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Review

Dental Microstructural Imaging: From Conventional Radiology to In Vivo Confocal Microscopy

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Abstract: The innovative perspectives of the modern medical era aim to reach the highest performance and accuracy of therapeutic processes carried out for the evolution of diagnostic pathways. Digital planning and real-time diagnosis represent the hottest topics for researchers and clinicians in dentistry and oral medicine. To date, radiology is the gold-standard method for caries detection in the clinical setting. However, radiology poses a series of clinical limitations due to the use of ionizing radiation and its incapacity to recognize and detect enamel defects or early caries. In addition, radiology also presents issues surrounding its responsiveness to remineralizing agents and the microscopic gaps between the tooth and restorative treatments. To date, the evaluation of these conditions is only permitted ex vivo, with common methods in clinical practice not being applicable for establishing the actual condition of every case in every single patient. This work aims to develop state-of-the-art knowledge on conventional and unconventional innovative dental imaging techniques, focusing on those that not only promise to pursue the early and less invasive detection of dental disorders but also those that could be applied in clinical practice, with a particular interest in real-time in vivo confocal microscopy.

Keywords: clinical dentistry; confocal microscopy; dental imaging; diagnostic imaging; enamel defects; new technologies; innovative imaging; in vivo imaging; optical biopsy

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1. Introduction

The oral cavity displays the entry path into the human organism, and it is a complex system unit involved in several disorders of both soft and hard oral tissues [1]. Dental tissues can be subject to various pathological processes caused by environmental or genetic factors [2]. Enamel defects, for example, are commonly verifiable in clinical practice; they may be aberrations in the quality and quantity of dental enamel causing early damage to the enamel organ, predisposing to plaque accumulation and caries [3]. Among them, *Enamel Hypomineralization* is a qualitative defect of systemic origin with reduced mineralization, which shows discolored enamel with standard thickness and regular shape of the involved teeth [4]. Histologically, enamel hypomineralization shows a picture of less distinct prismatic sheaths and a lack of arrangement of the enamel crystals [5]. The demineralized enamel has lower mechanical properties, as the hardness and elastic modulus have lower values than those found in normal enamel, requiring complex management for

tooth restoration [6]. In comparison, *Enamel Hypoplasia* is a quantitative defect predisposing dimpled teeth with reduced enamel thickness, but enamel quality is unaffected [7].

Furthermore, a very high rate of deciduous teeth, ranging from 10% to 80%, may result in dental erosion, which determines altered dental shape and aesthetics and the loss of occlusal vertical dimension [8]. A broad picture of hereditary disorders, such as *osteogenesis* and *amelogenesis imperfecta*, may also affect oral hard tissues, thus leading to bone and dentin defects [9].

Certainly, tooth decay is the most common dental disease in children and young adults, severely affecting their quality of life. Dental caries recognizes bacterial plaque as the major etiological factor, organized as a biofilm matrix that can damage several host tissues [10]. Furthermore, recent investigations seem to suggest interesting data regarding some microorganism salivary levels and general health: the oral microbiota is increasingly assumed to play an important role in clinical and scientific interests, hosting a vast complexity of microorganisms, namely bacteria, archaea, protozoa, fungi, and viruses [11]. With the increasing definition and profiling of the oral microbiota, growing evidence has emerged regarding the bidirectional association between systemic and oral pathologies; for example, the correlations between osteoporosis and peculiar oral dysbiosis are supported by some cariogenic or periodontal pathogenic bacteria [12]. Salivary flow plays a crucial role in maintaining microbiome homeostasis; altered salivary secretion could generate an imbalance between the host and microbiota, thus leading to dysbiosis and dysfunctional anomalies [13]. Thus, the microorganisms' crucial role has always shown a considerable weight in both periodontal and endodontic disorders [14]. The most demanding challenge for clinicians lies in correctly identifying the most appropriate diagnosis to better manage patients suffering from those disorders [15].

For this purpose, dentistry has constantly renewed how to classify caries, its direct effects on the stomatognathic apparatus, and its indirect effects on the patient's and their family's well-being [16]. In recent decades, caries detection and assessment have been standardized by an international team of caries researchers that developed an integrated clinical scoring system called ICDAS (International Caries Detection and Assessment System) [17]. The ICDAS is helpful in clinical practice, research, and epidemiological purposes, simplifying caries management and its outcomes.

Parallel to the research and development of scoring systems and clinical assessment, a strong interest has been raised in the empowerment of technological tools in diagnosing dental affections. In fact, clinically similar lesions of the dental crown surface can underlie completely different conditions that require different therapeutic approaches. The most striking example is the difficulty in distinguishing the white opacities of the enamel of different origins on a sole clinical base. Caries-related white spots, enamel opacities, enamel hypoplasia, and molar incisors hypomineralization (MIH) have an overlapping clinical appearance [18], but the therapeutic approach and responsiveness to remineralizing treatments strongly differ from each other. In this case, a more appropriate side chair diagnostic adjunct may support the dentist's more appropriate treatment choice.

