The ultraviolet (UV) spectrum is traditionally divided into three UV bands: UV A (315-400 nm, also termed black light, long wave or near UV), UVB (280-315 nm, also termed middle UV - responsible for the sunburn response), and UVC (200-280 nm, also termed short wave UV). Having the lowest photon energy of the three ultraviolet wavebands, UV A has little effect on microbial pathogens and virtually no effect on human tissue with short-term exposures.

UVA is the important band in terms of diagnostic applications in dentistry. The ability of UVA to make biological materials fluoresce is well known in medicine and dentistry as well as in industry. For example, UVA sources have application for identifying materials such as dyes, inks, minerals, chemical and various biological materials (such as blood, when used in forensic science). Light in the visible violet and UV A wave bands can also initiate chemical reactions which contribute to the photopolymerization of some dental composite materials, as well as adhesives, coatings, and resins used in fields other than dentistry.

**A primer on fluorescence**

The process of fluorescence is of particular interest for diagnostic applications in dentistry as well as for other areas of health care. Upon absorbing UVA light, certain molecules (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. At low concentrations, the emission intensity is linearly proportional to the concentration of the molecule present in the target tissue. Because of this feature, molecular fluorescence is very useful for quantification.

**Ultraviolet-induced fluorescence: shedding new light on dental biofilms and dental caries**

By Professor Laurence J. Walsh and Fardad Shakibaie

“The ability of UVA to make biological materials fluoresce is well known in medicine and dentistry as well as in industry...”

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. Many naturally occurring substances fluoresce, including minerals, fungi, bacteria, keratin, collagens and other components of body tissue; this is termed primary fluorescence or autofluorescence.

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 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. This relationship is known as Stokes’ law, named after Sir George Stokes, who published the first significant paper on fluorescence in 1852.
Fluorophores are excited by a range of wavelengths, and also emit over a broad range. Thus, for any fluorophore there will be some overlap between its absorption (excitation) spectrum and its emission spectrum. The difference between the absorption maximum and the emission maximum is known as the Stokes’ shift.

Light sources used for fluorescence must produce light within the absorption region of the fluorophore of interest, at sufficient intensity. Until recently, the types of UVA light sources used included nitrogen lasers, helium cadmium lasers, high pressure mercury lamps, high pressure xenon lamps, and metal-halide arc lamps. These light sources are large, expensive, and have limited lifespans. With advances in LED technology, UVA emitting LEDs have now been developed. Common UVA wavelengths in commercially available LEDs are: 375, 385, 395 and 405 nm. UVA LEDs are small, long lasting and very reliable, by comparison with other light sources. Examples of dual wavelength dental curing lights with visible blue LEDs and UVA LEDs are the Ultradent UltraLume 5™ and the GC G-Light™. UV A LEDs have also been incorporated into dental imaging systems and examples of this include the Durr Vistaproof™ and the Morita Penscope™. The LEDs used in such devices are spectrally broad and also produce some faint light in the visible violet range (400-440 nm).

To observe fluorescence, optical filters are used which pass the fluorescence wavelengths but not the excitation wavelengths. These filters can range from coloured glass or polymers (such as coloured spectacles) through to the more expensive interference filters made by depositing layer upon layer of dielectric materials onto a glass surface, each of which has different refractive indices. Constructive and destructive interference occurs with different wavelengths of light, causing some to be transmitted through and others reflected back (rejected). For instruments which measure fluorescence, interference filters are typically used.

**Plaque and calculus detection**

UVA light emits visible red fluorescence from deposits of mature dental plaque on the surface of teeth, restorations, or dental appliances. This has been studied since the seminal work of Bommer and Benedict in the mid-1920’s, who first reported the characteristic red fluorescence from dental plaque and dental calculus respectively, when using UVA light sources. The fluorescence arises because of the presence of porphyrin compound, particularly protoporphyrin-IX (PP9), in bacteria. PP9 and similar porphyrin molecules are derivatives of haemoglobin and are involved in the biosynthetic pathway for heme. Because PP9 is found in high amounts in Gram negative oral bacteria, and the levels of Gram negative bacteria increase as the dental plaque biofilm becomes more mature, red fluorescence is associated with mature dental plaque on teeth as well as on appliances such as dentures. Laboratory studies have shown that Actinomyces odontolyticus (found in dentine carious lesions), Bacteroides intermedius, Corynebacterium spp. and Candida albicans all emit at 620-635 nm and 700 nm when excited by 407 nm UVA light, while the Gram positive Streptococcus mutans, Enterococcus faecalis and various lactobacilli are weaker or negative for porphyrin fluorescence in the red spectral region.

Thus, the maturity of dental plaque, rather than the presence of cariogenic streptococci, is the basis for the red fluorescence which occurs with UVA light. The emission of the red light from dental plaque corresponds with known emission peaks of UVA-excited PP9 (Figure 1). As well, the rapid decay of fluorescence once the incoming UVA light is ceased confirms that the process is PP9 fluorescence rather than phosphorescence. PP9 is also excited by visible red light (655 nm) giving near-infrared emissions. This longer wavelength is used in the KaVo DIAGNOdent™ system, which measures fluorescence quantitatively.

In the mid-1920’s, Bommer detected an orange and red fluorescence of dental...
plaque in patients using a UVA light source (Wood’s lamp). Today, this process is fully understood at the molecular level. In short, when dental plaque or calculus is present, there is an increase in the absorption in the UVA spectral region at 350-420 nm, with the appearance of a fluorescence signal in the visible red spectral region at 590-650 nm. Using a polarizing element (to reduce reflection and scatter) and a long pass orange-red filter, these emissions can be seen with the naked eye, and can also be recorded with CCD or CMOS sensors in imaging systems.

