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Vuokko Anttonen

LASER FLUORESCENCE IN
DETECTING AND MONITORING
THE PROGRESSION OF
OCCLUSAL DENTAL CARIES
LESIONS AND FOR SCREENING
PERSONS WITH UNFAVOURABLE
DIETARY HABITS

FACULTY OF MEDICINE,
INSTITUTE OF DENTISTRY,
UNIVERSITY OF OULU

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Academic dissertation to be presented, with the assent of the Faculty of Medicine of the University of Oulu, for public defence in Auditorium I of the Institute of Dentistry (Aapistie 3), on November 23rd, 2007, at 12 noon

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Abstract

This study focuses on the clinical use of laser fluorescence compared to visual inspection (VI) for detecting and monitoring the progress of caries lesions during a one-year follow-up period and for screening subjects with unfavourable dietary habits causing demineralization of teeth. The effect of professional cleaning on laser fluorescence was also studied. The study groups were comprised of schoolchildren (n = 259), and altogether 3 651 occlusal tooth surfaces were examined visually and by using laser fluorescence.

Laser fluorescence was found to be useful as an adjunct to visual inspection in detecting dental caries lesions during routine dental check-ups. The variation of laser fluorescence values in each visual category excludes its use as a primary or only method for caries detection. It rather functions as an alarm for a closer or more thorough examination. In addition, it can be a useful tool when deciding on the intervention method and the length of the recall-interval. The best cut-off point for considering operative intervention was found to be 30/99.

Laser fluorescence was also found to be useful in monitoring lesion progression. Monitoring can be done through clear sealants. High laser fluorescence values (> 20) of sound tooth surfaces may predict decaying within a period of one year.

Professional cleaning increased laser fluorescence values of molars, especially second molars. The increase was significant in molars with a visual score of > 0 or when visually detected initial and dental caries lesions were included. Professional cleaning was most efficient when using only a rubber cup and water spray without paste.

A computer-based questionnaire on dietary habits was used to evaluate the cariogenicity of children's dietary habits. The laser fluorescence values of tooth surfaces of children with harmful dietary habits were found to be higher than among children with favourable dietary habits. Laser fluorescence can be used for screening children whose current dietary habits may harm their teeth.

Keywords: caries, dietary habits, laser fluorescence, monitoring caries, professional cleaning

“Do not despise the day
of small beginnings”
(Zech 4:10)

*To Kalle, Harri, Antti and Elina,
Emma and Nooa
To all children*

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Abbreviations and definitions

AC	Alternating current
AF	Autofluorescence
AUC	Area under curve (ROC)
BW	Bitewing radiography
CFV	Combined FOTI and visual method
dmf(t)	Number of decayed, missed, and filled deciduous teeth
DMF(T)	Number of decayed, missed, and filled permanent teeth
dt	Number of decayed deciduous teeth
DT	Number of decayed permanent teeth
D0	No or only slight changes caused by caries
D1	Caries lesion in the outer half of the enamel
D2	Caries lesion in the inner half of the enamel
D3	Caries lesion in dentine
D4	Deep dentinal caries lesions
DC	Direct current
DELFL	Dye-enhanced laser fluorescence
DIFOTI	Digital imaging fibre optic transillumination
ECM	Electric Caries Meter, a commercial device for detecting demineralization of teeth. The function is based on difference in electrical resistance of sound and demineralized tooth surfaces
FOTI	Fibre optic transillumination
ICC	Intra-class correlation coefficient
LF	Laser fluorescence
ROC	Receiver operating characteristic curve (graphical presentation of the relationship between true and false positive rates)
VI	Visual inspection
VIM	Visual inspection using magnification
VIP	Visual inspection using probing
QLF	Quantified laser fluorescence

DIAGNOdent® is a commercial device based on laser fluorescence used for quantifying demineralization of teeth

Suggested caries status by the detection method	Actual caries status		Total
	Caries	No caries	
Caries detected by the method	a	b	a + b
Caries not detected	c	d	c + d
Total	a + c	b + d	n

Sensitivity (Se) the proportion of positive findings (in this context tooth surfaces detected to have demineralization of teeth) of all true positive cases (in this context all teeth with caries) $(a/(a + c)) \times 100$

Specificity (Sp) the proportion of negative findings (in this context tooth surfaces detected to be sound) of all true negative cases (in this context all sound teeth) $(d/(b + d)) \times 100$

Youden's index (sensitivity) + (specificity) – 100; Interpretation: value 100 means that the test is unimistakeable and value 0 that it has no diagnostic value

Positive predictive value $a/a + b \times 100$

Negative predictive value $d/c + d \times 100$

Kappa (κ) Cohen's kappa coefficient is a statistical measure of agreement of repeated measures

Chromophore any chemical group, whose presence gives a decided colour to a compound

Porphyrin a macromolecule derived from four pyrrole-like subunits interconnected via their carbon atoms via bridges (=CH-). It is a highly conjugated system and deeply consequently coloured

List of original papers

This thesis is based on the following original articles, which are referred to in the text by the numerals I–IV:

- I Anttonen V, Seppä L & Hausen H (2003) Clinical study of the use of the laser fluorescence device DIAGNOdent for detection of occlusal caries in children. *Caries Res* 37: 17–23
- II Anttonen V, Seppä L & Hausen H (2004) A follow-up study of the use of DIAGNOdent for monitoring fissure caries in children. *Community Dent Oral Epidemiol* 32: 312–318
- III Anttonen V, Seppä L & Hausen H (2005). Clinical study on the effect of professional cleaning of occlusal tooth surfaces on laser fluorescence measurements. *Caries Res* 39: 280–283
- IV Anttonen V, Hausen H, Seppä L & Niinimaa A. Effect of dietary habits on laser fluorescence values of visually sound occlusal surfaces among Finnish schoolchildren. *Int J Paed Dent* (In Press).

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1 Introduction

During the past decades in the industrialized world, a decline in the caries rate has occurred even to a point where caries has no longer been considered as a threat for oral health. Even the association between eating sugar and sweets and dental caries incidence has been reported to be weak or unclear during the period of the greatest decline in the prevalence of dental caries (Bagramian *et al.* 1974, Richardsson *et al.* 1977, Sundin *et al.* 1983).

Dental caries is not a disease that has been eradicated (Alanen 2005). In Finland, dental health has not improved since the 1990s, rather the opposite, especially among adolescents. The DMFT value for 12-year-olds has remained around 1.2 (Nordblad *et al.* 2004). Despite the fact that the sale of sugar has remained stable during the past 20 years, the sale of sweets has increased between the years 1985 and 2001 from 7.0 to 12.5 kg/person/year. There is also a new pattern of diet, which is replacing meals with comfort eating and fast foods. Drinking beverages is very common even in schools. In Finland, the tooth brushing frequency of adolescents is among the lowest in Europe (Kuusela *et al.* 1997, Poutanen *et al.* 2005).

With the decrease in caries prevalence, the progression of dental caries lesions has become slower. Occlusal caries in molars predominates during adolescence (Espelid *et al.* 2003). Conventional methods for detecting occlusal dental caries lesions have been based on inspecting lesions visually together with a tactile examination. Methods commonly combined with the visual tactile method are fibre optic transillumination and bitewing radiography. All these methods are capable of detecting established cavities. Since the 1990s, much effort has been put into developing new methods for detecting caries lesions at the non-cavitated stage. If lesions are detected in time, non-invasive procedures and oral self-care can be used to stop the progression of the lesion. Assessing the activity of lesions is challenging, but essential for the clinical decision concerning the method of intervention. Understanding the caries process should cause the emphasis to move from operative intervention towards early intervention for controlling dental caries.

International Consensus Workshop on Clinical Caries Trials (ICW-CCT) recommended the following criteria for caries detection methods: the method should be capable of capturing the manifestations of the caries process in dental hard tissues at any given time, be able to monitor changes in manifestations of the

caries process over time, and be able to differentiate states of lesions (initiation, progress, arrest, regression) (Pitts 2004).

There is a need for caries detection methods that are objective and valid and can be used for monitoring lesions and evaluating the effectiveness of prophylactic means. There is also a need for methods that can differentiate the lesions suitable for non-invasive care from those lesions for which operative care is needed. For monitoring, a lesion must be inspected more than once for detecting its progression or arrestment. In the summary of the European Organization for Caries Research (ORCA) Symposium in 1997, it was stated that the main research priorities for the coming 10 years are to study caries diagnostic tools and diagnostic tests, to transfer evidence-based clinical guidelines on diagnostic knowledge to general practitioners, to study the relationship between diagnosis and treatment decisions, and to assess the effect of diagnostic and treatment decisions on the outcome and cost-effectiveness of care (Verdonschot *et al.* 1999).

The aim of this study was to test clinically one of the new caries detection methods, laser fluorescence (LF, DIAGNOdent®), for detecting and monitoring dental caries and screening children with unfavourable dietary habits.

2 Review of the literature

2.1 Dental caries

Dental caries is a disease, which leads to destruction of the tooth structure and eventually to infection of the dental pulp and even surrounding tissues. Factors contributing to the progression of the disease include diet (mainly fermentable carbohydrates), microbes, and the host (amount and constituents of the saliva, habits). The progression of dental caries lesions needs time. Fluoride protects the teeth from dental caries by influencing the tooth structure.

The progression of a caries lesion is a dynamic process with periods of dissolution and redeposition of minerals in the dental hard tissue. When there is a net loss of minerals, caries develops or progresses. Remineralization occurs when redeposition predominates (Nyvad *et al.* 1999). Factors that determine the balance of the reactions and thus the likelihood of mineral loss or gain and the rate at which it occurs, are composition and thickness of the biofilm covering tooth surfaces, the diet, the fluoride ion concentration, and the salivary secretion rate (Kidd & Fejerskov 2004). It is widely recognized that progression of an incipient dental caries lesion can be arrested and a lesion can be remineralized. This knowledge has resulted in the development of several techniques for early detection and quantification of carious lesions.

Caries has become strongly polarized, which means that the majority of an age cohort have no history of dental caries and no present signs of decay, whereas the minority has a history of dental caries and the majority of present caries lesions are concentrated in their teeth. Vehkalahti *et al.* (1997) evaluated how the size of a high-caries group changed between 1976 and 1993 among the 5- to 15-year-old children in the city of Helsinki. The mean dmft of the 5-year-olds decreased from 4.6 to 0.8, and the mean dt + DT from 0.9 to 0.6. In 1993, 78% had their dmft = 0, whereas only 8% accounted for 76% of all decayed teeth. Among the 15-year-olds, the decrease was even greater: DMFT fell from 12.1 to 3.0, and dt + DT from 3.1 to 0.8. However, 26% had their DMFT = 0, and 55% of all dt + DT occurred in 10% of patients. The results showed a strong polarization and a need for new preventive strategies.

2.1.1 Progression of dental caries lesions

Caries process causes porosity. In the *in vivo* studies by Holmen *et al.* (1985, 1987), tooth surfaces were exposed continuously to dental plaque. Using histological validation visible changes were seen on air-dried enamel after two weeks, and without drying (opaque, matte surface) after 3 to 4 weeks. Children with high caries experience in deciduous teeth also tend to have higher caries experience in permanent teeth (Helm & Helm 1990). Permanent and deciduous teeth have different macro- and micro morphological characteristics: i.e. thickness of primary enamel is about half of the thickness of that of permanent teeth (Kennedy 1979), and in primary enamel there are some prismless areas. Mineral content is also relatively lower in primary teeth. Primary teeth have higher susceptibility to demineralization compared to permanent teeth (Shellis 1984).

While the occurrence of dental caries has reduced, the proportion of occlusal caries lesions has increased. It has been reported that 60% of all lesions are occlusal, whereas the total area of occlusal surfaces accounts for 12.5% of all tooth surfaces (Lussi 1991). In children, most caries lesions occur on occlusal surfaces. The higher susceptibility of fissures to dental caries compared with other surfaces of the tooth is a consequence of the morphology and the structural irregularities of pits and fissures as well as of a long eruption time, allowing biofilm to develop and remain in the fissures. Detection of occlusal caries lesions is difficult due to the initiation of the lesions in visually restricted areas of fissure walls and base (Ricketts *et al.* 1997a, Hall *et al.* 1997). Smooth surface lesions are frequently detected among patients with increased risk of getting dental decay and among orthodontic patients after removal of orthodontic brackets (Shi *et al.* 2001b).

With the decline of dental decay and slower progress of dental caries, the occurrence of non-cavitated lesions has increased. Operative opening of fissures often reveals that lesions are deeper than what was expected from the clinical appearance. Sawle & Andlaw (1988) found in their longitudinal study that 32% of the caries lesions in the first and second molars appeared as radiolucency in a radiograph and were not detected clinically (the so-called hidden caries). The reason was assumed to be the effect of regular use of fluorides causing greater opacity of enamel and thus obscuring underlying lesions in dentine.

Estimating the activity of lesions is essential for early intervention and arrestment of the caries progression. Recent data indicate that it is possible to diagnose clinically active and inactive caries lesions with a high degree of

reliability (Nyvad & Fejerskov 1997, Nyvad *et al.* 1999). Yet, the results of the clinical study by Ekstrand *et al.* (2005) suggest that a great proportion of lesions requiring non-invasive treatment are unlikely to receive it, on the basis of a single examination by a dentist not specifically trained for evaluating changes in the activity of caries lesions. Periods lasting only some weeks are too short for a clinician to learn to differentiate visually between active and inactive and regressing lesions.

The origin of the discolouration of dental caries lesions is somewhat unclear. It may be Maillard pigment (glycation or non-enzymatic browning), or it may be caused by melanin or food dyes. Iron, zinc, copper, and manganese are found in higher concentrations in carious lesions than in sound tissue. Some bacteria are known to form pigments, e.g. *Porphyromonas* and *Actinomyces*, which have also been detected in carious lesions. During the caries process discolouration precedes the bacteria that penetrate the demineralized dentine. It is likely that this colour change is brought about by compounds diffusing ahead of the bacteria. Maillard reaction probably occurs in anaerobic and acidic environments of the caries lesion front with small aldehydes deriving from bacterial metabolism. When the lesion progresses and oxidation becomes possible, melanin and lipofuscin may also cause discolouration in the outer layers of the lesion. (Kleter 1998). Discolourations may interfere with caries detection.

2.1.2 Association of dental caries with diet, tooth brushing habits, and the use of fluoride and xylitol products

So far, attempts to identify in advance those individuals who are likely to develop dental decay in the near future i.e. have a high caries risk, have not been very successful (Hausen 2003). Among children and adolescents, the estimation of caries risk can be based on evaluating factors contributing to the progression of dental caries lesions, e.g. oral hygiene level, counts of micro-organisms, fluoride history, diet, sucrose intake, and nocturnal drinking as infant (Kidd & Fejerskov 2004). In addition, some socio-economic parameters, such as parents' education and income level, have been employed for the assessment of the risk of getting dental decay. The course of the caries disease from active to inactive can be turned by affecting any of the factors contributing to the progression of the lesions (Nyvad *et al.* 2003).

Gustafsson *et al.* (1954) were the first to find a positive correlation between sweets consumption and dental caries occurrence in a longitudinal clinical study

on institutionalized patients in Vipeholm, Sweden. The fact that the caries rate has declined since the 1970s while sweet consumption has increased at the same time, gave reason to believe that sugar no longer should be considered as an important causal factor of caries as earlier (Sundin *et al.* 1983). In 1974, Bagramian *et al.* and in 1977, Richardsson *et al.* found only a low correlation between caries and diet or oral hygiene among schoolchildren. They also presented possible explanations for this lack of association: answers to the dietary questionnaire may not have been representative of the diet, or the survey covered a very short period whereas the DMFT index is a contribution of many years of the disease and the treatment. Furthermore, they conclude that there could have been uncontrolled variables, i.e. the food ingredients reported having low level of carbohydrates may have contained enough cariogenic foods to promote caries, or teeth reported clean may still have had a sufficient amount of plaque to promote dental caries.

During the last few decades, the consumption of sweets has increased worldwide causing related health risks, such as obesity and its consequences and dental caries, to a degree to have become an issue of major concern (Sheiham 2005). In Finland, the amount of candies and chocolate consumed yearly per person has increased from 7.2 kg in 1985 to 12.5 kg in 2001¹. In recent health studies, many children have been reported to drink soft drinks and eat snacks rather than eat free meals offered at school² (Nordblad *et al.* 2004). The results of a recent study on Finnish children's eating behaviours showed that drinking water for quenching thirst has been replaced by drinking soft drinks, juice, and concentrate juice (Kasila *et al.* 2005). In addition, sugary chewing gums were used frequently. The remarkable increase in sweets sales (Nordblad *et al.* 2004) has not been observed in earlier studies on diet (Honkala *et al.* 2003).

