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Physics for Surgeons-Part 5: Optics for Surgeons

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ABSTRACT

Optical techniques create a great impact in the biomedical field. Recent advances in the optical techniques (advances in photonics, biomaterials, genetic engineering, and nanotechnology) which are currently used in clinical practice to diagnose and treat the disease. In the present review, we highlight the fundamentals of light and its interaction with matter, applications of optics in the recent techniques so that surgeons can better understand the pattern of disease and find the best way to treat the disease.

Keywords: Fundamental of light, disease, diagnosis, Laser.

1 Introduction

Optics has affected the life of peoples by changing the approach to major health problems such as treatment of heart disease, cancer, kidney stone and eye diseases. Recent advances in biomedical optics have enable sophisticated technologies particularly in nanotechnology, biomaterials, genetic engineering integrated photonics. The use of light and optical fibre has led to less invasive ways of treating disease from open surgery to minimally invasive therapies. The use of light in the field of medicine began in 19th century with involvement of understanding of nature of light and its interaction with matter [1]. With interdisciplinary background, study of light can be divided into two areas of application in biomedical field: diagnostics or therapeutic. In diagnostics area, application of light involves development of new technologies and methods for detecting the biological state of healthy and abnormal tissue. The primary goal of this area of light is to detect the disease at its earliest stage when it is easily treated and cured well. In therapeutic area, application of light involves the treatment of disease or altering the biological process. Typically, light radiation with high energy is used to treat the disease. This high energy light radiation changes the chemical or mechanical properties of cells and tissue resulting in the desire outcome of cell death like ablative treatment of tumour. Scientist around the world continues to advance, innovate, and create new technology as well as discover new science.

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2 Interaction of Light with Matter

When light interact with matter, various phenomenon's can occurs depending upon the relationship of wavelength of light to the interacting particle size. Light radiation will reflect, refract, transmit, absorbed, or scattered (Figure 1) depending upon the transparency or translucency of the material and its surface quality. In tissue, when light interact with biological matter it passes through various processes like absorption, reflection, refraction, scattering etc.

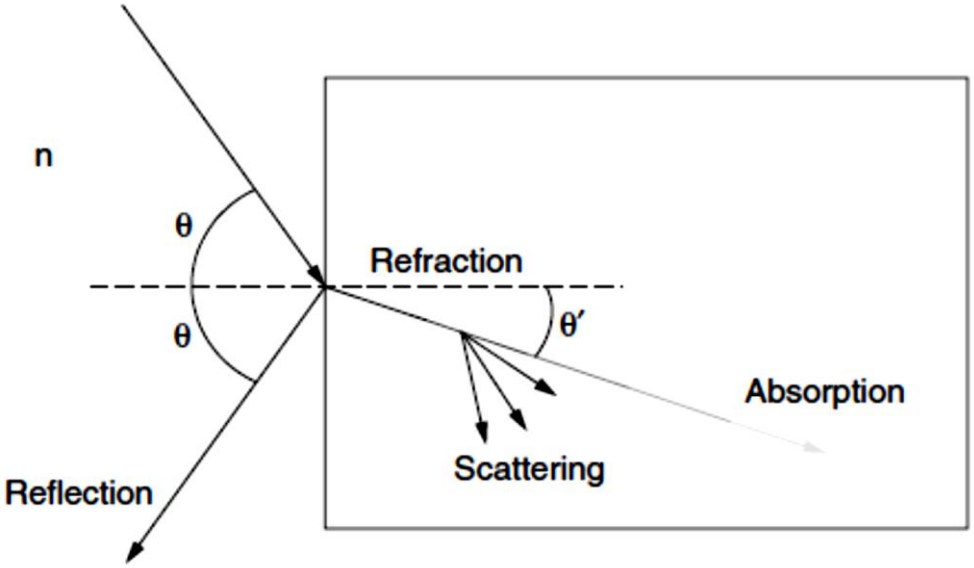

Figure1: interaction of light with matter

2.1 Absorption

Absorption is a process by which light is absorbed by the material and converted in to energy. Absorption depends on the frequency of light used and the nature of material. Therefore, absorption is directly proportional to the frequency of light used. When light is absorbed heat is generated. Absorption occurs when the frequency of photon matches with the molecules energy transition. Electron absorbed energy of light and transform it into vibrational motion. Electron interacts with the neighbouring atom to convert vibrational energy in to thermal energy. Absorption spectra can serve as a fingerprint of the molecule. Diagnostics application of absorption is in tumour detection and other physiological assessment (pulse oximetry). Therapeutic application of absorption is in laser surgery and tattoo removal [2,3]. Table 1 indicates different absorbers of light in the tissue.

Table 1: Absorber and scatterers in the tissue

Light tissue interaction	Source
Absorption	Hemoglobin, water, lipid
Raleigh Scattering	Nuclei, Mitochondria, collagen fibers
Raman Scattering	Cell cytoplasm, cell nucleus, fat, collagen, lipid, water.

2.2 Refraction

Refraction is the bending of light passing from one medium to another. This bending is caused by the change in speed experienced by the light when it travels from one medium to another medium. The amount of bending depends on the refractive index (n) of two materials. The refractive index of tissue is in the range of 1.35-1.55. Refraction follows Snell's Law which states that "ratio of sin of angle of incident to the sin of angle of reflection is equal to the ratio of phase velocity (v_1/v_2) in the two medium or equivalent to ratio of refractive indices of two media (n_1/n_2) [4]

$$\frac{sin\theta_1}{sin\theta_2} = \frac{v_1}{v_2} = \frac{n_1}{n_2} \qquad (i)$$

2.3 Scattering

Scattering is a physiological process in which light changes its direction when interact with the matter. Light scattering in tissue depends on the wavelength of light and the size of interacting particle. There are two types of scattering: Elastics scattering (no change in energy) and inelastic scattering (change in energy).

2.3.1 Elastic scattering (no energy change)

Frequency of the scattered wave = frequency of incident wave

In elastic scattering photons are mostly scattered by the atoms whose size matches with the wavelength (also called Rayleigh scattering). Elastic scattering gives the insight structure of the materials. In Rayleigh scattering photons penetrate in the medium whose particles size much smaller than the wavelength of incident light. Elastic scatterers in tissue are nuclei, mitochondria and collagen fibers [5]. Elastic scattering is effective in detecting epithelial pre- cancer [6,7] because of change in nucleus size, nucleus –cytoplasm ratio and collagen fiber density in cancer cells.

2.3.2 In-elastic Scattering (change in energy)

Frequency of scattered wave ≠ Frequency of incident wave

Frequency change occurs in the inelastic scattering. If the frequency of scattered photon decreases, then scattering is called Stokes Raman scattering and if the frequency of scattered photon increases then it is called anti-Stokes Raman scattering [8]. Inelastic scattering provides information about vibrational bonds of the molecules. Internal energy levels of the molecules and atoms are excited in the inelastic scattering [2, 3]. Raman scatterers in tissue are cell cytoplasm, cell nucleus, fat collagen, lipid and water [9].

