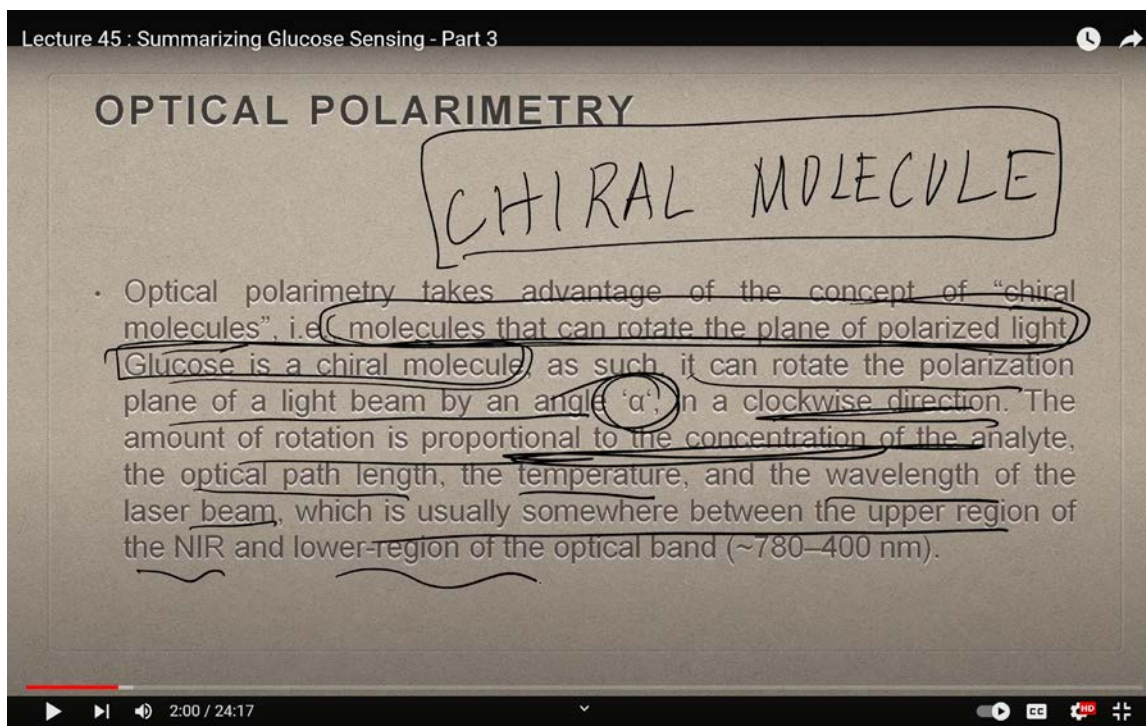


Design for Biosecurity
Prof. Mainak Das
Department of Design
Indian Institute of Technology, Kanpur
Lecture 45
Summarizing Glucose Sensing - Part 3

Welcome back to class! In our previous session, we delved into fluorescence resonance energy transfer, or FRET, and explored its application in glucose detection. Prior to that, we discussed the surface plasmon resonance (SPR) technique. Today, we will continue on this path and introduce another intriguing technique known as optical polarimetry.

So, what is optical polarimetry all about? It harnesses the concept of chiral molecules. I trust you all remember what chiral molecules are from your high school studies, but I encourage you to review the definition if needed. Chiral molecules are those that can rotate the plane of polarized light.

(Refer Slide Time: 02:00)



Lecture 45 : Summarizing Glucose Sensing - Part 3

OPTICAL POLARIMETRY

CHIRAL MOLECULE

- Optical polarimetry takes advantage of the concept of "chiral molecules", i.e. molecules that can rotate the plane of polarized light. Glucose is a chiral molecule, as such, it can rotate the polarization plane of a light beam by an angle (α), in a clockwise direction. The amount of rotation is proportional to the concentration of the analyte, the optical path length, the temperature, and the wavelength of the laser beam, which is usually somewhere between the upper region of the NIR and lower-region of the optical band (~780–400 nm).

2:00 / 24:17

Glucose, specifically, is a chiral molecule. In the absence of other chiral molecules, glucose can be detected quite easily because it can rotate the polarization plane of a light beam by an angle α in a clockwise direction. The degree of rotation is directly proportional to the concentration of the analyte, as well as the optical path length, temperature, and the wavelength of the laser beam. This wavelength typically falls within the upper region of the near-infrared spectrum and the lower region of the optical band, ranging from about 400 to 780 nanometers. This forms the basic principle of optical polarimetry.

However, there are significant challenges. The minimal optical rotation associated with physiological levels of glucose, combined with the presence of other active molecules and the high degree of light scattering in skin and tissue, makes it impractical to use optical polarimetry on the skin. Unlike the SPR technique, which can be applied directly to the skin, optical polarimetry finds a more suitable application in the aqueous humor of the anterior chamber of the eye.