In recent studies, high sugar consumption has again been found to be associated with children's and adolescents' increased caries occurrence. Karjalainen *et al.* (2001) showed that the daily sucrose intake of Finnish children who developed dental caries by the age of 6 was significantly higher at the age of 3 than that of children who stayed caries-free. The analysis of Ruottinen *et al.* (2004) revealed that constantly high sugar consumption increases the risk of dental caries. In the study of Mattila *et al.* (2005), poor dental health at the age of 10 was associated with children's nocturnal juice drinking at the age of 18 months as well as with frequent eating of sweets, infrequent tooth brushing, and plaque

¹ www.etl.fi/tilastot/pdf/myynti

² www.info.stakes.fi/kouluterveyskysely

and caries on teeth at the age of 3. In a cross-sectional study on children between 1.5 and 4.5 years old in Britain in 1992–1993, Gibson & Williams (1999) found that the amount and the frequency of eating candy and money spent on confectionary by the family influenced caries prevalence significantly. At the age of 36 months every 1% of dietary energy derived from sugar confectionary accounted for 1% increase in caries increment. Eating sweets every day instead of once a week was associated with approximately 7% increase in caries prevalence. Jamel *et al.* (2004) studied the influence of reduced sucrose consumption on 6- to 15-year-old children's dental health in Iraq during the United Nations Sanctions. Sugar consumption dropped from 50 kg/person/year in 1990 to 12 kg/person/year in 1994. Reduction in sugar availability was related to marked caries prevalence reductions among Iraqi children over a period of 5 years. In an American longitudinal study (Marshall *et al.* 2003); the teeth of the children of a birth cohort of an Iowa fluoride study were examined at 4 and 7 years of age. Diet was defined by nutrient adequacy ratios (NAR) and calculated as the ratio of nutrient intake to recommended dietary allowance/adequate intake. Subjects with caries had lower median intake of milk at 2 and 3 years of age than children without caries. Soft drinks, regular powdered beverages, and 100% juice were associated with increased caries risk. In general, inadequate intakes of nutrients (riboflavin, copper, vitamin D, vitamin B12) were associated with increased caries experience.

The main reason for the decrease in the occurrence of caries lesions during the past decades in the industrialized world has been suggested to be the use of fluoride products, mainly dentifrices (Bratthall *et al.* 1996, Birkeland *et al.* 2000). In Finland, fluoride is nowadays only seldom used as tablets or lozenges, whereas in the 1970s and the 1980s fluoride tablets were recommended for every child from the age of 6 months. About one third of the schoolchildren have reported brushing their teeth twice a day, which would give them enough fluoride to protect their teeth against demineralization (Kuusela *et al.* 1997, Poutanen *et al.* 2005). Among Finnish schoolchildren, the rate of brushing teeth twice a day has been one of the lowest in Europe (Kuusela *et al.* 1997). Karjalainen *et al.* 2001 reported that 94% of Finnish 3-year-olds brushed their teeth daily but only 1% twice a day and 5% even less than daily. At the age of 6 years, only 20% brushed more than once a day, 78% once a day, and 2% less than once a day. Poutanen *et al.* (2005) reported that unfavourable knowledge, attitudes, beliefs, and behaviour tended to accumulate in the same children. Ashley *et al.* (1999) studied the association between tooth brushing habits and caries experience among British

adolescents who had used 1000 ppm fluoride toothpaste for three previous years. About 80% brushed their teeth at least twice a day. Their DMFT was 3.5 compared to 4.8 of those who reported brushing less than once a day. In a three-year clinical trial by Chesters *et al.* (1992), 12-year-old children brushing two times a day had significantly lower caries levels than those brushing once a day.

In Finland dental education for schoolchildren has been reduced during the last decades. As part of a nationwide research, the Adolescents Health and Lifestyle survey (Honkala *et al.* 2002), the data on oral hygiene instructions received by the participants were collected. Since 1977, a questionnaire has been sent to a representative sample of 12-, 14-, 16-, and 18-year-olds entitled to free oral health care. In 1997 less dental health counselling was reported having been given than in 1989. Boys who were 12-16 years old and brushed their teeth once a day or less frequently were most likely to receive instructions. Only 20% reported having received dietary sugar advice, and there was a slight decrease in dietary counselling between the years 1989 and 1997 (Honkala *et al.* 2002).

The caries inhibitory effect of xylitol was first reported in Finland in the 1970s (Scheinin *et al.* 1975). In a field study in Ylivieska, Finland, a long-term reduction of caries was reported after two to three years xylitol chewing gum intervention (Isokangas *et al.* 1989, 1993). The reduction was associated with the regular use of the gum and the effect was greater in the teeth erupting during the first year of the trial than in other teeth (Isokangas *et al.* 1989). It has also been reported that regular maternal use of xylitol chewing gum can prevent the onset of dental caries lesions in children by prohibiting the transmission of mutans streptococci from the mother to the child (Isokangas *et al.* 2000). It is widely acknowledged that sufficient evidence exists to support the use of xylitol for reducing caries.

2.2 Methods for detecting occlusal dental caries

Dental care based on inaccurate diagnosis may lead to over or under treatment. The diagnosis of early caries lesions can be regarded as a cornerstone of the cost-effective and high-quality dental care (Verdonschot *et al.* 1999, Tranaeus *et al.* 2005). International Caries Detection and Assessment System (ICDAS) is currently being developed and piloted, and the plan is to publish it in the Internet in order to offer available criteria for the caries detection and the evaluation of caries activity (Pitts & Stamm 2004).

Detecting and diagnosing early demineralization allows the use of minimal intervention dentistry (Iwami *et al.* 2004). Preventive intervention should be targeted more and more at the surface level, rather than at the dentition or patient level (Verdonschot *et al.* 1999). Detection of incipient pit and fissure lesions allows selective application of sealants on teeth and sites with increased risk of caries development (Li *et al.* 2003).

Because this thesis focuses only on the detection of occlusal lesions, which are of most importance in the initiation of dental caries progression, mainly methods for occlusal caries detection are introduced.

2.2.1 Visual methods

Visual inspection with probing and magnification

Visual inspection aided by a probe (VIP) to find “sticking”, i.e. demineralized sites, used to be the method of choice for caries detection before and during the 1970s. In an *in vitro* study by Lussi (1991), 34 dentists inspected 61 tooth fissures visually with and without a probe using histological validation. Sensitivity was reported to be 60% when the probe was used and 65% when it was not. The specificities were 87% and 83%, respectively. The difference between the techniques was not statistically significant. It was concluded that the use of a probe does not improve the validity of the diagnosis of fissure caries when compared to visual inspection alone. Penning *et al.* (1992) studied the sticking of the probe in fissures with a force of 500 g using X-ray validation. Twenty-four percent of the lesions were detected by probing which indicated low sensitivity, but the probe was seldom retained in a sound fissure indicating specificity > 90%. Sensitivity was even lower than was presumed, which was an argument against the further use of the method. Loesche *et al.* (1979) showed that there is a danger of transmitting mutans streptococci with an explorer from infected to uninfected sites. Ekstrand *et al.* (1987) showed forceful probing to be destructive on demineralized dentine.

An *in vitro* study by Lussi (1993) compared the methods of visual inspection (VI), VI with a magnifying glass (VIM), VI and BW-radiography, VI and light pressure probing (VIP), and BW-radiography for caries detection. Twenty seconds were used for inspecting the tooth surface by each method. Histological validation was used as a gold standard. Sensitivity varied from 12% (VI) to 49%

(VI + BW) and specificity from 84% (BW radiography alone) to 88% (VI). Magnifying (sensitivity 20%) or probing (sensitivity 14%) did not improve sensitivity of VI significantly. Lussi *et al.* (2003) showed that also in primary teeth VIP performed worse than VI alone or VIM (Lussi & Francescut 2003).

Visual inspection

Visual inspection (VI) is the main detection method of caries lesions. In the *in vitro* study by Lussi (1991), the proportion of visually correctly diagnosed teeth was 42%. The study also supported the finding that dentists are more likely to leave decayed teeth untreated than to restore sound teeth (sensitivity was 62% and specificity 84%). Occult caries or hidden caries is difficult to detect visually, sensitivity ranging from 3% to 50% and specificity beyond 90% (Lussi 1991, Lussi *et al.* 2001, Kidd *et al.* 1992, Verdonschot *et al.* 1992, Weerheijm *et al.* 1992, Lussi 1993, Chong *et al.* 2003, Lussi & Francescut 2003).

Detecting caries is dependent on fissure morphology. (Lussi 1991) found no significant relationship between fissure morphology and specificity (83%), but it was easier to detect enamel lesions of wide fissures (sensitivity 80%) compared with lesions in narrow fissures (sensitivity 52%). For dentinal caries, the sensitivity in wide fissures was 31% compared with 4% of narrow fissures. Opacities on the tooth surfaces (white lesions caused e.g. by fluorosis) masked lesions, decreasing sensitivity by 35%.

Visual inspection with categorizing lesions and estimation of lesion activity

In a laboratory study by Ekstrand *et al.* (1997), occlusal surfaces of hundred teeth with different kinds of caries lesions were examined visually with a ranked scoring system and by using histological lesion depth as a gold standard. The sensitivity of the visual examination was 92%–97% and specificity 85%–93%. Intra- and inter-examiner reproducibility for the method was reported to be excellent (Ekstrand *et al.* 1997). The method can be used in a “field” setting, but it is time consuming to learn and requires cleaning of teeth prior to the inspection (Table 1). Reproducibility of recording lesions using the criteria presented in Table 1 has been found to be adequate (Ekstrand *et al.* 1997, Sheehy *et al.* 2001, Tranaeus *et al.* 2005, Huysmans *et al.* 2005, Angnes *et al.* 2005).

Table 1. Criteria for different visual categories in the Ekstrand's ranked system for assessing dental caries lesions (Ekstrand *et al.* 1997).

Visual category	Criteria for the visual category
0	No or slight change in enamel; translucency after prolonged air drying (5s)
1	Opacity or discolouration hardly visible on wet surface, but distinctly visible after air drying
2	Opacity or discolouration distinctly visible without air drying
3	Localized enamel breakdown in opaque or discoloured enamel or greyish discolouration from the underlying dentine
4	Cavitation in opaque or discoloured enamel exposing the dentine

In 1999, Nyvad *et al.* (Table 2) introduced criteria to evaluate the activity of cavitated and non-cavitated caries lesions on the basis of visual and tactile examination. Two examiners monitored the activity of cavitated and non-cavitated lesions of 50 children over a period of 3 years. Probes were used for removing bacterial plaque and for feeling the texture of the surface. The agreement over diagnosis was 94%–96%. The kappa values ranged between 0.74–0.85 for intra-examiner examinations and 0.78–0.80 for inter-examiner examinations. The reproducibility of the system was high. The authors suggested that the use of such system would help considering the dynamic nature of the caries disease.

Table 2. Criteria by Nyvad to evaluate the activity of cavitated and non-cavitated caries lesions (Nyvad *et al.* 1999).

Category of activity	Criteria for the activity
Sound surface	Enamel normal; fissures can be discoloured
Active caries surface intact	Enamel surface is chalky and mat and rough and generally covered by plaque. The surface is still intact, no visible tissue damage; On smooth surfaces usually near gingival margin; in fissures the texture is intact, but the white area spreads to the sides of the cusps
Active caries surface discontinuity	As in class 1 and no visible tissue damage; Discontinuity can be felt by moving the probe on the tooth surface
Active caries cavitation	Visible tissue damage in the enamel/dentine; the surface of the lesion feels soft/leathery by moving the probe on the tooth surface; the lesion may have reached the pulp
Inactive caries surface intact	Enamel appears white, brown or black and may be shiny and the texture feels hard and smooth
Inactive caries surface discontinuity	As in the class 4 and local tissue damage (micro cavitation)
Inactive caries cavitation	Visible cavitation in enamel or dentine; The lesion surface feels smooth and hard by moving the probe on the tooth surface; the lesion has not reached the pulp

2.2.2 FOTI, DIFOTI

FOTI is a qualitative optical method used in caries detection since the 1970s. It does not give any numeric value to describe the status of the lesion. White light from a cold-light source is passed through a fibre to an intra-oral fibre-optic light probe that is placed on the buccal or lingual/palatal side of the tooth. The surface is studied by inspecting the transmitted light. Demineralized areas appear darker than the surrounding sound tissue.

In the clinical study of Verdonschot *et al.* (1992), 88 occlusal lesions on 23 teeth of 13 children were examined using visual inspection, FOTI, radiography, and Electric Caries Meter (ECM). Validation was done by operatively opening the fissures. They found that kappa values for inter-examiner reproducibility were good for both VI and FOTI. FOTI did not improve the results of the clinical examination (Verdonschot *et al.* 1992). In the *in vitro* study by Cortes *et al.* (2000), visual inspection, fibre-optic transillumination, and bitewing radiographs were compared in detecting occlusal caries and evaluating lesion depth. Histological validation was used. FOTI, VI, and radiography all showed good correlation with the histology, but had difficulty in distinguishing lesions located deep in enamel and in the outer third of the dentine. All methods, however, were able to detect lesions reaching deeper than to outer third of dentine. FOTI was as accurate as VI in detecting occlusal lesions. The intra-class correlation coefficient for intra-examiner reproducibility varied between 0.75–0.82 and for inter-examiner reproducibility between 0.75–0.79. The highest correlation was found between VI and the histological score, followed closely by FOTI and the histological score, and the lowest was found for bitewing radiography and the histological score (Cortes *et al.* 2000). In an *in vitro* study by Cortes *et al.* (2003), FOTI and visual inspection were compared with other diagnostic methods for the detection and evaluation of the depth of occlusal caries by using histological validation. FOTI added to the determination of the caries lesion depth compared to the visual method alone (Cortes *et al.* 2003). The equipment is cheap and portable, but the FOTI equipment must have a high-intensity point source of light.

Images of teeth obtained through visible light can be acquired with a digital CCD camera and sent to a computer for an analysis with dedicated algorithms. In this method, which is called DIFOTI, or Digital Imaging Fibre-Optic Transillumination, the images are processed digitally. The method is also quantitative, which is necessary for monitoring lesions.

In an *in vitro* study by Schneiderman *et al.* (1997), both occlusal and approximal caries lesions at the dentine caries level were examined using VI, DIFOTI, and radiography with histological validation. For approximal lesions, DIFOTI was more than twice as sensitive as radiography, the specificity was about 10% lower. For occlusal caries, sensitivity was about three times higher than that of radiography; specificity was about 10% lower. For smooth surfaces, the sensitivity of DIFOTI exceeded the sensitivity of radiography more than 10-fold, specificity being about 10% lower. DIFOTI was superior to radiographic imaging of proximal, occlusal, and smooth surface lesions.

2.2.3 Radiography

Radiography is useful in detecting approximal dentine caries, but has little value for detecting occlusal caries. Occlusal radiography (as well as FOTI and VI) has shown fairly good correlation with the histology of caries lesions, but has difficulty in distinguishing lesions located deep in enamel and in the outer third of dentine (Cortes *et al.* 2003, *in vitro*). Caries assessment should always precede a radiographic examination. There is no evidence that radiographic screening will benefit populations with low caries experience (Pitts 1996).

In the *in vitro* study of Cortes *et al.* (2000), visual inspection, fibre-optic transillumination, and bitewing radiographs were compared for detecting occlusal caries and evaluating the lesion depth by using histological validation. Radiography as well as other methods showed good correlation with the histology. The intra- and inter-examiner reproducibility was excellent for all methods. For dentinal lesions, the area under the ROC curve (AUC) was the highest for the radiographic method and good for all methods.

There are some limitations for radiography: it cannot distinguish between active and arrested lesions or between small cavitated and non-cavitated lesions. The depth of the lesion is difficult to estimate precisely from radiographs. The value of bitewing radiography in the detection of early occlusal lesions is questionable (Verdonschot *et al.* 1992), but it is valuable in detecting approximal lesions.

A new method for caries detection spread widely among clinical practitioners is digital radiography, most likely not due to superior diagnostic ability but due to other advantages above conventional radiography (Verdonschot *et al.* 1999). Digitized radiography was developed to improve the value of radiography for detecting dental caries lesions. There are two basic types for digital radiography:

storage phosphor plates, which appear like films and must be processed (scanned) after the exposure and solid-state sensors (Charge-Coupled Device [CCD], in which a cord connects the receptor and the computer. The solid-state sensors are not optimal because of their smaller size than number 2 of a radiographic film size, thickness, and the cord sticking out of the mouth. Phosphor plates are the same size as conventional films, and their holders are similar to film holders. When sensors and phosphor plates are compared, more positioning errors take place with the sensor plates. More retakes are reported with the CCDs (28%) than conventional films (6%) (Versteeg *et al.* 1998). Wenzel *et al.* (1993) concluded that digital imaging techniques performed as or even more accurately than conventional radiography particularly in case of large occlusal dentinal lesions.

