BM E76 - MEDICAL OPTICS

UNIT-I

2 MARKS

1. **Define refractive index of the medium.(Nov-2018)**
   - Refractive index, also called index of refraction, measure of the bending of a ray of light when passing from one medium into another.
   - Refractive index is also equal to the velocity of light c of a given wavelength in empty space divided by its velocity v in a substance, or n = c/v.

2. **Define Lambert-Beers law.(Nov-2018)**
   - The Beer-Lambert Law (also called Beer's Law) is a relationship between the attenuation of light through a substance and the properties of that substance.
   - In this blog post, the definitions of transmittance and absorbance of light by a substance are first introduced followed by an explanation of the Beer-Lambert Law.

3. **What is meant by Photoabalation?(Nov-2018)**
   - Photoablation effect is defined as a pure ablation of a material without thermal lesions at the margins, such as one would make with a scalpel.
   - As a physical process, medical photoablation is a volatilization of tissue by ultraviolet rays emitted by excimer laser at 193 or 308 nm.

4. **Define fluorescence.(Nov-2019)**
   - Fluorescence is the emission of light by a substance that has absorbed light or other electromagnetic radiation.
   - It is a form of luminescence. In most cases, the emitted light has a longer wavelength, and therefore lower energy, than the absorbed radiation.

5. **What do you mean by the term speckle?(May -2019, Nov-2019)**
   - The term speckle refers to a random granular pattern which can be observed e.g. when a highly coherent light beam (e.g. from a laser) is diffusely reflected at a

surface with a complicated (rough) structure, such as a piece of paper, white paint, a display screen, or a metallic surface.

6. What are optical filters?(Nov-2019)

- An optical filter is a device that selectively transmits light of different wavelengths, usually implemented as a glass plane or plastic device in the optical path, which are either dyed in the bulk or have interference coatings.

7. What is a polarizer? How it helps in light detection?(May-2019, Nov-2019)

- A polarizer or polariser is an optical filter that lets light waves of a specific polarization pass through while blocking light waves of other polarizations. It can filter a beam of light of undefined or mixed polarization into a beam of well-defined polarization, which is polarized light.
- Polarizing filters or sensors reduce the glare; reduce surface reflections; and, increase the clarity of structures, defects, and shapes. The filters eliminate reflections and light refractions, in a manner that is similar to sunglasses.

8. State the law of refraction.(May-2019)

- The first law of refraction states that the incident ray, refracted ray and the normal all lie in the same plane.
- The second law of refraction states that the ratio of sine of angle of incidence to the sign of angle of refraction is constant to the interface of any two-given medium.

9. What are xenon lamps?(May-2019)

- A xenon arc lamp is a highly specialized type of gas discharge lamp, an electric light that produces light by passing electricity through ionized xenon gas at high pressure.

11 MARKS

1. Describe the various types of interactions of ultrasound with tissues. (May-2019)

- When an ultrasonic pulse travels through the tissues of the body, it undergoes continuous modifications, which depend on characteristics of sound waves as well as tissues.

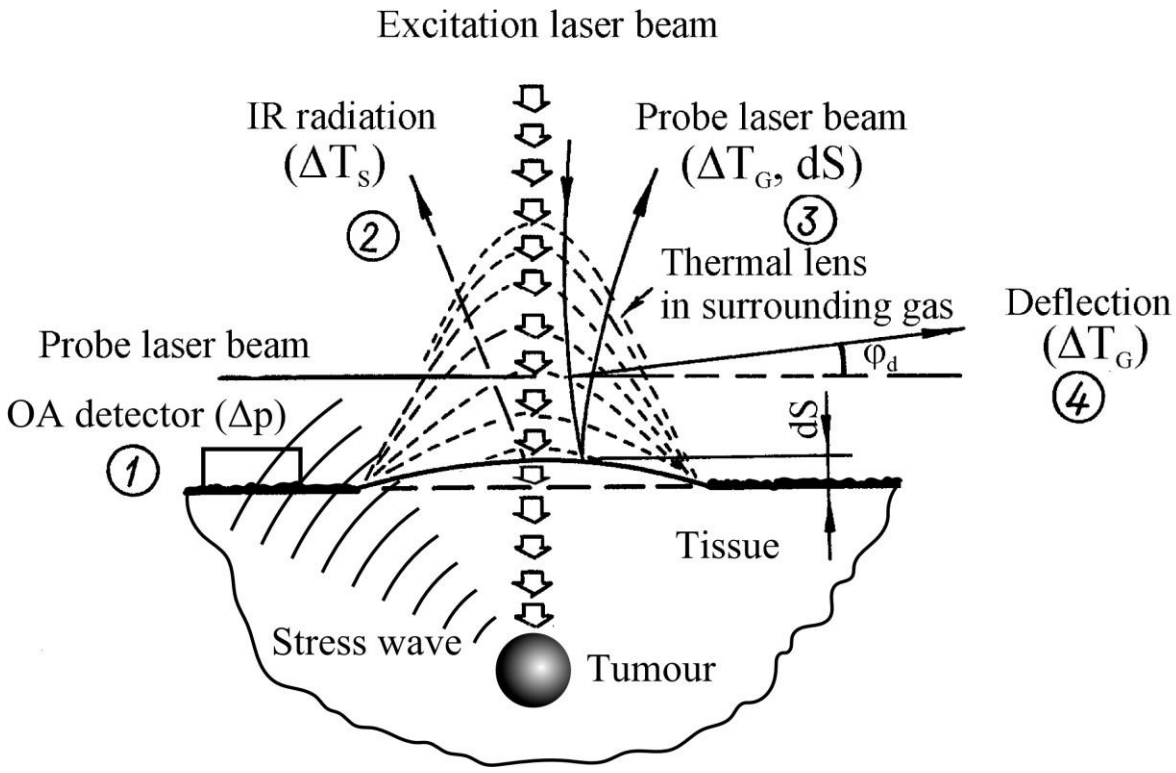
- This section describes some important parameters of ultrasound/tissue interactions. Velocity describes the speed at which ultrasound travels through a medium depends on the density and compressibility of the material.
- The more solid the material, the greater is the velocity of sound. The below table shows the values for biological tissues. As seen from values for water at different temperatures, the velocity increases with the temperature.
- It also depends on condition of the tissue, e.g., dead or living. In ultrasonics for tissue characterization, there are a few situations, listed below, in which the knowledge of the velocity is relevant.
    1. For conversion of pulse-return time into the depth of tissue.
    2. To calculate the acoustic impedance of tissue, this allows echo size to be estimated.
    3. Refraction (deviation of ultrasonic beam) occurs at tissue interfaces when velocity differs in two tissues.
    4. To produce B-scan images of tissues, an average value for the velocity of sound in the examined tissue, rather than the exact velocity for each individual tissue, is taken.
- This can create errors, typically about 2mm in range of 20 mm for abdominal scanning.
- Using this fact, velocity profile imaging techniques have recently emerged, producing tomograms of spatial distribution of velocities in tissues from their time-of-flight properties.
- The below table show that Mean velocity values for selected biological tissues.
- Acoustic Impedance and Reflection Acoustic impedance of tissue is the resistance exerted by tissue to the sound propagation; it is given by the product of tissue density (p) and the velocity of sound (c) for the tissue.
- An echo is generated at a tissue interface if the acoustic impedances of two tissues on either side are different. Echo size is determined by magnitude of the difference in the impedance.
- The ease with which any mass, e.g., a tumour, is detected in diagnostic ultrasonics is highly dependent on its acoustic impedance relative to that of the surrounding tissue.

Tissue/material	Mean velocity (m/sec)
Air	330
Aqueous humour	1500
Blood	1570
Bone (skull)	4080
Brain	1540
Breast	1510
Fat	1450
Kidney	1561
Lens of eye	1620
Liver	1550
Lung	658
Muscle (skeletal)	1585
Soft tissues (average)	**1540**
Vitreous humour	1520
Water (20^o C.)	1480
Water (50^o C.)	1540

- Specular reflector is the term used for a large, flat surface reflecting a perpendicularly (or normally) incident beam.
- Here, the reflected beam is also perpendicular to the surface, so the same transducer can receive it.
- Specular reflection is very common in abdominal scanning; examples are capsules of the liver and kidney, the gall bladder, and the aorta.
- The size of echo due to reflection at a particular interface is expressed as the ratio of reflected wave amplitude to the incident wave amplitude. This ratio is also known as reflection coefficient (R).

2. Explain the optothermal interactions of light with tissues? (May 2019, Nov2019)

The time dependent heat generation in a tissue at interaction with optical radiation is known as optothermal (OT) effect. This interaction also induces a number of thermoelastic effects in a tissue in particular causes generation of acoustic waves. Detection of acoustic waves is a basis for optoacoustic (or photoacoustic) method. The informative features of this method allow one to estimate tissue thermal, optical and acoustical properties which depend on tissue structure peculiarities.



Two main modes can be used for excitation of tissue thermal response:

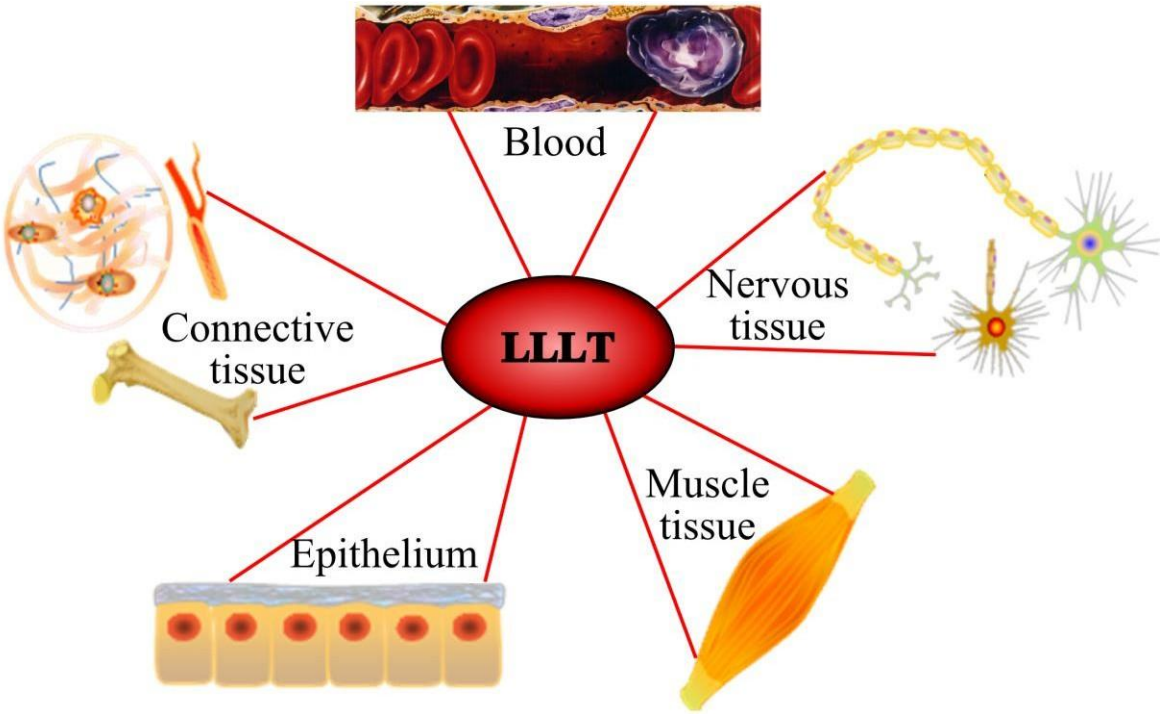
1) a pulse of light excites the sample and the signal is detected in the time domain with a fast by acoustic transducer attached to a wide band amplifier (signal averaging and gating techniques are used to increase the signal-to-noise ratio) 2) an intensity modulated light source is used and the phase sensitive signal detection for the selected modulation frequency is provided.

In every case the thermal waves generated by the heat release result in several effects which have given rise to various techniques:

1. Optoacoustics (OA) or photoacoustics (PA)

2. Optothermal radiometry (OTR) or photothermal radiometry (PTR) Photorefractive techniques, and etc.

When a laser beam falls down to the sample surface and the wavelength is tuned to an absorption line of the tissue component of interest the optical energy is absorbed by the target component and the most of the energy transforms to heat.



The time dependent heating leads to all of above mentioned thermal and thermoelastic effects. In OA or PA techniques, a microphone or piezoelectric transducer which are in acoustic contact with the sample are used as detectors to measure the amplitude or phase of the resultant acoustic wave. In OTR technique, distant IR detectors and array cameras are employed for the sample surface temperature estimation and its imaging.

Two widely spread therapeutic applications:

- Photodynamic therapy (PDT)
- Low level light therapy (LLLT)

3. Write in detail about any two excitation light sources.(May-2019)

Xenon arc lamp:

- A **xenon arc lamp** is a special type of gas discharge lamp. Xenon arc lamps produce light by passing electricity through ionized xenon gas at high pressure.
- It produces a bright white light that closely mimics natural sunlight, which extends its applications into the film, and daylight simulation industries.

Working:

- Here, when voltage is applied across the electrodes, the gas discharge phenomenon starts in the xenon gas in the gap between electrodes. There are always present some free electrons in the gas.
- Due to the applied electric field across the electrodes, the free electrons get accelerated and collide with xenon atoms. Due to these collisions electrons from the outer orbit of the xenon atoms get detached from their position and come to the higher energy level.
- Due to the electrostatic attraction of anode or positive electrodes, the free electrons originated due to the ionization process ultimately comes to the anode and returns to the source.
- Due to the attraction of the cathode, the positive ions ultimately collide with the cathodes front surface and generate positive metal ions, neutral xenon atoms, and free electrons.
- These electrons are called secondary emitted electrons. These electrons help to continue the gas discharging process. As the cathode is not additionally heated for electron emission, the cathode of xenon arc lamp or xenon lamp is known as a cold cathode.

Xenon arc lamps are used for:

- Specialized uses in industry and research to simulate sunlight
- Searchlights
- Movie projectors in theaters

The light source used in a fluorescence microscopy experiment must emit the specific wavelengths of light that excite the fluorophores present in the sample. When imaging live cells, lower intensities of excitation light are best to avoid damaging the cell through photo bleaching and photo toxicity.

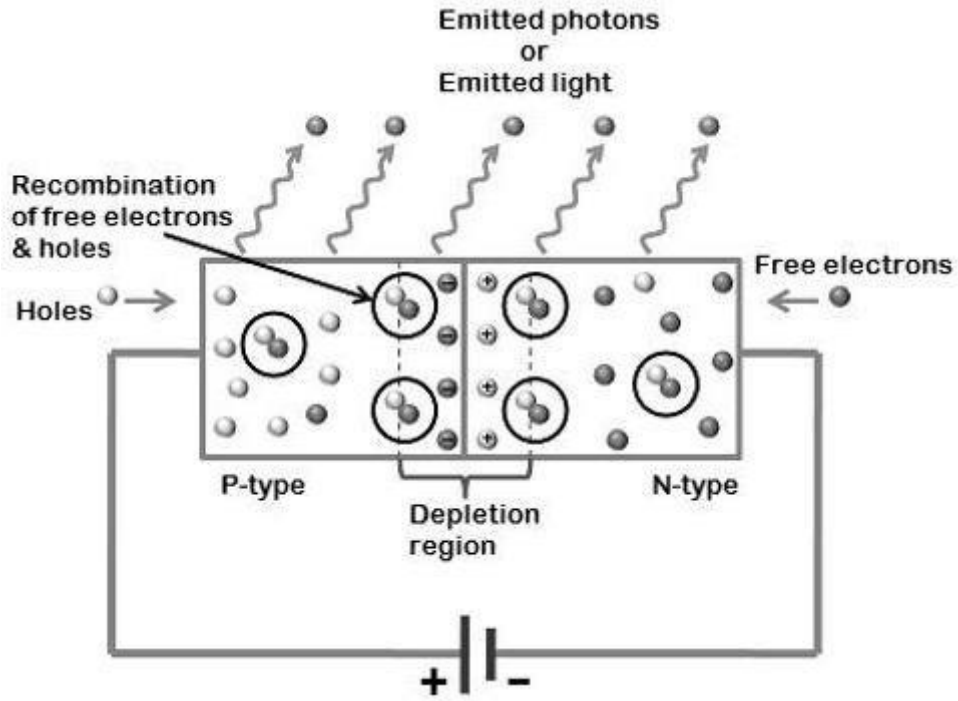
LIGHT EMITTING DIODE (LED):

- A light releasing diode is an electric component that emits light when the electric current flows through it.

- It is a light source based on semiconductors. When current passes through the LED, the electrons recombine with holes emitting light in the process.
- It is a specific type of diode having similar characteristics as the p-n junction diode. This means that an LED allows the flow of current in its forward direction while it blocks the flow in the reverse direction. Light-emitting diodes are built using a weak layer of heavily doped semiconductor material.
- Based on the semiconductor material used and the amount of doping, an LED will emit a colored light at a particular spectral wavelength when forward biased.

Working Principle of LED

- Like an ordinary diode, the LED diode works when it is forward biased. In this case, the n-type semiconductor is heavily doped than the p-type forming the p-n junction.
- When it is forward biased, the potential barrier gets reduced and the electrons and holes combine at the depletion layer (or active layer), light or photons are emitted or radiated in all directions.
- A typical figure blow showing light emission due electron-hole pair combining on forward biasing.



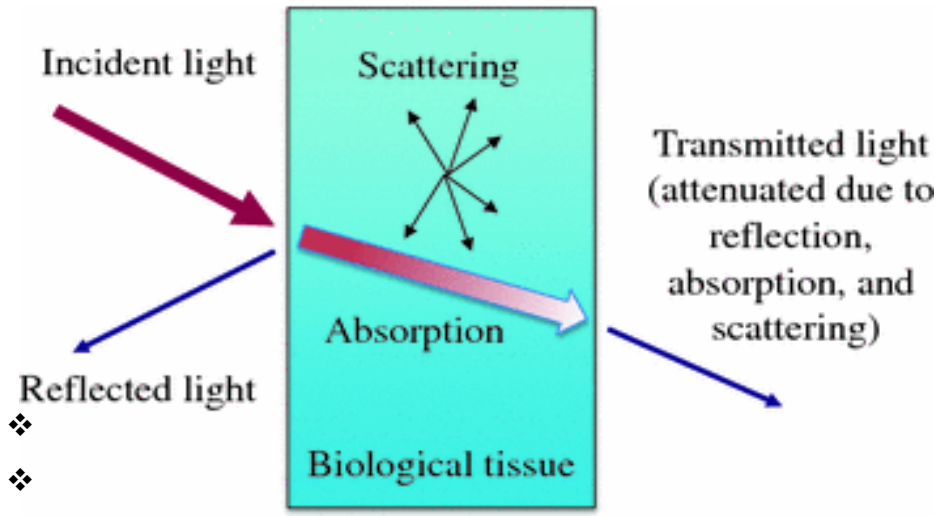
- The explanation behind the emission of photons in an LED diode lies in the energy band theory of solids.

- According to this theory, whether the electron-hole combining will give out photons or not depends on whether the material has a direct band gap or indirect band gap.
- Those semiconductor materials which have a direct band gap are the ones that emit photons.
- In a direct band gap material, the bottom of the energy level of conduction band lies directly above the topmost energy level of the valence band on the Energy vs Momentum (wave vector 'k') diagram.
- When electrons and hole recombine, energy E = hν corresponding to the energy gap o (eV) is escaped in the form of light energy or photons where h is the Planck's constant and ν is the frequency of light.

4. Highlight the photon transport theory to explain the light interaction with tissues.(Nov-2018,2019)

The light-tissue interactions in the diagnostic approach must by contrast be non-destructive and the main goal is to study the physiology or pathology of the tissue.