2.4 Interaction of Light with Tissue

Optical properties of tissue were studied [10] and found that most tissue is inhomogeneous with multiple absorbers like melanin (the primary pigment in skin), oxyhemoglobin (a constituent of blood) and proteins. Wavelength of light used in medicine extends from ultraviolet to infra red of the electromagnetic spectrum. Penetration depth of blue light in the tissue is least, whereas red and infrared light penetrate more deeply (see Figure 2). Wavelength region between 600 nm to 1200nm is called the optical window of tissue [11].

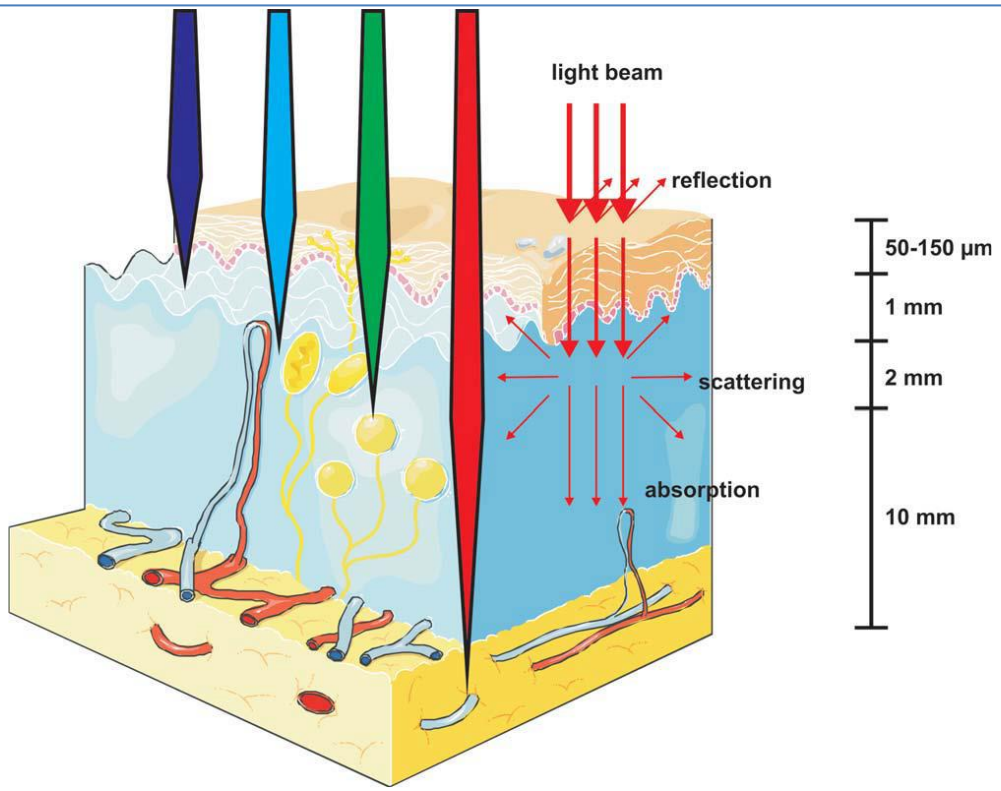

Figure 2: penetration depth of visible light in tissue.

New clinical treatments grew from deep understanding of light - tissue interaction. X-rays and gamma rays are used in the computed tomography (CT) and positron emission tomography (PET), radio waves and magnetic field used in the magnetic resonance imaging (MRI). Light in the electromagnetic spectrum comprises from deep blue to near infrared (NIR) with unique energy range 0.5-3eVand can provide distinct advantages because these energies fall in the window with safe interaction of light with organic molecules. Bond dissociation (>3.6 eV for C-C and C-H bonds) and ionization (>7eV) can occurs at higher energies. Water absorption increases at lower energy which prevents the specific targeting of molecules [1]. Application of light can be categories in to three categories: Optical diagnosis, Laser Surgery, and light activated therapies (Figure 3).

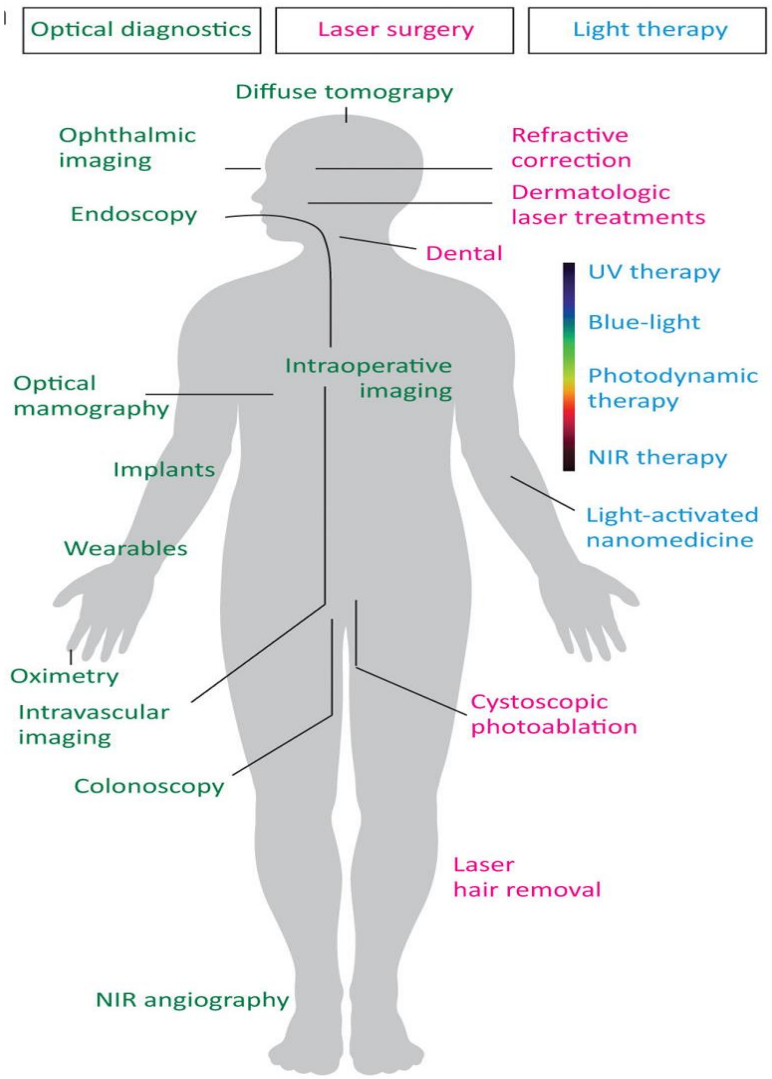

Figure 3: Application of light in different areas: Green color indicates for diagnosis and imaging, magenta color for surgery and cyanine color indicates the therapy [1].

