Early detection of proximal caries is receiving much attention because preventive and minimally invasive therapies can minimize the need for restorative treatments. Caries beneath the proximal contact is frequent, but the diagnosis is often difficult (1). Visual–tactile examination of the interproximal space is often impeded by the adjacent tooth and may not provide sufficient information for a therapeutic decision. Visual assessment alone [e.g., according to the International Caries Detection and Assessment System II (ICDAS II, https://www.iccms-web.com)] may therefore not be sufficient for detecting approximal caries (2). Bitewing radiographs have been used for many years as additional diagnostics for either partially visible or undetectable clinical lesions (3); their advantages are ease of use and immediate availability of results, particularly when digital radiography is employed. Carious lesions can be visualized, and information on their extension to the pulp can be provided. The ability to detect proximal lesions by X-ray examination increases with the depth of the decay; accordingly, dentin lesions are significantly easier to detect than enamel lesions (2, 4). Radiography is of especially limited value for the detection of early initial lesions, and the ionizing radiation limits its repeatability. Therefore, digital bitewing radiography (BWR) is not appropriate for monitoring initial proximal lesions. Consequently, alternative methods for caries detection, which are also suitable for monitoring, and for use in pregnant women and low-risk patients, must be evaluated and validated. In the search for such alternatives, laser fluorescence, which does not require short-wave ionizing radiation, was brought into focus. Devices that use laser fluorescence to diagnose caries include DIAGNOdent (KaVo, Biberach, Germany) and SiroInspect (Sirona, Bensheim, Germany) (5, 6). Other diagnostic devices that use even longer wavelengths (e.g., in the infrared range) have recently become commercially available; one such device is the DIAGNOcam (KaVo), which uses near-infrared (NIR) light of 780 nm for transillumination of posterior teeth (7–9). Lederer et al. (10) have shown that NIR transillumination performs similarly to, or even better than, digital radiography for proximal caries detection, as its sensitivity values for detection of enamel and dentin lesions were almost twice as high as those of BWR. Another diagnostic tool, which uses light of 850 nm wavelength, is the VistaCam iX HD with the Proxi interchangeable head (Dürr Dental, Bietigheim-Bissingen, Germany). This device is based on near-infrared reflection (NIRR) rather than NIR.
transillumination. With additional interchangeable heads, this device can be used also for fluorescence measurements at 405 nm and as a conventional intraoral camera using white light. The Proxi interchangeable head of the VistaCam iX HD was developed for detection of proximal caries in posterior teeth and is equipped with a positioning holder and a complementary metal oxide semiconductor sensor with an autofocus system, which is mounted between two 850 nm infrared light-emitting diodes. The emitted light can transilluminate sound enamel and is scattered and reflected by carious lesions in enamel and by dentin (Fig. 1) (8, 11). Reflected light is captured by the sensor, and the digital images generated can be evaluated using the VISTASOFT software (Dürr Dental) on a separate monitor. The light reflected from a carious lesion and the dentin becomes visible as bright areas, while sound enamel appears dark.

Classic NIR transillumination has already been investigated in many in-vitro and in-vivo studies. However, there are few studies that evaluate the principle of NIRR as used by the Proxi interchangeable head of VistaCam. In a prior in-vivo study by Jablonski-Momeni et al. (12) NIRR was shown to yield comparable results to BWR in detecting proximal enamel lesions. An in-vitro study by Tonkaboni et al. (13) compared the diagnostic performances of ICDAS, BWR, and NIRR for proximal caries detection, with histology as the reference standard. This study assessed the proximal areas of permanent posterior teeth divided into three segments between the contact area and the cemento–enamel junction and finally concluded that NIRR showed significantly higher overall sensitivity of all three methods, at the cost of a slightly lower specificity.

The aim of the present study was to assess the in-vitro validity of NIRR for detecting proximal caries and to compare its diagnostic performance and reliability with that of BWR. To compare BWR and NIRR on an equivalent basis, we used micro-computed tomography (µCT) as the reference standard. The null hypothesis of this study was that BWR and NIRR exhibit similar diagnostic performances.

