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Exploring the boundaries of caries detection

• Two advanced methods evaluated •

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To my mother, Þórdís Einarsdóttir

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Abstract

Background:

Caries detection methods require thorough validation. This should include studies which clarify what characteristics of the caries lesions are being measured, the limitations of the method and comparison of performance with conventional caries detection methods.

The outcome of validation tests has important clinical implications, such as interpretation of the data at the cut-off points used by the clinician to differentiate between lesions requiring invasive and non-invasive intervention.

Aim:

The overall aim of the thesis was to evaluate the characteristics, technical efficacy and diagnostic accuracy/efficacy of two advanced methods for caries detection, laser-induced fluorescence (LF) and digital imaging fiber-optic transillumination (DIFOTI), with special reference to implications for clinical treatment decision-making at the threshold between non-invasive or invasive management of the lesion.

Material and methods:

In **Study I** the LF method was evaluated for occlusal caries detection and quantification *in vivo*. The method was validated by comparing LF readings to actual lesion depth, determined by opening the fissures. Reproducibility was tested by comparing readings from four different LF instruments for each site and repeatability by comparing two readings from the same instrument. The LF readings were also correlated with the microbial flora cultured from the measured site. **Study II**, *in vitro*, tested the ability of the LF instrument to measure fluorescence from the two major components of the caries lesion, demineralized enamel and cultivated caries-associated bacterial flora. Sound enamel was demineralized and the change in mineral content verified by quantitative light-induced fluorescence (QLF). LF readings were taken before and after demineralization, to determine the influence of the demineralization on LF readings. LF readings were obtained from cultures of caries-associated bacterial species, with and without surrounding culture media: the influence of the age of the culture on LF readings was investigated. In **Studies III** and **IV** the DIFOTI

method was validated for approximal and occlusal caries detection and quantification. Diagnostic accuracy was validated by comparison with actual lesion depth determined by microradiography and histology. Reliability was tested in terms of inter- and intra-observer agreement, by comparing the results of scoring recorded by eight different observers.

Results

Validation: For three out of the four LF instruments tested there was a weak but significant correlation between LF reading and actual lesion depth (**Study I**). A different cut-off level was however obtained for different instruments, meaning that no general cut-off threshold could be used to differentiate between enamel and dentin caries.

The DIFOTI method showed generally good correlation with actual lesion depth, especially for approximal surfaces, with good diagnostic accuracy and efficacy at both the diagnostic thresholds tested (**Study III**). Compared to film and digital radiography, DIFOTI showed better performance at diagnostic threshold D1, and similar performance at diagnostic threshold D3. For occlusal caries detection (**Study IV**), DIFOTI showed moderate to good diagnostic accuracy and efficacy at both diagnostic thresholds. Performance was better than for both types of radiographs, but similar to visual inspection.

Reliability: The LF method showed very good reliability in terms of both intra-observer and inter-instrument correlation (**Study I**). For the DIFOTI method, good to very good intra-observer agreement was achieved for approximal caries detection (**Study III**) but for occlusal caries detection there was greater variation, agreement ranging from low to very good (**Study IV**). For approximal caries detection, inter-observer agreement was good (**Study III**) while for occlusal caries detection agreement was generally lower, ranging from low to good (**Study IV**).

Characteristics of the LF method: No change in LF readings was observed with respect to demineralization of dental enamel. LF readings > 20, generally accepted as indicative of dentin caries, were recorded only for mutans streptococci cultured on mitis salivarius bacitracin agar (MSB) and young colonies of *Prevotella spp*. The readings increased as the colonies of mutans streptococci aged and decreased when the colonies were moved to glass slides (**Study II**).

Conclusions:

The LF method can be useful for occlusal caries detection and quantification, under certain conditions: a) no general cut-off threshold can be recommended due to inconsistency between instruments. b) the method does not give information on demineralization or specific information on bacterial content of lesions, but rather responds to the synergistic effect of the caries process.

The results of the *in vitro* investigations suggest that the DIFOTI method may be of value for caries detection and quantification on both approximal and occlusal surfaces. The method shows superior performance to both film and digital radiography, especially for detection of early caries lesions on approximal surfaces. Thus the method shows promise as a means of monitoring early caries lesions and warrants further investigation.

List of Publications

I. Ástvaldsdóttir Á, Holbrook WP, Tranæus S. **Consistency of DIAGNOdent instruments for clinical assessment of fissure caries.** *Acta Odontol Scand 2004;62:193-198.*

II. Ástvaldsdóttir Á, Tranæus S, Karlsson L, Holbrook WP. **DIAGNOdent measurements of cultures of selected oral bacteria and demineralized enamel.** *Acta Odont Scand 2010;68:148-153.*

III. Ástvaldsdóttir Á, Åhlund K, Holbrook WP, de Verdier B, Tranæus S. **Approximal caries detection by DIFOTI; *in vitro* comparison of diagnostic accuracy/efficacy with film and digital radiography.** *Caries Research, under revision.*

IV. Ástvaldsdóttir Á, Silfverberg M, Holbrook WP, de Verdier B, Tranæus S. **Occlusal caries detection by DIFOTI: *in vitro* comparison of diagnostic accuracy/efficacy with visual inspection, film and digital radiography.** *Caries Research, under revision.*

Contents

List of abbreviations

AUC	*Area under the receiver operating characteristic curve*
D1 diagnostic threshold	*Lesion extension limited to enamel*
D3 diagnostic threshold	*Lesion extension through DEJ*
DEJ	*Dentino-Enamel Junction*
DIFOTI	*Digital Imaging Fiber-Optic Transillumination*
FAA agar	*Fastidious anaerobic blood agar*
FOTI	*Fiber-Optic Transillumination*
K_w	*Weighted Kappa*
LF	*Laser Fluorescence*
MSB	*Mitis salivarius bacitracin agar*
QLF	*Quantitative Light-induced Fluorescence*
r	*Spearman's rank order correlation coefficient*
UV light	*Ultra violet light*
VI	*Visual inspection*

Introduction

The science of cariology has undergone a paradigm shift in recent years. Dental caries is now recognized as a dynamic disease process where an imbalance leads to net mineral loss [Featherstone 2004; Selwitz *et al.*, 2007; ten Cate *et al.*, 2003]. Lesions are therefore regarded as evidence of an advanced stage of the disease, rather than the disease itself. The dynamic interaction between the tooth surface and the surrounding biofilm leads to a process of de- and remineralization [Baelum and Fejerskov, 2003; Featherstone, 2000]. It is therefore assumed that very few individuals are truly unaffected by this disease and can be referred to as caries free [Pitts, 2004]. On the other hand, the question arises as to what threshold of net mineral loss should be termed "caries".

Over the past few decades, there have been pronounced changes in both the epidemiology and the course of the disease [Hugoson *et al.*, 2005; Marthaler *et al.*, 2004]. The overall decline in caries prevalence and improved understanding of the pathology of the disease have led to a change in therapeutic approach. There is an increased focus on patient-centered, individual treatment plans, rather than a mechanistic focus, in which many patients receive very similar care plans, despite different states of disease activity and different patterns of behavior and needs. Modern management of the disease is evidence based: in order to design an appropriate treatment plan, clinicians need to obtain and process information not only about the presence of lesions and lesion activity, but also general information about the individual patient. At lesion level, the importance of early detection and preventive intervention before the stage of irreversible damage is now generally acknowledged [Pitts, 2004].

Caries diagnosis has changed from an exercise in detection of manifest caries, to be treated restoratively, towards identification of the early lesion and staging of the lesion, as a part of caries assessment and diagnosis for the individual patient [Baelum *et al.*, 2006]. Methods used for caries detection have thus progressed from recording irreversibly decayed teeth in a population of high caries prevalence, towards the more demanding identification of early lesions in a population with low prevalence and slowly progressing disease.

Caries detection methods are themselves somewhat uncertain, as absolute dif-

ferentiation between diseased and sound tissue is not possible [Kidd *et al.*, 2003]. As the severity of the disease decreases and detection becomes more difficult, this uncertainty increases. For clinicians to make treatment plans for the individual patient and to determine the most appropriate intervention for each individual lesion, it is important to take into account the level of uncertainty/certainty associated with the detection method being applied.

When evaluating caries detection methods, the efficacy of the method should be measured in terms of its effect on clinical management of the disease at patient level. However, many steps are involved: each "component" of the specific detection method has to be validated.

