Application of biophysical technologies in dental research.

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ABSTRACT

There is a wealth of evidence to indicate that if dental caries can be recognised at an early stage, it is possible to halt its progression or even reverse it. This has led to an increased interest in the development of diagnostic techniques capable of visualising caries at an early stage in addition to providing clinicians with an aid to diagnosis. Several techniques are available for research and clinical applications for detecting early demineralisation. This manuscript has reviewed some of the techniques currently available to determine their advantages, whether they have any limitations and their applicability to dental research and clinical dentistry. No one method is the perfect choice in all situations but what is clear, is that the development and application of biophysical technologies has allowed major advances to be made in dental research as well as in clinical dentistry. With continued developments these technologies will play an important role in the future management of dental disease.

I. Introduction

For many years there has been interest in the development of new technologies for use in the oral environment. The focus for a number of methods has been directed towards research, although with the ultimate goal often applicability to the clinical situation. The paradigm shift in dental caries management from restoration to prevention has led to interest in the ability to detect carious change early. In addition it is important to be able to monitor the caries process together with the effect of any strategies aimed at preventing the progression of caries in the clinical situation.

Diagnosis therefore not only involves the ability to detect the presence of caries but also to attempt to determine the activity of the lesions that are present[1]. It is against this background that novel applications of existing, and also newer biophysical technologies have emerged as aids for diagnosis of dental diseases.

In particular there has been a demand for techniques which allow caries, periodontal diseases, erosion, toothwear, plaque and calculus to be quantitatively assessed. The development of appropriate techniques offers the research community the opportunity to elucidate in detail, not only the disease processes but also the efficacy of agents and therapies to prevent or halt the progression of dental disease. This paper will outline some of the most useful and most popular current biophysical techniques and evaluate their application for dental research.

II. Quantitative Light- Induced fluorescence (QLF™)

QLF is an optical technique which employs the principle that the carious process gives rise to changes in the auto-fluorescence of enamel. Although this observation was made around 80 years ago[2] , it was not until 1982 that the first study was published that reported a reduction in fluorescence when enamel demineralises [3] and is thought to be due to the increase in porosity of carious lesions when compared with sound enamel. There is an associated uptake of water and decrease in the refractive index of the lesion resulting in increased scattering and a decrease in light-path length, absorption and autofluoresence[4]. *In vitro* studies to use QLF to detect early caries were initiated by Sunstrom[5] and following the introduction of software to quantify the degree of mineral loss in lesions[6] , major advances in this technology have been possible. Probably the most significant of these advances was the switch from laser to light sources, since lasers caused concern over safety. The introduction of a small portable system for intra oral using a light source and filters[7] paved the way for clinical applications.

Initially all studies involving QLF reported only green fluorescence indicating demineralisation whereas more recently red fluorescence is used increasingly.

Green fluorescence is observed when blue light illuminates teeth and excites green intrinsic fluorophores in enamel, dentine the enamel-dentine junction (EDJ). When a lesion is present, the light travels a shorter distance in the tooth and the passage of light to the EDJ is blocked resulting in a dark area of reduced fluorescence surrounded by bright green fluorescence[8].

Red fluorescence results from the excitation of red fluorescent fluorophores from bacterial metabolites when illuminated with blue light. These fluorophores are thought to originate from porphyrins found in some bacterial species and are often those associated with gingivitis. Interestingly red fluorescence is often observed in advanced dental lesions and progressive white spots[8] and may be of clinical significance and as a diagnostic aid to help in clinical decision making in relation to timing of restorations and restoration replacements (Fig 1).

There are a limited number of methods available to quantitatively assess demineralisation and remineralisation parameters and the majority of these are destructive removing any possibility of monitoring the same lesions over time. One of the major strengths of QLF is that it is non-destructive and has the ability to longitudinally monitor and detect early caries in teeth *in vivo*. The technique has been validated by a number of studies (mainly *in* vitro) which have indicated that QLF is a reliable and valid technique to detect early demineralisation during the onset and development of smooth surface caries[9].