Material and methods

The experimental procedures were approved by the Ethics Committee of the Medical Faculty, Ludwig Maximilians University in Munich, Germany (488-15 UE).

Selection and preparation of samples

One-hundred teeth were selected from a pool of extracted permanent molar and premolar teeth from anonymous patients from Munich and its environs. Teeth with restorations, hypomineralization, non-caries-related tooth damage, and frank cavities were excluded, as were third molars. Based on visual examination, healthy teeth (i.e., teeth with an ICDAS score of 0) and teeth with exclusively proximal carious lesions (i.e., teeth with ICDAS scores of 1–5) were selected in approximately equal proportions; thus, 54 sound and 46 carious teeth were identified as appropriate for use in this study. The distribution of teeth according to ICDAS score (e.g., in the range of 1–5) was left to chance. For each tooth, either the mesial or the distal ICDAS scored surface was chosen to be included in the sample, photographed, and assigned a unique number. The teeth were stored in distilled water at 4°C and were cleaned before assessment.

Examiners and training

Two examiners (A.L. and F.L., with 3 and 7 years of clinical experience, respectively, as dentists at the University Hospital of Munich and having trained dental students in practical courses during this time) were instructed in the use of ICDAS based on an ICDAS e-learning course consisting of 40 photographs of sound and carious surfaces. For training in BWR (14) and NIRR classifications (Fig. 2), 50 examples of proximal surfaces, with and without caries, were collected and evaluated on bitewing radiographs and on NIRR images. This training was repeated three times, and differing diagnostic decisions were discussed in the group. Prior to the practical training sessions, the examiners were personally trained by an experienced expert (K.H.K.).

Visual inspection

Proximal surfaces were scored according to the ICDAS criteria by directly looking at the proximal surface without an adjacent tooth (15). Fifty-four of the surfaces were deemed sound (code 0), 12 surfaces showed the first visual changes of enamel (code 1), 14 surfaces showed a distinct visual change, nine surfaces demonstrated localized enamel breakdown (code 3), an underlying dentin shadow was present on five surfaces (code 4), and six surfaces had a distinct cavity with visible dentin (code 5).

Digital bitewing radiography

The teeth were fixed individually with silicone (Optosil; Kulzer, Hanau, Germany) in a sensor holder with the...
X-ray beam passing in a buccolingual direction and a tube-to-sensor distance of 6 cm. Using a Heliodent DS Dental X-ray unit (60 kV, 7 mA, 0.06 s exposure; Sirona, Bensheim, Germany) and an 'Intra-Oral II' charge-coupled device sensor (sensor size 30.93×40.96×7.0 mm; Sirona) digital bitewing radiographs were taken using the paralleling technique. Images were analyzed in SironaSIDE-XIS XG software (V2.63 2016; Sirona). The radiographs were classified, according to the Marthaler classification (14), as: absence of radiolucency (0); radiolucency in the outer or the inner half of enamel (1 or 2); or radiolucency in the outer or inner half of dentin (3 or 4).

NIRR examination

To examine the teeth using NIRR, the tooth roots were fixed with wax (Boxing Wax; Kerr, Biberach, Germany) in a transparent plastic container (1.5×4 cm). The tooth under investigation was flanked by two teeth chosen arbitrarily from the samples with the aim of mimicking adjacent teeth with a contact area on the mesial and the distal surfaces. Subsequently, the proximal area of interest was examined using the Proxi interchangeable head of VistaCam iX HD. All teeth were dried according to the manufacturer’s operating instructions to minimize reflections and they were examined in a darkened room. Images were captured using the Vist.soft software and were exported as Portable Network Graphics files. The proximal surfaces were evaluated following an existing classification of NIR transillumination according to the following criteria: sound (code 0); caries limited to enamel (code 1); caries with a single point of contact to the enamel–dentin junction (code 2); caries with extensive contact to the enamel–dentin junction (code 3); and caries visible in dentin (code 4) (16).

Validation with µCT

The teeth were vertically mounted in a cylindrical, 16.5-mm-diameter water-filled plastic container and scanned three-dimensionally using a µCT40 fully shielded cone-beam desktop µCT scanner (Scanco Medical, Bassersdorf, Switzerland). The scanner was operated at 70 kV and 114 µA with a 16.5 mm field of view. The scanning resolution was 512×512 points with a 0.032 mm pixel size.