Hence, this work aims to summarize the description of conventional dental imaging techniques and to report on advanced and novel technologies, focusing on those that not only promise to pursue the early and less invasive detection of dental disorders but also those that could be applied in clinical practice.

2. Conventional In Vivo Radiological Imaging

The in vivo imaging of oral hard tissues has deep-rooted origins [19]. Straight after Wilhelm Conrad Roentgen discovered X-rays in 1895, the application of radiology in medical fields immediately found widespread diffusion [20]. Only 14 days after Roentgen's intuition, Otto Walkhoff experienced the first application of X-rays during the making of dental radiographs [21]. Oral hard tissue radiographic techniques have been integral to clinical and diagnostic practice for over 100 years [22].

The most used and typical intraoral radiographs are periapical and bitewing radiographs: the former capture an image of the whole tooth, including its root apex and the alveolar bone; the bitewings, on the other hand, provide radiographs of dental arches, interproximal contacts, and the alveolar crest, approximately up to the coronal third of the root [23]. To date, intraoral radiographs have reached very high image definition levels to guarantee elevated sensitivity and specificity values for identifying dental tissue disorders [24].

Apart from intraoral radiographs, dental panoramic radiography, also called orthopantomography (OPG), constitutes a cornerstone in oral radiology [25]. OPG offers two-dimensional dynamic range visualization, providing complete imaging of the maxillary and jaw bones, the maxillary sinuses, and dental arches [26]. The large availability of the technique associated with the vast imaging information has made OPG an irreplaceable weapon for clinicians' daily practice [27].

In any case of intraoral radiography and OPG, X-rays pass through the anatomical structure to evaluate it and are then captured behind the object by a detector, usually a photographic film. In this case, the shadow of the structures is impressed on the film and visualized as negative images on a scale of greys after a precise and complex process of developing, fixing, washing, and drying the impressed analogical pellicle, then applied onto a lightbox to be fully appreciated by the clinician. However, during recent decades, digital detectors have increasingly replaced classical films, thus simplifying and shortening the imaging procedure. In this case, reusable X-rays–sensitive plates directly capture data during X-ray irradiation and immediately transfer it to a computer system that instantly displays the digital radiograph on a monitor. This avoids chemical processing, thus eliminating the cost and production of chemical waste to be disposed of, enabling the ability to apply special image processing techniques that enhance the overall display quality of the image, and is significantly time-saving. Finally, not to be overlooked, less radiation is used to produce an image of similar contrast to conventional radiography [28].

Recently, 3D radiography has increased in popularity to overcome the limitations of traditional two-dimensional dental imaging. It accurately imagines the superimposable details of maxillofacial bone structures and surrounding soft tissues [29]. Cone beam computed tomography (CBCT) has been introduced as a digital device capable of volume tomography depiction, aiming to reproduce imaging for the three-dimensions anatomy of dental and maxillary structures [30]. CT technology is based on the rigid connection between the X-ray tube and the detector placed on the other side of the subject to scan. The tube and detector move over the subject, sending a narrow X-ray beam through one thin slice at a time [31]. Then, the attenuation values of the transmitted X-rays are instantly processed by integrated specific software algorithms that rebuild the image of adjacent slices of the imaged volume [32]. This imaging technology offers applicability in almost all branches of dentistry and clinicians have several evidence-based guidance notes available [33].

Although CBCT provides higher radiation doses to patients than OPG or intraoral radiography, all the classical radiological methods based on conventional radiology are indistinctly considered invasive instrumental investigation methods due to exposure to X-rays [34]. Furthermore, seven years after the publication of the European SEDENTEXCT guidelines for radiation protection in CBCT for dental radiology investigations, the optimization of dental and maxillofacial CBCT imaging has not yet reached desirable maturity [35,36]. Indeed, both CBCT and all radiographic techniques expose the patient to ionizing radiations, so the number of radiological examinations must be limited and not always applicable in every clinical condition, despite the use of protective screens. Since there are no universal indications yet, in clinical practice, we follow the principle of using X-ray doses at the lowest possible threshold that allows the clinician to deliver the correct diagnosis [37].

3. In Vitro Imaging Techniques

Until a few years ago, the only way to determine and study dental microarchitecture was conventional microscopy, which inevitably works on extracted and laboriously processed, decalcified, and sectioned teeth in order to be observed under the microscope to analyze the features of enamel defects and the interface of various remineralizing compounds in vitro. Later, other ex vivo techniques were developed and used, thus simplifying the preparation of samples and allowing a better definition of the dental microarchitectural details.