(Refer Slide Time: 05:21)

Lecture 45 : Summarizing Glucose Sensing - Part 3

Unfortunately, the minimal optical rotation associated with physiological level of glucose, the presence of other active molecules, and the high degree of light scattering in the skin and tissue, make it unfeasible to use of optical polarimetry in the skin. However, it is possible to use it on the aqueous humor in the anterior chamber of the eye due to its excellent optical properties. The method consists of polarizing the light emitted by a light source before reaching the eye. The reflected light is then analyzed to determine its angle of rotation and intensity. Such technique has the potential of detecting small amounts of glucose as long as issues such as sensitivity to temperature and motion and others, can be addressed positively.

SKIN X

DIABETIC RETINOPATHY

CORNEA LENS AQUEOUS HUMOR OPTIC NERVE R RPE L BP

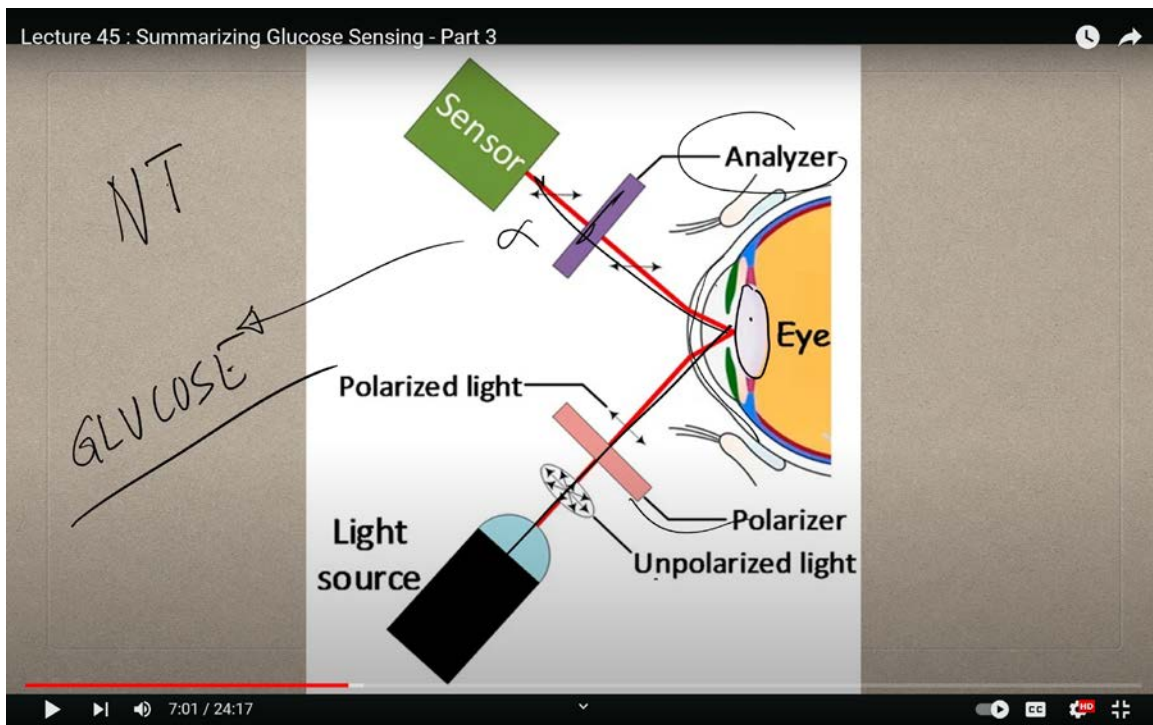
5:21 / 24:17

To visualize this, let's consider the structure of the eye: you have the retina here, connected

to the optic nerve. Surrounding it are the ciliary muscles, the lens, and the cornea, all encasing the aqueous humor. Additionally, we have the retinal pigment epithelial (RPE) cell layer adjacent to the aqueous humor. This anatomical context clarifies the potential for using optical polarimetry in this environment. Feel free to consult any textbook for detailed diagrams and explanations of the eye's structure; it will all make much more sense!

The method involves polarizing the light emitted from the light source before it reaches the eye. Once the light is reflected, it is analyzed to determine both the angle of rotation, denoted as α , and the intensity of the light. This technique holds the potential to detect even small amounts of glucose, provided that various factors related to tissue sensitivity, temperature, motion, and others can be effectively managed.

