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Objective identification of dental abnormalities with multispectral fluorescence imaging

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Abstract

Sensitive methods that can enable early detection of **dental diseases (caries and calculus)** are desirable in clinical practice. Optical spectroscopic approaches have emerged as promising alternatives owing to their wealth of molecular information and lack of sample preparation requirements. In the present study, using multispectral fluorescence imaging, we have demonstrated that dental caries and calculus can be objectively identified on extracted tooth. Spectral differences among control, carious and calculus conditions were attributed to the porphyrin pigment content, which is a byproduct of bacterial metabolism. Spectral maps generated using different porphyrin bands offer important clues to the spread of bacterial infection. Statistically significant differences utilizing fluorescence intensity ratios were observed among three groups. In contrast to laser induced fluorescence, these methods can provide information about exact spread of the infection and may aid in long term dental monitoring. Successful adoption of this approach for routine clinical usage can assist dentists in implementing timely remedial measures.

Keywords

Fluorescence spectroscopy; Multispectral imaging; Dentistry; Biophotonics

1. Introduction

The tooth surfaces, under normal physiological conditions, are essentially indestructible. However, their integrity is attacked to a major extent by myriad bacterial challenges. Indeed,

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dental abnormalities due to microbial infections are considered as one of the most common afflictions globally [1]. These infections can lead to irreversible solubilization of tooth minerals causing tooth decay. Caries and calculus (tartar) are direct consequences of dental bacterial plaque activity. Fermentation of sucrose and other sugars to lactic acids by *Streptococcus mutans* is the leading cause of tooth decalcification with proteolysis resulting in caries [2, 3]. If not treated or diagnosed in time, accumulation of dental plaques and evolving microflora may also cause gingivitis. Such conditions may progress to damage the periodontal membrane (chronic periodontitis) and lead to tooth loss. Despite its non-life threatening nature, the discomfort and substantial treatment costs offer compelling reasons to pursue superior detection and treatment options than those available currently [2].

The diagnosis and treatment strategies for these conditions are mostly directed towards early identification and eliminating bacterial species that appear to be overt pathogen in the dental plaques. Clinically, dental decay is a measure of cavitation on the tooth surface. However, the pathogenesis of cavitation is a long-term phenomenon, which is preceded by clinically detectable subsurface lesions known as 'white spots'. Prior to this stage, only microscopic changes occur in the process of demineralization³. In an ideal diagnostic scenario, all the lesions should be detected at the white spot stage. As dental diseases generally manifest in slow lesion progression, it offers a window of opportunity for therapeutic intervention. Early diagnosis of these abnormalities can help in monitoring lesion progression and therefore be of assistance to informed decision-making [4, 5]. Current clinical techniques for detection and identification of dental diseases include visual and radiographic examination. However, lesions can be visually detected only at relatively advanced stages whereas radiographic methods are suitable for proximal surface caries, due to low sensitivity they have limited effectiveness for early stage lesion diagnosis [3].

In this milieu, optical spectroscopic methods, due to the molecular specificity and non-perturbative nature, can provide an attractive alternative [6]. **Laser induced fluorescence (LIF)** spectroscopy has been explored extensively in study of dental diseases [7]. Alfano *et al.* utilizing three different excitation wavelengths (350 nm, 410 nm, 530 nm) had demonstrated that relative intensity of fluorescence in the red region (**below 540 nm**) was high in caries in comparison to controls [4]. Subsequently, using a nitrogen laser (**337 nm**) induced porphyrin autofluorescence differentiation between caries and early demineralization was achieved [7]. A study by Riberio *et al.* on extracted tooth specimen using 405 nm laser demonstrated that different stages of caries development could be readily identified and discriminated on the basis of intensity ratios [8]. Their findings suggests that emission maximums around 480–500 nm and 620–640 nm can be used for objective identification of sound teeth and caries [8]. Furthermore, Zezell *et al.* reported that areas associated with 455 and 500 nm bands have significant differences among caries and control tooth [9]. Subhash and co-workers have also shown that sensitivity of nitrogen laser induced fluorescence for identification of control and caries can be enhanced using Gaussian curve-fit analysis [10]. Based on the fitting coefficients, the exact positions of porphyrin bands (403.80, 434.20, 486.88, 522.45 nm) were identified. On the basis of intensity ratios they successfully identified both dentine and pulp level caries [10]. Another study by the same group using a 404 nm diode laser excitation, coupled with survival analysis, has

demonstrated the feasibility of classification between controls and cavitated/noncavitated caries [11].