- Reflection
- Refraction
- Absorption
- Fluorescence
- Scattering



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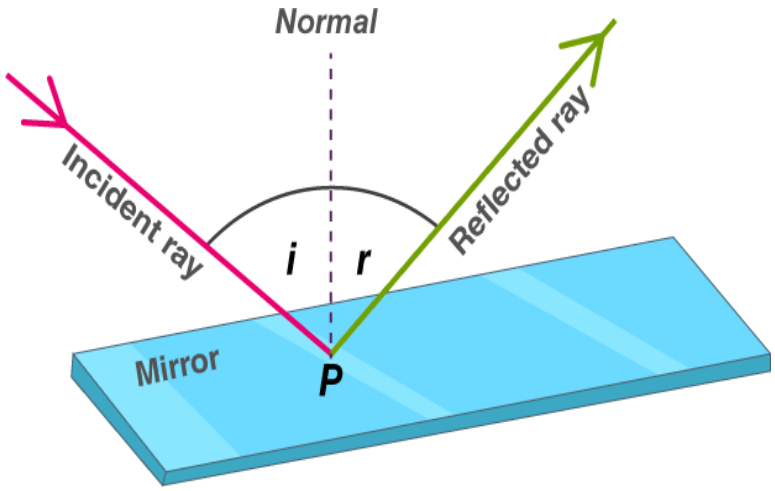
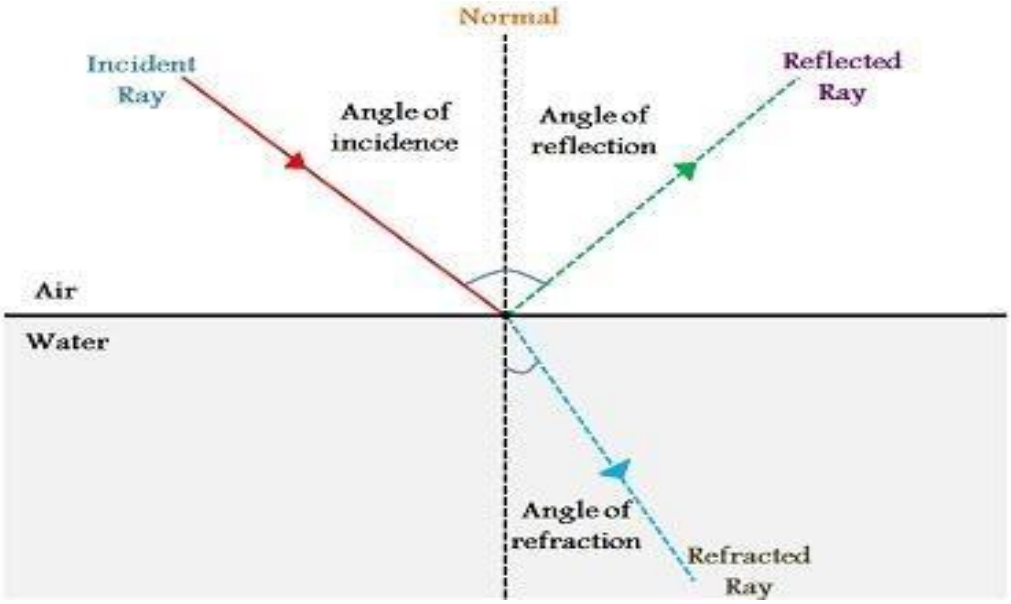
- Biological tissue is an aggregate of similar cells and cell products (tissue fibres and gels) forming a definite kind of structural material in normal or abnormal state.
- There are three major types of tissues, such as epithelial, connective, and glandular. More specifically tissues characterized as areolar, reticular, fibrous, tubular, and elastic.



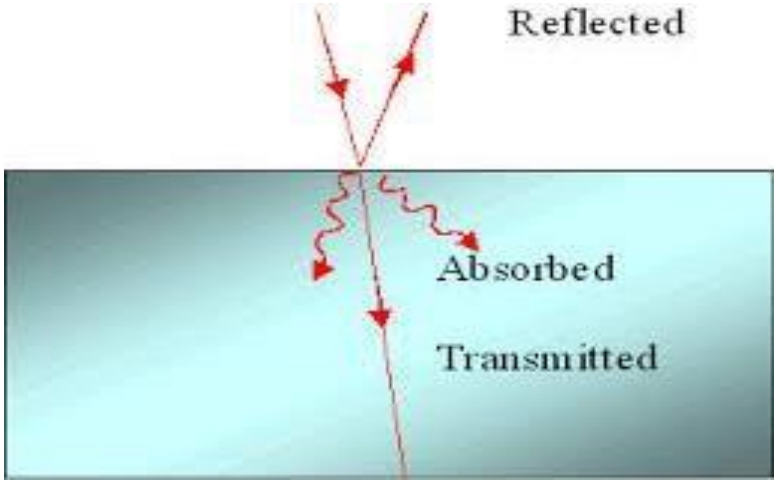
- Tissues are also could be identified as belonging to a specific organ or system, i.e., mucosal, muscular, nervous, cervical, eye, skin, lymphoid, and adipose (fat), or to pathology state, i.e., scar tissue and malignant tissue.
- Reflection is a change of direction of a wave front at an interface between two different media so that the wave fronts returns into the medium from which it is originated.
- Refraction is the bending of a wave when it enters a medium where its speed is different. Refraction is the change in direction of a wave passing from one medium to another or from the gradual change in the medium.

  Refraction of light is one of the most usually observed phenomena which include refraction of light through prism, but other waves like sound waves and water waves.



Absorption is defines as the process of absorbing something or of being absorbed Extraction of energy from light by a molecular species

- **Diagnostic applications**
- **Therapeutic applications**



Transitions between two energy levels of a molecule that are well defined at specific wavelength could serve as spectral fingerprint of the molecule. Various types of chromophores (Light Absorbers) in tissue. Wavelength – dependent absorption.

Tumour detection and other physiological assessments. Eg: Pulse oximeter.

Absorption of energy is the primary mechanism that allows light from source (LASER) to produce physical effects on tissue for treatment purpose.

Eg: LASIK(Laser Assisted in Situ Keratomileusis)
eye surgery ,tattoo removal.

5. **Explain about the solid state detectors with emphasis on time resolved and phase resolved detectors.(May-2019, Nov-2018,2019)**

- ❖ Relating to or denoting a spectroscopic technique in which a spectrum is obtained at a series of time intervals after excitation of the sample.
- ❖ Additional evidence for spectral relaxation comes from the time-resolved emission spectra that usually shows time-dependent shifts to longer wavelengths.
- ❖ Creating a phase-resolved spectrum for a periodic source allows the user to compare the spectrum from different portions of the cycle and look for changes that may occur as the source rotates or orbits.
- ❖ Previous versions of this thread used a different technique to filter the event file on PHASE that left the user to compute the correct exposure time.
- ❖ This new technique provides a way to filter the data using good time intervals (GTIs) so that the Datamodel can compute the exposure time automatically.
- ❖ DEFINITION: spectroscopy where time-dependent signals are measured Time-resolved spectroscopy (also called ultrafast spectroscopy) includes a wide range of spectroscopic methods which involve the measurement of time-dependent signals.
- ❖ Partly, the actual purpose is to study dynamic processes in certain materials on times scales between seconds and femtoseconds, or sometimes even shorter.
- ❖ In other cases, time dependencies only occur as part of a measurement method, while the actual quantities of interest are not time-dependent.

TYPES OF TIME RESOLVED DETECTORS USED SPECTROSCOPY

- ❖ Time-resolved photoemission spectroscopy and 2PPE
- ❖ Time-resolved fluorescence spectroscopy
- ❖ Time-resolved infrared spectroscopy
- ❖ Transient Absorption Spectroscopy
- ❖ Fluorescence Decay Measurements
- ❖ Coherent Time-resolved Methods
- ❖ Attosecond Pulse Spectroscopy

- Terahertz Spectroscopy
- Cavity Ring-down Spectroscopy

TYPES OF PHASE RESOLVED DETECTORS USED SPECTROSCOPY

- Phase-resolved surface plasmon interferometry of graphene
- Phase-resolved terahertz self-detection near field microscopy
- Pulse phase resolved spectroscopy
- Orbital phase resolved spectroscopy
- Phase-resolved optical emission spectroscopy

Time-resolved infrared spectroscopy (TRIR):

Three independent femtosecond laser systems used for generating ultrafast pulses in the mid-IR (and far infrared:THz) through the UV; infrared (InSb and MCT) and visible CCD multi-element focal plane array cameras and instrumentation for capturing transient spectra of samples with a single laser pulse; detection systems capable of making accurate time-resolved amplitude and phase measurements on THz pulses as they interact with samples; Optical parametric Amplifiers (OPA) and various nonlinear crystals for frequency conversion.

Time-resolved and phase-resolved vibrational spectroscopy by use of step-scan FT-IR:

Step-scan FT-IR is one of the most versatile and conceptually simple means of applying the power of interferometry to the study of time dependent phenomena. The fundamental advantage of the step-scan method is the separation of the spectral multiplexing from the time domain by collecting the data while the retardation is held constant. This permits impulse- response (time domain, or time-resolved) studies with temporal resolution limited only by the signal strength, detector rise time and speed of the electronics, as well as modulation- demodulation (frequency domain, or phase-resolved) studies at essentially any modulation frequency or by use of multiple simultaneous modulation frequencies, unhindered by the Fourier frequencies of the continuous-scan FT-IR method. Time-resolved fluorescence polarization spectroscopy and optical imaging of smart receptor-targeted contrast agents in tissues for cancer detection.

This detector focuses on time-resolved spectroscopy and enhanced near infrared (NIR) imaging using receptor-targeted contrast agents for prostate cancer detection. Two smart receptor-targeted contrast agents, Cybesin and Cytate, in aqueous solution and cancerous and normal prostate tissues were investigated using time-resolved spectroscopy. The time evolution of the fluorescence emitted from the contrast agents in solution was described using a set of first-order linear differential equations. An analytical polarization model was developed and used to extract rotational times and fluorescence anisotropies of the contrast agents in prostate tissues. Differences of rotational times and polarization anisotropies were observed for Cybesin (Cytate) in cancerous and normal prostate tissue, due to the preferred bonding of contrast agents with cancerous tissue cells, which offers high-contrast smart agents for imaging and distinguishing cancerous prostate tissues from normal tissues.

6. Describe the principle and working operation of high pressure arc lamp. (Nov-2019)

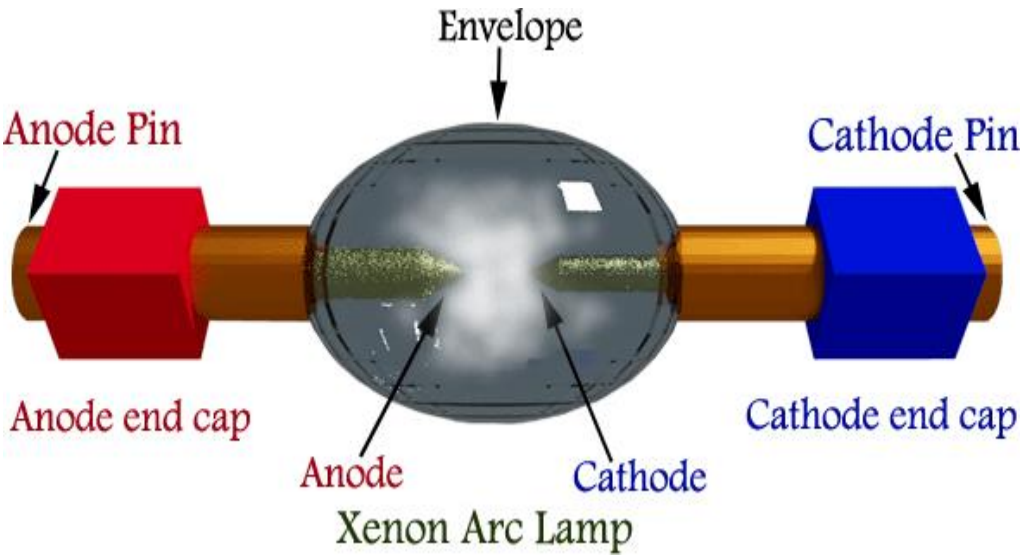
Xenon arc lamp:

A xenon arc lamp is a special type of gas discharge lamp. Xenon arc lamps produce light by passing electricity through ionized xenon gas at high pressure. It produces a bright white light that closely mimics natural sunlight, which extends its applications into the film, and daylight simulation industries.

Construction:

Thoriated tungsten is the tungsten added with 1 to 2% thorium to give extra strength to the arc by enhancing the electron emission capability of tungsten. Fused silica is also called quartz. It is non crystalline transparent silicon dioxide glass which provides extra strength and almost zero thermal expansion. It can withstand high pressure at high temperatures. The envelope or bulb is filled with xenon gas in very high pressure. The pressure inside the bulb is about 30 bars. Here, when voltage is applied across the electrodes, the gas discharge phenomenon starts in the xenon gas in the gap between electrodes. There are always present some free electrons in the gas. Due to the applied electric field across the electrodes, the free electrons get accelerated and collide with xenon atoms. Due to these collisions electrons from the

outer orbit of the xenon atoms get detached from their position and come to the higher energy level. Atoms with electrons of higher energy levels are called excited atoms.



When in the excited atoms, the electrons return from its higher energy level to its previous energy state, the extra energy is released as a photon. The wavelength of energy emitted through photons is within visual range. The color of the light of xenon arc light is like daylight. Due to the electrostatic attraction of anode or positive electrodes, the free electrons originated due to the ionization process ultimately comes to the anode and returns to the source.

Working:

Here, when voltage is applied across the electrodes, the gas discharge phenomenon starts in the xenon gas in the gap between electrodes. There are always present some free electrons in the gas. Due to the applied electric field across the electrodes, the free electrons get accelerated and collide with xenon atoms. Due to these collisions electrons from the outer orbit of the xenon atoms get detached from their position and come to the higher energy level.

Due to the electrostatic attraction of anode or positive electrodes, the free electrons originated due to the ionization process ultimately comes to the anode and returns to the source.

Due to the attraction of the cathode, the positive ions ultimately collide with the cathodes front surface and generate positive metal ions, neutral xenon atoms, and free electrons.

These electrons are called secondary emitted electrons. These electrons help to continue the gas discharging process. As the cathode is not additionally heated for electron emission, the cathode of xenon arc lamp or xenon lamp is known as a cold cathode.

Xenon arc lamps are used for:

- Specialized uses in industry and research to simulate sunlight
- Searchlights
- Movie projectors in theaters

7. Explain in detail the thermal effects of laser radiation.(Nov-2018)

- ❖ A laser can deposit a great amount of energy within a very small area (spot size), due to its collimation property, that allows the emission of non-divergent, parallel rays to generate minimum beam spread as they propagate over a distance.
- ❖ The diameter of the beam influences the amount of energy delivered by the laser, as light energy gets concentrated with the reduction of the beam diameter.
- ❖ Ordinary light is non-collimated. As it travels, its diameter spreads out, the beam spot size increases in diameter, and the light loses energy on its way.
- ❖ With non-collimated light beams, it is difficult to quantify the energy dosage delivered to its target from a distance, unless the beam is in direct contact with the tissue.

Thermal properties of the biological tissues

Boulinois classified laser effects into 4 groups, according to their biological interactions:

(1) Electromechanical effect

(2)Non-thermic effect: (photochemical, photophysical)

(3) Photo ablative effect

(4) Photothermal effect:

1. Vaporization
2. Coagulation
3. Protein Denaturation

Important aspects of laser-tissue interaction to be considered in biomedical studies are the thermal properties of the tissue and the thermal changes, caused by the interaction of light with the tissue.

- The thermal properties are related to the temperature distribution in the tissue. This is well explained in the study by Ansari., et al. where they state that, The transportation of thermal energy in biological tissue includes different phenomenological mechanisms such as thermal conduction, convection, radiation, metabolic activities and phase change, and further point out that a laser can induce multiple effects like coagulation, vaporization, carbonization or melting.
- They emphasize that these effects don't depend only on the thermal properties of the biological tissue, but also on the peak power and wavelength of the laser . Their explanation of the process is given as follows: "
- The vaporization of water occurs at 100°C. In the vaporization, or thermomechanical procedure, the temperature of tissue does not alter within the vaporization phase, and gas bubbles are formed.
- The propagation of these bubbles, along with the alteration of their volume, causes thermal decomposition of tissue fragments.
- If all water molecules are vaporized, carbon atoms are released and the adjacent tissues are blackened, causing the presence of smoke. This stage is called carbonization. Finally, beyond 300oC melting might occur.
- The distinct interaction principles of photophysical, photochemical, and photobiological mechanisms and other aspects of laser-tissue interaction is extensive and is also well covered in the literature.

BM E76 - MEDICAL OPTICS

UNIT-2 INSTRUMENTATION IN PHOTONICS

2 Marks:

1) Define Lambert-Beer's law (Nov/Dec 2018)

- The Beer-Lambert Law (also called Beer‟s Law) is a relationship between the attenuation of light through a substance and the properties of that substance.
- The Beer-Lambert law is a linear relationship between the absorbance and the concentration, molar absorption coefficient and optical coefficient of a solution:
- **A= ϵcl**

  Where **A** is Absorbance, **ϵ**is molar absorption coefficient in**M $^{-1}$cm^{-1}, c** is molar concentration in **M**, **l**is optical path length in **cm.**

2) What is meant by photoablation? (Nov/Dec 2018)

- **Laser ablation** or **photoablation** is the process of removing material from a solid (or occasionally liquid) surface by irradiating it with a laser beam.
- At low laser flux, the material is heated by the absorbed laser energy and evaporates or sublimates. At high laser flux, the material is typically converted to a plasma.
- Usually, laser ablation refers to removing material with a pulsed laser, but it is possible to ablate material with a continuous wave laser beam if the laser intensity is high enough.
- Excimer lasers of deep ultra-violet light are mainly used in photoablation; the wavelength of laser used in photo ablation is approximately 200 nm.

3) What are optical filters? (Nov/Dec 2019)

- An **optical filter** is a device that selectively transmits light of different wavelengths, usually implemented as a glass plane or plastic device in the optical path, which are either dyed in the bulk or have interference coatings.
- The optical properties of filters are completely described by their frequency response, which specifies how the magnitude and phase of each frequency component of an incoming signal is modified by the filter.

4) What is polarizer? How it helps in light detection? (Nov/Dec 2019, May 2019)

- **POLARIZER:**
  - A polarizer or polariser is an optical filter that lets light waves of a specific polarization pass through while blocking light waves of other polarizations.
  - It can filter a beam of light of undefined or mixed polarization into a beam of well-defined polarization, that is polarized light.
- **POLARIZERS IN LIGHT DETECTION:**
  - Distinguishing different directions of light – Polarizers can help to identify the normal direction of light and differentiate between vertical and horizontal light.
  - This feature is helpful to determine the source of light. Therefore, it is possible to eliminate both reflected light, and identify other properties of the light source.

5) What are xenon lamps? (May 2019)

- A xenon arc lamp is a highly specialized type of gas discharge lamp, an electric light that produces light by passing electricity through ionized xenon gas at high pressure.
- Xenon headlamps in automobiles are actually metal-halide lamps, where a xenon arc is only used during start-up to correct the color temperature.

