Quantitative Diagnosis of Small Approximal Caries Lesions Utilizing Wavelength-dependent Fiber-optic Transillumination

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Abstract. The instruments clinically available for the diagnosis of approximal caries lesions are inadequate to detect lesions early and quantitatively. The aim of this study was to investigate whether wavelength-dependent light scattering and absorption of carious tissues may be utilized for the quantitative diagnosis of these small approximal caries lesions. Seventeen extracted premolar teeth were transilluminated at an approximal surface with a glass fiber, which transported the light from a halogen light bulb. Seven approximal surfaces contained a naturally developed small white-spot lesion, and 5 surfaces a small discolored lesion. Five teeth were sound. The occlusal surface was imaged with a CCD camera. Light in the blue and red portions of the electromagnetic spectrum was selected by means of Schott glass filters. From the obtained images, average effective decadic optical thickness differences were determined. These were plotted as a function of average mineral loss assessed by means of wavelength-independent microradiography. The correlation coefficient between the average effective decadic optical thickness difference and average mineral loss was $r = 0.79$ (95% CI: 0.47...0.93). Different sources of variation that influence the observed correlation were defined and quantified. From these measurements, the correlation coefficient between average effective decadic optical thickness difference and 'true' average mineral loss was estimated to be $r = 0.92$ (95% CI: 0.77...0.97). The results indicate that early and, in principle, also quantitative diagnosis of approximal caries lesions is feasible when wavelength-dependent light propagation through carious tissues is utilized.

Key words: caries (approximal), diagnosis (optical, quantitative).

Introduction

The instruments clinically available for the diagnosis of approximal caries lesions, i.e., visual inspection and bitewing radiography, are inadequate for the early and quantitative detection of lesions. Early detection may lead to the arrest, or even regression, of lesion size when preventive measures are reinforced, e.g., by topical fluoride application and improved oral care (Angmar-Månsson and Ten Bosch, 1987). Quantitative diagnosis facilitates an objective assessment of lesion severity and the monitoring of lesion progress. Thus, there have been numerous attempts to improve bitewing radiography and to develop new techniques such as, e.g., electrical resistance and computer-aided radiography methods (see review by Angmar-Månsson and Ten Bosch, 1993). However, to date, reliable detection still occurs at a stage were damage is irreversible, nor has any method been demonstrated to correlate well enough with, e.g., lesion depth to monitor lesion progress reliably in vivo (Angmar-Månsson and Ten Bosch, 1993).

A promising alternative for the detection of approximal caries lesions is tooth transillumination. Clinically, teeth are being transilluminated with fibers, which transport the light from a halogen or tungsten projection lamp to the tooth, and lesions are diagnosed when dark spots or 'shadows' are perceived. After the introduction of fibers as a light source in dentistry (Friedman and Marcus, 1970), several clinical studies have been conducted to estimate the performance of what is being designated as “fiber-optic transillumination” (FOTI). A wide range of diagnostic sensitivity values has been reported, and several explanations have been proposed (Verdonschot et al., 1991). Nonetheless, there are indications that FOTI might at least be a valuable supplement to the diagnostic armamentarium (Pieper and Schurade, 1987; Peers et al., 1993). Hence, to establish the limitations of FOTI or to improve the technique, research into factors that influence the performance of FOTI is worthwhile.

An important issue is the suitability of FOTI for the detection of small lesions, i.e., non-cavitated lesions restricted to the enamel. Some studies signify that FOTI may be suitable for the exclusive detection of lesions that have progressed into
between two effective decadic optical thicknesses at two red. In Appendix 1, we have outlined that use of the difference is assumed that, in the case of small lesions, the effective optical thickness increases linearly with mineral loss in the visible spectrum, this increase is stronger than in the (Ten Bosch). 