The efficacy of a caries detection method can be assessed by applying the hierarchical model by Fryback and Thornbury [1991], which has six levels:

Level 1 – technical efficacy: testing the technical properties and quality of the detection method

Level 2 – diagnostic accuracy/efficacy: evaluating diagnostic accuracy of the method, sensitivity and specificity as well as interpretation of the results/images

Level 3 – diagnostic thinking efficacy: investigating the effect of the detection method on the examiner's thinking

Level 4 – therapeutic efficacy: as a continuation of level 3, *i.e.* the change in patient care/treatment decision based on the change in examiner's thinking

Level 5 – patient outcome efficacy: evaluating the improvements in patients' oral health achieved by applying the specific detection method

Level 6 – societal efficacy: measuring the cost benefits of using the detection method from a societal viewpoint.

In the literature on caries-diagnostic methods, there are few published studies in which the main outcome is patient outcome efficacy or societal efficacy [Mialhe *et al.*, 2009; Norlund *et al.*, 2009]. There has been major emphasis on finding the "real truth", in terms of accuracy of the method, in both *in vitro* and *in vivo* studies. Evaluation studies of diagnostic methods therefore generally present the diagnostic accuracy/efficacy of the method in question in terms of sensitivity and specificity,

and reliability/reproducibility.

In the era of evidence based dentistry, the accumulated information on the efficacy of different methods is presented in the form of systematic reviews. The need to systematize study design and presentation of outcome for validation studies of caries detection methods has been addressed in some studies [Bader *et al.*, 2002; SBU, 2007]. Lack of uniformity among earlier published studies has led to difficulty in reaching valid conclusions from the accumulated literature. Several approaches to this issue have been proposed: an instrument for quality assessment of diagnostic studies has been introduced, a tool for standardizing study design [Whiting *et al.*, 2003]; and descriptions of how the outcome of such a study should be presented [Bossuyt *et al.*, 2003]. When planning a study to evaluate diagnostic methods, these tools should be kept in mind, to ensure that the study yields appropriate information.

The procedure of clinical caries detection almost always starts with visual inspection (VI), traditionally using the operative light and dental mirror. The problem of reliability and reproducibility of the VI method has long been recognized [Topping and Pitts, 2009]. VI has in general shown low sensitivity, though with great variation, associated with high specificity and limited reliability [Bader *et al.*, 2002; Ismail, 2004; SBU, 2007]. These systematic reviews concluded that VI provides finite reliability and validity for detection of lesions without severe breakdown at both the D1 and D3 diagnostic thresholds. For approximal caries at the D3 threshold on posterior teeth, it was concluded that the method was not accurate enough for detection of dentin caries, with sensitivity of less than 0.4.

A sharp-ended instrument, the dental probe or explorer, has also traditionally been used to aid visual inspection, in order to increase the sensitivity of the method. Resistance to withdrawal of the probe when pressed into fissures or suspected lesions has been interpreted as a sign of manifest caries. More recently this procedure has been questioned, as it does not seem to increase the sensitivity of VI [Lussi 1991] and may actually be contraindicated due to the risk of iatrogenic damage, causing microfracture of demineralized enamel overlying the early lesion [Ekstrand *et al.*, 1987, Künisch *et al.*, 2007, Yassin 1995].

In order to increase the diagnostic performance of VI, several visual inspection systems have been introduced in recent years [Topping and Pitts, 2009; Ismail,

2004]. These systems are intended to systematize VI in order to make the scoring decisions more objective, thereby increasing the sensitivity, reliability and reproducibility of the method. Studies on these new VI systems have reported improved sensitivity and accuracy [Braga *et al.*, 2009; Ekstrand *et al.*, 1997; Nyvad *et al.*, 1999 and 2003; Topping and Pitts 2009].

Apart from the use of the dental probe, radiographs are the most common aid to VI for caries detection, particularly for approximal surfaces, where visual access is limited. Radiographs have generally shown high specificity, with a wide range of sensitivity for all surfaces [Bader *et al.*, 2002; SBU, 2007; Wenzel, 2004]. On the approximal surfaces, radiographs have been found to detect earlier lesions than VI alone [Bloomendale *et al.*, 2004], but for enamel caries, validity is limited on approximal surfaces and low on occlusal surfaces [Bader *et al.*, 2002; SBU, 2007].

The complex three-dimensional anatomy of the occlusal surfaces complicates the interpretation of the two dimensional images of radiography, making the early enamel lesion difficult to detect on radiographs. However, using VI alone, the enamel overlying a dentin lesion can appear macroscopically intact and progression of the lesion into dentin can thereby go undetected [Ricketts *et al.*, 1997]. Thus, compared to separate use of each method, a combination of VI and radiography shows increased accuracy in detection of D3 occlusal caries lesions [Lussi, 1993]. However, associated with radiographic examination of occlusal dentin caries is decreased specificity, especially in low prevalence samples [SBU, 2007]. Comparison of digital and film radiographs has shown only non-significant differences [Wenzel, 2006, 2004].

Although radiographic examination is widely used in clinical practice, exposure of patients to ionizing radiation is a matter of concern. In the current context of low caries prevalence and slow progression of new lesions [Marthaler *et al.*, 2004; Mejàre *et al.*, 2004], it is suggested that radiographs are no longer routinely required for all patients: adequate selection criteria should be applied to determine when radiographs are indicated [Espelid *et al.*, 2003; Mejare 2005]. Such an approach, however, precludes frequent monitoring of early lesions and the response to interventions intended to reverse or arrest lesion progression.

A further limitation of bitewing radiographs is that they offer only limited information on surface integrity, thereby decreasing their therapeutic efficacy [Pitts

1997]. It was previously accepted that lesions, shown on radiographs to extend through enamel and into the outer half of the dentin, should be treated invasively. This concept is now obsolete. A study by Pitts and Rimmer [1992] showed that out of all lesions radiographically denoted as extending into dentin, 59% of the surfaces of permanent molars and over 70% of the surfaces of deciduous molars were still intact. Thus bitewing radiography can be associated with a relatively high proportion of FP scores [Wenzel, 1995] and in order to contribute to valid treatment decisions, complementary information from other sources is necessary.

The conventional methods, VI and radiography, have been quite extensively evaluated in terms of sensitivity/specificity and reliability/reproducibility. Such evaluations give dichotomous answers at a specific caries detection threshold, for example the D3 diagnostic threshold, disclosing the ability of the method to differentiate between enamel and dentin caries. In the context of the modern approach to caries management, it is obvious that such a test does not give information on the method's ability to assess the lesion on a continuous scale.

The problems in achieving information on the continuum of the caries process are twofold. Firstly, the traditional detection methods, discussed above, give at their best, categorical data and are by nature too crude to provide detailed information on small changes of the order needed for evaluation of the effect of non-invasive intervention. Secondly, the available reference standards used for validation of detection methods, especially in clinical studies, do not offer continuous scale information. This problem has been addressed in a number of studies [Huysmans and Longbottom, 2004; Imrey and Kingman, 2004].

In view of the shortcomings of the conventional methods, particularly for detection of early enamel lesions, for monitoring the effects of preventive interventions, and the possible hazardous effects of aggressive probing and exposure to the ionizing effect of radiographs, a search for alternative methods has resulted in the development of a number of advanced methods for caries detection and monitoring. Two such methods are laser fluorescence (LF) and digital fiber-optic transillumination (DIFOTI). Both are commercially available and have been in clinical use for over a decade. Both methods are based on the interaction of light with the dental hard tissues and changes in the optical properties induced by lesion progression.

The following is a brief review of the basic concepts:

THE INTERACTION OF LIGHT AND THE DENTAL HARD TISSUES

The ways in which light can interact with the dental hard tissues are shown in Fig. 1. The light rays can either be reflected (a) at the tooth surface or travel through the tooth tissues in different ways [Analoui *et al.*, 1996; Vaarkamp *et al.*, 1995]. When passing through the dense structure of enamel and dentin, the light ray is either transmitted through the material (c), or is scattered (b) or absorbed (d,e). If light is transmitted, the light ray maintains its direction through the medium and the material appears transparent. Scattering results as the light rays interact with small particles in the medium, forcing them to change direction. As a result of the scattering, the surface can appear clinically as "chalky": opaque and white. Absorption takes place as the energy of the light photon is taken up by the matter it is traveling through, and is transformed to another kind of energy, for example heat (d). This absorption can also be transformed into light (e), which typically has a longer wavelength than the absorbed radiation. This is called fluorescence. Clinically, absorption appears as a dark shadow. As the light rays travel between two different media, such as air and enamel, the beam bends at the interface. This change of the light rays as they enter the new medium is referred to as the Refractive Index.

Sound enamel is microporous and translucent. As the early lesion forms, the porosity of the subsurface enamel increases, due to demineralization. This leads to a change in optical properties of the enamel and a lower refractive index [Friedman and Marcus, 1970]. This can be observed as a loss of translucency and a change in light refraction, only appearing when the surface is dehydrated. If the lesion progresses, further reduction in the refractive index occurs as the scattering and absorption of the light rays increases in the carious tissue. The opacity of the surface, or even a shadow, is clearly visible without air drying the surface. Thus, to detect initial demineralization, the surface has to be clean and dry, whereas the more advanced lesion is readily visible to the naked eye, even when covered with saliva.

