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# Optical pen-size reflectometer for monitoring of early dental erosion in native and polished enamels

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**Abstract.** Application of the specular reflection intensity was previously reported for the quantification of early dental erosion. Further development of the technique and assembly of the miniaturized pen-size instrument are described. The optical system was adjusted to fit into a handy device which could potentially access different positions in the oral cavity. The assembled instrument could successfully detect early erosion progression in both polished ( $n = 70$ ) and native ( $n = 20$ ) human enamels. Different severities of enamel erosion were induced by varying incubation time of polished enamel in 1% citric acid (pH = 3.60, 0.5 to 10 min), while the native incisors were treated in the commercial orange juice (Tropicana Pure Premium®, pH = 3.85, 10 to 60 min). The instrument provided a good differentiation between various severities of the erosion *in vitro*. The size of the measurement spot affected the erosion monitoring in native enamel (human incisors). The erosion measurement in the 0.7-mm (diameter) cervical spots showed systematically lower reflection intensities compared with the analysis of central and incisal small spots. The application of larger spot areas (2.3 mm) for the erosion monitoring revealed no effect ( $p > 0.05$ ) of the spot position on the reflection signal. High variation of the teeth susceptibility toward *in vitro* erosion was detected in native enamel. © The Authors. Published by SPIE under a Creative Commons Attribution 3.0 Unported License. Distribution or reproduction of this work in whole or in part requires full attribution of the original publication, including its DOI. [DOI: 10.1117/1.JBO.18.11.117009]

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## 1 Introduction

Dental erosion is a multifactorial disease<sup>1</sup> which is often related to the excessive consumption of acidic beverages (juices and energy drinks) in combination with abrasion of the enamel. The overlapping of these processes is the reason why it is called erosive tooth wear today. Dietary acids demineralize calcium-deficient hydroxyapatite of the enamel, causing reduced tissue hardness and increased vulnerability toward the mechanical impact such as attrition or abrasion.<sup>2</sup> Gradual loss of the enamel results in its irreversible thinning and can lead to a hypersensitivity and dentine exposure in severe cases.<sup>3</sup> Because the prevalence of dental erosion is growing steadily and shifting toward young ages,<sup>4</sup> it is important to diagnose its initial stages to timely apply the preventive measures and to postpone the tooth wear.

Among numerous analytical techniques for the erosion assessment in the laboratory studies,<sup>5–7</sup> only a few were tested for clinical erosion diagnostics and quantification. Optical coherence tomography (OCT) is one of them; it was successfully used for the erosion assessment in patients with gastroesophageal reflux disease.<sup>8</sup> OCT was proven to be a promising technique, but it is highly dependent on the severity of the erosion.<sup>9</sup> Nevertheless, this method is constantly under improvement emerging different OCT variations<sup>10,11</sup> such as polarization-sensitive OCT or Fourier-domain OCT. Quantitative light fluorescence (QLF) is another promising technique for the

erosion diagnostics,<sup>12</sup> although there were conflicting results among various studies.<sup>13</sup> In addition, chlorhexidine or fluoride applications were shown to affect the QLF results,<sup>14</sup> so that further technique development is required for its clinical application, especially in the early stages of erosion. Fried et al. demonstrated a new near-infrared (IR) reflectance imaging method<sup>15,16</sup> which could be potentially used for the clinical localization of the demineralized tissue. Thomas et al. showed a gradual increase of the diffuse reflectance during tooth erosion,<sup>17</sup> though the sensitivity of the signal to erosive changes was poor. Linear decrease and increase of the backscattered light intensity during demineralization and remineralization processes were reported by Kishen et al.<sup>18</sup> Change of the specular and diffuse reflection intensities during erosion progression was investigated in our previous *in vitro* studies.<sup>19,20</sup> Particularly, sharp decay of the specular reflection was observed already in the early stages of enamel erosion *in vitro*, while the less pronounced increase of the diffuse reflection intensity was measured, which correlated well with the results of Thomas et al.<sup>17</sup> Further investigation revealed a strong correlation between the decrease of the specular reflection and continuous surface roughening.<sup>19</sup> Significant change of the specular reflection was detected even in eroded and abraded surfaces. A prototype device (reflectometer) comprising the illuminating and measurement arms with the 45-deg angles of incidence and reflection, halogen lamp, and the spectrometer was used in the above-mentioned studies. Although these former studies provided an important methodological basis, a handy version of such an instrument was required to shift from lab-based setup to a chair-side clinical device.

Considering the practical requirements, a fiber-optic design with a measurement head of 15 to 17 mm maximum was

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targeted to ensure the access to all positions within the oral cavity (for example, buccal sites of upper molars). Moreover, a pen-like shape of the instrument was considered to be advantageous due to easy and light handling similar to a dental bur design. Software was created to provide fast and easy signal read-out, which contributed to the overall reduced analysis time. This article presents assembly of the miniaturized reflectometer version and its application for the quantification of early dental erosion in both polished and native human enamels.