Materials and methods

Changes in optical properties accompanying mineral loss

In incipient white-spot lesions, mineral loss is accompanied by an increase in light scattering. In older, discolored lesions, light absorption is also enhanced. In a set-up as depicted in Fig. 1a, the induced effect at the occlusal surface is caused by a combination of material properties and the distance light propagates through tooth material from the light source to the detector. This combination will be called “effective decadic optical thickness” and is dependent on the light wavelength. It is assumed that, in the case of small lesions, the effective decadic optical thickness increases linearly with mineral loss (Ten Bosch et al., 1984; Brinkman et al., 1988). In the blue part of the visible spectrum, this increase is stronger than in the red. In Appendix 1, we have outlined that use of the difference between two effective decadic optical thicknesses at two different wavelengths in the blue and red portions of the visible spectrum may lead to suppression of variations introduced by refractive index transitions at the outer enamel surface. Contrary to the absolute signal quantity radiance, $L \left[ W.m^{-2}.sr^{-1}\right]$, the average effective decadic optical thickness difference, in the remainder of the text abbreviated to optical thickness difference, is a relative signal quantity. 

Natural and simulated lesions

For computation of the required number of approximal surfaces in the sample to show an existing effect, the parameters effect size, significance level $\alpha$, and the power $1 - \beta$ are required (Cohen, 1977). For approximation of the effect size, it was assumed that the correlation between the optical thickness difference and ‘true’ average mineral loss is, in principle, perfect, i.e., $r = 1$, but that due to, e.g., de- and remineralization processes, the correlation is reduced to $r = 0.95$. In addition, since it has been stated that “all correlation coefficients of 0.7 - 0.8 deserve further study” (Angmar-Månsson and Ten Bosch, 1993), $H_0: r \leq 0.7$ was chosen as the null hypothesis. This results in an effect size of 0.25. The significance level and the power were put to $\alpha = 0.05$ and $1 - \beta = 0.95$, respectively. Given the above, at least 15 approximal surfaces are required.

Optical and microradiography measurements were performed on 17 freshly extracted premolar teeth. Seven approximal surfaces contained a naturally developed small white-spot lesion, and 5 surfaces a small discolored lesion. Five teeth were sound. The use of extracted teeth conforms to the protocol of the University of Nijmegen, Subfaculty of Dentistry. Informed consent was obtained according to the standards of this subfaculty.

After the optical measurements had been performed, mineral loss was determined from microradiography measurements. To this end, the enamel layer at the approximal surface was cut from the tooth, at approximately the dentino-enamel junction (DEJ). Upon visual inspection, it was ascertained that the lesions had not progressed into the dentin. The teeth, and later the samples, were stored in water at room temperature between measurements.

Additional optical measurements were performed on 5 other freshly extracted sound premolars with simulated approximal lesions. To simulate approximal caries lesions, we prepared cylindrical cavities with a diameter of 1.15 ± 0.1 mm at the approximal surface, at or below the largest diameter of the tooth crown, and up to the DEJ. White-spot lesions were modeled with Intralipid (20%, Kabi Pharmacia AB, Sweden; scatter coefficient approximately 32 mm$^{-1}$ at $\lambda = 633$ nm) and discolored lesions with coffee (absorption coefficient approximately 1 mm$^{-1}$ at $\lambda = 633$ nm) as filler fluid. To model subsequent stages of the lesion process, we used from 1 to 32 times the fluid dilutions. Sound enamel was modeled with water as filler fluid. This model has been described in more detail elsewhere (Vaarkamp et al., 1995).

Natural lesions: wavelength-independent microradiography measurements

Lesion mineral loss was assessed by means of wavelength-independent microradiography, WIM (Herkströter et al., 1990;
To quantify the advantage of using the optical thickness of natural lesions: optical measurements obtained a lower limit of the optical thickness difference, we set fewer than 600 pixel values larger than zero were measured. To by averaging the 600 lowest pixel values.

The WIM measurements were performed twice, and Pearson's product moment correlation coefficient, \( r \), was calculated. The symbol "^" indicates that the population quantity \( r \) is being estimated by use of a finite number of samples. One sample with a discolored lesion and two of a sound surface were so severely damaged during preparation that no WIM measurements could be performed. To determine the magnitude of variation in an obtained mineral loss value, we calculated the standard deviation over 6 sound samples. Since only 3 sound samples had remained, an additional 3 samples were prepared.

