IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 15, Issue 3 Ver. IX (Mar. 2016), PP 65-75
www.iosrjournals.org

Fluorescence And Dentistry

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Abstract: *In natural dentitions, fluorescence is a function of organic components of the enamel and mostly the dentin part, while in dental restorative materials (composite, ceramic, acrylic); fluorescence is a function of the illuminants or fluorescent materials that mostly are rare earth elements like: europium, cerium and ytterbium oxides, these fluorescent additives incorporated in the composite materials to increase the esthetic qualities of dental restorations. As a result, the dental materials used by the dentist had to fluoresce at different light sources (daylight or fluorescent lamp light) in a manner similar to that of natural dentitions otherwise dental restorations will appear dull, non-vital and easy to point out from the natural teeth structure.*
Keyword: *emission, fluorescence, organic matrix, spectrophotometer, LIFDT.*

I. Introduction

Composite restorations became the restorative material that mostly demanded in practice for both anterior and posterior restorative work, so that put many requirements on dental composite materials to fill. Anterior composite restorations that gave esthetic satisfaction to both dentist and patient must acquire as much optical properties as natural dentition; color, metamerism, opalescence, fluorescence, all these physical optical properties are mandatory to consider.

II. Fluorescence Definition:

Fluorescence by definition is the absorption of light by a substance and its emission at the same time at a longer wavelength. Such a substance emits more visible light than it receives, making it appear brighter than a non-fluorescent substance which, at best, can only reflect the visible light that is falling on it (1)

Fluorescence (flәres´әns): the emission of radiation of a particular wavelength by certain substances as the result of absorption of radiation of a shorter wavelength (2).

III. Physical Principle Of Fluorescence:

Fluorescence occurred when a molecular system absorbed some type of energy (an electron absorbed energy) and got excited to a higher quantum level then after a lag period, the excited electron lost its energy as photons and the electron relaxed back to a lower quantum level, so the emitted photons had lower energy than the absorbed energy, this phenomenon caused molecules to fluoresce.

Fluorescence occurred when an orbital electron of a molecule, atom or nanostructure relaxed to its ground state by emitting a photon of light after being excited to a higher energy level by a type of energy (3).

Excitation: $S0+hv_{ex} \rightarrow S1$ ….. equation 1

Fluorescence (emission): $S1 \rightarrow S0+hv_{em}+heat$ …. Equation 2

Hv: is the photon energy with h: known as planks constant and V: light frequency. The frequency of light depended on the excited system. State S0 referred to the ground energy level of the fluorophore's molecule where S1 referred as its first excited level.

Quantum yield: The fluorescence quantum yield gave the efficiency of the fluorescence phenomenon. Quantum yield defined as the ratio of the photons number which emitted to the photons number which absorbed.

$$\Phi = \frac{\text{Number of photons emitted}}{\text{Number of photons absorbed}}$$ ….. equation 3

The highest fluorescence quantum yield is 1.0 (100%) when each photon absorbed resulted in a photon emitted (4).

The Jablonski diagram at Figure (1) described most of the mechanisms of relaxation for excited state molecules. This diagram showed how fluorescence occurred after the relaxation of excited electrons of a molecule (3).

Jablonski diagram, after an electron absorbed a high energy photon the system was excited electronically and vibrationally and then the system relaxed vibrationally, and eventually fluoresced at a longer wavelength.

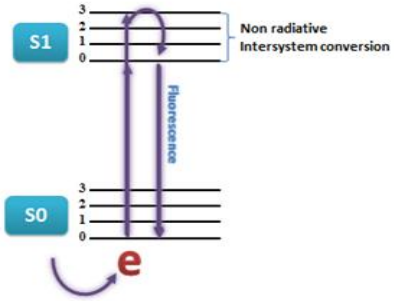

Fig (1): Jablonski diagram.

IV. Fluorophores

This term used to describe certain components or pigments that gave a neon color like illusion upon excitation of their molecules, those pigments referred as to fluoresce by Ralph M. Evans; scientists believed that fluorescence was because of the color property and the resultant fluorescence belonged to white component of the color, until they found out that the fluorescence occurred due to the energy shift of certain molecules.

