Design for Biosecurity

Prof. Mainak Das

Department of Design

Indian Institute of Technology, Kanpur

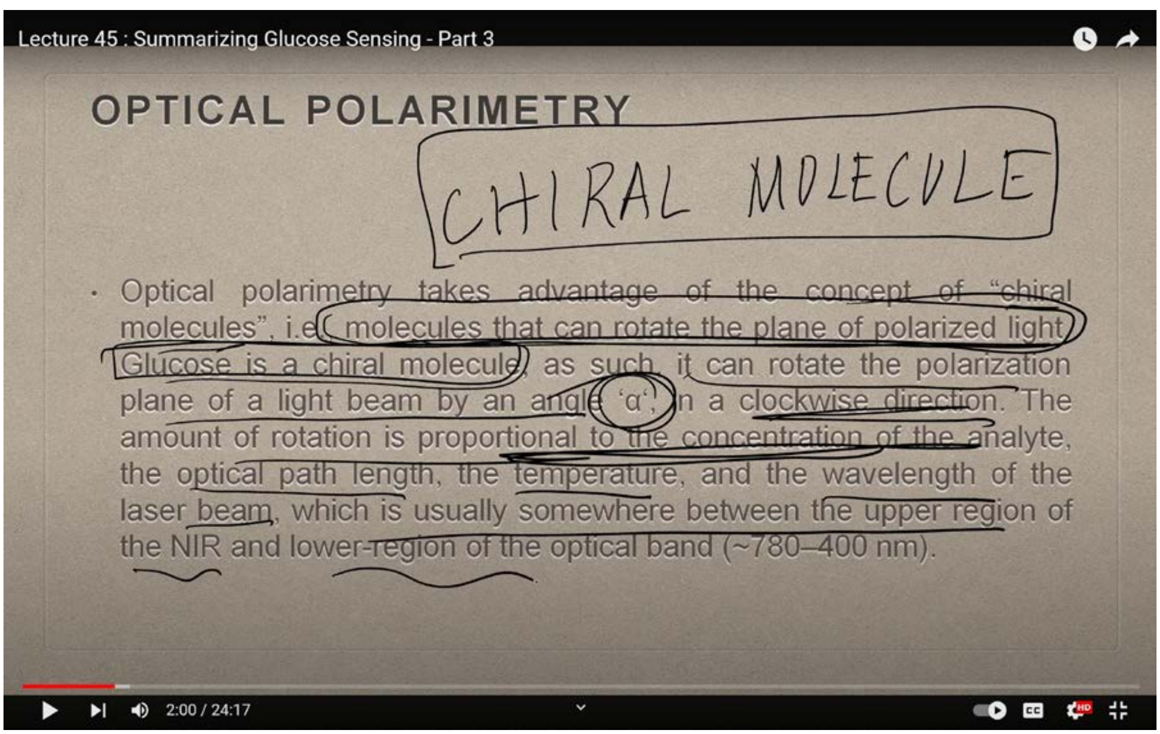
Lecture 45

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.

