

# Using an Optical Reflectometer to Measure Caries Lesion Activity on Enamel

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## Research Article

**Keywords:** Reflectometry, enamel caries, initial caries, caries progression, ICDAS, red laser

**Posted Date:** November 11th, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-1053628/v1>

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# Abstract

**Background:** Specular reflection can be used to quantify dental erosion, and might also provide similar results for caries. This study assessed the possibility of using specular reflection intensity (SRI; quantitative lesion activity assessment) to measure the progression of initial enamel caries lesions.

**Methods:** Two hundred native enamel specimens and flat ground enamel specimens (n=100 each) were subjected to a 4-species biofilm caries model during 2, 4, 6, 8, and 10 weeks (n=20 each), to induce 'active' enamel caries lesions. Afterwards, to induce 'inactive' lesions, all specimens were continuously remineralized and brushed twice daily for 2, 4, 6, 8, and 10 weeks. Change in specular reflection intensity (%SRI), visual caries detection (ICDAS) and visual caries severity assessment were performed for all active lesions and during the remineralization phase. Scanning electron microscopy (SEM) images were taken for qualitative analysis.

**Results:** For active lesions, %SRI dropped from 100% to about 80% in native enamel, and to about 15% in polished enamel. Remineralization/brushing increased %SRI in native enamel, but not in polished enamel. The comparison with visual caries scores yielded a better linear relationship of %SRI with early enamel lesion where caries was induced for up to 6 weeks.

**Conclusion:** The use of the optical reflectometer for the assessment of caries lesion activity seemed to work better for early caries lesions and for polished specimens.

## Highlights

- Good rater reliability is observed when using the optical reflectometer on sound surfaces and on caries lesions, on both native and on polished enamel;
- On both native and polished enamel, caries lesion formation causes to a decrease in surface reflection intensity (SRI);
- On native enamel: remineralization/brushing increases SRI for early caries lesions (2-6 weeks formation); but decreases SRI in more established lesions (8-10 week formation);
- On polished enamel: remineralization/brushing decreases SRI for early caries lesions (2-6 weeks formation); while values remain unchanged for more established lesions (8-10 weeks formation).

## Background:

Enamel surface specular reflection has been shown to be a valid method of quantifying dental erosion *in vitro* (1). It is based on the fact that when superficial and near-surface demineralization occurs, the loss of minerals from the tooth will lead not only to a softening of the surface, but also to a roughening of the tooth surface. Based on the principle of specular reflection intensity, a pen-size device was developed (2) and validated *in vitro* (3, 4) for early enamel erosion. However, untreated dental caries

remains a more globally relevant and widespread disease (5). In order to treat the disease, its early detection in enamel (6) and its progression rate (7) are crucial parts of caries diagnosis.

In 1999, Nyvad et al. (8) recommended a caries assessment criteria including the classification of lesions according to their activity, based on a visual/tactile characterization, where the lesions with a shiny/glossy, smooth surface are classified as inactive, and those with an opaque, matt/chalky surface are classified as active lesions (8). The latter aspect is due to the progressive demineralization of the enamel surface, which causes a differential quality to the enamel surface and offers a crucial feature for enamel lesion activity assessment. 'Active' enamel lesions are more likely to progress and turn into cavitated lesions than inactive lesions (9).

Neuhaus et al. (10) analyzed visual mattness/glossiness and perpendicular reflection intensity (PRI) of purportedly active and inactive lesions and observed, that inactive lesions presented the same glossiness and roughness as sound enamel, whereas active lesions presented significantly different roughness and PRI values. A criticism of that study is that the authors studied enamel lesions in extracted teeth and unanimously judged their degree of activity by their visual appearance. However, if perpendicular reflection measurements were able to objectively differentiate active from inactive lesions, it may be suggested that specular reflection could also provide similar results. In this case, the optical pen-size reflectometer may be used for such assessments, since the optical reflectometer has shown strong correlation to enamel surface roughness (1, 11). It could therefore be hypothesized that the optical reflectometer may also be used to differentiate active from inactive caries lesions. However, it is known from earlier experiments that the original surface texture of the teeth (12) or the original curvature of natural teeth could hamper optical reflection measurements (10), i.e., ground-polished flat enamel surfaces might behave differently from native enamel surfaces. Hence, the aim of this study was to evaluate the reflection intensity measurements to assess caries activity/inactivity for both ground-polished flat enamel and for native enamel. We hypothesized, that active caries lesions have a lower specular reflection intensity compared to sound surfaces, and that inactivation of enamel caries by remineralization/tooth brushing increases the specular reflection intensity.

## **Methods:**

### *Specimen preparation*

Two-hundred extracted molars with an intact buccal surface were used for this study. The teeth were taken from a pool of extracted, irreversibly anonymized teeth stored in 1% chloramine solution at 4°C. For projects using irreversibly anonymized samples, the local ethics committee waives the requirement of previous authorization because the data can no longer be assigned to a specific individual (KEK: Req-2016-00332). The roots were separated from the crowns using a high speed diamond. The pulp tissue was removed mechanically and the remaining crowns were cleaned using Prophyflex powder. The crowns were then embedded in self-curing acrylic resin (Paladur, Heraeus Kulzer GmbH, Wehrheim, Germany) using metal rings.

The teeth were then divided into 2 groups to obtain 100 native enamel surfaces (I) and 100 ground-polished surfaces (II).

The specimens of group II were then ground using rotating silicon carbide paper discs (Knuth-Rotor, Struers, Copenhagen, Denmark) with decreasing grain sizes of 60, 30, 18, 8 and 5  $\mu\text{m}$ . Further polishing steps included the use of 3 $\mu\text{m}$  and 1 $\mu\text{m}$  diamond polishing paste (LaboPol-6, DP-Mol Polishing, DP-Stick HQ, Struers, Copenhagen, Denmark) and a rotating felt disc. Between each abrading and polishing step, as well as after the final polishing, all enamel slabs were sonicated for 1 min in water. Photographs of all specimens were taken at 12x magnification (Leica M420, Heerbrugg, Switzerland). These photographs were used to mark a  $\pm 2$  mm diameter spot on the surface of each specimen. These spots were later used to mark the exact position of all future surface reflection intensity measurements. The specimens were then stored in 100% humidity in single lockable containers until use.