Fluorophores presented with abundance in nature (fishes, insects, flowers, minerals, gemstones, etc.), as examples of fluorophores in nature: many types of calcite and amber would fluoresce under longwave UV shortwave UV, and visible light spectrum. Rubies, emeralds, and diamonds exhibited red fluorescence under long-wave UV, blue and also emitted green light on some occasions; diamonds had the property of emitting light under X-ray radiation (4).Other materials like vitamin B12 gave yellow fluorescence, highlighter ink also fluoresced because of pyranine which used as fluorophore, fluorophores nowadays used as dyes or markers for biomedical agents, nucleic acids or enzymes substrates which traced down by spectroscopes or X-Ray devices.

Luminescence: this term described the emission of radiation, which combined two phenomena fluorescence (short termed) and phosphorescence (long termed), in addition to bioluminescence which occurred in living organisms by which the chemical interactions produced light. The difference between fluorescence and phosphorescence that in fluorescence, energy levels transitions did not have changes in electron spin, while in phosphorescence, an electron span through the intersystem crossing from the excited state (S1) to another excited state just below S1 (5). In addition to that fluorescence emissions remained only as long as the stimulating radiation was continued, on the other hand, phosphorescence process, which had an "afterglow" after the stimulating radiation turned off (6).

V. Fluorescence In Dentistry

Fluorescence and natural dentitions:

In 1911, Stubel1 used UV light to examine rabbits teeth and observed that teeth gave an intense blue color in matter of second to the eye, in 1924 policard mentioned the difference in fluorescence between carious and sound teeth; in 1928, Benidect mentioned that dentin fluoresced much more than enamel and cementum showed fluorescence similar to dentin but still with less intensity, Benidect also reported that carious dental tissues wither it was enamel or dentin eventually they had been losing their fluorescence and appeared black or dark brown under UV light illumination.

Benidect also extracted the organic components of dentin using nitric acid and found their strong fluorescence, on the contrary when he extracted the inorganic components of dentin by sodium hydroxide's addition he observed that they did not fluoresce, Benidect also suggested that the white spot lesions were the beginning of carious process because white spot lesions did not fluoresce either and added that fluorescence of dentin increased with age. In 1953, Hartles and Leaver further examined the fluorescence of different tooth structures and reported that enamel exhibited "normal" fluorescence with a bluish white appearance, occasionally tinged with yellow (7).

In 1963 Armstrong investigated the autofluorescence characteristic of different dental tissues samples, since 1980 many researchers tried to discriminate between carious and sound tooth structures using their fluorescing properties, Albin, Alfano and Buchala all studied the fluorescence behavior of sound and carious dental tissues by applying various UV light emission wavelengths and each reported fluorescent peaks and excitation range (5).

Fluorometric investigations revealed that collagen crosslinked with the hydroxyapatite considered as the main fluorescing compound within the dental tissue. When the organic structure of a tooth was affected by(for example: caries or pulpal necrosis) the tooth lost its vitality under both natural and UV light and appeared dark brown. Therefore, less fluorescence correlated with lesser value (8).

According to (9), fluorescence didn't have significant difference between the maxillary and mandibular teeth nor gender. All teeth in the same individual showed no difference in fluorescence intensity. With aging

odontoblast lie down more collagen matrix and the thickness of dentin icreased while enamel was undergoing wear and abrasion process and also became more mineralized with time, as aresult fluorescence of teeth increased as people advanced in age (10).

Fluorescence and dental materials:

In restorative dentistry, esthetic outcomes became the ultimate purpose in making any treatment plan, however fluorescence was neglected so far in dentistry and dental materials as an effective optical property, although it was fluorescence that provided the vital look and hided the metameric difference between the restoration and natural dentition no matter what light condition used and especially at dark lightening conditions. Normal dentitions had their autofluorescence property due to mainly dentin that contained amino acids as tryptophan that found at the collagen fibers substrates which made dentin fluoresce three times more than enamel. In the meanwhile composite resins fluoresced because of the luminescent (fluorophores) incorporated in, luminescent elements such as europium, cerium, and ytterbium (rare earths) oxides. Composite fluorescence meant to be very similar to that of tooth structure was obtained through the elements belonging to groups (III, IV, and V) in the periodic table. This fluorescence, however, presented as highly dependent on the type of the material which they were incorporated in. Uranium oxide used as a fluorescent for years, but its use abandoned because it released radiations (7).