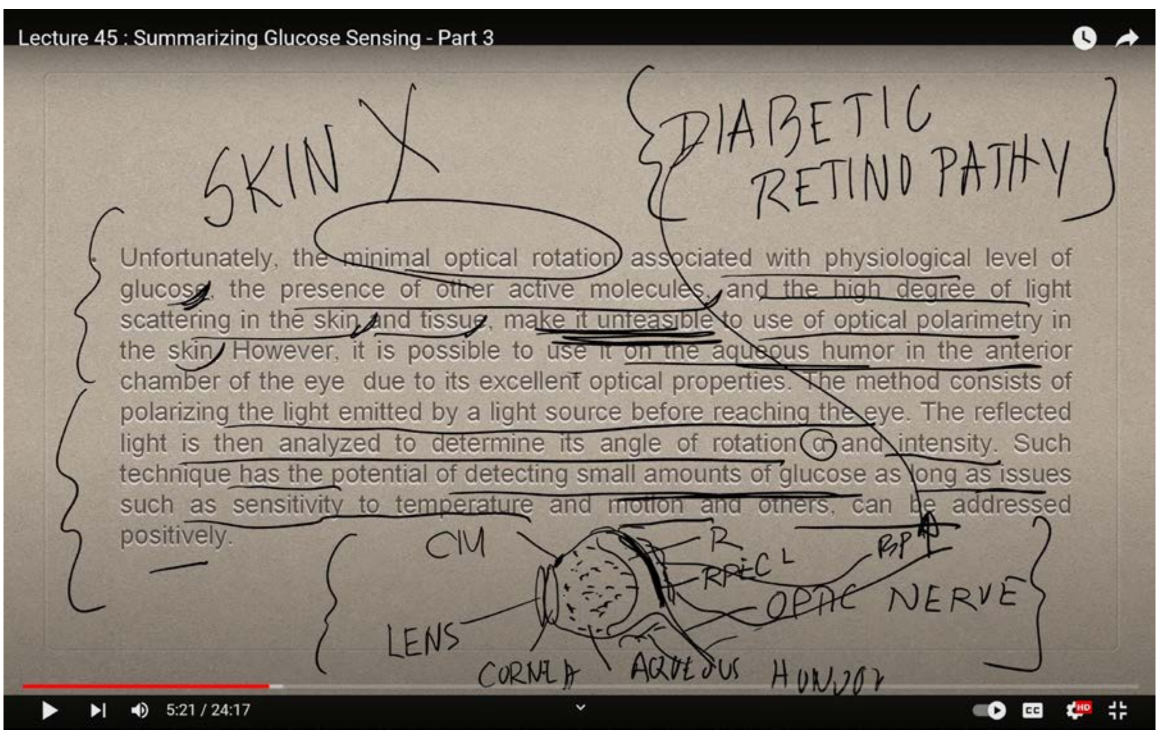
(Refer Slide Time: 02:00)



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.

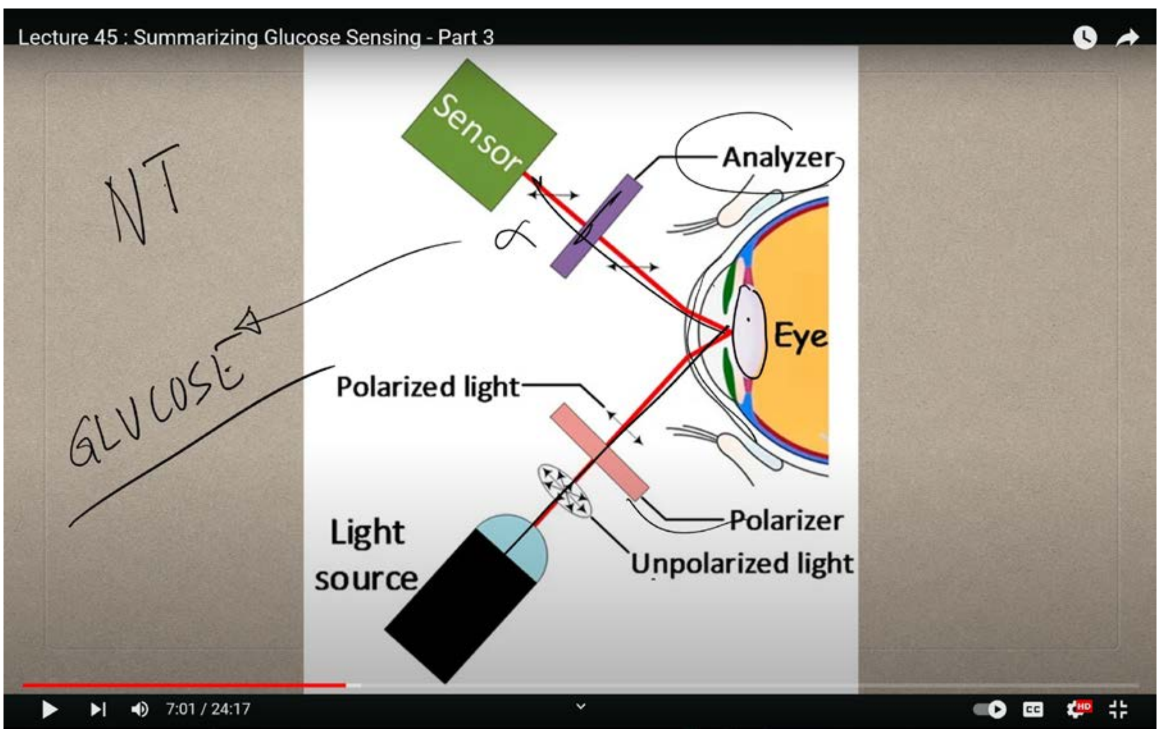
(Refer Slide Time: 05:21)



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.