The two methods evaluated in this thesis use these changes in optical properties to differentiate between sound and carious tissue, though in quite different ways. The LF method is based on the observation that when illuminated with a light of a certain wavelength (655nm), absorption and fluorescence in the 700-

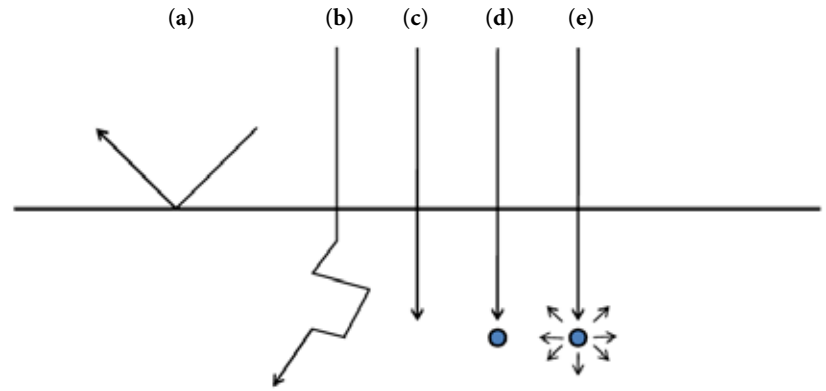

Figure 1. The interaction of light and the dental hard tissues

800nm wavelength region increase with the depth of the caries lesion. The DIFOTI method uses fiber-optic visible light to disclose the difference in refractive indices between sound and carious tissue.

LASER INDUCED FLUORESCENCE

In 1998, Hibst and Gall described the successful use of red light (655nm) to differentiate between sound and carious tissues and on this basis, the DIAGNOdent™ system (KaVo Biberac, Germany) was developed. When using light with an excitation wavelength of 655nm, more intense fluorescence in the 700-800nm wavelength region was observed from a carious lesion compared with a sound spot on enamel [Hibst *et al.*, 2001]. The instrument operates with light from a diode laser that is

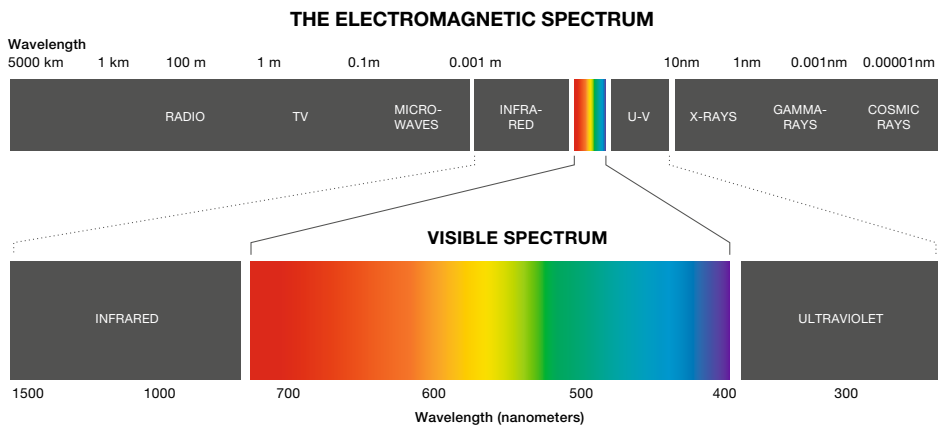

Figure 2. The electromagnetic spectrum

transmitted though a descending optical fiber to a hand-held probe. The probe is placed close to the measured surface, thereby illuminating it with the laser light. The tooth tissues absorb the light, and fluorescence within the near infrared spectra occurs. The emitted fluorescence is collected though the tip, and passed in ascending fibers to a detector where the signal is processed and finally presented on a display, as a number between 0-99. Thus, theoretically the instrument gives quantitative information on the caries lesion, thereby allowing quantitative caries monitoring.

It has been speculated that the difference in fluorescence between sound and carious tissue is attributable to the bacterial content of the lesion and not to demineralization. König *et al.*, [1998] described the similarity between the fluorescence spectrum from bacterial porphyrins and from carious lesions. Other studies have since confirmed these findings [Buchalla, 2005; Hibst *et al.*, 2001]. Porphyrins, produced by bacterial metabolism, are therefore thought to be responsible for the increase in DIAGNOdent readings accompanying carious lesion formation. Thus the back-scattered fluorescence is thought to be proportional to the severity of bacterial infection and lesion depth.

Because of the possible bacterial origin of the fluorescence measured with the LF method, new areas of application for the instrument have been discussed. The possibility of assessing caries activity has been suggested [Ribeiro *et al.*, 2005], as well as using the instrument during cavity preparation, to verify that all infected tooth substance has been removed prior to restoration [Lennon *et al.*, 2002].

Photo by Sofia Tranæus

Photo by Alfheiður Ástvaldsdóttir

Figure 3
a) The DIAGNOdent instrument; lesion depth is displayed as digits
b) Taking a DIAGNOdent reading on a clean, air-dried surface

The instrument has been quite extensively investigated, both *in vitro* and *in vivo* [Abalos *et al.*, 2009; Akarsu and Koprulu, 2006; Angnes *et al.*, 2005; Bader and Shugars, 2004; Burin *et al.*, 2005; Chu *et al.*, 2009; Khalife *et al.*, 2009; Lussi *et al.*, 2004; Neuhaus *et al.*, 2010; Reis *et al.*, 2006; Ricketts, 2005; SBU, 2007]. Published studies have generally shown that the LF method is more sensitive for occlusal caries detection than the conventional methods, but that specificity is inferior to clinical visual examination.

Some studies have compared the bacterial content of lesions with DIAGNOdent measurements [Iwami *et al.*, 2004; Iwami *et al.*, 2010] but the origin of the fluorescence signal has not been clarified. The ability of the oral microflora to emit red fluorescence in response to different excitation wavelengths has been described [Buchalla, 2004, 2005]. Cultures of single species of caries-associated bacteria were reported to emit red fluorescence at an excitation wavelength of 405 nm (± 20nm) [Lennon *et al.*, 2006]. Other studies have, however, concluded that some interaction between bacterial species and their surroundings is needed so that red fluorescence can be measured [Shigetani *et al.*, 2008; Coulthwaite *et al.*, 2006]. There are very few studies comparing the response of cultures of different bacterial species to the laser light of the DIAGNOdent instrument. Thus the origin of the fluorescence on which the LF method is based has yet to be determined.

DIGITAL IMAGING FIBER-OPTIC TRANSILLUMINATION

DIFOTI is a refinement of its predecessor, Fiber-Optic Transillumination (FOTI). The FOTI instrument uses an intense light source which, through a descending optical fiber and a fine probe, is placed on one side of the tooth, allowing the light transmission to be observed from the other side or from the occlusal aspect. This induces the differences in light scattering and absorption between sound and carious tissue, thereby making the carious lesion appear as a dark shadow on a light background. The method has been applied as an aid to visual examination, but yields qualitative information that is non-reproducible and needs subjective interpretation. The major advantage of the method is that in contrast to exposure to ionising radiation of radiography, it is non-invasive.

Studies on the FOTI method have shown somewhat contradictory results [Neuhaus *et al.*, 2009]. The instrument has been validated for approximal caries, with

some studies reporting sensitivity for dentin lesions to be significantly lower than for bitewing radiographs [Vaarkamp *et al.*, 2000], while in others FOTI has shown similar or even superior performance [Peers *et al.*, 1993]. In a validity study on occlusal caries detection, the method has shown good specificity but quite low sensitivity [Grossman *et al.*, 2002]. The combination of visual inspection and FOTI has been found to increase diagnostic accuracy compared to using the FOTI method alone [Côrtes *et al.*, 2003]. It has, however, been pointed out that in order to achieve good sensitivity, substantial training is essential [Peers *et al.*, 1993].

The DIFOTI system was designed to overcome the limitations of FOTI by providing digital image capture and analysis. Such images can be stored in digitized form and compared with previously acquired images. The DIFOTI system therefore offers a semi-quantitative method for detecting and monitoring change over time in early lesions.

To date the method has not undergone extensive investigation. It has been applied in only a few *in vitro* studies, with somewhat conflicting results. Schneiderman *et al.*, [1997] reported that compared to conventional film radiography, DIFOTI had greater sensitivity for detection of approximal and occlusal caries lesions. However, in a study on artificial caries lesions, Young and Featherstone [2005] reported that DIFOTI was more sensitive than film radiography to initial surface changes, but failed to yield accurate quantitative information.

