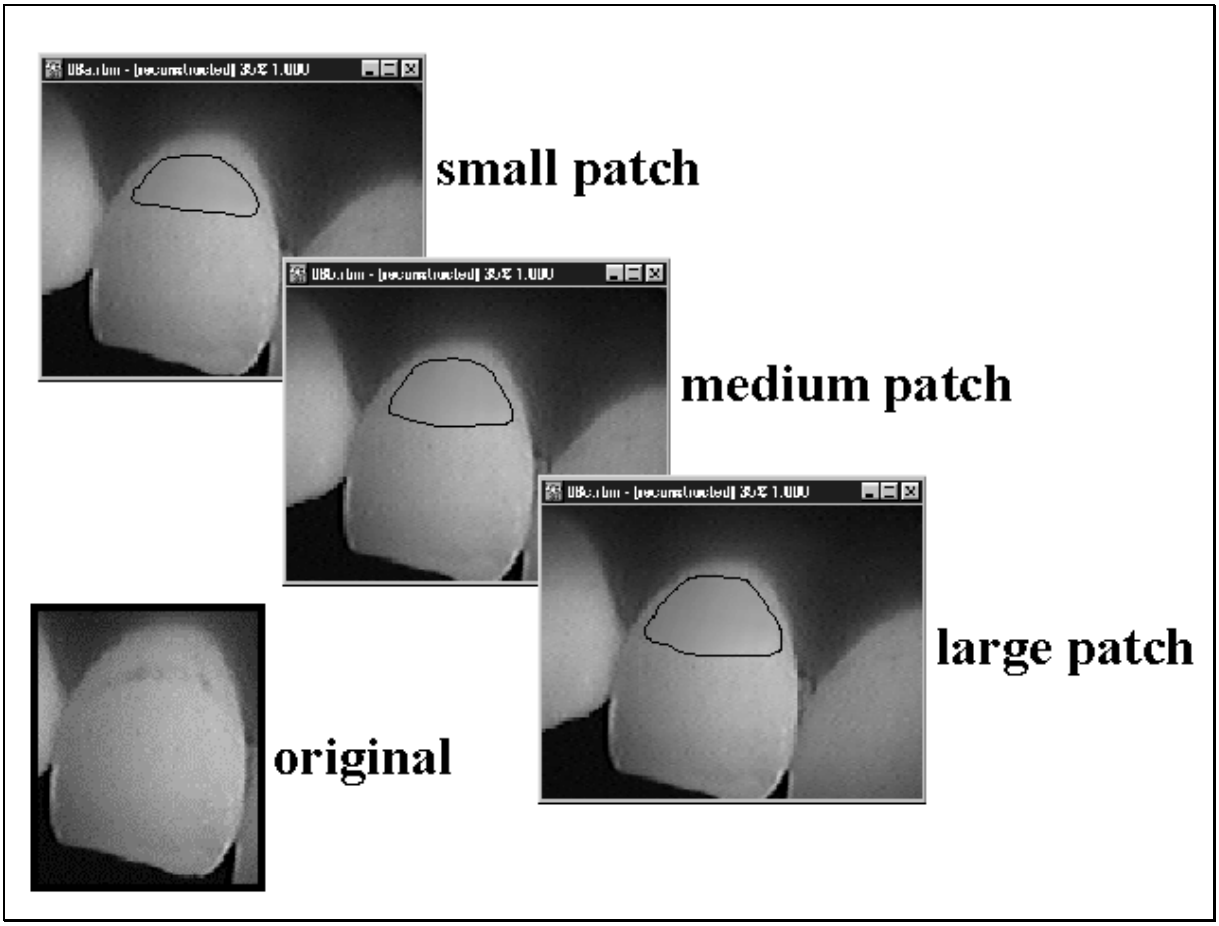


Fig. 5 Three different patch sizes used to illustrate their influence on the reconstruction of sound enamel fluorescence. By courtesy of Karger, Basel, Switzerland.

**Paper V**

The study was approved by the Ethics Committee at Huddinge University Hospital, Huddinge, Sweden (155/01 and amendment 00-01-05 to 430/97), and conducted according to ICH/GCP regulations.

*Occlusal lesions*

*Subjects*

The subjects comprised 17 patients, 18-42 years of age, with a total of 33 test teeth. All examinations were made independently by the two operators, who were calibrated before study start. A third person handled all information collection

including the DIAGNOdent readings, which were unavailable to the operators. All teeth incorporated in the study were documented with a digital camera, Nikon COOLPIX 990, before the lesion was opened, at the end of caries removal/cavity preparation, and after restoration. These images provided a permanent record of the appearance of the teeth and the extent of the lesions, as well as a guide for repositioning. The images were digitally stored, browsed, and processed throughout the study.

*Visual inspection and bitewing radiography*

An initial visual inspection (using magnification at 2.6x), with or without the explorer, was performed according to the following criteria and classification (modified Ekstrand criteria):

0 = no or slight change in enamel translucency after prolonged air drying (>5s).

1 = opacity or discoloration hardly discernible on wet surface, but distinctly visible after air drying, or opacity or discoloration distinctly visible without air drying,

2 = localised enamel breakdown in opaque or discoloured enamel and/or greyish discoloration from the underlying dentine.

Bitewing radiographs were taken of all teeth according to standard clinical protocol, unless the patient had recent bitewing x-rays (<6 months old). The radiographs were then evaluated according to the following criteria:

0 = no radiolucency visible, 1 = radiolucency visible in the enamel, 2 = radiolucency visible in the outer dentine, just beyond the DEJ, and 3 = radiolucency visible in the inner dentine, clearly beyond the DEJ.