In terms of early caries detection QLF has been shown to be particularly useful in patients who have undergone orthodontic treatment[10] (Fig 2 and Fig 3) and the technique was shown to detect caries at an earlier stage than that by visual examination. The suggestion that QLF may be useful *in vivo* during orthodontic treatment emerged from *in vitro* studies[11,12] investigating enamel demineralisation around orthodontic brackets bonded to extracted teeth. QLF is particularly valuable for investigating effects of different treatment regimes[14] were able to longitudinally monitor single white spot lesions where treatment was a 950ppm fluoride dentifrice versus a placebo at baseline and three monthly intervals for one year. Importantly they concluded that QLF had the ability to quantify the effect of a treatment in a smaller number of patients and in less time than would have been deemed appropriate in a conventional clinical trial suggesting evidence that could be a viable alternative to the traditional clinical trial design, a view endorsed by Higham et al[15].

The QLF technique is particularly well suited in the field of paediatric dentistry as it is pain free and the camera is easily accommodated in small mouths. The added bonus that children are able to view their own teeth on the monitor and the non-threatening nature of the camera all bodes well for the facilitation of use in children[16] .

The early detection of failing margins around restorations is of major interest to restorative dentists. When using traditional diagnostic techniques, it is recognised that secondary carious lesions are often only detected at a relatively advanced stage when further tissue loss has already occurred [17]. A number of trials have investigated the use of QLF to detect and monitor secondary caries . Demineralisation adjacent to restorations *in vitro* were reported to be detected and monitored longitudinally by Pretty et al[18] and similar findings were also reported by Gonzalez-Cabezas at al[17]. In a study investigating various techniques to detect natural secondary caries adjacent to amalgam restorations in extracted teeth, QLF was found to have the highest sensitivity and it was concluded that QLF is an appropriate technique for early lesions and those lesions up to a depth of 400 µm[19].

There is no doubt that QLF has many applications in dentistry for analysis of early sub surface caries on smooth surfaces of enamel but it is less clear at the present time whether it will prove to be as useful and popular on other surfaces such as occlusal, interproximal, dentinal lesions and eroded surfaces.

Until recent years there have been relatively few reports on the use of QLF to detect caries on the occlusal surface of teeth. It is an area that deserves more attention since occlusal caries often accounts for the majority of caries in many patients. Occlusal surfaces are notoriously difficult to diagnose due to confounding factors such as stain, compacted food and plaque. In addition radiographs are of limited use for early lesions since they are often only visible once they extend into dentine [20]. As diagnosis can be difficult, Higham et al [15] reported the *in vitro* development of an occlusal caries index using QLF. The ultimate aim, following further modification and validation *in vivo*, is to be able to provide clinicians with an interpretive index which may be useful as a guide to decision making regarding the management of occlusal caries in their patients. Kuhnisch et al [21] have recently reported on the *in vivo* detection of non-cavitated caries on occlusal surfaces using visual inspection and QLF. They found that QLF detected more lesions and smaller lesions than visual inspection but that QLF was more time consuming than visual inspection and concluding that QLF was not really practical for use in dental practice. However it must be realised that the QLF system used in this trial was developed as a research tool for very detailed examination and analysis. This clearly gives more information than is required by practitioner and patient. With this in mind systems are being developed for the dental practice setting.

Approximal caries detection and analysis provides a major challenge because of the inaccessibility of these sites. This has provided difficulties for the use of QLF in vivo to assess these lesions *in vivo*. Buchalla et al[22] have attempted to optimise camera and illumination angulations to overcome these problems and were able to detect advanced lesions although concluded that it was unlikely that QLF would be able to detect lesions at approximal sites at early stages in development. More recently they have reported findings of an *in situ* study in which they used QLF to assess approximal caries in contact with restorative materials. The contact point was flattened over an area of 1mm to simulate the shape of a contact point between molars or premolars. This had to be created between a flat and semispherical sample and not a rounded surface as found in the natural contact points[23]. Clearly more work is warranted in this area but the fact that QLF can be used to monitor simulated approximal caries *in situ* is a major advance for research in this area.

The optical properties of dentine differ from those of enamel and this has made evaluation of root caries by QLF problematical[24,25]. Progress has been made however by introducing the use of dyes to stain dentine *in vitro* [26] but further work is needed if QLF is to fulfil its potential to become a useful method for the assessment of root caries *in vivo*.