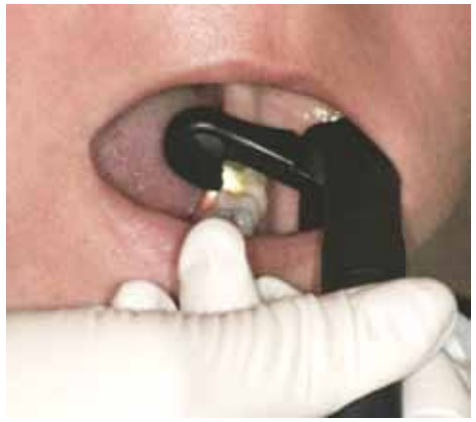

Photo by Áfheiður Ástvaldsdóttir

Figure 4
a) A monitor displaying a DIFOTI occlusal view image of a molar tooth
b) Clinical use of the DIFOTI approximal handpiece

The only clinical study on the diagnostic accuracy of DIFOTI concluded that sensitivity could be increased by use in conjunction with digital or film radiography [Bin-Shuwaish *et al.*, 2008]. Further studies are therefore warranted to validate the method.

The above overview highlights the importance of critical evaluation of new methods for detecting early caries lesions. Study design should comply with the stringent standards applied in systematic reviews.

The outcome of validation tests of a caries detection method has important clinical implications: only after thorough validation can the clinician apply the method with confidence, with a clear understanding of what is being measured, the limitations of the method and informed, appropriate interpretation of the evidence recorded with respect to clinical decision-making in the individual patient, such as the data at the cut-off points used to differentiate between lesions requiring invasive and non-invasive intervention.

Validation of caries detection methods should therefore include studies which clarify what characteristics of the caries lesions are being measured, the limitations of the method and comparison of performance with conventional caries detection methods.

Moreover, the efficacy of the method should be evaluated in the context of its effect on clinical management of the disease at patient level. Technical efficacy and diagnostic accuracy/efficacy can be extrapolated in order to predict the potential effect on patient outcome and societal efficacy, *i.e.* the cost benefits, at community level, of using the detection method. Thus evaluation of technical efficacy and diagnostic accuracy/efficacy in comparison with conventional methods of caries detection is also of fundamental importance.

GENERAL AIM

The general aim of this thesis was to evaluate the characteristics, technical efficacy and diagnostic accuracy/efficacy of two advanced methods for caries detection, laser-induced fluorescence (LF) and digital imaging fiber-optic transillumination (DIFOTI), with special reference to implications for clinical decision-making at the threshold between non-invasive or invasive management of the lesion.

SPECIFIC AIMS

- The aims of **Paper I** were: firstly, to evaluate the LF method for detection of occlusal caries *in vivo* and compare it with visual examination and bitewing radiographs, and to evaluate the consistency of data recorded under clinical conditions by four different LF instruments; secondly, to correlate LF readings with the microbial flora cultured from the measured site.

- The aims of **Paper II** were to measure the fluorescence of the normal cultivable caries-associated bacterial flora and typical porphyrin-producing bacteria with the LF method and to investigate the effect of demineralization of enamel on LF readings.

- The aim of **Paper III** was to compare the diagnostic accuracy/efficacy *in vitro* of DIFOTI and conventional digital and film radiography in detection of approximal caries at two different diagnostic thresholds, using histology and microradiography as standard references.

- The aim of **Paper IV** was to test the *in vitro* diagnostic accuracy/efficacy of DIFOTI for detection of occlusal caries lesions at diagnostic thresholds for enamel and dentin caries, compared with conventional visual inspection and film and digital radiography.

Material and methods

The studies presented in **Papers I** and **II** were approved by the Ethics Committee, Huddinge Hospital (402/02), Stockholm, Sweden and the National Bioethics Committee in Iceland (02-151-S2). The studies presented in **Papers III** and **IV** involved biological material which could not be traced to an individual donor: the regional Ethics Committee in Stockholm, Sweden determined that these studies were not subject to the law of ethical approval (2006/3:4).

A brief summary of the studies is presented below. The papers are reproduced in full in the appendix to this thesis.

PAPER I

Subjects

Thirty-four occlusal lesions were examined in subjects aged between 18 and 30 years. The lesions were scheduled for restorative treatment at the Dental School Clinic, University of Iceland.

Visual inspection and radiographic examination

Two examiners evaluated all surfaces visually, with gentle probing, and on bitewing radiographs. Lesions were scored according to following criteria:

Scores for visual examination according to modified Ekstrand criteria [Ekstrand *et al.*, 1987].

0 = Little or no change in enamel translucency after prolonged air drying (>5 sec)

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

2 = Localized enamel breakdown in opaque or discoloured enamel and/or grayish discoloration from the underlying dentin

Scores for radiographic examination

0 = No radiolucency visible

1 = Radiolucency visible in the enamel

2 = Radiolucency visible in the outer dentin, just beyond the DEJ

3 = Radiolucency visible in the inner dentin, clearly beyond the DEJ

LF measurements

One examiner carried out all LF measurements, and another person recorded the LF readings. Before measuring each subject, LF instruments were calibrated on the ceramic standard provided and the standard value for each tooth was determined by measuring a sound spot.

Each site was measured using four LF instruments in random order. To test intra-observer agreement, each instrument was used twice. The site was washed and air dried between measurements in order to standardize the humidity.

Bacterial samples

Bacterial samples were collected from all surfaces. The initial sample was collected after application of rubber dam and thorough cleaning of the surface, before any invasive treatment. The surface was dried with compressed air and a sterile paper point was inserted into the lesion. The second sample was collected after opening of the fissures, where the opening was big enough to fit a no.10 round bur. The samples were collected by placing a sterile round bur directly beneath the DEJ. Each sample was placed in 1mL reduced transport fluid for transport to the laboratory and inoculated onto four different culture media: blood agar for determination of the total cultivable count, Rogosa agar for lactobacilli, Mitis-salivarius-bacitracin agar (MSB) for *S. mutans* and Veilonella agar (Difco) for *Veilonella sp.*

Validation of lesion depth

After visual and radiographic examination, DIAGNOdent measurements and collection of the first bacterial sample, the fissures were opened with a bur of appropriate size. After excavation of all carious tissue, the true depth of the caries lesion was determined by two independent examiners, according to the following criteria:

0 = Sound

1 = Caries in enamel

2 = Shallow dentin caries, caries extending just beyond the DEJ

3 = Deep dentin caries, caries extending clearly beyond the DEJ

PAPER II

Bacterial samples

Bacterial samples were collected from five occlusal caries lesions, all with dentin involvement, verified by visual and radiographic examination and by LF readings >20. Samples of carious dentin were collected and handled in the same manner as described in **Paper I.** Further analysis was then carried out in the laboratory.

The study design is shown in the flowchart in figure 5 on page 26.

LF measurements

All LF measurements were recorded immediately after an appropriate incubation time for each culture. The LF instrument was first calibrated against a ceramic standard. The standard value for the underlying material was determined by measuring colony-free parts of the agar and glass slides. The tip was then placed as close to the colony as possible without direct contact. Two readings were taken for each colony. All bacterial species were measured directly on agar plates. Lactobacilli and mutans streptococci were also measured after transferring colonies to glass slides. The one MSB agar plate containing cultures from all samples was used to measure colonies of mutans streptococci after incubation for 24, 48 and 72h.

Teeth

The material comprised 15 visually sound premolar teeth, extracted on orthodontic indications, from young adolescents.

The tooth surface was rinsed and air-dried for 5s. The instrument was calibrated and baseline measurements were recorded. All teeth were then embedded in wax (Tenax wax; S.S. White materials, Prima Dental Group, Gloucester UK), leaving only a small window of accessible enamel surface for demineralization. The teeth were immersed in a demineralization solution for 2 weeks, the wax removed and an LF reading was taken at the demineralized spot. QLF images were captured at baseline and after demineralization to verify artificial lesion formation. All QLF images were analysed by one operator using Inspektor™ Pro intra-oral fluorescence system (Inspektor Dental Care Research and Development, Amsterdam, The Netherlands).