The resulting raw data sets (RSQ files) were converted into three-dimensional (3D) data sets (ISQ files). For further image processing and the evaluation process, these data sets were imported into Fiji (NIH, Bethesda, MD, USA) (17) using the plugin ‘KHKs_Scanco_ISQ_FileReader’ (18). When evaluating the images, a distinction was made between surfaces lacking radiolucency (code 0), surfaces radiolucent in the outer (code 1) or inner (code 2) half of the enamel, and radiolucency in the dentin (code 3).

Image evaluation

The evaluation of all digital radiographs, NIRR, and µCT images was performed in a darkened room on a calibrated monitor (Windows 7 ‘Display Color Calibration’; Microsoft, Redmond, WA, USA). Results that differed were discussed until a consensus was reached. Radiographs and NIRR images were analyzed and described separately in two evaluation cycles with a randomized order using the aforesaid classifications for BWR and NIRR. This was repeated with a minimum interval of 2 weeks to prevent recall bias.

Statistical analysis

To compare the scores for BWR and NIRR with those of µCT, the recordings were grouped for the assessment of caries vs. no caries, or sound vs. enamel lesions vs. dentin lesions. In radiographs and NIRR images, a code of 0 was rated as sound tooth structure and all codes of >0 were rated as carious: codes 1–2 represented enamel lesions; and codes 3–4 represented dentin lesions. The thresholds for NIRR have already been used in a previous study on transillumination (Table 1) (16). The overall accuracy was given as a percentage of the correctly classified diagnostic findings (both healthy and diseased) in relation to the total diagnostic findings. Inter- and intra-rater reliability were measured using linear weighted Cohen’s kappa (wk), in which a one-category difference could be considered as less severe than a two-category difference (19). Weights ranged from 0 to 1. The weight for cells for which the raters showed perfect agreement was 1. For cells in the lower-left
or upper-right corner (i.e., those with the largest disagreement) the weight was 0. Each weight for any particular cell was calculated using the formula $W_{xy} = 1 - (|x - y|)/z$, in which $W$ is weight, $x$ and $y$ are the categories, and $z$ is the total number of categories.

The statistical analysis was performed using SPSS version 24 (IBM, Armonk, NY, USA).

Results

Validation of the 100 proximal surfaces with $\mu$CT found 64% sound surfaces, 16% enamel lesions, and 20% dentin lesions, while visual inspection indicated 52% sound surfaces and 48% altered surfaces (Table 2).

Overall accuracy was 0.78 for BWR and 0.76 for NIRR. Sensitivity and specificity values for both methods are presented in Table 3. Both methods exhibited moderate sensitivity for caries detection in general (0.50 for NIRR and 0.53 for BWR). Concerning enamel lesions, sensitivity values were even lower, especially for NIRR, while specificity was high for both methods.

For all methods, the inter- and intra-rater reliability estimates showed weighted Cohen’s kappa values which would correspond to ‘almost perfect’ agreement (Table 4) (20). Inter-rater reliability ranged from 0.89 to 0.93 and intra-rater reliability from 0.80 to 0.89.

In several images, generated using NIRR, a white border appeared around the tooth in the area of the marginal ridge, which can be seen in Fig. 2. Surfaces with overexposed areas that could not be accurately scored were rated as code 0. Three-quarters of the surfaces could be assessed unambiguously.

Discussion

This in-vitro study investigated the diagnostic performance of NIRR for detection of proximal carious lesions in relation to radiographic diagnostics and $\mu$CT. Approximal caries is one of the most common oral diseases in humans. The incidence of hidden carious lesions of direct origin below the proximal interface has increased since the addition of fluoride to drinking water, the availability of fluoridated dental-care products, and better oral hygiene education (1, 21). It is frustrating for dentists and patients that the two worldwide standard methods – visual inspection and radiological examination – do not permit reliable, early detection of carious enamel lesions, which may result in possible further preventive treatments not being exhausted and invasive therapy not being optimally prevented (22). The NIRR technique addresses this problem and we hypothesized that BWR and NIRR would give similar diagnostic outcomes. However, we found significant discrepancies between the diagnostic methods tested and therefore the initial hypothesis must be rejected.