Observations during the use of QLF *in vivo* noted that heavy plaque residues had the ability to fluoresce red or orange [27,28] (Fig 3). This fluorescence may be due to the presence of porphyrins in certain oral plaque species , in particular gram negative anaerobes which are more numerous in late than in early plaque[29] . Coulthwaite et al [30] demonstrated that red, orange and green fluorescing colonies could be isolated from plaque which had accumulated on dentures. The responsible species identified via DGGE were *Prevotella melaninogenica, A Israelii, C albicans* (red and orange fluorescence), and *F nucleatum* and streptococci species (green fluorescence). In addition to the fluorescence detected in plaque biofilm red fluorescence has also been seen within caries lesions. This has been proposed as a potential marker for the presence of bacteria or their products within carious tooth tissue. Further investigations are required to elucidate what the clinical significance of this type of fluorescence may be. It has been suggested that this could be a surrogate marker for bacterial activity[30].

III. Digital Imaging Fibre-Optic Trans-illumination (DiFoti)

The interface of large posterior teeth produces contact areas that are difficult to visualise by clinical inspection. In an attempt to identify early decay, Fibre-Optic Trans-illumination (Foti) has been used to pass a light from the cheek side of the tooth through the contact surface and visualise the image from the tongue/palate side of the tooth. Foti has been found to be as accurate as detailed visual inspection in detecting occlusal caries [31] . Digital Imaging Fibre-Optic Trans-illumination (DiFoti) is a development that uses a charge-coupled device digital intraoral camera to collect the images generated. DiFoti has been reported to have a sensitivity of 0.56, specificity of 0.76 and repeatability of 0.25[32]. However, Ando [33] suggests that for non-cavitated caries detection, DiFoti has a low sensitivity and a high specificity.

DiFoti is a method of caries detection that has the potential of obviating the need for assessment by ionising radiation [34]. This provides a method of non-invasive assessment.

DiFoti is not a replacement for the use of radiographs that in a clinical context can assess not only the presence of early dental caries but also bone levels and other pathologies. The technique is useful in epidemiological practice, particularly, for following the development of interproximal caries. Occlusal caries can be efficiently monitored by detailed visual inspection [35]. DiFoti is a useful adjunct to caries assessment in conjunction with other techniques [34].

IV. Laser profilometry

Profilometry measures the bulk or mass of a surface. Non-contact optical profilometry permits the non-destructive study of surface detail. The measurement of the sample is made on a computer controlled traversing stage upon which the sample is systematically scanned by either a laser beam [36] using triangulation of the captured image or white light using chromatic aberration. The wavelength of the light focused onto the surface measures distances between the sensor and the sample to depict surface detail in three dimensional form.

Non-contact profilometry is useful for the laboratory based assessments of the detail on small surfaces. Reproducibility has been found to be at the same level as confocal microscopy [37].

The technique is useful as a method of measuring small changes in the surface topography of teeth in three dimensions. This allows the depth of the surface to be factored into assessments of surface change. It has been used in the measurement of erosion [38], abrasion, abfraction [39], early dental cavitation[40], wear of dental restorations [41], loss of dental enamel during orthodontic treatment as a result of biofilm demineralisation [42] or enamel bonding techniques [43], and changes to the form of soft tissues subject to facial prosthetic repair [44]. Small samples can be placed in-situ for the experimental phase and then measured in the laboratory [45].

Direct measurement of samples that cannot be removed from the mouth is not possible. Thus, indirect measurement of, for example, the wear of dental restorations becomes necessary. This indirect approach introduces another confounding variable to the accuracy of the technique. The technique measures flat surfaces more efficiently and this leads to in situ samples being ground flat before use. Measurement of a surface that has a natural curve becomes more problematic.

It is very useful in combination with other techniques, particularly when the conditions under examination are themselves compound conditions. Wear evaluations can be undertaken with multiple techniques [46] of which one technique is profilometry. The combined presentations of erosive demineralisation [47], remineralisation of abraded, eroded enamel [48] and ultrasonification to study subsurface demineralisation of eroded enamel [49] have been effectively studied by using profilometry in combination with assessments of mineral loss or gain. An example is given of combined techniques. (Fig 4.) The applications for profilometry in Oral Research have been demonstrated to contribute to any analysis that needs an evaluation of surface topography. The inclusion of an ability to make direct intra oral measurements would be a valuable next step.

V. Transverse microradiography (TMR)

Transverse microradiography (TMR) is a technique that allows the mineral content of the hard tissues to be quantified using x-rays. It is based on a concept that has been developed following the work of Angmar *et al*. [50] and the use of densitometry [51,52]. The densitometer based systems have been subjected to thorough error analysis [53]. Further important modifications were made possible following the development of image analysis systems comprising video (CCD) cameras and dedicated software [54].

