

Aus der Poliklinik für Zahnerhaltung und Parodontologie
Klinikum der Ludwig-Maximilians-Universität München
Direktor: Prof. Dr. Reinhard Hickel

**DIFOTI (DIGITAL FIBEROPTIC TRANSILLUMINATION):
VALIDITÄT IN VITRO**

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CECILIA GUTIERREZ
aus
Manila, Philippinen

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der Universität München

Berichterstatter: Prof. Dr. med. dent. Karl-Heinz Kunzelmann

Mitberichterstatter: Prof. Dr. Wolfgang Plitz

Dekan: Prof. Dr. med. Dr. H.c. M. Reiser, FACR, FRCR

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...to him whose face I have sought both in the Rhine and in the Alps and in the world of machines.... In the heights and in the deep, in all the mathematical equations, I am the variable and you are the constant.

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1. INTRODUCTION



Fig. 1. Diagnostic Imaging in the Dental Practice with the DIFOTI System [Electro-Optical Sciences, Inc., 2004].

Dentists have been in search of the ideal method for diagnosis of incipient carious lesions [Convissar, 2001]. Caries prevalence has decreased in most industrialized countries as a result of improved oral hygiene and/or changes in diet combined with regular fluoride exposure. It has become difficult, at the same time, to detect carious lesions. Fluoride-containing dentifrices have changed the pattern of the disease so that: a carious lesion in dentin may progress beneath a clinically intact enamel surface with cavitation occurring later, or arrest of incipient lesions may occur. Consequently, the conventional methods for diagnosing the disease are inadequate [Bamzahim *et al.*, 2002; Pine and *ten* Bosch, 1996; Shi *et al.*, 2000; Stookey *et al.*, 1999; Verdonshot *et al.*, 1999]. Caries diagnostic methods should allow for early detection of the lesion and for all pathologic changes attributable to the disease [Stookey *et al.*, 1999]. These are important in primary teeth most especially because of the rapid progression of caries which results from the reduced enamel thickness. Small carious lesions may not require local anaesthesia for caries removal and thus do not call for treatment which could cause fear in young patients [Attrill and Ashley, 2001]. More importantly, caries is now recognized as a dynamic process. In the initial stage, the caries process can still be halted and even reversed by non-surgical therapy or

minimally invasive restorative techniques. In non-invasive therapy, fluoride application serves to arrest demineralization and facilitate remineralization of the caries lesion before it becomes irreversible [Bühler et al., 2005; Malmö University Department of Cariology, 2004]. In dental practice, however, clinicians still measure the caries process as a dichotomous variable of presence or absence of disease. Diagnostic methods which are objective and quantitative are needed. Scientific innovations in dental diagnostics are potential aids in the decision-making whether lesion must be restored or preventive techniques may still be employed. Moreover, obtaining accurate measurements of lesions over time, monitoring, allows assessment of the behavior of lesions in response to fluoride treatments. Such concepts stimulate efforts in developing caries diagnostic methods [Bamzahim *et al.*, 2002; Pitts, 1997; Pitts, 2004; Stookey *et al.*, 1999]. Radiographs, although they could still be considered a useful diagnostic tool, pose concern over the use of ionizing radiation. These initiated investigation of transillumination as a means of caries detection and studies on the potential of fiber-optic transillumination (FOTI) to detect early carious lesions. However, due to the perceived shortcoming of FOTI, diagnosis of which by eye has been reported to have led to a high level of intra- and inter-examiner variability, it was then combined with a digital CCD (charge-coupled device) camera [Stookey *et al.*, 1999]. DIFOTI (Digital Imaging Fiber-Optic Trans-Illumination), a modern technology in dental diagnostic imaging, demonstrates potential for detection and monitoring of said lesions without the harmful effect of radiation [Electro-Optical Sciences, Inc., 2004; Schneidermann et al., 1997; Keem and Elbaum, 1997] and attempting to overcome the reported large inter- and intra-examiner variability encountered with its predecessor [Stookey *et al.*, 1999].

1.1 Background

Basic guiding concepts in Cariology are stated below in Table 1 and are divided into previous and latest knowledge. The latter has led to the development of modern clinical caries management and thus diagnostic devices such as DIFOTI.

	Previous Knowledge	Latest Knowledge
Caries definition [Ismail, 2004; Warren, 1997]	categorical disease	process
Diagnosis definition [ten Bosch and Angmar-Månsson, 2000]	determination of diseases by their signs and symptoms	evaluation of the disease activity
Diagnostic question [ten Bosch and Angmar-Månsson, 2000]	description of the present state	prediction of the future progress
Fissure morphology (as a basis of caries behavior) [Milicich, 2000]	V-shape	like a Coke bottle in cross-section (cavitation occurring beneath a sound-looking enamel surface)
Stage of lesion management is initiated [Verdonschot <i>et al.</i> , 1999]	frank caries lesion	incipient caries lesion
Prognosis of the “disease” [Electro-Optical Sciences, 2004a; Stookey <i>et al.</i> , 1999]	irreversible	may be reversible, arrested or irreversible

Table 1. Comparison of previous and latest knowledge in Cariology.

	Traditional	Modern
Basis of caries diagnosis [Angmar-Månsson <i>et al.</i> , 1996; de Josselin de Jong <i>et al.</i> , 1995; Huysmans <i>et al.</i> , 1998; Keem and Elbaum, 1997; Pitts, 2004; Pretty, 2002; Shi <i>et al.</i> , 2001; Stookey, 2004; Vaarkamp <i>et al.</i> , 1997]	presence or absence of lesion (dichotomous or yes-or-no decision)	quantitative measurement (lesion depth, mineral loss, electrical conductance)
Diagnostic methods [Stookey <i>et al.</i> , 1999; Bamzahim <i>et al.</i> , 2002; Sheehy <i>et al.</i> , 2001; Schneiderman <i>et al.</i> , 1997; Pretty <i>et al.</i> , 2002]	visual, tactile, visuo-tactile radiography	laser fluorescence (DIAGNOdent, QLF), electroconductivity (ECM) measurements light transillumination methods (FOTI, DIFOTI)
Treatment for early dental decay [Attrill and Ashley, 2001; Bühler <i>et al.</i> , 2005; Electro-Optical Sciences, 2004a; Stookey <i>et al.</i> , 1999]	aggressive cavity preparations	non-surgical or minimally invasive restorative techniques

Table 2. Developments in clinical caries management.

1.2 Objectives of the Study

1.2.1 To evaluate the validity of the DIFOTI (Digital Fiberoptic Transillumination) in terms of sensitivity and specificity.

1.2.2 To further assess the diagnostic performance of the system by a statistical approach, namely, the ROC-curve.

1.2.3 To correlate lesion depth of histological tooth sections and light penetration of the DIFOTI device through carious lesions.

1.3 Scope and Limitation

This study aims only to achieve results concerning the parameters mentioned above in the objectives, namely sensitivity and specificity of the DIFOTI system, diagnostic performance of the device as assessed by the ROC curve and correlation of lesion depth of teeth and light penetration of the DIFOTI through the caries. However, it gives information also about the device beyond the scope of the main interest of this research, such as the advantages and disadvantages DIFOTI may provide in clinical practice. It discusses briefly other available diagnostic methods, as knowledge of other diagnostic modalities for caries lesions may indirectly provide increased understanding of the device. And it provides some concepts in caries diagnosis relevant to the research. This study is limited to assessing the ability of the device in the diagnosis of enamel caries. Dentin and root caries plus marginal or recurrent caries have not been objects of study nor have been included in the review of pertinent literature.

Forty-five teeth have been used in the study. Additional specimens would give more credible results. Lack of available sound teeth required for the experiment, however, has compelled this limitation. Artificial lesions created on sound tooth surfaces to take the place of true initial caries pose the question of whether they do actually simulate natural carious lesions. Validity of the DIFOTI has been

evaluated in terms of sensitivity and specificity by comparing observations of caries lesions histologically and with the DIFOTI images and performing the necessary computation. To obtain light penetration values of the device through the lesions, a software specifically designed for the study, has been used to analyze the images taken with the DIFOTI. The author then performed statistical analysis with the aid of the computer program SPSS, to determine diagnostic performance of the device, and, in addition, the accuracy of the study test.

1.4 Definition of Terms

Sensitivity. The percentage of correctly diagnosed tooth caries [El-Housseiny, Jamjoum, 2001; Ashley et al., 1998].

Specificity. The percentage of correctly detected sound surfaces caries [El-Housseiny, Jamjoum, 2001; Ashley et al., 1998].

Validity. Ability of the device to measure what it claims to measure; comparison of the new method with the current gold standard within the field is the usual method of testing this [Pretty et al., 2002].

Receiver Operating Characteristic Curve. A method of summarizing the performance of a diagnostic test. It indicates the relationship between the true positive rate and the false positive rate of the test at various thresholds used to distinguish disease cases from non-cases [Walter, 2002]

Area Under the ROC Curve (AUC). One way to summarize an ROC-curve in a single number [Tax, 2004].

Confidence Interval. The likely range of the true value [Hopkins, 2001]. It gives an estimate of the amount of error involved in our data [Becker, 1999].

Standard Error. An estimate of how much error there is in a test [“Standard Error of Measurement”, n.d.]. It describes the typical amount of difference between the target population and the sample tended to see for this sample size [U.S. Census Bureau, 2002].

1.5 Working Hypothesis

1.5.1 The DIFOTI system as used for visual interpretation of digitized images has good sensitivity and specificity properties for buccal and occlusal initial lesions. For proximal superficial lesions, however, due to the contact between adjacent teeth, the DIFOTI has poor sensitivity and specificity properties.

1.5.2. For initial buccal and occlusal lesions, DIFOTI has good diagnostic performance as assessed by the statistical approach ROC-curve.

However, the ROC-curve shows the system has poor performance for initial proximal lesions.

1.5.3 Light penetration of the DIFOTI device through the carious lesion, as quantified by the View3D software, is proportional to the depth of the carious lesion.

The system is manufactured as a dental diagnostic imaging instrument. Kunzelmann *et al.*, 2002 has provided additional benefit to the DIFOTI by incorporating to the device a software program, View3D, which allows quantification of light scattering through the lesion.

In the digitized images, lesion is perceived as a dark area surrounded by the whiter sound tissue. The dark area is determined and is taken as the region of interest (ROI). With the View3D program, light intensity in the ROI is reconstructed based on the interpolation of the light intensity of sound tissue next to the lesion. The decrease in brightness in the region of interest is obtained from the percentage difference between the actual and reconstructed light intensity [de

Josselin de Jong et al., 1995]. Based on the concept that an imaged demineralized or carious tooth surface will transmit less light than surrounding sound tissue, the researcher *et al.* intends to achieve with the View3D statistical analysis results that correlate well with microscopic measurements. Furthermore, within the ROI, a histogram of light scattering could be drawn. Decrease in transillumination values of gray-level DIFOTI images are given cut-off points called quantile. In the histogram of the ROI, the object of search is a diagnostic threshold below the light scatter. In this study, decrease in brightness of 1% and median quantile images is examined. Hypothetically, by statistical analysis with the mentioned computer program, 1% quantile gray-level images will bring about increased tendency to overlook lesions. Median quantile images, setting the threshold too less discriminating, will however result to a great number of false positive values [Kunzelmann, 2005].

1.6 Schematic Framework of the Study

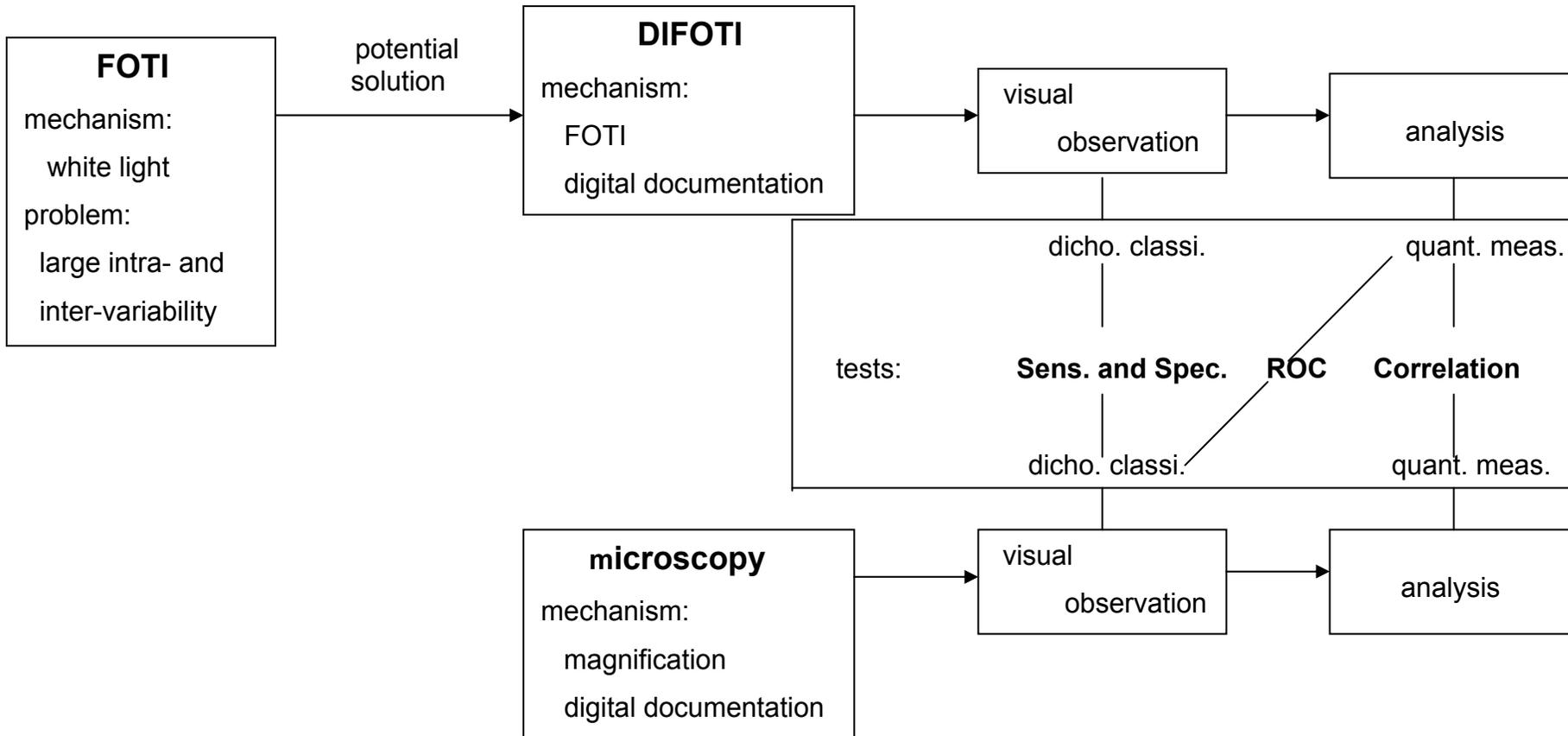


Fig. 2. Schematic Framework of the Study

* dicho. classi.=dichotomous classification; quant. meas.=quantitative measures; sens.=sensitivity; spec.=specificity; ROC=Receiver Operating Characteristics

2. LITERATURE REVIEW

The term *dental caries* comprises a range of different sizes of lesions from subclinical surface changes to macroscopic cavities [Pitts, 1997; Huysmanns and Longbottom, 2004]. Caries diagnostic methods therefore should allow detection of the disease from earliest stages to all pathologic changes attributable to the disease. Caries prevalence has decreased in most industrialized countries as a result of improved oral hygiene and/or changes in diet combined with regular fluoride exposure. It has become more difficult, at the same time, to detect carious lesions. Fluoride-containing dentifrices have changed the appearance of caries. A carious lesion in dentin may progress beneath a clinically intact enamel surface with cavitation occurring later or arrest of incipient lesions may occur. Caries is recognized as a dynamic process. The process of demineralization-rem mineralization may bring about progression, stabilization or regression of a lesion. Use of clinical criteria such as color, “softness,” and “resistance to removal” and tools like sharp explorer and dental radiographs are limited to detection of lesion. Methods for quantification of lesion are needed and devices for the this purpose are being developed [Angmar-Månsson *et al.*, 1996; Stookey, *et al.*, 1999].