(Refer Slide Time: 07:01)

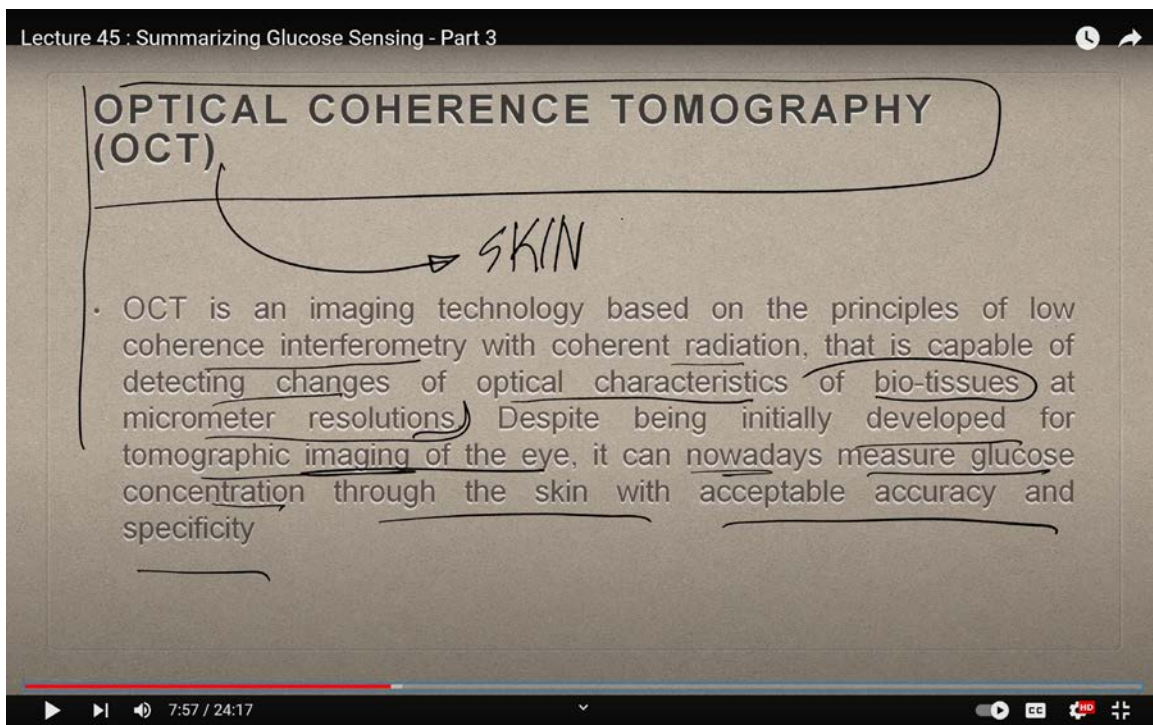


Now, this brings us to a particularly intriguing point: the condition known as diabetic retinopathy. Diabetes can lead to damage in the retina due to the elevated pressure in the blood vessels that supply blood to the eyes. This increased pressure, a consequence of diabetes, can cause damage to the retinal tissues, allowing glucose to infiltrate the aqueous

humor. By fine-tuning optical polarimetry for use in the eyes, we can address the challenges posed by diabetic retinopathy more elegantly than with current skin applications. The scattering of light in the skin presents significant difficulties, whereas the eye offers a much clearer view, making it easier to direct the beam accurately. The system should be able to quantify the amount of glucose present, and that's precisely how this technique operates.

Let's visualize how it works: the light source emits light that passes through the cornea and lens. The sensor and analyzer are positioned to capture the light. Unpolarized light passes through a polarizer, allowing us to analyze the angle α , which is proportional to the glucose concentration in the aqueous humor. This illustrates the functioning of the optical polarimeter.

(Refer Slide Time: 07:57)



Lecture 45 : Summarizing Glucose Sensing - Part 3

OPTICAL COHERENCE TOMOGRAPHY (OCT)

SKIN

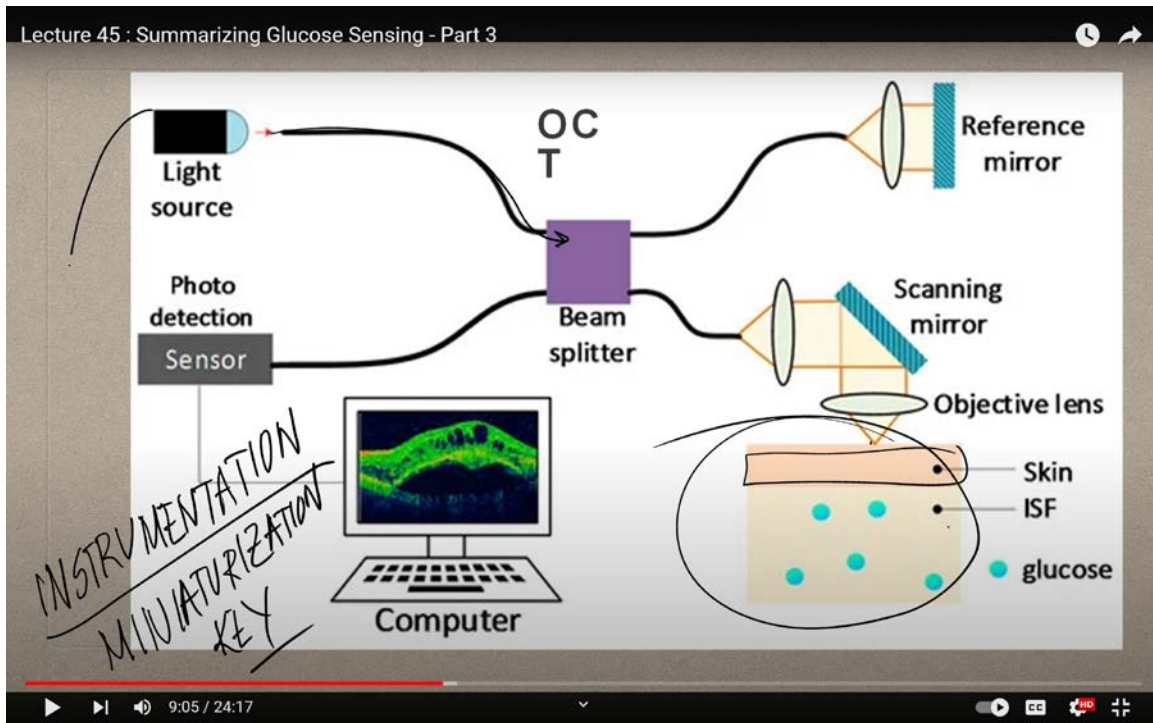
- OCT is an imaging technology based on the principles of low coherence interferometry with coherent radiation, that is capable of detecting changes of optical characteristics of bio-tissues at micrometer resolutions. Despite being initially developed for tomographic imaging of the eye, it can nowadays measure glucose concentration through the skin with acceptable accuracy and specificity.