3 Nature of Light

Light travel in a straight line. It does not need any medium to travel in the vacuum. Speed of light in vacuum is 299,792,458 metre per second. Light transverse in nature i.e; its electric and magnetic vector is perpendicular to each other and perpendicular to the direction of propagation. Basic properties of light are intensity, directionality, propagation wavelength or frequency. Light consists of packets of energy which comes from the sun in all directions. These packets are called Photons. Photons have wave as well as particle like characteristics. Each photon has certain frequency or wavelength which is related to the energy as:

$$E = h\nu = \frac{hc}{\lambda}$$

Where "h" is the Planck's constant ($6.62606 \times 10^{-34} Js$), ν is the frequency, c is the velocity of light and λ is the wavelength of photon. Shorter is the wavelength then higher

will be the frequency and greater will be the energy. Natural source of light on earth is Sun. Sun emit broad range of electromagnetic radiations (EMR). EMR is classified into different bands according to their wavelength or frequency (shown in figure 4) like radio-waves, microwaves, infrared, visible light, ultraviolet, X-rays and gamma rays which is known as electromagnetic spectrum.

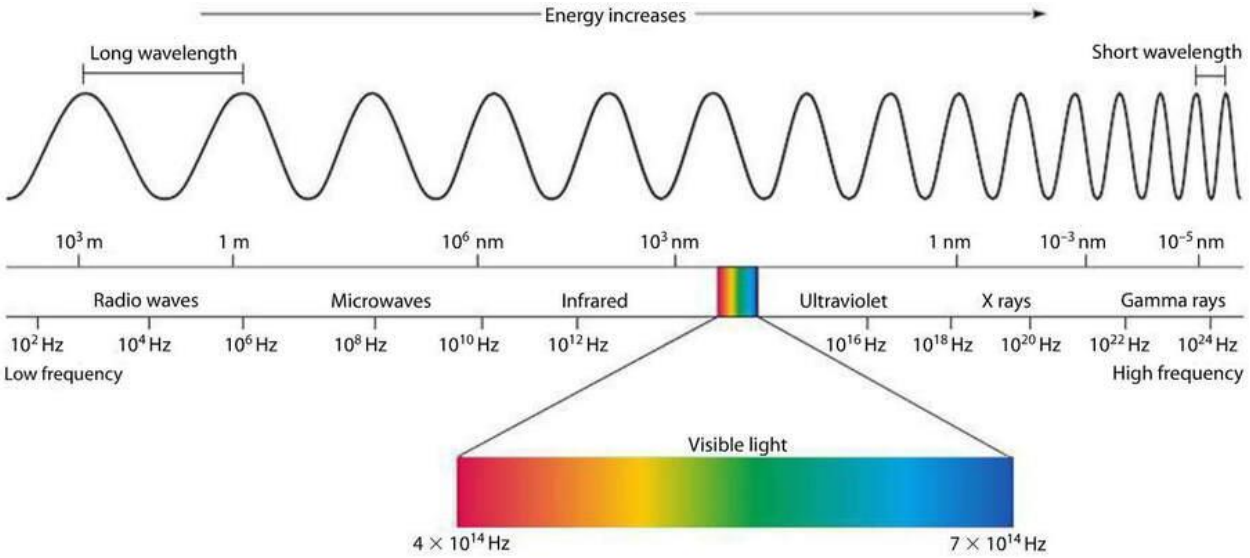

Figure 4: Spectrum of electromagnetic radiation

Each bands have different characteristics like their interaction with matter and its applications. Extreme ultraviolet, X-rays and gamma rays are classified as ionizing radiations because of high energy photons. They can ionize the atoms causing chemical reaction. Exposure to these radiations cause health hazard and can cause DNA damage and cancer. Radiation with low frequencies like visible, infra-red does not have enough energy to ionize the atom. So they are called as non- ionizing radiations [10]. When light travels through different medium like air, water, and glass then its frequency will remain same but its wavelength and speed reduces by factor "n" refractive index of the material (using relation (i)). Reflection, refraction and transmission are the boundary phenomenon, when electromagnetic wave at the boundary from one medium to another. Table 2: illustrate the different regions of electromagnetic radiations with their wavelength range and applications in different regions of biomedical field.

Table 2: Different regions of electromagnetic spectrum [12]

Type of wave	**Wavelength**	**Frequency**	**Application**	**Issues**
Radio waves	1cm -100km	10^3-10^{10}	Communication, Remote control, MRI	Requires control for band used
Microwaves	1mm-1cm	10^{10}-10^{11}	Communication, Oven, Radar, Cell Phone	
Infrared	700nm-1mm	10^{11}-10^{14}	Thermal Imaging, Heating	Absorbed by atmosphere

Visible	400-700nm	10^{14}-10^{15}	Photosynthesis, Human Vision	
Ultraviolet	10nm-400nm	10^{15}-10^{17}	Sterilisation, Vitamin D Production	Ozon depletion, Cancer Causing
X-rays	0.1nm -10nm	10^{17}-10^{20}	Security, Medical Diagnosis, cancer Therapy	Cancer Causing
Gamma Rays	< 0.1nm	10^{20} -10 ^{23}	Nuclear Medicine, Medical diagnosis, Cancer Therapy	Cancer causing, radiation Damage

3.1 Radio Wave

Radio waves of electromagnetic spectrum have the longest frequency range from 300 Giga hertz (GHz) to 3kHz. They are generated by accelerating charged particle such as time varying electric current [13]. Radio wave was first predicted by James Clerk Maxwell [14] in 1867. Spectrum of radio waves is divided into number of bands on the basis of frequency allocated for different used. In vacuum Radio waves travels with speed of light [15, 16]. When they pass though a medium, they slow down depending on the permeability and permittivity of the medium. Radio waves are related to the frequency and wavelength by the relation:

$$\lambda = \frac{c}{f}$$

Where λ is the wavelength, c is the speed of light and f is the frequency of wave. Radio waves are non-ionizing radiation i.e; they do not have enough energy to ionize the electron from the atom or molecules causing chemical reaction or DNA damage. Radio waves heat the material when it is absorbed. Polar molecule vibrates forth and back by oscillating electric field of the wave which cause the generation of heat in the material. Radio waves can penetrate the surface and deposit their energy inside the material or tissue. Penetration depth decreases with frequency and also depends on material sensitivity and permeability.

Radio waves are used in the diathermia for deep heating of body tissue to promote blood flow and healing. They also used in hyperthermia, a most recent technology for generation of higher temperature to kill cancer cells. The three main application of radiofrequency in medicine are: Magnetic resonance imaging (MRI), radiofrequency ablation (RFA) in cardiology and tumour therapy and localized dielectric heating (short wave diathermy) used in physiotherapy. Low temperature of radiofrequency as compared to electro-surgery or laser surgery to remove, shrink or sculpt soft tissue while simultaneously sealing blood vessels. Radiofrequency work well on connective tissue, which is primary, composed of collagen which shrinks when heated. Details of instruments used in electro-surgery are available in Physics for surgeon's part 4 [17].