Natural teeth had peaks in fluorescence in the range of wavelength (410-500nm), dental composites manufacturers nowadays claimed that the fluorophores they used, meant to allow composite restorations had the same fluorescence peaks as natural teeth giving whitish blue color in wavelength range between 410-500nm which favored the masking of restorations and consequently, the achievement of unnoticed restorations under UV illumination. Fluorescence of dental composites was more achieved within the last layer of composite restorations because unlike the natural dentitions the enamel layers of composite fluoresced much higher than the dentin or the opaque layers of composites so at the layering techniques of composite, higher fluorescence lied within the last layer of composite or by the combination of dentin and enamel layers if intermediate fluorescence values seeked (12).

VI. Application Of Fluorescence In Dentistry

Fluorescence concept used widely in dentistry for various applications in restorative, endodontic dentistry also in oral surgery and histopathologic investigation, most of these applications happened to be of diagnostic nature (13).

Fluorescence in dental researches:

Fluorescing agents long ago used in different fields of dental research for nearly 50 years to investigate a wide variety of issues as microleakage, characteristics and structures of the bonded restoration, adaptation of bonded restorations to the prepared walls, hybrid layer, or the morphology among different types of restorative materials at tooth restoration interface.The use of fluorescent dyes in microscopy considered as efficient investigative tool. These compounds traced down the path or at their locations since they had the capability of being detected at dilute concentration (13).

As an improvement over SEM techniques, confocal microscopy imaging offered the ability to visualize distinct components of bonding systems adding fluorescent tracers, assisting to determine which component was responsible for the formation of resin tags and the hybrid layer. Fluorescent confocal microscopy that used for the analysis of the interface of restorations and teeth structures was first analyzed by Watson and Boyde. These authors advocated the use of fluorescent dyes by mixing them with the adhesive systems to make the bonded interface highlighted and so on this technique used usually by researchers but following widely varying methodologies. Confocal microscopy had also been used to evaluate the hybrid layer micropermeability by incorporating fluorochromes in to the pulp chamber (14).

The sealing ability of the bonding systems also could be evaluated using fluorescent molecules permeation into microporosities from the pulpal direction. Cavosurface margin (microleakage to external fluids) assessed by fluorochromes using conventional light microscopy at lower magnification (15).Rhodamine B mostly used as fluorochrome for these different applications. Rhodamine B excited using green light (540 nm) and emitted red in return (590 nm). Rhodamine B was effective in very low concentration, fairly labile, moved freely across the bonded interface, and easily detected microscopically with appropriate filters. Another fluorochrome, sodium Fluorescein used in combination with conventional light and confocal microscopy (13).

According to (12) fluorescing agents addition methodologies were not consistent and still needed standardization and when fluorescent dyes added, their exact concentrations should be determined upon addition, these dyes might interfered with the resin polymerization, also the strength of bonded restorations might be jeopardized, so more studies recommended regarding the application of fluorochromes in dental researches.

Examples of fluorescence applications in dental diagnostics:

1) Diagnodent.
2) Quantitative light induced fluorescence (QLF).
3) Spectra.
4) FACE (fluorescent aided carious excavation):

A) SIROInspect.
B) Facelight.

5) LIFEDT—Light-Induced Fluorescence Evaluator for Diagnosis and Treatment:

A) SOPROLIFE.
B) SOPROCARE.

Diagnodent:

In the very first utilization of the fluorescence phenomenon in dental diagnosis, Angmar- Mansson at 1980 used argon laser for examining tooth structure and found that the coherent fluorescent light caused a yellow fluorescence of appetites while less fluorescence concentration noticed in the demineralized area. With time, argon laser which was expensive and complex to use replaced by diode laser which was cheaper and simple to use, Diagnodent released by KaVo at 1999 using diode laser (655nm wavelength) as the first commercial tool who took advantage of fluorescence in dental diagnosis (16).

Diagnodent concept:

Diagnodent operated by sending light with 655nm via descending optic fibers and collected backscattered light through the tip to the ascending optic fibers then transmitted to the filter where excitation light and short wavelength light smothered and fluorescent light sent to photo diode detector, the signals finally displayed on a scale from 0-99 as shown in Figure (2) (17).