These systems have largely replaced the densitometer based systems predominantly due increased ease of use.

TMR is a destructive technique that requires the samples to be cut into thin sections, polished to give planoparallel sections, orientated perpendicularly to the anatomical tooth surface. The sections together with a calibration step wedge are irradiated by monochromatic x-rays, the absorption of which is directly related to the optical density of the photographic film or plate and used to calculate mineral content [55].

TMR is a valuable research tool as a method for directly measuring the mineral content of the dental hard tissues. It is recognised to be a practical and reliable technique appropriate for quantifying not only mineral change, but also mineral distribution in enamel (Fig 5 and Fig 7)) , dentine and cementum [56]. As such it has been widely used for *in vitro* and *in situ* studies [57,58,59]. Unlike profilometry however, which relies heavily on the need for flat surfaces, TMR is not restricted to non-curved surfaces since several scans can be made for each microradiograph section (Fig. 6) Thanks to recent advances in TMR software, curved surfaces can be mathematically flattened to allow accurate mineral content estimates to be made [60] (Fig 6 and Fig 9) . TMR has also proved to be very useful in the measurement of enamel loss by erosion *in vitro* or *in situ* following a 2 step analysis described by Amaechi et al [61].

The fact that TMR requires thin sections is clearly a disadvantage in terms of preparation time, destruction of the samples and the associated limitations with experimental design. Like profilometry, TMR is unable to be used to assess samples that cannot be removed from the mouths of patients or subjects and has to employ experimental designs where lesion parameters can be assessed *ex vivo.* In addition there is a need to compare control and experimental samples rather than longitudinal analysis of the same tissue [62].

TMR has become an extremely valuable tool in dental research since it allows very detailed examination of the degree of mineral loss as well as depth and width of lesions.

VI. Optical Coherence Tomography (OCT)

Optical coherence tomography (OCT) is a non-destructive imaging technique with applications in medicine for use mainly in ophthalmology [62,63], but has application for imaging other transparent structures as well as semi- transparent structures such as teeth[64]. OCT is able to quantitatively monitor mineral changes in a caries lesion on a longitudinal basis in bovine teeth *in vivo* [65]*.* The OCT system used a wavelength of 850nm but other systems use 1310nm [66] resulting in image depths of 0.6 mm to 2.0mm. Jones et al.[66] used OCT to successfully evaluate artificial caries severity and depth in human teeth *in vitro.* As the application of OCT to dentistry is relatively new, there is much work still to be done to assess its full potential. Of clinical relevance is the development of prototype hand pieces for intra oral OCT although no *in vivo* data has been reported and as with all optical techniques it is likely that stain will be a confounding factor [64] .

VII. Electronic Caries Monitor (ECM)

The concept that electrical conductance may be used to detect carious changes in teeth is not new; it was mentioned as long ago as 1878 [67], there was a considerable delay before it was suggested that resistance measurements from teeth may be used in caries diagnosis [68] . The underlying principle is that of applying an electrical current through the tooth and determining the extent to which the current is conducted. Each of the component mineralised tissues of teeth displays differing conductivity; dentine, for example, is more conductive than enamel [69]. Sound tooth surfaces have been described as having little or no conductivity, while in contrast, demineralisation of the tooth, as in the caries process, leads to an increase in conductance [70]. At the simplest level the carious process leads to loss of mineral, which in turn is manifest as an increase in porosity of the carious tissue. The resulting pores contain ion rich fluid derived from the oral environment which results in increased conductivity or conversely a decrease in impedance.

Commercially available devices, such as the electrical caries monitor, consist of a probe from which a single fixed frequency alternating current (23Hz) is passed through the tooth, together with a contra-electrode normally held by the patient. The probe can be applied to any exposed enamel or dentine surface. Evaluation of ECM as a means of caries diagnosis has followed the initial path of a series of in-vitro investigations aimed at determining how electrical conductance of carious teeth may be affected by different experimental factors. These experiments have highlighted that various factors relating to the teeth themselves and the conditions under which measurements are made may influence electrical measurements, including dehydration state, tooth maturation and presence of stains [71, 72,] There are a limited number of clinical studies which have used ECM as a means of caries diagnosis. Given the potential for confounding factors to influence caries diagnosis using ECM it is perhaps not surprising that the few studies conducted have highlighted the need to take into account factors relating to the teeth and also the techniques used in applying this technology . Predicting occlusal caries using the ECM[73] indicated that surface specific electrical measurements may be of value compared with conventional detection methods in the 3 year clinical caries trial (CCT) reported. The CCT reported by Chesters et al [74] highlighted the possibility for confounding factors to operate when using site specific caries detection with ECM.