11 Marks:

1) Give an account of the near field imaging of the biological structure (Nov/Dec 2018)

BASIC PRINCIPLES OF NEAR-FIELD OPTICAL MICROSCOPY

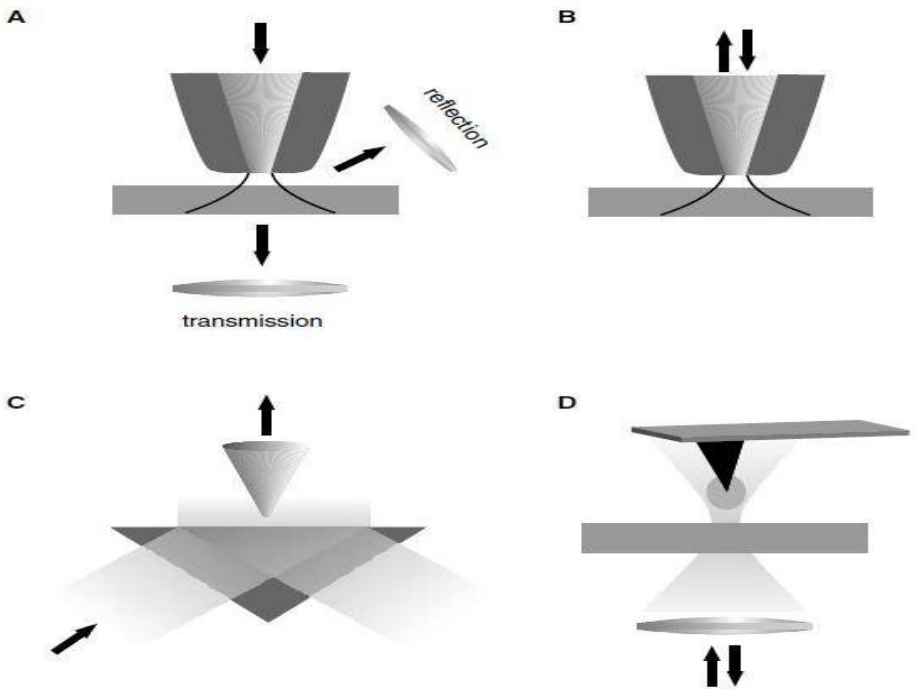
- Image formation in a classic (far-field) microscope is achieved by specimen illumination using a monochromatic plane wave. The object scatters the light in a characteristic way.
- The light is then collected by transmission or reflection and focused on a detector. The lens is several wavelengths away from the object, in the optical far-field.
- High spatial frequencies correspond to the fine details of the specimen and generate Fourier components of the field that decay exponentially along the normal object. Such frequencies cannot be collected by the lens.

- This effect is the well-known Abbé limit of diffraction, $\Delta x = 0.61.\lambda /NA$,
  where Δx corresponds to the smallest resolvable distance between two points,
  λ is the wavelength of the light, and
  NA is the numerical aperture of the microscope objective or the lens.
- If a confocal setup is used instead of wide-field illumination, the resolution increases slightly; multiphoton techniques can also improve the resolution.
- In contrast to far-field microscopy in which the light source is confined by a lens, in near-field optical microscopy the light source is confined by a metal aperture.
- Within a short distance beyond the screen, the size of the illuminated spot is limited only by the dimensions of the aperture.
- This area is the so-called optical near-field. If such a small light source is scanned above a surface and the distance between aperture and sample is in this near-field region, all scattering or absorption phenomena must originate from that small illumination spot. Consequently, aperture size and distance determine the resolution.

INSTRUMENTATION

General Considerations

- The key development for practical application of near-field optics was the fabrication of a functional sub-wavelength aperture. Instead of a hole in a planar metal screen, an aperture was formed at the apex of a pointed glass tip by coating it with a metal.
- This design ensured an easy way to approach the sample and keep it in close proximity to the surface, while still satisfying the optical requirement for the aperture size.
- The following figure shows some of the most important setups using aperture tips:
- Figure A shows the most common setup. The sample is illuminated through the aperture and the light is collected by reflection or transmission by standard optical techniques.
- Figure B shows an example in which the light is also collected back through the aperturein the illumination–collection mode.
- Figure C shows a more specialized arrangement that uses adifferent illumination by evanescent waves.
- Figure D illustrates a relatively new development without an aperture. Here, the light source is generated at the tip apex and can therefore be even smaller than any aperture. The most commonly used setup is the design depicted in A.
- The entire instrument is built around the core shown. It can be described as a microscope in which the standard illumination source has been replaced by a near-field probe.



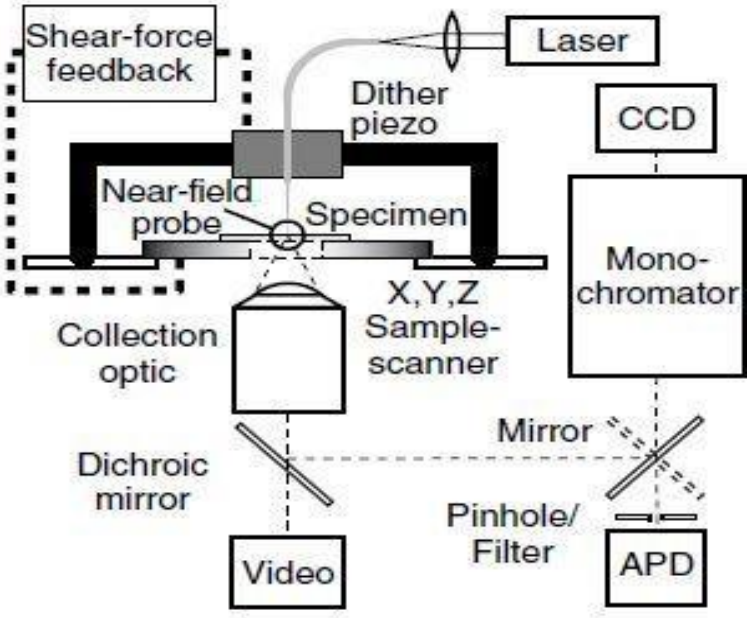
Different modes for near-field scanning optical microscopy:

(A) Illumination through the aperture, detection reflection or transmission via standard optics;

(B) Illumination–collection mode, both via the near-field aperture;

(C) Photon scanning tunneling microscope (PSTM);

(D) Apertureless or probe-enhanced techniques. The illuminated tip enhances the field and serves as a subwavelength light source.

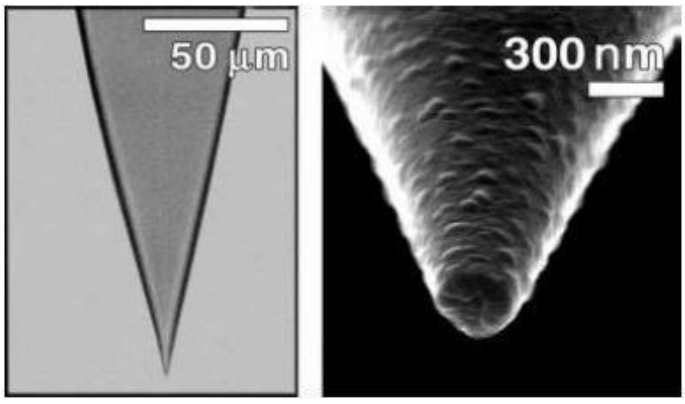


Standard experimental setup for transmission of NSOM. For specific analytic requirements different detection schemes can be applied.

- The above figure sketches the major parts of a modern NSOM. Laser light is coupled into the back end of the fiber probe, which is held above the sample at a distance of 5 to 15 nm by a shear-force feedback arrangement.
- This is a noncontact AFM mode where the tip is vibrated at its resonance frequency. As soon as the tip "feels" the surface this vibration is damped.
- The damping serves as the feedback signal for the tip–sample distance control and accordingly allows topographical mapping synchronously to the optical signal acquisition.
- The light is collected in transmission by an appropriate objective and, depending on the experimental requirements, simply imaged onto a detector.
- If spectral information is also desired, the collected light is dispersed by a spectrograph and then imaged onto a multichannel detector.
- To maintain the alignment of near-field probe and collection optics the sample is scanned underneath the tip; hence, the image is built up, point by point.

Near-Field Optical Probes:

- The fabrication of near-field optical probes is a crucial prerequisite for the experiment. Aperture size, efficiency of light transmission, and the damage threshold are considerabions in probe fabrication.
- Because large taper angles result in a higher transmission, the goal is to produce probes with a large angle that are still able to approach the sample surface and yield a useful topographical image.
- The straightforward approach would be a microfabrication similar to the process used to manufacture AFM cantilevers.



Optical (left) and scanning electron microscope image (right) of a "tube-etched" near-field probe

- The above figure is an example of a tube-etched near-field probe and a zoom into the aperture region.Finally, the near-field tip must be mounted into the scanning probe head.
- Here the dithering necessary for the feedback loop will be applied by piezoactuators or quartz tuning forks.

2) Explain about solid state detectors with emphasis on time resolved and phase resolved detectors (Nov/Dec 2018, Nov/Dec 2019)

- **TIME RESOLVED**
  - Relating to or denoting a spectroscopic technique in which a spectrum is obtained at a series of time intervals after excitation of the sample.
  - Additional evidence for spectral relaxation comes from the time-resolved emission spectra that usually show time-dependent shift to longer wavelengths.
- **PHASE RESOLVED**
  - Creating a phase-resolved spectrum for a periodic source allows the user to compare the spectrum from different portions of the cycle and look for changes that may occur as the source rotates or orbits.
  - Previous versions of this thread used a different technique to filter the event file on PHASE that left the user to compute the correct exposure time.
  - This new technique provides a way to filter the data using good time intervals (GTIs) so that the Datamodel can compute the exposure time automatically.
- **TIME-RESOLVED SPECTROSCOPY**
  - DEFINITION: spectroscopy where time-dependent signals are measured
  - Time-resolved spectroscopy (also called ultrafast spectroscopy) includes a wide range of spectroscopic methods which involve the measurement of time-dependent signals.
  - Partly, the actual purpose is to study dynamic processes in certain materials on times scales between seconds and femtoseconds, or sometimes even shorter.
  - In other cases, time dependencies only occur as part of a measurement method, while the actual quantities of interest are not time-dependent.
- **TYPES OF TIME RESOLVED DETECTORS USED SPECTROSCOPY**
  - Time-resolved photoemission spectroscopy and 2PPE
  - Time-resolved fluorescence spectroscopy
  - Time-resolved infrared spectroscopy

- Transient Absorption Spectroscopy
- Fluorescence Decay Measurements
- Coherent Time-resolved Methods
- Attosecond Pulse Spectroscopy
- Terahertz Spectroscopy
- Cavity Ring-down Spectroscopy

➢ **TYPES OF PHASE RESOLVED DETECTORS USED SPECTROSCOPY**

- Phase-resolved surface plasmon interferometry of graphene
- Phase-resolved terahertz self-detection near field microscopy
- Pulse phase resolved spectroscopy
- Orbital phase resolved spectroscopy
- Phase-resolved optical emission spectroscopy

➢ **TIME-RESOLVED INFRARED SPECTROSCOPY (TRIR)**

Three independent femtosecond laser systems used for generating ultrafast pulses in the mid-IR (and far infrared:THz) through the UV; infrared (InSb and MCT) and visible CCD multi-element focal plane array cameras and instrumentation for capturing transient spectra of samples with a single laser pulse; detection systems capable of making accurate time-resolved amplitude and phase measurements on THz pulses as they interact with samples; Optical parametric Amplifiers (OPA) and various nonlinear crystals for frequency conversion.

➢ **TIME-RESOLVED AND PHASE-RESOLVED VIBRATIONAL SPECTROSCOPY BY USE OF STEP-SCAN FTIR**

- Step-scan FT-IR is one of the most versatile and conceptually simple means of applying the power of interferometry to the study of time dependent phenomena.
- The fundamental advantage of the step-scan method is the separation of the spectral multiplexing from the time domain by collecting the data while the retardation is held constant.
- This permits impulse- response (time domain, or time-resolved) studies with temporal resolution limited only by the signal strength, detector rise time and speed of the electronics, as well as modulation- demodulation (frequency domain, or phase-resolved) studies at essentially any modulation frequency or by use of multiple simultaneous modulation frequencies, unhindered by the Fourier frequencies of the continuous-scan FT-IR method.

- **TIME-RESOLVED FLUORESCENCE POLARIZATION SPECTROSCOPY AND OPTICAL IMAGING OF SMART RECEPTOR-TARGETED CONTRAST AGENTS IN TISSUES FOR CANCER DETECTION**
    - This detector focuses on time-resolved spectroscopy and enhanced near infrared (NIR) imaging using receptor-targeted contrast agents for prostate cancer detection.
    - Two smart receptor-targeted contrast agents, Cybesin and Cytate, in aqueous solution and cancerous and normal prostate tissues were investigated using time-resolved spectroscopy.
    - The time evolution of the fluorescence emitted from the contrast agents in solution was described using a set of first-order linear differential equations.
    - An analytical polarization model was developed and used to extract rotational times and fluorescence anisotropies of the contrast agents in prostate tissues.
    - Differences of rotational times and polarization anisotropies were observed for Cybesin (Cytate) in cancerous and normal prostate tissue, due to the preferred bonding of contrast agents with cancerous tissue cells, which offers high-contrast smart agents for imaging and distinguishing cancerous prostate tissues from normal tissues.

3) Describe the principle and working of high pressure arc lamp. (Nov/Dec 2019)

- **HIGH PRESSURE ARC LAMP**
    - A xenon arc lamp is a highly specialized type of gas discharge lamp, an electric light that produces light by passing electricity through ionized xenon gas at high pressure.
    - Xenon headlamps in automobiles are actually metal-halide lamps, where a xenon arc is only used during start-up to correct the color temperature.
- **PURPOSE OF ARC LAMP**
    - Invented decades before it could be used, the first type of electric light was so brilliant it was used for lighthouses and street lamps.
    - An arc lamp produces light by the sparking (an electrical arc) of a high current between two conducting electrodes, usually carbon rods.
- **TYPES OF LAMPS**

- Incandescent lamps
- Low pressure mercury vapour lamp
- Lamps (Fluorescent tubes)
- Halogen lamps
- Sodium vapour lamp
- High pressure mercury vapour lamp
- Compact Fluorescent Lamps
- Metal Halide Lamps
- LED Lamps
- Neon Signs

➢ **INCANDESCENT LAMPS (FILAMENT LAMPS)**

- The electrical light source which works on the principle of incandescent phenomenon is called **Incandescent Lamp**.
- In other words, the lamp working due to glowing of the filament caused by electric current through it, is called **incandescent lamp**.

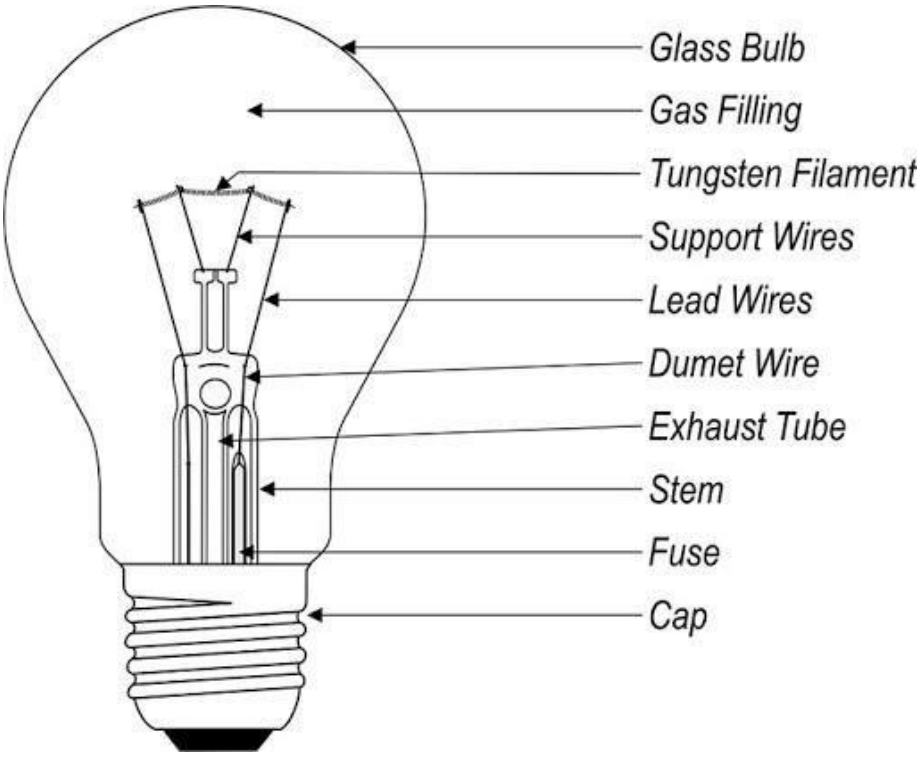
➢ **WORKING OF INCANDESCENT LAMPS**

- When an object is made hot, the atoms inside the object become thermally excited. If the object does not melt, the outer orbit electrons of the atoms jump to higher energy level due to the supplied energy.
- The electrons on these higher energy levels are not stable, they again fall back to lower energy levels.
- While falling from higher to lower energy levels, the electrons release their extra energy in a form of photons.
- These photons are then emitted from the surface of the object in the form of electromagnetic radiation.
- This radiation will have different wavelengths. A portion of the wavelengths is in the visible range of wavelengths, and a significant portion of wavelengths are in infrared range. The electromagnetic wave with wavelengths within the range of infrared is heat energy and the electromagnetic wave with wavelengths within visible range is light energy.
- Incandescent means producing visible light by heating an object. An **incandescent lamp** works in the same principle.

- The simplest form of the artificial source of light using electricity is an incandescent lamp.
- Here we use electric current to flow through a thin and fine filament to produce visible light. The current rises the temperature of the filament to such extent that it becomes luminous.

➢ CONSTRUCTION OF INCANDESCENT LAMPS

- The filament is attached across two lead wires. One lead wire is connected to the foot contact and other is terminated on the metallic base of the bulb.
- Both of the lead wires pass through glass support mounted at the lower middle of the bulb. Two support wires also attached to glass support, are used to support filament at its middle portion. The foot contact is isolated from metallic base by insulating materials.



Incandescent lamp

- The entire system is encapsulated by a colored or phasphare coated or transparent glass bulb. The glass bulb may be filled with inert gases or it is kept vacuum depending upon rating of the incandescent lamp.
- The filament of **incandescent lamps** is air-tightly evacuated with a glass bulb of suitable shape and size.

- This glass bulb is used to isolate the filament from surrounding air to prevent oxidation of filament and to minimize convention current surrounding the filament hence to keep the temperature of the filament high.
- The glass bulb is either kept vacuum or filled with inert gases like argon with a small percentage of nitrogen at low pressure. Inert gases are used to minimize the evaporation offilament during service of the lamps. But due to convection flow of inert gas inside the bulb, there will be greater chances of losing the heat of filament during operation.
- Again vacuum is a great insulation of heat, but it accelerates the evaporation of filament during operation. In the case of gas-filled incandescent lamps, 85% of argon mixed with 15% of nitrogen is used.
- Occasionally krypton can be used to reduce filament evaporation because the molecular weight of krypton gas is quite higher.

➢ **FILAMENT OF INCANDESCENT LAMP**

- In present days, **incandescent lamps** are available in different wattage ratings such as 25, 40, 60, 75, 100 and 200 watts etc.
- There are different shapes of bulbs, but basically, all are rounded in shape. There are mainly three materials used for producing the filament of incandescent lamps, and these are carbon, tantalum, and tungsten.
- Carbon was previously used for filament material, but presently tungsten is used most for the purpose.
- The melting point of carbon filament is about 3500^{o}C, and the operating temperature of this filament is about 1800^{o}C hence the chance of evaporation is quite less. Because of that carbon filament, incandescent lamps are free from darkening due to filament evaporation.
- Darkening of filament lamp occurs when molecules of filament material are deposited on the inner wall of the glass bulb due to evaporation of filament during operations.