7:57 / 24:17

Next, we transition to another optical technique that has garnered considerable attention due to its non-invasive nature: optical coherence tomography (OCT). OCT is an imaging technology based on the principle of low coherence interferometry, utilizing coherent

radiation to detect changes in the optical properties of biological tissues at micrometer resolution. Originally developed for tomographic imaging of the eye, it can now measure glucose concentrations through the skin with acceptable accuracy and specificity.

(Refer Slide Time: 09:05)



Unlike optical polarimetry, which faces challenges in skin applications, optical coherence tomography can effectively be utilized on the skin. But how do some of these optical techniques help overcome traditional challenges? Here's how it works: there's a light source and a beam splitter that directs light to a reference mirror and a scanning mirror. Another beam splitter is positioned on the skin's surface, where the interstitial fluid (ISF) containing glucose molecules is located, facilitating the detection process.

OCT emerges as a powerful tool for continuous glucose monitoring, but, as I mentioned in class, advanced instrumentation is essential. This is where miniaturization plays a critical role; the smaller your setup, the more marketable your device becomes. The technology involves radiating the skin with coherent light within the wavelength range of 800 to 1000 nanometers.

(Refer Slide Time: 10:29)

Lecture 45 : Summarizing Glucose Sensing - Part 3

OCT

↑ GLUCOSE ↑ Refractive Index.

- The technology consists of radiating the skin with coherent light, with a wavelength between 800 and 1300 nm. The backscattered radiation generated is then combined with a reference to produce an interferometric signal that is sensed by a photodetector, as shown in Figure. Hence, if an increase of glucose occurs, it will increase the refractive index and decrease the scattering coefficient, creating a mismatch reduction of the refractive index between the medium and the reference, proportional to the glucose concentration.

↓ Scattering coefficient

10:29 / 24:17

The backscattered radiation generated during the process is then combined with a reference beam to produce an interferometric signal, which is detected by a photodetector, as illustrated in the accompanying figure. This detection process involves numerous backscattered signals, and here's the crucial point to grasp: when there is an increase in glucose levels, the refractive index of the system also increases. This is an essential aspect for you to understand. As the glucose concentration rises, the refractive index of the medium increases, while the scattering coefficient decreases. This change results in a reduced mismatch of the refractive index between the medium and the reference, which is directly proportional to the glucose concentration.

This, in essence, outlines the fundamental principle of using optical tomography for glucose detection. Now, let's discuss near-infrared spectroscopy (NIR), a well-known technique, though it comes with its own set of challenges. Near-infrared spectroscopy operates in the wavelength range of 780 to 2500 nanometers and relies on the absorption and scattering of light due to molecular vibrations. We've covered this in previous classes, but let's briefly revisit the concept of bond rotations within molecules.