*Treatment decisions*

On the basis of visual inspection, bitewing evaluation, and clinical judgement, a decision was made as to whether to treat the tooth invasively or not. If the tooth was judged to be sound, and not in need of opening, it would be classed as sound clinically. However, if the DIAGNOdent reading indicated that the tooth had a

hidden lesion (a reading of 15 or higher), then the surface was "minimally explored" with a fine bur.

*Measurements with DIAGNOdent*

Two DIAGNOdent devices were tested separately. The device was used prior to opening the tooth, according to the manufacturer's instructions. The instrument was calibrated before every patient against the supplied standard, and zeroed on sound enamel of each test tooth to allow for intrinsic variation in fluorescence of individual teeth. Before the DIAGNOdent was used, the surface of the tooth was cleaned with bicarbonate spray utilising the Prophy Flex 2 device (KaVo, Germany). The tooth surfaces were air-dried for 5 s with compressed air prior to measurement. Each site was measured twice, with water spraying and air-drying in between to standardise the humidity. The measurement time was standardised to 10 s.

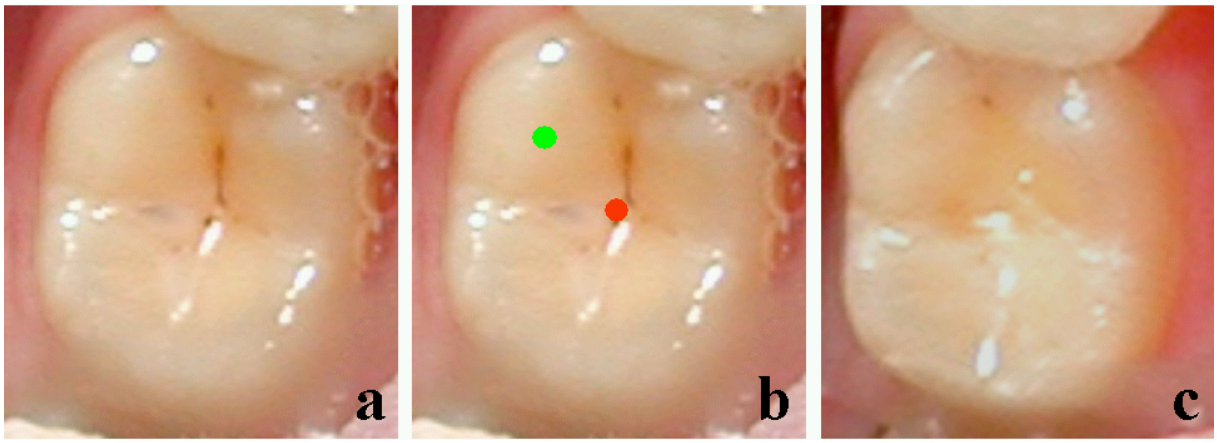


Fig. 6 Example of images of one lesion in the study, a) initial raw-image, b) image with a red mark for the experimental site and a green for the enamel reference point, and c) after careful caries excavation. In this case, the DIAGNOdent readings were between 27 and 36 and the lesion depth was in the outer dentine, just beyond the DEJ.

*Validation of lesion depth*

When the suspicious or definitely carious tooth was opened with the appropriate bur, the observer determined the extent of the lesion according to the following scale: 0 = no caries, 1 = caries in the enamel, 2 = caries in the outer dentine, just beyond the DEJ, and 3 = caries in the inner dentine, clearly beyond the DEJ.

A Starlite #2 piano wire explorer or equivalent was used to check for any remaining soft dentine. All opened teeth were finally restored with an appropriate material, depending upon the size of the lesion and the extent of the cavity. Routine clinical restorative procedures were followed.

*Smooth surface lesions*

*Subjects*

Thirty patients, 13-15 years of age with a total of 30 test teeth were included. The test teeth were standardised to the lower left first molar (tooth no. 36), with active incipient enamel lesions on the buccal smooth surface.

*Measurements with QLF and DIAGNOdent*

Firstly, images of the test sites were captured with the QLF method. The images were digitally stored until the study was completed and then analysed with special software (Inspektor QLF 1.99m). After the QLF image capturing, all teeth were examined with the DIAGNOdent independently by the two operators in a standardised way and according to the manufacturer's instructions. The instrument was calibrated before the measurements of every patient, and zeroed on sound enamel of each test tooth. The tooth surfaces were cleaned and dried with a cotton roll prior to measurements by the DIAGNOdent device. The operators performed the measurement cycle twice at each site, with water spraying in between to standardise the humidity. The QLF data (ΔF) were subsequently used as reference standards for validation purposes.

**Statistical methods**

In ***Paper I***, mean ($\bar{x}$) and standard deviation (SD) were used to test the repeatability of clinical image capturing as well as test of the reconstruction for all three parameters, lesion area, mean change and maximum change in fluorescence.

In ***Paper II***, a two-way ANOVA (parametric) with repeated measures on one factor was used to analyse the changes from baseline. 95% confidence intervals were calculated for the mean changes from baseline across visits. Estimates for these calculations were extracted from ANOVA. The mean ($\bar{x}$) and standard error of measurement (SEM) were also calculated.