To date there has been a single study which has demonstrated the potential for ECM to be used in the evaluation of preventive strategies in dentine (root) caries, and this has demonstrated that ECM may be of value in this type of CCT [75].

The significant limitation of using a single fixed frequency current includes the possibility of polarisation and this has led to electrical impedance spectroscopy (EIS) being developed commercially for caries diagnosis. The underlying idea is that EIS scans a range of electrical frequencies and provides information on capacitance together with impedance. Work on this new application of electrical impedance is ongoing, although there are reports of excellent differentiation between sound, non-cavitated and cavitated approximal tooth surfaces *in vitro* [76] recent work has highlighted that there may be limitations when evaluating restored teeth using this type of technology [77] and therefore the clinical application must be carefully considered.

VIII. X-ray Microtomography (Micro CT)

Micro CT is a development of computed tomography, now familiar technology in medical diagnostics, which has much finer resolution (in the region of 5μm) allowing x-ray scanning of thin slices of small objects, the threshold of the detectability of the smallest detail is potentially between 1-2μm. Essentially a 360 degree radioscopic image is generated from an object placed on a sample table in the scanner. This is obtained by using an X-ray image intensifier while the irradiated object is rotating on the sample table. This data is used for reconstruction of a three dimensional image of the object which is generated via dedicated computer software and can be displayed on a computer screen (Fig 9 and Fig 10). Significant advantages are that the technique is non-destructive and specific sample preparation is not required, on the other hand scanning may take an extended period of time and this may be a limiting factor, although newer systems have significantly reduced scanning times.

Conventional CT scanning involves a significant radiation dose to the subject being examined and also has insufficient resolution for the often small scale changes that are of interest in dentistry in general and more specifically cariology and restorative dentistry. These factors have led to interest in the use of micro CT which has the potential for studying the carious process *in vitro* in three dimensions. This is in contrast with some current materials and methods which often evaluate extremely small scale changes in mineralisation of the dental hard tissues in very thin specimen sections (e.g. standard transverse radiography) and produce a two dimensional image. Ethical issues relating to human experimentation, together with the expense involved in using human subjects in clinical studies, has led to the use of model systems to study various preventive and restorative treatments, and it is in this arena that micro CT potentially has a role. To date this relatively new technology has been used in a number of in vitro evaluations of the dental hard tissues, dental caries and restorative procedures and also as an aid to teaching students[78, 79, 80,81].

Early studies involving micro-CT demonstrated that striking images could be produced which might be of value as teaching aids and also for in-vitro studies involving endodontic treatment of extracted teeth. However, a note of caution was sounded about the ability of these early systems to be sufficiently discriminative between the mineralised dental tissues, particularly as the resolution was limited to approximately 40µm by the equipment used at that time. More recent technological advances have seen the resolution approach 5-6µm which led to a resurgence of interest in the use of this method, particularly for evaluating mineralised tissues [82,83]. The particular advantages are that the greater resolution permits changes in mineral to be determined in thinner sections than has previously been possible using physically prepared thin specimens (nominal 80µm thickness). The value of micro-CT has been demonstrated in the longitudinal evaluation of the in vitro demineralisation of enamel by Dowker et al [83], who suggested that this technique had the ability to provide measurements of X-ray attenuation coefficients for micrometre sized volumes for the specimens being evaluated. At the X-ray energies used in this study (40 KeV) the linear attenuation coefficient (LAC) was approximately directly proportional to mineral concentration [84]. In turn it has been assumed that mineral concentration is approximately proportional to the fractional volume occupied by the mineral. This is fundamental to the process of demineralisation in which pore spaces are created in carious enamel. However, in a more recent development of the micro-CT technique involving the use of a Synchrotron beam Dowker et al [85] have suggested that neither enamel porosity or fractional changes in porosity that occur in demineralisation can be reliably determined by recourse to analysis of LAC alone .

It is apparent that micro-CT offers a novel approach to the evaluation of in vitro demineralisation, the non-destructive nature of the technique permits longitudinal evaluation without recourse to destruction of the sample being evaluated. There is the possibility to reveal features in the development of carious lesions that would not otherwise be apparent using 2D model systems.