2.1 CARIES DIAGNOSTIC METHODS

2.1.1 Conventional Caries Diagnostic Methods

2.1.1.1 Visual inspection

Vision alone as a diagnostic tool is insufficient. Studies of visual inspection for diagnosis of occlusal caries have shown that the sensitivity ranged from 0.12 to 0.80 and the specificity from 0.67 to 0.97 [Ie and Verdonschot, 1994; Sheehy *et al.*, 2001]. Lately, a different ranked visual scoring system has been devised [Ekstrand, *et al.*, 1997; Sheehy *et al.*, 2001] which showed a high

correlation with a histological validation scoring system. This system offers promise for occlusal caries diagnosis [Sheehy *et al.*, 2001].

Score	Criteria
0	No or slight change in enamel translucency after prolonged air drying (>5 s)
1	Opacity hardly visible on the wet surface, but distinctly visible after air drying
1a	Discoloration hardly visible on the wet surface, but distinctly visible after air drying
2	Opacity distinctly visible without air drying
2a	Discoloration distinctly visible without air drying
3	Localised enamel breakdown in opaque or discolored enamel and/or gray discoloration from the underlying dentin
4	Cavitation in opaque or discolored enamel exposing the dentin

Table 3. The criteria used to record the visual appearance of teeth [Ekstrand *et al.*, 1998]

Presence or absence of caries is determined based on observation of color, texture, translucency [Angmar-Månsson *et al.*, 1996] and morphological changes of tooth tissue, and which may be facilitated by tools such as mouth mirror, light and magnification aids.

2.1.1.2 Tactile examination with the use of dental explorer or probe

Use of sharp explorer to diagnose occlusal caries is no longer accepted because of the risk of transferring microorganisms from one site to another [El-Housseiny and Jamjoum, 2001]. Probing pressure may damage the fissures over subsurface carious lesions [Sheehy *et al.*, 2001] or the early demineralized areas [El-Housseiny and Jamjoum, 2001], thus traumatically extending the lesions from incipient to frank caries [Ekstrand *et al.*, 1987; Schneiderman *et al.*, 1997].

2.1.1.3 Combination of visual and tactile examination

Only visual inspection with a good light source has been recommended for use in routine clinical examinations, but no explorer. The use of dental explorer does not

improve the validity of the diagnosis of fissure caries when compared to that of a visual inspection alone use [El-Housseiny and Jamjoum, 2001].

2.1.1.4. Radiography

There are various views regarding the use of radiography to detect early carious lesions. It is still held that incipient lesions can be seen radiographically [Schneiderman *et al.*, 1997] but it has been proven to be of no value in diagnosing caries in an early stage (initial enamel lesions) or for detecting approximal dentinal lesions [Stookey *et al.*, 1999]. The bitewing radiograph is said to detect demineralization in dentin and not the enamel lesion [Sheehy *et al.*, 2001]. In early caries diagnosis, it may be too that in radiographs, a small amount of demineralization at one site may be masked by the radiodensity of the surrounding sound enamel [El-Housseiny and Jamjoum, 2001]. However, even radiographs could still be considered a useful diagnostic tool, their many limitations should be considered: the need for ionizing radiation, the limitations of dental film, the physical limitations based on anatomic considerations and the high degree of inter- and intra-examiner variability [Stookey *et al.*, 1999].

2.1.2 Alternative Caries Diagnostic Methods/Non-invasive Techniques

Three other newer methodologies in caries diagnosis aside from DIFOTI, have been chosen for consideration in this review, namely QLF, DIAGNOdent and ECM.

With non-invasive, objective, quantitative diagnostic methods, it is possible to monitor lesion changes related to preventive measures over time [Angmar-Månsson *et al.*, 1996; Huysmans *et al.*, 1998]. Lesion monitoring is used at a series of examinations when lesions are less advanced than the particular stage judged to require operative intervention. A comparison of serial measurements over time permits an assessment of the behavior of lesions to be made and thus allows the efficacy of preventive care aiming to either arrest or reverse lesions to

be determined [Pitts, 2004]. Systems that permit monitoring of the results of preventive treatments and advice are not new. Backer-Dirks *et al.*, 1951 described a “reproducible method for caries evaluation” which graded the caries process into 12 levels of severity in order that longitudinal assessments of lesion activity might be made. Standardized grading systems which go beyond the DMFT index are important [Pitts, 1997]. A number of systems of scoring the behavior of individual lesions over time have been reported but few have been taken up [Hollender and Koch, 1969; Pitts, 1984a, 1985, 1997, 2004; Kingman and Selwitz, 1997]. Lesion behavior can traditionally be monitored with standard clinical examinations or with serial radiographic examinations [Pitts, 2004, 1997]. Later on, the need to measure small changes in lesions over time with objective, more reproducible and quantitative methods has been recognized [Pitts, 2004, 1984b, Angmar-Månsson and Ten Bosch, 1987]. Attempts to use computer-aided image analysis of serial radiographic images were made [Pitts, 2004, 1986; Pitts and Renson, 1987], but these were harmful due to the ionizing radiation and were not developed commercially. Further need for new quantitative methods has led to attempts to develop aids to the diagnosis and monitoring of lesions [Pitts, 2004].

2.1.2.1 Optical methods

Potential of these quantitative optical diagnostic methods could be better explained by an understanding of light transport through dental tissue. Tubules are the predominant cause of scattering in dentin. And because of the orientation of the tubules, from pulp towards the dentinoenamel junction, the anisotropic structure of dentin may result in a directionally dependent light propagation. In enamel, it is the hydroxyapatite crystals which contribute significantly to scattering [Vaarkamp *et al.*, 1995]. In incipient caries, mineral loss is accompanied by an increased light scattering [Vaarkamp *et al.*, 1997] (by hydroxyapatite crystals) within the lesion, making it look whiter than the surrounding sound enamel. This occurs because the remaining small mineral particles in the lesion are embedded in water rather than in mineral-rich sound

enamel [Angmar-Månsson, *et al.*, 1996; Pine and Ten Bosch, 1996]. In older, discolored lesions, light absorption is also enhanced [Vaarkamp *et al.*, 1997].

Results of selected studies from 2001 to the present on QLF, DIAGNOdent and DIFOTI are summarized and presented in two tables. Table 10 contains selected in vitro studies and table 11 in vivo.

The fundamental bases for the detection and quantification of caries by means of optical methods are the physical properties of the caries lesion. Available data on the nature of early caries lesions in enamel can be summarized [Arends and Christoffersen, 1986; Angmar-Månsson and ten Bosch, 1987] in the following conclusions:

- The surface layer covering an enamel lesion is a porous but still mineral-rich area;
- The subsurface area of the lesion is low in mineral content;
- The surface morphology of the initial lesion is slightly different from that of sound enamel.

2.1.2.1.1 FOTI

Fiberoptic transillumination (FOTI) is a simple, easily repeatable, nonhazardous method, which may supplement the clinical examination [Davies *et al.*, 2001; Cortes *et al.*, 2000; Vaarkamp *et al.*, 1997]. A narrow beam of bright white light is directed across areas of contact between approximal surfaces [Davies *et al.*, 2001]. And the resultant changes in the light distribution, as the light passes through the teeth, produce the image for analysis. Studies showing that the use of this technique leads to a high level of intra- and inter-examiner variability and has led to the development of the new methodology (DIFOTI) [Stookey *et al.*, 1999].

2.1.2.1.2 The DIFOTI System



Fig. 3. The Complete DIFOTI System [Ganz, 2003].

Description of the Method:

DIFOTI (Digital Imaging Fiber-Optic Trans-Illumination) is a dental diagnostic imaging instrument. It is manufactured by Electro-optical Sciences, Inc. which claims that the device is able to detect early carious lesions on all tooth surfaces. And DIFOTI is able to diagnose the disease even before the lesion appears in radiographs and can also discover marginal caries. Keem and Elbaum, 1997 and Schneidermann *et al.*, 1997 state that the DIFOTI is one of the new methods that has the potential not only to detect but also to monitor incipient carious lesions. Fractures, too, are discernible through the use of the device. In addition, it provides real-time imaging during caries excavation and monitors treatment and restorative materials post-operatively [Keem and Elbaum, 1997]. DIFOTI is an attempt to overcome the reported large inter- and intra-examiner variability of diagnosis of FOTI by eye. FOTI was then combined with a digital CCD (charge-coupled device) camera [Stookey *et al.*, 1999]. As a new device, DIFOTI requires validation in terms of sensitivity and specificity for different caries sites and of its diagnostic accuracy.

DIFOTI in the commercial form is not able to perform the function of quantitative monitoring. To make this possible, a software, View3D, particularly designed for the DIFOTI and this present study, is added to the system and utilized. View3D

analyzes images acquired by DIFOTI similar to the QLF evaluation by de Josselin de Jong et al., 1995 (Inspektor, model QLF 1.0).



Fig. 4. DIFOTI Control Box, Handpiece and Foot Pedal [Electro-Optical Sciences, Inc., 2001].

System Components:

1. handpiece
2. electronic control box
3. software
4. customized image capture card
5. foot pedal
6. disposable mouthpieces – 2 kinds: occlusal surface mouthpiece
proximal surface mouthpiece

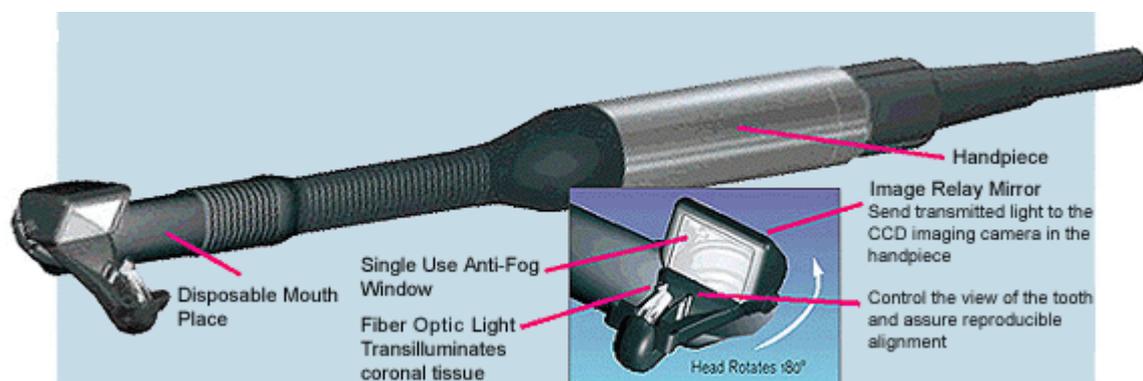


Fig. 5. DIFOTI Handpiece and Integral Parts of the Mouthpiece [Electro-Optical Sciences, Inc., 2004].

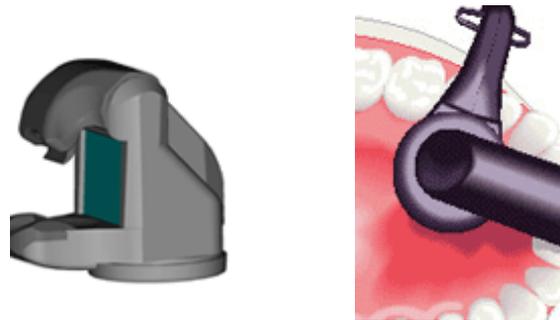


Fig. 6. The occlusal mouthpiece (left) transilluminates from both sides of tooth and images the occlusal surface. The proximal mouthpiece (right) transilluminates on one side of the tooth and images the other side [Electro-Optical Sciences, Inc., 2004].

Mechanism:

The DIFOTI device uses safe white light [Electro-Optical Sciences, Inc., 2004], or in other words, standard light [Ganz, 2003]. With the proximal surface of the tooth, light is shined from one surface, through the tooth, and captured on the opposite side by the camera on the other side of the mouthpiece. With the occlusal surface, the mouthpiece illuminates the tooth at angles through both the facial and lingual surfaces and images the light emerging from the top of the tooth [Electro-Optical Sciences, Inc., 2004]. The images of teeth acquired with the camera are sent to a computer for analysis with dedicated algorithms. These algorithms help to diagnose and locate the carious lesion [Schneidermann et al., 1997]. The system instantly creates high-resolution digital images of occlusal, interproximal and smooth surfaces [Electro-Optical Sciences, Inc., 2004]. Magnification is approximately 16x. Images can be acquired repeatedly, can be processed to create contrast between normal and carious tissues and to quantify the features of carious lesions for diagnosis [Keem and Elbaum, 1997], are reproducible, can be saved as file (on CD, for example) and are printable and are transmittable through e-mail. Dental practitioners can learn to interpret the images in a matter of hours [Electro-Optical Sciences, Inc., 2004].

Minimum system requirements of the DIFOTI system [Electro-Optical Sciences, Inc., 2004]:

1. Open PCI slot
2. Game port
3. Windows 98 or Windows Millenium Edition, NT, 2000 or XP operating system
4. 64 MB memory
5. 100 MHz processor speed or higher
6. 4 GB hard drive
7. Back-up storage device
8. Sound card

Advantages:

The advantages of DIFOTI over radiography include: no ionizing radiation, no film, real-time diagnosis, and higher sensitivity in detecting early lesions not apparent to x-ray, as demonstrated in vitro [Keem and Elbaum,1997], and not seen visually or through use of an explorer [Electro-Optical Sciences, Inc., 2004]. Furthermore, its unique advantage over other caries monitoring methods is its ability to monitor quantitatively selected lesions over a period of time [Keem and Elbaum, 1997].

Disadvantage:

Handling of the device may pose a problem as the camera may be bulky to be manipulated in younger patients' mouth.

Stage of Lesion: Lesion	Incipient Caries Lesion (<i>Surface Intact</i>) REVERSIBLE	Initial Caries Lesion (<i>Surface Intact</i>) IRREVERSIBLE	Frank Caries Lesion (<i>Surface Broken</i>) IRREVERSIBLE
Imaging Modalities for the Detection of Caries Lesions:	-----DIFOTI-----		-----Radiography-----
Lesion Management Options:	Preventive Therapy Oral Hygiene Instruction (Fluoride Treatments Sealants Surveillance)	Preventive Therapy and/or Minimal Surgical Intervention (Flowable Composites Sealants Amalgams)	Surgical Intervention

Table 4. Comparison between DIFOTI and radiography as imaging modalities for caries diagnosis [Electro-Optical Sciences, Inc., 2004].