As in-vivo validation with radiography as the reference standard is not sufficient because of the low sensitivity of X-rays, an in-vitro study with a reference standard based on histology or $\mu$CT is necessary. In this study, $\mu$CT was used as the reference standard, which is also described in previous studies (23, 24). Histological sections and $\mu$CT are both suitable for determining the depth of caries lesions in vitro (25, 26). However, $\mu$CT has some advantages over histological sections. Instead of slicing and destroying the samples, these remain available for further experiments. Furthermore, with the 3D information of the whole sample provided by the $\mu$CT image, caries depth can be evaluated reliably and repeatedly. However, both $\mu$CT and histology are themselves tests and therefore do not necessarily represent any ‘truth’. In this study, visual inspection with ICDAS revealed 12% more initial lesions than the micro-tomographic examination. This raises the question of whether our reference standard may not detect carious lesions in their very early stages, which, by contrast, can easily be detected by visual

| Diagnostic method | Sound surface | Enamel lesion | Dentin lesion | Carious surface |
|-------------------|---------------|---------------|---------------|----------------|
| ICDAS             | 0             | 1             | 3             | 6              | 16             |
| BWR               | 0             | 1             | 2             | 6              | 14             |
| NIRR              | 1             | 3             | 2             | 4              | 9              |
| $\mu$CT           | 0             | 1             | 1             | 2              | 4              |

BWR, bitewing radiography; ICDAS, International Caries Detection and Assessment System; $\mu$CT, micro-computed tomography; NIRR, near-infrared reflection.

| Score   | 0  | 1  | 2  | 3  | Total |
|---------|----|----|----|----|-------|
| ICDAS   | 52 | 1  | 0  | 0  | 54    |
| BWR     | 62 | 9  | 2  | 6  | 79    |
| NIRR    | 63 | 8  | 4  | 6  | 81    |

| Score   | 0  | 1  | 2  | 3  | Total |
|---------|----|----|----|----|-------|
| ICDAS   | 8  | 3  | 0  | 1  | 12    |
| BWR     | 4  | 4  | 1  | 5  | 14    |
| NIRR    | 0  | 2  | 3  | 4  | 9     |
| $\mu$CT | 0  | 0  | 1  | 4  | 5     |

Cross-tabulation of the scores given for the International Caries Detection and Assessment System (ICDAS), digital bitewing radiography (BWR), and near-infrared reflection (NIRR) against the corresponding micro-computed tomography ($\mu$CT) scores.
examination. We assume that the disparate findings for \(\mu\)CT and ICDAS may also be a result of persistent discoloration of the surfaces of some of the teeth, which may have led to false-positive visual results during assessment by ICDAS.

Our investigation was influenced by the idealized conditions of an in-vitro examination that possibly led to better performance of all diagnostic methods than in a clinical scenario. Visual inspection was conducted by looking directly at the specific surface, without simulation of a proximal contact with an adjacent tooth, because it was impossible to reconstruct the proximal contacts of the adjacent teeth as they had been arranged in the clinical context prior to extraction. Therefore, we did not consider ICDAS as a third test method but utilized it to describe the clinical composition of the sample (Table 2). For the radiographic assessment of caries, the teeth can be X-rayed either as a single tooth or as a simulated approximal contact by mounting another tooth in contact with the tooth under investigation. The simulation of proximal contacts is preferred if the overall diagnostic possibilities of bitewing radiographs are to be assessed. In our case, however, the aim was to create the best conditions possible to permit an accurate diagnosis when assessing the radiographs. For this reason, simulation of a proximal contact was omitted, as superimpositions with the neighboring tooth would have resulted in a higher incidence of false-negative findings or situations where no assessment could be made. If only one tooth is X-rayed, the automatic brightness control of the SIDEXIS software can lead to relative overexposure of this tooth as a result of the high proportion of dark background pixels. This would only be problematic if the teeth were saturated (high number of pixels with gray value 255). All radiographs were therefore checked using the histogram function of ImageJ, and no saturation in the area of the individual tooth could be observed in any of the specimens.