3.1. Conventional Microscopy

There is a broad spectrum of in vitro methodologies for dental microscopic dental assessment, such as enamel demineralization and bioactive substances' remineralization capacity [38]. For example, in 2020, Ali et al. investigated the potential remineralization of enamel in vitro by applying different bioactive glass-based fluoride toothpastes [39]. The authors employed a fluoride ion selective electrode to perform micro-hardness analysis of the enamel specimen blocks. The deep resolution of the imaging technique resulted in the generation of high-definition images, despite the fact that the tool could not demonstrate any in vivo or real-time feasibility.

3.2. Other Microscopic Techniques

Other than conventional microscopy, dental imaging histology can also be assessed with different techniques that can scan the sample's bulk or surface without the need for slicing or decalcification but offering details at microscopic resolution. The most applied techniques for dental imaging are reported below.

Confocal microscopy (CM) allows the imaging of horizontally oriented virtual slices of a sample by point-by-point illumination of various optical layers parallel to each others, whose microscopic image is instantly rebuilt by an integrated software to appreciate the microarchitecture with no need to physically cut, reduce, and slice the sample [40]. In CM devices, a nonharmful incident laser light at specific wavelengths stimulates the emission of refracted light from the dental tissue proportionally to the refractive indices specific to their organic and mineral compounds. Then, the refracted light emitted from the tooth is collected point by point by a detector, the signals are transduced into grayscale pixels, and integrated software then rebuilds en-face optical sections at microscopic resolution in a grayscale frame visible on a monitor [41]. Through this optical sectioning, it is possible to study the dental histology, its composition, and the disposition of each compound in the space with high resolution and contrast [42]. Furthermore, additional software offers the possibility of three-dimensional image reconstruction [43].

Images similar to those obtained with CM but cross-sectional oriented can be obtained with optical coherence tomography (OCT) that penetrates deeper than CM through the layers and utilizes near-infrared light to produce high-resolution images. OCT also avoids processing specimens, thus shortening and simplifying the imaging process. In detail, OCT uses incident infrared light, similar to CM, but detects the low-coherence interference of the light backscattered from tissues, similar to ultrasound-based technology: incident light diffuses through the tissues, which reflect it with different delay times according to their different compounds; then, signals are detected and converted to pixels on monitors, such as CM [41]. OCT is advantageously used in the studies of dental microscopic anatomy and to appreciate the marginal dental-restoration interface in studies on dental materials [44,45].

Scanning electron microscopy (SEM) scans the surface of a sample with a focused beam of electrons and offers striking micrographs with a considerable depth of field, thus presenting the characteristic three-dimensional appearance of the surface structure of the sample. In this case, SEM sends a focused flow of electrons over a surface; the electrons interact with the sample's atoms which emit secondary electrons that are attracted to a positively charged detector. Then, signals with different intensities are transmitted and integrated by software to display the surface's topography and composition [46]. In this way, SEM also offers the

possibility to visualize and measure microscopic details of dental defects and restorative materials in vitro [47] and, in some studies, also in vivo, on dental replicas [48].

Although the working principles are similar to SEM, in *transmission electron microscopy* (TEM), electrons pass through ultrathin slices of biological tissues. Then, the information obtained on the microstructural details is recorded through changes in the momentum and phase of the electronic waves scattered by the tissues' thin slices, thus offering high-resolution images of dental defects and caries [49,50] and restorative compound interfaces [51].

In many cases, the limitations of each microscopic technique are balanced by complementary ones to offer notable and significant details of the analyzed enamel microstructures from various points of view. For example, TEM can be coupled with other imaging devices for synergic use, as reported in 2019 by Schwendicke et al. The authors offered a comprehensive ex vivo picture of enamel microstructure imaging and evaluated ion-releasing materials' mineralization potential on artificial dental caries using TEM and CM [52].

Similarly, other studies reported the results from multi-instrumental imaging performed by the complementary use of SEM or TEM and other tools, such as *transverse microradiography* (TMR) [53]. TMR exploits the projection and absorption of unidirectional X-rays in thin slices of mineralized tissue to provide imaging and quantitative assessment of differently mineralized areas in the sample, revealing the heterogeneous/inhomogeneous pattern of tissue mineralization [54].