DIFOTI provides clear signatures of different types of frank caries on all types of teeth and provides signatures of occlusal caries on premolars and molars. As determined by the gold standard (visual inspection under x4 magnification, explorer and histological section), DIFOTI can detect incipient and recurrent caries even when radiological images fail to show their presence [Schneiderman, *et al.*, 1997].

Schneiderman *et al.*, 1997 evaluated the sensitivity, specificity and repeatability of a DIFOTI laboratory prototype for different tooth surfaces and compared it with radiographic images. For smooth surfaces, DIFOTI obtained 0.43, 0.87 and 0.12 for the mentioned parameters, respectively. The sensitivity of DIFOTI exceeds that of conventional radiograph, while the specificity is 10% lower. However, x-ray proved almost twice superior in repeatability than the DIFOTI. Sensitivity, specificity and repeatability values of the DIFOTI in detecting occlusal caries were 0.67, 0.87 and 0.52, respectively. DIFOTI's sensitivity is higher than that of x-ray, while the specificity is again $\approx 10\%$ lower. Its repeatability, compared with

radiography, does not show significant difference. For approximal caries, DIFOTI was reported to have sensitivity, specificity and repeatability values of 0.56, 0.76 and 0.25, respectively. The sensitivity of DIFOTI is more than twice as high as for radiography, also with a $\approx 10\%$ penalty in specificity. Its repeatability too was proven inferior to that of x-ray.

2.1.2.2 Fluorescence-based devices

Fluorescence may be induced when enamel, dentin and substances in caries lesions are exposed to (laser) light of a specific color. This principle is the basis of two caries diagnostic methods: DIAGNOdent and Quantitative Light-induced Fluorescence (QLF) [Verdonschot and van der Veen, 2002].

2.1.2.2.1 QLF (Quantitative Light-Induced Fluorescence)

Description of the Method:

Use of quantitative light-induced fluorescence (QLF) makes detection of very early demineralization and quantifying of mineral loss possible [Pretty, 2002a]. QLF is based on the fluorescence decrease in demineralized enamel upon exposure to blue-violet (laser) light. The intensity of the emitted light is related to the amount of mineral loss in the caries lesion [Verdonschot and van der Veen, 2002]. A charge coupled device (CCD) microcamera contained in a handpiece captures the tooth image. The tooth under examination is displayed on a PC screen and this image is saved on a disk. The image is then analysed by a software [Pretty *et al.*, 2002a, b].

The technique was initially developed using lasers and was demonstrated by Bjelkhagen and coworkers in 1982 [Pretty *et al.*, 2002b, Bjelkhagen *et al.*, 1982]. Concerns over the intraoral use of lasers led to the development of a system using filtered visible light by de Josselinn de Jong *et al.* The principle behind the technique is that enamel will auto fluorescence under certain lighting conditions.

Demineralized enamel will fluoresce less and this loss of fluorescence can be detected, quantified and longitudinally monitored using QLF [Pretty *et al.*, 2002b, 2001; van der Veen and de Josselin de Jong, 2000]. The contrast between sound and demineralized enamel is increased by a factor of 10 [Pretty *et al.*, 2002b, 2001b].

Advantages:

The QLF technique is user-friendly [Pretty *et al.*, 2002a]. It is suited to children because it does not cause pain and can easily be fitted into their mouths. Since it is non-threatening and the child can see his teeth on the computer screen, it helps to gain the child's cooperation and obtain high quality images [Pretty *et al.*, 2002b].

Disadvantage:

Lesion fluorescence decreases with dehydration, thereby influencing the result of the measurement in clinical application [Pinelli *et al.*, 2002]. There is difficulty in repositioning the optical monitor probe at the same measuring points at different times when used longitudinally, especially when the lesion area might change over time. There are also problems with sterilization of the instrument and limitations related to the size of the bulky laser equipment presently used as the light source [Angmar-Månsson *et al.*, 1996]. Daylight interferes with measurement. Research by Pretty *et al.*, 2002b states that currently, the QLF device is unable to accurately identify interproximal lesions.

A study has determined, *in vitro*, high intra- and inter-examiner agreement in the use [Pretty *et al.*, 2002a] and capability of the device in detecting the demineralization process [Pretty *et al.*, 2002b]. Various studies have also shown that this technology can assess the ability of caries-preventive measures to arrest or remineralize caries lesions [Stookey, 2004]. A review of 7 articles (Lagerweij *et al.*, 1999; Ando *et al.*, 1997; Al-Khateeb *et al.*, 1997; Hall *et al.*, 1997; Emami *et*

al., 1996; Hafström *et al.*, 1992; Shi *et al.*, 2001] on caries detection on smooth surfaces reported sensitivity, specificity and ROC results were very good, while correlation with gold standards were between 0.63 and 0.91 [Stookey, 2001, 2004]. Ferreira Zandoná *et al.*, 1998 reported results on caries detection on occlusal surfaces. Sensitivity, specificity and ROC results for QLF were 0.49, 0.67 and 0.78 respectively. On detection of approximal caries, Eggertsson *et al.*, 1999 reported the values 0.56-0.74 for sensitivity and 0.67-0.78 for specificity. Data concerning the diagnostic performance of QLF in terms of sensitivity and specificity, in comparison with another system, DIAGNOdent, are also available. In the trial done, two QLF devices, one with an argon ion laser and the other with non-coherent light from a xenon lamp, combined with a filter system, were compared with the DIAGNOdent [Shi *et al.*, 2001].

	DIAGNOdent			QLF, laser			QLF, lamp		
	cut-off	sens.	spec.	cut-off	sens.	spec.	cut-off	sens.	spec.
Dentinal caries	9	0.75	0.96	25%	0.83	0.98	20%	0.94	1

Table 5. Diagnostic accuracy with respect to dentinal caries: sensitivity (sens.) and specificity (spec.) of QLF and DIAGNOdent [Shi *et al.*, 2001]

In the above study, the „gold standard“ was determined by histopathology and microradiography. In addition, the correlation for lesion depth and mineral loss between the gold standard and the two methods was also assessed. The results showed that for lesion depth, the correlation with the gold standard was similar for QLF and DIAGNOdent: about 0.85. For mineral loss, Spearman's rank correlation coefficients for QLF and DIAGNOdent were 0.76 and 0.67 respectively, implying that QLF had closer correlation with mineral changes. Compared with DIAGNOdent, QLF showed higher sensitivity too. But for specificity, both were of equal merit [Shi *et al.*, 2001].

QLF is a reliable method for quantifying mineral loss from enamel and subsequent monitoring of this loss [Pretty *et al.*, 2002b]. But this high reliability has to be seen when analyzing *in vivo* lesions, thus, further research is required [Pretty *et al.*, 2002a]. And currently, it is unable to accurately identify

interproximal lesions [Pretty *et al.*, 2002b]. The reproducibility of the analysis stage of the device is good [Pretty *et al.*, 2002a; de Josselin de Jong *et al.*, 1995]. But new users of the technique are recommended to make repeated analyses of their images to reduce measurement error [Pretty *et al.*, 2002a].

The use of the QLF technology must be combined with a visual clinical examination. Since QLF detects any hypocalcified area (e.g. developmental defects, including dental fluorosis), it is essential to eliminate obviously non-carious hypoplastic areas with a visual clinical examination.

2.1.2.2.2 DIAGNOdent

Description of the Method:

DIAGNOdent (Kavo, Biberach, Germany) is a laser-based system [Stookey *et al.*, 1999; Shi *et al.*, 2001]. The tooth is illuminated by red laser light (wavelength 655 nanometers) which is absorbed by both inorganic and organic components within tooth substance [Sheehy *et al.*, 2001]. Bacterial porphyrins evoke fluorescence and the intensity of the emitted light is related to the size of the carious lesion [Verdonschot and van der Veen, 2002]. Hibst and Paulus, 1999, proposed that the enhanced fluorescence in the presence of caries results from the integration of bacterial metabolites rather than crystalline disintegration. Clean healthy tooth structure exhibits little or no fluorescence, resulting in very low scale readings on the display [Kavo, n.d.]. As the carious process advances, changes within the tooth substance cause the amount of this fluorescent light to increase and this radiation is registered and evaluated by the instrument [Sheehy *et al.*, 2001]. A fiber-optic ring around the tip returns the light to the unit where it is quantified. The unit is calibrated to a standard which then allows reproducible results. An increase in reading between examinations indicates treatment should be considered [Milicich, 2000].

The unit can be calibrated to be tooth specific. Some teeth have a natural fluorescence, and this can simply be calibrated out of the reading so that the information being supplied relates to increased fluorescence in the tooth due to decalcified tooth structure. Tooth-specific calibrated readings in excess of 20 indicate caries developing in the dentine. Clinically, laser diagnosis not only indicates the presence of a lesion, it can also indicate, by rotation of the tip within the fissure, the direction in which the caries is developing. The reading varies when the laser beam irradiates different sections of the fissure, giving an excellent indication of the direction of the caries [Milicich, 2000].

Advantages:

The DIAGNOdent is a flexible and mobile unit (battery operated). It provides simple, fast and painless examination. The findings are assisted by visual and acoustic signals [Kavo, n.d.].

Disadvantage:

In the clinical situation, false positives in results may occur for changes in physical properties of the tooth structure, namely presence of stains, calculus or dental plaque, disturbed tooth development or mineralization, result to increased reading of the device [Pinelli *et al.*, 2002]. Interpretation of DIAGNOdent reading in the context of therapeutic consequences is not validated.

Both in vitro and in vivo (under daily practice conditions) examinations reported good intra-examiner reproducibility of DIAGNOdent on occlusal and smooth surfaces [Lussi *et al.*, 1999, 2001; Attrill and Ashley, 2001; Shi *et al.*, 2001; Lussi and Francescut, 2003; Sheehy *et al.*, 2001; Heinrich-Weltzien *et al.*, 2002; Pinelli *et al.*, 2002]. Likewise, study by Pinelli *et al.*, 2002, the only investigation which assessed inter-examiner reproducibility on smooth surfaces showed a substantial Kappa value of 0.77 [Lussi *et al.*, 2004]. In the same study by Pinelli *et al.*, sensitivity and specificity of the instrument on smooth surfaces were also determined, 0.72 and 0.73 respectively, using clinical evaluation of white spots as

the validation criterion (see Tables 6, 7, and 8). Another article reported sensitivity and specificity results and correlation coefficients with the gold standards for caries detection on smooth surfaces, 0.75, 0.96 and 0.67-0.86 respectively [Shi *et al.*, 2000; González-Cabezas, 2001]. For the use of the DIAGNOdent system on occlusal surfaces, sensitivity, specificity, ROC and accuracy results for lesions limited to enamel were 0.42-0.87, 0.72-0.95, 0.92 and 0.79-0.84, respectively. For lesions that involved dentin, the results reported for sensitivity, specificity, ROC and accuracy were 0.76-0.84, 0.79-1.00, 0.99 and 0.81-0.83, respectively. The correlation with the gold standard was 0.76-0.79 [Shi *et al.*, 2001; Lussi *et al.*, 1999; González-Cabezas, 2001]. In the research by Bamzahim *et al.*, 2002, DIAGNOdent when compared with electrical conductance measurements, for detection of non-cavity occlusal carious lesions, showed higher sensitivity, specificity and correlation with histopathology than the latter. It was also proven superior to ECM in reproducibility, expressed as ICC, 0.97 versus 0.71.

Reference	Methodology	Type of Teeth	Caries location (surfaces)	Gold Standard	Repeatability	Sensitivity	Specificity	Correlation with Gold Standard
Pretty et al., 2002	QLF	M	smooth	TMR	high (no exact value given)	-	-	-
Shi et al., 2001	QLF	PM	smooth	TMR and hist.	-	D=0.94	D=1.00	r, LD=0.85 r, ΔZ =0.76
Bamzahim et al., 2002	DIAGNOdent	PM	occ.	hist.	r=0.97	0.80	1.00	r=0.93
EI-Housseiny and Jamjoum, 2001	DIAGNOdent	PM, M	occ.	hist.	-	0.95	0.50	K=0.42
Shi et al., 2001	DIAGNOdent	PM	occ.	TMR	-	D=0.75	D=0.96	r, LD=0.85 r, ΔZ =0.67

Table 6. Summary of selected published in-vitro studies from 2001 to the present on optical diagnostic methods.

Reference	Methodology	Type of Teeth	Caries location (surfaces)	Repeatability	Sensitivity	Specificity
Tranaeus et al., 2002	QLF	mand. left 1st M	smooth	r=0.95-0.99	-	-
Sheehy et al., 2001	DIAGNOdent	1st perm. M	occ.	rho=0.89	-	-
Ando et al. 2004	DIFOTI	decid. M	smooth= occ.	-	smooth= 0.10; occ.=0.33	smooth= 0.99; occ.=0.83

Table 7. Summary of selected published in vivo studies from 2001 to the present on optical diagnostic methods.

2.1.2.3 Electrical Conductance Measurements

Description of the Method:

Electrical conductance measurements are being used experimentally for occlusal caries detection. The instrument commercially available for this purpose, the Electronic Caries Monitor (ECM, Lode, Diagnostic, Groningen, The Netherlands) measures occlusal fissures with a probe. A coaxial airflow isolates the measuring site from the surrounding tooth surface, resulting in a site-specific measurement [Huysmans *et al.*, 1998]. Recently, it was suggested that the fissure system could be covered with a conducting medium for a surface-specific measurement to be achieved [Huysmans *et al.*, 1998, 1995; Verdonschot *et al.*, 1995]. The theory behind the use of ECM is the observation that sound surfaces should possess limited or no conductivity, whereas carious or demineralised enamel should have a measurable conductivity that will increase with increasing demineralization [Huysmans *et al.*, 1997; Stookey *et al.*, 1999]. The high electrical resistance of sound dental tissue decreases when pores created by carious demineralization are filled with water and soluble electrolytes [Bamzahim *et al.*, 2002]. With decreasing thickness and increased porosity, the performance of electrical resistance has been reported to be as valid as or better than more traditional means of diagnosing fissure caries [Angmar-Månsson *et al.*, 1998; Stookey *et al.*, 1999].

Disadvantages:

Enamel maturation as the tooth tends to erupt with a porous surface, which becomes less porous over time, affects resistance readings. Temperature is also a factor affecting the measurements provided by the ECM. False positives and false negatives in results may also occur due to several reasons. The airflow used to dry the site could influence the readings. If the airflow is too low, the surface may not be adequately dried and surface conduction to the gingival margin may lead to false positive results. If it is too high the lesion may dry too rapidly, resulting in a false negative outcome

[Ellwood and Cortes, 2004]. Especially in children, the use of the ECM may pose difficulty as the examination with the device relies on good moisture isolation. False positives may also be due to defects in the enamel extending to the dentinoenamel junction and may happen if the crown was incorrectly dried and a short circuit took place. And a false negative may result from incomplete contact between the conducting medium and the tooth surface and possibly if a lesion has a very dense surface zone [Ashley *et al.*, 1998].