## 2 Experimental Part

### 2.1 Materials

#### 2.1.1 Chemicals

Chloramin T trihydrate solution ( $\geq 98\%$ , Sigma-Aldrich, Steinheim, Germany) was applied for the storage of extracted teeth as received. Citric acid ( $\geq 99.5\%$ , Merck KGaA, Darmstadt, Germany) was used for the preparation of erosive solutions. Commercial Tropicana Pure Premium® orange juice (Tropicana Products Inc., Zaventem, Belgium) was purchased in Coop, Berne, Switzerland.  $\text{CaCl}_2$  ( $\geq 99.5\%$ ),  $\text{KH}_2\text{PO}_4$  ( $\geq 99\%$ ), and  $\text{NaCl}$  ( $\geq 99.5\%$ ) were purchased from Merck KGaA and were used for the preparation of the mineral solution as received.

#### 2.1.2 Preparation of the polished human enamel

Caries-free human molars with no cracks on the buccal surfaces (microscopy examination, magnification 20 $\times$ ) were selected from a pool of extracted teeth. All teeth were extracted by dental practitioners in Switzerland (no water fluoridation, 250 ppm  $\text{F}^-$  in table salt) and were stored in 1% chloramin T trihydrate solution. Before the extraction, the patients were informed about the use of their teeth for research purposes, and consent was obtained.

The preparation of enamel specimens was identical to the previous experiments.<sup>19,20</sup> Briefly, teeth crowns were separated from the roots using Isomet® Low Speed Saw (Buehler, Düsseldorf, Germany). The buccal sites of the specimens were embedded into the resin (Paladur, Heraeus Kulzer GmbH, Hanau, Germany) in two planar parallel molds. The thinner mold (200- $\mu\text{m}$  thick) was removed, while the teeth in the thicker one (7.5-mm thick) were serially abraded under constant tap water cooling using Knuth Rotor machine (LabPol 21, Struers, Copenhagen, Denmark) with silicon carbide paper discs of grain size 18.3, 8, and 5  $\mu\text{m}$ , 60 s each. The embedded enamel blocks

were taken out of the molds before being polished for 60 s with 3- $\mu\text{m}$  diamond abrasive on Struers polishing cloth under constant cooling (LaboPol-6, DP-Mol Polishing, DP-Stick HQ, Struers, Copenhagen, Denmark). Between two polishing steps and after the final polishing, all slabs were ultrasonicated for 1 min in tap water and rinsed. All prepared specimens had a flat-ground enamel area with 200- $\mu\text{m}$  cut-off layer. Samples were stored in a mineral solution [1.5 mmol/l  $\text{CaCl}_2$ , 1.0 mmol/l  $\text{KH}_2\text{PO}_4$ , and 50 mmol/l  $\text{NaCl}$ ,  $\text{pH} = 7.0$ <sup>21</sup>] and underwent further polishing with a 1- $\mu\text{m}$  diamond abrasive (60 s, LaboPol-6, DP-Mol Polishing, DP-Stick HQ, Struers) immediately before the experiment. Part of the exposed enamel area was covered with light curing fixation adhesive tape (Technovit, Heraeus Kulzer GmbH, Germany) to preserve the original enamel surface from the erosive treatment [reference nontreated area, Fig. 1(a)].

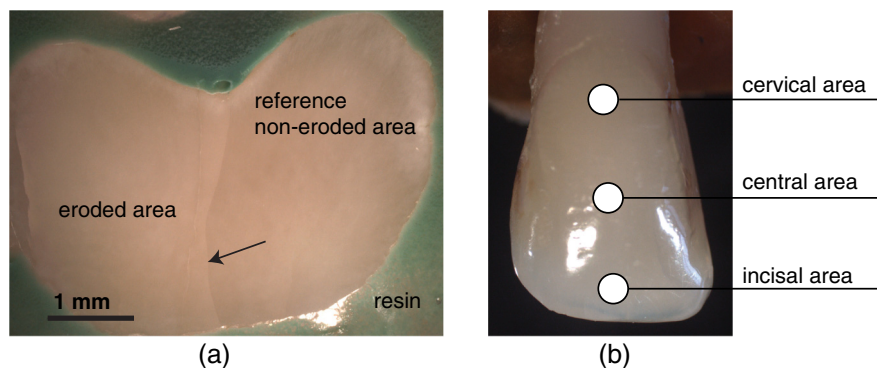
#### 2.1.3 Selection of teeth with native enamel

Human incisors without caries, visible cracks, and restorations were selected from the pool of extracted teeth. All teeth were extracted by dental practitioners in Switzerland (no water fluoridation, 250 ppm  $\text{F}^-$  in table salt) and were stored in 1% chloramin T trihydrate solution. Before the extraction, the patients were informed about the use of their teeth for research purposes, and consent was obtained. No history record of teeth, their age, or donors were recorded or remained.