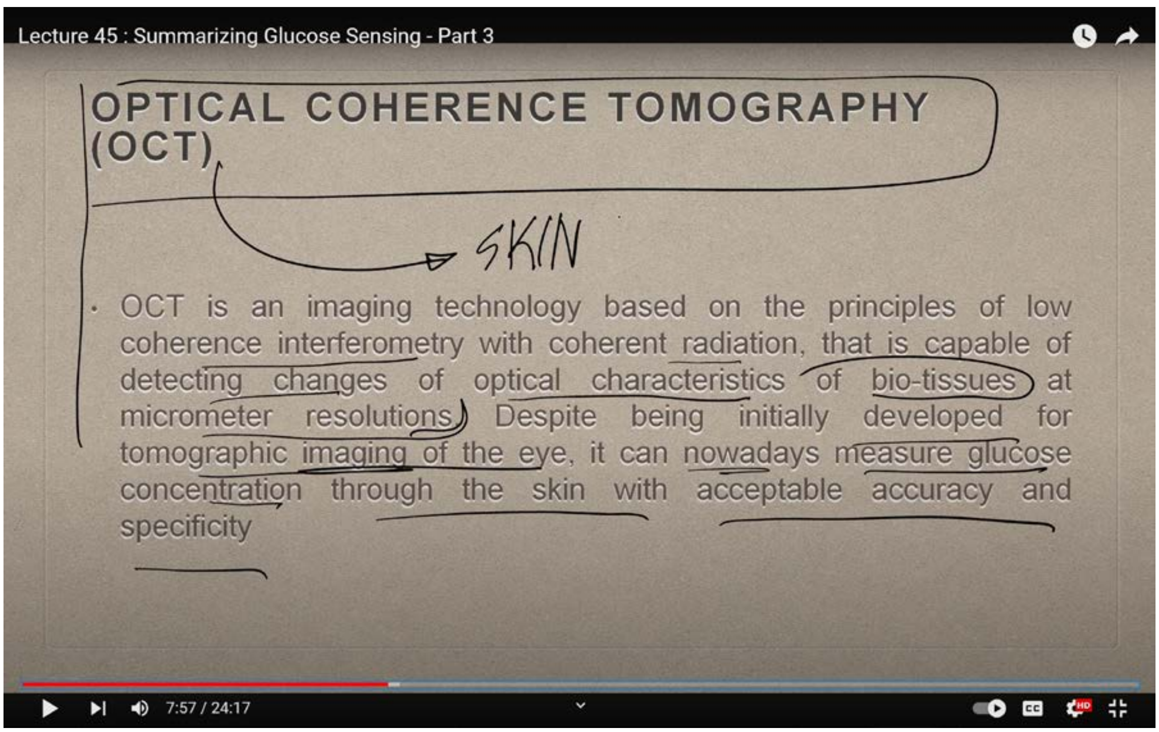
(Refer Slide Time: 07:01)



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.