In ***Paper III***, Spearman's rank correlation coefficient was used to calculate the correlation coefficient between the fluorescence methods and the lesion depth. Sensitivity and specificity of caries detection at $D_3$ level were calculated for each method. For enamel caries only, Spearman's rank correlation coefficient was applied to analyse the correlation between the fluorescence methods and TMR determination of mineral loss ($\Delta Z$). For all specimens, the readings by the two methods were correlated with lesion depth in μm measured by TMR, using Spearman's rank correlation coefficient. Additionally, the correlation between readings from the QLF lamp and laser devices was assessed using Pearson's correlation coefficient.

In ***Paper IV***, power transformations were used to reduce the (positive) skew distribution in the material used for image capturing tests. A log transformation reduced the skew distribution for the maximum change, but was not strong enough for the lesion area and the average change. For these variables a negative reciprocal transformation was needed. One-way ANOVA with repeated measures was used to analyse inter- and intra-examiner reliability. The intra-class correlation coefficient (r) was used as a measure of reliability and was calculated from the mean squares of the different sources of variation in the ANOVA model. An approximative 95%

confidence interval, using Satterthwaite's approximation (Fleiss, 1986), was used to test the power of the ICC calculations. Tukey HSD test was used for post hoc tests. The mean ($\bar{x}$) standard error of measurement (SEM) and the coefficient of variation (CV) were also calculated.

In ***Paper V*****,** one-way ANOVA with repeated measures was used to determine systematic differences between devices, operators, measurements, or systematic between-interactions. For inter-device and inter- and intra-operator agreements, Spearman's rank order correlation coefficient was used. Spearman's correlation coefficient was also used to determine the agreement between DIAGNOdent readings and the reference standard (QLF). To test the validity of occlusal readings, sensitivity and specificity were calculated using individual thresholds for each ranking system.

## RESULTS

### *Validation*

*QLF*

In *Paper III*, the correlation between lesion depth, derived from histopathology and microradiography, and readings from the two light sources of QLF were between 0.84 and 0.88. The sensitivity and specificity for the two light sources for caries detection at $D_3$ level on smooth surfaces were respectively: 0.83 and 0.98 for the laser (cut-off 25%), and 0.94 and 1.0 for the lamp (cut-off 20%). The correlation between mineral loss (ΔZ), lesion depth (μm), and the QLF readings varied between 0.74 and 0.77 for mineral loss, and between 0.87 and 0.91 for lesion depth. For maximum and average fluorescence loss, the correlations between the two light sources were 0.94 and 0.95 respectively.

In *Paper I*, test of the reconstruction showed a mean difference between actual and reconstructed surface of 1.6 ± 1.1% (mean ± SD) fluorescence loss.

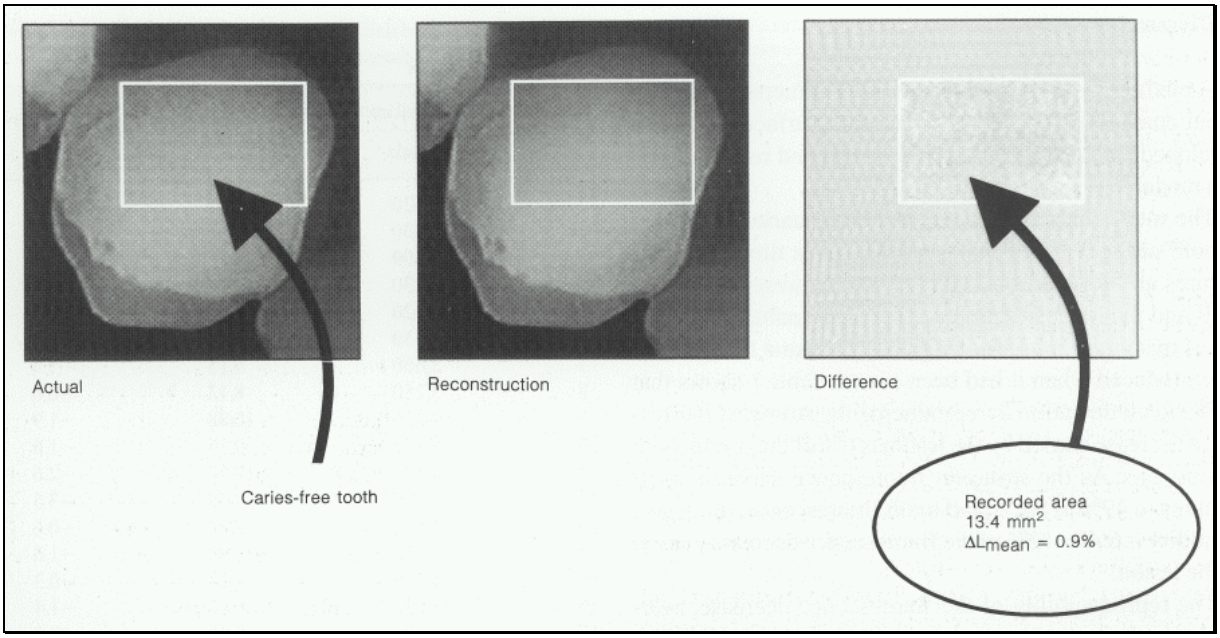


Fig. 7 Example of one measurement in the reconstruction test of the QLF method. By courtesy of Karger, Basel, Switzerland.