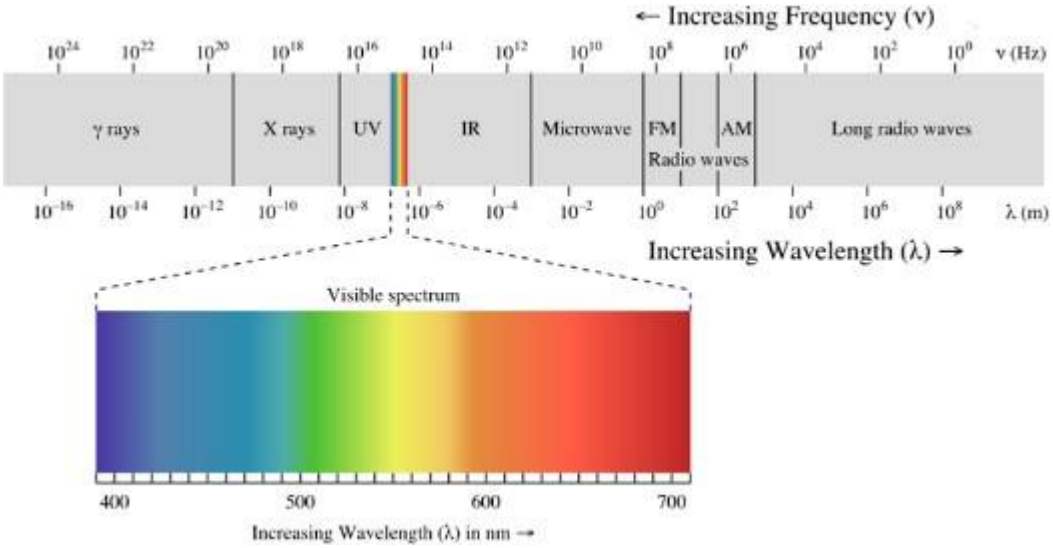
➢ **FILAMENT LAMPS MUST HAVE**

- It should have high melting point (3500^{o} C).
- It should have high resistivity.
- It should have low temperature co-efficient.
- It should have low vapour pressure.

- Mechanically Strong, ductile.
- Material used for filament is Carbon, Osmium tantalum and Tungsten.
- Gas used inside the lamp (Nitrogen or Argon)
- Life: 1000 Hrs working hrs.
- Lamp efficiency: 10 - 30 lumens/watt

4) Write in detail about any two excitation light sources (May 2019)

The light source used in a fluorescence microscopy experiment must emit the specific wavelengths of light that excite the fluorophores present in the sample. When imaging live cells, lower intensities of excitation light are best to avoid damaging the cell through photo bleaching and photo toxicity.



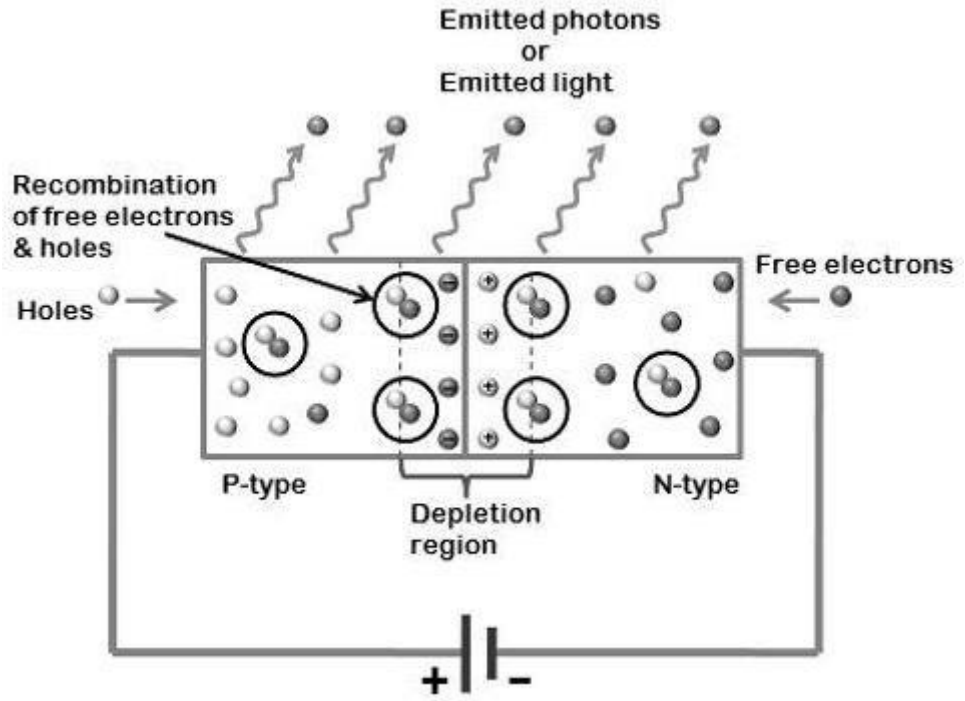
LIGHT EMITTING DIODE (LED):

A light releasing diode is an electric component that emits light when the electric current flows through it. It is a light source based on semiconductors. When current passes through the LED, the electrons recombine with holes emitting light in the process. It is a specific type of diode having similar characteristics as the p-n junction diode. This means that an LED allows the flow of current in its forward direction while it blocks the flow in the reverse direction. Light-emitting diodes are built using a weak layer of heavily doped semiconductor material. Based on the semiconductor material used and the amount

of doping, an LED will emit a colored light at a particular spectral wavelength when forward biased.

Working Principle of LED

Like an ordinary diode, the LED diode works when it is forward biased. In this case, the n-type semiconductor is heavily doped than the p-type forming the p-n junction. When it is forward biased, the potential barrier gets reduced and the electrons and holes combine at the depletion layer (or active layer), light or photons are emitted or radiated in all directions. A typical figure blow showing light emission due electron-hole pair combining on forward biasing.



The explanation behind the emission of photons in an LED diode lies in the energy band theory of solids. According to this theory, whether the electron-hole combining will give out photons or not depends on whether the material has a direct band gap or indirect band gap. Those semiconductor materials which have a direct band gap are the ones that emit photons.

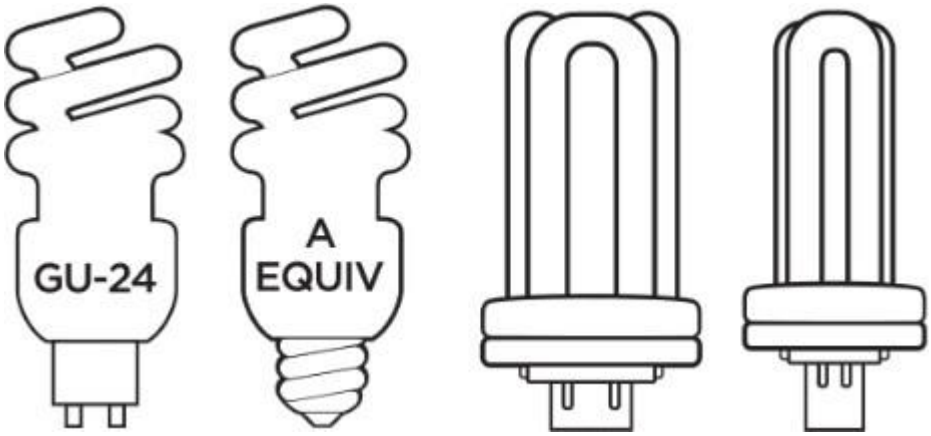
In a direct bandgap material, the bottom of the energy level of conduction band lies directly above the topmost energy level of the valence band on the Energy vs Momentum (wave vector „k“) diagram. When electrons and hole recombine, energy E = hν corresponding to the energy gap o (eV) is escaped in the form of light energy or photons where h is the Planck“s constant and ν is the frequency of light.

Compact fluorescent lamp (CFL)

A compact fluorescent lamp (CFL), also called compact fluorescent light, energy-saving light and compact fluorescent tube, is a fluorescent lamp designed to replace an incandescent light bulb; some types fit into light fixtures designed for incandescent bulbs. The lamps use a tube which is curved or folded to fit into the space of an incandescent bulb, and a compact electronic ballast in the base of the lamp.

Working principle of CFL:

A CFL uses vacuum pipe which is principle wise same to the strip lamps (commonly known as Tube light) . Tube has two electrodes on both ends which is treated with Barium. Cathode is having a temperature of about 900º C and generates a beam of electrons which is further accelerated by potential difference between electrodes.

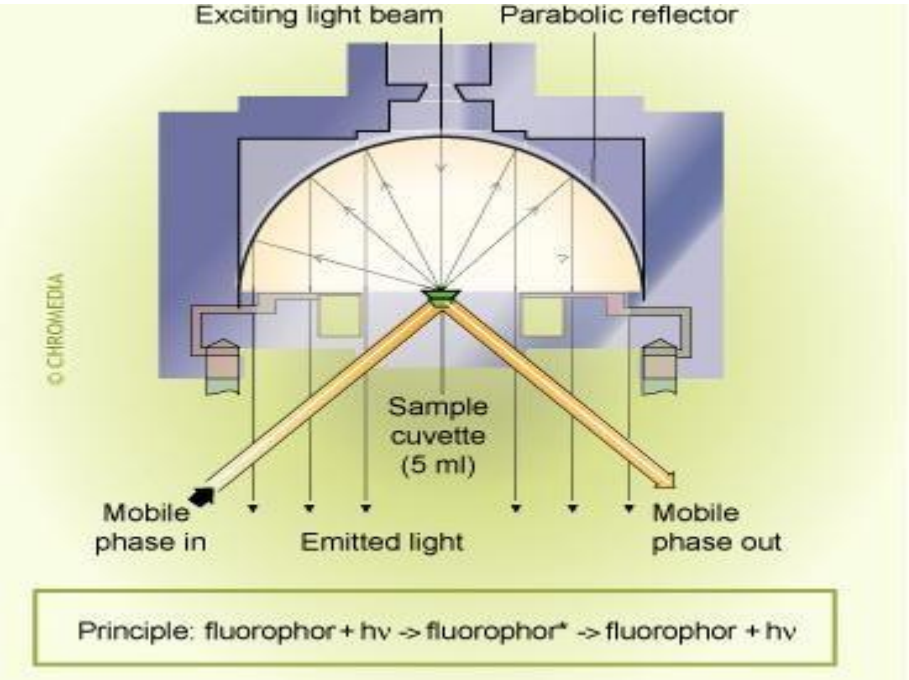


These accelerated electrons strike Mercury and Argon atoms which in turn results in the arise of a low temperature plasma. This process initiates the radiation of Mercury in Ultra violet form. Tube"s inside face contains „Luminophore" whose function is to convert Ultra violet light into visible light.

This tube is fed with AC power supply which facilitate the changing functionality of Anode and Cathode. The CFL also consists a switched mode converter. It functions on a very high frequency and acts as a replacement of ballast (choke) and starter assembly.

5) Briefly describe about the time resolved fluorescent detectors (May 2019)

A fluorescence detector excites the sample with excitation light and breaks up the emitted fluorescence light with a fluorescence monochromator. It extracts the required fluorescence wavelengths and measures the intensity with a photomultiplier.



The layout of a fluorescence detector cell is seen above. Detection is at 90 degrees to the incident light to minimize interference from scattered light. The parabolic reflector maximizes the collection of emitted photons.

Fluorescence detectors typically excite fluorophores with a specific wavelength (selected with either a filter or a monochromator), then monitor emission at a different (longer) wavelength selected with another filter or monochromator. The excitation light is removed by the second filter or monochromator, allowing only the emitted light to strike the transducer.

A limited number of compounds exhibit their own (native) fluorescence. Generally speaking, the more double bonds (especially conjugated double bonds) present in a molecule, the larger the fluorescence intensity. Because only a limited subset of molecules are fluorescent, fluorescence detectors are considered selective detectors.

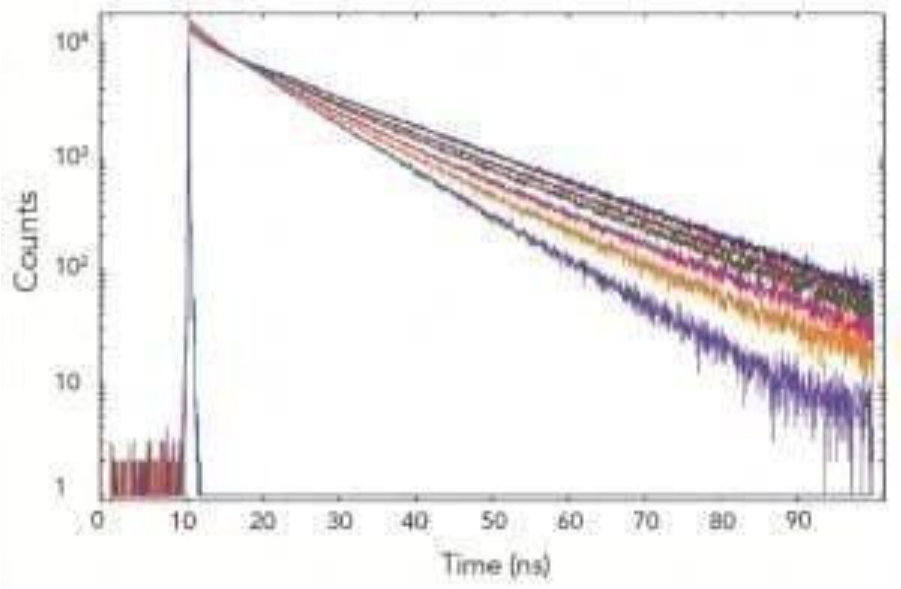
The selectivity of a fluorescent detector can be increased by proper choice of excitation and emission wavelengths. Ideally, interfering components are not detected because they do not absorb at the chosen excitation wavelength and/or do not emit at the

chosen emission wavelength. In addition to its selectivity, fluorescence is very well suited for trace analyses due to its signal to noise properties. Fluorescence detectors are often used in series with a variable wavelength UV detector, so both signals can be monitored for optimal sensitivity and selectivity.

Time-Resolved Fluorescence:

Time-Resolved Fluorescence (or Fluorescence Lifetime) Spectroscopy is an extension of Steady State Fluorescence. When we discuss time-resolved fluorescence or fluorescence lifetimes, what we are studying is the fluorescence of a sample monitored as a function of time after excitation by a pulse of light. Fluorescence lifetimes, occurring as emissive decays from the singlet-state, can also be approximated as those decays occurring in the time region from picoseconds to nanoseconds.

The time-resolution can be obtained in a number of ways, depending on the required sensitivity and time regions. Edinburgh Instruments employs the technique called Time-Correlated Single Photon Counting (TCSPC), for Time-Resolved Fluorescence, which is used for the acquisition of single photons and allows for time resolutions in the range of picoseconds(ps) to nanoseconds(ns).



This technique is a digital counting technique, counting photons that are time-correlated in relation to a short excitation light pulse. In TCSPC the sample is repetitively excited using a pulsed light source with a high repetition rate. During the measurement a probability histogram builds, which relates the time between an excitation pulse (START) and the observation of the first fluorescence photon (STOP). The fact that the time at which a

fluorescence photon is incident on the detector can be defined with pico second resolution is critical to the operation and precision of TCSPC. To study lifetime decays slower than this (ns to seconds time range) please see Phosphorescence Lifetime.

MEDICAL OPTICS BM E76

UNIT - III

APPLICATIONS OF LASERS

2 MARKS

1. Discuss the various advantages of laser induced fluorescence.(Nov/Dec 2018)

- LIF is a technique with high selectivity.
- LIF imaging is particularly attractive due to the strength of the resonant absorption process compared with the non-resonant Rayleigh and Raman techniques.
- Sensitivity and selectivity are the two main advantages of the LIF technique.

2. Discuss Laser in Dermatology. (Nov/Dec 2018)

Laser treatment can improve the appearance of acne scars, which are permanent and can be troublesome for some individuals. The light in this case targets different layers of the skin, helping to remove damaged tissue, while stimulating new collagen cells and boosting growth of fresh, healthy skin.

(Or)

In dermatology, the lasers are discussed through Rosacea, Thread veins, Hair removal, Acene Scars.

3. State the property of laser. (Nov/Dec 2019, May 2019)

- **Monochromatic:**

  It means that it consist of one color or wavelength

- **Divergence and directionality:**

  It means that the beam is well collimated and travels long distance with very little spread.

- **Coherence:**

  It means that all the individual waves of light are moving preciesly together through time and space.

- **Brightness:**

  The radiance of laser is an important factor. It is defined as the power emitted per unit surface area per unit solid angle.

4. List few applications of lasers. (Nov/Dec 2019)

- Lasers in medicine
- Lasers in communications
- Lasers in industries
- Lasers in science and technology
- Lasers in military

5. What are the benefits of OCT. (May 2019)

Optical coherence tomography (OCT) is an imaging technique that uses low-coherence light to capture micrometer-resolution, two- and three-dimensional images from within optical scattering media (e.g., biological tissue). It is used for medical imaging and industrial nondestructive testing (NDT).

6. List the benefits of laser tissue soldering? (Sep 2020)

- precise working with exact placing of the energy spot
- welding of complicated joint geometry
- low heat application, therefore minor changes in microstructure
- low thermal distortion
- cavity-free welds

- low post weld operation times
- Large working distance is possible (welding up to 500 mm distance and also to inaccessible parts).

7. Enumerate the various steps involved in selective photo thermolysis? (Sep 2020)

All in all, selective photothermolysis refers to using light to heat and destroy tissue in a selective area of the body.

Conditions Treated

- Severe inflammatory acne (light-absorbing particles are delivered into enlarged sebaceous glands.)
- Laser hair removal (photoepilation.)
- Tattoo removal.

11 MARKS

1. What are the applications of Lasers in ophthalmology. (Nov/Dec 2018 , May 2019)

Ophthalmology was the first medical field where lasers have found an application. Over more than five decades, use of lasers in ophthalmology has successfully shown effective and safe results in treating various eye conditions. Whether lasers are used to correct vision or repair damage due to degenerative diseases dictates optimal laser type, wavelength, and pulse length.

Refractive Eye Surgery

Since the introduction of lasers in refractive eye surgery, technology has continually evolved to refine the technique and minimize the risk of damage to the retina and optic nerve. Femto second lasers are used in conjunction with other technology in LASIK (laser in situ kerato mileusis) procedures to correct myopia (near sight edness). New alternatives utilize fem to second-only lasers to create lamellar flaps necessary for the procedure. Alternatively, an

approach used to correct hyperopia (far sight edness) and some forms of astigmatism is called Laser Thermal Keratoplasty. Here, a Ho:YAG laser is used to heat the cornea tissue, which contracts the collagen and reshapes the cornea in a controlled manner.

Cataract Treatment

Cataracts, or clouding of the eye's natural lens, are the most common cause of vision loss in the elderly population. Accordingly, cataract surgery is the most commonly performed surgical procedure in the world, Treatment involves removal of the lens for a prosthetic replacement. Lasers have recently been developed as a means to remove the lens via photodisruption. Femtosecond lasers are used in the treatment of cataracts and take advantage of the extremely high peak power densities to efficiently disrupt tissue with minimal surrounding thermal damage.

Retinal (Micro) Coagulation

Diabetic macular edema is the most common cause of visual loss in persons under 50 years of age in the developed world. Retinal photocoagulation has been developed and recognized as a standard of treatment. Recently, the procedure was perfected due to introduction of micro-coagulation technique employing pulsed diode lasers.

2. Explain How Laser is used in urology and otolaryngology and Dermatology.(Nov/Dec 2018)

Lasers in Urology

Over the course of the past five decades, this technology has evolved into a highly specialized entity, also finding a niche market in the field of urology. Lasers obtained from various lasing mediums producing amplified light of different wavelengths have been tested for urological applications. Today. these lasers are most commonly used in the surgical management of benign prostatic hyperplasia and as intracorporeal lithotripters. Other uses include ablation of various urologic tumors and incising strictures of the upper- and lower urinary tract. A continuous process of evolution of this technology is taking place, resulting in surgical lasers becoming ever safer, more effective, and more affordable.

Today, the types of lasers most commonly used in urology include:

- Nd:YAG;
- Ho:YAG (holmium:YAG);
- Thu:YAG (thulium:YAG); • CO. (carbon dioxide);
- KTP (potassium titanyl phosphate);
- LBO (lithium • diode laser.