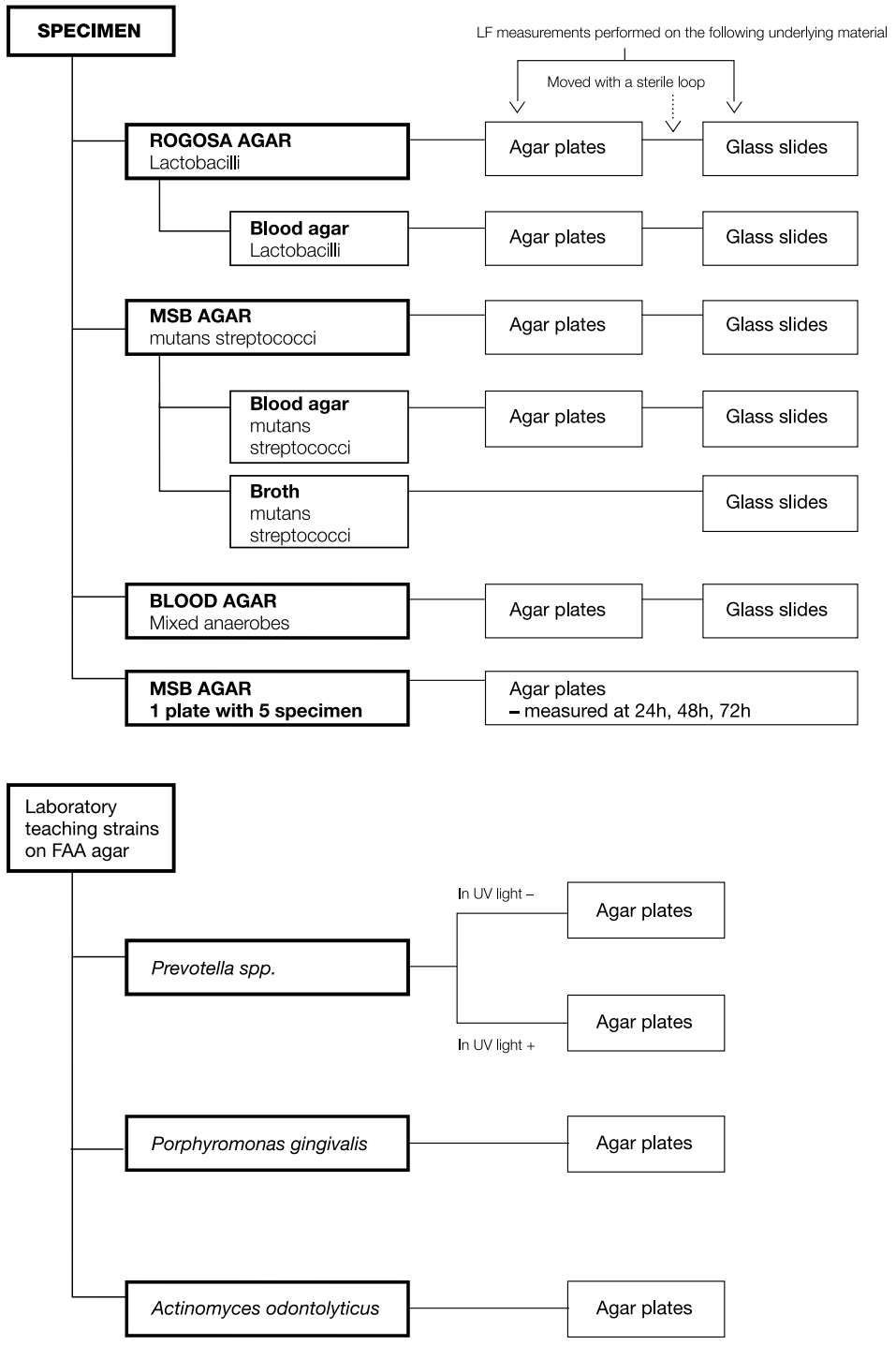

Figure 5. Study design for Paper II

PAPER III

Teeth

The material comprised 56 premolar teeth, extracted on orthodontic indications. The approximal surfaces of the selected teeth presented a range of conditions, from sound to non-cavitated and cavitated caries lesions.

Observers and examination

Eight observers examined all three sets of images twice, DIFOTI images, film and digital radiographs, scoring according to following criteria

0 = no caries

1 = caries lesion extending to outer half of the enamel

2 = caries lesion extending to inner half of the enamel

3 = caries lesion extending to outer half of the dentin

4 = caries lesion extending to inner half of the dentin

DIFOTI images and digital radiographs were viewed on a 15 inch monitor (Hewlett Packard L1520). Film radiographs were viewed on a table with a light source and a Mathsson binocular with 2 fold magnification.

Conditions were strictly standardized with respect to position of examiner, brightness and contrast of the monitor and light conditions in the room.

Before the DIFOTI examination, the observers underwent a 15 minute training session to become familiar with the method. No further calibration of the observers was undertaken.

DIFOTI images

Three images were captured for each surface: buccal, lingual and occlusal views. A trained operator captured the images under standardized darkroom conditions.

Radiography

A specially designed holder was used for both film and digital radiography, to standardize the projection geometry.

Film radiography:

Planmeca intraoral radiographic equipment (Planmeca, Helsinki, Finland) and

Kodak Ektaspeed films were used, with settings of 70kV and 7mA and an exposure time of 0.25s.

Digital radiography

Focus intraoral radiographic equipment, comprising a Sigma direct digital sensor and Cliniview software (Instrumentarium, Tuusula, Finland) was used. The settings were 60kV and 7mA, with an exposure time of 20s. The images were manipulated in order to standardize brightness and contrast, using ImageJ software (ImageJ, U.S. National Institutes of Health, Bethesda, Maryland, USA).

Histological validation

The teeth were sectioned, perpendicular to the enamel, into approximately 300μm thick sections. The sections were then exposed to Ni-filtered Cu Kα radiation at 20kV and 20mA, with an exposure time of 2h, using Kodak high speed holographic film SO 253. Three operators independently examined both microradiograms and tooth sections under a stereomicroscope, at a magnification factor of 16. Lesion depths were scored according to the following criteria

0 = no caries
1 = demineralization extending to outer half of the enamel
2 = demineralization extending to inner half of the enamel
3 = demineralization extending to outer half of the dentin
4 = demineralization extending to the inner half of the dentin.

PAPER IV

Teeth

The material comprised 80 premolar and molar teeth, extracted on orthodontic indications. The occlusal surfaces of the selected teeth presented a range of conditions, from sound to non-cavitated and cavitated caries lesions.

Study design

The design of this study was very similar to that described for **Paper III,** with the following differences.

Two DIFOTI images were captured for each surface: buccal and lingual views. For digital radiography, the exposure time ranged between 0.20-0.25s.

Visual inspection

The eight observers examined the occlusal surfaces visually and recorded a score on a scale of 0-4, as described for the DIFOTI, film and digital radiographic examinations in **Paper III**. The examination was made after approximately 5s air drying of teeth, under excellent illumination, with direct visualization, but without any magnification.

STATISTICAL ANALYSIS

In **Paper I**, Spearman's rank order correlation coefficient (r) was used to test intra-operator agreement and correlation of the different diagnostic methods with the reference standard. False positives and false negatives were calculated as percentages.

Bacteriological variables were ranked and Spearman's rank order correlation coefficient used to test the correlation between bacteriological variables and other diagnostic variables.

For greater clarity, a dissimilarity matrix was constructed to "map" the variables in a few dimensions. To do this, a hierarchical cluster analysis as well as multidimensional scaling was carried out.

In **Paper II** Wilcoxon Matched Pairs Test was used to analyse the effect of demineralization on LF readings and QLF measurements. Friedman's ANOVA and Kendall's coefficient of concordance were used to test the effect of maturation of mutans streptococci on LF readings. LF readings of different bacterial species were presented as medians, with minimum and maximum values.

In **Papers III** and **IV** Weighted kappa (K_w) was used to test intra- and inter-observer agreement for all methods. For inter-observer agreement, a mean value for each observer for each method was calculated and minimum and maximum values specified. Spearman's rank order correlation coefficient was used to correlate the

results of different detection methods with the reference standard.

Area under the receiver operating characteristic curve (AUC) was calculated to test the diagnostic accuracy of the different detection methods for each observer. Two cut-off levels, based on histology, were used for calculations of sensitivity, specificity and AUC. Cut-off 1 represents the D1 threshold: sound versus all caries lesions. Cut-off 2 represents the D3 threshold: sound and enamel caries lesions versus dentin caries lesions. Wilcoxon Matched Pairs Test was used to compare diagnostic performance of different methods.

The level of statistical significance was set at $p<0.05$.

Results

VALIDATION

LF method

In **Paper I**, three of the four LF instruments showed weak but statistically significant correlations with the reference standard ($r = 0.28$-0.51). Weak but significant correlations were also found for bite-wing radiographs ($r = 0.38$) and visual inspection ($r = 0.43$). The cut-off thresholds differed for the different LF instruments. However, if the cut-off threshold was adjusted for each instrument, the method showed good diagnostic accuracy/efficacy, in the form of low false positive/true positive fractions for dentin lesions.