3.3. Laser Speckle Imaging

The *laser speckle imaging* system is a non-destructive method based on the evaluation of the inhomogeneity of the microstructure of the dental surface in case of demineralization, erosion, or caries, accordingly with the different intensities of the backscattered coherent light detected from enamel and dentin irradiated with laser light [55]. Since the passage of light through the dental tissues changes according to the state of its regular/irregular mineralization, any change in intensity and direction of the light emitted from the dental surface helps to detect the degree of defect. The development of fiber-optic laser transmission systems has proven beneficial in the in vitro microscopic observation of carious lesions, which have a lower light transmission index, whereby a carious surface appears as a dark shadow that follows the spread of caries across the tooth, as reported by Angelini Sfalcin and colleagues [56]. Their in vitro analysis evaluated the remineralizing effects of different bioactive powders upon enamel blocks of bovine incisors with simulated caries lesions [56]. The specimens were irradiated by a HeNe Laser (at the wavelength of 633nm, with a power of 40mW), and the backscattered light was detected by a complementary metal oxide semiconductor (CMOS) sensor. The authors confirmed that their imaging methodology achieved microstructural data of the mineralization and remineralization status with good sensitivity. To date, preliminary in vivo works have demonstrated the applicability of a chairside prototype to discriminate gingival inflammation [57].

3.4. Pulse-Echo Ultrasonic Technique

In 1994, the first experiments by Hatton et al. evaluated the feasibility of ultrasound techniques for imaging the enamel structure's abnormalities [58]. Ultrasound devices are widely used in medicine to diagnose and image various body structures. In the case of teeth, a source of ultrasound is applied onto the dental surface, and a detector integrated with software calculates the enamel thickness by measuring the time interval between the transmission of an ultrasonic pulse on the surface of the enamel and the echo produced by the amelodentinal junction and using data on the average longitudinal velocity of sound within the enamel [59]. In 2021 Rodriguez-Sendra et al. performed an ex vivo study on extracted teeth to evaluate dentine demineralization involving a pulse-echo ultrasonic technique [60]. The ultrasonic system observed the exact amount of demineralization through the dental tissue surfaces. Hence, the authors also suggested future studies for its

in vivo application and feasibility. To date, the only study performed in vivo was that by Hatton et al. on dogs [58].

3.5. In Vitro Raman Spectroscopy

Raman spectroscopy (RS) is a non-invasive and non-destructive optical scattering technique used to explore the chemical arrangement of materials. RS is based on the principle that the matter irradiated by light produces molecular vibrations (the so-called "Raman effect") specific to each compound. In detail, the Raman effect ensues when a beam of light plugs the surface of a specimen, and some of the dispersed photons alter energy. Each molecule possesses distinctive vibrational modes, constituting the fingerprint of a molecule. Raman spectroscopy recognizes these vibrational differences and allows the identification of each specific molecule or tissue based on its vibration characteristics. [61].

Based on this principle, Zepeda–Zepeda and colleagues used micro-Raman spectroscopy to estimate dental fluorosis severity [62]. The authors collected 400 micro-Raman spectra images from 40 extracted teeth (30 suffering from dental fluorosis and 10 controls). Their data underwent accurate statistical analysis, which indicated a significant difference in fluorosis severity categories, such as endorsing micro-Raman in vitro techniques.

Recently, Condò et al. conducted RS analysis to observe orthodontic bonding composites [63]. Their in vitro investigation was performed on enamel discs, and the data obtained by the Raman device reveal unique variations in the spectrum among the examined materials.

Furthermore, RS revealed the intriguing prospect of being used for human gender recognition; in 2021, Gamulin et al. conducted an ex vivo investigation to detect the teeth's sexual dimorphism by Raman spectroscopy [64]. The authors observed good spectra classification accuracy, generating promising results for forensic scenario purposes.

The potential role of RS in dental tissue characterization comes from a study by Alturki et al., who aimed to standardize carious detection in clinical practice based on the subjective hardness operative perception of dentine [65]. This in vitro study employed high-resolution RS to determine the correlation between biochemical composition and the Knoop microhardness test of 20 carious dentine lesion samples. The authors found clear correlations between the results from the Knoop microhardness test and the Raman peak values of amide and phosphate moieties, supporting the potential of RS in identifying in vitro and in vivo carious lesions.

4. In Vivo Dental Imaging

A significant limitation of the techniques mentioned above is the need to work on extracted teeth. Despite oral mucosa biopsy being routinely performed, in the case of dental diagnosis, this option is not possible, thus limiting and hindering in vivo studies on the effectiveness of treatments or the dental–materials interface analysis in clinical conditions.

Therefore, in order to avoid X-ray-based tools to make dental imaging less invasive, as well as to improve the information regarding the microscopic architectures of the teeth, in recent decades, there has been growing interest in non-radioactive additional diagnostic tools for the advanced frontiers of non-invasive in vivo imaging of biological tissue layers.

4.1. In Vivo Raman Spectroscopy

Raman spectroscopy has delivered promising results in dental fields, mainly focusing on the study of periodontal tissues [66,67].