Extracted teeth (with roots) were rinsed in tap water and used without further modification. All teeth were stored in a humid chamber between the treatments and measurements.

#### 2.1.4 In vitro erosion of polished enamel samples

The same erosive conditions as in the previously published studies,<sup>19,22</sup> were applied due to a good correlation between the reflection signal and enamel microhardness under the given erosive regime. The prepared polished enamel specimens containing protected tape-covered areas (Sec. 2.1.2) were immersed into 30 ml of citric acid (1%,  $\text{pH} = 3.60$ ) either for 0.5, 1, 2, 4, 6, 8, or 10 min at 30°C. Samples were removed from acidic solutions, rinsed in tap water (20 s), and dried with oil-free air (5 s). Protective Technovit adhesive tape was removed after the erosion, so that each sample had eroded and noneroded areas for the reflection analysis [Fig. 1(a)]. Ten enamel specimens were used for each of the erosion time; 70 specimens in total.



**Fig. 1** (a) Image of the polished enamel specimen containing eroded (left) and noneroded (right) reference areas; arrow points to the border between two regions. (b) Assignment of the measurement positions in cervical, central, and incisal areas at the labial incisor surface.

### 2.1.5 *In vitro* erosion of native enamel

Human incisors ( $n = 10$ , 2 $\times$ ) were incubated in 100 mL of Tropicana Premium® orange juice [pH = 3.85, titratable acidity of 1.68 g/100 mL (0.35 mol OH<sup>-</sup>/l), titrated to pH = 7.00, freshly opened] for 10 min under constant agitation (100 rpm). Teeth were rinsed in tap water and dried with oil-free air (5 s). After the reflection measurements, the erosive procedure was repeated again to a total of six times, resulting in an overall of 60 min of exposure time in the orange juice (10 min, 6 $\times$ ). Twenty teeth were used in this cycling erosive experiment.

## 2.2 Methods

### 2.2.1 Assembly of the fiber-optic miniaturized reflectometer

A pen-size fiber-optic reflectometer system for the quantification of early dental erosion is illustrated in Figs. 2(b) and 3. The device was composed of two multimode fibers and an optical lens focusing the incident light onto the enamel surface and providing 23-deg angles of incidence and reflection. The illumination path was designed to achieve a numerical aperture (NA) of 0.12 and a measuring spot of 0.5 mm. For illumination, a 1.0-mW diode laser (FDS100, Thorlabs Inc., Newton, New Jersey) operating at 635 nm was coupled into a 100- $\mu$ m multimode fiber, where a small grin lens and a 1-mm prism were glued at the end of the tip for the collimation and the deflection of the illumination beam. The measuring path was designed with an NA of 0.25 and was composed of a 400- $\mu$ m fiber with a grin lens and a 2-mm prism coupling the light into a photodiode. The device had input and output fiber optic cables to the detection unit, which was connected to the PC over the USB port. Simple custom-made software was created to allow a signal recording from the reference area and area of interest with the possibility to save the sample name and erosive conditions.

The device was equipped with removable measurement tips (Fig. 3), so that different tip shapes and sizes could be tested, as well as single-use tips could be used to fulfil hygienic requirements. The length of the tip was fixed at 6 mm, while the diameters at the end of the tip were 0.7 and 2.3 mm (Fig. 3).

The device and tips were made by rapid prototyping with a three-dimensional printer (Objet Alaris30, VeroWhitePlus RGD835, Stratasys GmbH, Rheinmünster, Germany).

### 2.2.2 Measurements of the polished eroded enamel by the prototype and miniaturized reflectometers

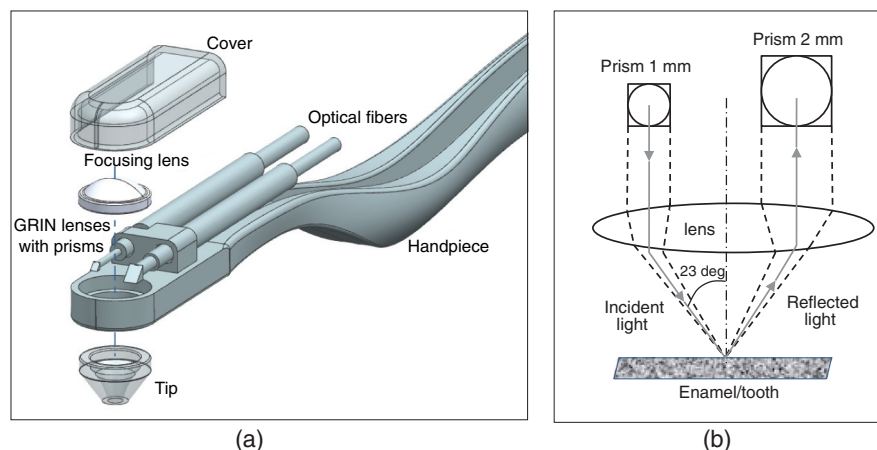
The assembly and description of the prototype optical reflectometer were reported earlier.<sup>19</sup> The same device was used for the comparison with new miniaturized version in this study. The prepared polished enamel specimen was positioned on the sample holder, so that the light spot was first localized on the noneroded reference enamel area [Fig. 1(a)]. The software was configured with a boxcar width (spatial averaging) of 10 nm and a scan average of 2. The position of the sample holder and the integration time were adjusted to obtain maximal reflection signal, i.e., 90% of the measuring range. The effect of the ambient light was subtracted by the elimination of the reflection spectrum recorded with the halogen light source being switched off (offset signal). Afterward, the light source was switched on, and the reflection signal from noneroded enamel ( $I_0$ ) was recorded in the wavelength range of 400 to 800 nm. Then the sample was moved, so that the light spot was localized on the eroded enamel area. The same measurement was performed for the eroded enamel after each subsequent erosive challenge ( $I_{er}$ ). The specular reflection intensity ( $I$ ) was calculated at 635-nm wavelength using Eq. (1):