A research was conducted, comparing the ECM with visual examination, fibre-optic transillumination, conventional and digital bitewing radiography (see Table 8). When detecting enamel lesions, it had the highest sensitivity (0.65) and the highest positive (0.78) and negative (0.58) predictive value. Visual inspection had a similar specificity to the ECM (0.73); both of which obtained lower specificity value than the other diagnostic systems. At the dentinal level, the ECM had the highest sensitivity (0.78) and the highest negative predictive value (0.87). It had, however, the lowest specificity among the diagnostic systems (0.80). The ECM was the most repeatable system, with kappa value 0.63 and weighted kappa 0.43 [Ashley *et al.*, 1998].

In the study concerning the ECM, Longbottom and Huysmans, 2004, states that correlations range from 0.47 to 0.82. In terms of traditional caries thresholds used in CCTs, there are sensitivity and specificity data for D1 (enamel) caries and D3 (dentinal) caries for both site-specific and surface-specific ECM methods. For site-specific D1 measurements, the sensitivity figures from different studies range between 0.70 and 0.92, with specificity values of 0.78 to 1.00. The equivalent figures for surface-specific measurements are 0.61 to 0.65 and 0.73 to 0.86. In this most previous study too, it is shown that ECM measurements have higher sensitivity but lower specificity than clinical visual methods and are currently limited to occlusal sites.

<i>Diagnostic System</i>	<i>Sensitivity</i>		<i>Specificity</i>		<i>Repeatability (Kappa)</i>
	<i>Enamel</i>	<i>Dentin</i>	<i>Enamel</i>	<i>Dentin</i>	
ECM	0.65	0.78	0.73	0.80	0.63
Visual	0.60	0.24	0.73	0.97	0.42
FOTI	0.21	0.14	0.88	0.95	0.51
Conventional bitewing	0.19	0.24	0.80	0.89	0.45
Bitewing with Digora system	0.24	0.19	0.80	0.89	0.44

Table 8. Sensitivity, specificity and repeatability (kappa) values of each diagnostic system when detecting caries extending into enamel and dentin [Ashley et al., 1998].

Compared with DIAGNOdent, ECM is found to be inferior in reproducibility, 0.71 versus 0.97. In detecting occlusal caries at the D3 level, it offers too lesser sensitivity and specificity than the former [Bamzahim *et al.*, 2002].

Although it has been proven that ECM compared with visual examination, fibre-optic transillumination and radiography, is the most valid and reliable diagnostic tool among these methods [Ashley *et al.*, 1998], later research proves that it has no increased accuracy over visual diagnosis. Therefore, visual examination should be preferred over ECM in caries detection [Ashley, 2000].

2.2 ARTIFICIAL CARIES PRODUCTION

As lesion production is part of the examination procedure, literature review is done to aid in selecting a demineralizing solution for the production. To facilitate this, first, different demineralizing solutions used in different studies were compared in terms of composition, the duration of application of the individual solution and remineralization ability (see Table 10). Then important points, such as the reason for the composition of the demineralizing solution and the depth of the lesion it produced, were gathered. Finally, analysis is made to come up with a decision as to which demineralizing solution is best fit for the purpose of this study.

At present, the two basic methods for providing an artificial cariogenic challenge to the tooth are: chemical systems and bacterial systems. The former allows regulation of the experimental environment and relatively economical but they do not simulate the in vivo situation as accurately as a bacterial system. The latter therefore permits more clinically relevant in vitro investigations. However, the choice of a model to be used for the creation of an artificial lesion still greatly depends on the purpose of the study [Fontana *et al.*, 1996]. In the literature, caries models have been designed for and generally limited to the study of specific topics. Thus, the selection of the demineralizing solution to be used in the study should be carefully done.

Wefel and Harless, 1984 made a comparative study of lesions produced by three artificial caries systems and natural white spot lesions using polarized light microscopy and microradiography. These three systems were: acidified gelatin gel, diphosphonate surface dissolution inhibitor, and a partially saturated buffer system. Results showed that among these three, acidified gel system reproduced the classical histological zones most frequently. Similarly, Damato *et al.*, 1988 compared the de-remineralization behavior of acidified undialyzed gelatin system and a buffered solution. It showed that the demineralization rates of the lesions, in terms of the total mineral loss, produced by the latter were greater than those created by the former. Another study supporting buffer solution is done by Theuns *et al.*, 1984. Acetate buffer containing system was shown to produce caries-like lesions in enamel without the use of surface dissolution inhibitors or gels.

In the study made by Schmidlin *et al.*, 2002, using a gel system (pH 4.8), after 15 weeks of subjecting to demineralization, all specimens showed clearly visible white spots after drying with air – caries-like subsurface lesions with an intact surface, which are histologically discernible with a depth of 0.1 mm. Similarly, using acidified gel-like, but with a different composition, Eberhard and co-workers [2000] demineralized teeth over a period of 6 weeks and the radiographic and histometric evaluation of the artificial lesions made after revealed extensions of 0.48 ± 0.25 and 0.54 ± 0.18 mm, respectively. Buskes *et*

al., 1985, by contrast, utilized an apparatus for the de- and remineralization in vitro under constant composition conditions (using an acetate buffer containing methylhydroxydiphosphate, MHDP) and the lesion depths produced are as stated below (see Table 13). This method showed ability to produce lesions with reproducible depths.

MHDP concentration, μM	Lesion depth, μm
2	145 \pm 12
6	118 \pm 7
50	52 \pm 11

Table 9. Lesion Depths (mean \pm SD) as measured by light microscopy for different MHDP concentrations in the liquid after 115h [Buskes et al, 1985].

Ten Cate et al., 1982, taking cross sections of the lesion, used the measure of hardness as a function of depth and microradiography. 0.376 (\pm 0.017 μmol)Ca/mm² and 0.208 (\pm 0.015) μmol PO₄/mm² were removed from the enamel during lesion formation in this study

It is important, too, to evaluate the composition of the de-/remineralizing solution. In the study of synthetic polymer gels by White, 1987, results showed that polyacrylic acid, a component of carbopol gels, is an effective surface-protective agent for the preparation of artificial carious lesions. MHDP was previously used too as a surface-protective agent during artificial carious lesion preparation because of its similar reactivity. However, due to the low molecular weight of the MHDP molecule, it could influence subsequent lesion reactivity toward fluoride or remineralization. Polyacrylic acid of a high molecular weight, on the other hand, reduces the concerns about hysteresis effects on remineralization and fluoride activity. In the same study, synthetic polymer system was found to be useful for the preparation of 'life-like' artificial incipient lesions.

In this current research on the validity of the DIFOTI system, the Damato protocol was decided upon to be used in the methodology for preparing artificial carious lesions. The succeeding chapter on Discussion states the reason for the choice of demineralizing solution.

AUTHOR	YEAR	COMPO. OF DEM. SOL.	DURATION OF APPLIC.	REMIN.PHASE (Y/N)
Schmidlin et al.	2002	0.1 mol/l acetate, 1.5 mmol/l calcium (Ca), 0.9 mmol/l phosphate (PO ₄), 150 mmol/l potassium (K) 60 g/l hydroxyethylcellulose (Natrosol RH 250) ph 4.8	(20 mg of the gel) 24 h before being rinsed off w/ 10 ml distilled water; repeated every day over 15 wk, while teeth were stored at 5 °C in a 50% humidity chamber	N
Itota, et al	2002	brain-heart infusion broth containing 1% sucrose inoculated w/ <i>S. mutans</i> IFO at 37 °C	14 days	N
Gray et al	2002	acidified gel (36% phosphoric acid)	5s	N
Eberhard, et al	2000	acidified gel (methylcellulose, acetate buffer, ph 4.8)	(teeth demineralized over a pd. of 6 wk)	N

AUTHOR	YEAR	COMPO. OF DEM. SOL.	DURATION OF APPLIC.	REMIN. PHASE (Y/N)
lijima et al.	1999	6 wt% carboxymethylcellulose gel (0.1 M lactic acid w/ 10 M KOH sol. eingestellt); 10 ml per sample; 37 °C	3 wk	Y
		REMIN. SOL.: 20 mM HEPES, 1.5 mM ca ²⁺ as CaC ₁₂ , 0.9 mM phosphate as KH ₂ P ₀₄ & 1 ppm F- as NaF; 37° C; ph 7.0	2 & 4 wk	
Gonzales-Cabezas	1998	50% saturated HAP/0.1 M lactic acid carbopol sol. (ph 5.0); 37°C	96 h = 40-70 µm deep lesions	N

AUTHOR	YEAR	COMPO. OF DEM. SOL.	DURATION OF APPLIC.	REMIN. PHASE (Y/N)
White	1997	synthetic polymer gels (Carbopol/ lactic acid gels) prepared from stock solutions of 1% polyacrylic acid & 1.0 mol/l lactic acid, respectively ph=4.5-5	various time periods	Y
Fontana et al.	1996	cultures of <i>Strept. Mutans</i> & <i>Lactobacillus casei</i> in dextrose-free trypticase soy broth, supplemented w/ 5% sucrose (TSBC), at 37°C, ph=4.1-4.5	7 or 12 days	N

AUTHOR	YEAR	COMPO. OF DEM. SOL.	DURATION OF APPLIC.	REMIN. PHASE (Y/N)
Herkströter et al	1991	3 mM CaCl ₂ ·2H ₂ O, 3 mM KH ₂ PO ₄ , 50 mM CH ₃ COOH in 20 liters demineralized water adjusted w/ KOH to ph 4.5	0.5 h	Y
		REMIN. SOL.: 1.5 mM CaCl ₂ ·2H ₂ O, 0.9 mM KH ₂ PO ₄ & 20mM Hepes at ph7, adjusted w/ KOH in 20 liters demineralized water		
		* The system is partly based on the setup of Buskes et al. [1985]		

AUTHOR	YEAR	COMPO. OF DEM. SOL.	DURATION OF APPLIC.	REMIN. PHASE (Y/N)
Damato et al.	1988	Gelatin Lesions: 10% Difco gelatin 1 mmol/L Ca ₃ (PO ₄) ₂ 80 mmol/L lactic acid ph 4.0 F ⁻ = 0.15 ppm	10-12 wk	Y
		Solution Lesions: 8.3 mmol/L CaCl ₂ 8.3 mmol/L NaH ₂ PO ₄ 50 mmol/L acetic acid NaOH to ph 4.0 F ⁻ <0.03 ppm	5 days	Y

AUTHOR	YEAR	COMPO. OF DEM. SOL.	DURATION OF APPLIC.	REMIN. PHASE (Y/N)
Katz	1986	concentrated <i>S. mutans</i> inoculum, that was overlaid w/ a nutrient layer of 15% agar, 15% glycerine & 5% sucrose; a filter paper and a thin layer of collodion was placed over the artificial plaque; incubated at 37 °C and continuously washed w/ artificial saliva (ph neutral) at a reg. rate	specimens removed daily & 0.24% sodium fluoride dentifrice applied for 3 min; repeated 5 days a wk; whole expt. for 8 wk	Y
Buskes et al.	1985	3 mM CaCl ₂ ·2H ₂ O, 3 mM KH ₂ PO ₄ , 50 mM CH ₃ COOH 2-50 μM MHDP ph=5 (ph brought to this value by adding 10 M KOH)	various demin. pd.	Y

AUTHOR	YEAR	COMPO. OF DEM. SOL.	DURATION OF APPLIC.	REMIN. PHASE (Y/N)
		(cont.) REMIN. SOL.: 1.5 mM CaCl ₂ ·2H ₂ O, 0.9 mM KH ₂ PO ₄ & 20mM Hepes at pH7 (adjusted by adding 10 M KOH)		
Clarkson et al.	1984	<i>Streptococcus mutans</i> FA1 cultured in thioglycollate broth containing 3.5% w/v dextrose & 2% w/v gelatin	6 wk	N
ten Cate, et al.	1982	2.2 mM Ca, 2.2 mM P 50 mM buffer (acetic acid/K acetate) pH=5.0, 37°C, nonstirred	0-14	Y

Table 10. Summary of Selected Published Studies on Demineralization Methods.

3. MATERIALS AND METHODS

3.1 *Tooth Preparation*

Forty-five extracted human molar or premolar teeth were used in the study. The teeth were gently pumiced, two coats of acid-resistant nail varnish (Max Factor, Proctor and Gamble, Surrey, UK) were painted on the buccal for the first 9 teeth, on the occlusal for the next 9 molars or premolars and on the proximal (either mesial or distal) for another 9 specimens, leaving an exposed window of enamel (approximately 3mm x 5 mm). The remaining 18 were left untouched and would not be exposed later to the demineralizing and remineralizing solutions.



Fig. 7. Sample tooth painted with nail varnish, leaving an exposed window of enamel.

3.2 *Lesion Production*

A pH cycling regime based on an in vitro pH-cycling study comparing solution and gel-prepared enamel lesions [Damato et al., 1988] was chosen for use in this

study. The reasons for the choice of demineralization/ remineralization method were: 1) advantage of buffered solution over acidified gel in creation of subsurface lesion [Damato *et al.*, 1988; Theuns *et al.*, 1984], 2) ease of set-up in comparison with other methods of demineralization, 3) acetate buffers produced lesions approximately 1½ times deeper than lactate buffers at the same pH and total concentration even though lactic acid is a stronger acid than acetic acid [Featherstone *et al.*, 1981].

The composition of the solutions were the following:

Demineralizing solution: 8.3 mmol/L CaCl₂
8.3 mmol/L NaH₂PO₄
50 mmol/L acetic acid
NaOH to pH 4.0

Remineralizing solution: 2 mmol/L Ca
2 mmol/L P
<0.03 ppm F⁻
pH 6.85

The teeth were exposed to the demineralizing solution for 16 hours (per day). After being rinsed with distilled water, the teeth were placed in a remineralizing solution for the remainder of the day. This procedure was repeated daily for 5 days. Then, they were left to air-dry for 30 minutes. The nail varnish was removed with acetone from the teeth and the teeth were individually mounted on Xantopren and Optosil Comfort (a silicone putty/wash impression system) (Heraeus Kulzer, Inc., Dormagen, Germany) to simulate the clinical situation. Each of the specimens from the group with the proximal window was mounted with an adjacent tooth (N=9, coming from the group of teeth unvarnished and unexposed to acid).

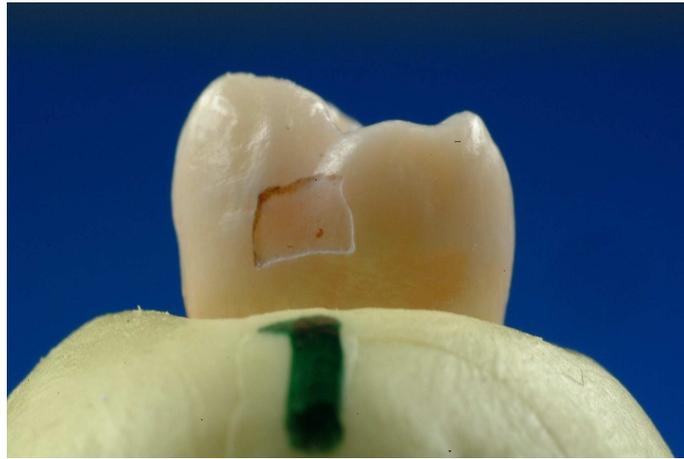


Fig. 8. Sample tooth with a smooth-surface artificial caries lesion created after demineralization.