A work by Bielfeldt and colleagues explains the utility of in vivo RS to evaluate the soft tissues of the oral cavity. In detail, they performed a novel in vivo approach with Raman spectroscopy for the chairside evaluation of the lip barrier to explore the potential of Raman resolution parameters for quantifying water content, natural moisturizing factors, and lip ceramide content to evaluate and cytoarchitecturally characterize the stratum corneum in a transepidermal vision of the oral and perioral mucosa [68]. Furthermore, the investigators compared the outcomes obtained through Raman measurements with those calculated through two already validated in vivo techniques: aquaflux and corneometry. Although

the achieved data show certain variability, the authors prompted further improvements and encouraged using Raman spectroscopy for the in vivo evaluation of the oral cavity's structures. Further in vivo studies have investigated the quality of alveolar bone in subjects suffering periodontitis [69], but specific works on in vivo RS for caries and dental surface evaluation are still lacking.

The most in-depth study reporting on the evaluation of decayed teeth is the work of Tabata et al. [70]. Based on the recent evidence of dental tissues pH measurements for early caries detection [71], the authors combined Raman spectroscopy with pH status analysis of decayed teeth surfaces [70]. In detail, the authors developed a combined system of wireless pH sensors with a handheld Raman spectroscope to validate the use and applicability of the system as a chairside diagnostic tool for daily dental practice. Despite still being ex vivo on extracted decayed teeth, their results reported the sensibility of the technique and the friendliness of the system, thus endorsing the method's applicability in cariology. However, it must be considered that, under clinical conditions, environmental factors such as light, vibrations, and body fluids (saliva) could interfere with the spectra by influencing the focus and image acquisition of Raman spectroscopy [72].

4.2. DIAGNOdent and Fluorescent Methods

With a different technical approach, and despite the heterogeneity of verifiable data, DIAGNOdent has been highlighted as a fluorescence-based method that demonstrates high values of sensitivity for the in vivo diagnosis of dental caries [73]. DIAGNOdent is a commercial device that works on the principle of tissue autofluorescence, similar to VELscope and other clinical tools used in dentistry and in oral medicine to establish the emitted fluorescence from oral soft tissues affected by suspicious lesions [41]. In general, "fluorescence is the property of some intrinsic molecules called fluorophores to absorb light at a particular wavelength and to re-emit it at a longer wavelength" [41]. DIAGNOdent consists of a pen-like probe that enlightens the tooth with a safe and painless laser beam at a wavelength of 655 nm to measure its internal fluorescence [74]. The same optic also has a fluorescence detector to quantify the amount of light reflected from the tooth. In detail, the fluorescence emitted by decayed tissue is higher than the sound enamel, and the entity is shown on the display of the device as a numeric value ranging from 0 to 99.

Teo et al. investigated this laser fluorescence-based device for detecting primary molar caries [75]. The authors performed both in vitro and in vivo analysis, achieving high validity data showing good repeatability for caries assessment.

Such fluorescence-based devices have been profoundly illustrated in the literature for their potential feasibility for in vivo visualization of caries lesions [76–79]. In 2020, Macey et al. performed a meta-analysis to evaluate the diagnostic accuracy of fluorescence-based devices for detecting and diagnosing enamel decays, including 133 studies [80]. That large amount of papers used led to non-uniform results concerning the sensitivity and sensibility of the different instruments and approaches. Nevertheless, one year later, the same authors conducted a similar meta-analysis investigation to assess the diagnostic accuracy of electrical conductance devices in evaluating non-cavitated coronal tooth decay [81]. On this occasion, their design included only seven studies; thus, their study and the reported data should be considered insufficient due to the lack of significant evidence.

In 2021, Achilleos et al. compared the DIAGNOdent pen™ device for the in vivo assessment of incipient caries on the occlusal surfaces of permanent teeth with visual examination according to ICDAS clinical criteria [17,82]. However, the accuracy values obtained through the use of the DIAGNOdent pen™ device did not prove to be able to contribute significantly in comparison with ICDAS visual examination.

Monea and colleagues sustained a similar investigation to the abovementioned one, comparing the validity of DIAGNOdent pen ™ data with clinical observations based on ICDAS criteria [83]. Their study evaluated pit-and-fissure non-cavitated lesions in young permanent molars; statistical analysis showed no difference between the ICDAS code and

DIAGNOdent pen™ measurements, highlighting a significant agreement between these two methods.

Lastly, in 2022, Sardana et al. compared DIAGNOdent pen™ examination and clinical photographs to detect white spot lesions in patients subjected to multi-bracketed fixed orthodontic treatment [84]. Their results showed higher sensitivity and specificity values for clinical photographs than the DIAGNOdent pen™, thus concluding that photographs deliver more relevant data in the diagnosis and estimation of the severity of demineralized lesions during multi-bracketed fixed orthodontic treatment.