Illumination and detection

Optical measurements were performed by the set-up depicted in Fig. 1a. The incandescent wire of a 20-W halogen light bulb was projected onto one end of a glass fiber (\( \phi 300 \mu m \)) with a 20x objective. For illumination of the teeth, the free fiber tip served as the point light source. Optical coupling between enamel and fiber tip was achieved with water. Light with a limited bandwidth in the blue and red portions of the electromagnetic spectrum was obtained by introduction of a blue (BG23) and a red sharp cut-off (RG610) Schott glass filter, respectively, between detector and occlusal tooth surface. Infrared radiation was removed with a heat-refracting mirror. The detector was a CCD camera (8 bits; 512*512 pixels; lens \( f = 135 \text{ mm} \)) imaging the occlusal surfaces of the teeth.

Estimation of optical quantities of natural lesions

We calculated the average radiance at the occlusal surface at the blue portion of the electromagnetic spectrum by averaging the 600 highest pixel values. It was observed that in doing so, we selected the pixels above the illuminated approximal surface, corresponding to approximately 1 mm². By multiplication with the detector response, this radiance was obtained in [W.m².sr⁻¹].

To determine the optical thickness difference, we calculated new images from the original images, using \( -\log_{10}(A_{\text{blue}} k L_{\text{occ}}(b)[i,j]/L_{\text{occ}}(rd)[i,j]) \), with [i,j] the pixel co-ordinates. The constant \( A_{\text{blue}} \) takes the wavelength dependence of the light source and detector sensitivity into account and was measured by direct illumination of the detector and the fiber. From these computed images, we calculated the optical thickness difference by averaging the 600 lowest pixel values.

In case of two discolored lesions illuminated with blue light, fewer than 600 pixel values larger than zero were measured. To obtain a lower limit of the optical thickness difference, we set the 'missing' pixels to a value of 1 and calculated the optical thickness difference as described.

Natural lesions: optical measurements

To quantify the advantage of using the optical thickness difference instead of the radiance, we had to compare the ratio of changes due to the presence of a caries lesion, i.e., the real effect, and variations due to the experimental procedure, e.g., the effect of positioning.

The range of optical thickness difference values occurring in the measurement set was approximated with the standard deviation over all 17 measured surfaces in the first measurement series. The magnitude of variation in an optical thickness difference value due to positioning was obtained from a duplo-measurement over the 17 surfaces studied and calculated from the standard deviation of the difference between the two measurement series divided by the square root of 2. In addition, the correlation coefficient of the two measurement series was computed. Similarly, the range and magnitude of variation in the radiance values were estimated.

To approximate the magnitude of variation attributable solely to biological variation in sound material properties and tooth geometry, we first approximated the total magnitude of variation in an optical thickness difference value as the standard deviation of the optical thickness difference values over the 5 sound surfaces. From the total variance and the variance due to positioning, the variance due to biological variation was obtained by subtraction.

Relation between optical and microradiographic data

The optical thickness differences were plotted as a function of average mineral loss, by use of the first measurement series in both cases. The correlation coefficient \( r \) was calculated, and the linear regression line was calculated by the Deming algorithm (Appendix 2).

From the magnitude of variation in both optical and microradiographic data, both degrading the correlation between measured optical and microradiographic data, the correlation coefficient between optical thickness differences and 'true' mineral loss was estimated (Appendix 2).

Simulated lesions: optical measurements

The relation between the optical thickness difference measured at the occlusal surface and the change in optical properties at the approximal surface was investigated with use of simulated approximal lesions with accurately known optical properties.

In the images obtained of each tooth with a simulated lesion, a fixed region of interest (ROI) was defined at the occlusal surface above the cavity (Fig. 1b). For blue and red light in that ROI, the average radiance was determined and the optical thickness difference was calculated according to the formula from the section 'Estimation of optical quantities of natural lesions'. The optical thickness differences were plotted as a function of the dye and particle concentration of the absorbing and scattering filler fluid, respectively. A straight line was fitted through all points, and correlation coefficients were calculated.

Results

In Table 1, the magnitude of the signal range and variation sources of the transillumination techniques and WIM are summarized. If two apparent outliers are disregarded (Cornbleet and Gochman, 1979), the correlation coefficient
of the repeated WIM measurements was $\hat{f} = 0.74$ (95% CI: 0.32...0.92).