Laser applications in urology

The ability of the laser to ablate prostatic tissue with minimal hemorrhage has concentrated most of the interest in urologically applied lasers to benign prostatic hyperplasia (BPH). Despite tremendous advances in the surgical and minimally invasive treatment of BPH, transurethral resection of the prostate (TURP) is still considered the 'gold standard" The risks of TURP are always mentioned when discussing the reasons for seeking alternative treatment modalities for BPH. Bleeding certainly remains a concern, especially in patients on some form of anticoagulation (heparin, coumarin related compounds, antiplatelet agents) or those with prostates in excess of 60-80 g. On the other hand, with the availability of transurethral reec tion in saline (TURIS), the TURP syndrome is a nowadays considered by many to be a relatively rare complication.

Although removal of benign prostatic tissue using a laser was first described in 1986, it was only in 1990 that introduction of the side-firing (deflecting device at the tip: 60-90) laser prompted more widespread use of this modality. The Nd:YAG laser was initially the laser most commonly used and is also the one most exten sively studied. One of the earliest techniques used for Nd:YAG laser treatment of BPH was called "visual laser ablation of the prostate' (VLAP).This involves lasing prostatic tissue in a noncontact fashion to create an area of heat-induced coagulative necrosis that extends about 10 mm into the tissue. The method is rea sonably simple to learn and perform, is safe in anticoagulated patients and carries no risk the TRUP syndrome.

Lasers In Otolaryngology

Otolaryngology - Head and Neck Surgery - was one of the first areas in surgery where lasers found application and transformed clinical care. The carbon dioxide laser (CO2) remains the workhorse in the treatment of disorders of the upper aero digestive tract in which non-contact, precise and hemostatic surgery is necessary. The role of the CO2 laser has expanded substantially in recent years and now is used to endoscopically remove advanced laryngeal and pharyngeal cancers, which previously required removal via a neck incision. Similarly, KTP: (Nd:YAG) and Ho:YAG lasers have been used to manage diseases in the nose, paranasal sinuses, larynx, and trachea.

Advances in lasers in Otolaryngology

Breathtaking advances in fiber laser technology and other non-conventional laser devices have led to new applications in office-based laryngeal and upper airway surgery:

- Pulsed dye lasers are being more broadly adopted for treatment of a wide range of true vocal fold and laryngeal disorders.
- Lasers are used to treat ear disease and cranial base disorders as well.
- In otosclerosis, which is a fixation of the stapes footplate, “optical drilling” facilitates the precise removal of bone to improve hearing.
- Lasers are also used to reduce or eradicate vascular lesions and neoplasms in infants, children, and adults that obstruct the airway.
- As facial plastic surgeons, otolaryngologists also apply various laser wavelengths to resurface the face, reshape cartilage of the ear and nose, and manage vascular malformations.

Currently, head and neck surgeons are at the forefront of using minimally invasive approaches such as imaged guided surgery, robotics and lasers to treat diseases within the head, neck, and upper airway.

Lasers in Dermatology

Wound healing Lasers operating at energies (low power lasers) that do not produce any thermal effect are able to produce photobioactivation. They are used for the treatment of leg ulcers and other chronic wounds**.**

Use of Laser in dermatology

Vascular lesions Flashlamp-pumped pulsed dye laser is used in the treatment of port wine stains. It is also used in superficial capillary haemangiomas. Replacing the Argon Laser, the Pulsed Dye Laser became the preferred laser for the treatment of vascular lesions. Including spider veins, strawberry birthmarks and port wine stains and a whole range of cutaneous vascular anomalies.

Removing of skin lesions For Removal of benign skin lesion, such as moles. warts, keratoses CO2 lasers are the preferred. They are used also for shaving, cutting, dermabrading and resurfacing sears.

Pigmented lesions Low-power continuous wave lasers of appropriate wavelength can be used successfully to treat melanin-containing lesions . The Q-Switched Nd: YAG is effective for treatment of pigmented lesions, including removal of black tattoos. Implanted dermal pigment may be treated with the carbon dioxide laser .The Ruby laser (emits red light is used in the treatment of tattoos and some other pigmented lesions including freckles. liver spots. Nevus of Ota, cafe-au-lait spots (in Q-Switched mode).

Hair removal The Alexandrite Laser removes the hair in millisecond-rangefin pulsed mode). Nd:YAG laser light is also effective for long-term hair removal.

Resurfacing Acne scars and extensive areas of skin damaged by photoageing have . good results with the use of carbon dioxide laser.

3. Explain The clinical application of lasers in urology and discuss it's mertis and demerits. (Nov/Dec 2019)

Laser applications in urology

The ability of the laser to ablate prostatic tissue with minimal hemorrhage has concentrated most of the interest in urologically applied lasers to benign prostatic hyperplasia (BPH). Despite tremendous advances in the surgical and minimally invasive treatment of BPH, transurethral resection of the prostate (TURP) is still considered the 'gold standard" The risks of TURP are always mentioned when discussing the reasons for seeking alternative treatment modalities for BPH. Bleeding certainly remains a concern, especially in patients on some form of anticoagulation (heparin, coumarin related compounds, antiplatelet agents) or those with prostates in excess of 60-80 g. On the other hand, with the availability of transurethral resec tion in saline (TURIS), the TURP syndrome is a nowadays considered by many to be a relatively rare complication.

Although removal of benign prostatic tissue using a laser was first described in 1986, it was only in 1990 that introduction of the side-firing (deflecting device at the tip: 60-90) laser prompted more widespread use of this modality. The Nd:YAG laser was initially the laser most commonly used and is also the one most exten sively studied. One of the earliest techniques used for Nd:YAG laser treatment of BPH was called "visual laser ablation of the prostate' (VLAP).This involves lasing prostatic tissue in a noncontact fashion to create an area of heat-induced coagulative necrosis that extends about 10 mm into the tissue. The method is rea sonably simple to learn and perform, is safe in anticoagulated patients and carries no risk the TRUP syndrome.

Merits of laser in urology

- Immediate relief of symptoms – Rapid evacuation during recovery.
- Fewer symptoms of irritation and side effects.
- Catheterization limited by all, to less than 24 hours (as opposed to other methods, where the catheter remains in the patient for 2-5 days).
- The patient usually leaves either the same or the next day.

- Workable even in outpatients.
- Rapid recovery: The patient returns to normal activities within a few days.
- Safe use of the laser in patients with implanted pacemakers or defibrillators.

Demerits of laser in Urology

It is used for incisions, resection, and ablation and can be passed through an optical fiber and thus through endoscopic instruments. One disadvantage of KTP laser energy is that tissue carbonization can be observed, rather than a true ablative effect.

4.List the Various types of lasers used in clinical side and specify it's applications. (Nov/Dec 2019)

1. Helium Neon Laser:

The first CW system was the helium neon (HeNe) gas mixture. Although its first successful operation was at an infrared wavelength of 1.15 μm, the HeNe laser is most well known operating at the red 633 nm transition. Some HeNe lasers today also can emit operate at other wavelengths (594 nm, 612 nm, 543 nm). Some earlier HeNe lasers were excited by radio frequency (RF) discharge but virtually all HeNe lasers today are driven by a small DC discharge between electrodes in the laser tube.

The HeNe laser operates by an excitation of the helium atoms from the ground state. This energy excess is coupled to an unexcited neon atom by a collisional process with the net result of an inversion in the neon atom population, thus allowing laser action to begin. Power levels available from the HeNe laser ranges from a fraction of a milliwatt to about 75 milliwatts in the largest available systems.

2. Argon, Krypton, and Xenon Ion Lasers

The family of ion lasers utilize argon, krypton, xenon, and neon gases to provides a source for over 35 different laser frequencies, ranging from the near ultraviolet (neon at 0.322 μm) to the near-infrared (krypton at 0.799 μm). It is possible to mix the gases, for example, argon and krypton, to produce either single frequency or simultaneous emission at ten different wavelengths, ranging from the violet through the red end of the spectrum.

The basic design of an ion gas laser is similar to the HeNe. The major difference is that the electrical current flowing in the laser tube will be 10-20 amperes; sufficient to ionize the gas. Population inversion is obtained only in the ionized state of the gas. An important feature of these lasers is the very stable (0.2%) high output power of up to 20 Watts/CW. Commercial models will normally have a wavelength selector (a prism) within the cavity to allow for operation at any one of the wavelengths available. In addition, approximately single frequency operation can be achieved by placing an etalon inside the optical resonator cavity.

3. Carbon Dioxide Laser

The carbon dioxide laser is the most efficient and powerful of all CW laser devices. Continuous powers have been reported above 30 kilowatts at the far infrared 10.6 µm wavelength.

An electrical discharge is initiated in a plasma tube containing carbon dioxide gas. CO(2) molecules are excited by electron collisions to higher vibrational levels, from which they decay to the metastable vibrational level occurs; which has a lifetime of approximately 2×10(-3) seconds at low pressure of a few Torr. Establishing a population inversion between certain vibrational levels leads to lasing transitions at 10.6 µm, while a population inversion between other vibrational levels can result in lasing transitions at 9.6 µm. Although lasing can be obtained in a plasma tube containing CO(2) gas alone, various gases usually added, including N(2), He, Xe, CO(2) and H(2)O. Such additives are used to increase the operating efficiency of CO(2) lasers. The most common gas composition in CO(2) lasers is a mixture of He, N(2) and CO(2).

Carbon dioxide lasers are capable of producing tremendous amounts of output power, primarily because of the high efficiency of about 30%, as compared to less than 0.1% for most HeNe lasers. The principal difference between the CO(2) and other gas lasers is that the optics must be coated, or made of special materials, to be reflective or transmissive at the far infrared wavelength of 10.6 µm. The output mirror can be made of germanium, which, if cooled, has very low loss at 10.6 µm.

4. ND:YAG Laser Systems:

One of the most widely used laser sources for moderate to high power uses a neodymium doped crystal Yttrium Aluminum Garnet (YAG), commonly designated Nd:YAG. In addition, other hosts can be used with Nd, such as calcium tungstate and glass. The Nd:YAG laser is optically pumped either by tungsten or krypton pump lamps and is capable of CW outputs approaching 2000 W at the 1.06 μm wavelength. The ends of the crystal, which is usually in the form of a rod, are lapped, polished, and may be coated to provide the cavity mirrors.

Nd:YAG lasers belong to the class of solid state lasers. Solid state lasers occupy a unique place in laser development. The first operational laser medium was a crystal of pink ruby (a sapphire crystal doped with chromium); since that time, the term "solid state laser" usually has been used to describe a laser whose active medium is a crystal doped with an impurity ion. Solid state lasers are rugged, simple to maintain, and capable of generating high powers.

5. Excimer Lasers:

High power ultraviolet (UV) lasers have been the desire of many in the laser applications community for over twenty-five years. Theoretically, such a laser could produce a focused beam of sub-micrometer size and, therefore, be useful in laser microsurgery and industrial microlithography. Also, photochemical processes which are dependent upon the shorter UV wavelength would be possible at significantly greater speeds because of the enormous UV photon flux presented by a laser beam.

Excimer lasers operate using reactive gases such as chlorine and fluorine mixed with inert gases such as argon, krypton or xenon. The various gas combinations, when electrically excited, produce a pseudo molecule (called a "dimer") with an energy level configuration that causes the generation of a specific laser wavelength emission which falls in the UV spectrum as given in Table II-2. The reliability of excimer lasers has made significant strides over the past several years. Now, systems operating at average powers from 50-100 Watts are commercially available. A typical excimer operates in a repetitively pulsed mode of 30-40 ns pulses at pulse rates up to 50 Hz with pulse energies of 1-2 Joules/pulse. Some systems use x-rays to preionize the excimer laser's gas mixture so-as-to enhance lasing efficiency and increases.

6. Semiconductor Diode Lasers

The semiconductor or diode injection laser is another type of solid state laser. The energy level scheme is constructed by charge carriers in the semiconductor. They may be pumped optically or by electron beam bombardment, but most commonly, they are pumped by an externally applied current. Although all of these devices operate in the near infrared spectral region, visible laser diodes are being made today. A useful feature is that many are tunable by varying the applied current, changing temperature, or by applying an external magnetic field. Laser diodes are used extensively for communications, in compact disc players, retail scanners, printer, and are beginning to be used in ophthalmology.

5. Write a Detailed account on the use of lasers in dermatology. (May 2019,Sep 2020)

- Dermatology is the branch of medicine dealing with the skin.
- It is a speciality with both medical and surgical aspects

TYPES:

- Medical Dermatology
- Surgical Dermatology
- Pediatric Dermatology
- Cometic Dermatology
- cutaneous lymphoma

Laser Treatment:

- **"LASER** - Light Amplification by Stimulated Emission of Radiation
- Lasers are sources of high intensity light with the following properties :

Monochromatic - Radiation of single wavelength

Coherent - Light beams are in phase

Collimated - Light beams travels in parallel

- Laser light can be accurately focused into small with very high energy.

Types of Laser used in dermatology:

- From decreasing wrinkles and age spots to improving acne scars and redness, Lasers can do amazing things for your complexion
- There are two types of Lasers

1. Ablative
2. Non -Ablative

- An ablative laser works by vaporizing the top layer of skin, while a non-ablative laser leaves the outer layer of skin alone .

Uses:

- wound Healing
- Vascular lesions
- Removing of skin lesions
- Pigmented Hair removal

I. Wound Healing

- Laser operating at low engeries are able to produce photo bio-activation.
- They are used for the treatment of Leg ulcers And other chronic wounds.

II. Vascular lesions

- Flash lamplamp-pumped pulsar dye laser is used in treatment of port-wine stains.
- They are also used in superficial Capilary haemagiomas.

III. Removing of skin lesions

- For removal of benign skin lesions, such as moles, warts, keratoses and CO2 lasers are preferred.
- They are used for shaving, cutting, dermotrading and resurfacing scan.

IV. Pigmented lesions

- Low-pressure continuous wave Lasers of appropriate wavelength can be used successfully to treat melanin-containing lesions
- The q-switched Nd-YAG is effective for treatment of pigmented lesions, including removal of black tatoos.

V. Hair removal

- The Alexandrite Laser removes the hair in millisecond range (in pulsed mode)
- Nd-YAG laser light is also effective for long-term hair removal.

VI. Resurfacing

- Acne scars and extensive scars of skin damaged by photo aging have good results with the use of CO2 laser.

6. Explain How Laser is used in urology? (Sep 2020)

Lasers in Urology

Over the course of the past five decades, this technology has evolved into a highly specialized entity, also finding a niche market in the field of urology. Lasers obtained from various lasing mediums producing amplified light of different wavelengths have been tested for urological applications. These lasers are most commonly used in the surgical management of benign prostatic hyperplasia and as intracorporeal lithotripters. Other uses include ablation of various urologic tumors and incising strictures of the upper- and lower urinary tract. A continuous process of evolution of this technology is taking place, resulting in surgical lasers becoming ever safer, more effective, and more affordable.

Today, the types of lasers most commonly used in urology include:

- Nd:YAG;
- Ho:YAG (holmium:YAG);
- Thu:YAG (thulium:YAG); • CO. (carbon dioxide);
- KTP (potassium titanyl phosphate);
- LBO (lithium • diode laser.

Laser applications in urology

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MEDICAL OPTICS

UNIT 4 OPTICAL TOMOGRAPHY

2 Marks :

1. What are the applications of laser in medicine ? [nov 2018]

Applications in medicine

- angioplasty.
- cancer diagnosis.
- cancer treatment.
- Dentistry.
- cosmetic **dermatology** such as scar revision, skin resurfacing, laser hair removal, tattoo removal.
- **dermatology**, to treat melanoma.

2. Define raman spectroscopy ? [nov 2018]

Raman Spectroscopy is a non-destructive chemical analysis technique which provides detailed information about chemical structure, phase and polymorphy, crystallinity and molecular interactions. **Raman** is a light **scattering** technique, whereby a molecule scatters incident light from a high intensity laser light source.

3. Define optical coherence tomography ? **[nov 2019]**

Optical coherence tomography (OCT) is a non-invasive imaging test. OCT uses light waves to take cross-section pictures of your retina.

With OCT, your ophthalmologist can see each of the retina's distinctive layers. This allows your ophthalmologist to map and measure their thickness. These measurements help with diagnosis. They also provide treatment guidance for glaucoma and diseases of the retina. These retinal diseases include age-related macular degeneration (AMD) and diabetic eye disease.

4. Write the applications of optical tomography towards clinical imaging? [nov 2019]

Optical coherence tomography (OCT) is an emerging technology for performing high-resolution cross-sectional imaging.

OCT is analogous to ultrasound imaging, except that it uses light instead of sound. OCT can provide cross-sectional

Using OCT in combination with catheters and endoscopes images of tissue structure on the micron scale *in situ* and in real time.enables high-resolution intraluminal imaging of organ systems.

5. What are the benefits of OCT ? [may 2019]

- The higher **OCT** resolution makes it easier to evaluate stent strut apposition against the vessel wall.
- It also allows clear imaging of neointimal stent strut coverage in follow-up exams and allows much better edge detection than IVUS.

6. What is limb prostheses ? [may 2019]

In medicine, a prosthesis or prosthetic implant is an artificial device that replaces a missing body part, which may be lost through trauma, disease, or a condition present at birth. Prostheses are intended to restore the normal functions of the missing body part.

7. Define electrograph. [Sep 2020]

- A phototelegraphic apparatus for the electrical transmission of pictures
- A device used for the etching or transfer of pictures or designs by electrolytic.

8. State the Principle of Doppler Optical Coherence tomography. [Sep 2020]

The basic principle of Phase-resolved Doppler OCT uses the phase change between sequential A-line scans for velocity image reconstruction. Using this principle, scanning speed is prominently increased. While decoupling the spatial resolution and velocity sensitivity in flow images, again it increases imaging speed.

11 marks :

1. **a) Explain the principle and working of optical coherence tomography (OCT)[nov 2018]**

Principle of OCT

OCT is often compared to medical ultrasound because of the similar working principles.

Both medical imaging techniques direct waves to the tissue under examination, where the waves echo off the tissue structure. The back reflected waves are analyzed and their delay is measured to reveal the depth in which the reflection occurred. OCT uses light in the near-infrared, which travels much faster than ultrasound. The delays of the back-reflected waves cannot be measured directly, so a reference measurement is used. Through the use of an interferometer, part of the light is directed to the sample and another portion is sent to a reference arm with a well-known length.

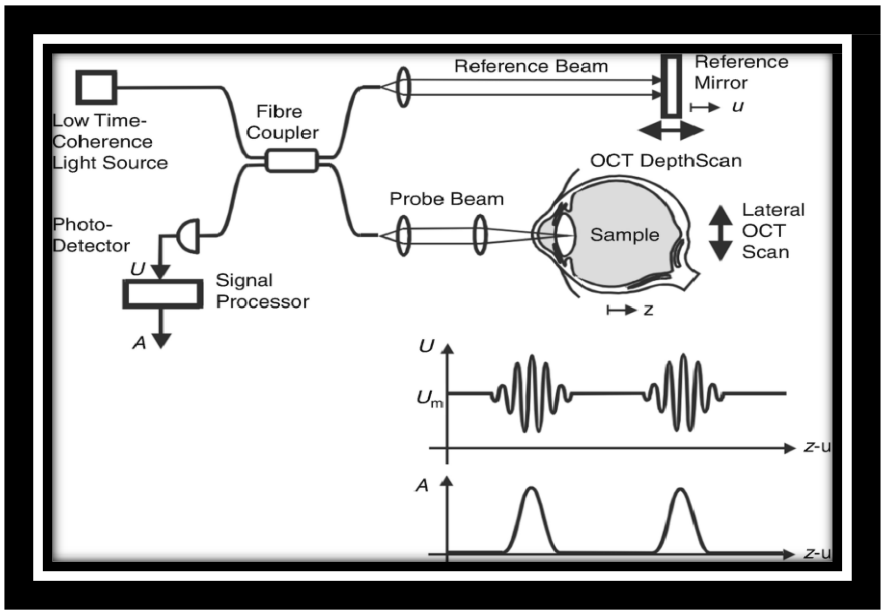
The idea of low-coherence interferometry is the underlying principle for all OCT implementations. Temporal coherence is a property of a light source and characterizes the temporal continuity of a wave train sent out by the source and measured at a given point in space. Wave trains emerging from a light source of low temporal coherence maintain a fixed phase relation only over a very limited time interval corresponding to a confined travel range, the coherence length or coherence gate. A light source with a broad spectral bandwidth is composed of a range of wavelengths. Such a broadband source has low coherence, while monochromatic laser light has a narrow spectral line and features a coherence length of at least several meters. An interferometer splits light, coming from a source, into two separate paths and combines the light coming back from the two paths at the interferometer output. There, under certain conditions, interference can be observed: coherent waves superimpose and their electromagnetic field amplitudes add constructively (i.e. they reinforce each other) or destructively (i.e. they cancel out each other) or meet any condition in between. The associated light intensity can be measured as an electrical signal using a photo detector.