3.2 Infrared Rays (IR)

Infrared light is the electromagnetic radiation with wavelength longer than the wavelength of visible light: ≥ 0.7 μm. Infrared (IR) radiation was discovered by sir William Herschel in 1800 [18]. They are produced by thermal motion, vibration and rotation of atoms. They are

also produced by electronic transition in atoms and molecules. IR radiations are not visible to human eye, because it starts from wavelength 750 nm [19]. Infrared spectrum is divided into three categories: Near infrared (NIR): (0.75µm-2.5 µm), Mid infrared (MIR): (2.5 µm-25 µm) and Far infrared (FIR) region: 25 µm -300 µm). NIR is use to study the harmonics or combination vibrations. MIR for the study of fundamental vibration and rotation-vibration structure of small molecules whereas FIR radiation used for the study of low heavy atoms vibration (metal–ligand or lattice vibrations).

IR spectroscopy is a non-distractive and can give structural information of the concerning molecules. Infrared rays are used for photo-biomodulation for wound healing, tissue repair and anti-inflammatory therapy. The mechanism largely attribute to the absorption of Near infrared (NIR) light by cytochrom C oxidase in to mitochondria, Which triggers the dissociation of inhibitory nitric oxide from protein complex which increases ATP synthesis and hence has direct beneficial effects on compromised and hypoxic cells [20]. In this techniques, the infrared light (1.8-2.2 µm) get absorbed by water results in increase of temperature that alters the membrane capacitance and depolarizes the target cells.

3.3 Visible Light (400-700 nm)

It's a narrow band of electromagnetic spectrum from 400 nm to 700 nm [3] of wavelength (the region up to which normal eye responds). Visible region of electromagnetic spectrum consist of different colors. Each color corresponds to specific wavelength range. The dependence of color on wavelength is shown in Table 3 and Figure 5.

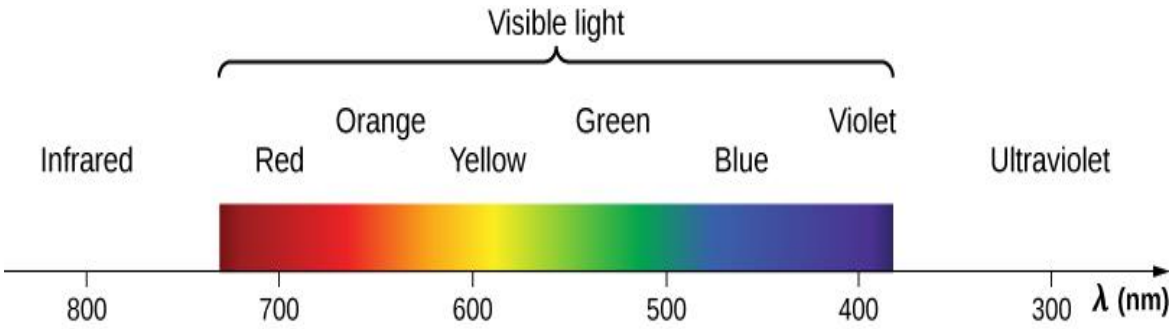

Figure 5: Wavelength distribution of colors of electromagnetic spectrum

Table 3: Wavelength dependence of color of visible spectrum.

Color	Wavelength (nm)
Violet	400-430
Blue	430-480
Green	480-560
Yellow	560-590
Orange	590-620
Red	620-700nm

Blue light (405nm-470 nm) used to treat broad range of bacterial infection including P.acnes associated acne vulgaris and H. Pylori gastritis in human as well as wound infection [21,22].In infant jaundice is commonly treated with phototherapy with light of wavelength 490 nm preventing serious sequelae of hyperbilirubinemia [23]. Bright light therapy effective for the treatment of non-seasonal major depressive disorders [24].

3.4 Ultraviolet (UV) light (100 nm- 400 nm)

The ultraviolet region starts from the wavelength of 400 nm down to 100 nm. UV light is further divided in to three categories: UV-A(320-400nm), UV-B (290-320nm) and UV-C (220-290nm). Most UV-B and all UV-C are absorbed by the ozone in the above atmosphere and 99% of UV sunlight reaching to the earth surface is UV-A. UV-B can directly damage the DNA and hence cause cancer. UV-A can also cause cancer through generation of DNA damage free radicals. Sunburn is cause by the exposure to UV-Band UV-C and repeated exposure increase the possibility of skin cancer. Tanning of skin is the defence mechanism of body by producing pigments in the inner skin layers to reduce the exposure of living cells [10].

Besides adverse effects UV radiation have some beneficial effects also. UV-B can activate the immune response through release of antimicrobial peptides and production of Vitamin D in the skin [12, 25]. Several studies suggest that the deficiency of vitamin D associated with the development of cancer (prostate, breast, colon) [26]. UV-B also suppresses the adaptive immune response by activation of regulatory T cells, B Cells and Mast Cells [26].

3.5 X-Rays

X-rays have wavelength range from 10 nanometer (nm) to 10 pico meter (pm) with corresponding energy 1241 eV to 145 eV. They have high energy than UV light, so they are more penetrating. X-rays with proton energy above 5-10eV (below 0.2-0.1 nm) are called hard X-rays while those with low photon energy are called soft X-rays [27]. Due to penetration ability hard x-rays are used in medical radiology for imaging the inside object. X –rays are used in checking broken bones, certain kind of disease [28]. Cancer and genetic defects can be induced by the X-rays because they effect on the rapidly dividing cells. X-rays can also be used to treat and cure cancer [10]. Since the size of x-rays are comparable to the size of the atoms. Therefore, x-rays are also used for determining the crystal structure. X rays have enough energy to ionize the atoms. This ionization energy is harmful for living tissue. A high dose radiation for short period of time causes radiation sickness while low dose increase the risk of radiation induced- cancer. Medical application of X-rays is projection radiography, computed tomography, Fluoroscopy, radiotherapy etc.

3.6 Gamma Rays

Gamma rays (γ-rays) have the smallest wavelength of electromagnetic spectrum with frequency above 30 exa hertz ($3 \times 10^{19} Hz$). They are the most energetic form of electromagnetic radiation with very small wavelength. γ-rays are ionization radiation therefore hazardous to life. It can damage bone marrow and internal organ due to high penetration power. So, large amount of shielding is required to reduce the level of harmful effect to living cells. γ-rays are best absorbed by high atomic number material and high density which contribute to stopping power. γ-rays are used to kill living organism in sterilization of medical equipment through process called irradiation. Despite of cancer causing property, γ-rays are also use to treat some kind of cancer because it also kills cancer cells. Gamma knife surgery is a process in which concentrated beam of γ-rays directed to the growth and kill the cancer cells. Beam of γ-rays are aimed at different angles to minimize

the damage to the surrounding tissue. γ-rays are also used in nuclear medicine for diagnostic purpose for example in PET scan and bone scan.

4 LASER (Light Amplification by Stimulated Emission of Radiation)

It is a narrow beam of light with single wavelength (monochromatic) which is in phase. To generate laser beam, population inversion, stimulated emission and pumping source (electrical or optical) these three processes must be satisfied. Medical application of optics spreads from ophthalmology to general surgery with the development of LASER. Today, various laser-based devices are available for therapeutic and diagnostic used. Potential application of Laser in medical field has been explore from the innovation of laser in 1960 which was the Ruby laser. LASER can emit radiation either in short pulse of light (pulsed Laser) or in continuous beam of radiation (Continuous Wave – CW laser) and their effect is also different. Table 4, indicates the different type of laser i.e; pulsed and CW laser. Carbon dioxide (CO2) LASER and Nd:YAG (Neodymium doped Yttrium-Aluminium Garnet) LASER have ability to cut the tissue while coagulation, led to their use in surgery as a surgical tool.