$$I = [(I_{er} - I_{offset}) / (I_0 - I_{offset})] \times 100. \quad (1)$$

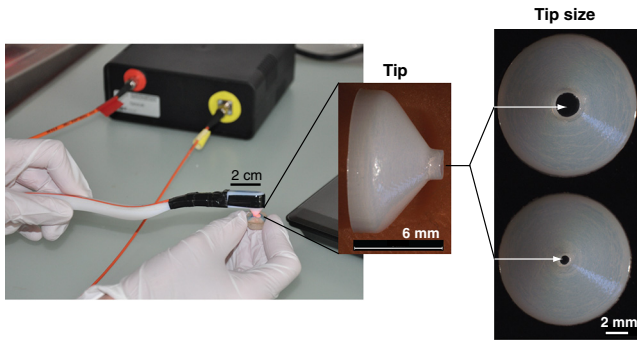
Furthermore, the same enamel sample was immediately measured by the assembled miniaturized instrument (Fig. 3). The tip of the device was first brought in contact with the noneroded reference enamel surface, and the specular reflection intensity was automatically measured during the entire contact duration. The custom-made software acquired the maximal reflection intensity measured in the reference enamel area and set the value as a reference signal ( $I_0$ ). Then, the specular reflection intensity was measured in the eroded enamel area ( $I_{er}$ ) using the same principle. The specular reflection intensity ( $I$ ) was calculated as a ratio ( $I_{er}/I_0$ )  $\times$  100 and saved in the database.

### 2.2.3 Monitoring of the erosive dose-response in native human enamel using assembled optical instrument

All selected teeth were photographed using light microscope (Leica M420, Leica, Heerbrugg, Switzerland), and the measurement spots were assigned to particular positions in cervical, central, and incisal labial tooth surfaces [Fig. 1(b)]. The positions



**Fig. 2** (a) Components of the assembled instrument; (b) optical scheme and the measurement principle of the miniaturized setup.



**Fig. 3** From left to right: photographs of the pen-size assembled reflectometer, measurement tip (6-mm long), and different sizes of the measurement spots (2.3 and 0.7 mm).

were marked on the photographs and were accurately verified before each measurement.

A tip with the measurement spot area of 0.7 mm (Fig. 3) was maintained in the miniaturized optical device. Defined positions within cervical, central, and incisal areas in healthy incisors were measured prior to erosive challenge. The obtained raw signals ( $I_0$ ) were used as reference reflection values for the subsequent measurements of the same areas after erosion. The tip was brought into a tight contact with the cervical area of the labial incisor, and the maximal reflection intensity was automatically recorded by the software and saved. The same was performed for the measurements of mesial and incisal areas. Afterward, a tip with a 2.3-mm diameter was placed into the device, and the same defined areas in healthy incisors (before erosion) were measured again. An identical measurement procedure was carried out using two different tips (0.7 and 2.3 mm) and in three defined positions in each tooth after every single erosive challenge ( $I_{er}$ ). The reflection intensities were calculated as ratios  $(I_{er}/I_0) \times 100$ , where  $I_{er}$  is the raw recorded reflection value after each of erosive treatment (six in total) in each of defined positions.

#### 2.2.4 Scanning electron microscopy

Representative specimens were mounted on aluminium stubs and sputtered with gold/palladium (100 s, 50 mA) using a sputtering device (Balzers SCD 050, Balzers, Balzers, Liechtenstein). Scanning electron microscopy (SEM) was performed with a Stereoscan S360 scanning electron microscope at 20 kV (Cambridge Instruments, Cambridge, UK). Equal digital SEM micrographs (of 2000 $\times$  and 5000 $\times$  magnifications, respectively) were generated for each specimen (Digital Image Processing System, version 2.3.1.0, point electronic GmbH, Halle, Germany).