Digital radiographic imaging provides many benefits compared with conventional radiography: the exposure times are shorter, no developing of films with chemicals is needed, and there is no need for packing and storing films. In addition, the method saves time and reduces the radiation dosage. Image analysis is somewhat different from the analysis of conventional films.

Computer aided image analysis has been explored since the 1980s and it is still under development. Wenzel (2004) reported that an automated caries detection programme (Trophy Radiologie Inc., Croissy-Beauorg, France), which has been marketed together with a digital sensor, lacked accuracy and consistency.

In the digital subtraction method information about the difference in mineralization of images taken at two occasions can be displayed leaving the unchanged areas neutral and showing only de- or remineralized areas. In a clinical longitudinal study by Wenzel *et al.* (2000), inter-examiner agreement for subtracted images was better than for comparing two pairs of bitewing radiographs.

2.2.4 Electrical conductance measurements

Measuring the conductance of teeth is a new method for detecting demineralization. Conductance measurements are done by using direct current. In impedance spectroscopy, alternating current is used, which inhibits polarization of the current. Appliances based on impedance spectroscopy are not yet commercially available. Intact enamel has high resistance, but when enamel becomes porous and a caries lesion contains water, the conductance increases. In

contrast to enamel, dentine has low resistance due to the porous nature of the tissue with many liquid containing tubules.

Continuous airflow during measurements is needed to remove superficial moisture and to prevent conduction to the gingival margin, thus interfering with the results. The relevant airflow on tooth surface while doing resistance measurements was studied by Ricketts *et al.* (1997a). A minimum airflow of 7.5 l/min was required to achieve the optimum sensitivity (92%) and specificity (87%) for dentine caries detection. The use of an air syringe or air from the ECM equipment showed similar performance (Cortes *et al.* 2003).

In a study by Huysmans *et al.* (1998), conductance measurements, BW-radiography, and VI were compared, and conductance measurements showed the best overall performance of the three methods studied. Studies of conductance measurements have shown good correlation with mineral content of enamel as well as with the lesion depth (Ricketts *et al.* 1997b). The commercial devices available [ECM® (Electronic caries monitor, Lode Diagnostics, Groningen, the Netherlands) and Caries Meter L (Onuki Dental Co. Ltd, Japan)] have shown equivalent diagnostic performance in a recent *in vitro* study by Kühnisch *et al.* (2006).

Ricketts *et al.* (1997) compared stable (3 seconds) ECM readings with cumulative resistance readings (continuous measurement for 10s) of the sites as to sensitivity and specificity and intra-examiner repeatability by using histological validation. For the stable resistance measurements, sensitivity and specificity were 92% and 87% respectively, and for cumulative resistance measurements 88% and 81% respectively. Stable conductance readings were achieved in a shorter period of time, and appeared to be more repeatable and most suitable for occlusal caries diagnosis. Stable readings are logarithmically and inversely related to resistance and they can be converted to resistance with units of MΩ. Stable readings can be recommended (Ricketts *et al.* 1997).

In the surface-specific method (one measurement/whole occlusal surface), the surface is dried and a contact medium applied on the fissure to provide contact between the probe tip and the tooth surface. The effect of different contact media has been studied. The strongest relationship between electrical conductance measurements and histology was found when using a lubrication jelly (KY-jelly), which was suggested to be the standard material for surface-specific measurements (Mosahebi & Ricketts 2002). Huysmans *et al.* (1998) reported that surface specific measurements showed higher reproducibility ($\kappa = 0.89$) than site specific (specific sites measured) measurements ($\kappa = 0.53$).

Cortes *et al.* (2003) found that the results of conductance measurements were impaired when staining was present. In the absence of staining, the performances of the methods were fairly similar for enamel and dentine thresholds. Ellwood & Cortes (2004) reported in their *in vitro* study with histological validation that the performance of the ECM might be improved by using different cut-off points to refer to demineralization in the presence or absence of staining.

Ie *et al.* (1995) studied first or second molars of 5–15-year-olds. VI and ECM measurements were repeated three times within 18 months. The ECM values obtained at the baseline from sites that developed decay were significantly higher than the values of the sites that remained caries free. Sites with dentinal caries had significantly higher ECM values than sites that were sound or had enamel caries. The ECM measurement had moderate sensitivity 77% and specificity 62%.

Schulte *et al.* (1999) studied post eruptive changes in conductance of 66 premolars of 18 children aged 8 to 14 years every 3 to 6 months over a period of 24 months. The mean resistance values increased continuously from eruption up to the 15th post-eruptive month. After 15 months, all sound teeth had resistance above 1000k Ω (1M Ω), which is the resistance of sound premolars of adults. When enamel matures in the oral cavity, pore volume and pore size, especially in outer enamel, decrease while the tooth is erupting. In outer enamel, there is also less water, but more Ca and P than in underlying layers. At 1 year, Na and Mg disappear, and the enamel becomes acid resistant. Low matured, porous enamel of recently erupted molars may cause high conductivity and thus increase the risk of false-positives measurements.

2.2.5 Caries detection methods based on fluorescence

Autofluorescence and fluorescence of dental tissues

Light interacts with dental hard tissues in different ways. Light can be reflected, scattered, transmitted through the material, or absorbed resulting in light energy transforming into heat, photochemical reactions or fluorescence. In fluorescence electrons of lower energy level are transmitted to a higher level due to external energy, e.g. light. When electrons return to their original level, energy is emitted in the form of light, fluorescence (König *et al.* 1998). During the last decade tooth fluorescence has been used for detecting initial caries.

Fluorescence values of whiter teeth are lower than that of darker teeth. To test the contribution of various calcium phosphates (e.g. tricalciumphosphate, dicalciumphosphate-dihydrate, calcium carbonate) to the fluorescence signal, Hibst *et al.* (2001) measured the fluorescence of pure pellets with excitation of 655nm. The relative fluorescence signals were 3% to 12% compared to human enamel. The cause of autofluorescence is not yet completely clear

Most of the autofluorescence is believed to be induced by organic chromophores. It has been proposed that fluorescence in dentine is caused by inorganic as well as organic complexes. In sound enamel, the path lengths of the light are long with a high probability that the photons will hit a chromophore (the light is absorbed by exciting the electron from the ground state into an excited state). Demineralization of dental hard tissues results in the loss of autofluorescence, i.e. the natural fluorescence of the tooth substance. Following factors may cause decrease in autofluorescence: light scattering in a lesion causes the light path to become shorter than in sound enamel; light absorption per volume is smaller in a lesion, and the fluorescence is weaker; light scattering in a lesion acts as a barrier for excitation light to reach the underlying healthy, fluorescing dentine, and a barrier for fluorescent light from dentine to reach the surface; fluorescence is quenched by a change in molecular environment of chromophores; and protein chromophores are removed by the caries process (Tranaeus *et al.* 2005). The QLF caries detection method is based on the autofluorescence of the tooth substance.

Taubinsky *et al.* (2000) found that carious cavities mainly fluoresced in the red with basic excitation wavelengths 620, 635 and 590nm, which correspond to fluorescence peaks of protoporphyrins, coproporphyrins, and zinc-protoporphyrins. König *et al.* (1998, 2000) continued that the fluorescence spectra of carious tissue are typical of fluorescent porphyrins. Measurements of fluorescence curves showed long autofluorescence life times of carious lesions. This is in line with the assumption that fluorescent porphyrins are responsible for the emission of carious lesions in the red spectral region. Endogenous porphyrins exhibit autofluorescence in the range of 600 to 800nm. They can be intermediate products in the synthesis of metaloporphyrins.

Examples of bacteria, which have been found to synthesize porphyrins, are *Porphyromonas gingivalis*, *Bacteroides*, *Propionibacteria*, *Clostridium*, *Pseudomonas aeruginosa*, and *Actinomyces* (Banerjee & Boyde 1998, König *et al.* 1998, 2000, Taubinsky *et al.* 2000, Mendes *et al.* 2004). Coulthwaite *et al.* (2006) added to the list *Prevotella intermedia*, *Corynebacterium* species, and

Candida albicans. Dental plaque may also fluoresce, and as plaque matures, autofluorescence alters from predominantly green to red area. Maillard reaction related fluorescence occurs with excitation wavelength 370nm and emission 440nm (Kleter 1998).

Banerjee & Boyde (1998) have also tried to identify the fluorescing agents. Major bacterial products associated with dental caries are lactic acids and proteolytic enzymes. Lactic acid producing bacteria like lactobacilli and streptococci show no typical porphyrin fluorescence in the red spectral region (Coulthwaite *et al.* 2006). Low pH does not cause enhanced autofluorescence, and neither do exogenous proteases. Banerjee & Boyde (1998) estimated that possible candidates are certain plasma proteins (for instance albumin and α_2 HS-glycoprotein) gaining access to the tissues through the circulatory system. Concentrations of such molecules as mentioned above increase once the degradative process has started (Banerjee & Boyde 1998).

Mendes *et al.* (2004) studied *in vitro* the effect on the LF values of storing carious primary teeth in saline versus 2% sodium hypochlorite for 24 hours. They found that there was a decrease in the LF values, and the LF values were statistically higher after soaking the teeth in NaOCl compared with saline solution, indicating an organic origin of LF. Dehydration increases the LF values, especially with long dehydration times (24 hours) (Shi *et al.* 2004), and the influence of dehydration time on fluorescence indicates that changes in fluorescence may not be caused only by microbial endogenous porphyrins (Francescut *et al.* 2006). Banerjee *et al.* (2004) hypothesized that during the carious process demineralization exposes more dental matrix to bacterial matrix interaction, refining the matrix chromophore responsible for the fluorescence signal detected in carious dentine (Banerjee *et al.* 2004).

Iwami *et al.* (2004) studied further the role of bacteria in enhanced fluorescence of carious dental tissue. They removed decayed dentine in the direction of the pulp chamber in layers of 300 μ m in 10 extracted molars with occlusal dentine caries and 3 extracted sound molars, after which dentine surfaces were evaluated by using laser fluorescence (LF). Dentinal tissue samples were collected by using a round bur. The rates of bacteria detected by PCR increased as the LF values increased. In the 10 specimens, the lowest LF value at which bacteria were detected was 15/99. At the LF values below that, no bacteria were detected. This study clarified the relationship between the LF values of dentine caries and the rates of bacterial detection. To test the role of bacteria in fluorescence, bacteria were incubated on blood agar, and the resulting colonies

were analysed by fluorescence microscopy (Lussi *et al.* 2004). Not only bacterial colonies but also the surrounding agar showed fluorescence. Agar fluorescence decreased with increasing distance from the colonies, indicating that there were diffuse bacterial metabolites, such as porphyrins, fluorescing on red-light excitation. Reassurance for the assumption that endogenous fluorescent porphyrins are responsible for the emission of carious regions in human teeth in the red spectral region was reported by König *et al.* (1999) in their study on time-gated autofluorescence imaging excited with continuous wave 407nm radiation of a krypton ion laser. Reflectance and fluorescence spectra were found to be typical of fluorescent porphyrins.

In vitro white spot lesions without bacterial involvement and very early *in vivo* white spot lesions have not been shown to cause an increase in fluorescence compared with sound surfaces (Lussi *et al.* 2001). Distinct fluorescence of the caries process in more advanced stages (D2, D3) supports the assumption that bacteria or their metabolites can contribute the fluorescence of dental caries lesions (Lussi *et al.* 2004).

Quantitative light-induced fluorescence

It has been known since the 1920s that intact and carious tooth substance appear different when excited by UV or short wave visible light. Bjelkhagen *et al.* (1982) found in an *in vitro* study that carious enamel caries appeared dark compared to sound enamel when using argon-laser with wavelength 488nm. The difference between carious and sound dentine substance was less. These findings were the base for the quantitative light-induced fluorescence (QLF) method.

The diagnostic capacity of QLF is based on the decreased intensity of natural fluorescence due to caries lesion (Angmar-Månsson & ten Bosch 2001). Yellow/orange fluorescence is induced by exposing the tooth to visible light in the blue green region from Argon ion laser $\lambda = 488\text{nm}$, or to blue light from a 50W Xenon microdischarge arc lamp equipped with an optical bandpass filter with a peak intensity of 370nm. The light illuminating the tooth is transported through a liquid-filled light guide. The fluorescence of enamel occurring in the yellow region is observed through a yellow high-pass filter, which filters out all reflected and back-scattered light. The fluorescent filtered images are captured using a colour CCD video camera and a framegrabber. Data are collected, stored and analysed by software (Inspektor Research Systems BV, Amsterdam, The Netherlands). Analysis of demineralization is based on a virtual reconstruction of

the lesion. The difference between measured and reconstructed values gives the loss of fluorescence in lesion. The mean fluorescence loss over lesion (%), the maximum fluorescence loss in the lesion (%), and the area of the lesion (mm²) are obtained (Heinrich-Weltzien *et al.* 2003, Tranaeus *et al.* 2005).

QLF was used for the first time in 1998, in a clinical trial, to detect caries on occlusal surfaces by Zandona *et al.*, who found that QLF is useful for this purpose together with visual inspection. In that study, it was estimated that the QLF measurements of occlusal, buccal and lingual surfaces take about 15 minutes and that it takes about 200 measurements to achieve expertise in the use of the device. QLF had been studied earlier in detection of smooth surface caries (Al-Khateeb *et al.* 1997). Al-Khateeb *et al.* (1997 and 1998), Tranaeus *et al.* (2001), Mendes *et al.* (2003), and Stookey (2004) have reported that the QLF method is useful clinically in monitoring lesions and can be used to evaluate preventive measures. In a clinical study by Tranaeus *et al.* (2002) where incipient lesions on smooth surfaces were studied, intra-examiner correlation coefficient varied between 0.93 and 0.99 and inter-examiner correlation coefficient 0.95–0.99. Meller *et al.* (2006) reported the results of a new method for the early assessment of caries *in vivo* in deciduous teeth. Conclusion was that the degree of demineralization by etching and its changes with time are associated with increased risk of getting dental caries lesions and that etching and the QLF analysis may be used for the early assessment of this risk. Eggertsson *et al.* (1999) reported that the dye-enhanced fluorescence method, DELF, was no more accurate than QLF and VI.

Hall *et al.* (1997) and Mendes *et al.* (2005) have shown good agreement between the QLF analysis and the lesion depth and mineral loss. Ando *et al.* (2001) concluded in their *in vitro* study that QLF quantifies mineral loss in early carious lesions slightly more accurately in deciduous than in permanent teeth.

Al-Khateeb *et al.* (2002) studied *in vitro* the effect of dehydration on the QLF values. Natural incipient lesions as well as artificially demineralized lesions showed a decrease in enamel fluorescence with increasing dehydration time. Tranaeus *et al.* (2005) have concluded that plaque, calculus and/or staining on the tooth surface, ambient light, office light, and the degree of dehydration of the tooth tissue may influence the outcome of the measurements. QLF appears to be a sensitive and reproducible method for quantification of smooth caries lesions. However, it seems to measure lesions limited to a lesion depth of only 400µm (Tranaeus *et al.* 2005).

Laser fluorescence (LF, DIAGNOdent®)

In 1998, Hibst & Gall showed that exposing a tooth surface to red light (638–655nm) can help differentiate between sound and carious tissue because fluorescence intensity caused by excitation in caries lesions exceeds that of healthy tissue. Red emission light as well as infrared fluorescence excitation radiation are less absorbed and scattered by enamel than light of shorter wavelength. Red light penetrates deeper in the tooth material, and it is therefore possible to detect fluorescence even from carious dentine under visibly sound enamel. On the basis of these findings DIAGNOdent® device was developed (KaVO, Biberbach, Germany) (Hibst & Gall 1998) (Figure 1). In 2001, Hibst *et al.* reported that the shapes of spectra for carious and sound areas are the same across the entire wavelength. Therefore, all fluorescence can be used for differentiating between sound and carious tissue without the need for any spectral analysis. The utilization of the total fluorescence light compensates in part for the lower intensities of emission compared with emission after excitation with shorter wavelengths.

DIAGNOdent® device (Fig.1) consists of a laser diode as the excitation light source (655nm, 1 mW peak power) and a photo diode combined with a long pass filter (transmission > 680nm) as the detector. The red laser excitation light ($\lambda = 655\text{nm}$) is transmitted through an optical fibre on the tooth. A bundle of 9 fibres arranged concentrically around the excitation light fibre transports light for detection. The emitted fluorescence, as well as back-scattered ambient light is collected through one tip and passed in ascending fibres to a photo-diode detector. A bandpass filter in front of the photo-diode detector absorbs the back-scattered excitation and short wavelength ambient light. To discriminate the fluorescence from ambient light, the laser diode is modulated. By amplifying only the modulated portion of the signal, the ambient light is suppressed. The signal of the fluorescence is finally processed and presented on the display as digits between 0 and 99. The display registers both real time and maximum value (Fig. 2). Individual calibration on sound, clean, and dry tooth surface and the ceramic standard block is carried out before scanning tooth surfaces. Units of the display are compared to a ceramic calibration standard. There are different shapes of tips for the hand piece. A tapered tip A has been designed for detection of fissure caries and tip B for smooth surfaces. In order to collect fluorescence from the maximum extension of carious lesions on occlusal surfaces, the tip must be tilted and turned around the site to be measured. This ensures that the tip picks up

fluorescence from the slopes of the fissure walls, where the caries process is believed to start. The acoustic signal with increasing digits helps the examiner to find the site of the maximum fluorescence value.