Leveraging these promising findings for dental abnormality detection, we seek to exploit multispectral fluorescence imaging (MSFI), which is considered as a synergistic combination of imaging and spectroscopy. MSFI represents a rapidly growing field with broad applications in astronomy, geology, agriculture, industry, and forensics [12]. Typical MSFI instrumentation consists of an illumination source and a camera to acquire spatial information while the spectra from each point of the image is obtained by scanning with a dispersive element. Broadband light sources, such as lamps or light-emitting diodes, are chosen on the basis of emission spectra of the biomolecules to be analyzed. Recent advances in MSFI have led to the replacement of mechanical scanning dispersive devices such as filter wheels or monochromators with electronic tunable filters, notably acousto-optic tunable or liquid crystal tunable filters (LCTF). In clinical and preclinical settings, MSFI has been used for drug response monitoring, tumor margin identification and *in vivo* imaging of animals [13]. Recently, these methods have also been applied for multiplex immunoassays and flow cytometry [14, 15]. In the absence of fluorescence labels, this class of methods can be applied to study autofluorescence as well. A study by Roblyer *et al.* using MSFI observed red autofluorescence in lesions in patients with histologically confirmed neoplasia [16]. A separate investigation on diabetic retinopathy has reported that, due to advanced glycation and collections of end products, 'lipofuscin' fluorescence is green-shifted in diabetic patients [17]. In dentistry, multispectral imaging using near infrared excitation for identification of caries have been recently reported. A study by Salsone et al has shown that spectral mapping from 1000 to 1700 nm can be used to generate a combine quantitative lesion map. They compared the results with gold standard histopathology and observed varying sensitivities and specificities of 72% and 91% for sound areas, 36% and 79% for lesions on the enamel, and 82% and 69% for lesions in dentine [18]. Fried and co-workers studied changes in the lesion contrast with severity and depth for near infrared, visible reflectance and fluorescence spectral regions. Their findings suggest that near-IR reflectance measurements at longer wavelengths coincident with higher water absorption are better suited for imaging early caries lesions [19]. These approaches are advantageous, as they are stain-free; however, their clinical efficacy still needs to be verified by larger sample cohorts and in early stage lesions.

Despite the improved understanding of dental diseases and availability of effective intervention, late detection of these lesions remains a major concern. Point diagnosis methods, such as laser-induced autofluorescence spectroscopy, have limited applicability in identifying lesions, elucidating infection spread and long term monitoring. Moreover, even though previous studies have shown the potential of LIF in identifying carious lesions in early stages, the applicability in identifying calculus has not been thoroughly investigated. As these lesions are coupled with microbial infection, we reason that autofluorescence of bacterial porphyrin pigment can be utilized for monitoring spread and deformity in the tooth. Thus, the present study aims at evaluating the potential of multispectral imaging in observer-invariant, objective diagnosis of dental caries and calculus. Pseudocolor maps and intensity ratios at standard porphyrin bands at 500 nm, 520 nm, 640 nm, 680 nm and 700 nm were analyzed. Successful adaption of this technique in routine clinical practice can help in

enhancing the availability of effective intervention strategies to minimize invasive tooth restoration methods.

2. Materials and Methods

Tooth specimens

Control, carious and calculus tooth specimens, identified by an experienced dentist (**Arja M Kullaa**), were collected from the educational dental clinic at Kuopio University Hospital, Finland. They had been extracted for various reasons including periodontitis or pericoronitis. A total of 30 specimens (10 each category) were obtained. After extraction the teeth were washed gently with physiological saline solution and transported to the laboratory in isotonic saline. Before spectral acquisitions tooth specimens were dried and stored at room temperature. Both visual and radiographic examinations were performed for characterization. Teeth with clear enamel or an intact surface were grouped as controls. Specimens with surface roughness and visible deformations in the enamel region were grouped as carious. In the third category calculus tooth specimens has distinct discoloration and ectopic calcified masses in the enamel regions with no visible cavitated areas.