Fig. 9. Sample tooth with an occlusal-surface artificial caries lesion created after demineralization.



Fig. 10. Sample tooth with a proximal-surface artificial caries lesion created after demineralization mounted with an adjacent tooth.

3.3 Image Acquisition

The detailed procedure followed for DIFOTI image production is described in the appendix section of this paper. Below are sample images of teeth taken with the DIFOTI device.

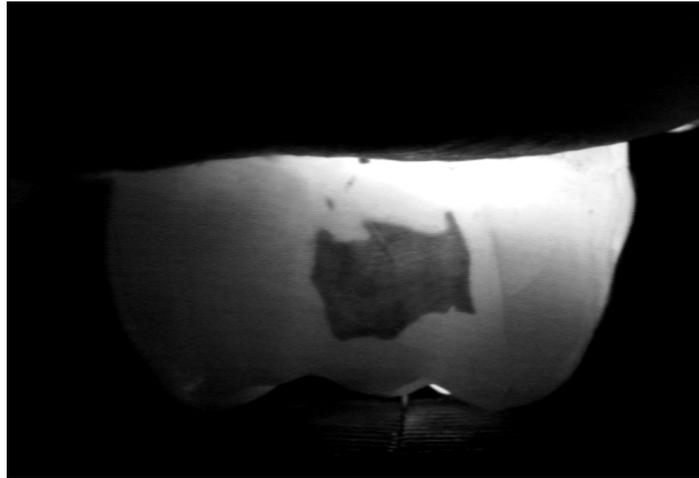


Fig. 11. DIFOTI image of a tooth with a smooth-surface caries.

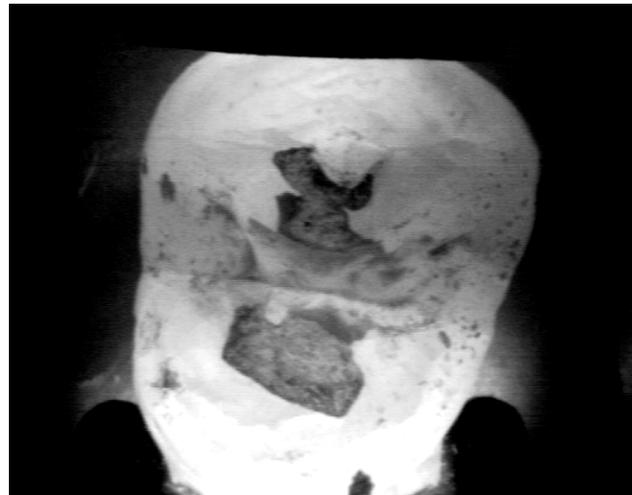


Fig. 12. DIFOTI image of a tooth with an occlusal-surface caries.



Fig. 13. DIFOTI image of a tooth with a proximal-surface caries.

3.4 Image Observations (Caries Detection)

Dichotomous measurement of the digitized images (presence or absence of caries) was done by visual observation and then recorded, a healthy tooth taking a “0” value and a carious tooth taking a “1” value. Lesions appear as darker areas than the surrounding sound enamel [de Josselin de Jong, *et al.*, 1995]. This diagnostic record was to be referred to at a later time for analysis.

3.5 Light Penetration Measurements or Absorbance Values

For tooth images both exhibiting and not exhibiting caries, light penetration of the DIFOTI device through the lesion or sound tissue was calculated with a software program, called View3D, particularly designed for this study, comparable to the one described by De Josselin de Jong *et al.*, 1995 to quantify light scattering.

Normally, lesion scatters and absorbs more light than surrounding healthy tissue. In images, therefore, lesion appears as a darker area against the more translucent brighter background of the surrounding sound surface [Electro-Optical Sciences, Inc., 2004]. Triangulations, consisting of 9-15 lines extending to gray areas beyond the boundary of the carious lesion, were made with the PC mouse on the lesion observed on the image. Each pixel value in the lesion image

corresponds to an amount of brightness. Sound enamel values on the triangles around the lesion were interpolated. The light penetration value is obtained from the percentage difference between the measured brightness (actual light intensity) and interpolated brightness (reconstructed light intensity) [de Josselin de Jong et al., 1995]. Based on the histogram of the observed gray levels, the 1% quantile and the median of the gray levels within the region of interest were selected for further evaluation.

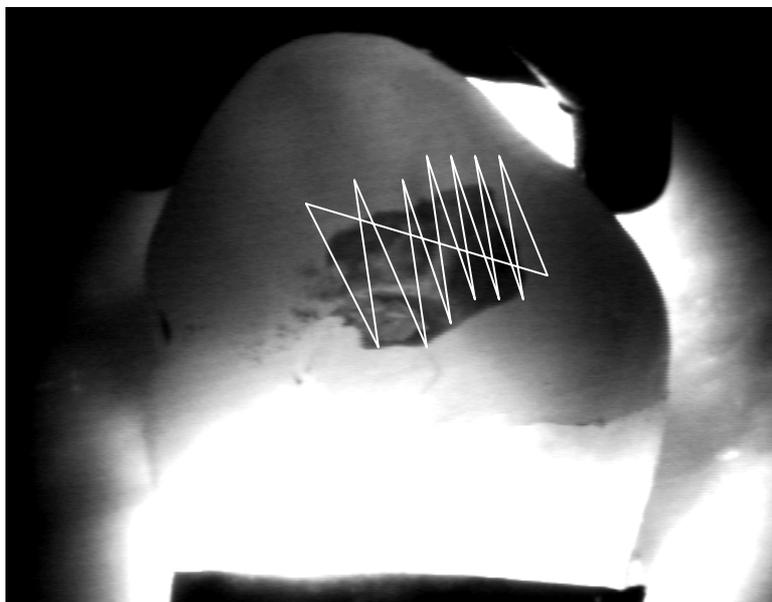


Fig. 14. Triangulations. Preliminary step to quantitative measurement of the lesion with the software program View3D.

A detailed description of how to operate the View3D software can be found at the appendix section of this paper.

3.6 Histological Sections

Specimens were mounted on self-curing resin Technovit [Heraeus Kulzer, Inc., 2004b]. The teeth were sectioned approximately 120-150 μm thick, using a saw

microtome (Leitz 1600, Germany) having a 300 μ m-thick annular frame. The tooth sections were stained and fixed to microscope slides.

The steps followed for staining were [American Registry of Pathology, 1960]:

1. Hydrate to water.
2. Mayer's hematoxylin for 15 minutes.
3. Wash in running tap water for 20 minutes.
4. Counterstain with eosin for 15 seconds.
5. Dehydrate in 95% and absolute alcohols, two changes of 2 minutes each or until excess eosin is removed.
6. Clear in xylene, two changes of 2 minutes each.
7. Mount in Permount.

The tooth slices were viewed under a light microscope (STEMI SV 11, Zeiss, Göttingen, Germany). Images were taken with a digital camera, then stored in hard disc for subsequent analysis.



Fig. 15. Sample histological tooth section with a smooth-surface caries.

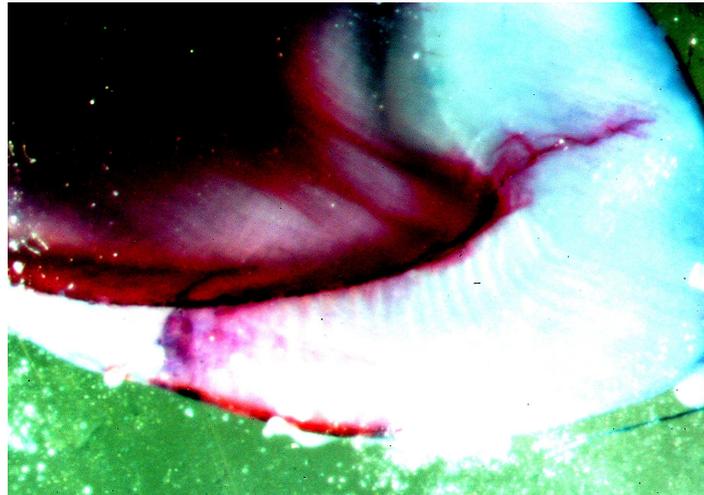


Fig. 16. Sample histological tooth section with a proximal-surface caries.

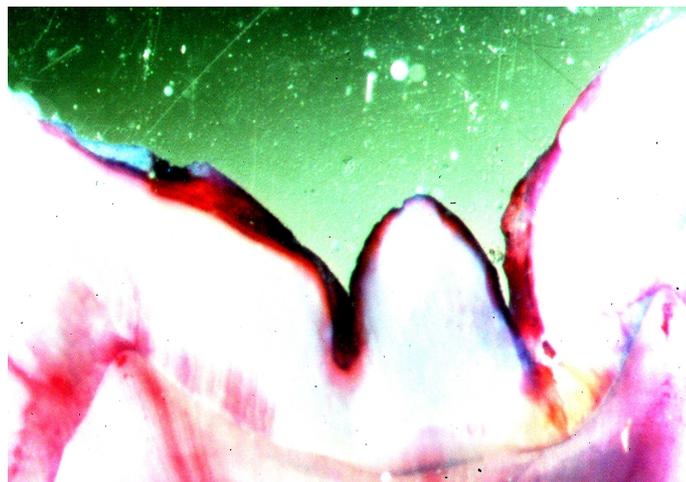


Fig. 17. Sample histological tooth section with an occlusal-surface caries.

3.9 Lesion Depth Calculation

Depth calculation of individual lesion was done with the aid of the computer program ImageJ (NIST, USA). Images of tooth sections were saved as JPEG files and imported one by one to ImageJ.

Prior to lesion depth measurements, the spatial scale of the images was defined, providing a known distance and corresponding distance in pixels of a region of interest on any of the images. Line measurements in millimeters would then be subsequently provided by the program. For each lesion, 10 lines were drawn by

the mouse evenly distributed over the lesion area, from the surface of the lesion to the enamel surface, taking note that the lines were perpendicular to the lesion surface. Line measurements for each lesion were thus obtained and averaged. The mean lesion depth of all the tooth sections was also calculated.

3.10 Validation of the DIFOTI Diagnostic System

3.10.1 Sensitivity and Specificity

The previous dichotomous classification of teeth as sound or carious by visual observation of digitized images was confirmed by the histological sections. Data consisted of values "0" and "1," the former meaning sound and the latter meaning carious as seen from the digitized images and by microscopy. The formula for computing sensitivity and specificity values could be found in the appendix section of this paper and was followed to obtain the degree of sensitivity and specificity of the DIFOTI system for the different caries sites.

3.10.2 Area Under the ROC Curve

Area under the ROC curve was calculated with the aid of the SPSS computer program, using the absorbance values obtained from prior statistical analysis made with the View3D program and dichotomous measurement of the gold standard. With the possibility of an artefact ranging from a value of 0-30 due to noise produced by the device, 1% and median quantile gray level DIFOTI image were selected for light penetration values. Cut-off values for 1% and median gray level evaluation were also determined. The upper and lower bounds on 95% confidence intervals and standard error were computed simultaneously with the AUC measurements.

3.10.3 Correlation of Light Penetration Values of the DIFOTI and of Lesion Depth of Teeth

Quantitative measurements of DIFOTI and the gold standard were correlated. With the lesion depth and 1% and median gray level image, Pearson's correlation coefficient was computed. Regression analysis, curvefit with linear model, was also done. However, these two statistical analyses, Pearson's correlation and curvefit, were performed only on data obtained from buccal lesion measurements of both imaging modalities.

4. RESULTS

4.1 Dichotomous Classification of Teeth Based on Visual Observations of the Digitized Images of DIFOTI and of Histological Sections (Qualitative Measurements)

Among the group of teeth that had undergone demineralization-remineralization (N=27), nine teeth did not exhibit surface changes on DIFOTI images and four teeth showed no sign of demineralization upon observation of histological sections. And among the initially-sound grouped teeth, teeth that had not undergone demineralization-remineralization (N=18), three were detected carious by both visual observations of the digitized images of DIFOTI and of microscopy.

Table 11 records the dichotomous evaluation of teeth by visual observation of DIFOTI images and by microscopy. As previously stated, values “0” and “1” signify absence and presence of demineralization, respectively.

Tooth Surface	Tooth Signification	Histology	DIFOTI
	1	1	1
	2	0	0
	3	1	1
	4	1	1
	5	0	0
	6	1	1
	7	0	0
Buccal	8	1	1
	9	1	1
	10	0	0
	11	0	0
	12	0	0
	13	0	0
	14	0	1
	15	0	0
	16	1	1
	17	1	1
	18	1	1
	19	1	1
	20	1	1
	21	1	1
Occlusal	22	1	0
	23	1	1
	24	1	1
	25	1	0
	26	0	0
	27	0	0
	28	1	1
	29	0	0
	30	0	0
	31	0	1
	32	1	0
	33	1	0
	34	1	1
	35	1	0
	36	1	1
	37	1	0
Proximal	38	1	1
	39	1	0
	40	0	0
	41	0	0
	42	0	0
	43	0	0
	44	0	0
	45	1	1

Table 11. Dichotomous evaluation of teeth by visual observation of DIFOTI images and by microscopy. Basis for calculation of sensitivity and specificity values.

4.2 Lesion Depth of Teeth and Light Penetration Percentage Values of the DIFOTI (Quantitative Measurements)

Teeth exhibiting presence of caries were further subjected to evaluation. Lesion depth was obtained, where demineralization was visible on a tooth. Table 12 lists the lesion depth of individual tooth. Teeth diagnosed sound histologically were given “0” value. From this recorded data, average depth of the lesions was computed: 62.39 μ m. Lesion depth of teeth would be subsequently correlated with the corresponding absorbance values derived from the 1% and median quantile gray level DIFOTI images. Alongside the computed lesion depth of teeth on the table are the light penetration percentage values computed from the 1% and median quantile DIFOTI images.

Tooth Surface	Tooth Signification	Lesion Depth (Histo.)	Light penetration values (DIFOTI)		
			1%quant	50%quant	
		1	66	93.91	27
		2	0	54.28	14.18
		3	50.61	86.1	72.68
		4	64	70.63	37.04
		5	0	99.92	48.29
		6	22	46.29	0.39
		7	0	99.89	81.95
Buccal		8	68	59.7	39.53
		9	46	74.72	51.01
		10	0	90.36	10.19
		11	0	57.05	13.34
		12	0	38.84	23.88
		13	0	24.87	15.26
		14	0	54.47	9.28
		15	0	79.08	21.06
		16	289	67	23.97
		17	98	61.3	30.56
		18	176	57.92	14.62
		19	320	62.79	29.67
		20	117	48	0.66
		21	127	53.83	12.74
		22	31	45.78	13.51
Occlusal		23	87	70.84	30.6
		24	151	57.58	12.42
		25	40.057	60.36	32.23
		26	0	46.35	32.59
		27	0	20.5	19.38
		28	20.143	48.14	3.84
		29	0	31.83	6.16
		30	0	39.93	9.8
		31	0	94	33
		32	26.73	48.73	16.02
		33	186	23,58	1.61
		34	29	47.1	23.87
		35	69	69.44	47.4
		36	292	66	28.69
		37	47	99.96	38.84
Proximal		38	42	79.74	44.6
		39	58	83.21	55.37
		40	0	8.86	37.17
		41	0	45.25	1.35
		42	0	7.85	17.6
		43	0	41.06	7.85
		44	0	15.16	6.96
		45	284.91	55.8	1.63

Table 12. Lesion depth and light penetration percentage values computed from 1% and median quantile DIFOTI gray-level images.