Fig. 1. DIAGNOdent®, KaVo (by permission from KaVo).

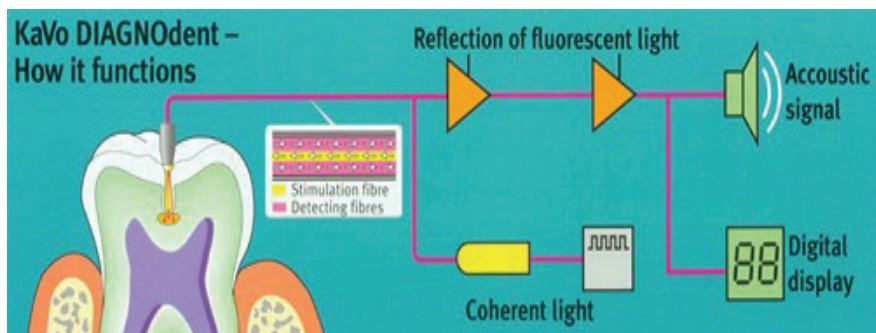


Fig. 2. The principle of the laser fluorescence device DIAGNOdent® (by permission from KaVo).

There are guidelines provided by the manufacturer (KaVo 1998a³, b⁴, 1999⁵, 2001⁶, and 2002⁷) and researchers (Lussi *et al.* 1999, 2001, Shi *et al.* 2000, and Pereira *et al.* 2001) for cut-off points of the laser fluorescence (LF) readings

³ KaVo (1998a) Clinical Guidelines Biberbach: KaVo, First German Issue

⁴ KaVo (1998b) Clinical Guidelines Biberbach: KaVo, German Issue III

⁵ KaVo (1999) Clinical Guidelines Biberbach: KaVo, German Issue III

⁶ KaVo (2001) Information 2001 CD-ROM. Biberbach: KaVo

⁷ KaVo (2002) Clinical Guidelines Biberbach: KaVo, German Issue II

referring to respective degree of dental caries status and giving guidelines for clinical procedures (Table 3). The effect of using different cut-off points of the LF measurements has been examined in several studies. In a clinical study, Heinrich-Weltzien *et al.* (2002), compared the cut-off points recommended by the manufacturer with the clinical status of the tooth using Ekstrand's criteria for detection of caries. Minimal operative intervention was used as a validation method. When correctly diagnosed lesions were considered, the optimal cut-off point for superficial dentinal lesion was 18 with sensitivity of 95% and specificity of 58%, and > 37 for deep dentinal lesion with a sensitivity of 84% and specificity of 70%. When the LF readings and the clinical status were compared, 9% of the superficial enamel and dentine lesions and 21% of the deeper dentine lesions were misdiagnosed when using the manufacturer's cut-off guidelines. At both diagnostic levels, LF overestimated the caries status in about 50% of the cases. Significantly different LF values were observed for different lesion depths: mean value 20.8 SD 11.4 for enamel lesions, 39.9 (SD 20.5) for superficial dentine lesions and 67.8 (27.2) for deep dentinal lesions. Deery *et al.* (2006) found in their *in vitro* study that at D3 level using the cut-off-points recommended by Lussi, sensitivity was 90% and specificity was 33%, and using cut-off points recommended by the manufacturer sensitivity was 90% and specificity 73%. Suggested ranges of the LF values by the manufacturer and in selected clinical and *in vitro* studies for different visual categories are presented in Table 3.

Table 3. Ranges of LF values suggested by the manufacturer and by selected clinical and *in vitro* studies for different visual categories.

Recommendations	Ranges of LF values for different lesions			
	Sound	Enamel lesion	Dentine lesions	
	D 0	D 1–2	D3	D4
According to a clinical study				
Lussi <i>et al.</i> (2001)	0–13	14–20	21–29	> 30
According to the manufacturer				
Clinical guidelines 1998a	0–4	5–10	11–20	> 20
Clinical guidelines 1998b	0–4	5–10	11–24	> 24 or > 30
Clinical guidelines 1999 and 2002	0–4	5–25	26–34	> 34
Clinical guidelines 2001	0–9	10–17	> 17	> 17
According to some <i>in vitro</i> studies				
Lussi <i>et al.</i> 1999	0–4	5–10	11–18	>18
Shi <i>et al.</i> 2000	0–7	8–21	> 21	
Pereira <i>et al.</i> 2001		> 10	> 10	

Correlation between the LF values and bacteria was studied *in vitro* by Iwami *et al.* (2004). Dentine was removed from extracted molars (3 visually sound and 10 with occlusal caries), and the LF values were measured after each removal. Bacterial DNA was detected in the dentine removed. Bacteria were detected when LF values were ≥ 15 . There was a positive correlation between the LF values of dentine caries and bacterial detection. Yet, it can be concluded that studies available at the moment are not conclusive, and more research is needed.

2.2.6 Other caries detection methods under development

Frequency-domain laser infrared photothermal radiometry for detecting dental caries was developed in the beginning of the 21st century. Sensitivity of the method was reported to be higher than that of VI and LF. Specificity was similar to that of LF. It has been shown that detection of deeper subsurface lesions is possible by using the longer wavelength laser source (830nm) (Jeon *et al.* 2004). There is also a method where a caries lesion is illuminated by using a light-emitting diode and the reflected infrared radiation (IR) is measured. Because of the scattering of the light in demineralized areas, the IR reflectance is decreased. These changes in IR are a result of continuous changes in the crystals at the enamel surface during the processes (Anderson *et al.* 1996). This method is suitable for *in vitro* use, e.g. for testing dental materials. Optical coherence tomography has its use in medicine producing cross-sectional images of internal biologic structures including ocular, intravascular, gastro-intestinal, skin, and oral soft and hard tissues. Polarization-sensitive optical coherence tomography uses linearly polarized incident light and can record the polarization information from the backscattered signal from two separate channels. This is an optical method, and uses near-infrared light to produce depth resolved. It can be used on smooth surfaces. Studies have demonstrated an increase in backscattering and changes in polarization due to demineralization. The principle behind this has not been defined. The technique can define the severity of the lesion (Jones *et al.* 2006). Handzel & Maryska (2006) have introduced a technique where micro defects in initial carious lesions can be detected and monitored *in vivo*. A replication technique using acetyl cellulose foil allows detecting changes of enamel with lateral $> 1\mu$ resolution. For the analysis of the replica, an optical microscope and computer image processing has been used. The method allows monitoring de- and remineralization.

2.3 Laser fluorescence

2.3.1 Laser fluorescence and occlusal caries

Permanent teeth

Sensitivity and specificity. In the first clinical study on LF by Lussi *et al.* (2001), seven dental practitioners examined a total of 332 occlusal surfaces of 240 patients (mean age 19.8, SD 8.4 years). Only molars and premolars with macroscopically intact surfaces were included. Plaque remnants were removed from the fissure if necessary. When operative intervention was needed, extent of caries was determined (classification D1 to D4) by opening the fissure. The highest sensitivity (92%) and specificity (86%) were found, when LF value of 14 was considered indicative of enamel caries. There was deviation of values for deep dentinal caries, but the highest sensitivity and specificity were achieved for cut-off values from 27 to 30. Heinrich-Weltzien *et al.* (2003) examined 248 first molars and 175 second molars of 94 15–19-year-olds. Teeth were professionally cleaned before scanning. At D3 level, sensitivity was 95% and specificity 58% at the cut-off value 18. At D4 level the values were 84% and 70% respectively at the cut-off value 37. Sensitivity and specificity obtained in selected *in vivo* studies for using the LF method in caries detection are shown in Table 4.

Good sensitivity and specificity values have been reached in clinical studies using histological validation when teeth have been scheduled for extraction after the LF scanning. In the study by Alwas-Danowska *et al.* (2002), two observers studied 45 occlusal sites of 13 patients (18–25 years) by using two DIAGNOdent® devices. The sensitivity ranged 93%–100% and specificity 47%–59% (using the threshold > 21 for carious dentin). Angnes *et al.* (2005) examined 110 sites of 57 molars of 38 adults (19–35 years). According to their study, the best LF readings for D0 or D1 were < 15, D2 15–20, and > 20 for D3 and D4. Sensitivity of LF ranged between 69–81%, specificity 56–54%, and AUC 64%–69%. In the study by Reis *et al.* (2006), 110 sites of 57 molars of 38 adults were examined after professional cleaning. The sensitivity and specificity at D2 level *in vivo* were 65% and 60% (cut-off > 19) and *in vitro* 73% and 56% (cut-off > 13). At D3 *in vivo* level sensitivity and specificity were 69% and 58% (cut-off > 22) and *in vitro* 78% and 63% (cut-off > 20). The mean LF values were significantly higher *in vivo* than *in vitro*. Sheehy *et al.* (2001) studied a total of 170 first

permanent molars of 179 children (mean age 6.9 years) using VI according to Ekstrand's criteria for validation. The agreement between VI and LF was 48% using cut-off points according to Lussi *et al.* (1999) and 78% according the manufacturer (Table 4). They also reported high LF values of hypo-mineralized sites and some high LF values without any visual reason for it.

Table 4. Validity of the LF measurements of occlusal tooth surfaces according to selected clinical studies.

Study	Number of teeth	Type of teeth	Classification used in VI	Validation; threshold	Threshold of LF for dentine caries	Se (%)	Sp (%)
Lussi <i>et al.</i> (2001)	332	Premolars and molars	D0–D4	Visual score; \geq D3	> 20	92	86
Sheehy <i>et al.</i> (2001)	170	Molars	E	Visual score; \geq D3	> 19 > 21	94 94	89 90
Alwas-Danowska <i>et al.</i> (2002)	45	Molars					
Heinrich-Weltzien <i>et al.</i> (2003)	248	Molars	E	H; superficial dentine caries H; deep dentine caries	> 18 > 37	95 84	58 70
Angnes <i>et al.</i> (2005)	57	Third molars	E	H; caries in middle third of dentine or deeper	> 19	75	55
Rocha <i>et al.</i> (2003)	30	Primary molars	E	H; score \geq D3	\geq 21	73	95

Se = Sensitivity, Sp = Specificity, E = Ekstrand's visual scoring system, H = Histological validation

In the *in vitro* studies at D2 level (cut-off point between 4 and 16); sensitivity has varied between 42% to 87% and specificity 68% to 95% (Lussi *et al.* 1999, Shi *et al.* 2000, Lussi & Francescut 2003, Baseren & Gokalp 2003, Burin *et al.* 2005). At D3 level (cut-off points > 10), sensitivity has varied between 75% and 100% and specificity between 71% and 100% (Lussi *et al.* 1999, Shi *et al.* 2000, Bamzahim *et al.* 2002, Baseren & Gokalp 2003, Lussi & Francescut 2003, Burin *et al.* 2005, Deery *et al.* 2006). (Table 5)

Using a reduced cut-off point 10–11 for D3, Pereira *et al.* (2001) obtained in their *in vitro* study sensitivity of 17%–20% and specificity of 98%. With the cut-off point 1, sensitivity was 98% at D3 level and 93% at D1 level, specificity being 24%. An *in vitro* study by Souza-Zaroni *et al.* (2006) reported that sensitivity of

LF was between 39%–45% and specificity 82%–99% (the cut-off value for D2 was 11–20 and D3 21–26). Francescut & Lussi (2003) reported sensitivity to be 77% at D2 level and 73% at D3 level but specificity only 49% at D2 level and 65% at D3 level when the cut-off values were 6 for the level D2 and 10 for the level D3.

Table 5. Validity of the LF measurements of occlusal tooth surfaces according to selected in vitro studies.

Study	Number of teeth	Type of teeth	Classification used VI	Storing method	Validation; threshold	Threshold of LF for dentine caries	Se (%)	Sp (%)
Shi et al. (2000)	76	Premolars and molars	E	Thymol	H; dentine caries	≥ 22	81	100
Attrill and Ashley (2001)	58	Primary molars	E	Formalin	H; dentine caries	> 18	79	84
Alwas-Danowska et al. (2002)	49	Molars		Water	H; dentine caries	> 20	95	52
Lussi and Francescut (2003)	95	Primary molars	D0–D4	Formalin	H; dentine caries	> 20	82	85

Se = Sensitivity, Sp = Specificity, E = Ekstrand's visual scoring system, H = Histological validation

Reproducibility. In the clinical study by Lussi *et al.* (2001), the intra-examiner correlation coefficient of the LF measurements was 0.93, and in the study by Sheehy *et al.* (2001) 0.94. In the study by Alwas-Danowska *et al.* (2002), the inter-observer correlation coefficient varied from 0.85 (DIAGNOdent device manufactured in 1998) to 0.90 (DIAGNOdent device manufactured in 1999). In the longitudinal study by Sköld-Larsson *et al.* (2004), LF was used for monitoring the effect of local prophylactic means on occlusal surfaces of 32 adolescents over a period of 48 months. Intra-examiner agreement were good ($r = 0.79$ and 0.83) for the two operators of the study. In the clinical study by Astvaldsdottir *et al.* (2004), 34 lesions scheduled for restorative treatment in subjects, aged 18–30 years, were studied. The intra-examiner correlation coefficient ranged between 0.85–0.98. Tranaeus *et al.* (2004) examined 52 occlusal sites with suspected caries by using two DIAGNOdent® devices. The intra-examiner correlation coefficient was 0.80–0.92 and the inter-examiner correlation coefficient 0.71–0.87. In the study by Angnes *et al.* (2005), the inter-examiner correlation

coefficient was 0.53. Reis *et al.* (2006) found the inter-examiner correlation coefficient for the LF measurements *in vivo* to be 0.57 and *in vitro* 0.62.

In the *in vitro* study of Lussi *et al.* (1999), intra-examiner correlation coefficient was 0.88 at the level D2 and 0.90 at the level D3. For inter-examiner reproducibility correlation coefficient values were 0.65 (D2) and 0.73 (D3). In addition, in other *in vitro* studies on extracted teeth with states of occlusal surfaces varying from sound (D0) to decayed (D4) and using histological validation, reproducibility has been found to be good (Kordic *et al.* 2003, Baseren & Gokalp 2003, Braun *et al.* 2005, Burin *et al.* 2005).

Lesion depth. In an *in vitro* study by Shi *et al.* (2000), the LF values increased slightly with the increased depth of the enamel lesion. The increase was most pronounced when the lesion depth penetrated the enamel-dentine junction. Wicht *et al.* (2002) found *in vitro* good correlation between the LF values and the lesion depth. Hibst *et al.* (2001) reported in their *in vitro* study that correlation between the lesion depth and the LF values was found to follow the exponential function. Heinrich-Weltzien *et al.* (2003) reported in their *in vitro* study that LF overestimates lesion extent.

In clinical studies by Tranaeus *et al.* (2004) and Astvaldsdottir *et al.* (2004), correlation between the lesion depth and the LF values was weak. However, in the latter study it was statistically significant. In a clinical study by Hamilton *et al.* (2006), 49 occlusal surfaces of teeth scheduled for restoration due to dental caries were scanned in 25 adults using LF after professional cleaning of the teeth. The LF values were compared with the cavity preparation volume, which was quantified after making a two-step impression using polyvinyl siloxane. No statistically significant correlation between the LF readings and the depth or volume of the final cavity preparations was found.

Primary teeth

Sensitivity, specificity, and reproducibility. Primary and permanent molars have different physical properties, which may affect the performance of the laser fluorescence method. Greater enamel porosity in primary teeth scatters the light more, and consequently a smaller fluorescence signal can be expected. On the other hand, enamel in primary teeth is thinner than in permanent teeth thus masking less the underlying dentinal fluorescence (Lussi & Francescut 2003).