(Refer Slide Time: 11:23)

Lecture 45 : Summarizing Glucose Sensing - Part 3

NEAR-INFRARED SPECTROSCOPY

NIR

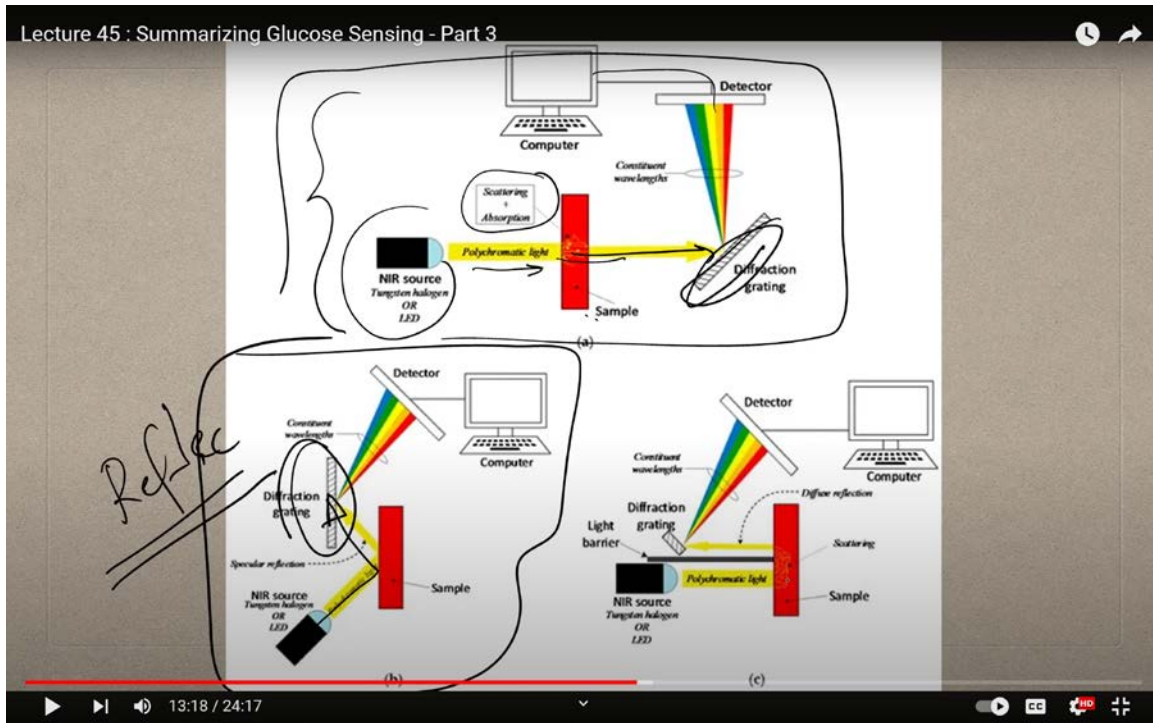
Near-Infrared spectroscopy (NIRS) technology relies on the absorption and scattering of wavelengths in the 780 nm to 2500 nm range due to molecular vibrations and rotation of bonds inside of the molecule. It uses three basic measurement modes: transmittance, reflectance (including diffuse reflectance), and intercalance. However, they all rely on the same core technology, a dispersive spectrometer.

11:23 / 24:17

NIR employs three primary measurement modes: transmittance, reflectance, and differences in reflectance. However, all these modes are built upon the same core technology, the dispersive spectrometer. In transmittance mode, scattered light irradiates polychromatic light onto the sample. To illustrate this process, consider the NIR polychromatic light that scatters and absorbs as it interacts with the sample, with the diffraction grating directing the resulting signals to the detector. This is how one variant operates: a light source emits polychromatic light onto the sample, and a diffraction grating on the opposite side splits the transmitted radiation into its constituent wavelengths, which are then sensed and analyzed by a detector and computer.

In reflectance mode, depicted as part B, the setup is slightly different. Here, the diffraction grating and detector are positioned on the same side as the light source to capture the reflected light. This mode detects specular reflection, the reflection occurring at a specific angle from the sample. You can visualize it this way: the light passes through, reflects off the sample, and then the diffraction grating captures the reflected light before it continues to the detector. This intricate dance of light and measurement forms the basis of how near-infrared spectroscopy functions in detecting glucose levels.

(Refer Slide Time: 13:18)



(Refer Slide Time: 14:00)

Lecture 45 : Summarizing Glucose Sensing - Part 3

In transmittance mode (Figure), a light source irradiates polychromatic light onto the sample, and a diffraction grating on the other side splits the transmitted radiation into its constituent wavelengths before being sensed and analyzed by a detector and computer respectively. In reflectance mode (Figure part b), the diffraction grating and detector are on the same side of the source to detect the specular reflection, i.e., reflection at a definite angle, from the sample. Similarly, interactance mode (Figure part c), also senses the reflected light from the sample, but it uses a light-barrier between the incident and the reflected beams to separate the field of view of the detector from the illuminated area. All modes are suitable for measuring absorption/transmittance and scattering in the sample, and the preference for one of them is based only on the type of media. For example, transmittance mode is preferred for analyzing fluids and very thin or transparent samples, whereas reflectance and interactance, are preferred with dense solids or thick samples.

Handwritten note: "SKIN" with arrows pointing to the sample area in diagrams (b) and (c).

14:00 / 24:17

Do you notice the distinction? This is the reflectance mode we just discussed. Now, let's delve into a third mode known as the interaction mode. This mode also senses the reflected light from the sample but employs a light barrier to separate the incident beam from the reflected beam, effectively isolating the detector's field of view from the illuminated area. Let's explore this configuration further: it operates similarly to the reflectance mode, yet the separation helps reduce system noise.