In Fig. 2, the average effective decadic optical thickness differences are plotted as a function of average mineral loss. The bars in horizontal and vertical directions denote two times the standard deviation in an average mineral loss and an optical thickness value, respectively. Correlations between the optical thickness and mineral loss are summarized in Table 2. The mean average lesion depth approximated from average mineral loss was 168 (S.D. 112) µm for the white-spot lesions and 370 (S.D. 372) µm for the discolored lesions.

A typical example of the optical thickness difference as a function of dye and particle concentration of the simulated lesions is depicted in Fig. 3. The correlation coefficients were $f = 0.999$ and $f = 0.992$, respectively.

![Figure 2. Average effective decadic optical thickness differences determined by use of fiber optic transillumination as a function of measured average mineral loss of 7 premolars with white-spot lesions, 4 premolars with discolored lesions, and 3 sound premolars. Mineral loss is determined by wavelength-independent microradiography and is expressed in kg m$^{-2}$, with 1 kg m$^{-2}$ = 3.19 x 10$^{-8}$ Vol% µm. Through the measured points, a regression line was fitted by the Deming algorithm. The bars denote two times the standard deviation in a measurement.](image)

### Discussion

**Lesion characterization**

To characterize lesion progress, we used WIM to determine average mineral loss. This method has the advantage that the entire lesion is characterized by the data obtained. To enable us to measure differences in mineral content induced by caries lesions, we used an interpolation algorithm which reconstructed the sound situation. Due to the curved tooth surface, some offset may occur, which explains the negative mineral losses in Fig. 2. Table 1 and the duplo-measurement indicate that the effect of the curved outer enamel surface probably has a strong variation-enhancing effect. The sample quality may be another factor causing the standard deviation determined in a WIM measurement to be considerably larger than the standard deviation value of approximately 0.05 kg m$^{-2}$ reported by Herkströter et al. (1990). Some samples were damaged during preparation, some had hypoplastic enamel near the lesion, and some were partially covered with a thin dentin layer, which makes the mineral loss value obtained less accurate.

In cases of large variations, a large number of samples is required for a reliable value of quantity to be obtained. In this respect, the estimated total variation in mineral loss value in Table 1 is not reliable. Using expressions similar to those given in Appendix 2 (A2.1 and A2.2), with mineral loss instead of the optical thickness difference, we found the obtained total variation in a mineral loss value to be approximately 0.12 kg m$^{-2}$. This approximation is more reliable because it is based on more samples. In addition, there is no systematic effect due to variations in sound material properties, since, inherent to the method, these effects are suppressed because mineral loss is determined relative to the sound environment.

From mineral loss, lesion depth was also approximated. The expression is based on the assumption that, initially, only the interprismatic enamel is dissolved (Arends et al., 1987). We derived that expression by pooling the results of 8 artificial demineralization and remineralization studies. Brinkman et al. (1988) obtained a similar result with naturally developed lesions. However, a large study by Theuns (1987) on the relation between mineral loss and lesion depth, also using naturally developed lesions, suggests that average mineral loss is only a rough indication of the order of magnitude of lesion depth.

### Table 2. Correlations and 95% confidence interval between the average effective decadic optical thickness difference and measured 'true' average mineral loss, arising from different assumptions according to the formulae in Appendix 2

<table>
<thead>
<tr>
<th>Correlation between</th>
<th>$r$</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optical - measured mineral loss</td>
<td>0.79</td>
<td>(0.47...0.93)</td>
</tr>
<tr>
<td>Optical - 'true' mineral loss</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A2.1</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>A2.2</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td>A2.3</td>
<td>0.92</td>
<td>(0.77...0.97)</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Variation</th>
<th>Signal Range and Variation A [kg.m$^{-2}$]</th>
<th>Signal Range and Variation B [kW.m$^{-2}$.sr$^{-1}$]</th>
<th>Signal Range and Variation C [-]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>0.20</td>
<td>0.59</td>
<td>0.45</td>
</tr>
<tr>
<td>Positioning</td>
<td>- 0.05b</td>
<td>- 0.35</td>
<td>- 0.14</td>
</tr>
<tr>
<td>Biological</td>
<td>- 0.19</td>
<td>- x</td>
<td>- 0.09</td>
</tr>
</tbody>
</table>

* $x = $ Missing data.
  