#### 1<sup>st</sup> measurement of surface reflection intensity (baseline)

Before induction of caries lesions, the specimens were bathed in water and exposed to for 20 min at 121°C (Laboklav ECO, SHB Steriltechnik, Detzel Schloss, Germany). Surface reflection intensity (SRI) was measured using the pen-sized hand-held optical Reflectometer. This reflectometer has a laser diode source of 635 nm, and the laser beam is coupled into a 105  $\mu\text{m}$  fiber, and the angle of incidence is fixed at  $\sim 23^\circ$  (2). The device was connected to a computer running a specific software program used to register SRI. The optical reflectometer has a rubber tip of 2.3 mm diameter that was brought in contact with the surface of the specimen at the predefined spot. The tip was adjusted on the surface of the sample, and the point of highest reflection intensity was registered. SRI measurements at baseline were carried out in duplicate with a 1 week interval.

All specimens were then submitted to A) microbiological assays in order to induce “active” enamel white spot lesions and B) remineralization/brushing treatments in order to convert the lesion into “inactive” white spot lesions.

#### A) Induction of “active” white spot lesions (caries induction)

The enamel surfaces were pre-treated with porcine mucine (type II; Sigma Aldrich, Buchs, Switzerland) for 2 hrs in order to create a pellicle. Initial enamel caries was then created using a 4-species biofilm model consisting of *Streptococcus mutans* ATCC 25175, *Streptococcus sobrinus* ATCC 33478, *Actinomyces naeslundii* ATCC 12104 and *Lactobacillus acidophilus* ATCC 11975. Bacteria were suspended in 0.9 % w/v NaCl and adjusted to McFarland 4. Each 0.5 ml of the suspensions with streptococcal strains and 1 ml of the other strains were given into 100 ml of nutrient broth. The specimens of both groups I and II were divided into five subgroups of 20 specimens each, according to the incubation time. The specimens were then incubated groupwise for 2, 4, 6, 8, and 10 weeks at 37°C in a microaerophilic atmosphere. During the experiment, the specimens were placed in nutrient broth for 6 h resulting in acidic conditions, and in a remineralizing solution for 18 h and during weekends (Table. 1). The pH of in the media was measured twice a day, each before exchange. Once a week, the bacterial growth was controlled.

Table 1: Composition of nutrient broth and remineralizing solution

<p>Nutrient broth (100 mL):</p> <ul style="list-style-type: none"><li>- 50 mL twofold brain heart infusion broth (2x BHI)</li><li>- 5 mL fluoridated distilled water (dH<sub>2</sub>O) 20 ppm</li><li>- 45mL sucrose solution (1 g sucrose in 45 ml dH<sub>2</sub>O)</li></ul>
<p>Remineralization solution (100 mL):</p> <ul style="list-style-type: none"><li>- 50 mL twofold brain heart infusion broth (2x BHI)</li><li>- 5 mL fluoridated distilled water (dH<sub>2</sub>O) 20 ppmF</li><li>- 45 mL buffering solution (130 mg KH<sub>2</sub>PO<sub>4</sub> and 100 mg Na<sub>2</sub>PO<sub>4</sub> in 45 ml dH<sub>2</sub>O)</li></ul>
<p>Bacteria diluted in nutrient broth (100 mL):</p> <ul style="list-style-type: none"><li>- 1 mL of <i>Actinomyces naeshlundii</i> ATCC 12104 suspension (McFarland 4)</li><li>- 1 mL of <i>Lactobacillus acidophilus</i> ATCC 11975 suspension (McFarland 4)</li><li>- 0.5 mL of <i>Streptococcus mutans</i> ATCC 25175 suspension (McFarland 4)</li><li>- 0.5 mL of <i>Streptococcus sobrinus</i> ATCC 33478 suspension (McFarland 4)</li></ul>

After the incubation periods, the biofilm was completely removed using maximum vortex and cleaning with running tap water. The test sites were then cleaned with cotton pellets soaked in 3% sodium hypochlorite by gently scrubbing across the surface.

2<sup>nd</sup> measurement of SRI and visual caries assessment (after caries induction)

After the induction of “active” caries lesions, SRI measurements were carried out in duplicate on the predefined spot, as previously described.

The same spot was additionally rated with two different visual caries detection systems. The first applied system was the International Caries Detection and Assessment System (13). Score 0 represented a healthy, unchanged surface, score 1 an initial white spot lesion that became visible after drying with compressed air, and score 2 was applied when the white spot lesion was visible on the moist specimen. Score 3 represents a local enamel breakdown. The second applied system was the Andersson score (14) in order to describe the severity of the white spot lesions in greater detail: Score 0: No visible colour change; score 1: Slight white colour change, only visible after air drying; score 2: Slight colour change with certain marked white areas; score 3: White consistent colour change; score 4: Distinct white colour change.

Between the experimental steps, the specimens were stored in 100% humidity.

## *B) Conversion into “inactive” white spot lesions (remineralization/brushing)*

For inactivation of the lesions, all specimens were incubated in a remineralizing solution (15) at 37°C for 10 weeks. Twice daily, the specimens were removed from the solution and they underwent controlled tooth brushing. This consisted of immersing the specimens for 2 min in toothpaste slurry (elmex Caries Protection toothpaste; 1450 ppmF, mixed in 1:2 ration with water), and, within this time, the specimens were manually brushed for 10 s, with a controlled force of  $1.50 \pm 0.2$  N (15). Tooth brushing occurred twice daily (during 5 days per week), for a total of 10 weeks. The toothbrush was soft and consisted of rounded nylon bristles with filament diameters of 0.15 mm.

3<sup>rd</sup> measurement of SRI and visual caries detection (after remineralization/brushing).

After conversion into “inactive” white spot lesions, SRI measurements were again performed on the predefined spot, and ICDAS and Andersson score were also applied.