4.1 Properties of Lasers

Lasers have following three properties:

- Monochromatic
- Coherence
- Directionality

4.2 Types of Laser

- **Pulsed Laser:** When light comes in the form of short duration. Such Lasers are called *Pulsed Laser.*
- **Continuous Laser**: They are capable of producing ***continuous beams***.
- There is various type of laser depending upon the active material used for lasing action like solid state laser, gas laser, and liquid laser. Surgeons select appropriate laser to excise or coagulate the tissue.
- ***Radiance:*** It is the power density. It is the amount of power per unit surface area during a single pulse or exposure

4.3 Laser-Tissue Interaction

4.3.1 Photochemical interaction

In this type of interaction laser beam of low intensity applied for longer duration which causes a photochemical changes either by slow transfer of energy as heat or by specific chemical reaction as in photodynamic therapy (PDT) and in Lasik vision correction. Excimer laser have wavelength in UV range which have ability to break the covalent bond in protein. Therefore, are used to modify the shape of cornea in Lasik surgery.

4.3.2 Thermal interaction

Thermal Interaction is based on the concept of a less intense- longer pulse of laser will cause a rapid heating. When laser light is absorbed by the chromophore, it converts in to heat leading to denaturation of protein at 42-65℃. Tissue vaporization, coagulation or both depends on the exposure time. CO_2 laser use to cut and vaporize the tissue which is mostly consists of water. When laser irradiate the tissue, generated heat causes the water in tissue in to steam hence ablate the tissue. Short exposure time is necessary to minimize the thermal damage and maximize the ablation. This can be done by pulsed laser with less thermal relaxation time. Thermal relaxation time is the time taken by target to dissipate 50% of energy absorbed to the surrounding whereas thermal containment time is the time in which no heat (no thermal effect) is dissipated to the surrounding tissue and it is roughly one quarter of the thermal relaxation time [29].

4.3.3 Photo-ablation

Photoablation is a process in which chemical bonds break without heating the material. Condition for breaking chemical bond by electronic excitation is that when photon energy is greater than or equal to the bond energy then only chemical bonds will break. This can be achieved by UV laser i.e; excimer Lasers. Along with breaking of chemical bond thermal energy also exist which cause rapid detachment of molecules in to gas phase hence ablation of material. Advantage of photoablation is, it is well localized to the irradiation region hence no thermal damage as coagulation or vaporization. Disadvantage is photoablation is limited to UV light only. Pure photoablation is achieved at wavelength 193 nm of ArF excimer Laser.

4.3.4 Photo-electromechanical Interaction

high intensity laser pulse with short duration will cause explosive expansion of tissue called photomechanical reaction of photodisruption. Ultra short laser pulse of high intensity cause extremely rapid heating of the target with expansion of plasma. As the plasma collapse the shock waves causes' mechanical disruption of target. This photomechanical disruption is utilized in the removal of tattoos, disruption of stones and certain pigment in the skin. When laser fluence is high electric field of magnitude 107 to1012 Vm^{-1} may attain this leads to breaking and ionization of chemical bonds this is the origin of the shock wave. In biological material plasma expand rapidly hence electroacoustic shock waves develop which is used to destroy solid gains. When laser beam is absorbed by the plasma hence diminishing the target this technique is used in ophthalmology to destroy opaque of vitreous humor without damaging retina [29,30]. List of Laser used to irradiate the variety of tissue, their wavelength, therapeutic interaction has been indicated in Table 4.

Table 4: Common laser and their clinical applications.

S.No	Name of Laser	Type Pulsed /CW	Active Material	Wavelength (nm)	Applications
1	ArF Excimer Laser	pulsed	Gas	193	Refractive surgery,
2	Argon ion LASER	CW	Solid state laser	488, 514	Retinal photocoagulation
3	Nd:YAG, frequency doubled laser	pulsed	Solid state laser	532	Tissue cutting and coagulation, tattoo removal
4	Pulsed Dye Laser	pulsed	Liquid laser	577	Removal of vascular lesion
5	Continuous Dye Laser	CW	Liquid laser	630-690	Photodynamic Therapy
6	Visible diode Laser	pulsed	Solid state laser	650-690	Photodynamic therapy
7	Ruby LASER	pulsed	Solid state laser	694	Removal of tattoos
8	Infrared Diode Laser	CW	Solid state laser	800	Tissue welding, retinal photocoagulation
9	Nd:YAG LASER	pulsed	Solid state laser	1016	Tissue cutting and coagulation, many surgical applications.
10	CO_2 LASER	pulsed	Gas laser	10600	Tissue cutting and coagulation, skin resurfacing

4.4 Application of Laser in Medical Sciences

4.4.1 LASER Application in Surgery

With the understanding of light tissue interaction, Laser could be used to produce tissue effect other than purely thermal ones used in early laser surgery. Pulsed Laser has ability to cause number of mechanical effects. These mechanical effects found clinical used in ophthalmology for “photo disruption”, a procedure used to treat the side effects of cataract surgery [31], destruction of skin lesion [32]. Laser induced mechanical effect also used in fragmentation of urinary tract calculi (stone) in patients called “Laser Lithotripsy”. In Laser Lithotripsy, optical fibre is used to deliver the light to stone for fragmentation which could not be accessed by shock wave lithotripter because pelvic bone blocked the acoustic pulses. Additionally, in non-thermal use of laser in cancer treatment by injecting the drug in to the patient and selectively illuminating the area of interest which leads to photo-thermal destruction of tumour called photodynamic therapy (PDT) [33].

4.4.2 LASER Application in ophthalmology

ArF excimer (λ=193 nm) laser is used to reshape the cornea for refractive error corrections [34]. Peptide bonds of collagen fibre in the cornea were breaks with UV radiation hence expelling a discrete volume of corneal tissue from the surface [35]. A single pulse of excimer laser (with power 0.25J/cm^2) with spot size of 1mm removes ~0.25 µm of tissue.

For cataract surgery femtosecond laser technology is used. Nd-YAG laser used for capsulotomy to treat posterior capsule opacification in cataract surgery [36].

4.4.3 LASER Application in Dermatology

Laser are effectively used for removal of unwanted skin marking, tattoos, birth marks, stretch marks, acne scars and leg veins [37]. Cutaneous laser surgery, revolutionaries the concept of selective photothermolysis [38], in this technique pulsed laser with optimal parameters (wavelength, duration, energy) can selectively destruct target within the skin, mimimizing the damage to the normal tissue. Dye laser (585nm, 0.4-2ms, 5-10J/cm^2) used for skin marking by selectively targeting haemoglobin with red blood cells [39]. For deeper dermal penetration and destruction of larger vessels spot size of 2-10 mm are used. CO2 laser (10.6µm, 1-10ns, 5J/cm2) is use for ablative therapy [40].