4.3 Validity of the DIFOTI System

4.3.1 Sensitivity and Specificity

Site-specific sensitivity and specificity values of the DIFOTI for the detection of initial caries are listed in Table 13.

	Sensitivity	Specificity
Smooth	1	0.88
Occlusal	0.82	1
Approximal	0.44	0.83

Table 13. Sensitivity and specificity values of DIFOTI in the detection of smooth, occlusal and approximal incipient caries.

4.3.2 ROC Curves

The previous absorbance values obtained from the 1% and median quantile DIFOTI images and the dichotomous classification of teeth based on visual observation of histological sections were used as data for computation of AUC (area under the ROC curve) determining the diagnostic accuracy of the DIFOTI system for initial lesions.

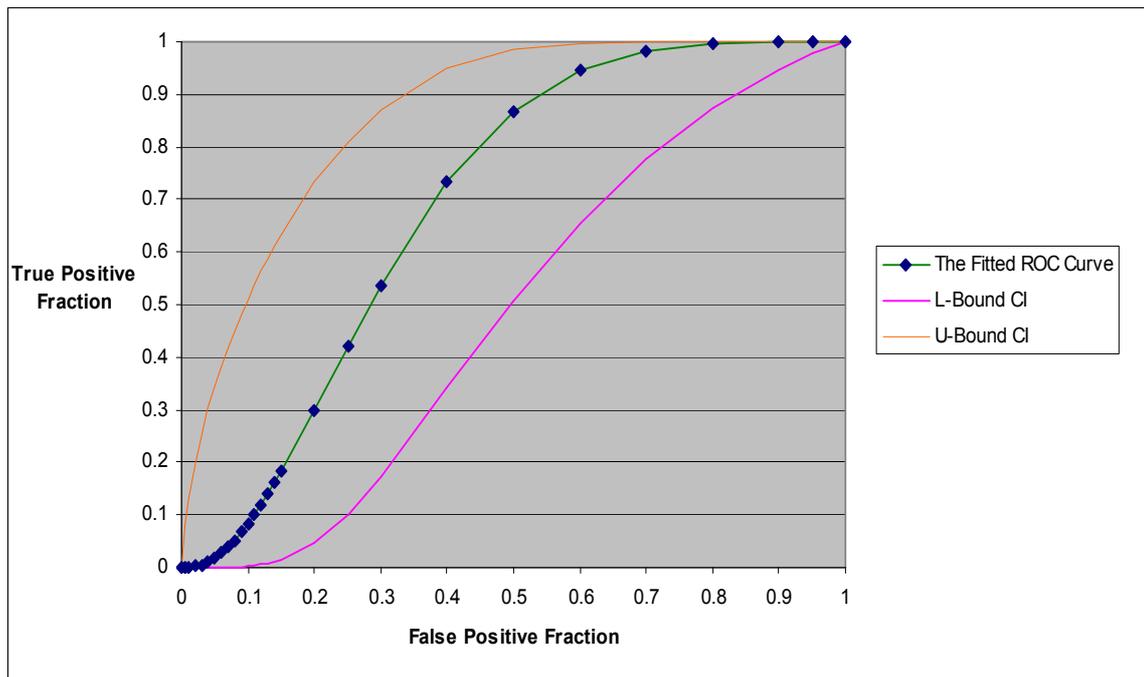


Fig. 18. ROC curve for the diagnosis of superficial artificial caries by the DIFOTI system (corresponding to 1% quantile gray level DIFOTI image).

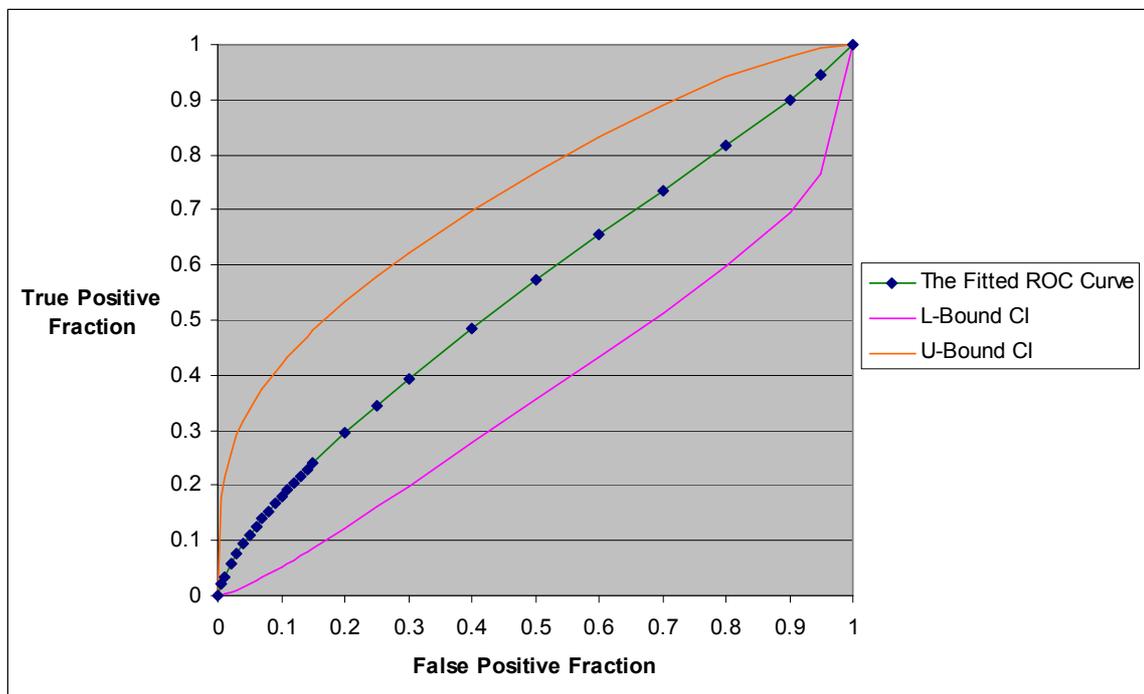


Fig. 19. ROC curve for the diagnosis of superficial artificial by the DIFOTI system (corresponding to median quantile gray level DIFOTI image).

	AUC	Standard Error	95% CI	
			L-bound	U-bound
1% quantile	0.69	0.09	0.51	0.87
median quantile	0.59	0.09	0.42	0.76

Table 14. Area under the curve, standard error and lower- and upper-bound 95% confidence interval for the 1% and median quantile absorbance values.

With no preference for a high sensitivity or specificity at the cost of the other, the cut-off value for the light penetration percentage on 1% quantile gray level DIFOTI image is 47.1, with calculated sensitivity and specificity of .74 and .79 respectively. Selected cut-off value for light penetration percentage on median quantile gray level DIFOTI image is 23.87, with calculated sensitivity of 72.72 and specificity of 56.52.

4.3.3 Correlation of Lesion Depth of Teeth and Light Penetration Percentage Values of the DIFOTI

Lesion depth and light penetration values were plotted to determine their correlation. Pearson's correlation coefficients obtained from the quantitative measurements of buccal lesions by both imaging modalities, .199 and .318 corresponding to 1% and median quantile gray level image respectively, signify no relationship between the two test variables. Similarly, significance values from the curvefit show no correlation between lesion depth and light penetration values.

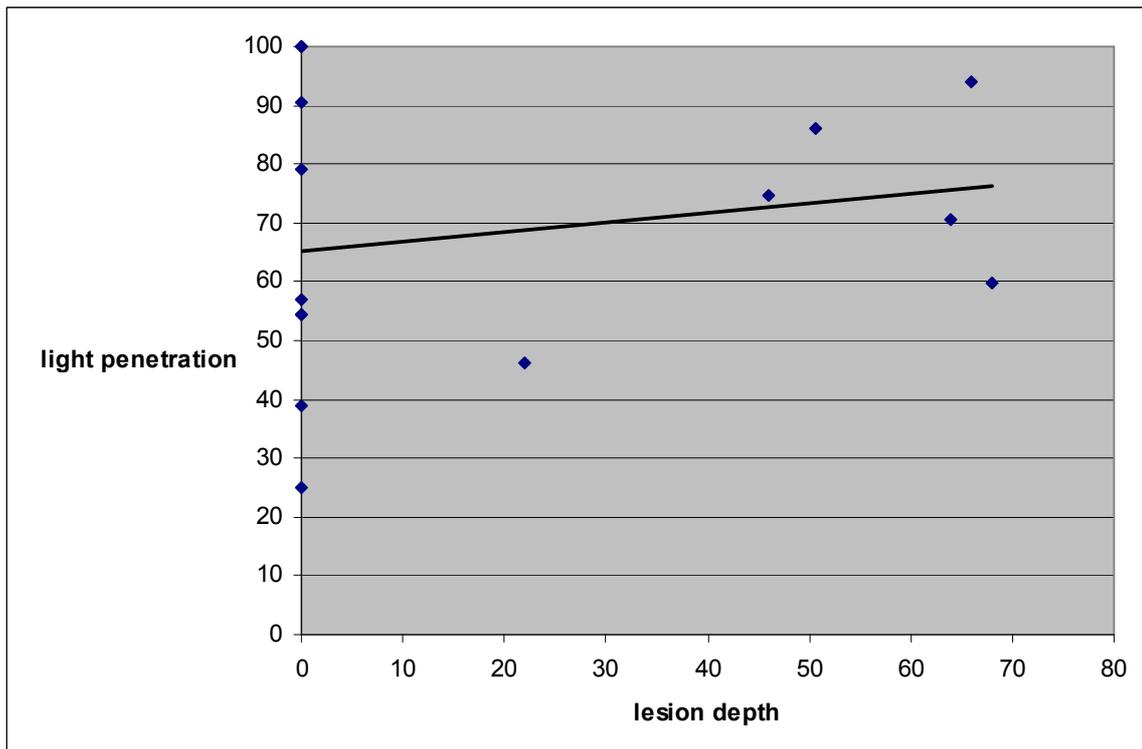


Fig. 20. Correlation of lesion depth and light penetration values on 1% quantile gray level image.

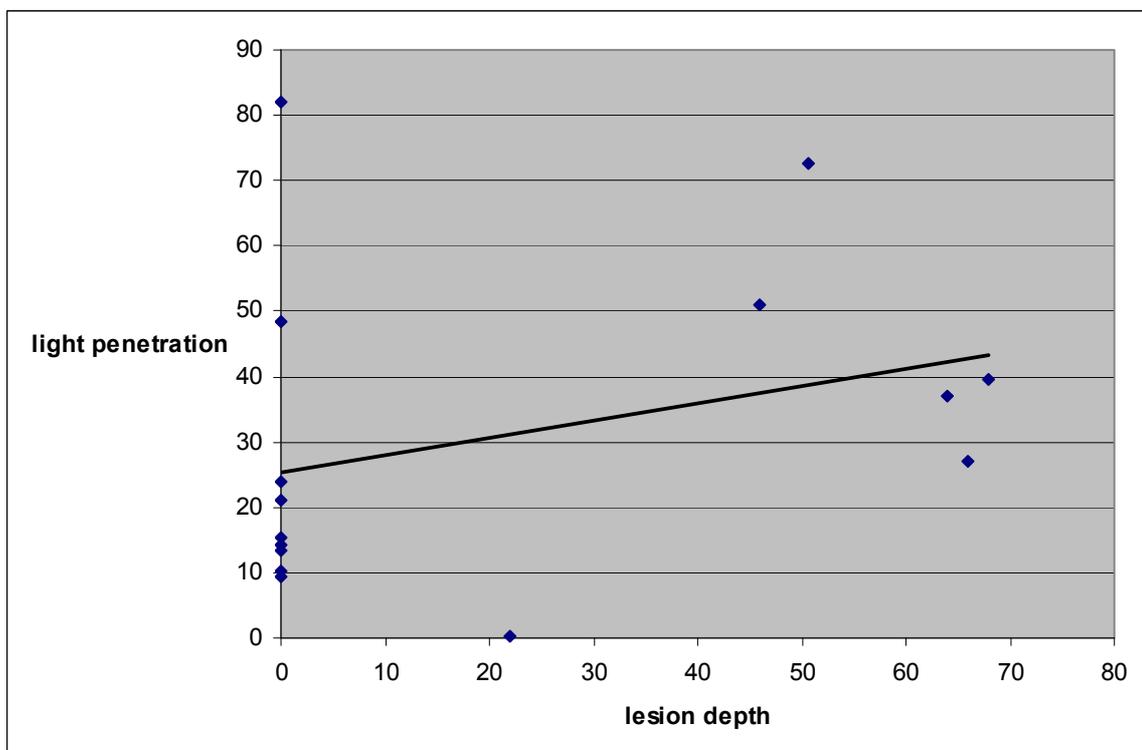


Fig. 21. Correlation of lesion depth and light penetration values on median quantile gray level image.

5. DISCUSSION

Caries, a dynamic process, is usually measured as a dichotomous variable of presence or absence of disease by current methodologies. Caries monitoring tools must be developed that would allow comparison of serial measurements over time and the assessment of the status of lesions like arrest or reversal of the demineralization [Pitts, 2004; Stookey et al, 1999].

5.1 In-vitro Evaluation of the Validity of a Caries Diagnostic System

In-vitro studies should always precede in-vivo evaluation of new diagnostic systems [Pine and ten Bosch, 1996]. Therefore, in-vitro evaluation of the DIFOTI has been performed in this study to lead to clinical evaluation of the device in the future. They have, however, serious limitations. They are more difficult to generalize to the environment of dental practice. They permit careful selection of individual teeth or surfaces for assessment, rather than forcing the inclusion of a more representative set of teeth or surfaces. They also minimize many limitations imposed by working within the oral cavity [Bader *et al.*, 2001].

5.1.1 Microscopy as Gold Standard

To evaluate the validity of a caries diagnostic system, its outcome should be compared to the true state of the lesion, often referred to as *gold standard* [Pine and ten Bosch, 1996; Hintze and Wenzel, 1999, 2003]. Three universal criteria must be fulfilled for a robust gold standard. It should be [Hintze and Wenzel, 1999, 2003]:

1. established by a method that is itself precise, i.e. reproducible,
2. reflect the patho-anatomical appearance of the disease,
3. established independently of the diagnostic method under evaluation.

Microscopy has been chosen in this research for validation of the DIFOTI system as it fulfils the criteria for a gold standard stated above. Pine and ten Bosch, 1996, stated histological examination and quantitative microradiography as the preferred means of validation. In this study, microscopy has been used as the gold standard.

It is to be taken into consideration that in caries diagnosis, even the histological validation is open to errors related to tissue loss due to tooth sectioning and the subjectivity involved in assessing microscopic appearance of incipient lesions. Thus, there is difficulty in obtaining the objective, realistic and truly comparable validated estimates of sensitivity and specificity [Pitts, 1997]. A study on inter- and intra-assessor reproducibility of 3 scorers individually assessing histological sections was performed by Pitts *et al.*, 2001. Agreement was calculated using Cohen's kappa. Table 15 and 16 are a summary of the intra- and inter-assessor comparisons of all viewed tooth surfaces.

