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Evaluation of Perpendicular Reflection Intensity for Assessment of Caries Lesion Activity/Inactivity

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Key Words

Caries activity · Diagnostics · Enamel caries · Natural caries lesions · Non-cavitated caries lesions · Profilometry · Surface properties

Abstract

The aim of this study was to evaluate, using visual assessment, an experimental optical sensor measuring perpendicular reflection intensity (PRI) as an indicator of enamel caries lesion activity/inactivity. Forty teeth with either an active or an inactive enamel lesion were selected from a pool of extracted teeth. Each tooth was cut into halves, with a clinically sound half and a half with a non-cavitated enamel lesion. After gentle plaque removal, the teeth were kept moistened. The lesions were then photographed and a defined measuring site per lesion was chosen and indicated with an arrow on a printout. Independently, the chosen site was visually assessed for lesion activity, and its glossiness was measured with PRI assessment. Surface roughness (SR) was assessed with optical profilometry using a confocal microscope. Visual assessment and PRI were repeated after several weeks and a reliability analysis was performed. For enamel lesions visually scored as active versus inactive, significantly different values were obtained with both PRI and SR. PRI values of the clinically sound control surfaces were significantly different only from active lesions. Generally, inactive lesions had the same glossiness and the same roughness as the sound control surfaces. The reliabilities for visual assessment (κ = 0.89) and for PRI (ICC = 0.86) were high. It is concluded that, within the limits of this study, PRI can be regarded as a promising tool for quantitative enamel lesion activity assessment. There is scope and potential for the PRI device to be considerably improved for in vivo use.

As caries is a chronic disease of the dental hard tissues, monitoring of caries lesion behaviour over time is essential from a therapeutic perspective. Monitoring of caries lesion severity over time can be carried out visually by the use of the ICDAS II criteria [ICDAS 2009], which reflect progression and regression of lesion depth. The Nyvad criteria were proposed as an alternative visual-tactile caries lesion detection method [Nyvad et al., 1999], which in addition to assessing lesion severity by classifying lesions

L.J. holds a patent application for PRI measurement using a chromatic-confocal sensor.

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into non-cavitated and cavitated stages, include an activity score of the lesions. When the Nyvad classification was validated in longitudinal trials, it was shown that irrespective of the level of lesion severity, activity assessment was able to predict to a certain extent the future prognosis of the lesions [Nyvad et al., 2003]. Over a 3-year period, active non-cavitated lesions had a higher risk of progressing to a cavity or a filling than inactive non-cavitated lesions or sound surfaces. Because of their degree of prognostic properties, the Nyvad criteria may therefore serve as a useful guide for clinicians in decision-making [Nyvad, 2004].

According to the Nyvad criteria, lesion activity assessment is based on a visual/tactile characterization of the lesions. Active non-cavitated enamel lesions look opaque and show a matt/chalky surface. The surface texture of these lesions feels rough when the tip of a probe is moved gently across the surface. Active lesions are often covered by adherent plaque. The biologic background for the matt and rough surface of active lesions lies in progressive surface dissolution of the crystal structure in the outermost enamel surface [for a review, see Thylstrup et al., 1994]. By contrast, inactive non-cavitated enamel lesions may look whitish or brownish-black due to staining, they are shiny/glossy, and feel smooth on gentle probing. The shiny and smooth surface appearance of inactive lesions is probably the result of a combination of surface abrasion by toothbrushing and by remineralization as bacterial metabolism is reduced [Thylstrup et al., 1994]. Several recent caries detection approaches have now adopted similar key features for assessment of lesion activity, such as ICDAS II [ICDAS 2009], and UniViSS [Kühnisch et al., 2011], but without, as yet, longitudinal clinical validation.