#### 2.2.5 Statistical data analysis

A total of 70 polished enamel samples ( $n = 10$ /each of erosion time) and 20 human incisors (native enamel) were included in the study. Statistical data analysis was performed using a non-parametric Brunner–Langer model (F1\_LD\_F1)<sup>23</sup> and pairwise Wilcoxon rank sum tests. The level of significance was set at 0.05. The correlations between the specular reflection intensities measured by the prototype and miniaturized devices were calculated using Pearson rank coefficients ( $r^2$ ). The correlation coefficients were determined using all samples at all applied erosion times. Statistical Software R, version 2.15.1, was applied

for the estimation of spot-to-spot deviation when measured by different tip sizes (0.7 and 2.3 mm).

### 3 Results

This study can be divided into three parts. First, the explored optical setup for the reflection analysis was miniaturized into the pen-size device. Second, the assembled instrument was tested for the erosion quantification in polished enamel, and the results were compared with the reflection signal measured in the same samples by the previous prototype version.<sup>19</sup> Third, the same assembled miniaturized device was applied to detect the erosive dose-response in native enamel, and thus its applicability for the measurement of curved uneven surfaces was verified. The results obtained in each part of the study are presented in the below sections accordingly.

#### 3.1 Assembly of the Optical Pen-Size Device

The components of the miniaturized reflectometer were described in Sec. 2.2.1 and are shown in Fig. 2. The assembled device had the dimensions of a tooth brush with an entire length of 19 cm and the height of the measuring head of 1.5 cm (Fig. 3), which would allow access to all positions within the oral cavity. The measuring tips of the device were removable, so that the tips with different diameters of the measurement spot (0.7 to 2.3 mm) could be inserted and tested. Moreover, exchangeable tip design permits application of single-use tips in further clinical trial.

#### 3.2 Application of the Assembled Instrument for the In Vitro Erosion Analysis in Polished Enamel

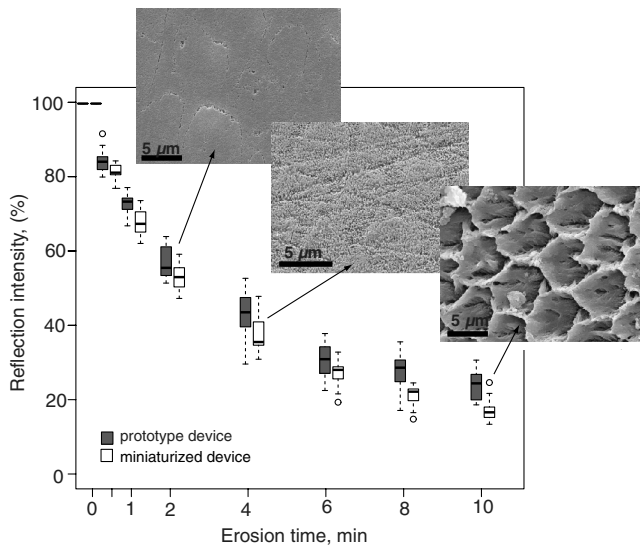
Similar decrease of the specular reflection intensity was measured with erosion progression using the old prototype and the miniaturized devices with the tip diameter of 2.3 mm ( $p > 0.05$ , Fig. 4). After 1 min of the erosive challenge, about 25% to 30% loss of the reflection intensity was measured in enamel samples using both devices (Fig. 4). After 4 min of erosion, only 40% to 45% of the reflection intensity remained, while subsequent erosive challenges resulted in a less prominent change of the reflection signal (Fig. 4). Around 75% to 80% of the reflection loss was measured after the total 10 min of the erosion. High correlation ( $r^2 = 0.968$ ) was found between reflection measurements by the two devices at all applied severities of the erosion.

SEM images also showed the advancing etching of the enamel with prolonged erosion time. First, the roughening of the originally smooth polished enamel occurred (Fig. 4, see 4 min), followed by the signs of the enamel prisms and their disclosure (Fig. 4, see 10 min).

Moreover, tips with different diameters of the measuring spot (0.7 and 2.3 mm, Fig. 3) were tested for the comparison. The same polished enamel samples and the same enamel areas were used for the comparative measurements by different tips. Obviously, within the tested spot sizes, there was no detectable effect of the tip size on the reflection signal in polished enamel ( $p > 0.05$ , graph is not shown).