4.4.4 LASER Application in Urology and Gastroenterology

In urologic disease photothermal, photochemical and photomechanical property of laser is used for treatment. High power laser (80-180W, 20ms, 400J/cm2) is used to remove the excessive tissue in benign prostate hyperplasia (BPH): is a non-cancerous condition. In this condition enlarged prostate squeezes or partially block the surrounding urethra [41]. Frequency doubled Nd-YAG laser (532 nm) delivered by optical fibre through cystoscope for photoselective vapourization is the rapidly growing laser surgical technique for prostate [42]. When ultrasound based shock waves lithotripsy cannot be used then holmium: YAG laser with flexible ureteroscope use to perform laser lithotripsy [43].

4.4.5 LASER Application in Cardio and Vascular Surgery

Laser assisted lead extraction is an effective technique for extraction of lead wire from peacemakers and defibrillators from coronary blood vessel [44]. Typically XeCl laser (100-200 ns, 3-6 J/cm2) is used for localized ablation of ~100 µm of tissue [45]. Excimer laser also used for ablation of plaques through fibre optic catheter [46]. High power continuous wave Laser: NIR diode laser (810 nm, 940 nm or 1470 nm 10-30 W) intravenously delivered through optical fiber used to ablate the varicose vein [47].

5 New Emerging Optical Technologies

Numerous new emerging optical techniques have been employed for the detection of various types of cancers. These techniques include optical coherence tomography (OCT), reflectance imaging, diffuse reflectance spectroscopy (DRS), fluorescence imaging etc.

5.1 Confocal Microscopy

Confocal microscopy is also known as confocal laser scanning microscopy (CLSM) or laser scanning confocal microscopy (LSCM). It's a new technique that allows optical sectioning of the sample with greater resolution and three dimensional reconstruction of the sample [48]. This technique allows real time visualization of fully hydrated, living specimen [49]. Confocal microscopic technique is based on the measurement of fluorescence intensity originating from limited volume in the imaged specimen. Intensity is quantized in the absolute unit and 3D micrograph is produced. Therefore we can obtain quantitative information on concentration of biomolecules in cell and tissue of imaged area. The main

function of a confocal microscope is to produce a point source of light and reject out of focus light. This provides the ability to image deep tissue with high resolution and optical sectioning for 3D reconstruction of imaged samples.

Components of Confocal microscope are pinholes, objective lenses, low noise detectors including fast scanning mirrors, filters for wavelength selection, laser for illumination (either argon and helium neon laser, solid state laser, or diode laser). In configuration, pinhole is placed in front of light source to provide a point light on the sample by objective lens. The second objective lens focuses the illuminated sample point to second pin hole in front of the detector. This double focussing system rejects the out of focus rays from the illuminated sample. X-Y stage used to scan the sample in x-y plane through the illuminated point to build the image. Figure 6, shows the schematics diagram of modern confocal microscope.

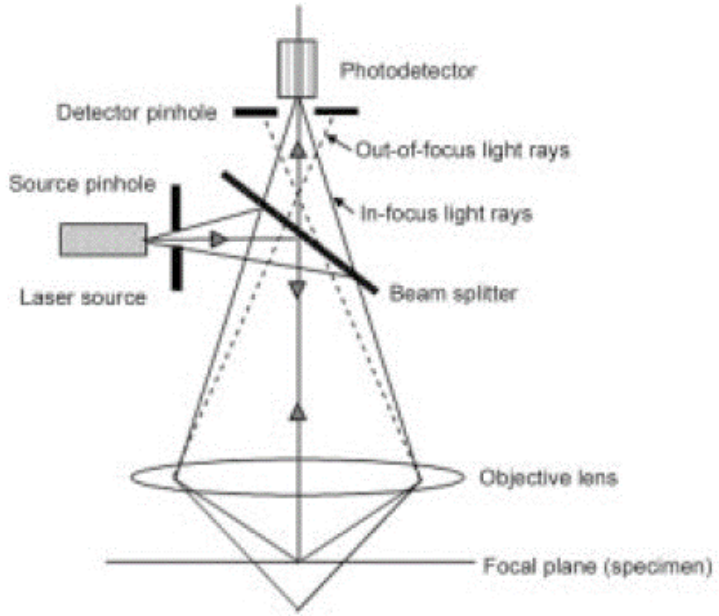

Figure 6: Schematic diagram of confocal microscope

The resolution of image depends on the numerical aperture (NA) of the objective lens, refractive index of the sample (η) and wavelength of light (λ) used for illumination. Lateral and axial resolution of image can be obtained by the following equation:

$$R_{lateral} = \frac{0.4\ \lambda}{NA}$$

$$R_{axial} = \frac{1.4\lambda\eta}{(NA)^2}$$

The best lateral resolution that can be obtained is ~ 0.2 µm and ~0.6 µm axial resolution. For dimly fluorescent sample, pinhole open wide to collect more light to improve the contrast [50].

5.2 Photodynamic therapy

Photodynamic therapy (PDT) becomes popular after getting regulatory approval to several photosensitizing drugs and light applicators worldwide. Lipson and Blades reported the neoplastic tissue containing photosensitizer (porphyrin mixture) fluoresce when irradiated by ultraviolet light [51]. Photofrin was the first photosensitizing drung which is FDA approved for clinical practice. Three main essential part of PDT are: photosensitizer, light and oxygen

[52, 53]. Individually none of these are toxic in nature but in combination they initiate a photochemical reaction that culminates in the generation of highly reactive singlet oxygen ($_1O_2$). This reactive singlet oxygen causes significant toxicity leading to cell death via apoptosis or necrosis [54]. Anti-tumour effect of PDT involves three inter-related mechanisms: direct cytotoxic effect on the tumour cells, damage to tumour vasculature, robust inflammatory reaction that leads to systematic immunity. Type of mechanism depends on the large extent on the type and dose of photosensitizer, time between administration of photosensitizer and light exposure, duration of light exposure, its fluorescence rate and concentration of tumour oxygen [55].

5.2.1 Photosensitizer

Most of the PDT photosensitizers have heterocyclic ring structure similar to that of chlorophyll or heme in hemoglobin. After exposure to light photosensitizer start a chemical reaction in the presence of molecular oxygen produces singlet oxygen (1O2) or superoxide (O2-), hence induces cell damage through direct and indirect cytotoxicity. Therefore, photosensitizer plays a crucial role in PDT. Photosenistizers can be classifying according to their structure. They can be divided in to three categories: (i) porphrin based photosensitizer (e.g; photofrin, ALA/PpIX, BPD-MA), (ii) chlorophyll based photosensitizer (e.g; chlorins, purpurins, bacteriochlorins) and (iii) dye (phtalocyanine, napthalocyanine). Currently most of the photosensitizers which are clinically approved are porphyrin based photsensitizers. For clinical practice, a photosensitizer should meet following criteria: It should be commercially available in pure chemical, low dark toxicity, strong photocytotoxcity, good selectivity towards the target cells, rapid removal from the body, ease of administration through various routs, long wavelength absorption [56]. Clinically approved photosensitizers are available in Table 5.