The fascinating aspect here is that all these modes, transmittance, reflectance, and interaction, are suitable for measuring absorption, transmittance, and scattering in samples. The choice of mode is dictated primarily by the type of medium being analyzed. For instance, transmittance mode is preferred when examining fluids and very thin transparent samples, while reflectance and interaction modes are more suitable for dense, solid, and thick samples. In practical terms, when we discuss reflectance and interaction modes, we are often referring to applications involving skin. In contrast, transmittance mode is better suited for clear or translucent fluids and thin tissue slices. The development of these technologies is tailored to meet such specific needs.

(Refer Slide Time: 15:53)

Lecture 45 : Summarizing Glucose Sensing - Part 3

MIRS

- Mid-infrared spectroscopy (MIRS) also called fingerprint spectroscopy, is a vibrational spectroscopy technique. Hence, it relies on the same system configuration and absorption principles of NIR spectroscopy, but used in the mid-infrared region, approximately between 120 THz (2.5 μm) and 30 THz (10 μm), although some claim 12 THz (25 μm) to be the lower limit in the frequency band.
- Due to the longer wavelength, there is less scattering of MIR radiation in the tissue, leading to higher absorption rates and specific sharp absorption lines in the spectrum, especially between 8–10 μm . This characteristic means that molecules have a unique spectrum in the MIR region, making it ideal for molecular identification. Unfortunately, the strong water absorption in this region does not let MIR signals to penetrate more than some micrometres into the tissue (100 μm approximately), making necessary the use of powerful MIR sources such as Quantum Cascade Lasers (QCL) and the use of complementary technologies, such as photoacoustic spectroscopy, to increase the sensitivity towards glucose detection.

QCL → H₂O

15:53 / 24:17

Now, shifting our focus to mid-infrared spectroscopy, which is often referred to as fingerprint spectroscopy, this technique relies on vibrational spectroscopy principles. While it mirrors the system configuration and absorption principles of near-infrared spectroscopy (NIRS), mid-infrared spectroscopy operates within the mid-infrared region, approximately between 120 and 30 terahertz. Some sources even cite 12 terahertz as the lower limit of this frequency band.

One significant advantage of mid-infrared (MIR) radiation is that its longer wavelengths result in less scattering within tissues, leading to a higher absorption rate and distinctly sharp absorption lines in the spectrum, especially within the range of 8 to 10 micrometers. This characteristic means that each molecule exhibits a unique spectrum in the MIR region, making it an excellent tool for molecular identification.

However, challenges abound, particularly due to strong water absorption in this region. Water significantly limits the penetration of MIR signals, typically restricting it to just a few micrometers, around 100 micrometers at most. To overcome this limitation, powerful MIR sources, such as quantum cascade lasers (also known as OCL lasers), are essential. Complementary technologies, like photoacoustic spectroscopy, can also enhance sensitivity for glucose detection. Remember, water poses a considerable challenge in this context. All living organisms exist in an aqueous environment, where water is the predominant molecule suspending everything. So, as we explore these technologies, always keep in mind the critical role of water in biological systems.

Spectroscopic challenges abound, primarily due to the peculiar absorption characteristics of water. The properties of water can vary significantly, especially when its concentration is low, leading to substantial changes in how it interacts with light. Consequently, it's essential to recognize that interference from water molecules, along with other ions trapped within the water, can impact measurements.

Now, let's revisit a topic we've already touched on: Raman spectroscopy. This is another highly favored technology in the field. Raman spectroscopy, or scattering spectroscopy, revolves around the decrease in scattering of monochromatic light, a phenomenon known as the Raman effect. When single-wavelength light strikes a target, the scattered light

disperses in all directions. Most of this scattered radiation is termed elastic or Rayleigh scattering, which occurs at the same wavelength as the incident light. However, a smaller portion consists of inelastic scattering, known as Raman scattering, where the scattered light has a different wavelength.

(Refer Slide Time: 18:59)

Lecture 45 : Summarizing Glucose Sensing - Part 3

RAMAN SPECTROSCOPY

Raman scattering determines the degree of scattering of monochromatic light based on the Raman effect. When single-wavelength light hits a target, it produces scattered light travelling in all directions. The majority of this radiation, called elastic or Rayleigh scattering, has the same wavelength as the incident light, while the rest is just a small amount of scattered radiation with a different wavelength, called "inelastic scattering" or "Raman scattering". Such a wavelength difference is the Raman shift, and it represents the difference between the initial and final vibrational states of the molecule under study. As such, Raman spectroscopy is dependent on the rotational and vibrational states within molecules, and it can be used to detect specific absorption bands and quantify the corresponding molecules, meaning that peak locations in the Raman spectrum show the vibrational modes of each functional group within the molecule. Hence, indicating that the Raman shift (expressed in wavenumbers, cm^{-1}) will be the same regardless of the wavelength of the incident light. In case of glucose, the most representative vibration modes are those linked to the C—H stretching band, around 2900 cm^{-1} , and the C—O and C—C stretching bands between 800 and 1300 cm^{-1} .