### Scanning electron microscopy (SEM)

SEM images were obtained after induction of caries and after brushing/remineralization. The chosen specimens from each group were dehydrated in a desiccator for at least five days and then sputter-coated with gold/palladium (Balzers SCD 050, Balzers, Liechtenstein; 100 s, 50 mA). The enamel surfaces were evaluated under an SEM (JSM-6010PLUS/LV, Jeol, Tokyo, Japan). Count rates remained constant during the measurements, indicating that neither contamination nor loss of mass occurred. The magnification was 2,000× representing an area of 60 µm × 45 µm for qualitative analysis.

### Statistics

All SRI measurements had been taken in duplicate. The average of the two measurements were used as baseline and 2<sup>nd</sup> and 3<sup>rd</sup> measurements. The percentage differences in specular reflection (%SRI) between the baseline and later measurement moments were calculated. SRI at baseline were considered as 100% and the later values were calculated:  $SRI_i \times 100 / SRI_{baseline}$  where i is the SRI measurement either after caries induction or after remineralization/brushing.

Differences between the groups among the observed time period were analysed with a time-related non-parametrical ANOVA, according to (16). Additional analysis for the same variables among a shorter sub period was done in the same manner. The p values of all these global tests were adjusted according to Holm-Bonferroni’s method. As post-hoc tests, comparisons between the groups at some singular time points were done performing Kruskal-Wallis tests and Wilcoxon rank sum tests, respectively. These tests were not corrected for multiple testing and should be seen in an explorative context. These statistical results were calculated with R 3.2.2 (r-project.org, University of Vienna, Austria), using its packages “MASS”, “nparLD” and “exactRankTests”.

Intraclass correlation (ICC) was calculated for intra-rater reliability analysis using a two-way random effects model. For that, we used the SRI measurements made in duplicate. ICC values were calculated for

baseline (prior to incubation in the biofilm), and for measurements after caries induction (after incubation in the biofilm). These analyses were made using IBM SPSS Statistics v.24. The level of significance was set to 0.05.

## Results

### SRI measurements

Strong intra-rater reliability values were obtained for both native and polished enamel specimens. ICC values for native enamel specimens were ICC=0.813 and ICC=0.890 for SRI at baseline and after caries induction, respectively ( $p<0.001$ ); and polished enamel specimens yielded ICC=0.935 and ICC=0.995 for SRI at baseline and after caries induction, respectively ( $p<0.001$ ).

For native enamel, incubation in the biofilm caused a reduction of the %SRI from 100% at baseline to values between 80-90% after caries induction for 2 to 10 weeks, but there was no relationship to the duration of caries induction ( $p>0.05$ ) (Fig. 1A, light grey boxes). After remineralization/brushing, %SRI increased in early lesions, i.e., in specimens where caries induction took place for up to 6 weeks, we observed higher %SRI; whereas for specimens with longer caries induction periods (8 or 10 weeks) and where enamel lesions were more established, we observed a highly significant drop in %SRI after the remineralization/brushing period (Fig. 1A, dark grey boxes). A linear relationship of %SRI could not be detected for remineralization/brushing.

For polished enamel, caries induction with the biofilm model caused a highly significant drop of %SRI (Fig. 1B, light grey boxes). Longer caries induction periods caused greater decrease in %SRI, but there were no significant differences ( $p>0.05$ ) in %SRI, neither for the very initial lesions (formed after 2 or 4 weeks of caries induction), nor for more established lesions (formed after 8 or 10 weeks of caries induction). After remineralization/brushing (Fig. 1B, dark grey boxes), %SRI decreased significantly in initial lesions, in specimens that were exposed to caries induction for up to 6 weeks, but it remained largely unchanged in more established lesions (formed during 8 and 10 weeks of caries induction).

### ICDAS assessments

For native enamel samples, we observed no white spot lesions on specimens that underwent 2 or 4 weeks of caries induction (median ICDAS score 0). Longer caries induction periods produced more visible caries lesions and a highly significant increase in the ICDAS scores. After 6 weeks of caries induction, the lesions had a median ICDAS score 1, and after 8 and 10 weeks caries induction, even more established enamel lesions were observed, with median significantly rising to ICDAS score 2 (Fig. 2A, light grey boxes).

Remineralization/brushing decreased the median ICDAS score to 0 for the initial lesions formed after 6 weeks of caries induction ( $p=0.08$ ); and decreased the median ICDAS score to 1, for the established lesions formed after 8 and 10 weeks of induction ( $p<0.01$ ).

Likewise, for polished enamel, the white spot lesions became more visible as the caries induction period increased (Fig. 2B, light grey boxes). On specimens that underwent caries induction in for 6 weeks or more, the median ICDAS scores significantly increased to 1, and on specimens that underwent 8 or 10 weeks of caries induction, median ICDAS scores significantly increased to 2. We observed, however, no significant differences in the ICDAS scores between the specimens that underwent 8 or 10 weeks of caries induction. Remineralisation/brushing (Fig. 2B, dark grey boxes) did not affect the established caries lesions, that were formed after 6, 8 or 10 weeks of induction period. However, remineralization caused a significant decrease in the median ICDAS score in specimens when caries induction took place 4 weeks or less.

### Lesion severity assessments

Similar to the ICDAS, the native enamel specimens presented highly significant differences in lesion severity Andersson scores (Fig. 3A, light grey bars) after 4, 6 and 10 weeks of lesion formation ( $p < 0.01$ ), but not between 6 and 8 weeks of lesion formation ( $p = 0.21$ ). Remineralization/brushing led to a significant improvement of lesion severity scores ( $p < 0.01$ ), but did not affect the elder caries lesions ( $p = 0.72$ ; Fig. 3A, dark grey bars).

For the polished enamel samples, significantly increased Andersson scores could be observed for white spot lesions that were created during 6 and 8 weeks of caries induction, but not for the more established lesions developed after 10 weeks of caries induction (Fig. 3B, light grey bars). Remineralization/brushing apparently led to a decrease in the severity of the caries lesions formed up to 8 weeks of caries induction, but not for those formed after 10 weeks (Fig. 3B, dark grey bars).

### SEM analysis

The caries lesions on native enamel lesions had a flaky appearance, exposing areas of sound enamel and presenting 'focal holes' within a slightly eroded surface that are typical for enamel caries, where biofilm is allowed to grow undisturbed (Fig. 4A). Remineralization/brushing led to a clear shift towards a smoother surface, though still flaky, and the focal holes were no longer seen (Fig. 4B). In the polished enamel, only a small number of focal holes could be detected. Instead, the polished surface showed a more uniform eroded enamel surface (Fig. 4C). Remineralization/brushing apparently led to a slightly more scratched surface (Fig. 4D).