Caries	Assessor 1 t1 vs. t2	Assessor 2 t1 vs. t2	Assessor 3 t1 vs. t2
D1	0.58	0.74	0.82
D3	0.88	0.83	0.37

Table 15. Intra-observer agreement of three scorers each assessing histological sections.

Caries	Assessor 1 vs. Assessor 2	Assessor 1 vs. Assessor 3	Assessor 2 vs. Assessor 3
D1	0.58	0.63	0.46
D3	0.60	0.44	0.64

Table 16. Inter-observer agreement of three scorers each assessing histological sections.

Tables above show considerable variation in intra- and inter-observer agreement in grading caries on histological sections. This leads to the question: Is histology a valid gold standard? Qualitative classification of lesions based on visual observation of microscopic sections is subjective. Training of examiners is necessary prior to classifying lesions on samples. In assessing validity of new emerging diagnostic technologies, microradiography may be utilized as an alternative gold standard. It eliminates tooth tissue loss due to sectioning and produces high quality level of digitized images of the samples. Further investigation as to which is the best suitable gold standard to be used for assessing the validity of DIFOTI is still needed.

5.1.2 Artificial Caries to Simulate Natural Lesions

Wenzel and Hitze, 1999 disapprove that artificial lesions simulate natural caries and state therefore that they do not contribute to the validation of diagnostic tests, since there is no evidence that these tests detecting artificial caries will perform in an equivalent manner when used on carious teeth. However, difficulty in acquiring teeth with mere superficial caries has led the researchers to opt for artificial incipient caries in place of true initial caries for evaluation of the DIFOTI system. Careful selection of demineralizing-remineralizing solution was used though to create superficial artificial lesions. The previous chapter on literature review provides a comparison of demineralizing solutions used in different studies to serve as aid in the selection of the needed solution for the current research.

The solutions for producing artificial lesions decided upon to be used for preparing artificial carious lesions were based on the pH-cycling regime used by Damato et al., 1988 to compare the de-/remineralization behavior of lesions. The advantages and disadvantages of the Damato protocol are stated below:

Advantages:

1. The Damato protocol, a chemical system for producing artificial caries lesions, is chosen over representatives of the bacterial system as it allows regulation of the experimental environment and is relatively economical.
2. Damato *et al.*, 1988, in his study comparing the de-remineralization behavior of acidified undialyzed gelatin system and a buffered solution, has shown that the demineralization rates of the lesions, in terms of the total mineral loss, produced by the latter were greater than those created by the former. Another study supporting buffer solution is done by Theuns *et al.*, 1984. Acetate buffer containing system was shown to produce caries-like lesions in enamel without the use of surface dissolution inhibitors or gels. Buskes *et al.*, 1985, in addition, utilized an apparatus for the de- and remineralization in vitro under constant composition conditions (using an acetate buffer containing methylhydroxydiphosphate, MHDP) and was able to demonstrate the ability of this method to produce lesions with reproducible depths. Hence, a buffer solution, the pH-cycling regime used by Damato *et al.*, 1988, was selected to be used to produce artificial lesions.

Disadvantage:

1. In comparison to the bacterial systems of providing an artificial cariogenic challenge to the tooth, chemical systems such as the Damato protocol do not simulate the in vivo situation as accurately as the former. The bacterial systems therefore permit more clinically relevant in vitro investigations than the latter. However, the choice of a model to be used for the creation of an artificial lesion still greatly depends on the purpose of the study [Fontana *et al.*, 1996].

5.2 Parameters for Assessing Validity of the DIFOTI System

5.2.1 Sensitivity and Specificity

Sensitivity and specificity constitute the basic measures of performance of diagnostic tests [Park *et al.*, 2004]. Their main disadvantage, however, for evaluating the validity of caries diagnostic systems is the need to limit the classification of a carious lesion to be caries or sound. When using these parameters for assessing diagnostic accuracy, the sample should not contain large obvious lesions to avoid overestimation of sensitivity. Artificial incipient caries, therefore, ranging from 3-5 mm in size with average depth of 62.39 μm have been utilized in the evaluation of the DIFOTI in this study.

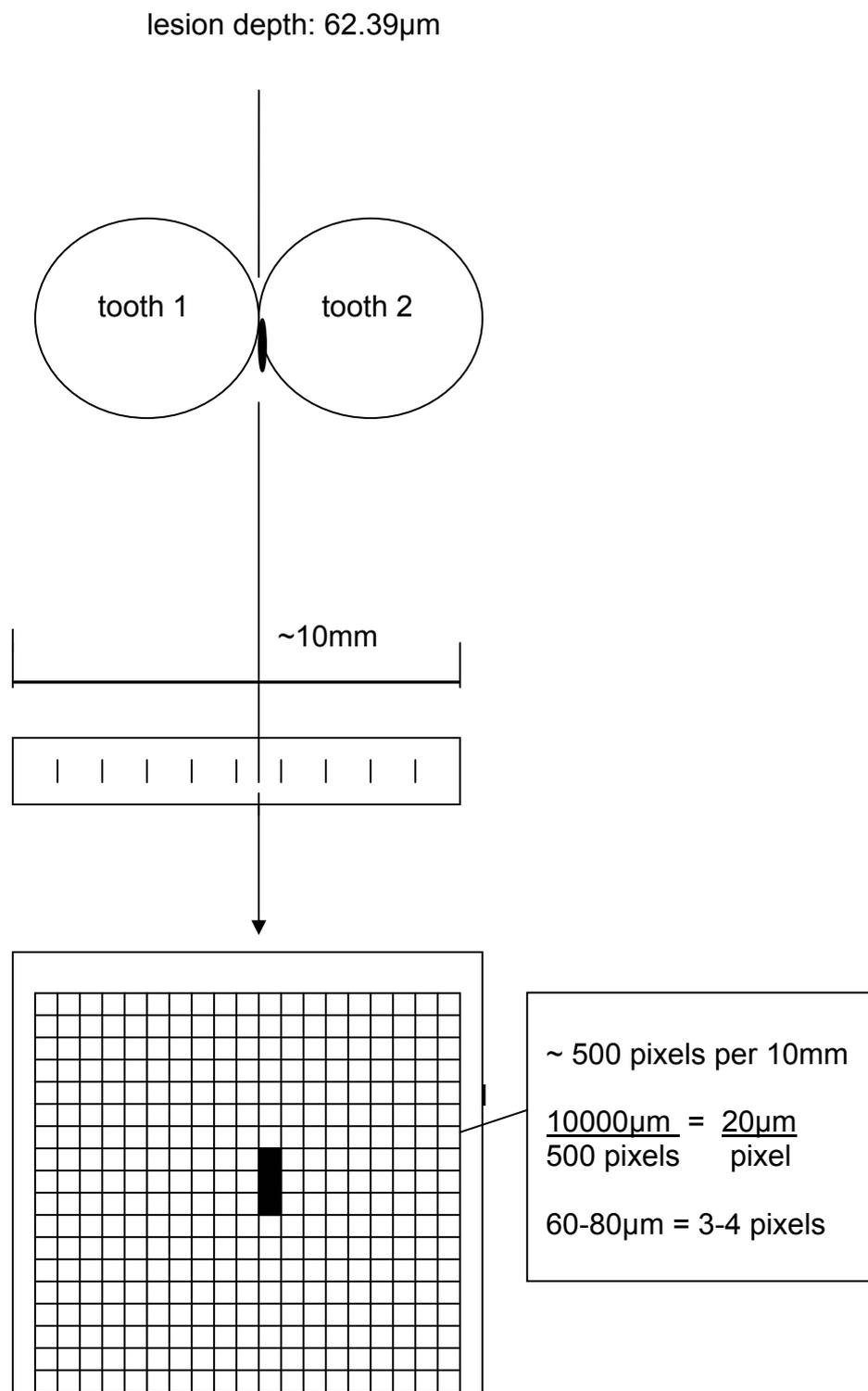


Fig. 22. Difficulty in viewing proximal caries with the DIFOTI system due to shallow lesion depth, which appear only ~3-4 pixels on the computer screen.

A comparison of sensitivity and specificity values of the DIFOTI system acquired by researchers and by this current study is stated below.

	Sens.		
	Schneiderman <i>et al.</i> , 1997	Ando <i>et al.</i> , 2004	Current study results
Approx.	0.69	-	0.44
Occlusal	0.80	0.33	0.82
Smooth	0.41	0.10	1

Table 17. Sensitivity values of DIFOTI from various research.

	Spec.		
	Schneiderman <i>et al.</i> , 1997	Ando <i>et al.</i> , 2004	Current study results
Approx.	0.73	-	0.83
Occlusal	0.87	0.83	1.0
Smooth	0.90	0.99	0.88

Table 18. Specificity values of DIFOTI from various research.

The results show the potential of DIFOTI for the detection of initial caries on smooth and occlusal lesions but it fails to diagnose early lesions on approximal surfaces; nevertheless, it still has good specificity in this regard. The lesion depth of the tooth specimens is approximately 62.39 μ m. When the tooth is digitized with a CCD chip, the distance of two pixels is approximately 50 μ m. The proximal lesion is then 3-4 pixels wide, hence, there is difficulty in seeing the lesion (see Figure 22). A previous study by Schneiderman *et al.*, 1997 agree with this present finding that: the system has good sensitivity in detecting occlusal lesions and poor sensitivity for approximal lesions. However, they demonstrate less

sensitivity for smooth caries (0.41) compared with the value obtained from this research (1.00). The most current published study by Ando *et al.* shows the lowest sensitivity values of the DIFOTI in detecting occlusal and smooth lesions; but the specificity values are in proximity to those obtained both from the study by Schneiderman *et al.* and this present study.

Based on visual interpretation of the digitized images, this *in vitro* study on superficial artificial caries has shown that DIFOTI has very good diagnostic properties concerning sensitivity and specificity on smooth and occlusal surfaces. However, DIFOTI, fails to detect incipient proximal caries satisfactorily. Nevertheless, it still has good specificity proximally.

5.2.2 Receiver Operating Characteristics Curve

Ten Bosch and Angmar-Månsson, 2000, suggested the receiver operating characteristics to validate a quantitative method with a dichotomous gold standard, with the requirement that the cutoff value should be determined in relation to the use of the method. Such statistical method has been applied in this study. In particular, the AUC (area under the curve), one of the summary measures of the ROC curve, has been used [Park *et al.*, 2004]. According to a rough guide for classifying the accuracy of a diagnostic test [Tape, 2004], the AUC measurements (0.69 corresponding to the 1% quantile DIFOTI image and 0.59 for the median) in general prove the system insufficient for initial caries detection.

Correlation between the dichotomous classification of teeth based on microscopic observation and quantitative measurements of the DIFOTI system as indicated by the upper and lower bounds of the 95% confidence interval is between .51 and .87 for 1% quantile DIFOTI image and between .42 to .76 for median quantile image. Basing on the general guideline that values above 0.75 indicate good reliability [Tranaeus *et al.*, 2002], the upper and lower confidence limits indicate good reliability of the result of the AUC measurement. By obtaining too the standard error, approximately .09 for both quantile images, good reliability of the

assessed value of the diagnostic ability of the DIFOTI system is confirmed [“Standard Error of Measurement”, 2004].

5.2.3 Correlation of Lesion Depth and Absorbance Values Obtained from DIFOTI Gray-level Images

Pearson’s correlation coefficients corresponding to both 1% and median gray level DIFOTI image signify no correlation between lesion depth and decrease of light penetration values. The curvefit confirms the non-significance of the correlation. DIFOTI, therefore, can not be recommended yet to be used for quantitative caries monitoring of initial caries with the computer program View3D; further investigation is needed.

5.3 Ideal Characteristics of Measurements

A review of the ideal characteristics of measurements by Pitts, 1997 may facilitate in evaluating the DIFOTI system.

1. non-invasive
2. provide simple, reliable, valid, sensitive, specific and robust measurements of lesion size and activity
3. based on biological processes directly related to the caries process
4. affordable, acceptable to dentists and patients
5. capable of early implementation into both clinical practice and research settings
6. its use should promote informed and appropriate preventive treatment decisions which deliver long-term oral health

With the View3D software incorporated into the DIFOTI system, it is the presupposition of the researcher that the DIFOTI has the potential to allow measurements of lesions over time. Quantitative measurements are possible leading then to objective results, decreasing variations in the judgements of clinicians in diagnosing caries. Thus, it could permit monitoring of changes over

time in mineral loss or gain resulting from preventive treatments and advice. However, accuracy of the images produced (e.g. free or minimal of artefacts) must first be assessed and ascertained. In addition, this study has proved no correlation of the results obtained from the statistical analysis of the View3D and of the lesion depth of the histological sections of teeth. Therefore it could not be used yet for monitoring of lesions. Nevertheless further examination of the ability of the software program to provide absorbance or light penetration measurements is recommended.

5.4 DIFOTI in Comparison with the Existing Diagnostic System/s (e.g. QLF, DIAGNOdent, ECM)

At present there is no single diagnostic method that can reliably detect small superficial lesions and monitor them [Stookey *et al.*, 1999]. Applying, therefore, multiple tests to detect caries in an individual patient increases the overall efficiency of the diagnosis [NIH Consensus, 2001]. DIFOTI must be compared too to the existing diagnostic system/s (e.g. QLF, DIAGNOdent, ECM) which it is to aid or replace [Pine and ten Bosch, 1996].

DIFOTI and QLF both offer the advantage of real-time imaging. The handpiece which contains a charge coupled device camera captures the tooth image. The tooth under examination is displayed on a PC screen, the image is saved and analyzed by the software [Pretty *et al.*, 2002b; Electro-Optical Sciences, 2004a]. It is said that for both DIFOTI, learning to interpret the images can take the dental examiners only a matter of hours [Electro-Optical Sciences, 2004a]. In contrast, a study in Indiana (Ferreira Zandoná *et al.*, 1999), determined that the use of the QLF required approximately 15 minutes for an examination of all tooth surfaces [Stookey, 2004]. In addition, both systems are user-friendly, owing to the advantage previously stated – the patient can see his teeth on the computer screen – and the painlessness of both techniques and the handpieces could easily be accommodated in the mouths of the patients. However, with the DIFOTI, this ease of accommodation into the mouth is only an assumption of the author

based primarily on the availability of different sizes of mouthpiece; clinical observation of patients is required to ascertain this.

The DIFOTI is not even of equal merit to the QLF system, and therefore could not replace the latter. Stookey, 2004, has stated that with current hardware and software refinements and the results of long-term clinical validation studies that are in progress, the QLF may be the future method of choice for caries clinical trials. A major advancement of the system, for example, is the integration of a markedly improved video repositioning system to the QLF instrumentation for reproducibly capturing the desired images of lesions at subsequent examination periods. DIFOTI, to equal this device in performance or exceed its ability, must also undergo various studies leading to its improvement, which may take years too. Various research then, however, about its present capabilities as a diagnostic tool must first be undergone by the device, before it advances to competing with other systems, such as QLF.