C-H / 2900 / 800 / 1300 / C-O / C-C INELASTIC / RAMAN

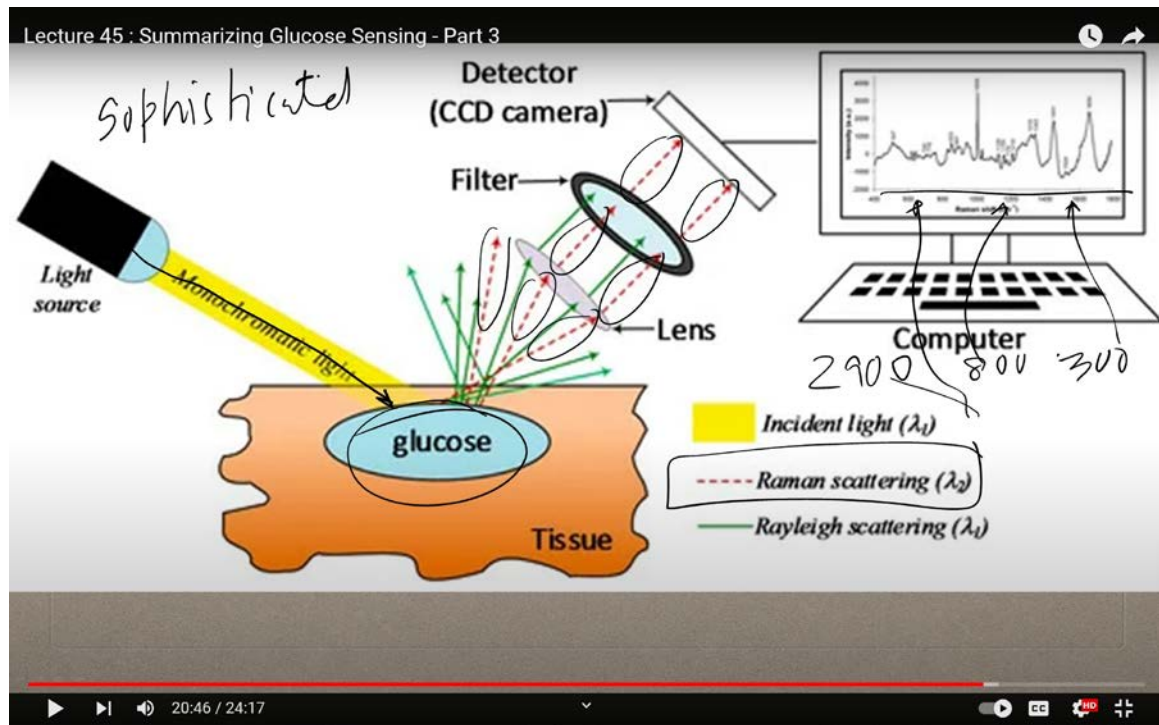
18:59 / 24:17

We're particularly interested in this inelastic scattering, as it yields what is known as the Raman shift. This shift represents the difference between the initial and final vibrational states of the molecule being studied. Therefore, Raman spectroscopy hinges on the rotational and vibrational states within molecules. It is capable of detecting specific absorption bands and quantifying the corresponding molecules. This means that the peak locations in the Raman spectrum correlate with the vibrational modes of each functional group present in the molecule. Notably, the Raman shift, expressed in either wavelength or wave numbers, remains consistent, irrespective of the wavelength of the incident light.

In the case of glucose, the most characteristic vibrational modes are associated with the OH—CH stretching band, which appears around 2900 cm^{-1} , and the CO and CC stretching

bands, located between 800 and 1300 cm^{-1} . These figures are crucial to remember when analyzing Raman spectra. When you observe these peaks, it suggests the possible presence of glucose. However, additional measurements are necessary to confirm its presence unequivocally.

(Refer Slide Time: 20:46)