  From Herkströter et al. (1990).

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Table 1. Magnitude of the signal range and sources of variation in a measurement of wavelength-independent microradiography (A) and the transillumination techniques with one (B) and two (C) wavelengths.
Quantitative optical diagnosis

From Table 1 it can be concluded that the use of optical thickness differences is superior to the use of radiances. This Table further shows that the determination of average mineral loss has a significant impact on the observed correlation between optical thickness and average mineral loss. This conclusion is supported by the results obtained with simulated lesions, which showed that it is possible to measure, at the occlusal surface, changes that occur at the approximal surface. However, these fluid holes are an ideal situation in which only one parameter is changed. In natural lesions, the shape of the lesion also varies considerably. Such variations affect the correlation between ‘true’ average mineral loss and the optical thickness difference.

Table 2 shows the extent to which a more precise validation technique may have resulted in a higher correlation. The small difference between the results of A2.1 and A2.2 illustrates the conclusion from Table 1 that repositioning is an important source of variation. Part of the repositioning effect in the optical measurement may be due to the fact that a caries lesion is a strong inhomogeneity and, hence, that the measured effect becomes partly dependent on the exact position and direction of the light source compared with the lesion. The small difference between the results of A2.2 and A2.3 indicates that, to a large extent, variations in lesion geometry are incorporated into the repositioning effect.

In conclusion, the results indicate that early and, in principle, also quantitative diagnosis of approximal caries lesions is feasible when wavelength-dependent light propagation through various tissues is utilized.

Acknowledgments

This work was financially supported by the University of Nijmegen and the State University of Groningen.

References


Appendix 1. Description of light propagation and signal definition.

For development of a quantitative optical transillumination technique, in this section the relation between optical changes occurring at the approximal surface and measurable quantities at the occlusal surface is derived within the framework of a simplified model describing light propagation through teeth.

In a previous study (Vaarkamp et al., 1995), light propagation through simulated approximal caries lesions was studied. Light extinction in cavities up to the dentino-enamel junction (DEJ) filled with light-absorbing fluids could be approximated by the equation:

\[ L_{\text{through}}(\lambda) = L_{\text{s}}(\lambda)10^{\mu_{\text{sc}}/\lambda} \]  
(A1.1)

with \( \lambda \) being a certain light wavelength. In this expression, \( L_{\text{through}} \) is the radiance contribution, estimated in a specific direction at the occlusal surface, of light that propagated through the lesion. \( L_{\text{s}} \) is the value of \( L_{\text{through}} \) obtained with a cavity filled with water, which simulated sound enamel. \( \mu_{\text{sc}} \) is the decadic absorption coefficient of the light-absorbing fluid and \( l \) the average path length through the cavity. When cavities were filled with a non-absorbing light-scattering suspension in low particle concentrations, a similar relation held, with \( \mu_{\text{sc}} \) replaced by \( \mu'_{\text{sc}} \), the decadic reduced scatter coefficient. \( \mu'_{\text{sc}} \) is defined as \( \mu'_{\text{sc}} = \mu_{\text{sc}}(1 - g) \), with \( \mu_{\text{sc}} \), the decadic scatter coefficient. \( g \) is the asymmetry parameter in the cavity which was not changed during the measurements. However, at higher particle concentrations, light extinction increased less than was to be expected based on expression (A1.1). This was probably due to multiple scattering.