*DIAGNOdent*

In *Paper III*, the correlation between lesion depth, derived from histopathology and microradiography, and readings from the DIAGNOdent were 0.85. The sensitivity and specificity for the DIAGNOdent for caries detection at $D_3$ level on smooth surfaces were 0.75 and 0.96 (cut-off 9), respectively. For carious lesions confined to the enamel only, the correlation between mineral loss (ΔZ), lesion depth (μm) and the DIAGNOdent readings were 0.67 for the mineral loss, and 0.86 for lesion depth.

In *Paper V (occlusal surface),* DIAGNOdent readings obtained from both DIAGNOdent devices (DD 1 and DD 2) showed a very weak correlation, ranging from 0.03 to 0.32. With a specificity >0.8, the sensitivity was as low as 0.14–0.43 for deep dentinal lesions. The corresponding values for VI and BW were at best 0.75 and 0.50 for sensitivity, and 0.84 and 0.84 for specificity, respectively.

In *Paper V (smooth surface)*, the correlation between readings from the DIAGNOdent device and the reference standard (QLF, ΔF) varied between 0.57 and 0.73.

***Repeatability and reproducibility***

*QLF*

In *Paper I*, when repeatability of "image capturing + analysis" was tested, the lesion area was found to be 0.56±0.20 $mm^2$, the mean change in fluorescence 17.6 ± 0.7%, and maximum change in fluorescence 49 ± 6%.

In *Paper IV (image capture)*, inter-examiner reliability among the three analysts for the three variables tested (lesion area, and average and maximum changes in lesion fluorescence) showed a correlation between 0.95 and 0.98 (Table 1).

In *Paper IV (image analysis),* intra-examiner reliability for the three analysts in measuring the three test variables ranged from 0.93 to 0.99 for the analyses with the first patch, as well as between the first and the second patch. Inter-examiner reliability among the three analysts for the three test variables ranged between 0.95 and 0.99 for all analyses (Table 1).

| **QLF**<br>*Intra-class correlation coefficient* | **Intra-operator agreement** | | | **Inter-operator agreement** | | |
|---|---|---|---|---|---|---|
| | ***Area*** | ***Average fluorescence change*** | ***Maximum fluorescence change*** | ***Area*** | ***Average fluorescence change*** | ***Maximum fluorescence change*** |
| ***Capture*** | | | | 0.98 | 0.95 | 0.97 |
| ***Analysis*** | 0.93 – 0.99 | 0.96 – 0.99 | 0.96 - 0.99 | 0.95 – 0.96 | 0.98 – 0.99 | 0.96 – 0.98 |

Table 1 Clinical intra- and inter-operator agreement for the QLF method.

| **DIAGNOdent**<br>*Spearman's rank order correlation coefficient* | **Intra-operator agreement** | | | **Inter-operator agreement** | | |
|---|---|---|---|---|---|---|
| | ***Smooth*** | ***Occlusal*** | | ***Smooth*** | ***Occlusal*** | |
| ***Device*** | | | | | 0.63 - 0.87 | |
| | | **DD 1** | **DD 2** | | **DD 1** | **DD 2** |
| ***Operator*** | | | | 0.79 - 0.87 | 0.78 - 0.82 | 0.63 - 0.83 |
| ***Measurement*** | 0.94 | 0.82 - 0.94 | 0.88 - 0.89 | | | |

Table 2 Clinical intra- and inter-operator agreement for the DIAGNOdent device.

*DIAGNOdent*

In *Paper V (occlusal surface),* intra- and inter-agreement on three levels (device-, operator-, and measurement level) for clinical DIAGNOdent readings showed correlation values from 0.63 to 0.87 for inter-device, 0.63 to 0.83 for inter-operator, and 0.82 to 0.94 for intra-operator agreement (Table 2).

In *Paper V (smooth surface),* intra- and inter-agreement on two levels (operator-, and measurement level) for clinical DIAGNOdent readings showed correlation values between 0.79 and 0.87 for inter-operator agreement, and 0.94 for intra-operator agreement (Table 2).

***Clinical application***

*QLF*

In *Paper II*, the results showed statistically significant inter- and intra-group differences. In the fluoride varnish group, there was a statistically significant change over time (baseline – 6 months) in both lesion area, p=0.011, and average change in fluorescence, p=0.002 (decreased lesion area, and increased fluorescence radiance). The corresponding changes in the professional tooth-cleaning group were not significant. There was a statistically significant difference (p=0.03) between the groups for average change in fluorescence in the lesion. For lesion area, there was no significant difference, but a tendency towards inter-group difference (p=0.055).