Inter-rater reliability was calculated according to the results of the second evaluation cycle and showed 'almost perfect' agreement for all methods. This agreement was even slightly better than that for intra-rater reliability, which is unusual. There was certainly a steep learning curve for the two examiners from the first to the second assessment cycle, which would have had a negative effect on their intra-rater reliability. In addition, both examiners had completed their education at the same university and therefore probably already had a high degree of calibration per se.

Another important aspect of the methodology of this study is the sample selection and size, which should also be considered in the context of caries prevalence in our study population. Caries prevalence has been estimated to be 20% in Germany (27). However, it is not the caries prevalence in the epidemiological sense which is relevant in this context, but the proportion of carious surfaces to all surfaces examined in this study. Approximately equal numbers of carious and sound surfaces were selected according to visual criteria (yes/no decision), corresponding to a proportion of carious surfaces of about 50% in our sample, while the \(\mu\)CT data indicated the proportion of carious teeth in the sample to be 36%. It must be considered that it is far removed from a typical clinical scenario to have a group of patients in whom 48% or even 36% of the posterior proximal surfaces would have caries.

According to BUIJANG et al. (28), for an estimated caries prevalence of 20%, a sample size of 250 would be required in order to distinguish a sensitivity of 0.50 from a sensitivity of 0.70 at \(z = 0.05\) and \(1 - \beta = 0.80\). However, a sample size of 250 would have exceeded our budget because of the high cost of \(\mu\)CT scans. For this reason, we based the sample size and composition on studies previously published on a comparable topic (29–32).

In previous studies, sensitivity values reported for BWR ranged from 0.23 to 0.53 for enamel caries, from 0.16 to 0.63 for dentin caries, and from 0.15 to 0.54 for any carious lesion (30, 33, 34). In our study, the sensitivity values for BWR were 0.31 for enamel caries, 0.55 for dentin caries, and 0.53 for any carious lesions and

### Table 3

| Carious surface | Enamel lesion | Dentin lesion |
|----------------|--------------|--------------|
| **BWR**       |              |              |
| Sensitivity    | 0.53         | 0.31         | 0.55         |
| Specificity    | 0.97         | 0.94         | 1.00         |
| **NIRR**      |              |              |
| Sensitivity    | 0.50         | 0.13         | 0.55         |
| Specificity    | 0.98         | 0.95         | 0.98         |

| Diagnostic method | Inter-rater  | Intra-rater |
|-------------------|--------------|-------------|
| Rater 1 vs. Rater 2 |              |             |
| ICDAS             | 0.90 (0.84–0.95) | 0.88 (0.82–0.93) | 0.86 (0.81–0.92) |
| BWR               | 0.93 (0.85–1.01) | 0.89 (0.80–0.99) | 0.80 (0.67–0.93) |
| NIRR              | 0.89 (0.79–0.99) | 0.84 (0.71–0.98) | 0.88 (0.78–0.98) |

Data are given as linear weighted Kappa values with the corresponding 0.95 CI in parentheses.
therefore are in agreement with previous results. For NIRr, our results showed sensitivity values of 0.13 for enamel caries, 0.55 for dentin caries, and 0.50 for any carious lesion. Especially in the detection of enamel caries, NIRr achieves poorer results than visual methods, BWR, and alternative diagnostic methods, such as fiberoptic transillumination or laser-induced fluorescence measurements (33). However, it should be emphasized that our results were compared with µCT as the reference standard, whereas previous in-vitro studies often used histology or even bitewing radiographs. This can lead to a reference test bias, which influences the interpretation of the study results (35, 36).

The cross-tabulation (Table 2) and the calculation of sensitivity and specificity values showed a tendency towards false-negative findings for BWR and NIRr. In particular, initial proximal lesions seemed to go undetected. Regarding BWR, this is a well-known fact and has been described in previous studies (29, 37–39). In addition, some difficulties were encountered in evaluating the NIRr images, which led to underestimation of the decay. In almost all NIRr images a white border appeared around the tooth in the area of the marginal ridge, as seen in Fig. 2. The light is probably refracted more strongly in this region because of the spherical shape of the marginal ridge and the proximal surfaces. Incipient enamel lesions located in the outer half of enamel, which are usually triangle-shaped and situated directly beneath the contact point, were, in many cases, not visible because of these artifacts. If a surface did not reveal any criteria of carious lesions, it was classified as sound (code 0).