## Discussion

In the mouth, active enamel lesions occur under undisturbed demineralizing conditions and show progressive lesion dynamics, whereas inactive lesions refer to the process of stagnation of progression due to lesion remineralization and brushing of the surface (removal of biofilm, polishing). To our best knowledge, this is the first study to use specular surface reflection intensity to discriminate caries lesion activity on both native and ground/polished enamel. Our results show that the handheld reflectometer was able to measure a loss of surface reflectivity after caries induction on both native and polished

specimens. However, specific differences regarding caries severity (time of caries induction) were not observed on native specimens, only on polished specimens.

Caries induction caused a decrease in %SRI to different degrees on native and polished specimens. Generally, SRI measurements are easier to accomplish on flat and polished enamel, since the flat, smoother surface allows for a more regular reflection of the laser (4), thus yielding higher SRI values as native enamel; whereas, the rougher native enamel surfaces tend to scatter the laser beam, but they are also less susceptible to acid demineralisation than polished enamel (12, 17), and this may explain why %SRI values did not decrease more than 20%, levelling out at a plateau of  $\pm 80\%$ , even in groups with longer caries induction times of 8 or 10 weeks. Also, native enamel may have a natural hypermineralized layer on its surface, and this may have also protected the surface against demineralization, leading to a slower progression of the lesion, which could, in part, also explain the differences in the results between native and polished surfaces. Glossiness of polished specimens also decreased during caries development, leading to a decrease in %SRI by more than 50% after 6 weeks, and over 80% after 10 weeks. Surprisingly, we were not able to detect a clear change of %SRI values after the remineralization and tooth brushing period on these specimens.

During the remineralization period, we expected that the combination of the abrasive forces of the toothbrush together with the mineral deposition in the white spot lesions would lead to an increase in enamel surface glossiness (polishing) and, thus, an increase in the %SRI values. On the contrary, toothbrushing actually caused a further decrease in %SRI in all polished specimens. An explanation for this significant decrease in %SRI after the remineralization period is that the initial caries induction period caused a demineralization of the enamel. Since polished enamel is more susceptible to demineralisation than native enamel, the caries induction weakened the enamel surface and caused an initial surface roughening. Subsequently, the brushing with toothpaste, during the remineralisation phase, actually removed part of this weakened surface, thus causing a further roughening of the enamel surface. This is supported by further experiments made in our laboratory. In the present experimental model, we brushed the specimens for a total of 1000 s (10 s twice daily, 5 days per week, for a total of 10 weeks), and when we brushed a sound (caries-free) polished tooth surface for 1000 s, it already caused some roughening of the surface, with an average decrease in %SRI of  $\pm 7\%$ . So, the act of brushing a polished specimen, even when not weakened by caries, already leads to a detectable roughening of the surface, which is exacerbated when the surface is demineralised during caries induction.

Contrary to the polished specimens, the remineralization period promoted remarkably positive results on native enamel with early caries lesions (2, 4, and 6 weeks of caries induction), where tooth-brushing caused an increase in surface glossiness and an increase in %SRI. So, the native enamel specimens with early caries lesions (2, 4, and 6 weeks of caries induction) behaved according to our expectations and clinical experience, where the caries lesions are apparently shinier (more reflective) when they are inactive (8). Interestingly, similarly to polished specimens, we also observed a decrease in %SRI after brushing/remineralization that had undergone caries induction for 8–10 weeks (where more established enamel lesions were formed – ICDAS score 2). In this case, we hypothesize that the early caries lesions

(induced for 2, 4, or 6 weeks) on native specimens were able to decrease the glossiness of the native enamel surface, thus causing a small, albeit distinct, decrease in %SRI, but this early demineralization was not able to weaken the enamel surface enough to allow a further roughening from tooth-brushing. On the other hand, longer caries induction (8–10 weeks) not only caused a decrease in glossiness, but it also caused a weakening of the enamel surface, which was later roughened by the brushing. Furthermore, our limited remineralisation phase (only 10 weeks) produced only a finite toothbrushing period. This was not enough to effectively smoothen the weakened enamel surface, accounting for the lower %SRI values. We can, therefore, also speculate that, in the clinical setting, the recurrent brushing periods that last several months could probably, in time, remove a great deal of the weakened outermost layer of demineralised enamel, thus leading to a smoother surface, with the traditional shiny/glossy appearance of inactive lesions (8). Unfortunately, because we used unpolished, native enamel specimens in this study, we were not able to precisely measure either surface hardness or roughness in the present experiment. Further studies are still necessary to verify our hypothesis, and to assess the relationship between these parameters and %SRI in a caries induction model.

Our caries model mimicking active and inactive enamel lesions worked, and it produced typical white spot lesions. One limitation, however, is that we did not use any histological or non-destructive methods to measure the depth of these lesions, or methods such as TMR to measure the remineralizing effect after toothbrushing. On the other hand, some of these methods are not easily carried out in native enamel surfaces, which we used in the present study. We did, however, use the ICDAS and the Andersson scores to confirm the presence and changes to the white spot lesions. ICDAS is a validated system that links visual appearance to lesion depth (18), while the Andersson score assessed the severity of ICDAS 2 lesions (14). A bacterial biofilm under cariogenic conditions not only leads to superficial enamel erosion, but also, and more importantly, to a subsurface demineralization. The longer and the more aggressive the cariogenic challenge, the deeper the demineralization, as could be confirmed by our results: ICDAS and Andersson in this study were sensitive enough to measure an increase of lesion depth and lesion severity during the caries formation stage. After the remineralization/brushing period of the experiment, the visual assessments were sensitive enough to detect a decrease of severity and, partially, lesion depth.