4.3. In Vivo Confocal Microscopy

Recently, a strong interest in the potential clinical role of in vivo *reflectance confocal microscopy* (RCM) in stomatology has arisen [85]. Conventionally, the in vivo RCM system functions using a diode laser at 830nm combined with a water immersion objective lens. The laser power provides a variable power setup ranging between 5 and 10 mW, based on the depth of the tissue to be examined and the nature of the tissue itself; the powers emitted do not cause any tissue damage or heating [86].

As the ex vivo counterpart, in vivo RCM allows optical scanning on horizontal virtual planes, layer by layer, with the ability to deeply penetrate both ex vivo samples and in vivo tissues. In the case of in vivo dental imaging, the penetration depth is strongly limited by the reflectance of the hard mineralized tissues that allow the imaging within the first layers of the enamel, up to 300 µm from the dental surface [87].

The first in vivo dental observations under RCM were executed by Watson et al. in 1992 [88] and by Ogaard et al. in 1996 [89], achieving promising results despite the prototypes' technical limitations.

In 2014, Contaldo et al. endorsed the feasibility of an RCM device (commercially available for skin imaging) for the in vivo microscopic imaging of dental surfaces [87]. The authors collected en-face dental images from 15 healthy volunteers, obtaining scanned horizontal slices of 500 × 500 µm, 5µm thick, and with a lateral resolution of 0.5–1 µm, similar to the histological standards. Despite producing very high microscopic resolution images, the device did not reach the most posterior sites of the oral cavity due to the rigidity of the probe. Nonetheless, the images obtained in their study show the regular prismatic and interprismatic structures of the healthy dental elements and identify the cracked enamel surfaces' fracture lines of the affected teeth. In addition, the device produced images capable of distinguishing the deep enamel structure from resin composite restorations.

One year later, Gentile and colleagues conducted a similar in vivo exploration study to investigate the microanatomic differences between deciduous and permanent enamels. For this purpose, they imaged permanent and deciduous teeth in adults and children, respectively, and reported the differential features observed during in vivo RCM scanning [90]. Lower mineralization percentage results signified lower mechanical strengths; such aspects of the deciduous teeth explained the irregular appearance of dark areas in which the architecture appeared optically empty.

Later, the same research group also applied in vivo RCM imaging to preliminarily identify and distinguish various kinds and degrees of enamel defects, such as hypoplasia and hypomineralization [91], as well as the remineralizing effect in vivo of amorphous calcium phosphate agents in a series of children with enamel defects. This study reported encouraging results on RCM's capability to monitor treatment and the patient's compliance [92].

Examples images of the hard and soft tissues of the oral cavity as they appear under RCM are shown in Figure 1.