It appears from the above that a visual (glossiness/mattness) and a tactile (roughness) evaluation of the surface features of caries lesions are crucial factors for enamel lesion activity assessment. However, surface roughness (SR) assessment with a probe is difficult, as was shown in a pilot study that was recently carried out on Teflon plates with five different standardized degrees of SR [Ando et al., 2010]. The authors showed that even after extensive calibration inter-examiner reliability equalled zero. Because one examiner's sensation of roughness is not necessarily shared by another, the authors concluded that there is a need for an objective method for assessing tooth SR.

Optical quantitative assessment of glossiness/reflection might be a feasible approach for objective assessment of lesion activity. Reflection is a phenomenon encountered in wave physics, particularly in optics. If a beam of light enters a plane, such as the surface of an enamel-like object, at a 90-degree angle, three main phenomena can be observed (excluding fluorescence). First, light is reflected to the light source (perpendicular or vertical reflection). Second, due to nano-scale or micro-scale surface irregularities and material properties, light is dispersed and takes a different direction (scattered reflection or scattered transmission with refraction at the surfaces). Third, some light energy gets absorbed and is conversed to heat [Karlsson, 2010]. Thus, perpendicular reflection intensity (PRI) is a measurable quantity of reflected light to be observed in light/object interaction. Surface properties such as roughness cause a different proportion of reflection, scattering and absorption. Scattering is also dependent on the wavelengths of the incoming light, because shorter wavelengths (blue-green spectrum) are scattered more at the tooth surface while longer wavelengths (red spectrum) penetrate deeper [Hall and Girkin, 2004].

A preliminary study showed considerable differences between surface glossiness and SR of active and inactive non-cavitated enamel lesions [Jaruszewski, 2008]. In this study, PRI using a white light source seemed to be capable of differentiating shiny and matt lesions; active lesions showed measurements ranging from 4 to 15%, and inactive lesions from 15 to 33%. Therefore, assessment of surface glossiness might be a step towards an objective assessment of lesion activity. The aim of this in vitro study was to evaluate, using visual assessment (Nyvad criteria without using a probe), an experimental optical sensor measuring PRI as an indicator of enamel caries lesion activity versus inactivity.

Materials and Methods

Sample Size Calculation

A pilot study was carried out using 38 teeth different from the teeth used in this study, with 19 active and 19 inactive non-cavitated lesions. A relevant difference in reflection intensity measurements of 12% was assumed. A power analysis was carried out in order to identify the required number of teeth for this study (α level = 0.05 with Bonferroni-Holm correction; power 0.9; 8 comparisons) (GPower 3.1, University of Dusseldorf, Germany). The sample size was calculated to be n = 72 (including carious sites and sound control tooth surfaces). Assuming the possibility of a loss of some specimens during the experiment, we decided to include 40 teeth (equalling 80 tooth surfaces) into our study.

Selection of Teeth

Forty teeth which each had on one surface a non-cavitated enamel lesion that had either a dull/matt appearance or a shiny/