Instrument

The spectral images were captured by using the Nuance liquid crystal tunable filter (LCTF) spectral camera (model N-MSI- 420–10 Cambridge Research & Instrumentation, Woburn, Massachusetts), Figure 1. The used spectral range of the camera was 440 to 720 nm with 10-nm sampling. The measurements were conducted in standard 45/0 measurement geometry (45 illumination angle, normal detection angle). An image is captured for each band with a pixel resolution 1392×1040 . On the basis of emission spectral profile of bacterial porphyrin, a 405 nm LED (M405L2, Thorlabs) with 410 mW output power was chosen for illumination. Spectral data was acquired using optimal integration times for each measured channel with the camera software (Nuance 1.6.8.2). Samples were illuminated as evenly as possible and measurements were performed in a dark room to avoid stray light interference.

Statistical analysis

The output results files are series of images, called as 'image-cube or spectral-cube'. It can be defined as a three-dimensional dataset consisting of multiple images using the same specimen field acquired at different wavelength bands, Figure 2. A single pixel location in the lateral image dimension can be examined along the wavelength. As shown in Figure 2, the intensity and/or color of the pixel changes as function of emission signal strength and wavelength. By plotting pixel intensity versus wavelength on a linear graph the emission spectral profile of the particular fluorophore can be easily identified. The image-cubes at different wavelengths ranging from 440–720 nm were further processed by a custom-made MATLAB program (MathWorks, Inc., Natick, Massachusetts) and pseudo color maps were generated. Spectra were acquired from clinically identified sound, carious and calculus areas. An optimal baseline function in form of a best-fit 2nd order polynomial curve was used and spectra were area normalized. Mean spectra were generated and standard deviations were calculated. Normalized spectra were used for computing the fluorescence intensities. Emission maxima of already reported porphyrin bands were chosen for

calculation of intensity ratios among three groups. One way analysis of variance (ANOVA) test was performed to determine if the intensity ratios were statistically significant among sound, carious and calculus teeth. All statistical test were performed using GraphPad Prism 6.0 software.

3. Results and Discussion

Spectral Features

Average spectra along with standard deviation extracted from clinically identified regions of tooth specimens are shown in Figure 3. Overall fluorescence intensities were lower in pathological samples with respect to controls. The lower intensities in carious and calculus specimens can be attributed to the reduction in the fluorophore content and/or structural changes. Normal structure of tooth enamel consists of mineral crystals in form of prisms and waveguides that assist in deep penetration of light. In abnormal specimen especially in caries this architectural arrangement is disturbed, leading to loss of fluorescence intensity [20]. In calculus, on the other hand, loss of intensity can be ascribed to accumulation of cell debris and food particles over tooth surface [5]. This observation is further supported by the progressive increase in the fluorescence signal in the red region and a corresponding decrease around 500 nm. As the accumulation of exogenous molecules increases during microbial infection, it can contribute to the enhancement of autofluorescence in the red region. In contrast to abnormal samples, the fluorescence intensity of control specimens was significantly lower in the red region. Emission maxima's around 455, 500, 520 and 575 nm in control and diseased specimens can be ascribed to natural enamel [5,21]. In carious specimens emission maxima of the sound enamel was red shifted (530 nm). In carious and calculus samples additional sharp features in the red region were also observed. Changes in the spectral shape can be related mostly to the appearance of new fluorophores, which are a product of bacterial flora metabolism e.g. additional bands in carious and calculus specimens around 640, 680 and 700 nm can be assigned to endogenous porphyrins, particularly protoporphyrin IX, mesoporphyrin and co-porphyrin of bacteria [5,22,23]. These pigments from both gram positive and negative bacteria are known to absorb in UV-blue spectral region and fluoresce in the red spectral region.