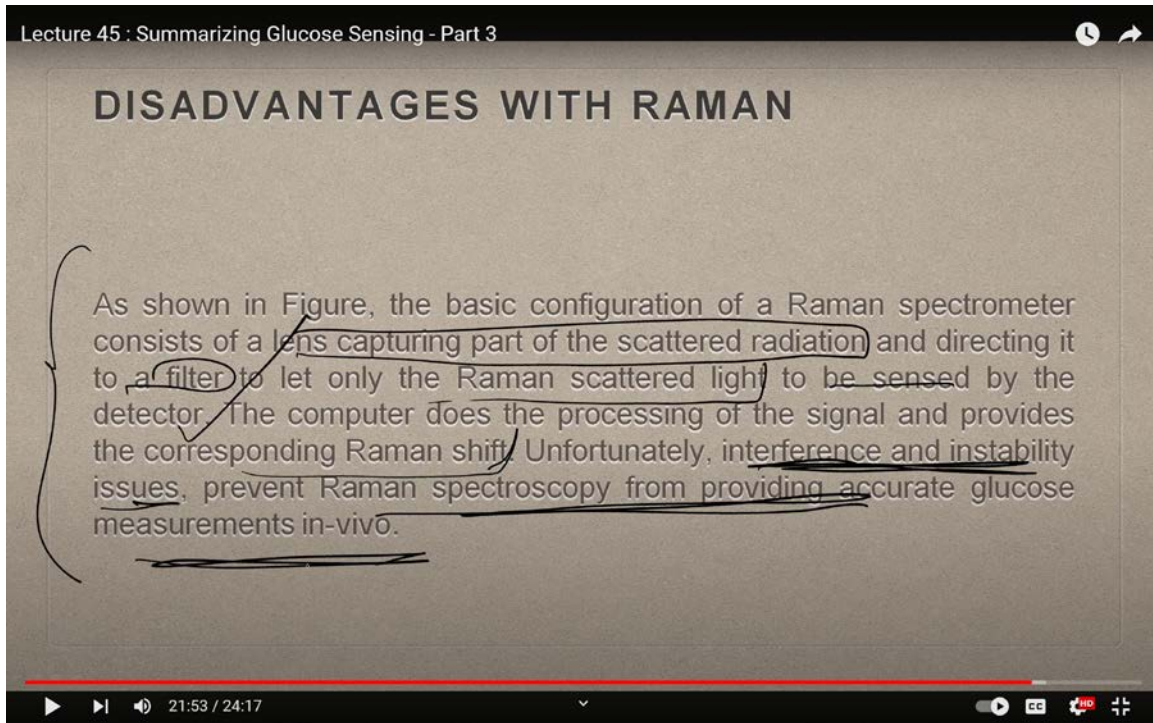


To visualize how Raman spectroscopy operates, consider that it employs monochromatic light, leading to various types of reflections. Our primary focus remains on inelastic scattering, specifically the wavelength differences that characterize the Raman shift. This shift effectively represents the transition between the initial and final vibrational states of the molecules in question.

In our discussion, we focus exclusively on the scattered radiation that exhibits a different wavelength, known as inelastic scattering. This is precisely what the Raman spectra reveal to us. Our primary concern lies with these distinct peaks, particularly the three key wave numbers: 2900 cm^{-1} , 800 cm^{-1} , and 1300 cm^{-1} . It's essential to pinpoint where these values appear in the spectrum, as they are critical for determining not only whether the sample

contains glucose but also the concentration of glucose present.

(Refer Slide Time: 21:53)



Lecture 45 : Summarizing Glucose Sensing - Part 3

DISADVANTAGES WITH RAMAN

As shown in Figure, the basic configuration of a Raman spectrometer consists of a lens capturing part of the scattered radiation and directing it to a filter to let only the Raman scattered light to be sensed by the detector. The computer does the processing of the signal and provides the corresponding Raman shift. Unfortunately, interference and instability issues, prevent Raman spectroscopy from providing accurate glucose measurements in-vivo.

21:53 / 24:17

Now, you must appreciate that achieving this level of specificity requires sophisticated instrumentation. The detection tools employed in Raman spectroscopy are exceptionally intricate, posing significant challenges in terms of optical sophistication.

However, it is crucial to acknowledge some inherent disadvantages associated with Raman spectroscopy. The basic configuration of a Raman spectrometer includes a lens designed to capture a portion of the scattered radiation. This lens directs the captured light to a filter, which eliminates the majority of Rayleigh scattering, allowing only the Raman-scattered light to be detected. The resultant signal is then processed by a computer, which generates the corresponding Raman spectrum.

Regrettably, interference and instability often hinder Raman spectroscopy from delivering accurate glucose measurements in vivo. While I have great admiration for this elegant technique, the extent of interference and ambiguity complicates its application in living systems. For effective measurements, we need to apply the technology to the skin without

adversely affecting the light we are directing onto the surface. Achieving this without introducing noise or other disruptive elements is incredibly challenging, even with the most advanced tools available. This remains a pressing challenge for researchers: how can we establish reliable detection limits, and how far can we advance this technology?

Moving forward, we will delve into additional technologies in our next class, starting with far infrared spectroscopy, followed by discussions on various other methodologies.

However, for now, it's important to realize the enormity of what lies ahead. We are concentrating on just one molecule, glucose, which is among the simplest of biomolecules, but when we consider the complexity of various biological systems, the challenge multiplies. Each of these systems requires its own unique signature, and developing concrete systems may take decades or even longer. So, I urge you to focus and think critically about this topic because a significant amount of literature surrounds glucose sensing.

By the end of this course, you should possess a holistic understanding of biosensor development and the myriad opportunities it presents. Thank you!