IX. Diagnodent

DIAGNOdent [DD] (KaVo, Germany) is a commercially available device employing red light emitted by a small laser at 655nm to detect caries. Specifically designed tips are employed for use on occlusal and smooth surfaces of the teeth. The tip emits the red laser light used to excite the tooth tissue and also gathers the resulting fluorescence. The development of DD followed the usual path of a series of in-vitro studies which aimed to establish the validity and reliability of the device, and these have been followed by a smaller number of investigations using the instrument in vivo.

Early in vitro studies showed promise with the device demonstrating sensitivity and good reproducibility and agreement amongst different users [86,87,88,89]. The small number of clinical studies undertaken suggest that the instrument has good sensitivity (0.75-0.96) but specificity is lower [90,91,92,93]. A recent systematic review [94] compared the ability of DD to detect caries in human teeth compared with a histological gold standard. This confirmed that DD had high sensitivity but lower specificity than visual assessment methods. This raises the possibility that a diagnostic device of this nature could potentially lead to sound sites being incorrectly diagnosed as being carious. This could be the result of various confounding factors that may operate in clinical situations such as staining, calculus, plaque and dryness of the surface. More recent publications have focused on the clinical use of DD and Alkurt et al [95]have concluded that LF may be a useful adjunct to visual examination, and that the diagnostic performance seemed to be good for the detection of occlusal caries. In contrast Silva et al have demonstrated that DD may be unable to monitor the changes that occur when caries has been clinically assessed as arrested in child patients after an interval of 60 days. Kuhnisch et al [96] have demonstrated the use of DD compared with ICDAS II in the detection of occlusal caries in an epidemiological study and concluded that although DD showed a positive relationship with higher ICDAS II scores the use of DD in field studies added a limited amount of additional information. There has also been speculation that DD may not be measuring the intrinsic changes in early enamel lesions but may in fact be detecting bacterial activity. There is some theoretical support for this premise in that the 655nm wavelength employed by DD induces fluorescence of bacterial porphyrins. Recently Arif et al [97]have used DD along with Ekstrand scores in the identification of carious teeth from which *Veillonella* spp. were isolated. However, there was no attempt to relate the DD values with the bacterial isolates or Ekstrand scores obtained.

It would seem therefore that DD may be a useful adjunct to clinical diagnosis and caries management, but this tends to depend on the ‘cut off value’ used to guide the practitioner and the careful control of any confounding factors that may be present.

X. Conclusions

There are several methods available to researchers and clinicians to detect early demineralisation, but all of them have some limitations which affect their application to the clinical setting mainly because of practicalities including the need for sample preparation or that they have other limitations which affect their diagnostic ability or cannot monitor change longitudinally or produce data in an archivable form. Some methods are unable to detect caries at a stage where it is early enough to allow non-invasive repair/remineralisation so have limited uses over and above visual inspection. Others produce data which is indirectly measured and may have arbitrary values. No one method is the perfect choice in all situations but what is clear, is that the application of biophysical technologies has allowed major advances in dental research and in the clinical setting. With further developments and modifications, these technologies, will continue to play an important and significant role in the management of dental diseases.

FIGURE LEGENDS

Fig 1 QLF image showing secondary caries around a composite restoration on a canine tooth

Fig 2 Example of QLF image of demineralisation around an orthodontic bracket following debonding (left) and a white light image of the same tooth (middle and right)

Fig 3 White light image of patient undergoing fixed orthodontic treatment (left) and QLF images of teeth showing red fluorescent plaque

Fig 4 Corresponding QLF image (left), TMR image (middle) and Laser profilometry scan (right) of an eroded bovine tooth

Fig 5 Microradiograph of a sub- surface artificial caries lesion

Fig 6 Single TMR scans performed by rotating the image for each scan

Fig 7 TMR analysis screen

Fig 8 Original TMR analysis of curved surface (right) and using new algorithm (left)

Fig 9 Diagrammatic representation showing the principles of micro CT

Fig 10. Micro CT 2D image of a tooth slice demonstrating an early caries lesion with sub-surface demineralisation (right) and 3D image of a tooth block demonstrating early caries on the wall of an occlusal fissure. (Courtesy of Dr. BT Amaechi, University of Texas Health Science Center at San Antonio)

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