In the *in vitro* studies by Attrill & Ashley (2001) and Lussi & Francescut (2003), LF was found to be a more accurate method for detecting dentinal caries

in primary molars than the visual and radiographic methods. Lussi & Francescut (2003) compared *in vitro* LF with other caries detection methods (VI, VIM, VIP, BW) in clinically intact deciduous teeth. The threshold values for LF were 5–12 at D2, and > 12 at D3 and D4 level. Sensitivity of the LF scanning at D2 level was 46% and at D3 level 82%, and specificity 68% and 85%, respectively. In the same study the intra-examiner kappa-scores ranged 0.76–0.86 (D2) and 0.77–0.85 (D3). The best cut-off points for dentinal caries were similar to those obtained in studies on permanent teeth. In the *in vitro* study by (Francescut & Lussi (2003), the cut-off values of LF at D2 level were 5, and at D3 level 13. They reported the sensitivity and specificity to be 75% and 68%, respectively, at D2 level and 82% and 85% at D3 level. In a clinical study by Rocha *et al.* (2003), 50 sites of primary molars of 29 patients were examined by two dentists, and the validity of LF in detecting occlusal lesions was evaluated using histological validation after exfoliation or extraction of teeth. The cut-off values of LF were 15–20 at level D2 and 21–99 at level D3. At level D1/ D2, sensitivity of LF was 60% and specificity 90%. At level D3/D4, sensitivity was 73% and specificity 95%. Mendes *et al.* (2005) studied *in vitro* sound smooth surfaces and surfaces with white spot lesions in primary molars. The cut-off points of LF were 3 at D1 level, 6 at D2 level, and 8 at D3 level. Sensitivity and specificity at D1 level were 51% and 96%, at D2 level 58% and 98%, and at D3 level 82% and 94%. The LF readings showed a good positive correlation with the lesion depth ($r = 0.78$), but a lower correlation with mineral loss ($r = 0.57$). According to these studies, the LF method is a useful addition to caries detection of primary teeth particularly at D3 level.

2.3.2 Laser fluorescence and smooth surface caries

Sensitivity, specificity and reproducibility. In an *in vitro* study by Shi *et al.* (2001a), sensitivity of the LF measurements was found to be 75% and specificity 96% for dentinal caries on smooth surfaces using the cut-off point of 9. Spearman's rank correlation coefficient for early enamel loss was 67% when histopathology and microradiography were used for validation. In an *in vitro* study by Shi *et al.* (2001b), for inter- and intra-examiner agreement intra-class correlation coefficient were 0.94 and 0.95, respectively.

In a clinical study by Pinelli *et al.* (2002), two examiners examined 220 teeth visually and by using LF twice at an interval of one week to evaluate the activity of the lesions. Sensitivity of the LF measurements was 72% and specificity 79%

when VI was used for validation (LF scores < 5 for visually inactive lesions and ≥ 5 for visually active lesions). The intra-examiner reproducibility of the examiners was 0.79 for one and 0.71 for the other. The inter-examiner reproducibility was 0.77. In a clinical study by Aljehani *et al.* (2006), intra-examiner reproducibility of the LF measurements for three examiners was determined on white spot lesions of orthodontic patients. Intra-class correlation coefficient for intra-examiner agreement ranged 0.91–0.98 and for inter-examiner agreement 0.69–0.82.

Lesion depth and mineral loss. In the *in vitro* study by Shi *et al.* (2001b), Spearman's rank correlation coefficient between mineral loss and the LF readings was 0.67. In another *in vitro* study by Shi *et al.* (2001a), Spearman's rank correlation coefficient varied between the lesion depth and the LF readings (0.78–0.85), and mineral loss in enamel and the LF readings (0.64–0.68) depending on the storing medium. Aljehani *et al.* (2004) reported Spearman's rank correlation coefficient to be 0.76 between histological lesion depth and the LF readings, and Pearson correlation coefficient to be 0.64 between mineral loss and the LF readings.

2.3.3 Laser fluorescence and secondary and approximal caries

Traditionally secondary and approximal lesions are detected visually, tactilely, radiographically, by using FOTI, or by using combinations of these methods. Recently some studies using LF for detecting secondary caries have been published. In the *in vitro* study of Bamzahim *et al.* (2004), the aim was to validate LF in detection of secondary caries around restorations, and to compare it with radiography and visual inspection. Amalgam and tooth-coloured materials showed no or little fluorescence. The filling material may obstruct the access of the laser light causing false-negative results. With the cut-off point 20 for dentinal secondary caries, the sensitivity of LF was 60% and specificity 56% when using fissure opening as validation and when the older model of the DIAGNOdent® device was used. Stains on tooth surfaces caused false positive findings. In a clinical study by (Bamzahim *et al.* 2005), the validity of LF and radiographs in the detection of secondary caries was assessed in teeth with amalgam restorations. A total of 51 posterior teeth of 21 patients (aged 19–45 years) with fillings needing replacement were examined. Opening the suspected site by removing the restoration was used for validation. Teeth were cleaned by using toothbrush and water-spray. Before removing the filling, the margins were scanned by using LF

and its conical tip A. When LF values > 30 indicated secondary dentine caries, sensitivity of the LF measurement was 61% and specificity 81%. All teeth with false-positive findings had stains. Sensitivity was the lowest on approximal surfaces. This was suggested to be due to the environment approximally: there could have been saliva, filling margins probably were not clean, and the tip could not reach the margins properly. Boston (2003) evaluated LF *in vitro* in detecting secondary caries around composite fillings using 15 extracted teeth and using histological validation. For dentine caries detection optimum threshold was ≥ 22 sensitivity at that level being 73% and specificity 84%

A new LF device DIAGNOdent pen® has been developed especially for detecting approximal caries, but it can also be used for detection of occlusal caries. The tip of the device has been designed to penetrate the approximal surface of the tooth. Similarly with the original version of the DIAGNOdent® device, energy is transported to the approximal surfaces via fibre tips in the form of light of the wavelength of 655 nm. The tips are sapphire fibre, which allows the end of the tip to be formed in a prismatic shape and approximal caries can be reached and detected. There are two types of tips: wedge-shaped and tapered. In an *in vitro* study, Lussi *et al.* (2006) assessed DIAGNOdent pen® and its tips (Table 6) in detection of approximal caries *in vitro* by using histological validation, and compared it with BW-radiography. Sensitivity and specificity of DIAGNOdent pen® at D2 level were 87% and 89% when using a wedge-shaped tip (WDG), and 84% and 89% when using a tapered wedge-shaped tip (TWDG). At D3 level sensitivity and specificity values were 89% and 82% (WDG), and 92% and 81% (TWDG). Intra-examiner reproducibility of the LF method was high ($\kappa > 0.74$). The authors concluded that the pen-type device was able to detect dental caries with a good accuracy, and may decrease the need of BW-radiography in detecting approximal caries.

Table 6. Suggested ranges of LF values using the DIAGNOdent Pen®, referring to different visual categories (Lussi *et al.* 2006).

Visual caries status	Range of LF values	
	Wedge-shaped tip	Tapered tip
D0	0 < LF ≤ 6	0 < LF ≤ 9
D1	6 < LF ≤ 9	9 < LF ≤ 13
D2	9 < LF ≤ 15	13 < LF ≤ 19
D3, D4	> 15	> 22

2.3.4 Laser fluorescence and caries detector dyes

In an *in vitro* study by Lennon *et al.* (2002), an examiner checked 40 teeth for residual caries using LF values < 15 for sound tissue after D2 caries had been removed. Detector dye using a fluorescent nucleic acid stain in conjunction with confocal microscopy was used for validation. Sensitivity and specificity of LF were 94% and 83% (cut off point 15 for caries), percentage of the correct scores was 89%, and the positive predictive value of the methods tested was about 80%. In the *in vitro* study by Yazici *et al.* (2005), LF and caries detector dye were compared for detecting residual dentinal caries. The cut-off points of LF for sound tissue was < 30 , and dentinal caries ≥ 30 . Three examiners studied extracted molars with deep dentinal caries lesions on approximal surfaces after caries had been removed chemo-mechanically using Carisolv®. Two measurements were done at the same sites two weeks apart. Validation was done by polarized light microscopy. Kappa value for inter-examiner reproducibility was 0.61 and intra-examiner reproducibility 0.94. LF showed higher sensitivity (63%) than caries detector dye (40%). The specificities of caries detector dye and LF were 100% and 86%, respectively. Low sensitivity of both methods may lead for lesions to remain undetected. In a clinical study by Boston & Sauble (2005) using the cut-off points of 11 or 12, the LF method was highly accurate in discriminating between dye-stainable and dye-unstainable surfaces, producing a correct classification proportion of over 98% and an AUC of 100% for all 176 surfaces included in the study.

2.3.5 Laser fluorescence and monitoring the progression of caries lesions

In 2004, Sköld-Larsson *et al.* (2004) studied whether the LF measurements can be used in evaluating the caries preventive effect of topical antibacterial treatments. In a split-mouth study, fissures in homologous pairs of second upper or lower molars of adolescents (12–18 years, mean age 14.1) were treated with either antibacterial chlorhexidine varnish or a placebo varnish every six weeks for 48 weeks. LF was measured every 12 weeks. The mean LF readings of the subjects in the placebo group increased significantly after 24, 36, and 48 weeks compared to the baseline values, but not in the group where the subjects received active antibacterial varnish. In the clinical study by Gokalp & Baeren (2005), 56 test sites and 28 control sites were randomly chosen from the occlusal fissures of

molars of 19 subjects. The sites were examined by using LF. Fluor Protector was applied on 27 test sites and Cervitec® on 29 sites. At 6 months, the LF values were lower in both test groups compared with the values at the baseline. There was no significant difference in the LF values between the two test groups at six months. The LF values of the control group were higher at 6 months than at the baseline ($p = 0.011$). In the clinical study of Schirrmester *et al.* (2005), mineralization of incipient occlusal caries lesions of 30 participants with the LF values of 10–20 were monitored for two weeks. The subjects brushed their teeth three times a day using dentifrices with 1450 or 5000 ppm fluoride ($n = 15$ in both groups). A decrease in the LF values was observed in both groups over the test period. The decrease was larger in the group using the dentifrice of 5000 ppm fluoride than the control group (21% difference). In the clinical study by Aljehani *et al.* (2006), the effect of two caries-preventive programmes was evaluated by using LF for longitudinal quantification of changes in incipient carious lesions on smooth surfaces. The subjects ($n = 12$, 127 test sites) were divided into two groups: one received repeated professional tooth cleaning combined with oral hygiene instruction, and the other received only repeated oral hygiene instruction. The white spot lesions were measured by LF at the baseline, and at 3, 6, 9, and 12 months thereafter. There was a difference between the LF readings at the baseline and during and after the follow-up period. However, there was no difference between the two treatment groups regarding the changes in the LF values over time. Ferreira *et al.* (2006) evaluated clinically the alterations of the LF values in active incipient caries lesions on 111 smooth surfaces of anterior teeth of 36 patients after 8 weekly applications with fluoride varnish. They found that the mean LF score decreased from 7.4 (SD 5.4) to 4.5 (SD 3.3) at the end of the trial.

Ijima & Takagi (2000) reported in their *in vitro* study a reduction in the LF values of artificial caries lesions in permanent teeth after remineralization by using the Ijima formula. In an *in vitro* study, Mendes *et al.* (2003), however, found that the LF values in primary teeth with enamel caries lesions did not change after exposure to a remineralizing solution (the formula of Ijima) for 28 days. The measured mean lesion depth in the experimental halves of the teeth was smaller than in the control halves, which had been exposed to water, suggesting remineralization in the study group.

2.3.6 Laser fluorescence and activity of caries lesions

Lussi *et al.* (2001) reported that *in vitro* white spot lesions without bacterial involvement and very early white spot lesions *in vivo* do not produce an increase in fluorescence compared with sound surfaces. In the study of Haak & Wicht (2004), caries lesions with a soft surface texture showed significantly higher LF values than leathery or hard lesions, and values also increased with increasing cavity depth. In the clinical study by Pinelli *et al.* (2002), two observers examined 220 caries lesions on smooth surfaces of 50 persons clinically and by using laser fluorescence, and repeated the procedures in 1 week. Using VI as validation for active or inactive white spot lesions (cut-off point 4), sensitivity of LF was 72% and specificity 73%. The kappa values for the two observers to measure intra-examiner reproducibility of laser fluorescence measurements were 0.79 and 0.71, and to measure inter-examiner reproducibility 0.79. Conclusion was that LF is a good auxiliary method for detecting activity of incipient caries lesions on free smooth surfaces. Ferreira *et al.* (2006) studied LF in monitoring alterations in activity of incipient smooth surface lesions in 8 weeks during which time fluoride varnish was applied weekly on the lesions. Nyvad's criteria (Nyvad *et al.* 1999) were used to estimate the activity of the lesions. In the beginning all lesions (n = 111) were active and mean LF was 7.4 (SD 5.1). At week 9, 55 lesions were still active, 45 inactive, and 11 were sound. Respective mean LF values were 5.7 (4.3), 3.6 (1.8), and 2.3 (SD 0.9). LF values were significantly lower in the end of the follow-up period than at baseline.

2.3.7 Laser fluorescence and oral health screening

Li *et al.* (2003) used LF to identify which parts of the molars are most susceptible to dental caries. The study group consisted of 6-year-old caries-free children and of children of the same age with caries lesions or history of caries (n = 170). Central fossae of first mandibular molars were the most demineralized sites in both groups. The LF values were higher in all test sites of children who had a history of dental caries or had caries lesions.

In a study by Tetuan *et al.* (2005), school nurses screened a convenience sample of 2622 children by using LF. The children were referred to a dentist if restorative care was considered necessary. Eighty-two percent of children referred were found to need restorative dental treatment. Aleksejuniene *et al.* (2006) also tested LF in a field setting and scanned same sites under different conditions

(naturally wet, dried and polished and dried). There was a systematic difference between mean scores in each case, but the difference was marginal and did not impact on the threshold for recording sites as carious. In two subjects, high, unexpected values were recorded on sound sites prior to the actual measurements, i.e. during the establishment of a standard value for a sound site.

2.4 Factors influencing laser fluorescence measurements

2.4.1 Patient related factors

Plaque

In an *in vitro* study, Lussi *et al.* (2001) suggested that plaque or calculus in the fissures may cause false-positive LF readings. Mendes *et al.* (2004) evaluated *in vitro* the influence of dental plaque on performance of LF on occlusal sites of primary molars. After baseline readings, plaque collected from children was placed on the selected sites and the readings were repeated. The LF values were lower after placing the plaque than at the baseline. At level D2, sensitivity decreased from 72% to 59%, and at level D3 from 86% to 29%. The AUC was statistically significantly higher in the absence of plaque, but only at the D2 level. The plaque was suggested to act as a barrier.

Discolourations

Sheehy *et al.* (2001) found in their clinical study that fissure staining was associated with high LF readings. Francescut & Lussi (2003) investigated the relationship between discolouration and the depth of caries lesions in primary and permanent teeth and the influence of discolouration on the LF values in an *in vitro* study. When there was dark brown or black discolouration on the permanent tooth surfaces, histological examination proved that 87% of the lesions were in enamel and 13% had reached the dentine. In primary teeth, 58% of such lesions were in enamel and 42% in dentine. The LF values were higher when discolouration was present irrespective of the histological depth.

Sealants

Takamori *et al.* (2001) evaluated the ability of LF to detect occlusal caries under sealants *in vitro*. Premolars and molars with occlusal caries were used. The LF measurements were done at the baseline and after chemical irrigation, acid etching and application of light-cured fluoride releasing sealant (clear, red, or white). Caries was detected in 90% of teeth with clear and red sealants and 54% of teeth with white sealants (Takamori *et al.* 2001). In the *in vitro* study by Hosoya *et al.* (2004), placement of sealants decreased the mean LF values. Sealants used in this study were opaque. In addition, in the *in vitro* study by Deery *et al.* (2006), the LF values decreased after placing a clear sealant on surfaces with different stages of caries. After placing a clear sealant, specificity was higher but sensitivity lower at levels D1 and D3. Both visual examination and LF underestimated the presence of caries lesions under clear sealants.

2.4.2 Procedure related factors

Calibration

Calibration of the device is an important part of the measuring procedure, and the LF readings are dependent on the mode of calibration. So-called individual calibration excludes the interfering fluorescence caused by sound tooth surface, and thus the digit value on the display represents the value of demineralized tissue. If calibration is done only on the calibration block, the fluorescence of the tooth substance is included in the LF value.

The manufacturer recommends only one arbitrarily chosen spot for calibration. Braun *et al.* (2005) evaluated in an *in vitro* study the influence of the mode of calibration of the LF device on the measurements. Values with standard calibration were significantly higher than those achieved using individual calibration for both small and more advanced lesions. The same study also showed that choosing referral sites may affect the LF measurements up to six units. The *in vitro* study of Braun *et al.* (2005) showed that mean LF values of the occlusal reading point following standard calibration were about 4–5 units higher compared to values measured after individual calibration. During individual calibration, a mean range of about 2 units could be observed when comparing the readings of the occlusal reading point with respect to the four different areas of each buccal surface. The results suggest that for monitoring as well as for clinical

decisions, individual calibration is necessary and that the same mode of calibration should be used always when employing the LF measurements for longitudinal caries monitoring.