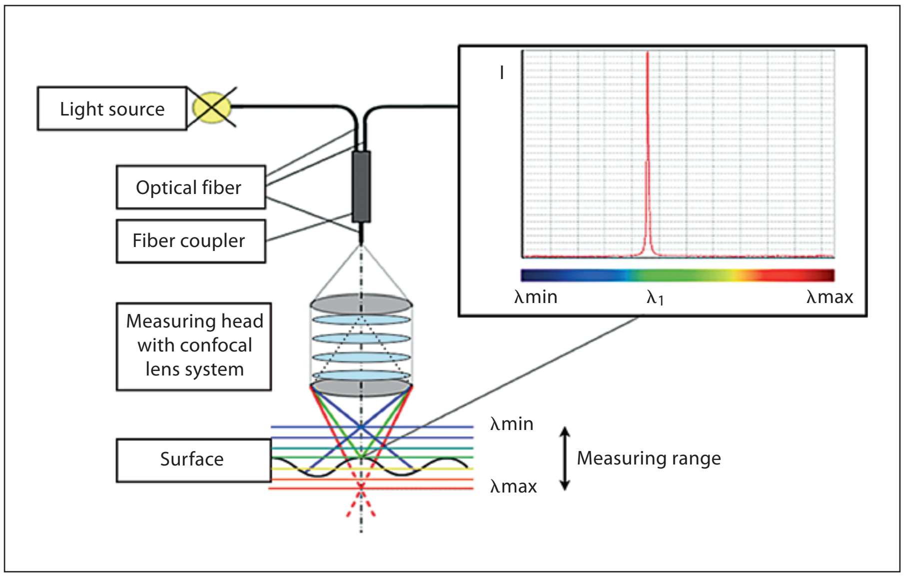

Fig. 1. Measuring principle of PRI measurements. The location of the peak (λ_1) on the X-axis corresponds to the punctual distance between surface and optics; its height on the Y-axis (I) corresponds to reflection intensity.

glossy appearance were selected from a pool of extracted teeth, stored in 1% chloramine solution at 4°C. Informed consent was obtained from the donors to use their teeth for scientific purposes. The carious sites chosen for examination were located on a smooth surface and showed no micro-cavities. Thirty-seven lesions were located on a proximal surface, three lesions were located buccally.

Processing of Teeth

The teeth were gently cleaned with a cotton pellet soaked in 1% sodium hypochlorite for 30 s in order to remove plaque remnants. Afterwards, the teeth were rinsed gently with water-air spray for 10 s, followed by air-drying with a 3-in-1-syringe for 10 s. The teeth were digitally photographed at ×5.8 magnification (Leica, Heerbrugg, Switzerland). The site of interest of the lesion was marked with an arrow on a printout of the photograph. The crown halves with the enamel lesion and their opposite clinically sound crown halves were separated by a low-speed diamond saw (Isomet 11–1180, Buehler, Switzerland). The crown halves were mounted on four plastic plates and subsequently numbered. Before and between measurements as well as for transport, the plates were stored in an airtight lockable container, at the bottom of which was placed 1 cm of rubber foam soaked in 1% chloramine solution.

Visual Lesion Activity Assessment

Two plates containing the tooth halves with enamel lesions were visually assessed for lesion activity according to the Nyvad criteria [Nyvad et al., 1999], but no probe was used to avoid scratching the surface. The examiner (B.N.) only assessed the site of interest at the tip of the arrow on the photographed lesion. The lesions were divided into 'active' (1) and 'inactive' (0) categories. This assessment was repeated after 3 months for the measurement of reproducibility. The two plates with the sound tooth halves were not visually assessed in the experiment.

Reflection Intensity Assessment

In order to quantify reflection intensity, a chromatic-confocal optical sensor was used. Chromatic-confocal sensors are mainly used in industries for high-precision distance measurements or for the purpose of profilometry. In contrast to confocal sensors, no punctiform focus at a defined distance is built. Instead, a multi-lens optical system constructs a linear focus ('measuring range'; fig. 1). In other words, within their measuring range, chromatic-confocal sensors have a permanent 'perfect' focus. PRI assessment was performed with an experimental sensor based on a pen-like non-contact chromatic-confocal sensor (IFS-2402-4, Micro-Epsilon Messtechnik GmbH, Ortenburg, Germany) combined with a spacer according to Jaruszewski [2005] defining the distance between sensor and tooth surface at 3.6 mm. The handheld optics has a dimension of 68 mm length and a diameter of 4 mm. The measured area has a diameter of 20 µm with a lateral resolution of 10 µm and a vertical resolution of 140 nm. The measuring range is 3.5 mm, the start of the measuring range is about 1.9 mm. The sensor operates on white light, the source of which is a halogen lamp located in the control unit (opto NCDT 2400, Micro-Epsilon Messtechnik GmbH, Ortenburg, Germany). The optics of this sensor consist of a confocal lens system with controlled chromatic deviation. The chromatic-confocal lenses disperse the incoming polychromatic white light in its monochromatic wavelengths. A defined distance is assigned to each wavelength by a factory calibration. The controller processes only the wavelength, which is exactly focussed on target surface. The specific beam reflection thus depends on the distance between surface and sensor front lens (fig. 1). The reflected wavelength passes a confocal aperture and a receiver, which periodically detects and processes the spectral changes and converts them a distance measurement. In combination with a distance holder, a defined focus in the blue-green spectrum within the measuring range is built for the assessment of reflection intensity. Figure 1 shows – on the X-axis – a hypothetical wavelength (λ_1) of the blue-green spectrum that is