#### 3.3 Detection of the Erosive Dose-Response in the Native Enamel and Effect of the Tip Size on the Spot-to-Spot Signal Variation

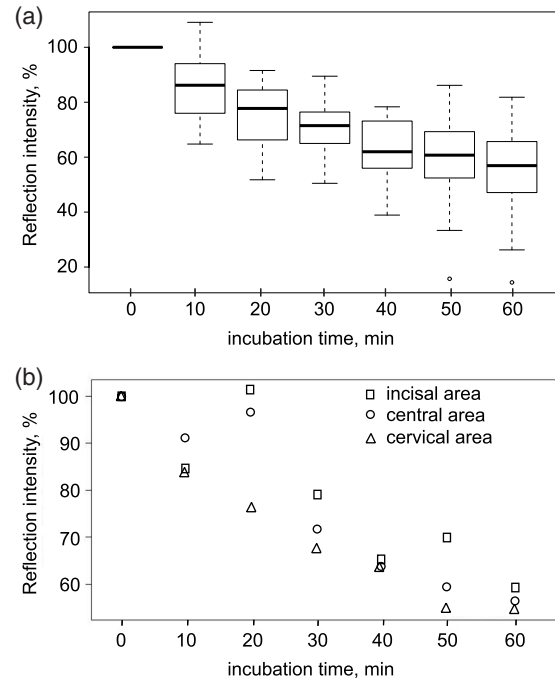
Extracted human incisors were incubated in the orange juice for the total time duration of 60 min (6 $\times$ 10 min cycles). The



**Fig. 4** Decrease of the reflection intensity during *in vitro* erosion progression in polished enamel measured by the prototype (gray boxes) and miniaturized (white boxes) reflectometers. SEM images demonstrate representative surface morphology after 2, 4, and 10 min of erosive treatments.

erosion analysis was performed on the cervical, central, and incisal areas of labial enamel surfaces [Fig. 1(b)] using tips with the diameters of the measurement spot of 0.7 and 2.3 mm (Fig. 3). In both cases (two tip sizes), a continuous decrease of the specular reflection intensity was observed with each subsequent incubation period in orange juice (Fig. 5). The results obtained by the application of 2.3-mm large tip showed no differences between the erosion assessment in cervical, central, or incisal areas ( $p > 0.05$ ); hence, the measurements were combined and the results are presented as a box plot [Fig. 5(a)]. The reflection signal reduced to a mean of 85% after the first 10 min of exposure, indicating the erosive effect of the juice. Further loss of the reflection intensity was detected down to 73% and 58% after total of 30 and 60 min of incubation in juice, respectively [Fig. 5(a)]. Statistical data analysis showed significantly different reflection signals measured after each of subsequent erosive dose, except of 40 versus 50 min (Table 1). Interestingly, the erosion assessment by the smaller tip size (0.7-mm diameter) showed significant variation of the results ( $p < 0.05$ ) depending on the choice of the measurement position, i.e., cervical, central, or incisal. Hence, median values of the reflection intensities were plotted for each of measured areas [Fig. 5(b)]. There was a general tendency toward lower median reflection intensities when measured in cervical areas of the teeth compared with the central and incisal spots [except 10 and 40 min incubation time, Fig. 5(b)]. Although, a similar decrease of the reflection intensity was measured after the total 60 min of erosion using both tips, the choice and definition of the measurement spot might be carefully considered if tips with small diameters (<2.3 mm) are used.

SEM images of the teeth revealed different degrees of the erosive etching on the enamel surface (Fig. 6). The images were taken after the entire erosive challenge was applied (60 min). The most [Figs. 6(d)–6(f)] and the least [Figs. 6(b) and 6(c)] eroded enamel tissues as well as different measured areas are presented in Fig. 6. Although data analysis showed reduced reflection signal in cervical areas compared with central and incisal ones, the example of the most eroded tooth sample



**Fig. 5** Change of the reflection intensity with erosion duration in native enamel measured by the tips with the diameters of 2.3 mm (a) and 0.7 mm (b).