Subsurface demineralization is characterized by a pseudo-intact surface that allows bacterial acids to penetrate the enamel through focal holes (Fig. 4A). Only at an advanced stage of caries does the net demineralization eventually lead to a breakdown of the surface. This was found in some of the native enamel specimens after 10 weeks of caries formation (Fig. 2A). Thus, the enamel specimens, both native and polished, behaved as expected under long-term cariogenic/demineralizing conditions and under remineralizing/abrasive conditions. In theory and from our clinical experience, enamel lesions under an undisturbed biofilm challenged by cariogenic conditions are 'active' lesions, while regular removal of biofilm by tooth-brushing, abrasion of the surface by toothpaste, and remineralization of the enamel by fluoride, calcium and phosphorous lead to characteristic surface changes (increase of glossiness, less chalky appearance) that are attributed to 'inactive' lesions (9). However, although we observed a change in lesion appearance mirrored in the ICDAS and Andersson scores, we were not able to detect changes of glossiness or mattness in our specimens throughout the duration of the study. This could be because the

caries model chosen was rather mild, or that we needed longer brushing periods to allow for further tooth surface polishing.

The visual appearance of enamel lesions is dependent on many factors, e.g. the level of magnification (19), the amount of light that is used during caries diagnosis (20), or on the examiner him/herself: In an *ex vivo* study on 104 extracted teeth with white spot lesions, only about half of the lesions were unanimously rated “matte” or “shiny” by 4 examiners (21). Therefore, an objective method would be desirable to measure the reflective light and thus to quantify mattness of a lesion. A first promising step was described some time ago using a chromatic confocal white light sensor that measured the perpendicular reflection intensity (PRI) with an angle of 0° between incoming and reflected light (10). Due to the nature of the confocal sensor, the wavelength of the incident light was not important for the measurements, but only its reflection intensity. In an *ex vivo* study, 43 white spot lesions were visually judged as being ‘active’ or ‘inactive’, and the PRI method was correlated well to these visual judgements. A true validation however was not possible, because it is error-prone to judge the status of lesion activity on extracted teeth without the necessary specific clinical information. Furthermore, some lesions can also be regarded as ‘mixed’ lesions, with surface characteristics of both active and inactive lesions. Therefore, instead of using extracted teeth with caries lesions, we have opted for a bacterial caries model to reliably simulate clinical conditions for caries development, thus standardizing the cariogenic impact. We set up a rather mild caries model, with a total fluoride content of 1 ppmF in the demineralization solution, in order to be able to better display a time-related relationship between caries formation and loss of SRI. Our caries model allowed for distinct differences in %SRI on the polished specimens, where we observe a gradual decrease in %SRI as the duration of caries induction increased.

Different to PRI, the Optipen device operates on SRI (specular reflection intensity), using a red laser light (635 nm) with entrance angle and reflection angle both set at 23°. Therefore, it could be speculated that PRI and SRI measure different reflection features. It was shown earlier that light absorption into human enamel increases with decreasing wavelengths (22). Therefore, absorption would not interfere at the chosen wavelength. In a former study by (23) the wavelength of 633 nm was reported to almost perfectly fit to a Monte Carlo curve. In this curve the scattering maximum is at 0°, while for angles >20° the curve reaches a plateau at log (Fract. scattered energy) of  $10^{-1}$ , indicating that scattering at the surface in enamel is almost negligible (23). So, under the parameters chosen in our experiment, the light beam of the Optipen purportedly is transduced into the enamel and is scattered at the body of the white spot lesion. The formerly established method of laser fluorescence for caries detection operates on a quite similar wavelength of 655 nm and uses the backscattered fluorescence signal to measure lesion depth (24). Thus, while PRI measures only reflection intensity of the surface, the Optipen probably also excites fluorophores of bacterial porphyrins that are located in the subsurface lesion (25). This also explains why the SRI measurements in our experiment remained stable in the native enamel group. PRI was found to correspond well with lesion activity in enamel caries lesions that were induced for 3, 6 and 9 days, respectively (26). However, in the latter study only flat polished enamel was used, and the results cannot be transferred to native enamel.

Because lesion activity is not only characterized by optical appearance (shiny or matte), but also by tactile surface features (rough or smooth), it would have been desirable to also measure roughness of our specimens. Although this was not possible in this paper, previous erosion studies using the SRI device have shown a strong correlation between surface roughness and SRI (27), so the decrease in %SRI observed in our specimens is most probably related to an increase in enamel surface roughness. Moreover, there is a strong correlation between SRI and surface hardness, where a decrease in %SRI is also related to a weakening of the enamel surface (3). This corroborates our initial hypothesis, that enamel specimens submitted to our caries model presented a decrease in %SRI, which is associated to a rougher and weaker enamel surface, and less gloss; while the subsequent remineralization period (tooth-brushing) further roughened the enamel surface, provoking an even greater decrease in %SRI values.

Despite the interesting positive results with the Optipen for early enamel lesions on native enamel, there are two main disadvantages to this device that could hinder its use in the clinical setting. The first is that the Optipen is highly surface-dependent, where the SRI values will depend on the surface micro-morphology of each tooth surface. This means that different teeth, depending on their surface micro-morphology and roughness, will yield very different SRI values. It follows that, we cannot generate a universal SRI range for “sound” and “demineralised” tooth surfaces, instead, each tooth surface will have its own “sound” and “demineralised” values. Therefore, the Optipen should rather not be used as a diagnosis tool, but as a monitoring device. The other disadvantage is that the Optipen is also, to some extent, rater-dependent, where different individuals using the device on the same tooth surface will obtain slightly different SRI values. This, however, can be counterbalanced by training, where trained individuals have obtained good agreements, with intraclass correlation of ICC = 0.77 and 0.86 for deciduous and permanent teeth, respectively (3).

## Conclusion

Our expectations to use specular surface reflection intensity to discriminate between active and inactive caries lesions were only partially met. While the hand-held reflectometer was able to distinguish between caries-free and caries-active enamel surfaces (before and after caries induction), it was neither able to fully discriminate lesion activity on polished specimens, nor to distinguish established caries lesions (formed for 8–10 weeks caries induction) on native specimens. Although optical enamel lesion activity assessment is desirable, Optipen, in its current form, would be suitable only for early enamel lesions on native enamel.