Table 1. Comparison of PRI (%) and SR (μm) on surfaces of lesions visually rated as 'active' and 'inactive'

	Results	p values		
PRI for 'active' lesions	15.4 (11.5–19.5)	0.0026*	0.0002*	
Control site	30.6 (25–35)			0.7187
PRI for 'inactive' lesions	26 (24–29)	0.0291		
Control site	29.8 (27–32.5)			
SR for 'active' lesions	0.805 (0.567–1.051)	0.0244	0.0051*	
Control site	0.369 (0.305–0.504)			0.8279
SR for 'inactive' lesions	0.411 (0.353–0.470)	0.2774		
Control site	0.365 (0.295–0.408)			

Data presented as means with nonparametric 95% CI in parentheses.
* Significant differences after Bonferroni-Holm correction (α = 0.05).

reflected as a result of the specific distance. In this study setup, not the distance measurement itself was analyzed, but the intensity of the vertically reflected light, i.e. the height of the peak of the excited wavelength (fig. 1, Y-axis). The display of the control unit shows values ranging from 0 to 50 according to the percentage value of PRI.

It is noteworthy that the optics only collects perpendicular (or vertical) reflection. The sensor should therefore ideally be positioned at a 90-degree angle to the tangential plane of tooth surface. In order to obtain this crucial position for maximal reflection, the distance keeper of the handheld sensor was placed on the area of interest of the caries lesion as indicated on the photograph. The sensor was positioned with cautious tilting movements and slightly rotated along its vertical axis until the highest PRI value was obtained.

The reflection intensity assessments were repeated by the same examiner after 2 weeks. The examiner was blind to the results of the visual assessment.

SR Assessment

The defined site of the enamel lesion was further assessed for SR using optical profilometry with a confocal microscope. The non-contact confocal 3D-microscope (μsurf explorer, NanoFocus, Oberhausen, Germany) with an optic module providing a lateral resolution of 0.7 μm and a vertical resolution of 4 nm (320 L, NanoFocus and scanned the point of interest as indicated on the photograph over an area of 320 × 320 μm. Perpendicular measurement was assured with individual positioning of the tooth half or the carrier plate. The SR profile was adjusted with a Robust Gaussian Filter at a cut-off of 100 μm in order to reduce measurement errors due to tooth curvature in the area of interest. The vertical scanning process resulted in an average SR of the respective area (R_a in μm). The examiner was blind to the results of the visual assessment.

Statistics

Statistical analyses were performed using R software (r-project, Institute for Statistics and Mathematics, University of Vienna, Austria). A reliability analysis was performed. For visual assessment, Cohen's κ statistic was chosen, whereas the intraclass correlation coefficient (ICC) was chosen for PRI [Shrout and Fleiss, 1979].

For statistical comparison, visual assessment served as the validator in this experiment. The Wilcoxon rank-sum test was applied for comparison of active and inactive lesions assessed with PRI and SR. The Wilcoxon signed-rank test was applied for comparisons with the clinically sound control halves. The level of significance was set at α = 0.05. The Bonferroni-Holm correction for multiple comparisons was applied. For PRI and SR, descriptive statistics, nonparametric 95% CI and Spearman's correlation were calculated.

Results

In this study, 13/40 lesions were visually rated as 'active', and 27/40 as 'inactive'.