When one is dealing with teeth with naturally developed lesions, only the light extinction by all material between light source and detector can be estimated. However, when a point light source, emitting at a certain wavelength, is used in the light source - detector geometry depicted in (A1.1), it is assumed that a first approximation light extinction in the enamel can also be described by an expression similar to equation (A1.1):

\[ L_{\text{through}}(\lambda) = L_{\text{s}}(\lambda)10^{\mu_{\text{sc}}/\lambda} \]  

With \( L_{\text{through}} \) being the radiance contribution, estimated in a specific direction at the occlusal surface, of light that propagated through the lesion. \( L_{\text{s}} \) is the value of \( L_{\text{through}} \) obtained with a cavity filled with water, which simulated sound enamel. \( \mu_{\text{sc}} \) is the decadic absorption coefficient of the light-absorbing fluid and \( l \) the average path length through the cavity. When cavities were filled with a non-absorbing light-scattering suspension in low particle concentrations, a similar relation held, with \( \mu_{\text{sc}} \) replaced by \( \mu'_{\text{sc}} \), the decadic reduced scatter coefficient. \( \mu'_{\text{sc}} \) is defined as \( \mu'_{\text{sc}} = \mu_{\text{sc}}(1 - g) \), with \( \mu_{\text{sc}} \), the decadic scatter coefficient. \( g \) is the asymmetry parameter in the cavity which was not changed during the measurements. However, at higher particle concentrations, light extinction increased less than was to be expected based on expression (A1.1). This was probably due to multiple scattering.

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\[ L_{\text{through}}(\lambda) = L_{\text{s}}(\lambda)10^{\mu_{\text{sc}}/\lambda} \]  

In the above equation:
\[ I_{\text{filter}}(\theta_{\text{approx}}, \varphi_{\text{approx}}) \] = Intensity emitted by the fiber
\[ \alpha_{\text{in}} \] = Function describing the effects of the change in refractive index at the enamel surface when light enters the enamel.
\[ \tau_{\text{eff}}(x, y) \] = Effective decadic optical thickness.
\[ \alpha_{\text{out}}(x, y) \] = Function describing the effects of the change in refractive index at the enamel surface when light departs the enamel.
\[ D \] = Imaging properties of the camera lens system.
\[ R_{\text{det}} \] = Response of the sensor.
\[ L_{\text{vac}}(x, y, \theta_{\text{vac}}, \varphi_{\text{vac}}) \] = Radiance at the occlusal surface.
\[ V_{\text{det}}(x, y, \theta_{\text{det}}, \varphi_{\text{det}}) \] = Detector output voltage.

With increasing mineral loss, the optical thickness of the enamel layer increases, as has been shown implicitly in the past with regard to white-spot lesions (Ten Bosch et al., 1984; Brinkman et al., 1988; Øgaard and Ten Bosch, 1994). Consequently, quantitative FOTI may be based on the measurement of increases in \( \tau_{\text{eff}}(x, y) \).

However, due to the tooth geometry, the measurement of \( \tau_{\text{eff}}(x, y) \) is subject to large variations. For assessment of whether part of these variations can be suppressed by the measurement of \( \tau_{\text{eff}}(x, y) \) at two different wavelengths and determination of the difference \( \tau_{\text{eff}}(x, y, \lambda_1) - \tau_{\text{eff}}(x, y, \lambda_2) \), the wavelength dependence of the quantities in expression (A1.2) is analyzed. \( \tau_{\text{eff}}(x, y, \lambda_1) \) can be written as:
\[ \tau_{\text{eff}}(x, y, \lambda_1) = \frac{1}{1 - \alpha_{\text{in}}(x, y, \lambda_1)} \] where \( \alpha_{\text{in}}(x, y, \lambda_1) \) is the source strength and \( \alpha_{\text{out}}(x, y, \lambda_1) \) the detector strength. In conclusion, in determining \( \Delta \tau_{\text{eff}}(x, y) \), variations in \( \tau_{\text{eff}}(x, y) \) induced by variations in optical coupling and small variations in angle between fiber and enamel surface are suppressed.

Using the above, \( \Delta \tau_{\text{eff}}(x, y) \) can be obtained from:
\[ \Delta \tau_{\text{eff}}(x, y) = \frac{1}{1 - \alpha_{\text{in}}(x, y, \lambda_1)} \tau_{\text{eff}}(x, y, \lambda_2) \] (A1.3)
in which \( \alpha_{\text{in}}(x, y, \lambda_1) \) is defined as \( V_{\text{source, det}}(\lambda_1) / V_{\text{source, det}}(\lambda_2) \), with \( V_{\text{source, det}}(\lambda_1) \) and \( V_{\text{source, det}}(\lambda_2) \) measured when the detector is illuminated directly with the fiber.