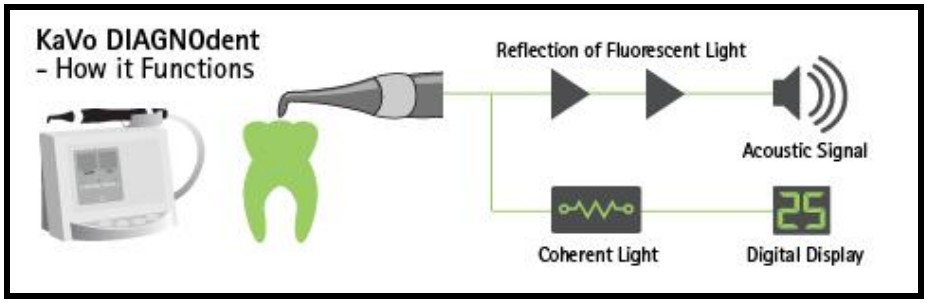

Figure (2) Diagnodent diagram (KaVo dental, 2015).

Clean healthy teeth structures gave low readings on the other hand carious lesions gave high readings. Porphyrins contents and bacterial metabolites in carious lesions considered as the reason behind high fluorescence readings (KaVo dental, 2001), these readings might determine the route of therapy as in Table (1).

Table 1: Therapeutic measures information according to KaVo Diagnodent

0 – 14	No special measures.
15 – 20	Usual prophylactic measures.
21 – 30	More intensive prophylaxis or restoration: indication is dependent on: *Caries activity. *Caries risk. * Recall interval, etc.
from 30	Restoration and more intensive prophylaxis.

Quantitative induced light fluorescence (QLF):

QLF device Figure (3) used for diagnostic purposes in monitoring dental caries, bacterial activity, stains, plaque, calculus and mineral content of teeth including white spot lesions; for either dental researches or clinical applications (18).

Figure (3) Quantitative induced light fluorescence (19).

Quantitative induced light fluorescence basic principle:

In 1978, Folke Sundstrom, a Swedish dentist found out that when illuminating teeth with a specific light spectrum in the blue region, teeth gave off a green fluorescence, based on this notification, QLF was built since sound teeth produced green fluorescent light also carious lesions gave less intense green fluorescence because carious lesion structure scattered the absorbed light more often than sound teeth structure which giving what was so called autofluorescence image of teeth (17).

According to (18) QLF showed rates of accuracy and specifity higher than visual and tactile diagnostic methods, (there are about two to tenfold more lesions detected comparing to visual, visual-tactile examination or visual-radiographs, depending on surface-type).

Quantitative induced light fluorescence device hardware:

Quantitative induced light fluorescence consisted mainly of intraoral camera connected to a computer where images analyzed by the system software. Special xenon arc-lamp used as light source. Dental mirror helped to illuminate areas uniformly as in Figure (4). Yellow-transmitting filter placed in front of a color CCD-sensor caused the video image completely reflections free (18).

Figure (4) QLF device with mirror (18).

Spectra:

Spectra concept (AIR TECHNIQUES) based on porphyrins. Cariogenic bacteria Streptococcus mutans produced special metabolites called porphyrins. These porphyrins fluoresced at 405-nm wavelength light that's when Spectra device detected their fluorescence and allowed their visualization. The red fluorescent signal would be more intense if the bacterial film was thicker. Spectra manufacturer claimed to be different from Diagnodent in that Diagnodent depended on the amount of light penetrated through enamel and dentin while Spectra depended on the presence of caries related porphyrins, an additional advantage of Spectra claimed by the manufacturer was capable of detecting caries adjacent to amalgam restoration which usually difficult for Diagnodent to detect because its emitted light would be reflected by the restoration ending in giving false results while this concern eliminated for Spectra system (20).

Spectra design:

Spectra looked like intra oral camera provided with white LEDs surrounding the lens emitting a 405 nm blue-violet light and rubber spacer as in Figure (5), Spectra connected to a computer via a USB connector and was operated by specific software (20). Different fluorescent reflections indicated different depths of involvement as referred in Table (2)

Figure (5) Spectra camera with spacer on (Kurtzman, 2010).

Table 2: Interpretation of Spectra data (Kurtzman, 2010).