Glossiness and roughness were significantly different for lesions visually scored as 'active' or 'inactive'. For PRI, the mean values with nonparametric 95% CI for lesions scored 'active' and 'inactive' were 15.4% (95% CI 11.5–19.5%) and 26% (24–29%), respectively (table 1). For SR, the corresponding values were 0.805 μm (0.567–1.051 μm) and 0.411 μm (0.364–0.5 μm), respectively. Glossiness of the clinically sound control surfaces was significantly different from 'active' lesions. 'Inactive' lesions showed the same degree of glossiness and roughness as the clinically sound control halves (table 1; fig. 2).

Reliability analysis: Cohen's κ for visual assessment was 0.89, intra-class correlation for PRI was 0.86. No reproducibility assessment was done for SR as this measurement was performed only once.

Correlations of medium strength were detected for PRI with visual assessment (r = –0.601) (fig. 3) and for PRI

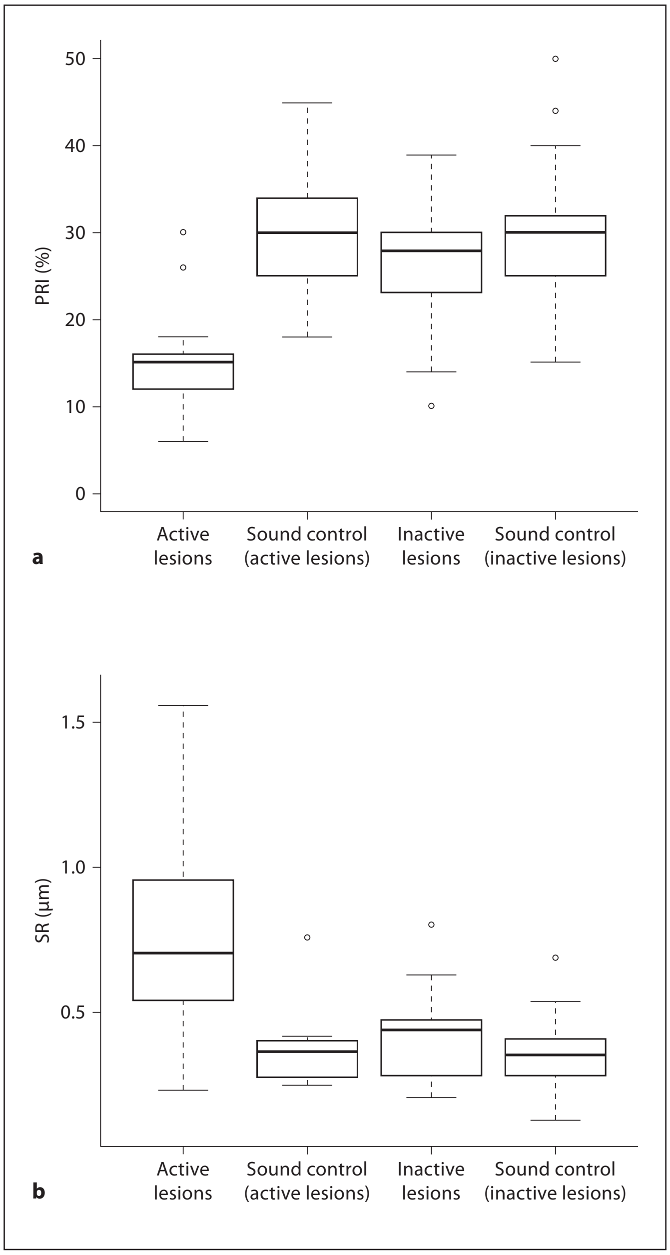

Fig. 2. Boxplots showing PRI (**a**) and SR (**b**) measurements of 'active' and 'inactive' lesions and their sound control tooth halves.