If it is assumed that, in the case of small lesions, \( \tau_{\text{eff}}(\lambda_1) \) increases linearly with average mineral loss, \( \Delta C_{\text{true}} \) (Ten Bosch et al., 1984; Brinkman et al., 1988), then estimation of \( \lambda_{\text{eff}}(\lambda_1) \) and \( \lambda_{\text{eff}}(\lambda_2) \) are fully equivalent as a measure of mineral loss. In the case of a linear relation between \( \tau_{\text{eff}}(\lambda_1) \) and \( \Delta C_{\text{true}} \), \( \tau_{\text{eff}}(\lambda_1) \) can be written as:
\[ \tau_{\text{eff}}(\lambda_1) = a(\lambda_1) + b(\lambda_1) \Delta C_{\text{true}} \] (A1.4)

As the value of \( \tau_{\text{eff}}(\lambda_1) \) measured when the approximate surface would be sound and \( b(\lambda_1) = d(\lambda_1) / dC_{\text{true}} \). In the following, \( a(\lambda_1) \) and \( b(\lambda_1) \) are expressed in terms of the optical properties of sound and carious enamel.

After defining \( \tau_{\text{true}}(\lambda_1) = \tau_{\text{eff}}(\lambda_1) - \tau_{\text{air}}(\lambda_1) \), being the intensity just after the enamel surface of entrance, and assuming a light-absorbing transparent medium, the irradiance at the enamel surface of departure \( E_{\text{true}}(\lambda_1, x, y) \) can be written as:
\[ E_{\text{true}}(\lambda_1, x, y) = 10^{-a(\lambda_1)} \tau_{\text{true}}(\lambda_1) \] (A1.5)

If the medium is also light-scattering, the irradiance consists of a component through and past the lesion, or \( E_{\text{true}}(\lambda_1) = E_{\text{true}}(\lambda_1) + E_{\text{sun}}(\lambda_1) \). In determining \( \lambda_{\text{eff}}(\lambda_1), \) the component \( E_{\text{true}}(\lambda_1) \) can be incorporated into \( a(\lambda_1) \) and is further neglected. If it is assumed that the lesion size, \( l_i \), is small compared with \( l \), then \( \tau_{\text{eff}}(\lambda_1) \) can be approximated as:
\[ \tau_{\text{eff}}(\lambda_1) = \int (\mu_{\text{eff}}(\lambda_1) + \mu'_{\text{eff}}(\lambda_1)) l_i \] (A1.6)

where \( l_i \) is the average path length in the sound enamel. If \( \mu_{\text{eff}}(\lambda_1) \) and \( \mu'_{\text{eff}}(\lambda_1) \) are the absorption and reduced scatter coefficient of sound enamel, respectively. \( l_i \) is the average path length in the carious enamel. Finally, in the case of white-spot lesions, \( d(\lambda_1) = d(\lambda_1) / dC_{\text{true}} = d(\lambda_1) / dC_{\text{true}} \) (Ten Bosch et al.)
In the case of discolored lesions, it is assumed that
\[ d\bar{\Delta}_{\text{True}}(\lambda_k) = \frac{1}{\mu_c(\lambda_k)} + \frac{1}{\mu_{cs}(\lambda_k)} \] and
expressing \( a(\lambda_k) \) and \( b(\lambda_k) \) in terms of the optical properties of sound and carious enamel, \( a(\lambda_k) = \frac{1}{\mu_c(\lambda_k)} + \mu_{cs}(\lambda_k) \) and \( b(\lambda_k) = \frac{1}{\mu_c(\lambda_k)} + \mu_{cs}(\lambda_k) \), then,
assuming that \( \text{Const}_{\text{True}}(\lambda_k) = \text{Const}_a(\lambda_k) \), then,

\[ d\bar{\Delta}_{\text{True}}(\lambda_k) = \frac{1}{\mu_c(\lambda_k)} + \frac{1}{\mu_{cs}(\lambda_k)} \] and

If a validation method is subject to variations which have the same order of magnitude as variations in the validated method, the application of least-squares linear regression is inappropriate. Furthermore, the correlation between the data sets deteriorates compared with the situation in which the correlation with some 'true' set of values would have been computed. In this Appendix, we address the issue of how the influence of variation sources in the validation technique can be resolved.