Professional cleaning

The manufacturer recommends careful removal of bacterial plaque from the tooth surface before the LF measurements, and this has been done in majority of studies. In the studies the teeth have been cleaned with an explorer (Lussi *et al.* 2001), a single-tufted toothbrush (Sheehy *et al.* 2001), a rubber-cup and polishing paste (Alwas-Danowska *et al.* 2002), and using an airflow device (Heinrich-Weltzien *et al.* 2002), which is also recommended by the manufacturer.

Rinsing after professional cleaning has been found to be necessary to avoid the false readings caused by the remnants of the paste or pumice. Lussi *et al.* (2005) found that 10 seconds rinsing with a 3-in-1 syringe was required after professional cleaning; 3 seconds was not long enough. Hosoya *et al.* (2004) studied fluorescence of ten polishing pastes and four dentifrices *in vitro*. The deepest occlusal pits of sound unsealed and sealed occlusal surfaces of premolars were measured using a LF device before and after cleaning with paste and water. Conclusion was that pastes, if not rinsed properly away, interfere with the LF values. In the study by Schirrmester *et al.* (2005), the LF values were measured after cleaning with a brush and again after using an airflow device. The difference in the LF values of the two groups was not significant.

Drying time

When the lesion is dried, incident light will be absorbed less, leading to an increase in the amount of light passing into deeper parts of the lesion, and consequently there will be less absorption of fluorescent light and the LF values are bigger. Drying times have varied in the studies: drying with a 3-in-1 syringe for 2 seconds (Lussi & Francescut 2003), 5 seconds (Attrill & Ashley 2001), 8 seconds (Shi *et al.* 2001a, b), 10 seconds (Bamzahim *et al.* 2002), and only briefly (Sheehy *et al.* 2001, Alwas-Danowska *et al.* 2002, Heinrich-Weltzien *et al.* 2002). In the studies of Lussi *et al.* (2001) and Pereira *et al.* (2001), the length of the drying time was not described.

Lussi *et al.* (1999) reported in an *in vitro* study that fluorescence signals from wet sites are only slightly different from the same sites after air-drying. Shi *et al.*

(2000) compared *in vitro* accuracy and reproducibility of the LF measurements on wet and dry, clinically intact occlusal surfaces twice with an interval of two weeks. Drying for two minutes increased the LF readings. Sensitivity was increased by drying but specificity was decreased. Reproducibility of LF was not affected by wetness of the surface. The results suggested that moisture does not seem to influence the diagnostic performance of LF. In 2005, Lussi *et al.* studied the subject further in a clinical setting. Three dentists examined visually 117 intact occlusal surfaces of 70 young adults. The LF values were recorded in the sequence: moist and uncleaned, dried and uncleaned, moist and cleaned, and dried and cleaned. LF values referring to a certain visual status were up to 5 units lower for moist and uncleaned surfaces compared with the other conditions. Drying seemed to increase the LF values of dentinal caries on uncleaned tooth surfaces.

Mendes *et al.* (2004) evaluated *in vitro* the effect of the drying time on the performance of LF in occlusal sites of primary molars. LF was measured on moist sites, on sites dried for 3 seconds and for 15 seconds with a 3-in-1 syringe, and on sites dehydrated for 24 hours. The mean LF values were significantly higher on dehydrated sites than on the moist sites and on the sites dried for 3 seconds. On dehydrated sites sensitivity was higher, but specificity was lower at level D3 when compared to moist sites.

Devices

Many studies have reported excellent reliability between separate DIAGNOdent® devices. In the study of Alwas-Danowska *et al.* (2002), two separate devices were evaluated *in vitro* and *in vivo*. The intra-examiner kappa value of the two examiners' LF readings using the two devices was 0.90 and 0.88, and the inter-examiner agreement was 0.85 for one device and 0.90 for the other. Baseren & Gokalp (2003) found high reliability between the two devices of the two examiners (0.90 and 0.88); inter-examiner reliability was 0.85 for one device and 0.90 for the other. In the *in vivo* study of Astvaldsdottir *et al.* (2004), the inter-device agreement of the LF readings of the four DIAGNOdent instruments used ($r = 0.81-0.92$) was significant ($p < 0.05$). In the study by Kühnisch *et al.* (2004), the intra-class correlation coefficient of the LF readings between devices was 0.89.

Storing of teeth in vitro

For *in vitro* studies, teeth are stored using different storing agents or by freezing. It is common knowledge that LF is affected by storing and that the results of *in vitro* studies cannot be applied to clinical practise without interpretation.

Shi *et al.* (2001b) reported that formalin caused a dramatic increase in the LF values, whereas thymol-saturated saline did not. This may be due to the fact that formalin denaturizes proteins, which alters the structure and quality of one of the organic components, and consequently causes an increase in fluorescence. Francescut *et al.* (2006) studied the change in the LF values of 40 teeth stored in 1% chloramines, in 10% formalin, in 0.02% thymol, or in -20°C for two years. After the storage, the frozen teeth showed a slight but a non-significant increase in the LF values, whereas the LF values of teeth stored in all other ways decreased significantly. For analysing, the teeth were defrosted. Lussi *et al.* (2006) also found that the cut-off values for caries detection decreased when teeth were stored in thymol, formalin or chloramines, but not when they were frozen.

2.5 Comparison of LF and other caries detection methods

Laser fluorescence and visual inspection

Sheehy *et al.* (2001) compared the LF values with a visual scoring system (Ekstrand *et al.* 1997) for *in vivo* detection of occlusal caries. One examiner did the examinations. The LF readings were interpreted using the manufacturer's cut-off recommendations and those based on *in vitro* studies, and using histological validation. A total of 132 mandibular 38 maxillary first molars in 170 children were examined (mean age 6.9 ± 0.6 years). Agreement between VI and the LF measurements was better when the manufacturer's recommendations for cut-off points were used. When the cut-off points recommended by *in vitro* studies were used, either LF was overscoring or VI was underscoring.

In a clinical study, Alwas-Danowska *et al.* (2002) found that sensitivity of the LF measurements was higher but specificity lower than that of VI, but overall accuracies of VI and LF were not statistically significantly different. This was also the finding of the *in vitro* study of Baseren & Gokalp (2003). According to the *in vitro* study by Deery *et al.* (2006), both visual examination and LF underestimated the presence of caries lesions under clear sealants. In the clinical study by Reis *et al.* (2006), which used histological validation, there was no

difference between VI and LF *in vivo* or *in vitro* at D2 level. At D3 level, VI had higher specificity than LF both *in vivo* and *in vitro*. The inter-examiner and intra-examiner reproducibility was higher for VI than LF. In their clinical study with histological validation, El-Housseiny & Jamjoum (2001) found that LF was superior compared with VI and probing.

Laser fluorescence and radiography

In the *in vitro* study of Shi *et al.* (2000), the diagnostic accuracy of LF was significantly better than that of radiography for detecting all types of occlusal caries lesions. In an *in vitro* study, comparing DIAGNOdent Pen® and BW-radiography in detection of approximal caries *in vitro* using histological validation, BW radiography was less accurate than LF. Positive likelihood ratios for BW-radiography were 2.0 (D1) and 4.2 (D3), and for DIAGNOdent pen® 13.3 (D1) and 4.8 (D3) (Lussi *et al.* 2001).

Laser fluorescence, visual inspection, and radiography

Lussi *et al.* (2001) reported in their clinical study superior sensitivity of LF compared with VI and BW-radiography. In the clinical study by Heinrich-Weltzien *et al.* (2002), untreated first and second molars having either visually sound occlusal surfaces or enamel/dentine lesions were inspected by using VI (Ekstrand's criteria), radiography, and LF. Fissure opening was used for validation in cases where caries was suspected based on VI. LF had the highest sensitivity and reproducibility. Specificity was highest for VI and radiographic methods. The results of this study suggest that the criteria by Ekstrand *et al.* (1997) created for third molars may not be applicable for the first and second molars. Rocha *et al.* (2003) compared in a clinical study VI, radiography, and LF in detecting occlusal caries in first and second primary molars. LF and radiography showed higher sensitivity and lower specificity than VI for dentine lesions. In the clinical study by Angnes *et al.* (2005) using histological validation, VI and LF had similar and superior sensitivities to BW-radiography. VI and BW-radiography showed similar specificities, which were superior to LF. The inter-examiner reproducibility was good for VI and BW and moderate for LF. Similar sensitivities for VI and LF in occlusal caries detection were reported in an *in vitro* study by Burin *et al.* (2005), and superior for BW-radiography. In this study, specificity was reported to be similar for VI, LF, and BW-radiography.

In a clinical study, Bamzahim *et al.* (2004) compared LF with radiography and VI in detecting secondary caries in restoration margins. The results showed that LF was better than the other two methods. In the clinical study by Bamzahim *et al.* (2005), LF was compared with VI and radiography in the detection of secondary caries in teeth with amalgam restorations. LF had higher sensitivity than radiography and VI. Specificity was lower for LF than for the other two methods. The overall performance of LF was better than that of radiography for detection of secondary caries. Concerning detecting approximal caries in primary teeth, sensitivity of LF was higher but specificity somewhat lower than that of BW-radiography.

Laser fluorescence and electric conductance measurements

According to Lussi *et al.* (1999) and Takamori *et al.* (2001), LF had higher validity for occlusal caries detection, and higher reproducibility for measurement of lesions than ECM *in vitro*. Hibst *et al.* (2001) reported that the reproducibility of LF was superior to that of ECM *in vitro*. LF was also superior to ECM in detection of non-cavitated lesions. LF was found to be very useful, because the whole surface could be scanned (Hibst *et al.* 2001). Katz & Huntington (2004) monitored changes in caries status using FOTI, radiography, LF, ECM, and combination of these in a one-year follow-up trial. The changes in the ECM and the LF values correlated positively with the changes in visual caries status, but correlation was not statistically significant.

Laser fluorescence and quantitative light induced fluorescence

According to the *in vitro* study by Shi *et al.* (2001a), the LF and QLF methods were equally valid for quantification of smooth surface lesions. Aljehani *et al.* (2006) found *in vitro* a stronger association between the lesion depth and the QLF values than the lesion depth and the LF values, which also was the finding in the clinical study by Tranaeus *et al.* (2004). The QLF values also reflected mineral loss better than did the LF values.

Laser fluorescence and fibre optic transillumination

In the *in vitro* study, Cortes *et al.* (2003) compared different methods for caries detection and evaluation of the depth of occlusal caries lesions (152 sites) using

histological validation. The effect of stain on caries detection was also assessed. The methods studied were combined FOTI/visual method (CFV), VI, FOTI, LF, and ECM. The correlation coefficients with histology ranged from 0.42/1 (LF) to 0.66/1 (CFV). FOTI added to the accuracy of caries detection compared to visual method alone. The AUC for CFV was significantly greater than those of VI, LF, and ECM. The exclusion of stain and brown spot lesions improved performance for all methods. It was concluded that CFV was useful for the determination of occlusal lesion depth and that in the presence of stain and brown spot lesions, different cut-off points may be required for the ECM- and LF-methods for dentine caries detection.

2.6 Summary

The results of these studies suggest that clinicians should not rely only on the LF measurements as the primary diagnostic method, but should use LF as a supplement to the traditional caries detection methods of VI, FOTI, and radiography. LF can refine questionable diagnosis and guide in choosing intervention method. The LF method is also useful in monitoring lesions. The LF values reflect better lesion depth than mineral loss.

The problem of validation in clinical studies leaves the researcher with queries: is the high LF reading dependable or is the lesion difficult or even impossible to detect visually? According to literature, in general, sensitivity of the method is higher than that of VI, but specificity is lower. Sensitivity and specificity of the LF method vary and depend on the cut-off point. The cut-off points recommended by the manufacturer and according to selected clinical studies are higher than those achieved in *in vitro* studies. There is also variation of the LF values inside visual categories, often without any explanation. *In vitro* studies are numerous compared to those *in vivo*. There are only few studies on monitoring the LF values – the existing studies monitor LF values after prophylactic intervention – and estimating the activity of lesions using LF. There are no studies estimating usefulness of the method in screening children e.g. for the harmfulness of their dietary habits on oral health.

3 The aim of the present study

By the time of the first studies of this thesis, the majority of studies on laser fluorescence had been done *in vitro*. However, *in vivo* studies are needed to get clinical guidelines for the method. Majority of dental caries exists on occlusal surfaces, and detection of occlusal caries still remains as a challenge for a clinician.

The hypothesis for this study were that laser fluorescence (LF), measured as a part of a routine dental check-up, is useful in detecting and monitoring dental caries lesions and that professional cleaning before scanning is not necessary for having valid and reliable LF measurements. It was also hypothesized that due to early demineralization of the enamel, children with unfavourable dietary habits have higher LF values on visibly sound occlusal tooth surfaces than children with favourable dietary habits. Therefore, LF scanning is useful in detecting children with current unfavourable dietary habits, before visible damage is caused to their teeth.

The aims of the present study were

1. to study, how the LF values of occlusal surfaces of molars and premolars measured as a part of a routine dental check-up of children correlate with the visual status of the teeth
2. to find out, which values of laser fluorescence could be used as cut-off points for considering operative intervention
3. to study the rate of progression of occlusal caries lesions over a period of one year as assessed by visual inspection and LF
4. to compare the LF values before and after professional cleaning of teeth with and without paste
5. to study if self-reported dietary habits are reflected in the LF values of visually sound occlusal surfaces of premolars and molars

4 Subjects and methods

4.1 Subjects (I, II, III, and IV)

This study comprises four clinical studies (I, II, III, and IV). There were two study groups, one in the studies I, II and III and another in study IV (Table 7).

Table 7. Number of subjects and teeth scanned with LF in the studies I, II, III and IV.

Study	Year	Number of children	Number of teeth		
			Primary	Permanent	Total
I	2001	109*	436	613	1049
II	2002	81*	315	423	738
III	2003	46*		643	643
IV	2005	150		1221	1221
Total			751	2900	3651

*the subjects were from the same group

Children participating in the study I were 1st graders (7–8-year-olds, n = 55) and 7th graders (13–14-year-olds, n = 54) in two schools in the city of Oulu, Finland. In the study II, individuals of the study I were re-called for a dental examination (n = 81). The mean interval between the two inspections was 1.2 years. Forty-six children from the original study group (6 of the younger and 40 of the older children) participated in the study III in their second re-call examination.

The clinical visual examinations of studies I and II were routine dental check-ups of the municipal public health care system. Occlusal surfaces without fillings were then scanned by using an LF device. Four hundred and thirty first molars, 183 second molars, and 436 primary molars were examined in the study I, and a total of 423 permanent and 315 primary molars in the study II following the same protocol. In the study III, 643 occlusal tooth surfaces were examined visually and by using a LF device in the same way as in studies I and II. After the LF scanning, the teeth were cleaned professionally using a rotating hand-piece, a rubber cup, and either polishing paste (20 children) or plain water spray (26 children). The occlusal tooth surfaces were then rescanned by using the LF device. A total of 308 teeth were cleaned with paste and 335 without paste.

Study IV was conducted in two phases. In the first phase, 462 children from the city of Oulu aged 13 to 14 years answered a self-administered questionnaire on dietary habits. Children were classified according to the total score of their answers. One hundred children with the most favourable and the most

unfavourable dietary scores were invited to the second phase of the study consisting of a visual inspection, an LF scanning of visually sound occlusal surfaces of premolars and molars, and an oral self-care questionnaire. Hundred and fifty children were inspected: 76 from a group with the most favourable and 74 children from a group with the most unfavourable dietary habits. A total of 1221 sound occlusal surfaces were scanned.

4.2 Visual examination (I, II, III, and IV)

One dentist (VA) examined the teeth visually in all studies. The criteria for the visual inspection in all studies are presented in Table 8. Visual inspection was done by using the light of the dental unit, a plane mirror, and a probe. No prior professional cleaning was done and no magnifying device was used. The teeth were lightly blow-dried using a 3-in-1 syringe before the examination, and the texture of the occlusal surfaces was felt tactilely without pressure by using a probe. FOTI was used to confirm visually suspected decay of any kind without recording the findings. Surfaces with fillings and opaque sealants were excluded from the analyses, but surfaces with clear sealants were included in studies I, II, and, III. A dental nurse recorded the data (I, II, III) manually. In the study IV, the findings were entered in patient files by using a computer programme designed for the study.

Table 8. Criteria for visual categories of caries lesions.

Scale	Category	Criterion
0	Sound	Normal enamel texture.
1	Inactive enamel caries	The fissure enamel is brownish or black. The surface feels hard and smooth. No loss of substance.
2	Active enamel caries	The fissure enamel is opaque with loss of luster. The surface feels rough. No loss of substance.
3	Dentinal caries	The fissure enamel is opaque and feels soft. The subsurface dentine around the fissure appears dark. Often loss of substance, but not necessarily.

