Applications of Laser induced Fluorescence in Dentistry
Fardad Shakibaie, Roy George, L.J.Walsh

Abstract
The laser-induced fluorescence can be used to detect and diagnose dental caries, calculus and bacterial biofilms in dental applications. As such the molecular fluorescence phenomenon has been illustrated scientifically and the literatures relating the fluorescence properties of dental tissues have been explored till the literature reviews ultimately led up to the development of the current Laser Fluorescence (LF) devices. These LF devices have been introduced clinically for dental caries, calculus and bacterial detection to replace inaccurate contemporary detection tools. The future for LF technologies in diagnostic dentistry has been indicated as accurate and potentially applicable in a wide range in restorative dentistry, periodontology and endodontics.

Key words: Diagnosis; Detection; Dental Applications; Fluorescence; Laser Fluorescence

Introduction
Diagnostic fluorescent methods are promising technologies can be used for detection of dental caries, plaque, calculus and oral tumours (1). While this technology is at its early research and clinical trial nevertheless it has produced incredible results particularly for detection of carious lesions (2-6). It can detect interproximal caries, primary occlusal caries or identify occult lesions beneath fissure sealants (7-9). This detection can be outperform very accurately, the application is easy and very safe as laser-induced fluorescence can avoid high risk ionising radiations(10, 11), (12).

This technique has been used to detect deposits of subgingival calculus (13). Laser-induced fluorescence can accurately and sensitively diagnose subgingival calculus compared to the conventional periodontal probing (14). Bacterial biofilms similar to dental caries and calculus can also be identified by fluorescent methods because of porphyrin derivatives present in all infected dental tissues (1, 15). Due to this, detection of bacteria within root canals can potentially be performed by fluorescence in endodontics (16).

The broad application of new innovative diagnostic fluorescence in dentistry has briefly described, clearly indicate an optimistic future for this new innovation in clinical applications. Therefore this paper attempts to introduce the science and technology, which led to the development of Laser Fluorescence devices. As such this paper primarily propose the principle of the fluorescent phenomenon, then explore the scientific background of fluorescent studies on dental tissues, and finally introduces the current laser fluorescence devices are clinically employed in dentistry.

Fluorescence Phenomenon
Fluorescence is the process of absorption of light of a short wavelength which results in emission of radiation at a longer wavelength. This emitted radiation is called fluorescence, as certain molecules (fluorophores) de-excite electronically from higher energy level to a lower energy level (17, 18). Upon absorbing light, fluorophores become electronically excited to high energy levels and then decay to lower energy levels by emitting radiation (emission or luminescence). Fluorescence occurs if the transition is between states of the same electron spin and phosphorescence if the transition occurs between states of different spin. Luminescence is a general term used to describe the emission of radiation, which incorporates both fluorescence (short lived) and phosphorescence (long lived), as well as other phenomena such as bioluminescence in living organisms in which chemical reactions generate light. The fluorescence and phosphorescence phenomena are graphically demonstrated in the Jablonski energy diagram of beneath Fig 1. Many naturally occurring substances fluoresce, including minerals, fungi, bacteria, keratin, collagens and other components of body tissue; this is termed primary fluorescence or autofluorescence.

The molecule absorbs energy from lower ground singlet state \(S_0\) to one of vibrational level at excited singlet state \(S_n\) (n=1,2,….) as shown in Fig 1 with blue arrow. The excited molecule positions itself in an unstable level therefore vibrates and loses energy partly through internal conversion (Red arrow) without photon emission. After that the molecule at excited singlet state will spontaneously return back to one of vibrational level of ground singlet state (Pink arrow) to fluoresce a lower energy photon (17, 19).
In fluorescence, energy levels transitions do not involve changes in electron spin, whilst in phosphorescence, an electron spin exchange firstly occurs through intersystem crossing from the excited singlet state ($S_1$) to an excited triplet state ($T_1$) just below $S_1$ (17). Molecular fluorescence emissions persists only as long as the stimulating radiation is continued, unlike the process of phosphorescence, which persists as an “afterglow” after the incoming exciting light has been turned off. If light emission occurs within one microsecond (one millionth of a second) of light exposure, the luminescence is fluorescence, whereas if light emission takes longer than this, the luminescence is phosphorescence.

In molecular fluorescence, the colour of the emitted light has a longer wavelength than the colour of the exciting light. For example, when a molecule absorbs UVA light, the emissions are often in the visible spectrum (e.g. visible red, in the case of porphyrins). This relationship is known as Stokes’ law, named after Sir George Stokes, who published the first significant paper on fluorescence (20). Fluorophores are excited by a range of wavelengths, and also emit over a broad range, therefore it is scientifically important to explore the excitation-emission ranges for each dental target tissue such as enamel, dentine, caries, calculus and plaque.