Table 5: Clinically approved photosensitizers

Photosensitizer	Structure	Wavelength	Application of PDT in treatment of cancer of
Porfimer sodium (photofrin)(HPD)	Porphyrin	630nm	Lung, esophagus, bile, duct, bladder, brain, ovarian
ALA	Porphyrin precursor	635 nm	Skin, bladder, brain, esophagus
Remoporfin (Foscan) (mTHPC)	Chlorine	652 nm	Head and neck, lung, brain, skin, bile, duct,
Vesteporfin	Chlorine	690nm	ophthalmic, pancreatic, skin
HPD: hematoporphyrin derivative, ALA: 5-aminolevulinic acid, mTHPC: m-tetrahyrdoxyphenylchlorin			

5.2.2 Light Sources and Light Delivery in PDT

Initially the source of light in PDT was non-coherent light (like conventional arc lamp). It was safer, easy to use and less expensive. This can excite various photosensitizers due to broad wavelength range. But also comprise disadvantages like significant thermal effect, low

light intensity and difficulty in controlling light dose. Nowadays most of the drawback can be overcome by carefully choosing light source for PDT like LED (light emitting diode) and Lasers. LEDs can generate the light of desire wavelength, range of geometries and size with high intensity. In case of brain tumor, the intraoperative PDT contains LED probe arranged in a cylindrical tip to fit in to the balloon catheter [57]. In minimal invasive interstitial PDT small flexible LED catheter can be implanted in to the tumour precutaneously [58, 59]. In case of flat surface where wide area of superficial lesion needs to illuminate then large LED array may be more suitable [60,61]. Most commonly lasers are used for PDT as light source. Lasers produce monochromatic light with high energy, specific wavelength with narrow bandwidth which can excite the specific photosensitizer. Focused laser light can be delivered through optical fibre directly to the targeted site.

5.2.3 IR Thermography

AT room temperature (290K), objects emit peak intensity radiation with wavelength 10μm (infrared). Since this is infrared radiation human eye can't see this radiation so we can only sense this as heat like electric fire radiate energy in the form of heat. Measurement of radiation emitted by an object is generally call infrared thermography. Infrared thermography or thermography is a process to detect radiation emitted by body in a long range of electromagnetic radiation (infrared radiation) ranges from 9000-14000 nm and creates a thermal image of the same. These thermal images are called thermograms. IR thermography is an optical technique which is non-contact, full field, real time, rapid and non- destructive [62]. Infrared Thermography is based on the principle that anybody with a temperature above absolute zero (-273.15 C) emits electromagnetic radiation which travel with speed of light. Intensity of emitted IR radiation is a function of temperature. Higher is the temperature higher will be the intensity of infrared radiation emitted by the object. Radiations are characterised by two parameters i.e; its wavelength (λ) and Intensity (I). Both these parameters are related by surface temperature [63] and can be describes as:

$$\boldsymbol{Q_\lambda = \frac{A}{[\lambda^5(e^{(B/\lambda T}-1)]}} \qquad (1)$$

Where $\boldsymbol{Q_\lambda}$ **is the** intensity of emitted radiation (W) at particular wavelength (λ), T is the surface temperature of the object (K) A and B are constants with value $3.74 \times 10^8\, \mu m^4 m^{-2}$ and $1.439 \times 10^4 \mu m.K$ respectively. When we integrate the equation (1) then we will get the total energy emitted by the body is:

$$\boldsymbol{Q_{total} = \sigma . T^4} \qquad (2)$$

Where σ is the Boltzman constant ($5.67 \times 10^{-8} W m^{-2} K^{-4}$). The energy radiated by a body is proportional to its emissivity and fourth power of its absolute temperature. Wavelength of emitted radiation and surface temperature of the body are related with each other. Lower the wavelength higher will be the temperature of the body. This relationship can be described by Weins displacement law:

$$\lambda_{max} = \frac{2.9}{T} \qquad (3)$$

$\lambda_{max}$is the peak wavelength (in mm unit) of emission at surface temperature T(in K unit). Intensity of emitted radiation also depends on the nature of the surface of the emitting material. Object which is a perfect emitter at all wavelengths is called black body. A perfect emitter is also a perfect absorber of the radiation at same wavelength. Absorptivity of radiation at a wavelength is equal to the emissivity of the material at the same wavelength. This phenomenon is called Kirchoffs law.

Biological materials in of 9-11µm range have absorptivity (and emissivity) in between 0.9 to 0.97 (infrared radiation) [64]. High absorptivity is probably due to biological material have high water level content (greater that 65% water) and water have absorptivity in infrared range (i.e; 0.96) [65]. For infrared thermography of biological material generally the surface emissivity assume to be fixed value in between 0.95 and 0.98.

Since infrared radiations are invisible to human eye so special cameras are required to acquire and process the information [66]. Infrared measuring device absorbed the infrared radiations emitted by the object and convert it into electrical signal [67]. The basic infrared device is a pyrometer. Pyrometer produces a single output using a single sensor whereas advanced devices have array of sensors to image the detailed information of the scene. Electrical signal obtained from the sensor are converted in to visible images by assigning a color to each infrared energy level. The results are the false color image called thermogram [68].

IR Thermography is used for multiple processes: as a health indicator in medical applications, as an indicator of heat loss in buildings. It provides accurate reading without the invasive procedure. It can be used body tumours [69], in diagnosis of diabetic neuropathy or vascular disorders [70], fever screening [71], skin diseases [72], dentistry [73] and heart operation [74]. IR thermography has two different approached to employ: passive IR thermography and Active IR thermography [75]. In passive IR thermography, radiations emitted by the object are measured without any external heat simulation. Whereas in active IR thermography, the specimen is subjected to the external thermal stimulation which results to the temperature difference on the surface of target radiation comes from the thermal response of the target to the external excitation [76, 62].

5.2.4 Infrared (IR) and Raman Spectroscopy (RS)

IR and Raman spectroscopy can provide the detailed molecular information about tissue. These spectroscopies have potential to generate the biochemical information about tissue diagnosis like characterization of atherosclerosis (plaque in arteries) and cancer diagnosis.

In IR spectroscopy we measure the absorption between the wavelength 2000 nm (~5000 cm^{-1}) and 100000 nm (100 cm^{-1}). In this wavelength range absorption occurs due to the transitions between vibrational energy levels of the molecule being probed. A biological molecule has several absorption bands due to multiple vibrations occurs within the single molecule. Pattern of absorption spectra strongly depends on the type(s) of molecule therefore; IR spectroscopy is very sensitive to the tissue biochemistry.

Raman spectroscopy (RS) is an inelastic scattering phenomenon. Inelastic occurs due to the difference between the incident and scattered light. Due to scattering, Energy of the scattered

light either increase (anti-stokes) or decrease (stokes) and is equal to the energy difference between the vibrational levels of the molecule scattering the light. Raman spectrum is the plot of the intensity of scattered light as a function of energy difference between the incident and scattered light. Raman spectrum provides the information of molecular vibration of the molecules.