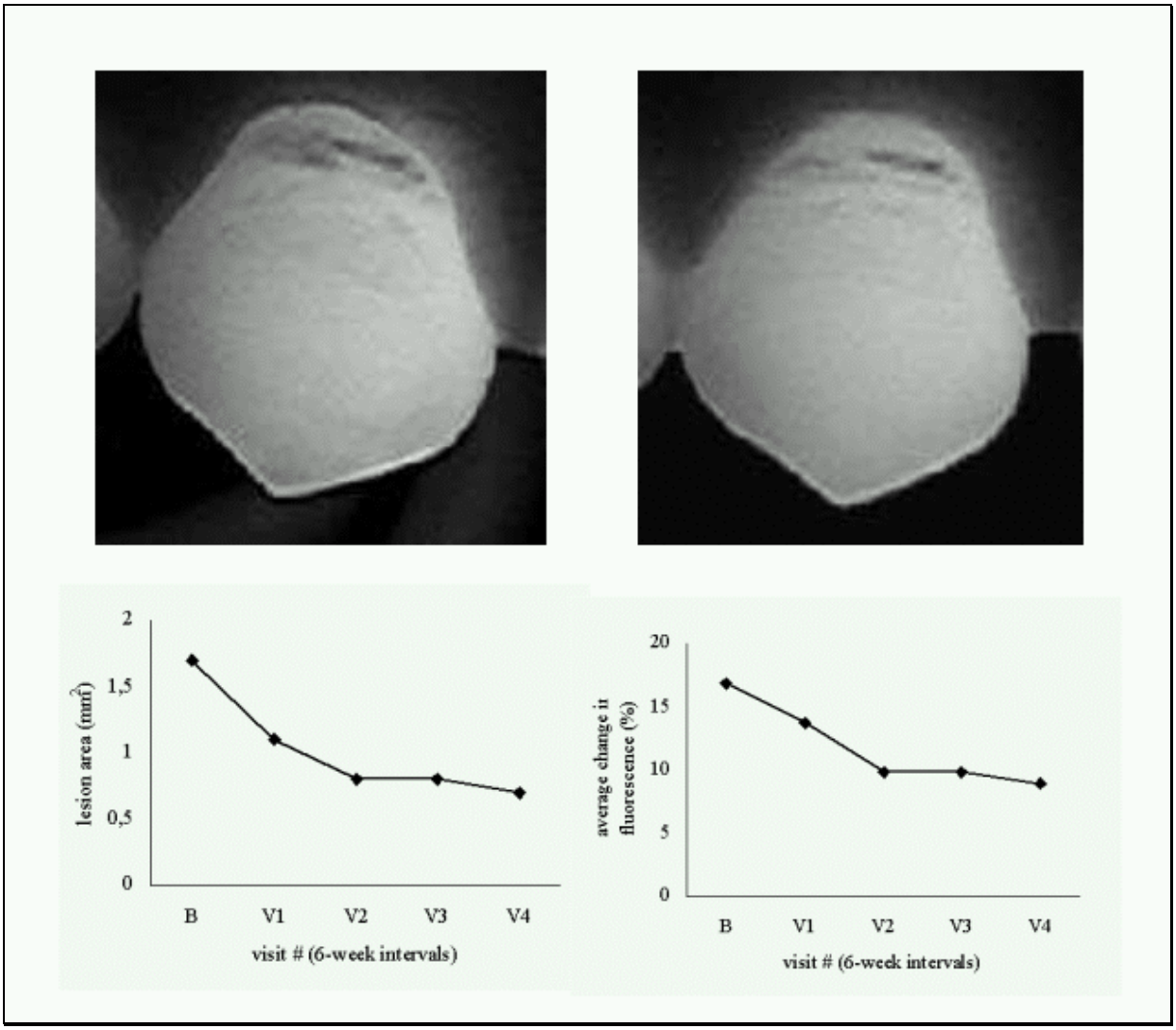


Fig. 8 An example of a lesion at study start (left) and at the end of the study (right). The graphs show the corresponding changes over time (6 months, baseline – visit 4) in lesion area (left) and average fluorescence change (right) for a lesion in the fluoride varnish group. By courtesy of Eur J Oral Sci, Munksgaard, Denmark.

## DISCUSSION

The National Institute of Health Consensus Development Conference (2001) on "Diagnosis and Management of Dental Caries Throughout Life" listed the following among major clinical caries research directions:

- "Research into diagnostic methods, including established and new devices and techniques, is needed. Development of standardised methods of calibrating examiners is also needed."
- "Clinical trials of established and new treatment methods are needed. These should conform to contemporary standards of design, implementation, analysis and reporting. They should include trials of efficacy."
- "Studies of clinical practice including effectiveness, quality of care, outcomes, health-related quality of life, and appropriateness of care are needed."

In the context of research into diagnostic methods, the studies on which this thesis is based provide important preliminary data on two laser methods, QLF and DIAGNOdent, intended for clinical detection and quantification of caries lesions. The investigations were broadly based, encompassing not only evaluation of the methods for detection of both smooth surface and occlusal lesions, but also for monitoring lesion progression after preventive intervention. Performance in the hands of less experienced operators was also evaluated. In the course of the investigation, many questions arose, but in summary it may be stated that in the quest for a sensitive, specific diagnostic system for detecting early demineralisation and possible progression of caries, before the stage of cavitation, QLF has proved very promising. In the clinical evaluation of the two DIAGNOdent devices, some inconsistencies emerged. Clarification would require further clinical investigation, on a larger scale.

When tested *in vitro* on smooth surfaces, correlation for maximum and average fluorescence loss in the lesions measured with the QLF laser and QLF lamp devices were 0.94 and 0.95, respectively (*Paper III*). This is in agreement with findings from

*in vitro* demineralisation studies (Al-Khateeb *et al.,* 1997a), indicating that the new generation QLF is equal to its laser predecessor. This result has important implications for clinical application. The correlation with integrated mineral loss was lower (0.74–0.77), however, than in a previous study on artificial lesions (Al-Khateeb *et al*., 1997a). This may be attributed in part to the fact that in natural lesions it is more difficult to obtain with certainty a tooth slice containing the deepest part of the lesion. This would influence the outcome of the microradiographic analysis.

Readings by the laser methods were also correlated with the lesion depths in µm registered by TMR. This correlation disclosed an interesting phenomenon. For lesions <800 µm deep, the scatter plots of the QLF methods showed a linear relationship between lesion depth in µm and mean fluorescence loss, but no such trend for lesions >800 µm deep. Together with the sensitivity and specificity calculations (at specific cut-off values), showing relatively high sensitivity and specificity for QLF, our results indicate that while the method could be helpful for detecting dentinal caries, it is inappropriate for quantifying de- and remineralisation in "deeper" lesions, *i.e.* >800µm.