**Excitation-Emission Properties of Dental Tissues**

Prior to the first World War, Stübel investigated the fluorescent properties of different biological tissues while irradiated by short wavelength ultraviolet (UV) light, and found that teeth brilliantly fluoresce an intense blue colour second only to the lens of the eye (21). Bommer detected an orange and red fluorescence of the “tooth film” (dental plaque and calculus) in patients by using a Wood’s lamp, which is a source of UV light (22). Benedict found that dentine fluoresces more brilliantly than enamel when using ultraviolet light (23). He used nitric acid to extract the organic component of dentine, and found that these organic compounds fluoresced strongly. In contrast, inorganic constituents of dentine extracted by 50% sodium hydroxide did not fluoresce. Interestingly, he reported that supragingival calculus emitted a reddish orange colour when excited by a long wavelength UV light source.

Several researchers came to the conclusion that the organic components of tooth structure can fluoresce under UV radiation (24-26), while Foreman (1980) claimed that the amino acid tryptophan was one of the major fluorophores of sound dentine (27).

In 1963, Armstrong investigated the autofluorescence characteristics of healthy and carious human dentine samples (28). Since the 1980s, research has focussed on discriminating carious lesions and healthy tooth structure by their differing fluorescent properties (29). Alfano used 488 nm excitation from an argon ion laser, and detected a maximum emission peak of 550 nm for carious and non-carious regions (30). Albin
similarly used 488 nm excitation and reported maximum fluorescence at 590 nm for carious lesions, and a 553 nm peak for surrounding sound surfaces (31). König excited carious and non-carious regions using a krypton ion laser at 407 nm (in the UVA region) and reported a strong fluorescence peak at 635 nm for carious regions (32). Alfano used 350, 410 and 530 nm excitation wavelengths, and reported distinct high fluorescence emissions in the visible red region which were not present in sound tooth structure (33). Buchalla also reported similar high peak fluorescence emissions between 600-700 nm when 360-580 nm wavelengths were used for excitation (34). Table 1 shows the excitation and emission of some major fluorophores found in dental tissues.

**Laser Fluorescence (LF) Devices**

Hibst undertook significant work which led to the development of clinical devices for dental practice based on LF. He varied the excitation wavelength over a broad range, and initially reported promising results with 406-488 nm excitation, since the fluorescence emissions were very high, and the emission patterns of a healthy tooth surface and a carious region could be distinguished easily (35). However, he then rejected the notion of developing an optical device using these excitations, as it required a powerful UV-violet light source and accurate spectral analysis. However, he was able to show that the fluorescent yield of a healthy surface decreases much more than the carious region, as excitation wavelength increases in the red spectral region (Fig 2) (35, 36).

This finding led to the development of the first dental chairside LF device by KaVo (Biberach, Germany) in 1999, called the DIAGNOdent. This utilizes an In:Ga:As:P diode laser emitting at 655 nm to detect occlusal caries.

Modern LF devices such as the DIAGNOdent typically irradiate targets with coherent and monochromatic laser light in the 600-700 nm range (e.g. red light at 655 nm), and then collect fluorescence radiation (36-40). A high-pass filter removes reflected light and ambient light (from daylight and operator lighting), such that only near infrared light (>680 nm) will pass (41). The longer wavelength fluorescence radiation is then evaluated using a detector. The intensity of the fluorescent radiation is presented to the operator as a digital value (on a 0-99 scale). As well as the DIAGNOdent “Classic” system, the DIAGNOdent Pen (Fig 4) and the In:Ga:As:P diode laser part of the KEY3 system (KaVo, Biberach, Germany) (Fig 4) also use the same LF diagnostic approach and use identical laser illuminators, detectors, and processing software.

Many studies have claimed that the DIAGNOdent LF method outperforms conventional approaches for detection of caries (6, 42-48). This improved sensitivity and specificity is thought to be due to the fluorescence characteristics of porphyrin derivatives which act as key fluorophores within dental caries (49-52).