In terms of aiding another caries diagnostic system, it is the opinion of the author that the DIFOTI is better complemented with the DIAGNOdent than with the QLF. Instead of image capture and analysis as they are with DIFOTI and QLF, DIAGNOdent evaluates the fluorescence and displays its readings on the display [Kavo, n.d]. DIFOTI can be confirmed by DIAGNOdent and vice-versa. Two devices of different approaches may work together better than devices of similar approach to caries diagnosis paired.

In comparison to ECM, a method based on electrical conductance measurement, DIFOTI or other optical caries diagnostic devices may be a better choice to be employed in the dental practice. Several factors may affect measurements provided by the ECM: enamel maturation, temperature, the airflow used to dry the site [Ellwood and Cortes, 2004], moisture isolation, defects in the enamel extending to the dentinoenamel junction, incomplete contact between the conducting medium and the tooth surface and possibly if a lesion has a very dense surface zone [Ashley *et al.*, 1998]. Fixed-frequency measurements have some limitations in the amount of information they provide on tooth structure and,

hence, on caries status. In addition, ECM measurements are currently limited to occlusal sites [Longbottom and Huysmans, 2004]. However, there is no evidence yet that it DIFOTI is a better choice than ECM for caries diagnosis in clinical practice, as the former still has to prove its sensitivity to detect caries on all surfaces of the teeth.

The DIFOTI device uses standard or safe white light [Ganz 2003; Electro-Optical Sciences, Inc. 2004a]. Light is a particularly suitable tool for the study of teeth. The regular structure of teeth ensures good propagation of light through the crystalline enamel and the tubules of dentin. The size of the structures is comparable with the wavelength of visible and near-infrared light. Disruption to the ordered structure of a tooth increases the likelihood of scattering of light that passes into the tooth. The uptake of fluid into pores created by demineralization – in addition to the uptake of exogenous stain, bacterial breakdown products, and other contaminants present as a result of the caries process – will change the normal interaction of light with tooth structure. In addition to scattering, these changes will include absorption and fluorescence.

The wavelength is important for certain interactions, in particular, as already mentioned, for absorption and scattering. The probability of scattering of a scattering event depends on the relative size of the wavelength and the scattering site. Scattering probability decreases with increasing wavelength. Hence, longer wavelengths scatter less than shorter wavelengths and therefore can penetrate objects more deeply [Hall and Girkin, 2004]. The wavelength of the DIFOTI light, as it uses visible light, ranges from 400nm to 700nm [Schneiderman *et al.*, 1997; Keem and Elbaum, 1997; Hall and Girkin, 2004] . The QLF's wavelength is within that range too mentioned (488 nm). As for DIAGNOdent, the tooth is illuminated by red laser light with irradiation at 655 nm [Lussi *et al.*, 2004].

The use of longer wavelengths for diagnostic techniques may help with the penetration through the tissue, but the counterbalance to this is that the resolution of an image is directly proportional to the wavelength. This means that,

as longer wavelengths are used, the ultimate resolution possible (the smallest feature that can be seen) falls. It can be noted that DIFOTI and QLF, both relying on imaging, compared with DIAGNOdent which evaluates the fluorescence and displays its readings on the display, have shorter wavelength than that of the latter. However, resolution, is normally not the factor limiting the size of feature that can be seen. Contrast in the image is needed to resolve the features. Both DIFOTI and QLF claim to create contrast between normal and carious tissues [Keem and Elbaum, 1997; Pretty *et al.*, 2002].

5.5 Response to New Concepts in Caries Management

“What seems to be lacking is a clear strategy on how to merge these new technologies in a way consistent with today's changing paradigm of caries management [Young, 2002].” Further studies assessing the potential of the DIFOTI, however, are first necessary if the system is intended to respond to the new concepts in Cariology.

6. CONCLUSION

In-vitro, for very superficial artificial carious lesions on smooth and occlusal surfaces, DIFOTI as a caries diagnostic device, has very good properties concerning sensitivity and specificity based on visual interpretation of digitized images. However, DIFOTI fails to detect incipient proximal caries. Nevertheless, it still has good specificity proximally. Based on the quantitative measurements of the DIFOTI system and on the gold standard (as valid dichotomous classifier of teeth and allowing quantitative measurements itself), subsequent statistical analysis, ROC curves and correlation of lesion depth and percentage decrease of absorbance values, have shown that with the currently used software, View3D, the system can not be recommended yet to be used for quantitative monitoring of initial caries. Further research on the device and on the said computer program is needed to prove its ability and/or for its development.

7. Summary

7.1 In English

Caries monitoring is an important trend in modern caries diagnosis. DIFOTI, digital fiberoptic transillumination, allows not only the documentation but has also the potential to monitor carious lesions. DIFOTI is a new device and therefore it requires validation. In addition its diagnostic performance concerning sensitivity and specificity has to be determined.

Sound teeth were randomly assigned to two groups. One group consisted of unaltered teeth (N=18). In the second group an artificial carious lesion was created (Damato *et al.*, 1988) on the buccal (N= 9), proximal (N= 9) and occlusal surface (N=9). To evaluate proximal caries a proximal contact was simulated. Images of teeth were made with the DIFOTI and analysed with View3D, a special software, performing an analysis comparable to the one described by De Josselin de Jong (Caries Res, 1995) to quantify loss of transillumination. The specimens were sectioned and evaluated histologically. The ROC curve was calculated and AUC was determined (for 1% quantile and median DIFOTI image). Quantitative measurements of DIFOTI (percentage decrease of absorbance values) and of the gold standard (lesion depth) were correlated.

Histological evaluation revealed an average depth of 62.39µm of the carious lesions. Sensitivity and specificity data based on visual examination of the digitized images were:

	Sensitivity	Specificity
Smooth surface caries	1	0.88
Occlusal surface caries	0.82	1
Proximal surface caries	0.44	0.83

Table 19. Sensitivity and specificity values of the DIFOTI for smooth, occlusal and approximal surfaces.

The AUC value for the 1% quantile DIFOTI image was 0.69 (SE=.092) and for the median 0.59 (SE=.087). Pearson's correlation coefficients show non-significance of the correlation of loss of transillumination and lesion depth, .199 and .318 corresponding to 1% and median quantile gray-level image respectively.

In-vitro, for very superficial artificial carious lesions on smooth and occlusal surfaces, DIFOTI has very good diagnostic properties concerning sensitivity and specificity based on the visual interpretation of the digitized images. However, DIFOTI fails to detect incipient proximal caries. Nevertheless it still has a good specificity proximally. Based on statistical analysis of the quantitative measurements of the DIFOTI, with the currently used software, however, the system is at present not suited for a diagnostic test, which means that it cannot still be used for quantitative monitoring of initial caries.

7.2 Auf Deutsch

„Karies Monitoring“ ist ein wichtige Trend in der modernen Kariesdiagnose. DIFOTI, Digital Fiberoptic Transillumination, erlaubt nicht nur die Dokumentation, sondern ermöglicht es auch, die Progression kariöser Läsionen zu überwachen. DIFOTI ist ein neues Gerät und folglich erfordert es Validierung. Zusätzlich muß seine Diagnoseleistung hinsichtlich der Sensitivität und der Spezifität festgestellt werden.

Die gesunden Zähne wurden nach einer Zufallsauswahl zwei Gruppen zugewiesen. Eine Gruppe bestand aus unveränderten Zähnen (N=18). In der zweiten Gruppe wurde eine künstliche kariöse Läsion (Damato *et al.*, 1988) bukkal (N=9), approximal (N=9) und okklusal (N=9) hergestellt. Um die approximal Karies auszuwerten wurde ein Kontakt zum Nachbarzahn simuliert. Bilder der Zähne wurden mit DIFOTI fotografiert und dann mit der Software View3D analysiert. Das Verfahren ist mit dem von DeJosselin de Jong vergleichbar (Caries Res, 1995), mit dem er den Verlust von Autofluoreszenz quantitativ bestimmte. Die Proben wurden histologisch geschnitten und

ausgewertet. Die ROC-Kurve wurde errechnet und AUC wurde festgestellt (für 1% und 50%quantil DIFOTI-Bild). Die Korrelation zwischen Läsiontiefe und Transilluminationsverlust wurde berechnet.

Die histologische Auswertung deckte eine durchschnittliche Tiefe von 62,39 μm der kariösen Läsionen auf. Die Sensitivität und Spezifität, die auf Sichtprüfung der digitalisierten Bilder basieren, sind:

	Sensitivity	Specificity
Bukkalkaries	1	0,88
Okklusalkaries	0,82	1
Approximalkaries	0,44	0,83

Tabelle 20. Sensitivitäts und Spezifitäts der DIFOTI berechnet für die Bukkal-, Okklusal- und Approximalflächen.

Der AUC-Wert für das 1%quantil des DIFOTI-Bilds war 0,69 (SE=,092), für den Median 0,59 (SE=,087). Der Pearsons Korrelationskoeffizient zeigt keine Signifikanz der Korrelation von Transilluminationverlust und Läsiontiefe, 0,20 und 0,32 korrespondieren mit dem 1%quantil bzw. Median.

Die Validierung von DIFOTI in-vitro hat ausgezeichnete Sensitivität und Spezifität für die bukkalen und okklusalen oberflächlichen künstlichen Kariesläsionen gezeigt. Es hat jedoch schlechte Fähigkeit, anfängliche proximale Karies zu ermitteln. Dennoch hat es noch gute Spezifität approximal. Das System ist zur Zeit noch nicht für einen quantitativen Diagnostest geeignet und benötigt weitere Untersuchungen.

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9.3 Preparing the DIFOTI System

With the software properly installed to the computer and the proper connections of the DIFOTI components done, manipulation of the system is ready. Power switches for the computer, monitor and control unit of the DIFOTI are turned on. A disposable mouthpiece is attached. Proximal mouthpiece is used for viewing or creating images of facial and proximal lesions and occlusal mouthpiece for occlusal lesions. A light is seen coming out of the mouthpiece.

A DIFOTI icon is visible on the desktop and this icon is double-clicked. By this, the *Welcome to DIFOTI System* screen appears. The Login Name *difoti* and Password *difoti* are typed in. Confirm by clicking *OK*. The *Patient File Cabinet* takes place. By clicking on the *Add* button on the said screen, the *Add new patient* entry form is created. And this filled-up accordingly, a patient file exists, as this is necessary before images can be taken.

9.4 Image Production

To take pictures, an icon for this purpose is selected. The *Take Pictures* screen appears and the *play* button clicked on. To change the tooth number (present in the *tooth chart* near the bottom of the screen and which refers only to the tooth, as used in the study and the designation given to it previously, but does not conform to which type the tooth is in reality) and imaging aspect (for proximal lesions, since *proximal* is not included among the choices in *View aspect selection*, *facial* is just selected), either the mouse or the foot pedal is used. The disposable mouthpiece (the occlusal mouthpiece is used for teeth with lesions on the occlusal and proximal mouthpiece for teeth with lesions on the facial or proximal surface) is placed over the tooth, the rubber tooth locator contacting the tooth to be imaged. The image being viewed on the monitor, the position of the mouthpiece is adjusted. When the image desired is obtained and the image stabilizes, as indicated by the turning-green of the *Image Ready* "pilot light," the central portion of the foot pedal is pressed, then quickly released, or the *Capture*

button is clicked on. The captured image appears in the (smaller) lower-left window. The number of images made for each tooth is varied.

The *Review* icon is selected to view again the images - their size, position, contrast are open to manipulation for viewing convenience and enhancement. The pictures which are no longer needed for the future image analysis are deleted; this is done by choosing the appropriate button on the *DIFOTI Database* screen which is present simultaneously with the *Review* screen.

The square red button at left side of the screen is clicked on to exit the DIFOTI program [Electro-Optical Sciences, Inc. 2001].

9.5 View3D Software Operation

For tooth images both exhibiting and not exhibiting caries, light penetration of the DIFOTI device through the lesion or sound tissue is calculated with a software program, called View3D, particularly designed for this study. Opening the ACDSSee 5.0, a program used to view the DIFOTI images, images can be transformed to TIFF, the format to be used for View3D to be set in function. Double-click the ACDSSee 5.0 icon, search for the data and click the selected images to be subsequently analyzed, click on *Extras* from the menu, scroll down and choose *Formatumwandlung* (to change the format). The window *Bildformatkonvertierung* shows up and *TIFF Tag Bilddateiformat* is selected when the mouse is scrolled down and the *OK* button then clicked. Small TIF windows of chosen tooth images consequently appear (with marked green *i* on the top portion of each TIF window). Close ACDSSee 5.0 window.

Open *Verknüpfung mit view3D-03-2002.exe* by double-clicking the icon. *View3D 2.4* window emerges. Click on *File* from the menu, then *Open TIFF* and a corresponding window appears where search for the file to be analyzed is done. Select the appropriate entry from the choices found in the *Suchen in* (Search in) on the top area of the window. To finally work on the chosen image, click *Öffnen*

(Open). TIFF file window, containing the tooth image, takes the place of the previous one. On the top portion, choose *Linear* for *Style*, and *All* for *Sel*. Create triangulations with the mouse on the lesion observed, any area where caries could possibly occur if tooth appears sound, consisting of 9-15 lines extending to gray areas beyond the boundary of the carious lesion. Left click is done every after line of the triangle is drawn and right click when all the triangulations on the lesion have been completed. Click *Process* from the menu, choose *Subtract triangles (perc.)....* A new window appears with the reconstructed image of the lesion. Choose *False color* for *Style* and *All* for *Sel*. Click *Z Range* from the menu, scroll down and choose *Histogram* so that another window appears. Move the cursor on the histogram sideways to adjust the z found at the bottom of the window to as closest to -5%, then right click. Return to the window containing the reconstructed image. Choose *Process* from the menu, scroll down, choose *statistics*. A window containing statistics data come out. Click *File* then *Save*. Another window with *Save Text* appears. A name on the *Dateiname* (Data name) on the lower portion of the window automatically becomes available and is ready to be saved (unless desired to be changed) by clicking *Öffnen*. Return to *View3D 2.4* to perform the analysis on the remainder of the tooth images. Repeat the above procedure for obtaining the statistics.

9.6 Sensitivity and Specificity

		GOLD STANDARD		
		<i>Positive</i>	<i>Negative</i>	
DIFOTI	<i>Positive</i>	<i>a</i> True positive	<i>b</i> False positive	<i>a+b</i>
	<i>Negative</i>	<i>c</i> False negative	<i>d</i> True negative	<i>c+d</i>
		<i>a+c</i>	<i>b+d</i>	<i>N=a+b+c+d</i>

$$\text{Sensitivity} = a/a+c$$

$$\text{Specificity} = d/b+d$$

Table 21. Formula for computing sensitivity and specificity values [Swets *et al.*, 2000].

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11. Lebenslauf

Name: Cecilia Gutierrez

Geburtsdatum: 15.12.1974

Geburtsort: Manila

Staatsangehörigkeit: Philippinin

Schulbildung:

1981 – 1987 St. Theresa's College, Quezon City
Abschluss Grundschule

1987-1991 St. Theresa's College, Quezon City
Abschluss Sekundarstufe

Studium:

1991-1998 Studium der Zahnheilkunde
University of the East, Manila
Abschluss Doktor der Zahnmedizin

1999 6-Monate Fortbildung in Kinderzahnheilkunde
Philippine Children's Medical Center

Berufstätigkeit: Privat Praxis