To calculate proper linear regression lines in situations as above, investigators have proposed several solutions, and the method suggested by Deming (1943) was found to be the most useful (Cornbleet and Gochman, 1979). Hence, the linear regression line was calculated by means of the Deming algorithm.

The deterioration introduced by variations in the validation method can be determined from assessment of the magnitude of variation sources, i.e., an estimate of the correlation \( r(\Delta_{\text{Wolff-efg}}(\Delta\bar{C}_{\text{True}}) \) between average decadic optical thickness difference, \( \Delta_{\text{Wolff-efg}}(\Delta\bar{C}_{\text{True}}) \) and average true mineral loss, \( \Delta\bar{C}_{\text{True}} \) can be obtained. Assuming a perfect correlation between \( \Delta_{\text{Wolff-efg}}(\Delta\bar{C}_{\text{True}}) \) and \( \Delta\bar{C}_{\text{True}} \) and that a value \( r(\Delta_{\text{Wolff-efg}}(\Delta\bar{C}_{\text{True}}) < 1 \) is caused only by random experimental variations, then \( r(\Delta_{\text{Wolff-efg}}(\Delta\bar{C}_{\text{True}}) \) can be determined from a duplo-measurement using the expression (Ferguson, 1976):

\[ r(\Delta_{\text{Wolff-efg}}(\Delta\bar{C}_{\text{True}}) = \sqrt{r(\Delta_{\text{Wolff-efg}}(\Delta\bar{C}_{\text{WIM}}) \cdot r(\Delta\bar{C}_{\text{True}}(\Delta\bar{C}_{\text{WIM}}))} \] (A2.1)

If biological variation in optical properties of the sound tooth also causes a \( r(\Delta_{\text{Wolff-efg}}(\Delta\bar{C}_{\text{True}}) < 1 \), then use of expression (A2.1) overrates \( r(\Delta_{\text{Wolff-efg}}(\Delta\bar{C}_{\text{True}}) \). In that case, \( r(\Delta_{\text{Wolff-efg}}(\Delta\bar{C}_{\text{True}}) \) should be determined from (Ferguson, 1976):

\[ r(\Delta_{\text{Wolff-efg}}(\Delta\bar{C}_{\text{True}}) = \frac{1}{\sqrt{1 + \left(\frac{\epsilon_{\text{Wolff-efg}}}{\sigma_{\text{Wolff-efg}}}\right)^2}} \] (A2.2)

\( \epsilon_{\text{Wolff-efg}} \) is the magnitude of variation sources in a measurement and \( \sigma_{\text{Wolff-efg}} \) the magnitude of the signal range. If variation in lesion geometry deteriorates \( r(\Delta_{\text{Wolff-efg}}(\Delta\bar{C}_{\text{True}}) \) too, or if \( \epsilon_{\text{Wolff-efg}} \) increases with lesion severity, then expression (A2.2) also overrates \( r(\Delta_{\text{Wolff-efg}}(\Delta\bar{C}_{\text{True}}) \). If \( r(\Delta\bar{C}_{\text{True}}(\Delta\bar{C}_{\text{WIM}}) \) is approximated using an approach as above, a third approximation of \( r(\Delta_{\text{Wolff-efg}}(\Delta\bar{C}_{\text{True}}) \) is obtained from (Ferguson, 1976):

\[ r(\Delta_{\text{Wolff-efg}}(\Delta\bar{C}_{\text{True}}) = \frac{r(\Delta_{\text{Wolff-efg}}(\Delta\bar{C}_{\text{WIM}})}{r(\Delta\bar{C}_{\text{True}}(\Delta\bar{C}_{\text{WIM}}))} \] (A2.3)

\( r(\Delta\bar{C}_{\text{True}}(\Delta\bar{C}_{\text{WIM}}) \) was approximated from a duplo-measurement.

When the above is applied, the effect of using a validation method which cannot be regarded as a "gold standard" can, to some extent, be resolved.