Multispectral Imaging

Pseudo-color maps of spectral bands ranging from 440 to 720 nm were generated and analyzed. Representative images of two clinically verified control, carious and calculus tooth specimens at 500 nm, 520 nm, 640 nm, 680 nm and 700 nm are shown in Figure 4. These spectral bands were chosen on the basis of the average spectra and porphyrin bands reported in the literature [5,7,11,20]. Sound enamel areas in all specimens can be easily identified by the intense fluorescence signals at 500 and 520 nm. No fluorescence in these regions were observed at 640, 680 and 700 nm. In the caries samples, sound and infected areas can be readily differentiated at each band. Boundaries of cavity-affected areas in the caries specimens can be easily marked in fluorescence maps. The best distinction was observed in the map of the 640 nm band. As mentioned loss of fluorescence in carious specimens can be attributed to disturbance in the structural arrangements leading to dental deformity. Similarly, in calculus tooth specimens', clear demarcation between sound and infected areas

were observed. Reduction of fluorescence signals stemming from deep cavitation has previously been demonstrated by Borisova *et al.* Using *in vitro* models they suggested that loss in intensity of cavitated areas can be ascribed to the change of the surface structure and to the accumulation of absorbers such as food particles, blood cells etc. in the cavity [5]. As mentioned in the present study tooth samples with no visible deformations or discoloration were considered as sound. Teeth with loss of lustre, surface roughness and visible cavitation were categorized as carious. There is a possibility that in carious samples, because of the irregular shape, tooth were not exposed evenly and also scattering losses were more as compared to smooth surfaces. In contrast to caries, where no fluorescence in these regions were observed, demarcation between sound and infected areas were more visible in calculus at 680 and 700 nm. Some of the porphyrin pigments from gram-positive bacteria such as *Candida* sp. and *Corynebacterium* sp. have fluorescence maxima around 700 nm region [5]. As can be seen from spectral maps at 680 and 700 nm of calculus specimens, they have better distinction from sound areas suggesting specific infection from these bacterial species. This finding further supports high specificity of the technique in identifying carious and calculus infections objectively.

As mentioned, one of the major limitations of the widely explored LIF in dental applications is that of discrete sampling, which constrains the diagnostic determinations to only a few points. Wide field imaging techniques such as MSFI based on endogenous fluorophores can offer a viable alternative. Efficacy of these methods using a hand held device in identifying normal and dysplastic areas in oral mucosa has been recently demonstrated [16]. Our findings here further reinforce the feasibility of extending the same approach to objective diagnosis of key dental pathologies. Our future studies will focus on identifying early stage caries especially at the 'white-spot' stage. Fluorescence maps at 680 and 700 nm of calculus collectively indicate that porphyrin signals originating from specific bacterial species can also be identified thereby assisting in the selection of interventional measures.

Intensity Ratio

Spectral features and color maps suggests that major differences among three groups could be analyzed by intensities of 520 nm, 640 nm, 680 nm and 700 nm bands. Therefore, as the next step, three mean spectral intensity ratios (520/640, 680/640 and 700/520) were computed, Figure 5. As can be seen, the ratio of bands originating from sound enamel (520 nm) and bacterial porphyrin (640 nm) were highest in control specimens. Corroborating findings of MSFI ratio of 680/640 was highest in carious specimens, suggesting effectiveness of this band in delineating infection boundaries. Further, ratio of gram-positive bacteria specific porphyrin (700 nm) and 520 nm was highest in calculus tooth specimens. One-way ANOVA analysis suggests the presence of statistically significant differences ($p < 0.0001$) among all three groups.

As the focus of treatment philosophy has undergone a shift to minimally invasive dentistry, several non-invasive methods have been explored for prospective adoption in dental research. An ideal detection tool should be able to differentiate between all stages of caries development, amenable to different kinds of surfaces and have minimum intra- or inter-observer discrepancies. The increasing attention in this area is evidenced by the emergence