Following professional prophylaxis, residual deposits of plaque and calculus appear as red fluorescing areas. As well as the obvious diagnostic benefits to clinical operators, UVA-induced fluorescence can be used to educate patients and to assist in oral hygiene instruction, since it is not necessary to use disclosing dyes (Figures 2 and 3).

If one wishes to use a dye, UVA is a powerful inducer of bright yellow fluorescence in sodium fluorescein or fluorescein diacetate. These dyes can be applied topically in the mouth or to dental appliances either intra- or extra- orally, and following rinsing the location of the retained dye can be used to assist in oral hygiene education. UVA can excite other dyes such as the red-maroon compounds rhodamine B and rhodamine 123, giving visible yellow emissions.

**Mineral loss and dysmineralization**

Prior to the first World War, Stubel in 1911 investigated the fluorescent characteristics of various biological tissues when irradiated by ultraviolet light and found that teeth brilliantly fluoresce an intense blue-green colour. Later work showed that the organic (protein) components of tooth structure were responsible for this, rather than the mineral components, with the amino acid tryptophan attracting attention as a natural fluorophore of sound dentine.

With excitation at 375 nm, emission peaks for human enamel occur at 460 (blue) and 560 nm (green). Because of this, areas of mineral loss are readily apparent because of their reduced positive signal. Thus, under ultraviolet light, enamel with white spot lesions is darker compared to the adjacent luminescent sound enamel. The same method will identify dysmineralization defects which occur during tooth formation, as well as dental fluorosis, which can have a texture and colour similar to those of initial caries lesions but another shape and location.

**Dental caries**

In white spot lesions, PP9 is trapped in small surface porosities and can be detected because of its fluorescence properties. A recent large scale clinical trial of this using the DIAGNOdent (which has a detection limit of 1 picomole) showed detectable fluorescence in buccal white spot lesions on deciduous teeth.

Similarly, once the overlying plaque is removed, dyes applied to the tooth surface will be retained in areas of porous enamel affected by early caries, assisting the identification of these areas by fluorescence, e.g. using sodium fluorescein.

UVA-induced fluorescence can detect more demineralized pre-cavitated enamel areas (white spot lesions) than a conventional visual examination. A significant decrease in the intensity of the fluorescence signal occurs in both demineralized teeth and in teeth with dentine carious lesions. For wavelengths from 400 to 420 nm, carious lesions with cavitations in dentine containing bacteria showed emissions at 600-700 nm typical for porphyrin compounds.

**Clinical applications**

The Durr Vistaproof system uses six LEDs emitting at 405 nm and is the ideal wavelength in the UVA waveband for revealing...
PP9 fluorescence. The image sensor is a 6mm CCD, and the camera optics are shrouded to excluded ambient light when examining a tooth, thus increasing the signal to noise ratio. The shroud also acts as a spacer, giving a constant distance (Figure 4). Captured images from the camera unit are ported back to a computer via a USB 2.0 interface for analysis (Figure 5). The camera is powered from its own source, not via the USB interface, thereby making it suitable for use with laptop computers as well as desktops. The DBSWIN analysis software highlights the intensity of the red signal from PP9, providing a numerical score that can assist treatment decisions, in much the same way as has been done with QLF (Inspector™) and DIAGNOdent. Toggling between view modes, and freezing images is available on this article for subscribers

### Table 1. UVA excitation and emission interactions

<table>
<thead>
<tr>
<th></th>
<th>Excitation</th>
<th>Emission</th>
<th>Colour of emission</th>
</tr>
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<tbody>
<tr>
<td><strong>Sound tooth structure</strong></td>
<td>337 nm</td>
<td>430-450 nm, 480-500 nm</td>
<td>Light blue</td>
</tr>
<tr>
<td></td>
<td>375 nm</td>
<td>460 nm, 560 nm</td>
<td>Light blue</td>
</tr>
<tr>
<td><strong>Bacteria (mature plaque, dental caries)</strong></td>
<td>320-380 nm, 407 nm, 360-580 nm, 655 nm</td>
<td>590-650 nm, 635 nm, 600-700 nm, 720-800 nm</td>
<td>Red, Red, Red (Infrared)</td>
</tr>
<tr>
<td><strong>Supra and subgingival calculus</strong></td>
<td>420 nm</td>
<td>595 nm, 635 nm, 650 nm, 695 nm</td>
<td>Red, Red, Deep Red</td>
</tr>
<tr>
<td><strong>Protoporphyrin IX</strong></td>
<td>407 nm</td>
<td>590 nm, 620 nm, 635 nm</td>
<td>Red, Red, Red</td>
</tr>
<tr>
<td><strong>Sodium fluorescein</strong></td>
<td>400-465 nm</td>
<td>520-530 nm</td>
<td>Green</td>
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</table>

References


### Table 2. Some dental applications of UVA light

- Detect mineral loss (white spot caries, dental erosion);
- Detect dysmineralization (developmental lesions, fluorosis);
- Detect carious lesions which involve the DEJ or dentine;
- Check caries removal during excavation;
- Demonstrate plaque and calculus during patient education;
- Visualize plaque and calculus remaining after debridement; and
- Reveal bound or trapped marker dyes (porosity/leakage).

About the author

Professor Laurence J. Walsh is the technology editor of Australasian Dental Practice magazine. He is also a noted commentator on and user of new technologies and is the Head of The University of Queensland School of Dentistry.

Dr Shakibaie is a PhD student in the QU School of Dentistry working in the field of biophotonics. A dental graduate of the University of Queensland, he completed his MPhil in 2006 working on laser-induced fluorescence for diagnostic applications.