Displayed Color	GREEN → BLUE → RED → ORANGE → YELLOW				
Displayed Number	1 → 5				
Depth of Involvement	Sound Enamel	Initial Enamel Caries	Deep Enamel Caries	Initial Dentin Caries	Deep Dentin Caries

FACE (Fluorescence Aided Caries Excavation) concept:

A clinical adjunctive tool in caries detection, healthy dental tissue exposed to a light spectrum around 405 nm fluoresced as green while carious areas were visibly red which due to porphyrin compounds arose during the breakdown of hard dental substance by cariogenic bacteria. Tools which used FACE concept usually consisted basically of handheld probe and goggles (21).

Tools used FACE:

a) SIROInspect (Sirona): Sirona focused on the handling of SIROInspect Figure (6). According to Sirona, dentists only had to do during the treatment was turning on the probe, putting on the diagnostic glasses and the check-up was ready to start. There were also two sterilizable, small-diameter light guides that enhanced the illumination of preparation site and good accessibility (22).

Figure (6) SIROInspect (22).

b) Facelight: by (W&H): Facelight Figure (7) used to illuminate an exposed cavity with violet light (wavelength405 nm), bacterial porphyrins which would show a red fluorescence indicating the areas for caries excavation (23). Facelight base body consisted of :

> Battery.
> Light rod 90°.
> Charging station.
> Power supply unit.
> Diagnostic goggles.
> Filter disc.
> O-ring.

Figure: (7) Facelight (23).

Caries excavation using FACE concept step-by-step:

1) Cavity preparation: in order to have an unlimited access to carious dentin, complete removal of the carious enamel strongly recommended as in traditional caries removal procedure.
2) Diagnosis of the caries: caries state checked after being exposed by illumination with the FACE utilized hand probe (carious areas had red fluorescence which should be excavated), caries removed layer by layer until green fluoresced healthy dental tissues appeared as shown in Figure (8). It would only necessary to check for the dentin hardness by a probe around the cavity margin at the end of the excavation (21).

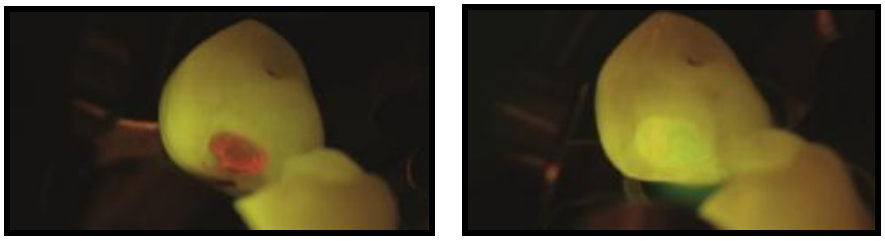

Figure (8) Photos showed cavity illumination with Facelight before and after caries excavation (21).

Some issues during excavation:

- A small amount of red-fluorescing dentin might be left in the areas close to the pulp chamber to avoid endodontic procedure so those areas near the pulp must be treated with calcium hydroxide before the restoration of the cavity.
- In other cases, the dentin at the foremost front of the spread of the carious lesion might be softened (demineralized) without the bacteria being actually penetrated that far. This bacteria-free dentin would appear green with FACE, but when checked with a probe found to be softer than normal dentin that was the reason why caries excavation should had final check with a dental probe. If the dentin at the cavity margin was still soft, it would be recommended to excavate all to hard dentin (21).

LIFEDT—Light-Induced Fluorescence Evaluator for Diagnosis and Treatment:

LIFEDT concept considered as dental therapy assisted by florescence utilized tools, (LIFEDT) based on the imaging of the difference in the autofluorescence of between healthy and carious dental tissues (24). As examples for LIFEDT tools:

1) **Soprolife: (Sopro – Acteon):**

fluorescence based camera system as in Figure (9) that utilized the principle of LIFEDT marketed as system to aid caries detection and to guide through cavity preparation. The camera captured the images in three different modes which were: daylight, diagnosis and treatment mode.

a) The day light option provided a white light image with a magnification exceeded fifty times the tooth surface. While the two other modes used the principle of autofluorescence (24).

Figure (9) SOPROLIFE camera (24).

b) In the diagnostic mode, a visible blue light wavelength (450 nm) illuminated the tooth surface, green fluorescence areas indicated healthy dental tissues; while carious lesions reflected red fluorescence, the red fluorescence might represent either deep dentinal caries or false signals coming from the organic deposits so for validation red fluorescence area should be cleaned off using sodium bicarbonate, if they were organic deposits on the tooth surface, red fluorescence would be disappeared (25).
c) The treatment mode filtered all colors from the reflection signal so soft tissues appeared in black/white scale such as gingiva, then the fluorescence signal came from the dentin amplified in color as in Figure (10). The only difference from the diagnosis mode that in treatment mode, the red wavelengths increased and the blue ones decreased (26).