In detecting smooth surface caries at $D_3$ level, the QLF lamp device had the highest sensitivity; specificity was the same for DIAGNOdent and the QLF devices. The QLF laser device had the highest correlation coefficient (r=0.74-0.77) and DIAGNOdent the lowest (r=0.67) when validated by mineral loss. This was not unexpected, as Hibst and Paulus (1999) hypothesised that the enhancement of fluorescence in the presence of caries is attributable bacterial metabolites, rather than to crystalline disintegration.

For DIAGNOdent, a two-degree polynomial function gave a better curve fit for lesion depth than linear function. The regression line indicates that the DIAGNOdent readings increased more steeply when the lesion extended into dentine. This is in agreement with the results of a previous study on occlusal surfaces (Shi *et al.*, 2000). Linear regression functions were applied to both QLF methods. The mechanism underlying QLF is believed to be related to changes in light scattering, due to the decrease in mineral content in a lesion (Angmar-Månsson and ten Bosch, 1987). The

factors underlying the increase in infrared fluorescence registered by DIAGNOdent in the presence of caries have yet to be clarified.

For *in vivo* measurements of lesions on occlusal surfaces, the correlation between lesion depth and readings from the two DIAGNOdent devices was very low (*Paper V*). The range of readings was very wide for both superficial and deep lesions of dentine: 15–95 and 12–99, respectively. Despite the authors' attempts to find balanced sensitivity and specificity values (with a specificity over 0.8, and a high overall performance of sensitivity+specificity) it was not possible to identify a suitable cut-off value. With a specificity >0.8, the sensitivity was as low as 0.14–0.43 for deep dentinal lesions. This is unacceptable for a caries quantification method. The corresponding values for VI and BW were at best 0.75 and 0.50 for sensitivity, and 0.84 and 0.84 for specificity, respectively.

In this context it should be noted that the number of observations has an impact on sensitivity and specificity calculations. A greater number of observations might therefore yield a more positive interpretation of the diagnostic performance of DIAGNOdent.

To date, there is only one published study where the DIAGNOdent device showed sensitivity and specificity values over 0.8 for superficial dentinal caries under clinical conditions (Lussi *et al.*, 2001). Some comments about this study are in order, as it differed in several respects from the present study and these differences preclude direct comparison of the results. In that study, the results for deep dentinal caries showed at best a sensitivity of 0.40, with a corresponding specificity of 0.85 (cut-off 50). True-negative (TN) data (sound surfaces) were used, but not validated to clinical lesion depth findings (*i.e.*, they were not opened). If a corresponding number of TN data is added to the present study, with specificity >0.8, the sensitivity rises by 10 percent units, the FP observations double, and the cut-off thresholds decrease by 10 units. However, there is a problem with not validated TN data. In that fraction, there might be false negative observations that will remain undetected. This will influence the sensitivity/specificity calculations.

In a recent study by Sheehy and co-workers (2001), the *in vivo* diagnosis of occlusal caries was assessed by DIAGNOdent and visual inspection using criteria by Ekstrand *et al.* (1998). The findings were not histologically validated, but the authors concluded that either the DIAGNOdent device was overscoring some lesions, or the visual method was underscoring them, and that the DIAGNOdent instrument probably should be used as an adjunct to a clinical examination, rather than the sole means of lesion detection. Because of the uncertainty of visual inspection of occlusal lesions, there is a need to validate DIAGNOdent against a more consistent reference.

In the present series of studies, our earlier results led us to select QLF as an appropriate non-invasive, quantitative reference standard for assessment of lesion depth of the smooth surface lesions (*Paper V).* The validity of the QLF method has been demonstrated in a number of studies. For incipient lesions on smooth surfaces, the correlation with mineral content, determined by transverse microradiography (TMR) is very good (Al-Khateeb *et al.*, 1997a; Lagerweij *et al.*, 1999; Shi *et al.*, 2001, this thesis). The method has also been tested for operator reliability and has shown excellent results (Tranæus *et al.*, 2002, this thesis).

In *Paper III*, DIAGNOdent readings showed a weaker correlation with mineral content (r=0.67) than QLF (r=0.74-0.77). In *Paper V*, the correlation between DIAGNOdent and QLF ranged from 0.57 to 0.73, which was considered to be satisfactory.

As with all methods involving technology, the QLF method is dependent on the operator's skill and previous experience of the equipment and technique. In *Paper IV*, the reliability of QLF in terms of inter-operator agreement was tested. For both image capture and analysis, inter-examiner reliability showed excellent intra-class correlation coefficients, r=0.93–0.98. For the image capture, there was a significant difference between analyst 2 and analyst 3 (p=0.004). However, analysts 2 and 3 were trying to reproduce the images captured by analyst 1, and had no access to any other images during the capturing process.

As lesion fluorescence decreases with dehydration, the degree of dehydration would influence clinical measurement. In this study, each image took approximately 5 s to capture, a time span brief enough to avoid dehydration. However, less experienced operators would probably take longer, causing greater dehydration of the lesion. Analysts 1 and 2 in the present study were highly experienced with the QLF method. Despite the lack of experience of analyst 3, all three achieved similar results.