Biological tissue which contributes to Raman and IR spectra are protein (including collagen), lipid, nucleic acid and water. Phospholipid, proteins and nucleic acid have their unique spectra. IR and Raman spectra of protein primary due to vibration of amid backbone [77-80]. Protein display band near 1450 cm^{-1} due to CH_2 and CH_3 scissoring [81] and also have band near 1400 cm^{-1} because of CH_3 deformations and vibration of COO^-[82]. Glycogen has vibrational frequency in the spectra region 950-155 cm-1[83]. IR and Raman spectroscopy both measure the vibrational spectra of the molecules. IR spectroscopy is more useful in the study of polar bonds (like C=O and OH) whereas Raman spectroscopy is more sensitive to nonpolar groups (such as C=C and S-S). Penetration of IR radiation is limited in case of in-vivo samples. In contrast, visible and near IR wavelengths used for excitation in Raman penetrate more deeply in the tissue. Therefore, Raman is preferred technique for tissue characterization. IR and Raman spectroscopy have potential to provide detailed information about biomolecular tissue. Significant application of Raman and IR spectroscopy is to investigate the chemical assay of atherosclerotic plaque and diagnosis of cancers and precancerous lesions [84]. Goal behind the vibrational spectroscopy for cancer diagnosis is to develop a method which is non invasive and give molecular information of tissue.

5.2.5 Fluorescence microscopy

Fluorescence microscopy is widely used research tool almost all disciplines of biological and biomedical science. Fluorescence microscopy provides a unique approach to study the fixed and living cells because of its high sensitivity, specificity and its versatility. By exploring the characteristics of fluorescence, various techniques have been developed for visualising and analysis of complex dynamics events in cells and organelles within the biological specimen. The describes techniques are Fluorescence recovery after photobleaching (FRAP), Fluorescence loss in photobleaching (FLIP), Fluorescence localization after photobleaching (FLAP), Förster or Fluorescence resonance energy transfer (FRET), Fluorescence lifetime imaging microscopy (FLIM).

Fluorescence is a phenomenon where fluorophore excited by certain wavelength of light and emit radiation of different wavelength. Fluorophore generally termed as fluorescent probe or dye and fluorochrome. Fluorophore conjugated covalently to biological macromolecules such as nucleic acid, lipid, or proteins. Fluorophore may be organic (dye), fluorescent proteins (green fluorescent protein) some inorganic luminescent semiconducting nano-particles, quantum dots introduce as label for biological assays, biomedical imaging purpose.

Fluorescence follows a series of discrete steps: When light of particular frequency hit the fluorophore sample, atom, molecules or ions absorb the quanta of specific light which push the valence electron from ground state GS_0 in to higher energy level, creating excited state ES_n. This process is very fast and in femtosecond range. After excitation, electron

quickly relax to the lowest possible sublevel (process is in pico second range) the energy decay occurs through intramolecular non radiative conversions and heat. When return to the ground state it dissipate the remaining energy in the form of photon with a longer wavelength i.e; fluorescence emission. The whole mechanism can understand by Jablonski diagram (see figure 7). Due to energy loss in the process and wavelength varies inversely proportional to the energy (Plank's energy equation 1) the emission wavelength always higher than the excitation wavelength. This is called red shift in the spectrum [85]. The wavelength difference between emission and excitation spectra is called "Stocke's shift. Anti-Stockes shift where emission wavelength is smaller than the excitation wavelength also possible in case of photon up conversion or two photon excitation. Anti-stockes fluorescence commonly observes in the fluorophore where absorption and emission spectra overlap significantly. Fluorophore not only have characteristic excitation spectra as well as characteristic emission spectra that are depends on their specific vibronic configuration and properties [86, 87].

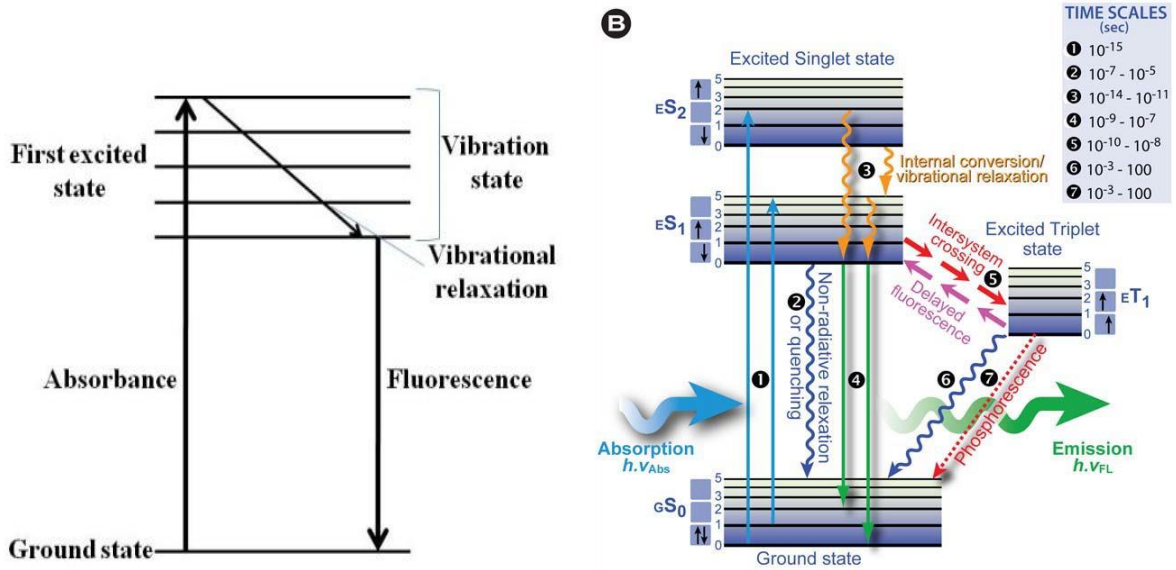

Figure 7: Jablonski diagram of Fluorescence [88]

Fluorescence imaging is a powerful tool that has been widely used for imaging the endogenous fluorophores such as collagen, FAD, NADH in the biological tissue. Fluorescence imaging has low detection limits and has high molecular sensitivity and specificity. Various studies indicates the use of fluorescence imaging for screening and detection of cancers in oropharynx and anogenital regions [89-93]. Recent advances in fluorescence imaging techniques have been reviewed by Shin et al [94] to detect oral cancer and its precursors. Nowadays Fluorescence microscopy is the standard procedure to study the normal and pathological cell biology in single cells or across a cell population. Major advantage of fluorescence microscopy is that it provides information with spatio-temporal resolution and less destructive as compared to other imaging technique like electron microscopy (EM).

6 Conclusion

Optics is the powerful tool for examination, diagnostic and therapeutic use on both sick and healthy person. New advancement in the optics drive the development of new generation of imaging tools. Optics and optical components are the at the core in the variety of modern optical devices and instruments. Power of light and its interaction with matter is extremely useful from removal of tattoos to eye surgery, tissue ablation and coagulations and up to emerging technologies for diagnosis and therapies for cancer treatment.

7 Competing Interests

The authors declared that no known conflict of interest exist in this work.

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