Figure (10) infected dentine reflected red signals, demineralized enamel reflected in black and white on the right part of the cavity (26).

2) SOPROCARE:

The "SOPROCARE" concept by also (SOPRO – ACTEON) was of LIFEDT developed with fluorescence technology called (SOPRO patent* – 2003). SOPROCARE manufacturer claimed this product as the first product on the market to expose gingival inflammation through PERIO mode option. SOPROCARE used wavelength of light between 440 and 680 nm. (27).

SOPROCARE had three operating modes as in Figure (11):

a) The PERIO mode informed practitioners about the presence of dental plaque while simultaneously enabling them to distinguish healthy from diseased gingival tissues as shown in Figure (12).
b) In CARIO mode, the camera used to observe and show patients a warning sign of enamel and dentinal caries as in Figure (13). The other healthy tissues are represented in black and white.
c) In DAYLIGHT mode, the Macrovision allowed the visibility of the unpredictable and observing the evolution of micro lesions (27).

Figure (11) SOPROCARE device with three opening modes (SOPRO – ACTEON, 2014).

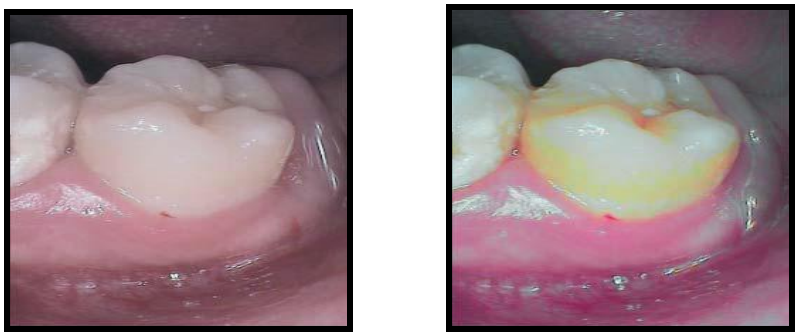

Figure (12) SOPROCARE in both daylight and perio modes. (A) Invisible plaque and inflammation in DAYLIGHT mode. (B) Plaque and inflammation PERIO mode. (28).

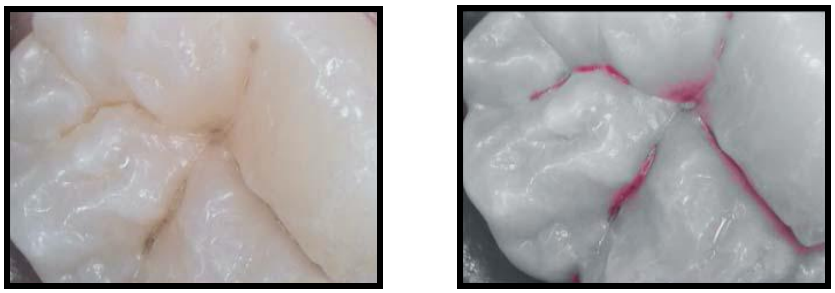

Figure (13) same surface in daylight and cario modes of SOPROCARE. (A) Carious lesion invisible in DAYLIGHT mode. (B) Carious lesion visible in CARIO mode (28).

SPOPROCARE didn't not only take the advantage of teeth autofluorescence to detect caries but also had the advantage of selective chromatic amplification using the absorption properties of the 'blue light', the selective chromatic amplification differentiated the color of the tissues. The hues of red indicated gingival inflammation and now clearly revealed by SOPROCARE (28).

VII. Fluorescence Measurement Tool (Fluorescence Spectrophotometer):

Fluorescence spectrophotometer is a tool aim to measure fluorescence of a sample using excitation light which usually an ultraviolet light causing the sample to emit light not necessarily an ultraviolet light. There were two types of fluorescence spectrophotometer: fluorimeter which isolated incident and fluorescent light using filters and spectrofluorometer which isolated incident and fluorescent light using diffracting graded monochromaters (29).