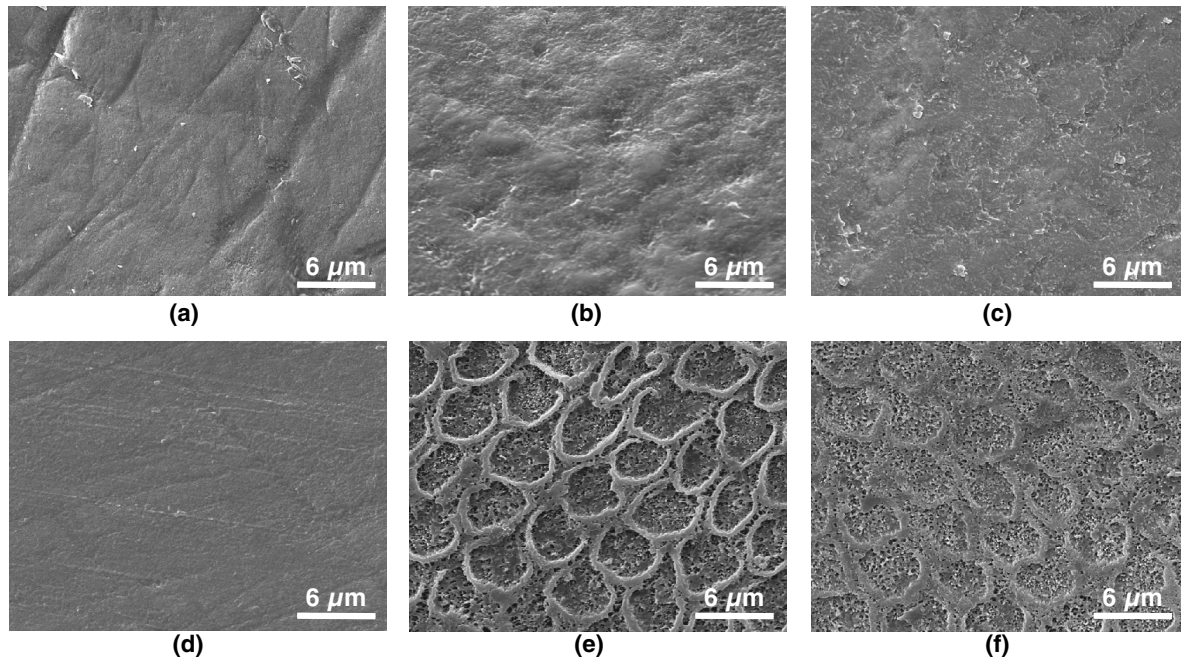
[Figs. 6(d)–6(f)] demonstrated relatively smooth cervical area [Fig. 6(d)], whereas the central area contained the most pronounced etched prism pattern [Fig. 6(e)], which could be clearly seen in the incisal position too [Fig. 6(f)]. These images demonstrated a high variability of the erosion progression in teeth and in different tooth areas. Note that the SEM observations correlated well with the reflection results obtained using the 2.3-mm large tip. Thus, the most and the least eroded samples also demonstrated relatively low and high reflection signals, accordingly, during the entire erosive experiment.

## 4 Discussion

The proposed method was developed and proven in our former *in vitro* studies.<sup>19,20</sup> This study presents a next step toward the

**Table 1** Differentiation of the reflection signals at the subsequent erosive doses in native enamel.  $p$ -values were corrected for a multiple testing. Significant differences were calculated to the level of significance = 0.05 and were marked with asterisk.

Time in the orange juice (min)	$p$ -value corrected for multiple testing
0 versus 10	<0.0001*
10 versus 20	0.0412*
20 versus 30	0.0412*
30 versus 40	0.0412*
40 versus 50	0.9746
50 versus 60	0.0004*
40 versus 60	0.0020*



**Fig. 6** SEM images of the initial noneroded native enamel surface of the incisor (a), central and incisal areas of the most resistant enamel sample (b and c, respectively), and cervical, central, and incisal areas of the most eroded tooth (d, e, f, respectively).

application of this technique in *in situ* and clinical studies. To make this step, a handy and simple instrument was required based on the same optical system utilized previously.<sup>19</sup> Thus, the entire optics was miniaturized into a pen-size instrument, though the angles of incidence and reflection had to be reduced to 23 deg (versus 45 deg in the original prototype setup<sup>19</sup>) to provide so small instrument head size that it could access all positions inside the oral cavity (Fig. 3). Moreover, the operating wavelength was fixed at 635 nm, which was in the optimal wavelength range (600 to 780 nm) as shown earlier.<sup>19</sup> As the reflection intensity is strongly affected by the distance to the measured sample surface, the assembled pen-like instrument had a fixed 6-mm long tip (Fig. 3), which should be brought into the direct contact with the analyzed tooth interface. The 7-mm thick head plus 6-mm long tip formed c. 13-mm large operating part of the instrument, which could be used to access the labial surfaces of molar teeth.

Due to variation in the angles and distances, the assembled device was tested in *in vitro* study with polished human enamel first. The results were compared with the prototype device which was applied for the erosion monitoring in the same teeth. The same erosive conditions and enamel preparation, as in the previously published *in vitro* studies,<sup>19,22</sup> were applied to polished enamel due to a good correlation of the reflection signal and enamel microhardness under the chosen erosive regime. It was proven that the assembled optical pen could be successfully applied for the detection of different erosion stages in polished enamel *in vitro*. In spite of the different light sources (laser diode versus halogen lamp) and the reduced angles of incidence and reflection, the signal of the specular reflection intensity significantly decreased during the total 10 min of erosive challenge and could be well differentiated at each of subsequent erosive treatments (Fig. 4). High correlation of the results obtained by the prototype device<sup>19</sup> and the current miniaturized version showed the potential applicability of the instrument in erosion studies. Although being a proof-of-

concept, the outcome of this experiment is significant for further development and improvement of the device. The measurement of the samples as well as data analysis was fast and simple.

Interestingly, the decrease of the measurement spot from 2.3 to 0.7 mm did not affect the reflection intensity values when measured at the same area in the polished enamel. The small size of the measurement tip can be potentially used for the clinical erosion analysis of the occlusion sites, while larger tips can be applied for the screening of the labial surfaces. Similar to the previous observations, the decay of the specular reflection intensity correlated with the increase of the surface roughness and appearance of the characteristic honey-comb topography of the *in vitro* eroded enamel (Fig. 4).