The largest inter-analyst differences occurred in lesion area. Creation of the patch is based on the analyst's visual judgement of good delineation of the reconstructed area. Within a narrow range of patch size, good delineation could be achieved. The test of the effect of varying patch size showed that the size of the lesion area tended to change within this range, which could explain the inter-examiner differences in area. A small patch results in a large lesion area, while a large patch results in a small lesion area. The average and maximum changes in lesion fluorescence also seemed to vary within this range, but not as much as lesion area. This phenomenon could be explained by a) tooth morphology; the tooth surface is not a flat surface, but slightly curved, and b) illumination, which is most prominent in the centre of the image and decreases towards the outer areas. In the QLF method, an individual patch is created for each lesion. This patch is then superimposed on all images in the same longitudinal series, *i.e.* the patch size does not change within the trial period.

Intra-operator agreements for DIAGNOdent measurements on occlusal surfaces have been tested *in vitro*, and have shown excellent results (Shi *et al.*, 2000). Under clinical conditions, such factors as humidity in the hard tissue and the tooth surface (Shi *et al.*, 2000), the presence of dental plaque and staining of various kinds can detract from the performance of the DIAGNOdent system. Intra-operator agreement *in vivo,* tested by Lussi *et al.* (2001), showed excellent results.

In *Paper V*, both intra- and inter-operator agreements *in vivo* were tested. The results showed a range of intra-operator agreements for both operators, of 0.82-0.94, and inter-operator agreements of 0.63-0.83. The agreement at device level was also tested, and was found to be 0.63-0.87.

These findings should not be viewed in isolation, but in the context of reliability performances of other new methods for quantification of caries on occlusal surfaces, *e.g.* the ECM (Electronic Caries Meter). The reproducibility for continuous measurements with the ECM device is reported to range from 0.76 to 0.93 (Huysmans *et al.*, 1998a, b). However, Ekstrand *et al.*, 1997, reported limits of agreement of about ± 2.6 steps on the ECM-scale for the most competent operator, and ± 5.5 for the least competent. The inter-operator limits of agreement were about ± 4.0 to 5.5. As the ECM-scale has about 14 points, these discrepancies between readings are not inconsiderable. The corresponding Kappa values were 0.59 to 0.92 and 0.50 and 0.57 for intra- and inter-operator reproducibility, respectively.

With respect to smooth surfaces, inter- and intra-operator agreements for DIAGNOdent have been tested *in vitro*, and have shown good results, 0.94 and 0.95 respectively (Shi *et al.*, 2001). Under clinical conditions on smooth surfaces, the above-described factors (moisture, plaque remnants and staining) would be expected to probably detract from the performance of DIAGNOdent. In *Paper V* the intra-operator agreement showed a correlation coefficient of 0.94, which was considered to be excellent. The inter-operator agreement ranged from 0.79-0.87, a result that was interpreted as very good. Compared to occlusal surfaces, it is quite easy to manage moisture and remove plaque on smooth surfaces, and application of the probe tip is also easier to control. Thus the differences in *in vivo* reliability performance were not unexpected.

Traditional diagnostic methods, such as visual inspection, appear to have very low sensitivity and high specificity in diagnosing occlusal caries (Wenzel *et al.*, 1991; Kidd *et al.*, 1993; Ie and Verdonschot, 1994). Although improvements of visual inspection with new scoring systems seem promising (Ekstrand *et al.*, 1997; Fyffe *et al.*, 2000a, b), further clinical validation is still necessary.

For this purpose, only a few non-invasive methods have been available. Techniques based on electrical impedance such as the ECM-device have shown

sensitivity values of 0.80-0.97, and specificity values of 0.56-0.89. (Rock and Kidd, 1988; Verdonschot *et al.*, 1992; Ricketts *et al.,* 1995). For caries lesions, a specificity >0.80 is preferred, in order to minimise the false positive fraction. For the technique to be of value under prevailing clinical conditions, this should preferably be combined with sensitivity >0.80.

To date, there is only one published study in which the clinical performance of the DIAGNOdent device has fulfilled that requirement (Lussi *et al.*, 2001). In *Paper V*, however, no unambiguous conclusion could be drawn with respect to validation of the DIAGNOdent device.

The clinical performance of two different DIAGNOdent devices has not previously been compared. The results in *Paper V* disclosed a significant systematic difference in readings from the two devices. This precludes making general recommendations with respect to cut-off values, for example as guidance for the general practitioner. On the other hand, the study showed promising intra-operator performance in the clinical setting and this is a factor of major importance for longitudinal measurements.

Despite the dramatic decline of caries incidence in children over the past 20 yr (Marthaler, 1990; Sundberg, 1996), there remains a proportion of the population which is susceptible to caries, either already caries active or at high caries risk. These individuals require intensive preventive measures. In Scandinavia, the measures most commonly applied in the clinical setting comprise a prophylaxis (professional tooth cleaning) followed by an application of fluoride varnish. The outcome is often difficult to evaluate with conventional clinical methods. In clinical practice, objective, reliable quantitative data on the outcome of efforts to arrest disease activity, *e.g.* longitudinal monitoring of lesion response to preventive measures, would allow flexibility in selecting intervention appropriate to the individual patient, before lesions progress to a stage requiring expensive invasive therapy. For this purpose, trials have been conducted of QLF, ECM and DIAGNOdent.