Technical realizations of OCT

In the first implementation of OCT , the reference length was modulated for each depth scan and the record of the intensity of the combined light at the sensor gave the reflectance profile of the sample. This variant is called time-domain OCT (TD-OCT) .

Working principle of TD-OCT : light from the light source is split into the reference beam and the central beam. Back reflected light from both arms is combined again and recorded by the detector. To record one depth profile of the sample (A-scan) the reference arm needs to be scanned. This has to be repeated for each lateral scan position.

As depicted, the light of a low-coherence source is guided to the interferometer, which in this example is a fiber-based implementation. In a system using bulk optics the fiber coupler is replaced by a beam splitter. The input beam is split into the sample beam and into the reference beam travelling to a mirror on a translational stage. The back-reflected light from each arm is combined and only interferes if the optical path lengths match and therefore the time travelled by the light is nearly equal in both arms. Modulations in intensity, also called interference fringe bursts, are detected by the photodiode. The amount of back-reflection or back-scattering from the sample is derived directly by the envelope of this signal.

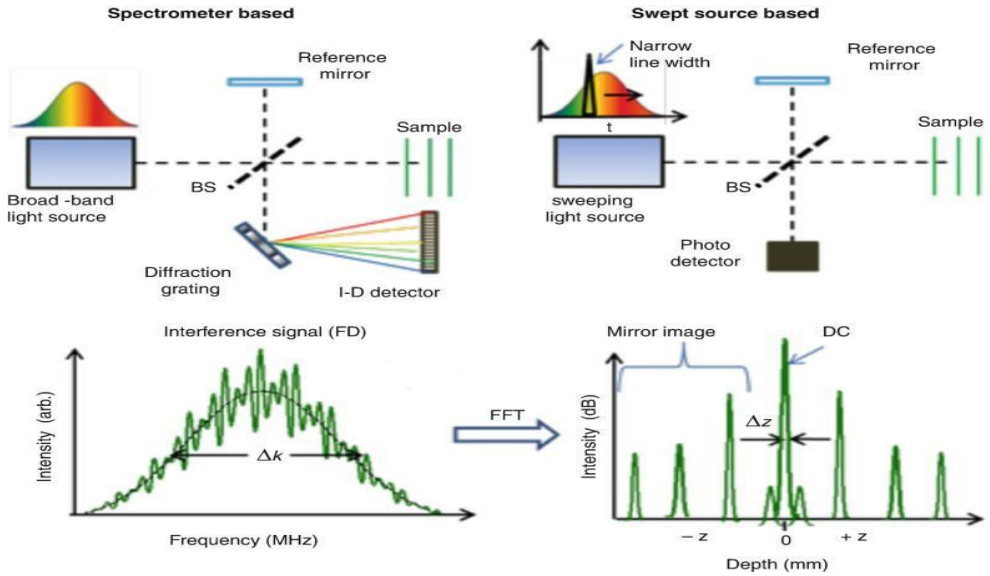


For each sample point, the reference mirror is scanned in depth (*z*) direction and the light intensity is recorded on the photo detector. Thereby a complete depth profile of the sample reflectivity at the beam position is generated, which—in analogy to ultrasound imaging—is called A-scan (amplitude scan).

To create a cross-sectional image (or B-Scan), the sample beam is scanned laterally across the sample. This abbreviation originated in ultrasound imaging, where B-Scan means brightness scan.

Fourier domain OCT (FD-OCT , also frequency domain OCT) is the second generation of OCT technology and provides a more efficient implementation of the principle of low-coherence interferometry.

Optical setup of spectrometer based OCT (SD-OCT) in the upper left inset and swept source OCT (SS-OCT) in the upper right inset. While SD-OCT uses a spectrometer for wavelength separation, SS-OCT features a light source which sweeps the wavelength in time. Both implementations record an interference spectrum which carries the depth in formation of the sample. FFT is used to transform the interference signal into the A-scan.



b) merits and limitations over other imaging modalities ? [nov 2018]

Advantages:

- **OCT provides a faster and easier method for diagnosis.** This technique gives results quicker as compared to other traditional medical diagnosing procedures.
- **OCT is a computerized procedure.** Since this is not operated mechanically, therefore chances of error are reduced.
- **OCT gives a better image of the test results.** This process follows higher number of scans and thus provides an image of higher resolution as the result of diagnosis.
- **OCT provides broad and dynamic range of operations.**
- **OCT involves rapid data acquisition rate.**
- **OCT uses simple catheter / endoscope.** The design of the catheter / endoscope installed in OCT is small and inexpensive.
- **The device used in OCT is portable.** The structure of the device is compact and hence makes it easy to carry.

Disadvantages:

- **OCT has a limited penetration power.** It can result in penetration of up to 2-3 mm, ideal case being 4 mm.
- **Femtosecond laser used in OCT is expensive.**
- **During OCT, the transverse resolution needs to be similar to axial resolution.** If this goes below10 μm, a short confocal parameter is needed which results in the focus falling off rapidly.

2. Give an elaborate account on the principle and applications of elastography ? [nov 2018 ,nov 2019 , may 2019]

The general principle of elastography can be summarized as follows:

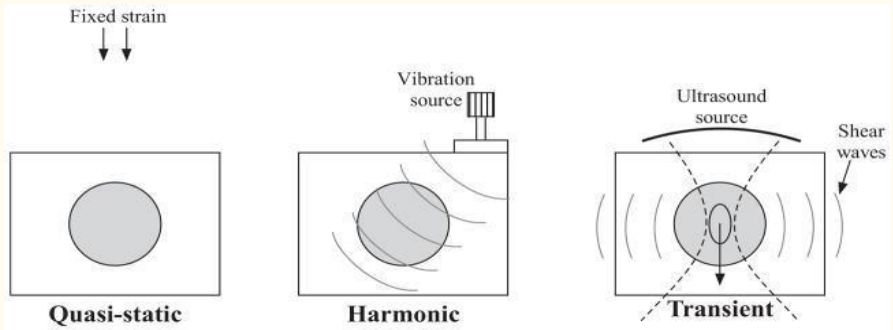
(1) perturb the tissue using a quasi-static, harmonic, or transient mechanical source;

(2) measure the resulting mechanical response (displacement, strain or amplitude and phase of vibration); and

(3) infer the biomechanical properties of the underlying tissue by applying either a simplified or continuum mechanical model to the measured mechanical response In this chapter, we will describe

(a) the general principles of quasi-static, harmonic, and transient elastography the most popular approaches to elastography and

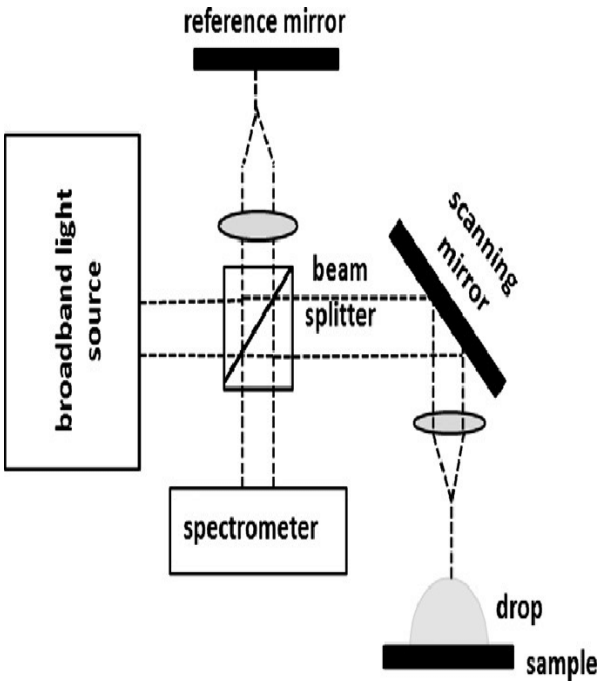
(b) the physics of elastography—the underlying equations of motion that governs the motion in each approach. We also provide examples of clinical applications of each approach.



Applications

- Elastography is used for the investigation of many disease conditions in many organs. It can be used for additional diagnostic information compared to a mere anatomical image, and it can be used to guide biopsies or, increasingly, replace them entirely. Biopsies are invasive and painful, presenting a risk of hemorrhage or infection, whereas elastography is completely noninvasive.
- Naturally, elastography sees use for organs and diseases where manual palpation was already widespread. Elastography is used for detection and diagnosis of breast, thyroid, and prostate cancers. Certain types of elastography are also suitable for musculoskeletal imaging, and they can determine the mechanical properties and state of muscles and tendons.
- Preliminary reports on elastography used on transplanted kidneys to evaluate cortical fibrosis have been published showing promising results.

3. With a neat schematic give an account on optical coherence tomography ? [nov 2019,may 2019, Sep 2020]



Visualize the flows in the drop we used a Thorlabs SR-OCT, a Fourier domain OCT, which combines a broadband low coherence super-luminescent diode light source with a Michelson interfer- ometer, a high speed spectrometer and a fast piezo-electric scan- ning mirror that enables a set of neighboring axial profiles to be collected to form a cross-section This system is ideal for imaging turbid samples and provides a direct measurement of scattered light along a vertical axis within a bulk sample. Fourier domain measurements enable each subsurface axial profile to be collected at the same time without time delay between the top and the bottom interface of a profile, which makes this technique very useful for internal flow observations. The backscattered light from various depths of the sample interferes with the light re- flected from the reference mirror to produce an interference pat- tern which is measure by a spectrometer. Fourier transform of this interference fringe as a function of wavenumber gives the depth profile. The central wavelength of the light is 930 nm and the spectral bandwidth (FWHM) is 100 nm. The imaging for our experiments is done at 2 fps with an axial resolution of 7 lm and lateral resolution of 9 lm. The maximum image depth and width are 1.6 mm and 4 mm respectively.

The dilutions are mixed with tracer particles (copolymer microspheres -ThermoScientific) 4.3 lm and 7.9 lm in size. Fig. 2 schematically shows the different scanning procedures of confocal microscope and OCT. Confocal microscope select horizon- tal slices of the droplet sequentially while changing the focal plane by shifting the position of the pin hole. In case of OCT imaging, the 1D vertical (z-axis) profiles are obtained without physical scanning and the horizontal scanning is done by the movement of the scan- ning mirror, which shifts the ray along the direction perpendicular to the x-z plane on the drop each time; with the help of a motor- ized table the substrate is moved in the x-axis direction (direction perpendicular to the slice) to gain a 3-D image. The advantage of OCT is to be able to observe the cross section of the whole droplet on one .

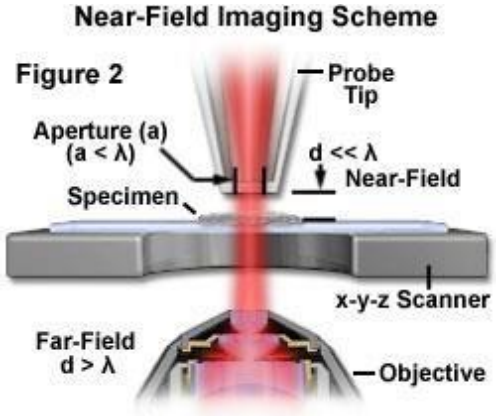
4). Explain in detail about various special near field techniques used for imaging biological structures. [Sep 2020]

A fundamental principle in diffraction-limited optical microscopy requires that the spatial resolution of an image is limited by the wavelength of the incident light and by the numerical apertures of the condenser and objective lens systems. The development of near-field scanning optical microscopy (NSOM), also frequently termed scanning near-field optical

microscopy (SNOM), has been driven by the need for an imaging technique that retains the various contrast mechanisms afforded by optical microscopy methods while attaining spatial resolution beyond the classical optical diffraction limit.

Near-field scanning optical microscopy is classified among a much broader instrumental group referred to generally as scanning probe microscopes (SPMs). All SPMs owe their existence to the development of the scanning tunneling microscope (STM), which was invented by IBM research scientists Gerd Binnig and Heinrich Rohrer in the early 1980s.

The theoretical resolution limit of conventional optical imaging methodology (200 to 300 nanometers for visible light) was the primary factor motivating the development of recent higher-resolution scanning probe techniques, such as STM and atomic force microscopy (AFM), and previously, transmission electron microscopy (TEM) and scanning electron microscopy (SEM). These and related techniques have enabled phenomenal gains in resolution, even to the level of visualizing individual atoms. However, prior to the development of near-field scanning optical methods, the superior resolution capabilities have come at the expense of the wide variety of contrast-enhancing mechanisms available to optical microscopy. Furthermore, the extreme specimen preparation requirements for most of the high-resolution methods have limited their application in many areas of study, particularly in biological investigations involving dynamic or in vitro measurements. The method of near-field scanning optical microscopy combines the extremely high topographic resolution of techniques such as AFM with the significant temporal resolution, polarization characteristics, spectroscopic capabilities, sensitivity, and flexibility inherent in many forms of optical microscopy.



Near-field scanning optical microscopy is continuing to grow in use, especially for the microscopist interested in obtaining the highest possible optical resolution. However, NSOM is not limited to serving solely as an imaging/microscopy instrument; it can also be utilized for specimen manipulation, fabrication, and processing on a nanometric scale. A wide variety of NSOM applications outside of the imaging realm are evolving, including precision laser machining, nanometer scale optical lithography, and localized release of caged compounds.

Some of the limitations of near-field optical microscopy include:

- Practically zero working distance and an extremely small depth of field.
- Extremely long scan times for high resolution images or large specimen areas.
- Very low transmissivity of apertures smaller than the incident light wavelength.
- Only features at the surface of specimens can be studied.
- Fiber optic probes are somewhat problematic for imaging soft materials due to their high spring constants, especially in shear force mode.

NSOM is currently still in its infancy, and more research is needed toward developing improved probe fabrication techniques and more sensitive feedback mechanisms. The future of the technique may actually rest in refinement of apertureless near-field methods (including interferometric), some of which have already achieved resolutions on the order of 1 nanometer. However, typical resolutions for most NSOM Instruments range around 50 nanometers, which is only 5 or 6 times better than that achieved by scanning confocal microscopy. This moderate increase in resolution comes at a considerable cost in time required to set up the NSOM instrument for proper imaging, and in the complexity of operation. The greatest advantage of NSOM probably rests in its ability to provide optical and spectroscopic data at high spatial resolution, in combination with simultaneous topographic İnformation. Combining atomic force measurements and near-field scanning optical microscopy has proven to be an extremely powerful approach in certain areas of research, providing new information about a variety of specimen types that is simply not attainable with far-field microscopy.

BM E76 – MEDICAL OPTICS

UNIT-5 SPECIAL OPTICAL TECHNIQUES

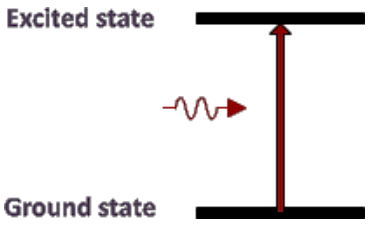
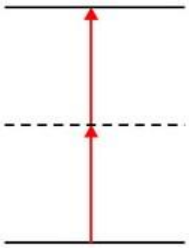
PART-A (2 MARKS)

1) a) What is Photo-Dynamic therapy (PDT)? (Nov/ Dec 2018) (or)

b) What is PDT? (May 2019)

- Photodynamic therapy (PDT) is a treatment that involves light-sensitive medicine and a light source to destroy abnormal cells. It can be used to treat some skin and eye conditions, as well as certain types of cancer.
- On their own, the medicine and light source are harmless, but when the medicine is exposed to the light, it activates and causes a reaction that damages nearby cells.
- This allows small abnormal areas of tissue to be treated without the need for surgery.

2) Compare the single photon absorption over the multiphoton absorption. (Nov/ Dec 2018)

SINGLE-PHOTON ABSORPTION	**MULTIPHOTON ABSORPTION**
Single-photon absorption (SPA or 1PA) is a linear **absorption** process whereby **one photon** excites an atom, ion or molecule from a lower energy level to a higher energy level, for example, from the ground state to the first excited state.	**Multiphoton absorption** is the term used to describe a process in which an atom or molecule makes a single transition between two of its allowed energy levels by **absorbing** the energy from more than a single photon.
 **SINGLE PHOTON ABSORPTION**	**Excited state**  **Ground state** **MULTIPHOTON ABSORPTION**

3) a) What is near field imaging? State its features. (Nov/ Dec 2019)

b) List the features of near field imaging. (May 2019)

NEAR FIELD IMAGING

- Near-field imaging occurs when a sub-micron optical probe is positioned a very short distance from the sample and light is transmitted through a small aperture at the tip of this probe.
- The near-field is defined as the region above a surface with dimensions less than a single wavelength of the light incident on the surface.
- Within the near-field region evanescent light is not diffraction limited and nanometer spatial resolution is possible.
- This phenomenon enables non-diffraction limited imaging and spectroscopy of a sample that is simply not possible with conventional optical imaging techniques.

FEATURES OF NEAR FIELD IMAGING

- The near-field optical (NFO) microscope involved a sub-wavelength aperture at the apex of a metal coated sharply pointed transparent tip, and a feedback mechanism to maintain a constant distance of a few nano-meters between the sample and the probe.
- **Near-field optics** is that branch of optics that considers configurations that depend on the passage of light to, from, through or near an element with sub-wavelength features, and the coupling of that light to a second element located a sub-wavelength distance from the first.
- Thus, when imaging at visible wavelengths, the smallest resolvable features are several hundred nano-meters in size (although point-like sources, such as quantum dots, can be resolved quite readily).
- Using near-field optical techniques, researchers currently resolve features in the order of tens of nano-meters in size. While other imaging techniques (e.g. atomic force microscopy and electron microscopy) can resolve features of much smaller size, the many advantages of optical microscopy make near-field optics a field of considerable interest.

4) What is the principle of fluorescent spectroscopy? (Nov/ Dec 2019)

- Fluorescence spectroscopy (fluorometry or spectrofluorometry), is a type of electromagnetic spectroscopy which analyzes fluorescence from a sample. It involves using a beam of light, usually ultraviolet light, that excites the electrons in molecules of certain compounds and causes them to emit light of a lower energy, typically, but not necessarily, visible light. This shift to longer wavelength is called stokes shift.

PART-B (10 MARKS)

1) (a) What are the ideal properties of a photo sensitizer? (Nov/ Dec 2018)

- One of the three crucial elements of PDT, apart from light and oxygen, is the presence of photosensitizers.
- These dyes are defined as substances capable of absorbing light with a specific wavelength, triggering photochemical orphotophysical reactions.
- As in each group of drugs, a set of characteristics and conditions describing the ideal photosensitizer can be distinguished:
  - High degree of chemical purity.
  - Stability at room temperature.
  - Photosensitive effect only in the presence of a specific wavelength.
  - High photochemical reactivity; the maximum absorption of light should be at wavelengths from 600nm to 800nm. Absorbance of light at a wavelength above 800nm does not provide enough energy to stimulate oxygen in its state of singlet and production of other reactive oxygen species.
  - Absorption minimum in the range from 400nm to 600nm. This prevents possible excessive photosensitivity caused by sunlight.
  - The absorption bands should not overlap the absorption band of other substances in the body, including endogenous dyes such as melatonin, hemoglobin or oxyhemoglobin.
  - Minimal cytotoxicity in the dark.
  - Easy solubility in the tissues of the body.