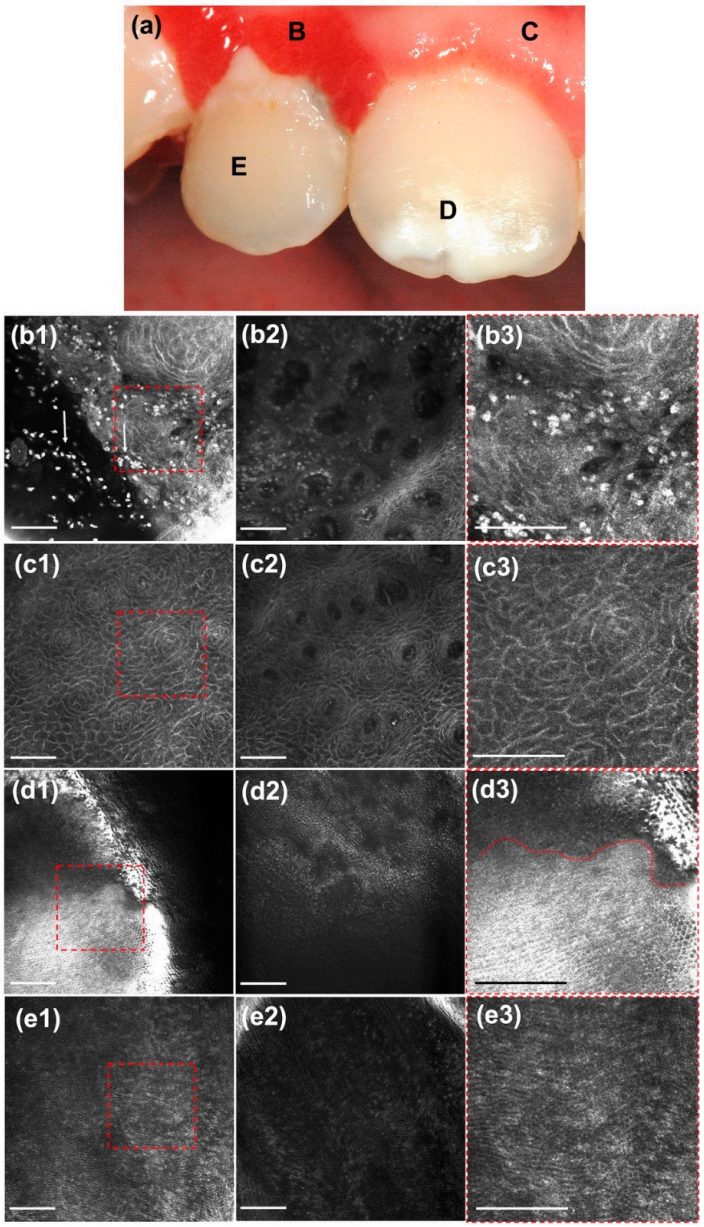

Figure 1. Examples of in vivo RCM images of the hard and soft oral tissues. (**a**) The clinical picture shows the points corresponding to the RCM frames: B, gingivitis, reported in b1-3; C, healthy gingiva, corresponding to frames c1-3; D, enamel defect, reported in d1-3; E, healthy enamel, shown in e1-3. (**b1**) Gingival epithelial surface, showing a massive presence of bacterial plaque (white arrows) covering the mucosal surface. (**b2**) Inflammation persists up to the basal epithelial layers with blood cells within the subepithelial and basal epithelial layers. (**b3**) Higher magnification of the bright roundish dental plaque bacteria from the picture in the red square of b1. (**c1**) Healthy mucosa at the intermedium epithelial layers. (**c2**) Healthy mucosa at the basal layer. (**c3**) Higher magnification of the regularly organized keratinocytes from the picture in the red square of c1. (**d1**,**d2**) Within the hypomineralized dental areas, a dark appearance and lack of interprismatic structures alternate with regular enamel through the enamel layers, magnified in (**d3**), where the red line has marked the boundaries between organized mineralized enamel and demineralized one. (**e1**,**e2**) The healthy enamel shows the classical architecture, given by the regular presence of enamel prisms and interprismatic substance, from the surface (**e1**) up to the last visible layer (**e2**). (**e3**) At a higher magnification, healthy enamel shows a bright interprismatic organic substance surrounding dark regular enamel prisms. Scale bars: 100 µm. [original pictures from the authors].

5. Discussion

This work aimed to overview the existing literature on conventional dental imaging techniques and to report the current knowledge on innovative methods, focusing on the ones that not only promise to pursue the early and less invasive detection of dental disorders but also those that could be applied in clinical practice.

The meaningful characteristics of the most representative promising in vitro and in vivo techniques are summarized in Table 1.

Although conventional radiology is still the gold standard for caries detection in the clinical setting, it is an invasive instrumental investigative method due to the use of ionizing radiations, thus presenting a series of clinical limitations [34]. Furthermore, enamel defects or early caries are not allowed to be detected with conventional radiology. This is also true when aiming to determine responsiveness to remineralizing agents or the microscopic gaps between the tooth and restorative treatments, which require ex vivo evaluation for research purposes, meaning radiology is not appliable in clinical practice for establishing the actual condition of every single case in every single patient.

Hence, dental research is currently investigating a series of alternative technologies that can be applied in clinical practice to overcome these limitations.

Despite the encouraging results from in vitro studies, most of the techniques described are not suitable for clinical use, mainly based on their principle of works, the need for thin sectioning, or the damage caused to living cells. This is the case of SEM and TEM, whose electrons may damage living tissues, and Raman spectroscopy, where examination may be affected by salivary fluid and imperceptible movements of the human being during the examination.

Among the techniques potentially applicable in vivo, laser speckle imaging systems have already proven their feasibility of evaluating the blood flux in gingivitis, and the pulse-echo ultrasonic technique could be considered to measure enamel and dental thickness in vivo. However, these hypotheses need to be concretized by expensive efforts in the research and development of handheld devices for clinical use, which are still never investigated to date.

DIAGNOdent remains the only unique device already available in this field, but there is a sizeable and contrasting body of work on its real and practical usefulness in recognizing caries; ICDAS seems to provide comparable or better results, which could discourage its use.

Among the investigated technologies, OCT and RCM seem to be the most promising and complementary to each other to offer a complete evaluation of the hard dental tissues at microscopic resolution in vivo. Indeed, both CM and RCM allow non-invasive real-time examination, are proven to be well tolerated by patients, are short-lasting, and are relatively simple for a trained operator [91].