<table>
<thead>
<tr>
<th>Fluorophores</th>
<th>Excitation(nm)</th>
<th>Emission(nm)</th>
<th>Calculus</th>
<th>Dentine</th>
<th>Caries</th>
<th>Plaque</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen (I,III,V)</td>
<td>300-340</td>
<td>420-460</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Copper</td>
<td>UV light</td>
<td>Orange-red</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Coproporphyrin</td>
<td>398,497,531,565,620</td>
<td>623,690</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cortisol</td>
<td>475</td>
<td>525</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cysteine</td>
<td>200,349</td>
<td>300,707</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Flavin</td>
<td>260,370,450</td>
<td>530</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NADH</td>
<td>260,340</td>
<td>470</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NADHP</td>
<td>260,340</td>
<td>470</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>257</td>
<td>282</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Protoporphyrin</td>
<td>406,505,540,575,630</td>
<td>633,700</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>220,280,288</td>
<td>320-350</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>220,275</td>
<td>305</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Uroporphyrin</td>
<td>404,501,533,568,622</td>
<td>624</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Water</td>
<td>308</td>
<td>344,430</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Zn-coproporphyrin</td>
<td>411,539,575</td>
<td>580</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Zn-protoporphyrin</td>
<td>421,548,585</td>
<td>593,646</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Apatite</td>
<td>UVC</td>
<td>Bright pink</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td>UVA and Blue</td>
<td>Bluish green and yellow</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 1. Fluorophores and their associated excitation-emission wavelengths for dental tissues.
Porphyrin derivatives, metabolite of bacterial byproducts, are also present within dental plaque and calculus therefore LF devices can be used to detect bacterial biofilms and dental calculus similar to the carious lesions (12, 18).

**Laser fluorescence in endodontics:** Laser fluorescence is an interesting diagnostic application of lasers that has potential for use in endodontics for detection of bacteria in the root canal, based on the fluorescence emissions of bacteria and their byproducts or metabolites. According to Hibst et al. (53), the main component responsible for fluorescence in dental caries and dental calculus is porphyrin derivatives.

When a fluorophore within a sample is exited, fluorescence will be emitted in all directions, and the intensity of fluorescence will be directly proportional to the intensity of the excitation source (54). Pini et al. (55) used laser fluorescence to detect residual pulp tissue within the root canal, using a 308 nm wavelength ultraviolet laser, while Sarkissian and Le (16) used 366, 405, and 440 nm wavelengths to distinguish remaining pulp tissue and bacteria from normal hard tissue in root canals.

Most work using fluorescence in dentistry has employed visible light as the excitation source. In 1993, Koenig et al. (56) used 407 nm wavelengths to detect various types of bacteria in culture using fluorescence. Subsequently, Hibst et al. (57) used the 655 nm wavelength for detection of dental plaque and dental caries, and this led to the development of the DIAGNOdent, a device which has become a widely used chair side diagnostic device in dental practice since 2000. Lussi et al. (58) later reported the use of a modified sapphire tip with the DIAGNOdent, for detection of proximal caries.

The DIAGNOdent assesses near infrared emissions from porphyrins and other molecules of bacterial origin. Recently, Sainsbury (59) has reported that the DIAGNOdent system could be adapted for the detection of bacteria in the pulp chamber and root canal. He identified that healthy dental pulp soft tissues and healthy dentine give minimal infrared emissions, whilst strong emissions occurred from canals which had been infected with bacteria either *in vivo* or *in vitro*.

Recently modified tips with a honeycomb fiber design had been reported by R. George and Walsh (REF). This honeycomb fiber is expected to be better for detection of fluorescence within the root canal when compared to a conical tip, because it emits and collects light from both lateral and forward directions (Fig. 5). Its higher lateral emissions should give a stronger signal from bacteria on the root canal walls, given that the intensity of fluorescence is directly proportional to the intensity of the irradiating light. Krause et al (60) reported that LED optical probes used to detect subgingival calculus need to be as perpendicular as possible to the surface, to increase sensitivity and specificity, however it would be difficult to achieve such angles in clinical settings.
and thus fibers such as the honeycomb fibers could be better suited for such a purpose.

The final issue worthy of comment is that of the "shelf life" of the modified fibers. No damage to tips would be expected with low intensity applications such as photodynamic therapy, disinfection, or detection. It has been found in practical usage in endodontics that when used at higher laser pulse energies (i.e., for ablation), conical tips sustain minor damage at the distal 0.5 mm of their terminus over 10 cycles of use, which does not appear to affect their optical performance. Re-etching for 5 minutes restores the geometry of the fiber tip. While little is known regarding the level of damage which may be sustained to honeycomb fibers, the point is of little practical importance since in clinical practice it would be appropriate to regard all such fiber tips as disposable (single patient use) items because of infection control concerns. Specific concerns are the inabilty of current medical instrument reprocessing methods to remove contamination at the cladding-fiber junction, and the risk of damage to the fibers during ultrasonic cleaning. Some commercial laser systems used for endodontics already employ single patient use optical fiber tips for these reasons (e.g., Biolase).

Fig. 5 Distribution of light in excitation and fluorescence.

Conclusion

The laser-induced Fluorescence is a great potential method for diagnosis in dentistry to be used widely beyond academic research centres in restorative works, periodontology and endodontics. There is no doubt that the LF technology may in near future replace conventional diagnostic tools. Therefore further studies are needed to accelerate the emergence of this innovative technology as they can provide the groundwork for advance exploration of principles that could be applied to improve LF systems and devices for clinical application.

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