DIFOTI method

Paper III revealed good correlation between the DIFOTI method and the reference standard ($r = 0.77$, $p < 0.001$). Both film and digital radiographs showed weaker but statistically significant correlations with the reference standard ($r = 0.45$, $p < 0.001$ and $r = 0.54$, $p < 0.001$, respectively). At diagnostic threshold D1, the DIFOTI method showed significantly better diagnostic accuracy, expressed as AUC (0.71-0.87) and sensitivity (0.46-0.89), than either type of radiograph. At diagnostic threshold D3, all methods showed similar performance. Specificity was good for all methods (spec = 0.82-1.0), but was significantly better for radiographs than for DIFOTI.

In **Paper IV**, DIFOTI showed good correlation with the reference standard ($r = 0.62$, $p < 0.001$). Correlation was weaker for both film and digital radiographs ($r = 0.37$, $p < 0.001$ and $r = 0.46$, $p < 0.001$, respectively), but similar for VI ($r = 0.63$, $p < 0.001$). The diagnostic accuracy, expressed as AUC, was significantly better for the DIFOTI method than for either type of radiograph at both D1 (AUC = 0.63-0.72) and D3 diagnostic thresholds (AUC = 0.73-0.89), but similar to visual inspection. For all methods, there was pronounced variance between observers with respect to sensitivity and specificity.

RELIABILITY

The LF method showed good reliability (**Paper I**) with respect to both intra-ob-

server and inter-instrument correlation (r = 0.85-0.98 and 0.81-0.92, respectively). For approximal surfaces (**Paper III**), the DIFOTI method showed good to very good reliability with respect to both intra-observer and inter-observer agreement (K_w = 0.74-0.94 and 0.62-0.76, respectively). Good reliability was also achieved for both types of radiograph. For occlusal surfaces (**Paper IV**), the DIFOTI method showed good reliability with respect to intra-observer agreement (mean K_w = 0.67), comparable with film and digital radiographs, but inferior to VI (mean K_w = 0.79). For all methods there was a wide range of mean inter-observer agreement.

CHARACTERISTICS OF THE LF METHOD

Of all the bacterial samples (**Paper I**), only lactobacillus counts from the first sample showed a statistically significant correlation with the reference standard (r = 0.41). The correlation between bacterial counts and the LF readings was non-significant. In **Paper II**, LF readings >20 were recorded only for mutans streptococci cultured on mitis-salivarius-bacitracin agar (MSB) and young colonies of *Prevotella spp*. The readings increased as the colonies of mutans streptococci aged and decreased when colonies were transferred from the surrounding agar to glass slides. The LF readings did not change following demineralization of the enamel.

Discussion

This thesis is based on a series of studies evaluating two advanced optical caries detection methods, LF and DIFOTI. The methods provide quantitative and semi-quantitative data intended to support clinical decision-making, such as whether a lesion should be treated invasively or non-invasively, or to confirm that non-invasive intervention has effectively arrested or reversed lesion progression.

Lack of uniformity of study design precludes direct comparison with the results of earlier studies. While it is recognized that both methods have limitations and disadvantages with respect to detection and measurement of fissure or smooth surface caries, neither method can be considered to have undergone thorough validation, even though they have been commercially available for a decade.

The outcome of validation tests of a caries detection method has important clinical implications: only after thorough validation can the clinician confidently interpret the evidence recorded with respect to clinical decision-making in the individual patient, such as the data at the cut-off points used to differentiate between lesions requiring invasive and non-invasive intervention.

It is also of fundamental importance to evaluate the efficacy of the methods, in terms of their influence on clinical management of the disease at patient level: data on technical efficacy and diagnostic accuracy/efficacy can be extrapolated to predict the potential efficacy at patient outcome and societal levels.

In **Paper I** the diagnostic accuracy/efficacy of the LF method was tested with respect to occlusal caries detection *in vivo*. **Paper II** presents a study on the technical efficacy of the LF, an attempt to analyse further the ability of the LF method to measure two major characteristics of the pathology of dental caries, cultivable cariogenic bacteria and demineralised enamel. In **Papers III** and **IV** the diagnostic accuracy/efficacy of the DIFOTI method was tested with respect to *in vitro* caries detection on both approximal and occlusal surfaces.

The results of these studies lead to the conclusion that both methods warrant further investigation.

Dentin involvement, *i.e.* lesion extension through the DEJ (diagnostic threshold D3), is generally recognized as a sign of manifest caries. However, according to the modern concept of caries management, this does not necessitate invasive interven-

tion and restorative treatment [Pitts, 2004]. Such a treatment decision should be based on more extensive diagnostic criteria, including data on surface integrity and/or lesion activity. In validating a detection method, it is of primary importance to determine the nature of the information provided by the specific method, *i.e.* to identify what characteristics of the caries process are being recorded.

With respect to the LF method, the information gained from the instrument is quantitative, or numerical, and according to the manufacturer's recommendations, readings above certain cut-off thresholds signify dentin involvement. Early studies reported that illumination with the 655nm wavelength laser light from the LF instrument resulted in emission of more intense fluorescence by carious tissue than by sound tooth structure [Hibst *et al.*, 2001]. Using spectroscopy, König *et al.*, [1998], described the similarity between the fluorescence spectrum of bacterial porphyrins and carious lesions. It was therefore hypothesised that the fluorescence of carious tissue was attributable to the bacterial content of the lesion and not to demineralization. This has, however, not been further investigated.

The initial study in this series, as presented in **Paper I**, was therefore designed to test the correlation between LF readings of carious lesions and caries-associated bacteria cultivated from the lesions. The correlation was non-significant ($r = 0.054$-0.38), meaning that increased counts of the bacterial species tested in the study did not result in increased LF readings.

This was further investigated in the second study, presented as **Paper II**, which tested the ability of the LF instrument to measure fluorescence emitted from bacterial cultures of species known to be associated with the caries process, such as *Lactobacilli*, mutans streptococci and *Actinomyces* and mixed anaerobic cultures. Typical porphyrin-producing bacteria such as *Prevotella spp.* and *P. gingivalis* were also tested. None of the bacterial species gave high LF readings, except for young colonies of *Prevotella spp.* and mutans streptococci on MSB agar. In contrast, mutans streptococcal cultures on blood agar did not give LF readings above 0. The results were therefore quite inconsistent and it was concluded that the LF instrument probably measures the synergistic effect of the caries process, rather than any specific pathological features of the lesion. LF readings should therefore be interpreted with considerable caution and further data about surface integrity and lesion activity should be collected before treatment decisions are made.

The DIFOTI method provides data in the form of digital images. Interpretation of these images can provide semi-quantitative information and then be stored for future reference. Subjective interpretation by the examiner, therefore, probably influences the outcome of the method, as has been described for radiographs [Mileman and van den Hout, 2002; Souza-Zaroni *et al.*, 2006]. Because it is an optical method, the DIFOTI images may provide more information about surface integrity than is available on radiographs. This applies especially to occlusal surfaces, where visual access is better. In **Papers III** and **IV**, the studies tested only the ability of examiners to estimate lesion extension within the tooth from DIFOTI images. It is important to note that the examiners were not required to make judgements on cavitation. This aspect therefore needs further evaluation before any firm conclusions can be drawn.

It is generally accepted that the earlier the stage at which a lesion is detected, the greater the potential for arrest or reversal of the caries process by non-invasive intervention. The primary function of caries detection methods is thus to enhance clinical diagnosis by indicating the presence of very early stages of the disease.

However, concern has been expressed that early detection may increase the risk of over-diagnosis and even overaggressive restorative treatment [Baelum, 2010]. Therefore, when evaluating the diagnostic accuracy of a detection method, it is important to apply clearly defined endpoints or thresholds. The diagnostic threshold which leads to a change in treatment, from non-invasive treatment to invasive intervention, can be regarded as the main threshold. As pointed out earlier, however, a treatment decision based on lesion severity requires detailed caries diagnosis [Pitts, 2004].

In the present studies of the diagnostic accuracy/efficacy of the LF and DIFOTI methods, only the outcome was considered *i.e.* the studies did not include treatment decisions based on the outcome. The evaluation therefore concerns quantitative aspects of these methods, their ability to differentiate between a sound surface and an initial caries lesion, the D1 threshold, and between the initial lesion (confined to the enamel), and the more advanced dentin lesion, the D3 threshold. Thus, the D3 threshold is regarded as the main endpoint in these studies, whereas the D1 threshold is a surrogate endpoint. These endpoints give an indication of the potential of these methods to influence treatment decisions, but no information on the thera-

peutic efficacy.