Sensitivity values for detection of dentin caries can also be explained by higher absorption and scattering of light in dentin (8). Previous studies assume that caries cannot be reliably visualized in dentin by NIRr transillumination if there is a healthy layer of dentin between the lesion and the occlusal enamel layer. However, dentin lesions directly under the occlusal enamel can be visualized (16). The distance between the occlusal surface and the lesion may also significantly influence the visibility, as NIR light is attenuated on its passage through the dental tissues. This was not considered in the study of Tonkaboni et al. (13), in which lesions below the contact area and below the cemento–enamel junction were also investigated. In a study by Jones et al. (9), it was shown that the contrast decreases significantly with increasing enamel thickness and is less pronounced in layers >4 mm. Therefore, the more cervically located lesions cannot be visualized.

Besides the occlusal morphology and the localization of the lesion, the opacity of the enamel can also have an influence on the potential of a carious lesion to be imaged. In many surfaces, caries detection was difficult because of highly opaque enamel, which appears lighter and causes low contrast differences. This was also described in previous studies on NIRr transillumination (40). The reasons for this could be the presence of hyper- or hypomineralized enamel or a change in the surface layer of enamel (e.g., the deposition of pigments) with increasing age. In addition, it has been found that hydroxyapatite crystals become larger with age, which has an influence on tooth brightness and might also affect the optical properties of the tooth (41–43). So far, no studies on the change in optical properties of enamel with increasing age have been carried out and therefore further research on this topic is needed.

Regardless, when using NIRr, some light is always reflected by the smooth tooth surface, in both dry and wet conditions. The manufacturer’s advice to remove saliva from the surfaces before assessment reduces, but cannot completely prevent, reflection. By repeated angulation of the laser beam, it is often possible to view a specific area without reflections, but in most cases, it is not possible to visualize the entire approximal area without reflections.

Reflections of the tooth surface are caused by the method itself and do not occur when teeth are transilluminated. Use of cross-polarization filter techniques, in which polarizing filters are attached to the light source and the camera, could probably reduce the reflections. This could influence not only the reflections but also the image brightness, necessitating stronger illumination. Research is also needed on the influence of varying wavelengths on NIRr. The optical properties of enamel have been investigated in detail, showing a decrease of extinction with increasing wavelength up to 1,310 nm (44). Use of higher wavelengths could improve the diagnostic potential of NIRr. Several studies have been carried out on NIRr transillumination at different wavelengths, but because of patents on NIRr transillumination at 795–1,600 nm, the manufacturers are only able to use lower wavelengths. As NIRr is not affected by these patents, NIRr devices with a higher wavelength could be generated.

Near-infrared transillumination does not result in the same limitations of image quality that are mentioned above for NIRr. Both NIRr and NIRr transillumination are based on the same physical principle. In both cases, light is scattered by the microporosities caused by caries. The difference between the two systems is the arrangement of the illuminator and the detector. In NIRr, the illuminator and the detector are arranged next to each other, whereas in NIRr transillumination they are arranged opposite to each other with the tooth in between. NIRr transillumination often results in a strong contrast between enamel, dentin, and a proximal carious lesion, and this may sometimes make it possible to provide a rough estimate of the extent of the lesion in the coronal–apical direction using the gray value of the lesion.

Near-infrared reflection, as used by the VistaCam iX HD, has several problems regarding image quality as many lesions cannot be visualized clearly. In general, NIRr alone does not seem to have high potential for proximal caries detection, as a result of optical principles. This applies especially to incipient lesions located under the white border artifact in the area of the marginal ridge. Advanced lesions are easier to detect. Although the approach of combining several diagnostic tools in one device is good, NIRr, as tested in this
study, is not suitable for reliable detection of proximal carious lesions.

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