Thinking about the clinical application of this instrument, it was important to include the native enamel samples into the study. Human incisors without restorations or initial erosive signs were selected and underwent cycling erosive challenges in the commercially available orange juice. It was decided to extend the erosive total duration up to 60 min ( $6 \times 10$  impacts), in order to verify the detection capacity of the instrument. In other words, if no erosive signs had been detected even after 60 min of exposure to juice, no good chance would be considered for the clinical assessment of the early erosion by the instrument. The obtained results proved that the proposed instrument could detect the reflection decrease in the response to erosive challenges (Fig. 5) and could even differentiate between each of subsequent erosive doses already after the first 10 min of treatment (Table 1). Furthermore, due to relatively large surface areas of the incisors, the choice of the measurement position was important. It was decided to monitor three different positions along the tooth crown, i.e., cervical, central, and incisal areas [Fig. 1(b)]. Similar to the study with polished enamel, tips with the spot diameters of 0.7 and 2.3 mm were used and compared in this study part as well. Although a nearly identical decrease of the reflection signal was measured during erosion progression (Fig. 5) using both tip sizes, the statistical data

analysis revealed significant differences between the results obtained at cervical, central, or incisal areas when the small tip of 0.7 mm was applied. Obviously, the native enamel surface of the samples was inhomogeneous, abraded, and with numerous scratches [Fig. 6(a)]. The smaller the tip diameter, the smaller the measurement area was; hence, a greater effect of the local inhomogeneity on the measurement results could be expected. This was not the case when the larger 2.3-mm tip was used for the measurement or when the measurements were carried out in flat-polished enamel specimens (Sec. 3.2).

The results obtained using a 0.7-mm tip diameter showed a tendency to slightly reduced reflection intensities measured in cervical areas of incisors. The reduced reflection values typically indicate more prominent erosion progression; however, faintly higher curvature of the cervical areas of the extracted incisors could also partially contribute to a less amount of the collected reflected light (incomplete contact of tip with the surface). On the other hand, several researchers indeed reported mechanical,<sup>24–26</sup> microstructural,<sup>25</sup> and chemical<sup>27</sup> site variations of the enamel tissue which could affect its susceptibility to demineralization. It is also known that the erosion progression might occur site specifically;<sup>28,29</sup> therefore, different erosion rate in cervical areas cannot be excluded. It should be mentioned that there was a large variation among the erosion susceptibility/rate in native enamel samples. While some incisors barely had a visible pattern of etched enamel after the total 60 min of treatment, others had open-hollow eroded prisms (Fig. 6).

It was interesting to observe different reflection functions over the erosion times, i.e., exponential<sup>19</sup> decrease in polished enamel or almost linear decay in native enamel (Fig. 5). Probably, change of the reflection signal was defined by the kinetics of the enamel demineralization; however, similar erosive conditions should be applied to different enamel tissues in future studies to explain this finding.

## 5 Considerations for the Application of the Instrument in the Laboratory and Clinical Experiments

The developed instrument can be easily used for the comparative monitoring of early dental erosion in the laboratory and *in situ* studies with polished and native dental tissues. It has the highest sensitivity toward the detection of softening phase of erosion. Several parameters should be taken into consideration when using this method in the erosion studies. One of the most important factors affecting the reflection analysis is the presence of a soft layer or any deposits on the enamel surface. Examples are salivary pellicle layer, CaF<sub>2</sub> and other deposits, and polymer films. These coatings might alter the surface roughness and should be removed from the enamel surface prior to analysis. For example, salivary pellicle can be cleaned from the enamel, which minimizes its effect on the reflection signal significantly.<sup>20</sup> Abrasion<sup>20</sup> or other mechanical impacts are factors which affect the enamel roughness and should be standardized/controlled during the application of the instrument.

## 6 Conclusions and Outlook

The present study described further development and assembly of the miniaturized pen-size reflectometer for the quantification and monitoring of early dental erosion. The new instrument was developed with modified angle of incidence and the light source compared with the prototype device. The dimensions of the instrument were maintained to access all positions in the oral

cavity and to provide a handy and simple measuring tool for the application in laboratory and *in situ* studies. *In vitro* study of the erosion progression in native and polished human enamels proved the applicability of the miniaturized device for the detection of early erosive stages in both enamel types.

Other studies are planned using the assembled reflectometer. Particularly, its applicability for the detection of different erosion rates will be investigated in the study with erosion-inhibiting marketed dental products. Generally, the proposed instrument allows monitoring of early dental erosion in native teeth at different sites and areas in a fast and simple manner. It can be a useful tool in both basic and clinical erosion research. Moreover, the utilized measurement principle is similar to other devices proposed for the detection of caries lesions.<sup>30,31</sup> We suggest that the assembled device can also be tested in the field of early caries diagnostics.

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