- High selectivity for neoplastic tissues: the photosensitizer should be slowly removed from the affected areas staying there for at least several hours, but be quickly eliminated from healthy tissues, thus minimizing the phototoxic side effects of the therapy.
- Inexpensive and simple synthesis and easy availability.

➢ The ideal photosensitizer would be a chemically pure drug with specific uptake by the target tissue, minimal dark toxicity (i.e., activated only upon irradiation), high photoactivity (high quantum yield of ROS), rapid clearance to avoid phototoxic side effects, and strong absorption at relatively long wavelengths (~630–800 nm). A number of synthetic or natural photosensitizers have been developed based on these properties.

(b) (i) Explain the mechanism of photodynamic therapy and its applications. (Nov/ Dec 2018)

(or)

(ii) Explain in detail about the importance of photodynamic therapy and specify its applications (Nov/ Dec 2019)

(or)

(iii) Explain how photodynamic therapy plays its role in cancer treatment. (May 2019)

❖ **Introduction**

➢ Photodynamic therapy (PDT) is a modern and non-invasive form of therapy, used in the treatment of non-oncological diseases as well as cancers of various types and locations.

➢ Good therapeutic results and the possibility of the parallel application of PDT with other therapeutic protocols make it more commonly used in many fields of medicine. PDT has been successfully used in dermatology, oncology, gynecology and urology.

➢ Photodynamic therapy is based on the local or systemic application of a photosensitive compound - the photosensitizer, which is intensely accumulated in pathological tissues.

➢ The photosensitizer molecules absorb light of the appropriate wavelength, initiating the activation processes leading to the selective destruction of the inappropriate cells.

- Photodynamic therapy is well tolerated by patients because of its selective action. Photodynamic protocols are painless, and the simplicity of their application allows for outpatient use.
- Photodynamic therapy is also used in the treatment of chronic inflammation and is an interesting alternative in the treatment of drug-resistant bacterial infections. The focus of this review is the anticancer application of PDT, its advantages and possible modification to potentiate its effect.
- Numerous studies indicating the use of photosensitizers in oncology have been carried out over last several decades.
- Despite the success of PDT, new compounds and innovative methods are still being researched and required to improve the effective use of photodynamic therapy in clinical oncology. Previous studies have led to a significant extension of the possible applications and combinations of photodynamic therapy against cancer cells.
- In addition to the traditional drug applications, the use of electroporation and nano carriers is considered to increase the local concentration of the photosensitizer, which results in better efficiency of applied therapy.

❖ **PDT mechanism**

- Molecular mechanism of photodynamic therapy is based on the three non-toxic components, which produce the desired effects within pathological tissues only by mutual interactions between: the photosensitizer (PS); Light with the appropriate wavelength; oxygen dissolved in the cells.
- There are two main mechanisms of the photodynamic reaction. Both are closely dependent on oxygen molecules inside cells. The first stage of both mechanisms is similar.
- A photosensitizer, after entering the cell, is irradiated with a light wavelength coinciding with the PS absorption spectrum and is converted from the singlet basic energy state S° into the excited singlet state S1 because of the photon absorption.
- Part of the energy is radiated in the form of a quantum of fluorescence, and the remaining energy directs a photosensitizer molecule to the excited triplet state T1 - the proper, therapeutic form of the compound.

- **Type I of mechanism of photodynamic reaction**
  - In the excited triplet state T1, the photosensitizer can transfer energy to the biomolecules from its surroundings. Between the photosensitizer in the T1 state and the cancerous tissue (substrate), a hydrogen or electron is transferred, which leads to the formation of free radicals and anion radicals of the photosensitizer and the substrate.
  - Electrons interreact with oxygen molecules, which remain in their basic energetic state. This process leads to the production of reactive oxygen species (ROS) - initially in the form of superoxide anion radical ($O2^{\bullet-}$), which creates further generation of ROS inside the cells.
  - The initiated cascade of reactions leads to the oxidative stress resulting in the destruction of cancer cells.
- **Type II of mechanism of photodynamic reaction**
  - As a result of the photosensitizer's transition into the excited triplet state, energy is transferred directly to the oxygen molecule in the basic energetic state (the basic triplet state). Direct energy transfer between molecules ($PS \rightarrow O2$) is possible because they have the same spins.
  - In this way excited oxygen particles - so-called singlet oxygen - are generated, which are characterized by extremely strong oxidizing properties. Most organic compounds are in the basic singlet state.
  - However, oxygen molecules are characterized by their triplet state (as the basis) and excitation into the singlet. Owing to this fact, excited photosensitizer particles do not damage organic cell structures and react only with oxygen molecules dissolved in the cytoplasm.
  - It is assumed that mechanism of type II is the most important process conditioning the efficiency of PDT.
  - Nevertheless, the ratio of the contribution of both mechanisms depends on many factors, including: oxygen concentration, tissue dielectric constant and pH and photosensitizer's structure. As the oxygen runs out, the first type of mechanism begins to prevail.

- Highly reactive oxygen species cause the photodamage of proteins, fats and other molecules in the photosensitized area. This leads to the direct death of tumor cells in the process of apoptosis and / or necrosis.
- The mutual contribution of different types of cell death depends on the intracellular location of photosensitizer. The damage of mitochondria can lead to apoptosis, cell membrane destructions and loss of integrity can induce necrosis, and damage of lysosomes or endoplasmic reticulum can provoke autophagy.

❖ **PDT selectivity**

- The described photocytotoxic reactions occur only within the pathological tissues, in the area of photosensitizer distribution, enabling selective destruction.
- Photosensitizers accumulate in significantly higher concentrations in cancer cells than in regular cells. The reason of such bio distribution may be the tendency of photosensitizers to combine preferentially with low density lipoproteins (LDL).
- The role of LDL is to supply tissues with the necessary cholesterol to create membranes during cell division. Vehemently dividing cancer cells show an increased uptake of LDL lipoproteins, which act as a "transporter" of the photosensitizer to the cancerous tissues.
- In addition, tissues with an increased mitotic activity reveal excessive expression of LDL lipoprotein receptors on the cell surface.
- The affinity of photosensitizers for serum lipoproteins, in particular for LDL, plays an important role in the delivery of these drugs to the tumor tissue. It is known now that PDT leads to a systemic anti-cancer response.
- Photodynamic therapy affects the vascular system of the tumor and stimulates the immune system. The process of destruction of an inappropriate tissue is complemented by the activation of coagulation processes (occlusion of tumor vessels) and local accumulation of inflammatory cells.
- Cancer cells that have escaped death by the direct photocytotoxic effects of PDT may still be destroyed via the indirect influence of PDT on tumor blood vessels. Reactive oxygen species damage of vascular endothelial cells activates clotting processes,

aggregate platelets and block vessels by forming thrombi. As a result of vascular occlusion, persistent hypoxia of tumor tissue leads to the cell death.

- Furthermore, the efficiency of the PDT-method is associated with systemic anti-cancer immune response of the body. PDT destroys the structure of the tumor and thus stimulates direct interaction between immune cells and cancer cells.
- Direct destruction in tumor tissue leads to the development of a strong inflammatory reaction and neoplasm infiltration by leukocytes.
- Membrane photo damages lead to the activation of phospholipases, and then cyclooxygenases, causing massive release of inflammatory mediators - lipid hydrolysis products and arachidonic acid metabolites. Photo-injuries of the blood vessel walls attract neutrophils and macrophages.
- Neutrophil degranulation as well as the release of lysosomal enzymes and chemotactic factors additionally contribute to the destruction of tumor tissue, exacerbating the destruction process initiated by the earlier irradiation.

❖ **Importance of Photodynamic Therapy**

- Photodynamic therapy is an unconventional treatment modality for neoplastic conditions and a promising treatment for recurrent cancers, depending on photochemical reactions and subsequent damage, and leading to cancer cell death.
- **Advantages of PDT**
  - It has no long-term side effects when used properly.
  - It’s less invasive than surgery.
  - It usually takes only a short time and is most often done as an outpatient procedure.
  - It can be targeted very precisely.
  - Unlike radiation, PDT can be repeated many times at the same site if needed.
  - There’s usually little or no scarring after the site heals.
  - It often costs less than other cancer treatments.

❖ Applications of Photodynamic Therapy

- **Acne**
    - PDT is currently in clinical trials as a treatment for severe acne. Initial results have shown for it to be effective as a treatment only for severe acne. A systematic review conducted in 2016 found that PDT is a "safe and effective method of treatment" for acne.
    - The treatment may cause severe redness and moderate to severe pain and burning sensation in some people. One phase II trial, while it showed improvement, was not superior to blue/violet light alone.
- **Cancer**
    - In February 2019, medical scientists announced that iridium attached to albumin, creating a photosensitized molecule, can penetrate cancer cells and, after being irradiated with light, destroy the cancer cells.
- **Ophthalmology**
    - Verteporfin was widely approved for the treatment of wet AMD beginning in 1999. The drug targets the neovasculature that is caused by the condition.
- **Photoimmunotherapy**
    - Photoimmunotherapy is an oncological treatment for various cancers that combines photodynamic therapy of tumor with immunotherapy treatment.
    - Combining photodynamic therapy with immunotherapy enhances the immunostimulating response and has synergistic effects for metastatic cancer treatment.
- **Vascular targeting**
    - Some photosensitisers naturally accumulate in the endothelial cells of vascular tissue allowing 'vascular targeted' PDT.

- Verteporfin was shown to target the neovasculature resulting from macular degeneration in the macula within the first thirty minutes after intravenous administration of the drug.
- Compared to normal tissues, most types of cancers are especially active in both the uptake and accumulation of photosensitizer agents, which makes cancers especially vulnerable to PDT. Since photosensitizers can also have a high affinity for vascular endothelial cells.

- **Antimicrobial Photodynamic Therapy**
  - Some photosensitizers have been chemically modified to incorporate into the mycomembrane of mycobacteria.
  - These molecules show promising in vitro activity and are potential candidates for targeted delivery of photosensitizers.
  - Furthermore, antibacterial photodynamic therapy has the potential to kill multidrug- resistant pathogenic bacteria very effectively and is recognized for its low potential to induce drug resistance in bacteria, which can be rapidly developed against traditional antibiotic therapy.

2) Construct a Laser Induced Fluorescence (LIF) set up and explain the use of Fluorescence Spectroscopy in tissue characterization. (Nov/ Dec 2018)

LASER INDUCED FLUORESCENCE (LIF):

Introduction

- Laser Induced Fluorescence (LIF) is an optical spectroscopic technique where a sample is excited with a laser, and the fluorescence emitted by the sample is subsequently captured by a photodetector.
- LIF can be understood as a class of fluorescence spectroscopy where the usual lamp excitation is replaced by a laser source.
- Whilst lasers are now routinely used as excitation sources in photoluminescence spectrometers, Laser Induced Fluorescence was not originally developed for a commercial instrument but as a standalone laser spectroscopy technique.

Types of LIF

There are different types of Laser Induced Fluorescence spectroscopy depending on the laser and detection system used. It is common to refer to the technique as *excitation* or *emission* LIF spectroscopy;

Figure 1 illustrates this concept. In the figure a laser is employed to excite molecules from their ground state into an electronically excited state. As the molecules relax back into the ground state, fluorescence is detected by a photomultiplier tube (PMT).

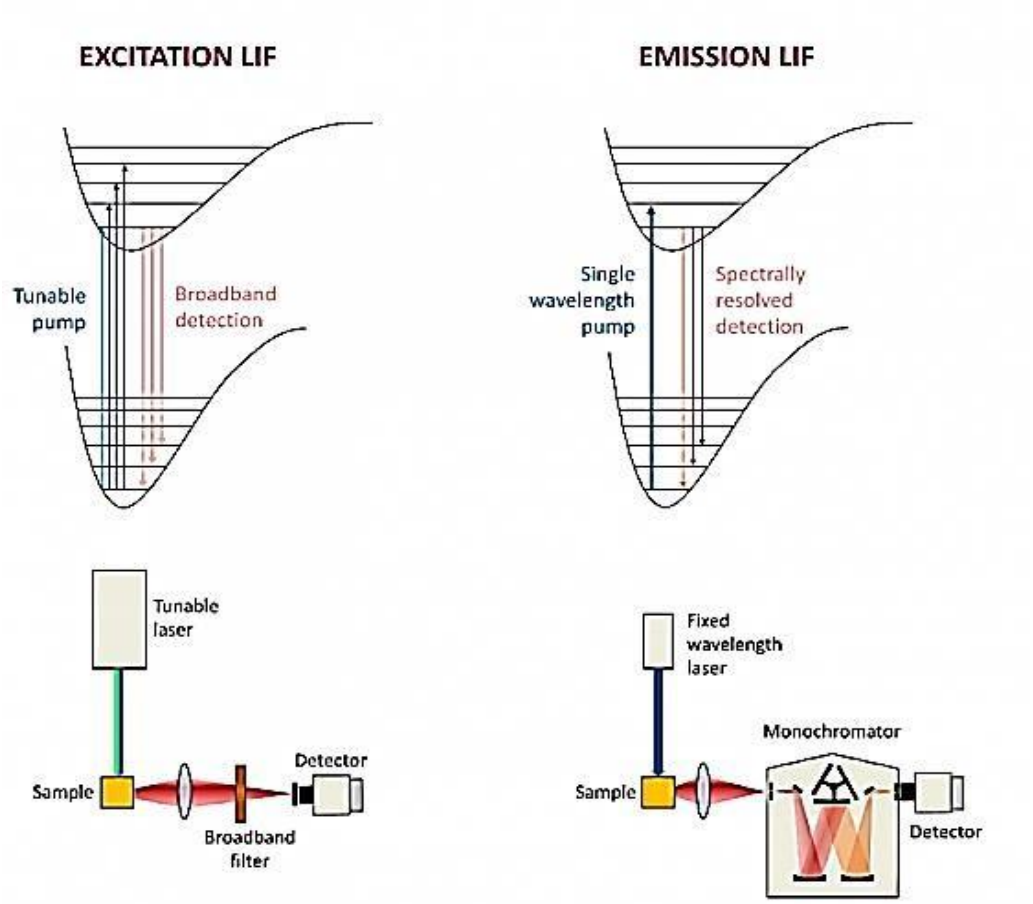


Figure 1. Schematic representation of excitation (left) and emission (right) Laser Induced Fluorescence spectroscopy.

In **excitation LIF**, the excitation wavelength is varied using a tunable laser which allows one to resolve the vibrational structure of the excited state. In a liquid sample, the molecules will

fluoresce from the lowest vibrational level of their excited singlet state, decaying to a series of vibrational levels in the ground state, however the emission spectrum is not resolved by the detection system. A bandpass filter is placed between the sample and PMT to detect all the emission from the sample whilst removing any scattered laser light.

In **emission LIF**, a fixed pump wavelength is used to excite the sample and the sample's emission spectrum is analysed utilising a monochromator to select the detection wavelength. The figure shows single-point detection with a PMT, but it is also possible to employ an array detector (CCD or CMOS) to capture the full spectrum in one shot.

FLUORESCENCE SPECTROSCOPY IN TISSUE CHARACTERIZATION

New diagnostic tools are needed to facilitate real-time (live) intra-operative diagnosis, guidance to surgery or other interventions. Optical techniques have demonstrated great potential for providing rapidly and non-invasively information about tissue anatomic, functional and molecular features. As reported by numerous research groups, fluorescence spectroscopy and imaging methods, in particular, have shown potential for tissue characterization and diagnosis with applications in numerous clinical areas including oncology, cardiology, and ophthalmology.

Time-resolve fluorescence measurements, in particular, were found very promising as they are less sensitive to presence of endogenous absorbers (e.g. blood) or changes in light excitation-collection geometries. Moreover, a recently reported multi-spectral time-resolved fluorescence spectroscopy (ms-TRFS) technique demonstrated potential for rapid acquisition of time-resolved fluorescence signal in multiple wavelength bands simultaneously with high photon throughput.

Importantly, such implementation allows for continuous scanning of tissue samples both in-vitro and in-vivo and recording of TRFS data from large surface areas from either planar or tubular structures. However, in these earlier applications, the TRFS data were first recorded from the entire area of interest, then analyzed to determine the actual fluorescence decay characteristics (e.g. fluorescence lifetime(s)), and finally the results carrying diagnostic information were displayed.

Typically such process requires a minimum of several seconds to a few minutes or off-line processing depending on the total number of data points and the method used for fluorescence decay analysis. While such approach represents a first step to implementation of a fast near-real-time tissue diagnostic tool it poses a few critical challenges.

First, the optical data from each measurement point or "pixel" needs to be co-registered with the location from where the measurement was actually taken. Such co-registration is difficult to achieve when the measurements are taken continuously in dynamic conditions such those encountered during *in vivo* studies where the location of the measurement may shift from the time of data recording to the time of data display.

Second, a robust means for fluorescence data acquisition is required to account for sudden variation of the intensity of auto-fluorescence signal so that the variation of fluorescence photon number has minimal impact on the time-resolved fluorescence detection. Such variation is due to a number of conditions including dynamic changes of fluorescence excitation-collection geometry, the broad range of quantum efficiencies exhibited by endogenous fluorescent molecules, non-uniform illumination due to uneven tissue surface profile, and the presence/absence of strong endogenous absorbers (e.g. blood).

These conditions are difficult to account for, in particular, when non-contact scanning fiber-optics probes are used and/or scanning of a large tissue surface area is needed. Third, the analysis of fluorescence decay data needs to be integrated in the data acquisition software platform in a manner that allows for near-instantaneously display of diagnostic data during tissue scanning.

3) Explain the special techniques used for near field imaging of biological structure. (Nov/ Dec 2019)

Near-field scanning optical microscopy (NSOM), also called scanning near-field optical microscopy (SNOM), is a scanning probe technique that overcomes the diffraction barrier in traditional far-field optical microscopy.

Conventional optical microscopy techniques are limited by the diffraction of light and the resolution is limited to roughly 250 nm, which make it very difficult to resolve the domains or clusters in the cellular membranes.

The basic idea in NSOM is to confine the illuminating light to nanometric dimensions to break the diffraction limit that cannot be achieved by traditional far-field optical microscopy. Thus, NSOM can produce high-resolution topographical and optical images to study biological membranes. Figure below shows the diffraction of light and the near-field in the NSOM fiber probe.

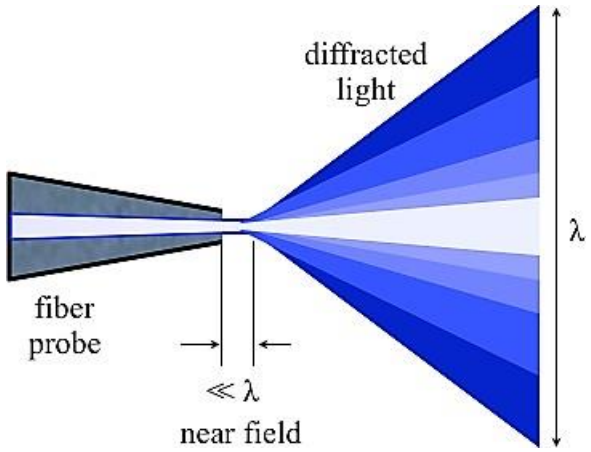


Figure 1 Diagram illustrating near field optics. Diffraction of light coming from NSOM fiber probe. Showing wavelength of light and the near-field. Image used with permission

Near-field Theory

In traditional far-field optical microscopy, the illumination source is a monochromatic plane wave. The lens collecting the scattering light is placed several wavelengths of the illumination light far away from the sample surface.

This causes the commonly known diffraction limit, that is far-field optical techniques are limited to resolve features approximately on the order of half of the wavelength of the illuminating light due to the diffraction of light.

However, the classical NSOM uses a tapered optical fiber probe and an aperture that is much smaller than the wavelength of the light. As shown in Figure 2, after light passes through the cantilever tip with nano-aparture, an optical near-field (or evanescent field) on the far side of

this tip can be created that is not diffraction limited. Then the resolution in near-field microscopy is directly affected by the size of the aperture and independent of the wavelength of the light.