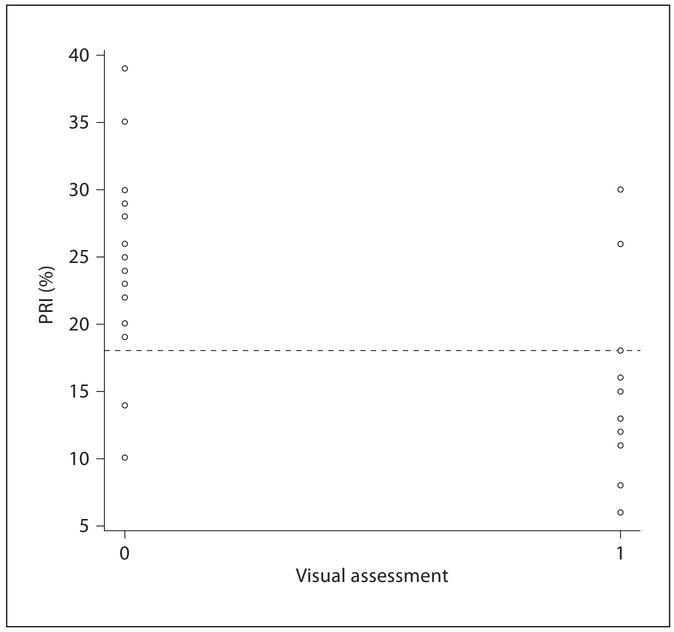

Fig. 3. A medium level correlation could be detected comparing PRI with visual assessment (r = –0.601). Visual assessment: 0 = lesions rated 'inactive'; 1 = lesions rated 'active'. The best cut-off value (dotted line) found for PRI was 18 resulting in 2 false-positive and 2 false-negative readings.

with SR (r = –0.553). The correlation of SR with visual assessment had lower strength (r = 0.463).

In order do differentiate 'active' and 'inactive' lesions by their degree of glossiness, the best cut-off found for the PRI device in this study was 18%. Two measurements ≤18% and two measurements >18% did not meet this cut-off. Baysean analysis of contingency tables without (and with) clinically sound control teeth showed a sensitivity of 0.85 (0.85), a specificity of 0.95 (0.94), a positive predictive value of 0.85 (0.73) and a negative predictive value of 0.95 (0.97).

Discussion

PRI measurements showed a significant difference between 'active' and 'inactive' lesions, and between 'active' lesions and clinically sound enamel. SR assessment also showed a significant difference between 'active' and 'inactive' lesions, but failed to detect a significant difference between 'active' lesions and clinically sound enamel. 'Inactive' lesions and clinically sound tooth surfaces did not differ in PRI and SR measurements.

The decreased PRI in lesions scored 'active' could be expected, because in active lesions the surface looks more matt and is rougher than in sound enamel [Nyvad et al., 1999]. For lesions scored 'inactive', no such difference could be detected. This means that the glossiness of lesions scored 'inactive' is about the same as sound enamel. This is also in line with literature stating that inactive enamel lesions look shiny [Nyvad et al., 1999].

Based on the findings of this study, for the new PRI device a cut-off value of ≤18% for 'active' and >18% for 'inactive' enamel caries produced optimal results. This resulted in a sensitivity of 0.85 and a specificity of 0.95 to discriminate 'active' from 'inactive' lesions. As this method is new, there is no other study for comparison of our results. However, the high positive and negative predictive values found in this study (0.85 and 0.95, respectively) suggest a high validity may be possible in vivo. Nevertheless, it is important to note that the cut-off value is relative and is dependent upon the specific sensor optics, the brightness of the light source, and on the scan rate of the control unit used. Because the device that was used in this study is a prototype, a different setup of optical sensor and controller of a future version of the device would result in a different cut-off.