## Abbreviations

PRI	Perpendicular Reflection Intensity
KEK	Cantonal Ethics Committee (Kantonale Ethikkommission)
SRI	Surface Reflection Intensity

%SRI	Relative Surface Reflection Intensity
ICDAS	International Caries Detection and Assessment System
SEM	Scanning Electron Microscopy
ICC	Intraclass Correlation
ppm	Parts per million

## Declarations

### Ethics approval

The present study used extracted teeth from a pooled biobank. The local ethics committee considers these specimens as “irreversibly anonymized”, weaving the requirement of authorization.

### Consent for publication

This study does not have the prerequisite for consent.

### Availability of data and materials

All data supporting the results and conclusions are reported within this article.

### Competing Interests

The authors (T.S.C; S.S., S.E., F.L. A.L, and K.W.N.) declare that they have no competing interests.

### Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

### Authors' contributions

Conceptualization: K.W.N. and T.S.C; Methodology: K.W.N. T.S.C, A.L, S.E.; Data acquisition and curation: S.S., T.S.C., K.W.N., S.E., A.L.; Writing—original draft: T.S.C. and K.W.N.; Writing—review and editing: S.S., S.E., A.L. and F.L. All authors have read and agreed to the published version of the manuscript.

### Acknowledgements

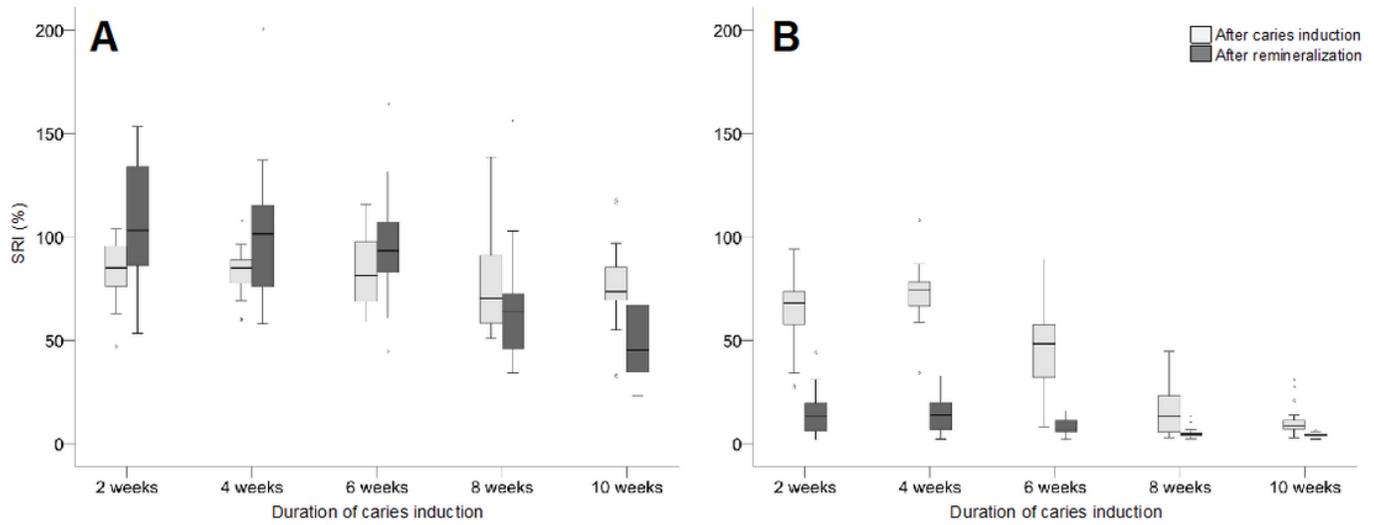
The statistics in this study were performed by Lukas Martig, significantis. Kathrin Tegel helped during the experimental phase of this study.

## References

1. Rakhmatullina E, Bossen A, Höschele C, Wang X, Beyeler B, Meier C, et al. Application of the specular and diffuse reflection analysis for in vitro diagnostics of dental erosion: correlation with enamel softening, roughness, and calcium release. *Journal of biomedical optics*. 2011;16:107002.
2. Rakhmatullina E, Bossen A, Bachofner KK, Meier C, Lussi A. Optical pen-size reflectometer for monitoring of early dental erosion in native and polished enamels. *Journal of biomedical optics*. 2013;18(11):117009.
3. Carvalho TS, Assuncao CM, Jost F, Burgin WB, Rodrigues JA, Lussi A. In vitro validation of a hand-held optical reflectometer to measure clinically observed erosive tooth wear. *Lasers Med Sci*. 2016;31(6):1105-12.
4. Carvalho TS, Baumann T, Lussi A. A new hand-held optical reflectometer to measure enamel erosion: correlation with surface hardness and calcium release. *Scientific reports*. 2016;6:25259.
5. Marcenes W, Kassebaum NJ, Bernabe E, Flaxman A, Naghavi M, Lopez A, et al. Global burden of oral conditions in 1990-2010: a systematic analysis. *Journal of dental research*. 2013;92(7):592-7.
6. Lussi A, Megert B, Longbottom C, Reich E, Francescut P. Clinical performance of a laser fluorescence device for detection of occlusal caries lesions. *European journal of oral sciences*. 2001;109(1):14-9.
7. Nyvad B. Diagnosis versus detection of caries. *Caries research*. 2004;38(3):192-8.
8. Nyvad B, Machiulskiene V, Baelum V. Reliability of a new caries diagnostic system differentiating between active and inactive caries lesions. *Caries research*. 1999;33(4):252-60.
9. Thylstrup A, Bruun C, Holmen L. In vivo caries models—mechanisms for caries initiation and arrestment. *Advances in dental research*. 1994;8(2):144-57.
10. Neuhaus KW, Nyvad B, Lussi A, Jaruszewski L. Evaluation of perpendicular reflection intensity for assessment of caries lesion activity/inactivity. *Caries research*. 2011;45(4):408-14.
11. Lussi A, Bossen A, Höschele C, Beyeler B, Megert B, Meier C, et al. Effects of enamel abrasion, salivary pellicle, and measurement angle on the optical assessment of dental erosion. *Journal of biomedical optics*. 2012;17:97009-1.
12. Ganss C, Klimek J, Borkowski N. Characteristics of tooth wear in relation to different nutritional patterns including contemporary and medieval subjects. *European journal of oral sciences*. 2002;110(1):54-60.
13. Banting D, Eggertsson H, Ekstrand KR, Zandoni AF, Ismail AI, Longbottom C, et al. Rationale and evidence for the international caries detection and assessment system (ICDAS II). *Ann Arbor*. 2005:1001-78.