of commercially available devices for caries identification. For instance, the KaVo DIAGNOdent (Biberach, Germany) works on a fixed wavelength of 655 nm and performs diagnosis on the basis of organic and inorganic components. Another device, the QLF system (QLF-clin, Inspektor Research Systems BV, Amsterdam, Netherlands), has an arc lamp (290–450 nm) and follows decrease in green fluorescence due to acid build-up. The Canary system (Quantum Dental Technologies, Toronto, Canada) utilizes a combination of photo-thermal radiometry and luminescence to access damages in the crystal structure of tooth. Finally, the Soprolife camera (Acteon, La Ciotat, France) operates by using LED lights between 440–650 nm to probe occlusal or interproximal decay through autofluorescence. Rechman et al. has compared diagnostic efficacy of these commercial systems against the International Caries Detection and Assessment System (ICDAS) criteria and observed varying efficacy [24]. With the DIAGNOdent, sensitivity and specificity of 0.87 and 0.66 were achieved in diagnosing healthy and abnormal teeth. In contrast, SOPROLIFE showed Sens/Spec of 0.93 and 0.63, respectively. The sensitivity of SOPROLIFE was slightly increased by changing the illumination wavelength. Other systems also showed a high sensitivity but low specificity. The variation in sensitivity and specificity of the commercial systems is reinforced by the findings of several other recent studies [25–28]. It is worth noting that even though high reproducibility is observed with commercial system in comparison to visual examination, efficiency of these methods is affected by several physiological factors. Studies have reported that stained dental materials affect DIAGNOdent reading and can lead to false positives. Additionally, dental fillings, prolonged drying, pits and fissures, bacterial plaques and calculus can also influence the detection accuracy of the commercial systems [29,30]. In this milieu, we reason that multispectral imaging, being sensitive to both architectural changes and bacterial metabolites such as porphyrin, can provide accurate information about microbial infection and demineralization together. By blending imaging capabilities with the molecular specificity of fluorescence spectroscopy, MSFI empowers the development of a complete morphological and biochemical tooth profile. In particular, a cut-off value for bacterial infection depending upon its area of spread over the tooth can be generated. This can aid in identifying the precise stage of infection and can help the dentist in treatment monitoring. Our ongoing studies focus on developing a multi-fiber based system for single-shot illumination of whole mouth cavity *in vivo*. Imaging algorithms will also be integrated in a bench top system to facilitate translation of this technique for routine usage following validation in a larger patient cohort. Engaging this nature of spectroscopic imaging tool will facilitate adoption of a non-invasive treatment monitoring approach and help in long-term surveillance for preventive measures.

4. Conclusion

Overall, our findings suggest that dental abnormalities can be visualized objectively with MSFI. In contrast to point spectroscopy, the extent of bacterial infection over tooth can also be identified. As carious, calculus and control tooth have different fluorescence properties, distinct areas in one tooth can be outlined. We reason that the decrease of total fluorescence emission in abnormal samples is principally the result of enamel surface damage. We further demonstrate that the intensity ratios of different porphyrin bands have significant differences

in autofluorescence signatures among control, carious and calculus groups, paving the way for large scale *in vivo* studies with the overarching goal of validating the diagnostic efficacy of MSFI.

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Figure 1. Schematic presentation of the measurement setup. Tooth samples were placed on a sample stage and illuminated with 405 nm LED. Spectral images were acquired with an LCTF camera in 45/0 geometry.

Figure 2.

(a) Multispectral image cube. Image are acquired at specific narrow wavelength bands. The intensity and/or color of the pixel P changes as a function of fluorescence emission signal strength and wavelength (b) By plotting pixel intensity versus wavelength on a linear graph, the emission spectral profile of the particular fluorophore spatially located at pixel P can be determined.

Figure 3. Average spectra along with standard deviation (shaded area) from clinically identified Control (a), Caries (b) and Calculus (c) areas. Spectra were baseline corrected and area normalized. White light photograph of representative tooth is shown in inset.

Figure 4.
Pseudo color maps of two representative clinically identified control, carious and calculus tooth specimens. Cavitated and calculus areas are marked. (CON- Control, CAL- Calculus, CAR-Caries)

Figure 5. Average spectral ratios for porphyrin bands (640 nm, 680 nm, 700 nm) for control, carious and calculus teeth. Statistically significant difference were observed after one way ANOVA (* $p < 0.0001$)