Confocal microscopy was used to verify SR. It is a well-established method in industries and serves as quality control of surfaces, e.g. for dental implants (personal information, NanoFocus). In this study, it was possible to detect significant differences between SR of 'active' and 'inactive' lesions. However, the comparison of roughness between 'active' lesions and their sound control surfaces only showed a tendency ($p = 0.024$, fig. 2b) and failed to reach statistical significance after Bonferroni-Holm correction for multiple comparisons (table 1). One reason might be the high sensitivity of confocal microscopy. For this method, measurement of a flat surface and measurements at a 90-degree angle are required. Although in this study only smooth surface caries lesions were used and the area of measurement was restricted to 320 × 320 μm, and although a mathematical alignment had been performed using a Robust Gauss Filter, the natural curvature of the teeth might have influenced the results. In an experimental in vitro model with bacteria-induced artificial caries on flat polished specimen, reflectometry and SR were assessed [Ando et al., 2009]. The authors detected a moderate relation between SR and reflection ($r = -0.63$), which is in the range of our findings ($r = -0.553$). In that study, an uninterrupted bacterial activity for 8 days was associated with increased mineral loss and with increased SR. The authors reported a mean SR of 0.15 μm for polished sound surfaces, 0.53 μm after 4 days and 1.22 μm after 8 days of demineralization. In our study, sound control sites showed a mean roughness of 0.37 μm, but they were not polished. The active lesions in our study showed a mean roughness of 0.8 μm (with a large confidence interval). Under the confocal microscope, signs of dental wear like scratches were regular findings in all specimens, which is in line with previous observations in vivo [Thylstrup et al., 1994]. SR is dependent on many more factors than acid exposure alone [Ganss et al., 2002].

Visual lesion activity assessment in our study was carried out on cleaned, extracted teeth. No information was available about the clinical history of the teeth, and no probe was used either. The degree of surface glossiness was the main clinical feature used to determine if the defined site of interest was 'active' or not. However, in spite of the artificial examination conditions, reassessment of the teeth after 3 months yielded an excellent agreement with the previous visual assessment ($\kappa = 0.89$). A high reliability could also be achieved with PRI measuring the same sites as visual assessment (ICC = 0.86). There is no information in the literature how the storage of teeth in 1% chloramine might influence SR or glossiness.

A potential weakness of the study setup might be the exact relocation of the assessed sites of the lesions. For all lesion assessments, the area of interest was given on a printout of a photograph ('at the tip of the arrow'). The relocation of this area was done visually in all three methods. Both the PRI sensor and the confocal microscope produce a visible point-like spot on the tooth surface allowing comparison to the defined area on the photograph. This practice is common in laboratory caries diagnostic studies, but in this particular setup comparisons might have been subject to unspecified measurement errors due to the relatively small size of the assessed area, especially with the PRI sensor. Furthermore, one could expect that if several measurements of the same lesion area had been performed, their average and range might have resulted in more precise estimates. However, repeatability with the PRI sensor was high supporting that possible variations of caries lesion surface topography over microscopic distances obviously did not affect the measurements. A likely reason is the tilting and rotating movement of the sensor necessary during the PRI measurement. Thus, PRI was not obtained by punctiform assessment of a single 20-μm spot, but instead was rather the result of multiple measurements of the area of interest as indicated on the photograph.

The activity assessments in this study were purposely performed on smooth surfaces, because it is relatively easy to obtain PRI measurements from these surfaces. However, measuring fissure entrances might be difficult due to 90-degree-angulation problems. Unpublished results with a modified PRI probe suggest that assessments of proximal surfaces would be technically possible. This experimentally modified probe had a diameter of 0.7 mm and a 90-degree beam angulation allowing for proximal measurements, which would be compatible with the space gained after temporary tooth separation [Novaes et al., 2010].

It should be appreciated that visual lesion activity assessment itself is not 100% prognostic, yet is still the only clinically validated method at hand. Within the limits of this study, it could be shown that PRI measurements have the potential to objectively assess surface glossiness of teeth. However, the PRI device used in this study needs significant improvement in practicability, such as a peak value holder in order to capture the maximum value of perpendicular reflection. Non-perpendicular measurements would result in too low readings, and might thus overestimate lesion activity. To further explore the prognostic value of the PRI device, a longitudinal clinical trial assessing visual lesion activity and accuracy of surface glossiness/mattness over time would be needed. Together with clinical findings, the particular information on the degree of surface glossiness could then be validated for its potential in instantaneous assessment of lesion activity.

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Disclosure Statement

The authors declare no conflicts of interest.

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