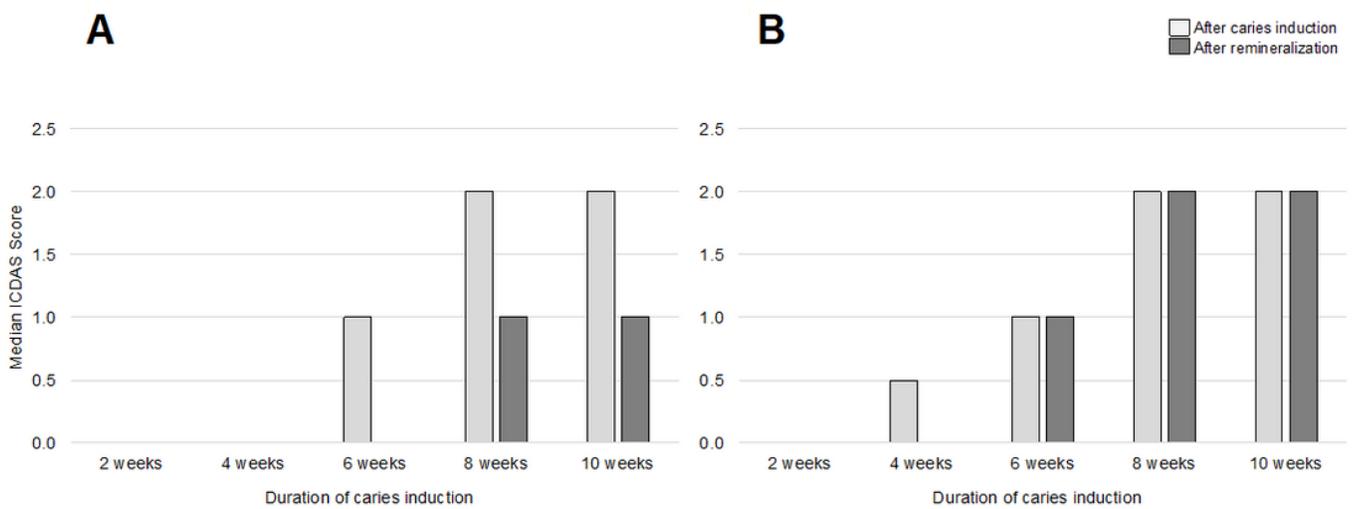
14. Andersson A, Skold-Larsson K, Hallgren A, Petersson LG, Twetman S. Effect of a dental cream containing amorphous cream phosphate complexes on white spot lesion regression assessed by laser fluorescence. *Oral Health Prev Dent.* 2007;5(3):229-33.
15. ten Cate JM, Arends J. Remineralization of artificial enamel lesions in vitro. II. Determination of activation energy and reaction order. *Caries research.* 1978;12(4):213-22.
16. Brunner E, Domhof S, Langer F. Nonparametric analysis of longitudinal data in factorial experiments. 1 ed. New York: John Wiley & Sons; 2002. p. 288p.
17. Sullivan HR. The solubility of enamel surfaces. *Journal of dental research.* 1954;33(4):504-10.
18. Topping GVA, Pitts NB. Clinical visual caries detection. *Monogr Oral Sci.* 2009;21:15-41.
19. Neuhaus KW, Jost F, Perrin P, Lussi A. Impact of different magnification levels on visual caries detection with ICDAS. *Journal of dentistry.* 2015;43(12):1559-64.
20. Neuhaus KW, Jasarevic E, Lussi A. Impact of Different Illumination Conditions on Visual Caries Detection with ICDAS. *Caries research.* 2015;49(6):633-6.
21. Neuhaus KW, Schlafer S, Lussi A, Nyvad B. Infiltration of natural caries lesions in relation to their activity status and acid pretreatment in vitro. *Caries research.* 2013;47(3):203-10.
22. Leuschner FW, Lotriet CJ, Denner BF. Spectral reflectance and transmittance in dental enamel for UV, visible, and NIR light. *Proc SPIE.* 1995;2323:317.
23. Fried D, Glens RE, Featherstone JD, Seka W. Nature of light scattering in dental enamel and dentin at visible and near-infrared wavelengths. *Appl Opt.* 1995;34(7):1278-85.
24. Lussi A, Imwinkelried S, Pitts N, Longbottom C, Reich E. Performance and reproducibility of a laser fluorescence system for detection of occlusal caries in vitro. *Caries research.* 1999;33(4):261-6.
25. Neuhaus KW, Longbottom C, Ellwood R, Lussi A. Novel lesion detection aids. *Monogr Oral Sci.* 2009;21:52-62.
26. Ando M, Fontana M, Eckert GJ, Arthur RA, Zhang H, Zero DT. Objective and quantitative assessment of caries lesion activity. *Journal of dentistry.* 2018;78:76-82.
27. Brevik SC, Lussi A, Rakhmatullina E. A new optical detection method to assess the erosion inhibition by in vitro salivary pellicle layer. *Journal of dentistry.* 2013;41(5):428-35.