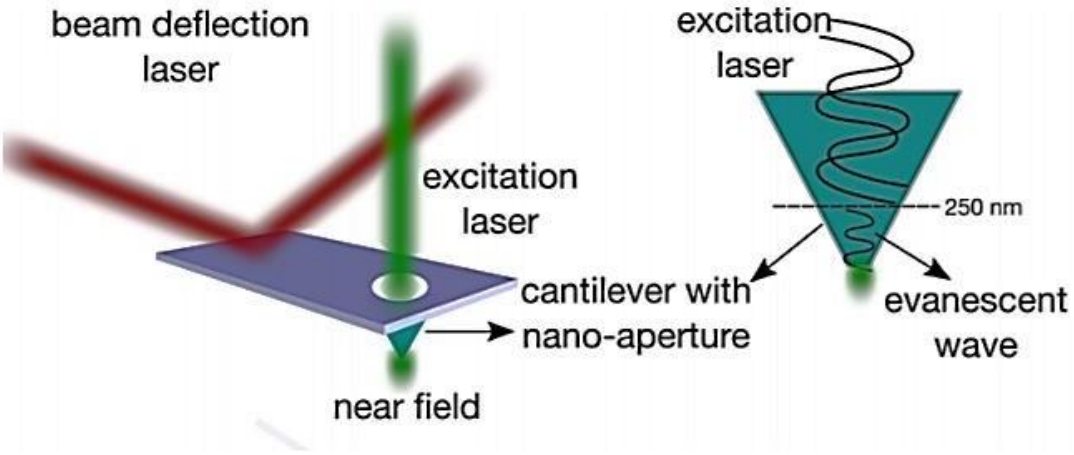


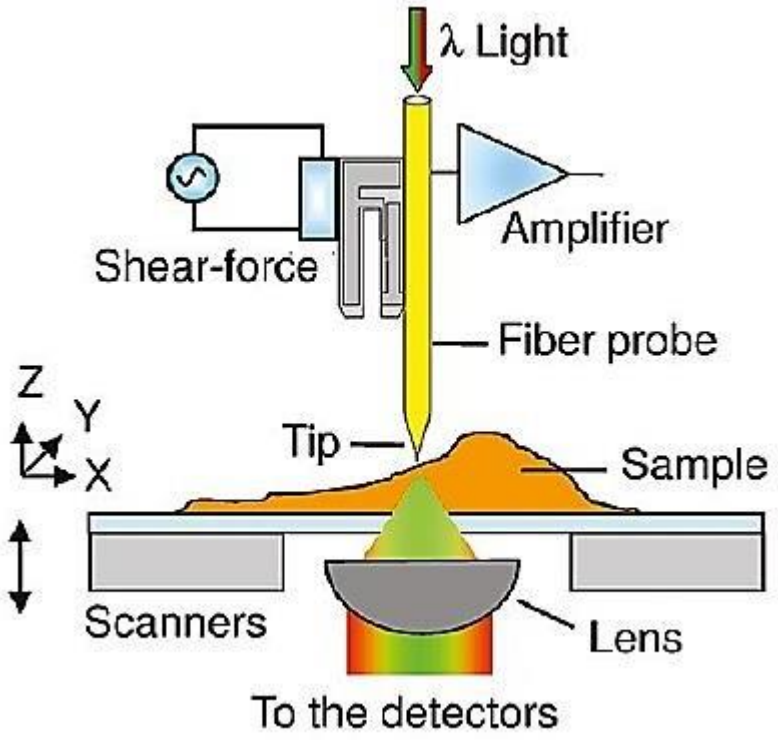
Figure 2: Near-field technique

NSOM Instrumentation

Standard NSOM normally contains three parts, the illumination unit, the collection and redistribution unit and the detection module. The illuminating light comes from the probe, goes through the aperture at the tip and interacts with the sample surface.

As light passes through the sample, the absorption or the fluorescence produced by the labeled molecules on the sample surface can be collected. During the scan, the probe can be brought to the sample at a very small distance (<10 nm).

The topographic and optical images showing the high spatial resolution can be generated simultaneously. The lateral resolution can be reached down to tens of nanometers, which is determined by the size of the aperture and sample-probe distance. The probe tip movement is monitored and controlled by the feedback system and the x-y-z scanner (usually piezoelectric) to keep the tip within the near-field.



General principles of NSOM instrumentation

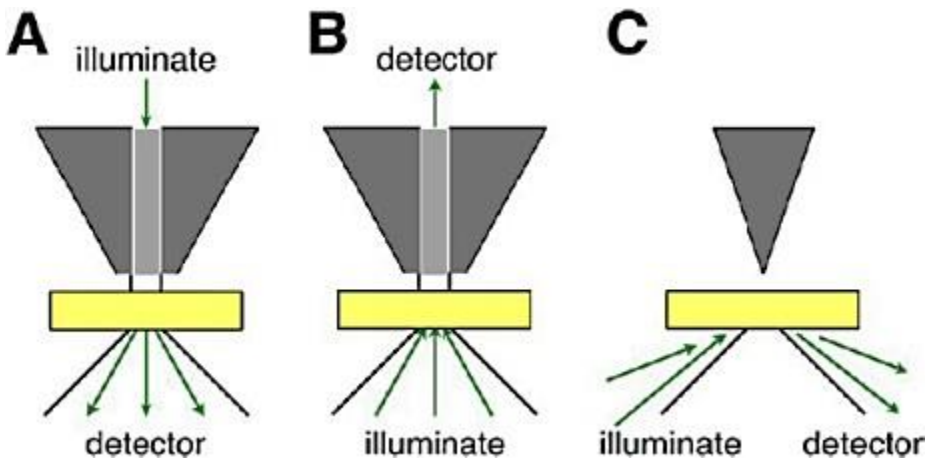
Figure shows three common modes of NSOM measurements, which are illumination mode, collection mode and scattering mode for aperture less NSOM.

In illumination mode, the evanescent field can be generated at the tip end when the illuminated light passes through the probe near the sample.

Then the scattered light from the probe-sample system is collected to generate the NSOM image. In collection mode, the evanescent wave near sample is acquired by the local probe within near-field of sample. The dashed line means the critical angle.

Besides aperture NSOM, the NSOM with apertureless probe was also developed, which use STM-like tip or AFM cantilever tip.

The probe tip is also brought very close to the sample, and the scattering light is induced near sample.



Three types of NSOM measurements

NSOM Fluorescence Imaging of Membranes

The combination of NSOM and the simultaneous fluorescence has been used to investigate the membrane systems such as lipid bilayers, lipid rafts, membrane receptor, clustering and other small domains on the membrane. The near-field technique makes it possible to detect the small domains with high resolution that cannot be achieved by the traditional optical microscopes.

Further, the specific assignments of detected domains can be made by comparing the simultaneous fluorescence mapping and the surface topography. Thus, the high-resolution images of NSOM combined with fluorescence can provide abundant information such as the size and specific features of different domains on the membrane, which is very helpful for us to understand the membrane organization and important function.

Supported lipid bilayers (SLB) studied by NSOM

Supported lipid bilayers (SLB) are phospholipid bilayers that contain different lipids, cholesterol or proteins. SLBs are significant model membranes that have been studied for a long time. NSOM help researchers to better understand the crucial factors in the small domains and structures in SLBs with high-resolution.

NSOM images of SLBs can identify different domains of dipalmitoylphosphatidylcholine (DPPC), dilauroylphosphatidylcholine (DLPC) on membranes.

Lipid rafts studied by NSOM

For the heterogeneous lipid domains in cell membranes, also called lipid rafts, NSOM has been used to visualize the nanolandscape of ganglioside GM1 after tightening by its ligand cholera toxin (CTxB). NSOM was able to show the nanodomains were smaller than 120 nm.

Membrane protein receptors studied by NSOM

The formation of receptors nanoclusters on membrane could also be investigated by NSOM. For example, the distribution of receptor DC-SIGN, a transmembrane protein in immature dendritic cells (DC) has been studied by NSOM. The "finger" domains are the main topographic feature of dendritic cells, where the colorful fluorescent areas indicate nanocluster distributions of DC-SIGN receptors.

NSOM has great advantages in studying biological membranes over conventional optical microscopy. The combination of near-field technique and scanning probe techniques utilized by NSOM can generate high spatial resolution images beyond the diffraction limit, which is not possible in conventional optical microscopy.

Combined with fluorescence technique, NSOM can show both topographic image and the simultaneous optical fluorescence image that can detect the small domains and the specific features in membrane systems.

4) Explain in detail the instrumentation of a fluorescent spectrophotometer. (May 2019)

Fluorescence spectrophotometry is a set of techniques that deals with the measurement of fluorescence emitted by substances when exposed to ultraviolet, visible, or other electromagnetic radiation. It has wide application in chemical and biological sciences as it can be used to analyze a biological system, by studying its interactions with fluorescent probe molecule.

Fluorescence

Fluorescence spectrophotometry is based on fluorescence, which is a photoluminescence event (photo = light; luminescence = the emission of light). In simple terms, it is the emission of light because of an exposure to (and resultant absorption of) light. Here, this exposure to and absorption of light is called excitation.

A common form in which photoluminescence is often observed, is as phosphorescence – what you see in glow-in-the-dark toys. This type of photoluminescence occurs when there is a long delay between the excitation and emission of light. A long delay here means about 10^{-6} seconds or longer. When the delay between excitation and emission is shorter (between 10^{-6} and 10^{-8} seconds), the result is fluorescence.

Phosphorescence can persist long after the initial exposure to light – hence you may see the glow-in-the-dark star against your bedroom ceiling even after the room had been dark for hours. Fluorescence, on the other hand, has a quick flash of emission (typically around 10 nanoseconds, but sometimes shorter than 1 nanosecond). Because of this, sophisticated electronics and optics are nowadays used to detect and measure fluorescence.

Excitation and Emission of Fluorophores

The physics behind fluorescence involves the different electronic and vibrational states that fluorophores can exist in. An electronic state is divided into multiple vibrational states. Photons that have energies in the ultraviolet to blue-green range of the spectrum can trigger an electronic transition from a lowest vibration in the ground state to one of the vibrational levels in a higher electronic excited state.

As soon as the energy input from the photon (in other words the excitation) stops, the fluorophore molecule relaxes into the lowest vibrational level of the excited electronic state. The fluorophore remains in this state for some time (around 10 nanoseconds, known as the fluorescence

lifetime) and then returns to the electronic ground state. This return to the ground state is associated with a release of energy, known as fluorescence emission.

The number of photons emitted by a fluorophore, relative to the number of photons absorbed, is called the quantum yield. A fluorophore with a large quantum yield, like rhodamine, will display a bright emission. The emitted radiation is always of a longer wavelength (lower energy) than the excitation radiation (higher energy).

For example, if the incoming light was blue (shorter wavelength), then the appropriate fluorophore will emit green light (longer wavelength). This observation, which was first described by Sir George Gabriel Stokes, and therefore today called the Stokes shift, is caused by the rapid return of the excited molecule to its ground state.

The process that happens between excitation and emission is illustrated using Jablonski diagrams (named after the father of fluorescence spectroscopy, Alexander Jablonski) (Lakowicz, 2013). These diagrams, such as the one in figure 1, show the different electronic (S_0 and S_1) and vibrational (0, 1, 2, 3) states of a molecule.

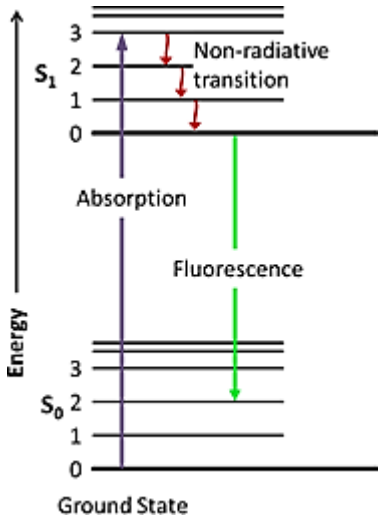


Figure 1. Jablonski diagram

Intrinsic fluorophores are molecules with a natural fluorescence, such as chlorophyll and the aromatic amino acids. Extrinsic fluorophores are those that are added to a sample to provide

fluorescence, or to change the spectral properties of the sample. Examples of extrinsic fluorophores are fluorescein and rhodamine, but there are many more.

Each fluorophore has its own characteristic properties, such as fluorescence lifetime, intensity, and position of the emission wavelength, thus, each fluorophore will yield a unique fluorescence spectrum. A fluorescence spectrum is a plot of the fluorescence intensity as a function of wavelength.

The environment in which the fluorophore finds itself will influence and modify its properties, hence reading the fluorescence spectrum of a fluorophore when immersed in different environments will give information about those environments.

For example, the intrinsic fluorophore tryptophan is an aromatic amino acid that is very sensitive to its local environment within the cell. When a ligand binds to it, or when it associates with a protein, or when a protein unfolds, tryptophan's fluorescence spectrum will change in particular ways.

By monitoring this change in spectrum, one can deduce information about the immediate cellular and molecular environment. In this way fluorophores, when linked to peptides, proteins, membranes, or DNA, can be used to study the structure, dynamics, and metabolism of living cells.

Fluorescence Detection and Measurement

While some fluorescence can be detected with the eye, sophisticated instruments have been built to detect even the faintest fluorescence emitted by a molecule.

The Filter Fluorometer

An older type of instrument for the measurement of fluorescence spectra, and one that is still used today, is the filter fluorometer. It consists of the following parts:

- an excitation source (like a lamp or laser),
- a primary filter,
- a sample chamber (also called a cuvette),
- a secondary filter, and
- a fluorescence detection system.

The filters only permit radiation of certain wavelengths (typically the primary filter permits short wavelengths needed for excitation and the secondary filter permits long wavelengths associated with emission) and serve to eliminate residual radiation scatter. The fluorescence detection system consists of photomultiplier tubes (PMT) that amplify the photon emission and record and display the signal electronically.

Modern Fluorescence Spectrophotometers

Most modern fluorescence spectrophotometers (also called spectrofluorometers) are more advanced instruments than the filter fluorometer in that they can detect fluorescence with higher precision and extraordinary sensitivity. They are superior in wavelength selectivity, flexibility, and convenience. A spectrofluorometer (see figure 2) is often equipped with the following:

- a high-pressure xenon arc lamp,
- monochromators,
- a sample chamber (also called a cuvette), and
- a fluorescence detection system.

The high-pressure xenon arc lamp, used as the excitation source, can provide an energy continuum that extends from the ultraviolet into the infrared.

Instead of filters, spectrofluorometers have monochromators which allow for the production of individual wavelengths from a broad-band light source. This makes it possible for spectrofluorometers to record both excitation and emission spectra.

The monochromators allow one to keep emission fixed at a single wavelength to obtain the excitation spectrum. Vice versa, it is possible to keep excitation fixed at a single wavelength, to record the fluorescence emission spectrum.

Like the fluorometer, the spectrofluorometer’s fluorescence detection system consists of photomultiplier tubes (PMT) for emission amplification. Electronic devices are then used to quantify the signal and display it electronically, usually as a graph.

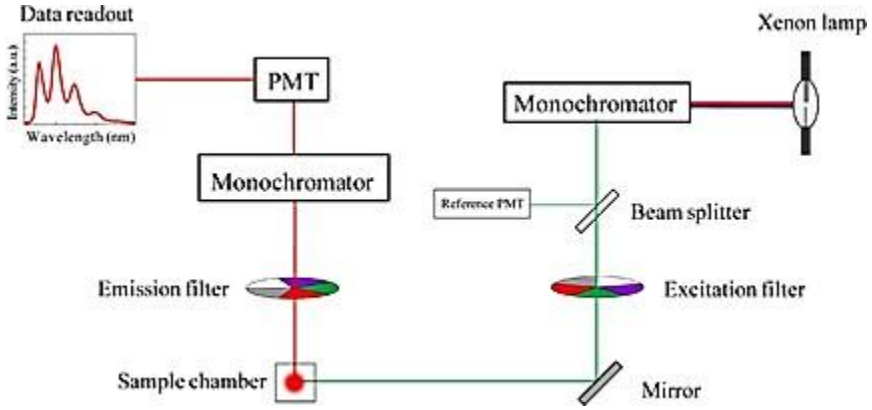


Figure 2. Schematic diagram of a spectrofluorometer

Fluorescence measurement

Fluorescent measurements can be classified into:

- Steady-state measurements or,
- Time-resolved measurements.

In steady-state measurements, a sample is illuminated with a continuous beam of light and the fluorescence intensity or fluorescence emission spectrum is recorded.

In time-resolved measurements, a sample is illuminated by a short pulse of light after which the intensity decay or anisotropy decay is measured.

Intensity decay refers to the fluorescence lifetime or the time that a molecule spends in the excited electronic state. How long the molecule's fluorescence lifetime is can provide rich information about the environment surrounding the fluorophore.

From the basic technology of fluorescence detection, as outlined above, a plethora of fluorescence technologies have evolved, such as multiphoton excitation, fluorescence correlation spectroscopy, single-molecule detection, fluorescence immunoassay, flow cytometry, and near-infrared fluorescence spectroscopy.

Applications of fluorescence spectroscopy

The applications of fluorescence spectroscopy are almost as wide as one's imagination. It is hard to imagine the chemical and biological sciences without this technique nowadays. Only

selected examples of application per sector, for some of the most common sectors it is used in, will be mentioned in the quick overview that follows.

- **Bioscience**
  - In the biosciences, one of the most frequent applications of fluorescence spectroscopy is the high precision quantification of DNA and RNA. An extrinsic fluorophore (often ethidium bromide) is added to a DNA sample and the sample is loaded into a fluorescence spectrometer to obtain a reading of the sample's concentration.
  - Another modern application is SMRT (single molecule real-time) DNA sequencing. In its ability to produce long read single molecules with high accuracy, it is predicted to be central to the next genetic diagnostic revolution.
- **Industrial**
  - Fluorescence spectroscopy is used in several industrial settings as a fast, noninvasive technique in the assessment of contamination.
  - For example, it has been used to detect contaminating organic compounds in groundwater, after hydraulic fracturing for gas exploration.
- **Chemical**
  - An important chemical application of fluorescence spectroscopy can be found in the field of nanoparticle synthesis for potential medical uses, such as drug delivery.
  - When nanoparticles are exposed to biological fluids, they become coated with proteins and other biomolecules (called the protein corona). The interactions between the nanoparticle and the protein corona has implications for its safe use in vivo.
  - Time-resolved fluorescence quenching and fluorescence correlation spectroscopy are used to study these interactions and understand nanotechnology better.
- **Environmental**
  - In environmental monitoring, the technique also has wide application. One example is in the treatment of water surrounding landfill areas. As rainwater percolates through waste

and liquid forms during the biodegradation of waste in a landfill, landfill leachates form. Leachate contains a mix of pollutants that can be harmful to the environment.

- High-resolution fluorescence spectroscopy and 3D-excitation emission matrix fluorescence spectroscopy are used to characterize dissolved organic matter in these samples and then based on that, optimize treatment processes for landfill leachate.

➢ **Pharmaceutical**

- Spectrofluorometric techniques are also used in the pharmaceutical field to analyze drugs. An example is the analysis of co-formulated tablets prescribed as cholesterol medication.
- Synchronous fluorescence spectroscopy provides a simple, fast, and accurate method for analyzing a tablet called Atoreza©, which contains both Ezetimibe and Atorvastatin calcium. The method is ideal for routine quality control of this medication.

➢ **Agricultural**

- In agriculture, spectroscopic techniques are also widely applied for instance in the identification of different crop varieties. Laser-induced fluorescent emission technique (LIFS) is an excellent tool used to identify citrus seedling varieties.
- Likewise, total luminescence spectroscopy can be used by tea manufacturers as a quick, affordable and objective alternative to employing trained tea tasters, to discriminate between similar types of tea.