In a caries clinical trial conducted in Lithuania in 2000 (Matuliene *et al.*, 2001), both ECM and DIAGNOdent failed to discriminate between the outcome on occlusal caries of use of a high- and a normal efficacy fluoride dentifrice in a 12-month test period. Clinical visual assessment (DSTM, at the D1 threshold) (Fyffe *et al.*, 2001a) disclosed a statistically significant 8% difference between the effect of the two products.

To date, a major disadvantage of such clinical studies on the efficacy of fluoride treatments has been the expense. These studies require not only a large number of subjects but also long trial periods, increasing the likelihood of dropout or non-compliance.

In *Paper II*, the QLF method was used to compare the effect of fluoride varnish application and professional tooth cleaning on remineralisation of white spot lesions in young teenagers. The subjects were all regular patients at the Department of Paediatric Dentistry at the Dental School, recruited locally from a suburb of low socio-economic status in south-west Stockholm. This region has a higher rate of caries risk patients than the average in Sweden. Tests of the baseline characteristics confirmed that the panellists had an increased caries risk. The preventive measures tested in this study were selected as appropriate to the target group.

No clinical changes in the lesions were detected by visual inspection. QLF offered the only means of detecting and quantifying the small changes that occurred during the brief study period. Despite the limited number of subjects, conclusions could be drawn from the results, showing statistically significant differences between the groups.

Participation in the study and the QLF method *per se* probably boosted motivation and compliance. The subjects were able to follow the measurements on the computer screen. They were interested in the appearance of their own lesions as well as in the modern camera- and computer technique. As the degree of interest shown seemed to be similar in the two trial groups, this factor probably had an even influence on the results.

Some increase of fluorescence in both trial groups might therefore be attributable to improved oral hygiene. While there is no indisputable evidence that good oral hygiene reduces caries experience, nor is there sufficient evidence to dismiss its value as a caries preventive measure (Sutcliffe, 1996).

In such a study design, the QLF method is clearly advantageous: fewer participants are required, and lesion changes can be measured at intervals of only 6 wk. The results of the above study (*Paper II*) confirm that the methodology warrants application to a larger material, to obtain more detailed information about the relative efficacy of different models for prevention.

The surface layer of an incipient caries lesion is, by definition, high in mineral content (Weatherell *et al*., 1977). After application of fluoride varnish, the mineral content of the surface layer increases (ten Cate *et al.*, 1981; Retief *et al*., 1983; Hattab *et al.*, 1987) and retards the diffusion of minerals to the inner part of the lesion (Larsen and Pearce, 1992). Whether remineralisation is occurring in the body of the lesion or in the surface layer cannot be disclosed by QLF. This question is unlikely to be resolved without further *in vitro* investigations into patterns of lesion remineralisation.

Recently, Alanen (2000) considered probable sources of bias in caries prevention studies. Two major sources of bias were identified, which influence both the number of lesions and the rate of lesion progression: underestimation of the preventive effect on incipient lesions, and overestimation of the preventive effect when observations were limited to reliably observed large cavities. Alanen concluded that the longer the study and the more sensitive the diagnostics, the smaller the bias attributable to variation in the decay rate in the test and control groups. Objective quantitative methods for detecting and monitoring lesion change would clearly have a valuable role in reducing the risk of bias in such studies.

The general objective of the studies on which this thesis is based was to evaluate the clinical performance of two laser fluorescence methods of quantifying dental caries, QLF and DIAGNOdent.

While the QLF method has many merits, because of its hard- and software components it is a rather expensive technique, and somewhat cumbersome in the conventional dental setting. While this is not a major disadvantage in clinical research**,** a less expensive, more compact device, such as DIAGNOdent, would probably find greater acceptance by clinicians, for application in longitudinal monitoring of lesion response to preventive measures in their caries risk patients. In the present studies, the data obtained by the DIAGNOdent device correlate satisfactorily with QLF, and show very good inter- and intra-operator agreements (*Paper V*). Financial aspects aside, however, the results offer no clear indication that DIAGNOdent would be an acceptable substitute for QLF on smooth surfaces in the clinical setting. To resolve this question, more extensive clinical evaluation is warranted. For research purposes, QLF with its closer correlation to mineral content, remains the preferred method for quantification of mineral loss on smooth surfaces.

## CONCLUSIONS

- Because of its excellent *in vivo* repeatability and reproducibility (for both image capturing and image analysis) of measurements of carious lesions on *smooth surfaces*, the Quantitative Light-induced Fluorescence method may be of value for longitudinal monitoring, *e.g.* for assessment of the effects of different caries preventive programmes.

- With the QLF method, measurements of carious lesions over six months showed that repeated fluoride applications had a favourable effect on the remineralisation of white spot lesions.

- Experience from a caries clinical trial showed that the QLF method *per se* probably boosted motivation and compliance by the subjects because they were able to follow the development and appearance of their own lesions on the screen.

- Although DIAGNOdent compared well with QLF in detection and quantification of natural *smooth surface* caries *in vitro,* QLF showed closer correlation with mineral content. QLF is therefore preferable for scientific purposes such as monitoring de- or remineralisation.

- Under clinical conditions, the DIAGNOdent device showed excellent intra-operator agreement and good inter-operator agreement for measurements of carious lesions on *smooth surfaces* and good intra- and inter-operator agreement for lesions on *occlusal surfaces*. Due to low inter-device agreement, it is recommended that the same device should be used throughout longitudinal measurements on a patient.

- With respect to clinical cut-off thresholds for dentinal caries on *occlusal surfaces,* no definite recommendations for DIAGNOdent could be made on the basis of data obtained in these studies.

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