Both the LF and DIFOTI methods were evaluated with respect to occlusal caries detection and DIFOTI was evaluated for approximal caries detection. Both the occlusal and approximal surfaces are regarded as predilection sites for caries, but the pronounced differences in dental anatomy influence detection of caries lesions. A different approach is necessary for interpretation of results for detection of fissure caries, on the occlusal surfaces, and those for smooth surface caries on the approximal surfaces.

The conventional methods for occlusal caries detection are radiographs and VI. Good visual access has led to adoption, with some reservations, of VI as the standard method. Radiographs are of limited use for occlusal caries detection, especially enamel caries, due to the complex three- dimensional anatomy of these surfaces [Ekstrand *et al.*, 1997, 2001; Espelid and Tveit, 2001; Pooterman *et al.*, 2000] and are usually regarded as an aid to VI. However, VI has generally shown low sensitivity and high specificity [Bader *et al.*, 2002].

In this context, VI and radiography are often used as standard methods for evaluating new methods for occlusal caries detection. Thus in general terms, validation of a new detection method requires diagnostic accuracy/efficacy as good as or better than the conventional methods; or the new method should provide additional information of some kind, needed to make valid treatment decisions.

For approximal caries detection in posterior teeth, radiography has been the standard method. Visual access to these surfaces is usually restricted, limiting the application of VI. At the D3 diagnostic threshold, the diagnostic accuracy/efficacy of radiographs is generally moderate, but better than VI [Bader *et al.*, 2002] The two major disadvantages of radiographs, however, are exposure to ionizing radiation and the limited information on surface integrity, which thereby detracts from their therapeutic efficacy. A requirement of new methods for approximal caries detection should therefore be diagnostic accuracy/efficacy equal to or better than that of radiographs.

Thus **Papers I** (LF method) and **IV** (DIFOTI) evaluated new methods for occlusal caries detection while **Paper III** evaluated the DIFOTI method for approximal caries detection.

In **Paper I** the diagnostic accuracy of the LF method for occlusal caries detection and quantification was tested in terms of correlation with actual lesion depth, *i.e.* the reference standard, and its ability to differentiate between enamel and dentin caries on occlusal surfaces (D3 diagnostic threshold/cut off threshold). For three out of four instruments tested in this study, the method showed weak but significant correlation with the reference standard ($r = 0.42$-0.51, $p < 0.05$), indicating that LF does not meet the requirements of a quantitative method. Correlation with the reference standard was similar to that for bite-wing radiographs ($r = 0.38$, $p = 0.026$) and VI ($r = 0.43$, $p = 0.01$). Thus, LF failed to demonstrate quantitative qualities superior to conventional detection methods.

Earlier published studies on the performance of the LF method have shown wide-ranging results. In the earliest *in vitro* studies, excellent correlation was reported for LF readings and actual lesion depth [Shi *et al.*, 2000; Lussi *et al.*, 1999]. The method showed high sensitivity ($r = 0.79$-0.82) and good specificity ($r = 0.84$-1.0). However, the results of *in vivo* studies were inconsistent. Under clinical conditions, it is difficult to control such factors as humidity of the dental hard tissue, dental plaque and staining of various kinds are difficult to control, especially on occlusal surfaces. It is recognised that these factors influence the fluorescence detected by the LF instrument [Francescut and Lussi, 2003; Lussi and Reich, 2005]. Thus the LF method has shown a greater range and generally poorer results *in vivo* than *in vitro* [Bader and Shugars, 2004; SBU, 2007]. The weak correlation disclosed in **Paper I** is therefore consistent with earlier results. The diagnostic accuracy of the LF method at D3 cut-off threshold was tested by calculating the percentage of false positive/true positive diagnoses at one given cut-off threshold. In an *in vivo* study, such as that presented in **Paper I**, surfaces denoted as sound by BW and VI cannot be confirmed by invasive means, for ethical reasons, and therefore no validation is possible. If TN data are not validated, FN observations are automatically included in the TN group and give erroneous results. Sensitivity and specificity were therefore not calculated: diagnostic accuracy was tested in the form of the percentage of false positive/true positive diagnoses at diagnostic threshold D3, using only validated surfaces.

An attempt was made to find the most accurate cut-off threshold for each instrument. Each device appeared to have an individual cut-off level and thus no absolute cut-off threshold could be determined for the method. Such inter-device inconsist-

encies were reported in a study by Tranæus *et al.*, [2004]. However, when different cut-off thresholds were used for each instrument, good specificity was observed (*i.e.* few FP recordings). The ability of the LF method to differentiate between dentin caries and enamel caries on occlusal surfaces was good, if the right cut-off threshold was used. This is of major importance in clinical application of the method, as the diagnostic threshold D3 represents the main endpoint in clinical diagnosis and thereby gives an indication of the method's ability to support decisions regarding selection of an invasive or non-invasive treatment approach.

Comparison of the present results with those of earlier studies is complicated by differences in study design, methodology and presentation of the results. Factors such as the number of surfaces included, the prevalence of dentin/enamel caries within the material, the reference standard used for validation, the LF threshold used for statistical analysis and statistical methods used for analysing results, can all influence not only the outcome, but also the interpretation of results. The percentage of FP/TP diagnosis presented in **Paper I**, is therefore not directly comparable with sensitivity/specificity results from earlier studies.

In **Paper IV**, the diagnostic accuracy of the DIFOTI method for occlusal caries detection and quantification was analysed in a manner similar to that applied to the LF method in **Paper I**, though on *in vitro* material. Correlation with actual lesion depth, *i.e.* the reference standard, and diagnostic accuracy at D1 and D3 diagnostic thresholds were tested. All surfaces included in the study could be validated histologically, allowing interpretation of the data in terms of sensitivity, specificity and AUC, at both diagnostic thresholds.

The DIFOTI method showed better correlation with the reference standard than digital or film radiography, but similar correlation as VI. Earlier studies have shown that radiographs are of limited value for detection of occlusal caries, especially enamel caries [Bader *et al.*, 2002; SBU, 2007]. VI has shown better results than radiographs but has, however, in general shown low sensitivity [SBU, 2007; Ismail, 2004; Bader *et al.*, 2002.]. The similar quantitative properties of VI and DIFOTI, revealed in **Paper IV**, therefore detract somewhat from the value of DIFOTI for quantification of occlusal caries lesions. There are no published studies correlating quantitative results from the DIFOTI method with the actual depth of occlusal caries lesions.

In **Paper IV**, testing diagnostic accuracy for occlusal caries, the DIFOTI value for AUC was significantly better than digital or film radiography at both D1 (AUC = 0.63-0.72) and D3 diagnostic thresholds (AUC = 0.73-0.89), but comparable with VI. Similar results have been reported previously at the diagnostic threshold D3 [Schneiderman *et al.*, 1997]. However, there are no published studies directly comparing DIFOTI and VI.

At the D1 threshold, sensitivity and specificity values for all methods showed pronounced inter-observer variation. These results highlight the problems associated with occlusal enamel caries detection. As mentioned earlier, factors such as caries prevalence in the material can influence the outcome. The high proportion of enamel caries in the present material might therefore have complicated caries detection and contributed to the very wide inter-observer range in outcomes for all methods.

Observer skills and experience influence caries detection outcomes and treatment decisions [Espelid 1987; Mileman and van den Hout 2002; Souza-Zaroni *et al.*, 2006]. The eight observers in this study were from diverse backgrounds and were not calibrated before study start. The outcome is therefore more likely to reflect the effectiveness of the detection methods under field conditions.

At diagnostic threshold D3, the results for sensitivity/specificity were more consistent. For all methods, specificity was good and differences in sensitivity between methods were not statistically significant. In general, however, the sensitivity was quite low for all methods. Thus sound surfaces tended to be correctly determined, but dentin caries lesions tended to be misreported as sound surfaces. These results indicate that with respect to occlusal caries, the DIFOTI method does not enhance sensitivity at diagnostic threshold D3.

It is important to note that the detection methods investigated in **Papers III** and **IV** were tested independently. Thus the results do not reveal any possible synergistic effect of using both methods to aid lesion detection. A combination of methods might give better results than individual application. In earlier studies, different combinations of detection methods have resulted in higher sensitivity than when each method is used individually [Souza-Zaroni *et al.*, 2006, Valera *et al.*, 2008]. Other authors have, however, expressed concern about decreased therapeutic ef-

ficacy (increased risk of treating enamel caries invasively) when treatment decisions are based the on results of mutiple detection methods [Pereira *et al.*, 2009].

In this context, the high specificity of DIFOTI at diagnostic threshold D3 for occlusal caries is an important advantage, as it decreases the risk associated with high numbers of FP surfaces at the D3 threshold.