4.3 Laser fluorescence scanning (I, II, III, and IV)

The LF scanning was done in all studies by one DIAGNOdent® device (KaVo, Biberach, Germany) from the year 1999. Scanning was done after the visual examination of teeth. The surfaces were blow-dried lightly before scanning. No

professional cleaning was done in studies I, II and IV. Teeth with clear sealants were scanned in studies I, II and III. The conical tip A was used, and the hand piece was run along the occlusal surface turning and tilting with the aid of the acoustic signal of the device to find the most demineralized sites of the surface. Three separate tips A were used during the four studies. The peak value of each surface was recorded by the dental nurse. The data of the gathered LF readings were recorded manually (I, II, III) and in patient files of a computer programme designed for the study (IV).

Calibration of the LF device was done in-between all patients. In studies I and II, a general calibration was done on free space (0) and on the ceramic standard according to the instructions given by the manufacturer at that time. In studies III and IV, individual calibration of the device was carried using the ceramic standard block and a sound labial surface of the upper incisor of each individual instead of free space. This way the sound tooth surface of an individual gave a 0 value. This was tested on the sound buccal surfaces of the molars and premolars to be scanned occlusally.

Teeth were cleaned professionally only in the study III. Tooth surfaces were scanned using the LF device before professional cleaning, after which, at the same appointment, the teeth were professionally cleaned by using a rotating hand-piece, a rubber cup and polishing paste (Cleanic®, Hawe Neos Dental Bioggio, Switzerland) or a rubber cup and plain water spray. The teeth were rinsed afterwards with copious water spray, and then blow-dried lightly. After cleaning, the tooth surfaces were rescanned by using the LF device. The cleaning paste gave an LF value of 5 when the tip of the calibrated (general calibration) device was placed on the surface of the paste.

4.4 Bitewing radiography (I)

Bitewings were taken of 13 to 14-year-old children, who had a clinical indication for a radiographic examination or for whom the parents had given their consent for the examination (n = 51). Bitewing radiographs were taken of 363 molars. Kodak Ultra Speed films size 2 with the exposure time of 0.5 s and the X-ray machine Planmeca Prostyle Intra (Planmeca, Helsinki, Finland) were used. The dentist who took the radiographs (VA) also examined them. She has been calibrated in reading and scoring bitewing radiographs by another researcher, an assistant professor and senior consultant in cariology and restorative dentistry. Occlusal radiographic status was recorded as follows: 0 = sound, 1 = enamel

caries, 2 = caries extending to the dentine-enamel junction or superficial dentine, 3 = caries extending to the middle third of the dentine, 4 = caries extending to the deep inner third of the dentine.

4.5 Questionnaires (IV)

For the study IV, a dietary questionnaire and a computer programme based on it, were designed. The computer-assisted questionnaire was piloted in Jyväskylä in 2004 (Kasila *et al.* 2005). Another computer-assisted questionnaire of oral hygiene and self-care was also developed. Of the hygiene questionnaire, only one question regarding tooth brushing was used in the current study. The children were given scores according to their answers on the dietary questionnaire. The maximum score referring to the best dietary habits was -86 and the minimum score was +90. One hundred children with the most favourable (score < -38) and the most unfavourable (score > -10) dietary habits were invited for the second phase (clinical part) of the study.

4.6 Validation (I)

To provide a gold standard for carious teeth, the depth of a lesion was determined visually (caries in dentine-enamel junction, middle-third of dentine, and caries near pulp) in cases which were judged clinically needing operative intervention (visual category 3, 51 teeth) (I). To assess the intra-examiner reproducibility for the visual inspection and the LF scanning, 102 teeth of 11 children were re-examined on a separate occasion following the protocol of the first examination. The time period between the two examinations was approximately four weeks. Intra-examiner reproducibility of radiographic caries detection was assessed by re-inspecting randomly chosen bitewing radiographs of 11 children.

4.7 Statistical analyses (I, II, III, and IV)

Means of the LF values were calculated in the studies I, II and III, and means of change parameters (the latter LF value minus the former LF value) also in the studies II and III. In the study IV, medians were calculated because of the skewness of the data. Skewness was noticed by inspecting.

Box-plots were used to illustrate the distribution of the LF values at different levels of the visual examination score (I, II), and the distribution of the LF values

among groups of tooth surfaces of children with favourable and unfavourable dietary and tooth brushing habits (IV).

Sensitivity, specificity, and Youden's index values (Youden, 1950) were calculated in order to evaluate the accuracy of classifications based on different threshold levels of the LF values (I). To evaluate the statistical significance of the differences in the LF values between unsealed and sealed teeth across visual categories, two-way analysis of variance was applied (I, II).

Unweighted kappa statistics was used for evaluation of the reproducibility of visual caries measurements (I). Extent and direction of systematic error was evaluated by comparing the mean values of the two measurements. Pearson correlation coefficient and intra-class correlation coefficient were used for assessing the extent of random error (I).

In the studies II and III, means of change parameters of the LF values were analysed. In the study II, a change parameter was calculated to illustrate the surface-specific differences between the visual examination score at the baseline and that at follow-up. A corresponding change parameter was used to describe the direction and extent of the surface-specific differences in the LF values between the two examinations (II, III) and in different teeth and age-groups (III). In the graphs illustrating the percentage distribution of tooth surface values were found value of the change parameter, the values were grouped into ten-unit categories. In the study III, ninety-five percent confidence intervals were calculated for the mean values of the change parameters.

Statistical testing and multivariate modelling for adjusting the dependency of the observations was done by using Repeated Measures ANOVA models with SAS PROC MIXED (SAS Institute INC., Cary, NC, USA) procedure (II, III, IV). The statistical software package SPSS 14.0.1 (Lead Technologies, INC., USA) was used to study the significance of dietary habits on the LF values in premolars and molars (IV). In the multivariate analysis, where the LF value was the response variable, a logarithmic transformation was applied to reduce the skewness of the original data.

4.8 Ethics (I, II, III, and IV)

The study protocols for the studies I, II and III were approved by the Ethical Committee of the Oulu City. The protocol in the study IV was within the guidelines of the Ethical Committee of the Northern Ostrobothnia Hospital District, which had given an approving statement for the study.

5 Results

5.1 Correlation of laser fluorescence values of occlusal surfaces of teeth with the visual status (I)

A total of 214 first molars and 436 primary molars of 6- to 7-year-olds and 216 first molars and 183 second molars of 13- to 14-year-olds were examined by using LF and visual examination with estimation of activity of enamel lesions. No differences in the distribution of the mean LF values were found between the two age groups or between the first and second molars. The mean LF values for sealed teeth did not differ from those of unsealed teeth in any of the categories of visual score, when transparent sealant was used. Teeth sealed with transparent sealants were included in the analyses of the studies I, II and III. The higher the visual score, the higher were the mean LF values. Variation among the LF values in each visual category was large. However, the mean LF values showed a steady gradient across the categories of the visual score. The mean LF values of visually sound teeth and of the teeth with initial lesions were lower in primary teeth than in permanent teeth. However, the mean values for dentinal caries were of the same order of magnitude as in permanent teeth. The number of initial lesions in primary teeth was small (20 out of 436). The mean LF values in different visual categories are presented in Table 9. The intra-class correlation coefficient for the LF readings measured in the study I was 0.79 and the kappa value for the intra-examiner reproducibility for VI was 0.85 (I).

Table 9. Means and medians of the LF values in different visual categories (I).

Visual category	Permanent molars								Primary molars			
	Unsealed				Sealed				Mean	SD	Median	n
	Mean	SD	Median	n	Mean	SD	Median	n				
Sound	17.9	13.6	14	225	19.6	16.8	14	159	4.2	4.6	3	399
Inactive initial caries	36.6	21.4	30	73	32.9	16.2	30	33	38.1	29.3	28	9
Active initial caries	43.9	23.3	40	61	47.8	24.0	43	13	29.7	23.3	30	11
Dentine caries	55.4	23.4	54	46	–	–	–	–	59.6	24.8	61	17

– Number of cases was < 5

5.2 Accuracy of laser fluorescence values (I)

When attempts were made to use laser fluorescence value for separating surfaces with dentine caries from sound surfaces or surfaces with initial caries, the best performance was achieved at the threshold level > 30 vs. ≤ 30 . Sensitivity was 92%, specificity 69%, and Youden's index value 60%. When only teeth with validated dentinal caries were considered carious (51 teeth), and teeth with a score of 0 in visual examination (384 teeth) were considered sound, sensitivity was 92%, specificity 82%, and Youden's index value 74% when LF values > 30 were considered indicative of a dentinal caries lesion. The kappa value for the intra-examiner reproducibility for the LF measurements was 0.85, Pearson correlation coefficient 0.74, and intra-class correlation coefficient 0.78. Ninety-six % of visually sound surfaces of molars without sealants had LF values ≤ 30 and 91% of surfaces with dentine caries had LF values > 30 (Table 10). Of the surfaces with inactive initial caries lesions, 63% had LF values ≤ 30 and of those with active initial caries lesions, 62% had LF values > 30 . Values < 10 were indicative of sound tooth surfaces.

Table 10. Distribution of permanent molars without sealants according to LF values (≤ 30 and > 30) and different categories of visual status.

Visual status	LF value ≤ 30	LF value > 30	Total
Sound	432	17	449
Inactive initial caries	70	41	111
Active initial caries	11	18	29
Dentine caries	2	20	22
Total	515	96	611

For the separation between sound/inactive and active/dentine caries, sensitivity was $38/51 \times 100 = 75\%$, specificity $502/611 \times 100 = 82\%$, positive predictive value $38/96 \times 100 = 40\%$, and negative predictive value $502/560 \times 100 = 90\%$.

Table 11 shows distribution of permanent teeth according to their LF values (> 30 vs. ≤ 30) and combined visual and radiographic status. The data in Table 11 is the same as in the study I, but here premolars are included and teeth with sealants are excluded.

Table 11. Distribution of permanent teeth according to LF values (≤ 30 and > 30) and different categories of status according to visual and radiographic examination.

Status according to visual and radiographic examination	LF value ≤ 30	LF value > 30	Total
Sound	419	22	441
Inactive enamel caries	38	27	65
Active enamel caries	28	27	55
Dentine caries	6	36	42
Total	491	112	603

For the separation between sound/inactive and active/dentine caries, sensitivity was $63/97 \times 100 = 65\%$, specificity $457/506 \times 100 = 90\%$, positive predictive value $63/112 \times 100 = 56\%$, and negative predictive value $457/491 \times 100 = 93\%$

5.3 Correlation of the changes in the visual scores and laser fluorescence values on occlusal surfaces over a period of one year (II)

Data comprised a total of 423 permanent molars and 315 primary molars, which were examined twice using visual inspection and a DIAGNOdent® device within a mean period of 1.2 years between the two examinations. For both primary and permanent teeth, the LF values were significantly higher at the follow-up visit than at the baseline (permanent teeth: $p < 0.0001$, primary teeth: $p = 0.026$). In 63% of the teeth, the visual status did not change, in 32%, the visual status became worse, and in 5%, the visual score improved. The means of the LF values at the baseline and after the follow-up period and the means of the changes in the LF values of occlusal surfaces of permanent teeth in different combinations of VI at baseline and at the follow-up are presented in Table 12.

Table 12. Mean LF values of permanent teeth at the baseline (LF1) and after the follow-up period (LF2), and the average change of the values (Δ LF) during the follow-up period in different categories of visual scores.

Visual score		n	LF1	SD	LF2	SD	Δ LF	SD	p
Baseline	Follow-up								
0	0	198	15.9	13.5	18.8	18.0	2.8	18.2	0.0348*
0	1	75	22.7	16.8	32.0	25.0	9.3	27.6	0.0170*
0	2	23	28.0	22.2	49.2	24.4	21.3	22.5	0.0004*
0	3	3	25.3	16.0	71.0	19.5	45.7	23.0	0.0752
1	0	11	30.2	16.3	20.9	17.1	-9.3	21.5	0.2507
1	1	45	39.4	23.1	34.3	22.0	-5.1	22.0	0.1704
1	2	11	31.5	12.4	35.1	25.2	3.6	31.3	0.7842
1	3	3	64.0	31.0	79.7	28.4	15.7	32.5	0.4913
2	0	8	42.8	27.3	29.0	21.9	-13.8	28.2	0.2447
2	1	13	42.4	29.7	34.9	15.5	-7.5	31.6	0.4143
2	2	11	47.7	18.7	50.5	23.4	2.8	30.4	0.7658
2	3	13	51.0	20.1	56.4	19.1	5.4	19.6	0.4589
Total		414							

9 teeth missing; those which had visual class 3 at the baseline and had been filled

For permanent molars with a visual score of 0 at both the first and second check-up, the mean baseline LF value was lower than for the molars with a visual score of 0 at the baseline and a follow-up score of 1, 2 or 3. This was also true for primary molars. The difference in the baseline values between teeth that remained sound and those that developed caries was statistically significant for both permanent and primary molars ($p = 0.001$ and $p < 0.0001$, respectively).

At the baseline, 53 permanent molars had a visual score of 0 and LF scores > 30 (mean 46). At the follow-up, the mean LF score for these teeth was 41. Of those teeth, almost 89% still had the visual score of 0 or 1, about 10% had the score of 2, and less than 1% had the score of 3.

All teeth with the visual score of 3 at the first appointment had been treated at the second appointment. In 32 permanent molars, the visual score had reversed from 1 or 2 to 0, or 2 to 1. For these teeth, the mean baseline LF value was higher than the follow-up value ($p = 0.086$) (Table 12). In the four primary molars with a visual reversal, a tendency for decreased LF values was also seen ($p = 0.716$).

5.4 Laser fluorescence values before and after professional cleaning of teeth with and without paste (III)

The mean LF values, and their differences before and after professional cleaning were calculated separately for teeth having visual score 0 (sound), and those having visual score > 0. For premolars, no significant differences in LF before and after cleaning of teeth were observed in any visual category. For permanent molars, the LF values tended to increase after cleaning. The increase was more distinct in teeth cleaned with water than in those cleaned with paste. The difference of the two measurements was statistically significant ($p < 0.05$) for molars cleaned with water and with the visual score > 0. The effect of cleaning was fairly similar for sealed and unsealed teeth.

5.5 Reflection of self-reported dietary habits on laser fluorescence values of visually sound occlusal tooth surfaces (IV)

The LF values of sound occlusal surfaces of premolars and molars of 13- to 14-year-old children whose dietary habits were favourable ($n = 76$) and of 13- to 14-year-old children whose dietary habits were unfavourable ($n = 74$) were analysed. A total of 1221 visually sound occlusal surfaces were scanned.

The median LF value in the teeth of children with favourable dietary habits ($n = 890$) was lower (4, lower quartile 2 and upper quartile 12) than that of children with unfavourable habits ($n = 717$) (6, lower quartile 2 and upper quartile 15).

The effect of dietary habits as well as the type of the tooth and the jaw on the LF values was statistically significant at least at $p < 0.05$ level when calculated using the repeated measures analysis of variance with the logarithmic LF value as the response variable and these factors as independent variables. The LF values were significantly higher for molars and teeth in the lower jaw and when the dietary habits were unfavourable.

6 Discussion

6.1 Correlation of laser fluorescence values and the visual status

The LF values and the visual status of permanent teeth seemed to agree fairly well for both enamel and dentine caries in studies I and II. The variation of the LF readings in each visual category was large (I, II, IV), as also found in the clinical study of Lussi *et al.* (2001). However, the mean LF values showed a steady gradient across the categories for visual score. Large deviation of values has also been reported in other studies (Sheehy *et al.* 2001, Heinrich-Weltzien *et al.* 2002, Li *et al.* 2003, Schirrmester *et al.* 2005,). This may be due to several reasons. Besides demineralization, elevated LF values may be caused by discolourations, hypomineralization, fluorosis, fillings, and remnants of polishing pastes (Lussi *et al.* 1999, Lussi *et al.* 2001, Sheehy *et al.* 2001, Mendes *et al.* 2004).

The mean LF values of active enamel lesions were clearly higher than those of inactive lesions. Differential diagnosis of active and inactive initial caries is very important in terms of management of the lesion. Although enamel caries cannot be validated in a clinical study, the compatibility of the LF values and visual scores suggests that the LF measurements can be helpful in making the diagnosis (I). Activity of occlusal caries lesions using LF has been studied by Schirrmester *et al.* (2005), who found change in the activity of occlusal lesions in a follow-up period of two weeks after a tooth brushing intervention with toothpastes. Lussi *et al.* (2001), Pinelli *et al.* (2002), and Haak & Wicht (2004) found LF to be a good auxiliary method for assessing activity of incipient caries lesions on smooth surfaces. The results of the study II suggest that estimating activity of lesions is possible by comparing the LF values of scannings in the beginning and in the end of a certain observation period. This information can be used in general practise when deciding on prophylactic and/or reparative procedures as well as the length of the recall-interval.