## Figures



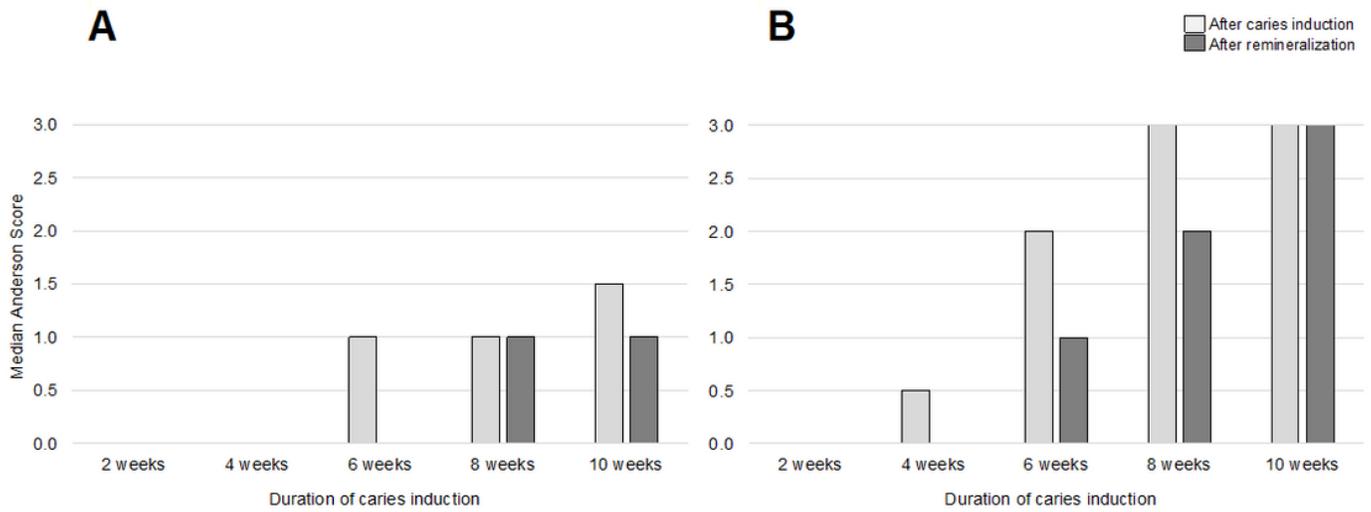
**Figure 1**

Surface reflection intensity (SRI) at each experimental period after caries induction (light grey boxes) and after remineralization (dark grey boxes): A) native enamel, B) polished enamel.



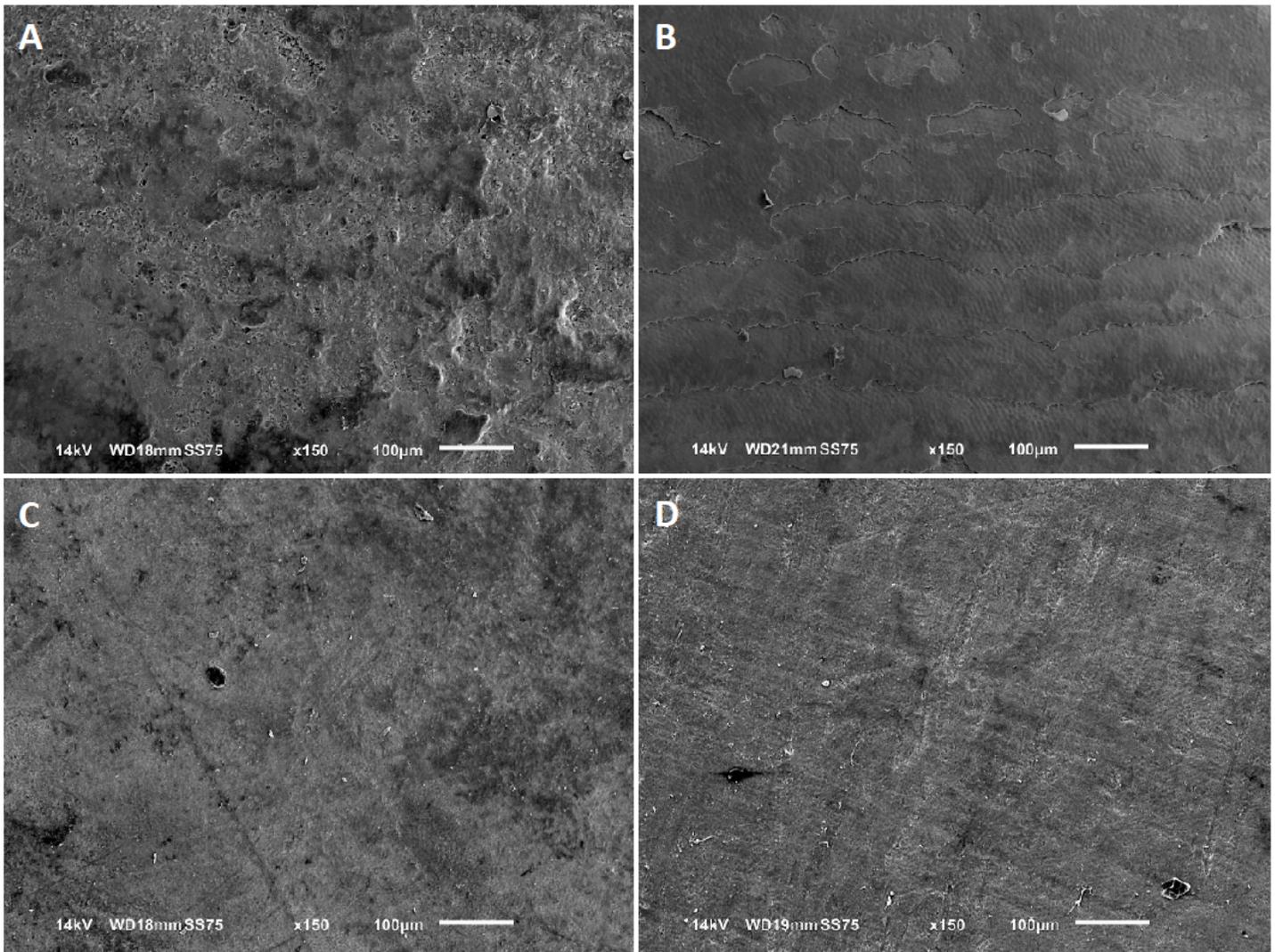
**Figure 2**

Median ICDAS score at each experimental period after caries induction (light grey boxes) and after remineralization (dark grey boxes): A) native enamel, B) polished enamel.



**Figure 3**

Median Anderson score at each experimental period after caries induction (light grey boxes) and after remineralization (dark grey boxes): A) native enamel, B) polished enamel.



**Figure 4**

Scanning electron microscopy images of the enamel surface. A) Native enamel after caries induction; B) Native enamel after remineralization/brushing; C) Polished enamel after caries induction; D) Polished enamel after remineralization/brushing.