Furthermore, OCT and RCM have already demonstrated their applicability in vivo on patients, including pediatric ones, with good collaboration and remarkable results from the image quality and monitoring of dental treatments. However, despite the feasibility of the procedures and the quality of the microscopic images, significant concerns related to the non-adaptability of these specific tools to image every oral cavity surface for limited access remain, thus limiting the potential use of existing de facto devices for the evaluation of the sole anterior teeth. Furthermore, these are costly devices and require a long learning curve, so their diffusion is unlikely, thus limiting their applications for research purposes or in hospitals and universities.

Last, the literature provides no data or publications on the specificity or reliability of these imaging modalities in clinical or research projects.

Table 1. Summary characteristics of the included dental imaging techniques.

Techniques	Type of Study	Dental Applications	References	Potential In Vivo Use for Dental Assessment #	Initial Costs and Learning Curve for the Dentist *
confocal microscopy	in vitro	en-face optical slices of the bulky tissues with microscopic resolution of dental histology and defects	[40–43,53]	- microscopical imaging of enamel layers - differential diagnosis of enamel defects - restoration-teeth interface imaging - monitoring of the effects of treatments or diseases	High purchase costs; long learning curve
	in vivo	en-face optical slices of the enamel defects with microscopic resolution in the clinical setting	[82–88]		
optical coherence tomography	in vitro	cross-sectional oriented virtual slices of bulky tissues with microscopic resolution of dental histology and defects and restorative materials	[44,45]	- microscopical imaging of enamel layers - differential diagnosis of enamel defects - restoration-teeth interface imaging - monitoring of the effects of treatments or diseases	High purchase costs; long learning curve
scanning electron microscopy	in vitro	Three-dimensional visualization of the surface from bulky tissues with microscopic resolution of dental histology and defects	[47,48]	not applicable	-
transmission electron microscopy	in vitro	high-definition microscopic details from ultrathin slices for dental histology and defects	[49–51]	not applicable	-
transverse microradiography	in vitro	evaluation of the degree of enamel mineralization	[53,54]	not applicable	-
laser speckle imaging and HeNe laser	in vitro	evaluation of the microstructure of the enamel surface and mineralization	[55,57]	- differential diagnosis of enamel defects	High research and developments costs; mean learning curve
pulse-echo ultrasonic technique	in vitro	evaluation of the dentin thickness	[58–60]	- differential diagnosis of enamel defects	High research and developments costs; mean learning curve
Raman spectroscopy	in vitro	evaluation of dental fluorosis, bonding composites, gender dental differences, and caries	[62–65]	- differential diagnosis of enamel defects	High purchase costs; long learning curve
	in vivo	evaluation of the alveolar bone with periodontitis, lip mucosa, and periodontal ligaments	[66–72]		
DIAGNOdent™ and fluorescent methods	in vivo	white spots and caries detection based on differential fluorescence	[73–84]	already used for differential diagnosis of enamel defects and caries detection	Affordable costs; low learning curve

Some devices cannot be used on living tissue and directly on patients. Scanning electron microscopy and transmission electron microscopy may destroy living cells; microradiography is based on ionizing radiations on thin slices. * The costs can be considered "affordable" for expenses lesser than USD 10,000.

6. Conclusions

The present overview offered a glance at the future of dentistry.

Although radiology is still the gold standard for diagnosing dental affections, the rise of clinical studies to establish the potential and the protocols of novel imaging techniques is desirable. The number of studies and technologies involved in the non-invasive imaging of such dental structures is a conquer not only for the diagnostic process of caries but also to establish, for the first time in vivo, under real clinical conditions, the degree of dental defects or caries in human beings. This progress will also allow us to monitor, in the future, the accurate and real responsiveness to remineralizing treatments or the quality of bonding materials, for example.

By definition, the ideal diagnostic approach should ensure the least invasiveness, ease, speed of execution, and, last but not least, reproducibility of the procedure.

Furthermore, we must also consider that the need for dental cures is presumably increasing. Indeed, the SARS-CoV2 pandemic era has produced reduced access to dental care and prevention over the last two years, as well as changes in habits, thus influencing and increasing the incidence of caries among the population [93,94]. In this context, dental practice has also changed drastically, facing challenges against infectious diseases spread by airborne transmission at the dental office [95] and a higher need for cures in those patients untreated for two years or more, who have changed their perception of oral health, considering dental appointments and screening "not a priority" and dental offices an "unsafe place" [96,97].

Dental clinicians cannot ignore the need to change their way of working, and they will prospectively benefit from the introduction of novel tools in clinical practice to support, shorten, and make the diagnostic process less invasive. Meanwhile, these promising techniques are still in their infancy. However, we optimistically expect the development of a novel method to communicate and interconnect various study groups by increasing telemedicine [98], exponentially increasing the quality and quantity of evidence in future studies. This globalization of research and the sharing of results would speed up the development and validation of dedicated, innovative devices, as we have seen in the COVID-19 pandemic, with the development of vaccines and targeted therapies in a short time.

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