In **Paper III** approximal caries detection by the DIFOTI method was evaluated. The results were analysed as described above for **Paper IV**. Correlation with actual lesion depth, *i.e.* the reference standard, and diagnostic accuracy/efficacy at diagnostic thresholds D1 and D3 were tested. The DIFOTI method showed high correlation with the reference standard ($r = 0.77$) Correlation with digital and film radiographs was weaker ($r = 0.45$ and $r = 0.54$, respectively). Thus the DIFOTI method showed good quantitative properties with respect to approximal caries detection. In contrast, Young and Featherstone [2005] reported that the DIFOTI method was unable to predict the actual lesion depth of approximal caries lesions and performance was inferior to film radiographs.

At diagnostic threshold D1, tests of the diagnostic accuracy of the DIFOTI method showed significantly greater AUC than digital and film radiographs, significantly better sensitivity than the other methods and similar specificity. Thus compared to radiography, the method seems to be able to identify lesions at an earlier stage, without increasing the number of sound surfaces incorrectly identified as carious. Previous studies have reported low sensitivity for radiographs in detecting initial lesions [White and Yoon, 1997]. The DIFOTI method has also been shown to be more sensitive than radiographs in detecting early changes in enamel [Young and Featherstone, 2005].

These results therefore suggest that at the D1 threshold, DIFOTI images provide more accurate information than radiographs. In the context of the current concept of caries management, with emphasis on early lesion detection and non-invasive treatment intervention, these properties are of great importance. A DIFOTI image from baseline measurement can be stored and retrieved for later reference, in order to validate the outcome of noninvasive intervention intended to arrest or reverse lesion progression.

At the D3 threshold, the differences in performance between DIFOTI and radiography were less pronounced. All methods showed good diagnostic accuracy in terms of AUC (0.65-0.98), and the differences were non-significant. Good specificity was noted for all methods (0.82-1.0), though significantly higher for radiographs. Sensitivity was moderate to good for all methods (DIFOTI=0.33-0.93; Film radiographs=0.27-0.53; Digital radiographs=0.40-0.60) and the differences between methods were non-significant. These results suggest that at the D3 threshold, diagnostic accuracy and efficacy are similar for all three methods.

Reliability tests were performed for both LF and DIFOTI method. The LF method has shown good reliability in *in vitro* studies but less consistent results *in vivo* [Abalos *et al.*, 2009; Akarsu and Koprulu, 2006; Angnes *et al.*, 2005; Chu *et al.*, 2009; Khalife *et al.*, 2009; Neuhaus *et al.*, 2010; Reis *et al.*, 2006]. In **Paper I**, the reliability of the LF method *in vivo* was tested by analyzing intra-operator and inter-device correlation. The intra-operator correlation coefficient ranged between 0.85-0.98, which was interpreted as very good. Inter-device correlation was also good, ranging between 0.81-0.92. However, due to the inconsistency in cut-off threshold, as discussed earlier, the reproducibility is questionable. Due to the statistical method chosen, the readings from different instruments may show good inter-device correlation even though one instrument may consistently give higher readings.

In **Paper IV**, the reliability of the DIFOTI method was tested *in vitro* with respect to inter- and intra-operator agreement for occlusal caries detection. The reliability was compared with the conventional methods, which are more familiar to most clinicians, visual inspection and film and digital radiographs. Eight examiners validated all sets of images and all occlusal surfaces visually. The best intra-examiner agreement was noted for VI, with good to very good agreement for all examiners. Other methods showed less impressive results: intra-examiner agreement was good to very good for only 6 examiners for film radiography, 5 for DIFOTI and 4 for digital radiography. With respect to inter-examiner agreement, the variance was even greater for all methods, although slightly better for VI than for other methods. Although the reliability for the DIFOTI method has not been tested in earlier published studies, great variance in results is reported in studies of the conventional methods, VI and radiographs [Bader *et al.*, 2002; SBU, 2007].

With respect to approximal caries detection *in vitro* (**Paper III**), the DIFOTI method showed good reliability for both intra- and inter-observer agreement (K_w= 0.74-0.94 and 0.62-0.76 respectively). Similar results were achieved for film and digital radiographs. The method therefore shows better reliability for detection of approximal caries than for occlusal caries.

This thesis presents studies which evaluated two advanced methods for caries detection. To guarantee patient safety and outcome efficacy and finally societal efficacy, these methods require further evaluation with respect to treatment outcome. Although many studies have been published on the accuracy of the LF method, both *in vitro* and *in vivo*, the therapeutic efficacy of the method has received little attention. The ability of the LF method to differentiate between enamel and dentin caries, if the right cut-off threshold is used, is a positive attribute, but the weak correlation with actual lesion depth limits the usefulness of the instrument as an aid to caries detection in clinical practice.

In contrast to LF, the DIFOTI method has not been extensively evaluated. The studies presented in **Papers III** and **IV** were planned as initial investigations of the accuracy/efficacy of the method for caries detection on occlusal and approximal surfaces. The positive data, especially for diagnostic threshold D1, thus support the potential application of the method for non-invasive detection and monitoring of early lesions on both occlusal and approximal surfaces.

However, the results of these *in vitro* investigations must be interpreted with caution. Histological validation is very sensitive and can detect early changes in the mineral content of the enamel surface. When the detection method being validated is less sensitive, the result is a high proportion of FN data. Such is the case, for example, for radiographs, which do not disclose early enamel lesions [White and Yoon, 1997]. Thus if the test material comprises a high prevalence of early enamel lesions, comparison of the DIFOTI method with radiographs tends to favor the new method.

In validation studies, such as those presented in this thesis, the prevalence of enamel and dentin caries in the material has an important bearing on the outcome. It has been pointed out that in *in vitro* studies, the caries prevalence in the test material is usually higher than in a normal patient population [SBU, 2007]. The results should therefore be extrapolated with caution and this limits the general conclusions that can be drawn from such studies. In this context, however, it is important to note that dental caries is a chronic, slowly progressing, dynamic disease process and that very few individuals are truly caries free. The high proportion of enamel caries presented in the material in **Papers III** and **IV** may therefore approach the true prevalence in the population, although the documented caries prevalence in epidemiological studies, evaluated by conventional detection methods, is much lower [Marthaler, 2004; Hugoson *et al.*, 2005].

The influence of caries prevalence in a given material or population is also relevant to patient outcome efficacy and societal efficacy. A substantial increase in the prevalence of dentin caries therefore favors the more sensitive method, given that the specificity is equal, even though the difference between test methods is not very pronounced. In the same manner, a method that may have shown good efficacy in a strictly controlled trial on a material with high caries prevalence does not necessarily perform with good effectiveness under the currently prevailing conditions of low caries prevalence in most populations.

The low caries prevalence has therefore resulted in higher detection costs per positive case detected. In order for a detection method to be cost effective at community level, there is a need for high sensitivity at diagnostic threshold D1, in order to increase the number of enamel caries lesions detected: such lesions can be treated non-invasively and spared restorative treatment. Moreover, this approach demands high specificity at diagnostic threshold D3, as false positive cases treated by restoration increase the total cost and lead to a decrease in patient outcome efficacy. In this context, interpretation of the LF method is complicated by the complexity of sensitivity and specificity calculations. In contrast, the DIFOTI method identified stages of lesion progression with high sensitivity at diagnostic threshold D1 and good specificity at D3. The method has therefore potentially high efficacy at higher levels of Fryback and Thornbury's hierarchical model [1991] and warrants further evaluation.

CONCLUSIONS

- The LF method is capable of differentiating between enamel and dentin caries, provided the correct cut-off threshold is used for each device. The method can, therefore, be applied to identify and differentiate lesions at diagnostic threshold D3, but is unacceptable as a quantitative method. For occlusal caries detection, performance was no better than the conventional detection methods, VI and radiographs.

- The fluorescence underlying the LF readings is not an indicator of enamel demineralization or specific bacterial content of the lesion, but is a response to the synergistic effect of the caries process. The results of bacterial analysis in **Paper I** and the *in vitro* investigation in **Paper II** do not support claims that the LF method can be used to detect or monitor levels of lesion infection or enamel demineralization associated with lesion activity.

- The *in vitro* investigation of the DIFOTI method for approximal caries detection implies good correlation with actual lesion depth and high accuracy/efficacy, especially at diagnostic threshold D1.

- For occlusal caries detection *in vitro*, the DIFOTI method showed more divergent results. At both D1 and D3 diagnostic thresholds, the accuracy was better than for film and digital radiography, but not superior to VI. Two important advantages of the method, its non-invasive character and the facility for storing digital images for future reference, suggest a potential role as an aid to VI.

- Thus the DIFOTI method shows promise as a means of detecting and monitoring early caries lesions and warrants further investigation.

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