Studying the effect of sealants on the LF values was not an objective of the present thesis. However, in study I, the mean LF values of sealed and unsealed surfaces were compared (clear sealants). Since no significant difference in the LF values of sealed and unsealed teeth could be detected, sealed teeth were included in studies II and III. In the few *in vitro* studies which have focused on studying the effect of sealants on the LF values (Takamori *et al.* 2001, Hosoya *et al.* 2004), even clear sealants have been found to decrease the LF values to some extent.

Deery *et al.* (2006) concluded, however, that monitoring can be done through them.

The conclusion, that the LF values were not influenced by the level of maturation of teeth was supported by the facts that there were no important differences between the two age groups (7- to 8-year-olds and 13- to 14-year-olds) nor between the first and second molars in the distribution of the LF values in different categories of visual scores (I). However, mean LF values at the baseline were somewhat lower than after the follow-up period, even when the clinical status did not change (II).

The LF values of premolars of the children were lower than those of molars (IV), which may be due to the simpler anatomy of the premolar fissures compared with molar fissures and fossae. The values for laser fluorescence of teeth in the lower jaw were higher than those in the upper jaw, which is in accordance with the findings of Li *et al.* (2003) and also of Shugars *et al.* (2005). Their three-year (2.9 years) follow-up study on asymptomatic third molars showed that mandibular third molars were more often attacked by dental caries during the follow-up time than maxillary molars. A retrospective study on the dental records of 483 children by Larmas *et al.* (1995) also indicated earlier caries onset on mandibular than maxillary molars. The reason for this can only be speculated. Possible explanations might be the earlier eruption of lower teeth and more difficult cleaning and self-cleaning of occlusal surfaces in mandibular molars, especially erupting ones. The anatomy of the occlusal fissures of mandibular molars may encourage more retention of plaque causing demineralization.

The LF values of sound primary molars in this study were significantly lower than those of permanent teeth. This is in accordance with the results of Lussi & Francescut (2003). In the primary teeth, there was no difference between the LF values for inactive and active enamel caries. This may be partly due to the small numbers of these lesions. It may also reflect the difficulty to discriminate visually between active and inactive enamel caries in primary molars as well as faster progression of caries into dentine in primary molars than in permanent molars (I) due to the anatomy.

6.2 Accuracy of laser fluorescence values

Sensitivity and specificity values of the LF method are highly dependent on cut-off points for different stages of caries progression. In the present study, the overall best performance for LF in detecting dentinal caries was found at the LF

value of about 30 by using both visual score and observed lesion depth for validation. Also Lussi *et al.* (2001) and Angnes *et al.* (2005) have reported that the LF readings > 20 indicate dentinal involvement, though not necessarily in need of operative intervention. The manufacturer's recommendations for cut-off points for different stages of occlusal caries have changed several times (1998a, 1998b, 1999, 2001, and 2002). Different recommendations have also been given in several studies (Lussi *et al.* 1999, Shi *et al.* 2000, Pereira *et al.* 2001, Lussi *et al.* 2001, 2005). The cut-off points recommended in clinical studies are higher than those of *in vitro* studies (Reis *et al.* 2006). The findings of this study agree well with the guidelines of the manufacturer and the clinical findings of Lussi *et al.* (2001) and Bamzahim *et al.* (2005). The conclusion in the literature is that LF should only be used as an adjunct to visual inspection (VI) (Bader & Shugars 2004), which was also supported by this study.

When visual status is used for validation, there always remains the question whether the surfaces diagnosed sound by VI really were sound. However, opening can only be done when dentinal caries is suspected. Radiography is known to be an inaccurate method for occlusal caries detection, until caries has progressed beyond the dentine enamel junction. Histological analysis would be the best validation method, but in a study like the present one, it is not possible to carry it out. In this study, sensitivity remained the same whether validation was done by VI or by opening of the fissure, but specificity was higher when fissure opening was used for validation. The results are in accordance with other clinical studies (Lussi *et al.* 2001, Alwas-Danowska *et al.* 2002, Heinrich-Weltzien *et al.* 2003, Angnes *et al.* 2005, Reis *et al.* 2006). When results of the visual inspection and the radiographic examination were used for validation of LF measurements, at the cut-off point 30, specificity was higher (90%), and sensitivity was lower (65%), than by using VI or VI and opening for validation. LF gives a numeric value for a lesion and the method can therefore be used for monitoring lesion progression. The results of this study are in accordance with the results of other studies, according to which present methods for caries detection rather underestimate than overestimate demineralization. VI is the method of choice for the final decision of the treatment, but LF can help making that decision. LF is useful in detecting initial enamel lesions and estimating their activity and changes in it.

The intra-class correlation coefficient for the LF measurements was calculated to be 0.78, which is in accordance with the intra-examiner reproducibility value of most clinical studies (Lussi *et al.* 2001, Sheehy *et al.* 2001, Sköld-Larsson *et al.* 2004, Astvaldsdottir *et al.* 2004, Pinelli *et al.* 2002).

6.3 Correlation of the changes in the visual score of occlusal surfaces and the laser fluorescence values over a period of 1.2 years

In the present study (II), changes in the visual score at the baseline and at the follow-up were compared with the changes in the LF values over a study period of 1.2 years. Changes in the LF values correlated positively with the changes in visual status. The value of the change parameter was significant in categories 0 → 0, 1, 2, where the number of the observations was big. The change in the LF value was most distinct when the clinical status changed from sound to initial caries in permanent teeth and from sound to dentine caries in primary teeth.

For teeth, in which caries had developed or progressed during the follow-up period, according to visual examination, the mean LF values were clearly higher at the follow-up examination than at the baseline. This was true for both permanent and primary teeth. The increase in values correlated positively with the change in the visual examination score, i.e. the change in the activity and depth of the lesion according to the visual examination. In primary molars, the increase was especially pronounced due to the low values for sound teeth.

An important finding was that in those permanent teeth which were visually sound at the baseline but had enamel or dentine caries at the follow-up, the baseline LF values were significantly higher than in teeth visually sound both at the baseline and at the follow-up (Table 12). These results suggest that, regardless of the visual appearance, teeth with baseline values of > 20 are more likely to become carious than those with a lower value. Many of these teeth probably had initial enamel caries, or even dentinal caries, already at the baseline, which had not been discovered visually. Based on this result, intensified self-care, prevention, and follow-up are indicated for children that have visually sound teeth but elevated LF values. More clinical studies are needed.

In permanent molars with a reversal from inactive or active enamel caries to a sound surface or from active initial caries to inactive as determined by visual inspection, the mean LF values had decreased considerably although, due to the small number of reversals, the difference was not quite statistically significant (Table 12). This suggests that LF may also be used for detecting the changes in caries status in both directions in permanent teeth, and that decreased LF values may be a sign of the success of preventive intervention or improvement of the self-care. In the four primary molars with a visual reversal, the follow-up LF value was only slightly smaller than the baseline value.

In the present study and in the study of Katz & Huntington (2004), the LF values have been monitored without intervention. In other studies the LF values have been monitored as a response to different prophylactic interventive means by using study periods from 2 weeks to one year (Sköld-Larsson *et al.* 2004, Gokalp & Baeren 2005, Schirmeister *et al.* 2005, Aljehani *et al.* 2006). The results of the study II agree with those of other studies suggesting that LF can be used for monitoring lesions even when scanning has been done as a part of a routine dental check-up. LF can be helpful in the practise of oral hygienists/dental auxiliary persons responsible for children's dental check-ups.

6.4 The laser fluorescence values before and after professional cleaning of teeth with and without paste

The aim of the studies I, II and IV was to follow the protocol of a routine dental check-up in a dental office, which does not include prior professional cleaning. Professional cleaning is time-consuming, and it was suspected that obligatory professional cleaning prior to a LF scanning may be an obstacle for using the method. Measuring the LF values without professional cleaning can be considered as screening tooth surfaces for demineralization. Even if studies show that Finnish adolescents are not frequent tooth brushers (Poutanen *et al.* 2005), visible plaque is seldom seen on occlusal tooth surfaces of fully erupted teeth. However, since professional cleaning is recommended by the manufacturer, its influence on the LF values was evaluated in the study III.

The LF values of most teeth increased after professional cleaning. This was true especially for permanent molars. The increase of the LF values after professional cleaning was statistically significant in teeth where the visual score was > 0 . Increase was more distinct when the teeth had been cleaned using only water without paste. An explanation might be that in spite of copious rinsing, remnants of paste are left in the bottom of fissures and thus fluorescence originating from the lesion is reduced. The results show that cleaning with the paste does not increase the accuracy of the LF values, rather the opposite, and that using a rotating rubber cup with a water flow and water spray afterwards is sufficient for cleaning the occlusal surfaces before measuring LF.

After professional cleaning, the increase of the LF values was most pronounced in second molars. The explanation is probably due to the fact that since second molars are more difficult to brush, there is generally more plaque on their occlusal surfaces than in the first molars or premolars.

Contrary to the present results, Cortes *et al.* (2003) found a decrease in the LF values after cleaning, and Mendes *et al.* (2005) found that dental plaque decreased the LF values on primary teeth. Cortes *et al.* (2003) suggested that stains in the fissures are a reason for high LF values. We did not keep record on stained fissures in our study, but staining is probably less common and less extensive in children than in adult teeth, which are commonly used in studies of extracted teeth.

The results of the study III are in accordance with the results of Lussi *et al.* (2005), who found that professional cleaning of teeth increased the LF readings to some extent. This may be of clinical significance in molars in borderline cases, where the need of operative intervention is considered. Therefore, cleaning with a rotating instrument and water spray before the LF measurements is recommended in teeth with visible plaque. Cleaning is advisable in cases where the LF readings approach threshold level for operative intervention.

6.5 Reflection of self-reported dietary habits on laser fluorescence values of visually sound occlusal surfaces of premolars and molars

The results of the study IV, where dietary habits of children were evaluated using self-reported questionnaires, showed that children with favourable dietary habits had, on average, lower values for laser fluorescence than did those with unfavourable habits. This is in line with the results of the study by Li *et al.* (2003) concerning caries active and inactive children, and indicates that LF reflects well recent dietary habits. LF of visually sound occlusal tooth surfaces has also been studied in the clinical studies by Lussi *et al.* (2001), Angnes *et al.* (2005), and Sköld-Larsson *et al.* (2004), but their focus was on the validation of LF in detecting and monitoring lesions rather than the validation of LF for estimating caries activity. In the present study the time interval between the questionnaire and the examination of the teeth was 0.5 years (SD 0.2). Consequently, for some subjects, the dietary habits at the time of the examination might not have been the same as those recorded at the baseline questionnaire. For those subjects, even the laser fluorescence of occlusal tooth surfaces might have been affected.

A written consent was obtained both from the parents of the children and from the children themselves. Because of this, the number of participants was low, meaning that less than half of those who were invited to the first phase of the study (1173) participated in it (462). 100 children with the most favourable and

100 children with the most unfavourable dietary habits were invited to the second phase of the study, and altogether 150 children came (74 with favourable and 76 with unfavourable dietary habits). It is very likely that the participants (462) had more favourable habits than did those who refused to participate. This is supported by the fact that the dietary score of the group of children who had the most favourable dietary habits and who participated in the phase 2 (74) agreed well with the whole invited group of children with favourable habits (100), whereas in the group with the most unfavourable dietary habits (76), the dietary score was lower (better) than in the whole invited group of children with unfavourable habits (100). This indicates that children with the most unfavourable habits did not participate in the phase 2 study. If the number of participants had been bigger, the difference in the LF values between the groups could have been even bigger, even now, it was statistically significant.

High LF values on sound tooth surfaces may be a reflection of harmful dietary habits. In those cases, professional dietary advice and motivation may help an individual to improve dietary habits, and as a consequence, oral health, and to turn demineralization of tooth surfaces towards remineralization before irreversible damage is caused.

6.6 Practical considerations

In the first study, standard calibration was carried out. The follow-up study had to follow the same study protocol to make the two consequent LF measurements comparable. In the last two studies, individual calibration was done. Individual calibration, today also recommended by the manufacturer, takes into consideration also the autofluorescence of the person's tooth substance. Values with standard calibration have been reported to be higher than those achieved by using individual calibration for both small and more advanced lesions *in vitro* (Braun *et al.* 2005). In addition, reference sites may affect the LF measurements up to six units. The empirical conclusion of the two first studies was that the effect of the autofluorescence was usually 0 to 2 units when labial sound, clean, and dried surfaces of upper incisors were used as reference sites having no notable influence clinically.

The manufacturer recommends professional cleaning before scanning. Professional cleaning was not done in study one, purposely, to find out the usefulness of the method as a part of a routine dental check-up without any extra procedures for the personnel except scanning. The results of our study (III and

IV) and those of Aleksejuniene *et al.* (2006) show that LF can be used for detecting or screening dental caries without professional cleaning and the method offers as such a second opinion or an alarm. If information that is more precise is needed, professional cleaning should be done. Cleaning can also be done by using a toothbrush simultaneously with dental education.

Because of the limited resources, responsibilities are given nowadays to oral hygienists and to dental auxiliary persons in doing check-ups and deciding, e.g. about children's dental treatment and recall-intervals. LF can be a helpful tool for them. The acoustic signal guides the examiner to find the site with the most demineralization, and is useful especially while working alone or doing screening. Acoustic signal may also be useful in patient motivation and oral health education.

There is a big deviation of values in each visual caries category, and no reason can always be given to remarkably big LF values. The final decision about intervention should always be based on VI. The numeric LF value helps monitoring activity of lesion progression.

In the old model of the DIAGNOdent® device, the tips wore down and the fibre points broke quite easily. In addition, the attachment became loose between the hand piece and the tip over time. One device and hand piece but several A tips were used during the present studies. There are no studies about the effect of changing the tips on LF the values.

7 Summary and conclusions

According to the results of the present studies, laser fluorescence was found to be useful as an adjunct to visual inspection in routine dental check-ups to detect demineralization of the occlusal surfaces of teeth. The visual inspection scores compared well with the LF values. The variation of laser fluorescence values in each visual category excludes its use as a primary or only method for caries detection. For considering operative intervention, the best cut-off point was found to be 30. The LF values of sealed and unsealed teeth were not significantly different.

LF can be used for monitoring changes in the state of demineralization of occlusal tooth surfaces. There is a positive correlation of the changes of the visual score of occlusal surfaces and the LF values as a function of time. Monitoring can be done through clear sealants.

Professional cleaning of teeth by using rotating instruments increases the LF values to some extent, especially in molars with the visual score > 0 . Professional cleaning does not affect the LF values of premolars. Professional cleaning by using a copious water spray is better for the LF measurements because remnants of cleaning paste may interfere with the results of the measurements. Screening teeth of children for caries by using LF can be done without cleaning if there is no visible plaque.

The LF screening may help to detect children with unfavourable dietary habits before damage is caused on their teeth. When high LF values are detected on several visually sound occlusal surfaces of molars and premolars, unfavourable dietary habits can be suspected and questioned. The LF screening may help to determine on prophylactic intervention and the length of recall interval and to monitor the situation.

More clinical studies are needed, e.g. in order to have evidence-based results of the changes of the LF values during longer monitoring periods and in order to find out the usefulness of the method in the practises of oral hygienists.

Based on the results, the following conclusions can be drawn:

1. LF scores correlate positively with the visual status, and LF can be used as an adjunct to visual inspection to detect demineralization of the occlusal surfaces of teeth.
2. The LF value 30 is recommended to be used as the cut-off point for considering operative intervention.

3. LF can be used for monitoring changes in the state of demineralization of occlusal tooth surfaces.
4. Screening teeth of children for caries by using LF can be done without cleaning if there is no visible plaque.
5. The LF screening may help to detect children's unfavourable dietary habits before visible damage is caused on their teeth.

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Original papers

- I Anttonen V, Seppä L & Hausen H (2003) Clinical study of the use of the laser fluorescence device DIAGNOdent for detection of occlusal caries in children. *Caries Res* 37: 17–23
- II Anttonen V, Seppä L & Hausen H (2004) A follow-up study of the use of DIAGNOdent for monitoring fissure caries in children. *Community Dent Oral Epidemiol* 32: 312–318
- III Anttonen V, Seppä L & Hausen H (2005). Clinical study on the effect of professional cleaning of occlusal tooth surfaces on laser fluorescence measurements. *Caries Res* 39: 280–283
- IV Anttonen V, Hausen H, Seppä L & Niinimaa A. Effect of dietary habits on laser fluorescence values of visually sound occlusal surfaces among Finnish schoolchildren. *Int J Paed Dent* (In press).

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