Fluorescence spectrophotometers scheme:

Fluorescence spectrophotometer which as shown in Figure (14) generally had:

a) Light source which could be laser, LED or mercury vapor but usually xenon arc used.
b) Filter or monochromater worked to adjust which wavelength to be transmitted.
c) Sample chamber and a cuvette allowed the placement of samples in 90 degree as shown in Figure (15) to eliminate the interference from the excitation light and reduced the noises. The optic component and photodetector, all connected at last to personal computer.

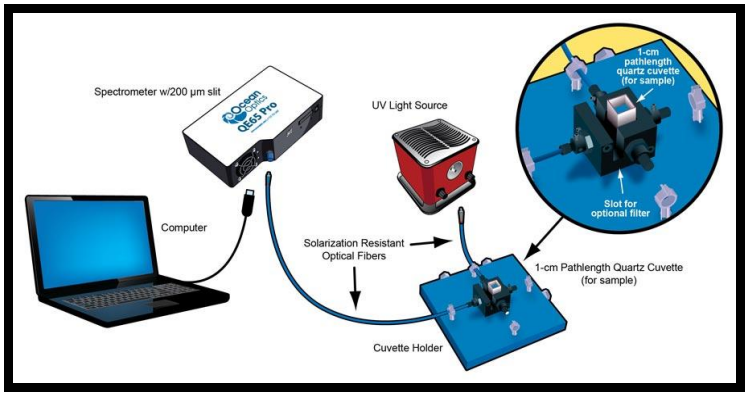

Figure (14) Fluorescence spectrophotometer scheme (29).

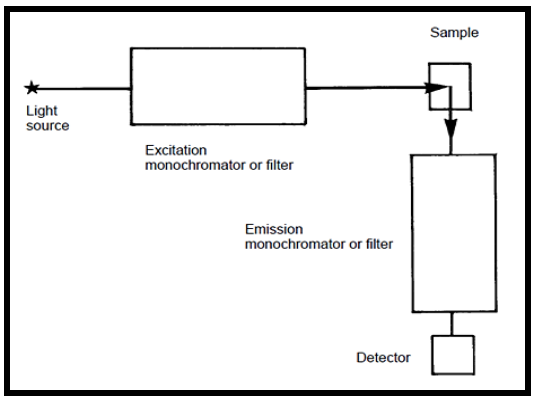

Figure (15) Essential components of fluorescence spectrometry (29).

Applications of fluorescence spectrophotometry:

Fluorescence spectroscopy utilized in various scientific aspects including biochemical, medical, and chemical research fields for analyzing organic contents. Studies about using spectrofluorometer in differentiating benign from malignant tumors were reported lately.

Atomic Fluorescence Spectroscopy (AFS) technique: used to detect specific components presented in water, air or others as example heavy metals such as mercury, fluorescence spectroscopy used as detectors also at analytical chemistry (30).

Factors that influence the analysis of data:

Factors that influenced the analysis of data by fluorescence spectrophotometer are either machine dependent or sample dependent.For machine dependent, first light source which was important to emit light with constant intensity and wavelength but this criteria considered difficult to obtain over time during the same experiment or between two experiments; as for the monochromators or filters, their light transition efficiency could be changed along with wavelengths changing or time passed and it was the same for the photodetectors which were responsible for photons catching, the percentage of photons detected varied with time and wavelength.

For sample dependent factors, the cuvette which used to hold the samples must be made of material of low light absorption values as quartz which enabled wide range of wavelengths (200-3800nm) to be transmitted with the least amount of absorption, also the photobleaching or photodecomposition of the samples which referred to the decrease in fluorescence intensity over time, another issues that might influence the data collected from spectrofluorometer were like the optics that used to direct radiations and reabsorption issues by the inner filter where some inner filter molecules reabsorbed wavelengths at which samples' fluorophores got excited, in

this case, fluorescence intensities lowered because small amount of emitted light photons didn't reach the detector system.As a result controlling the factors mentioned above were important to standardize the experiments and allowed calibrated data collection (31).

VIII. Conclusions

Fluorescence is an optical physical property to be recommended in the field of dentistry on many levels reflecting the ability of certain molecules to absorb energy and emit fluorescence in return like cariogenic bacteria by products or restorative materials. Different light conditionings contained UV light in its component and when light stroke the dental tissues it would cause fluorescence at different intensities, therefore fluorescence should be considered by dentists and clinicians.

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