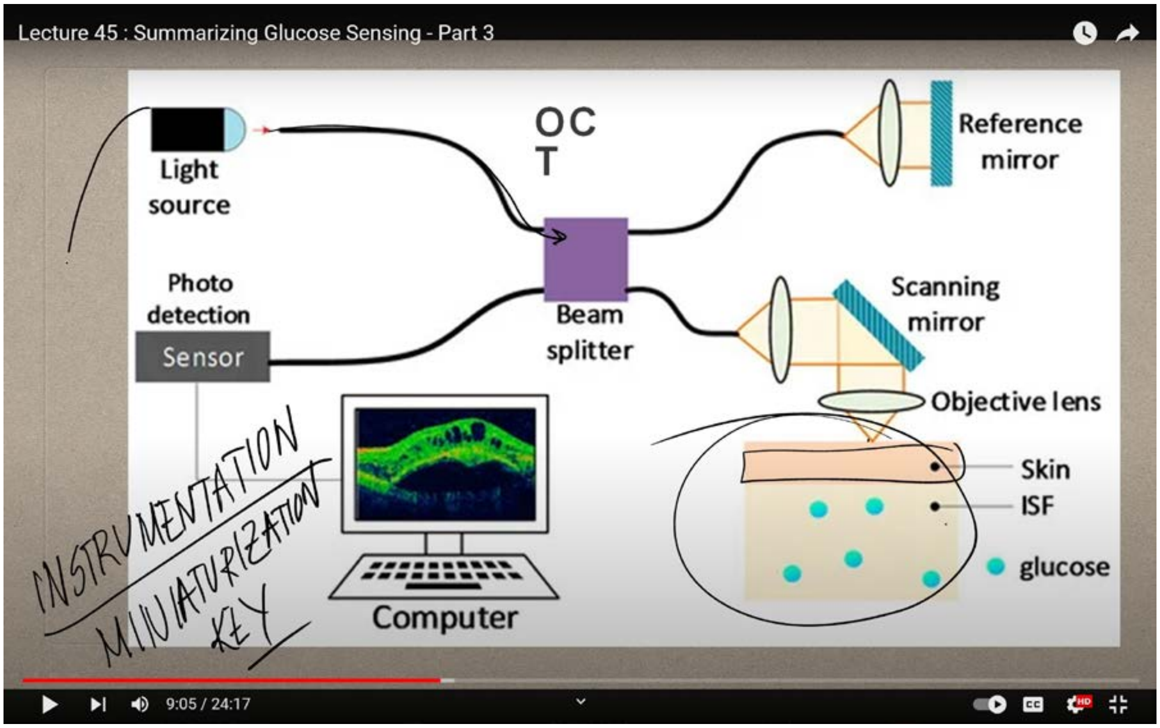
Let's visualize how it works: the light source emits light that passes through the cornea and lens. The sensor and analyzer are positioned to capture the light. Unpolarized light passes through a polarizer, allowing us to analyze the angle α, which is proportional to the glucose concentration in the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

(Refer Slide Time: 07:57)



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.

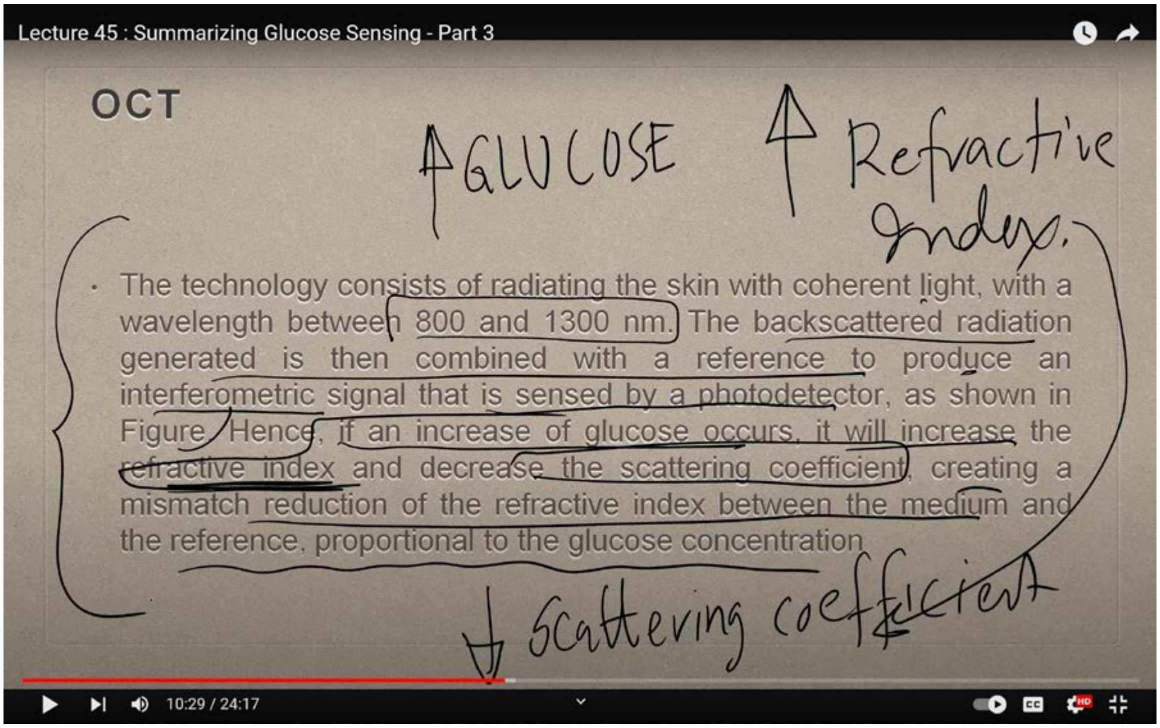
(Refer Slide Time: 09:05)



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.

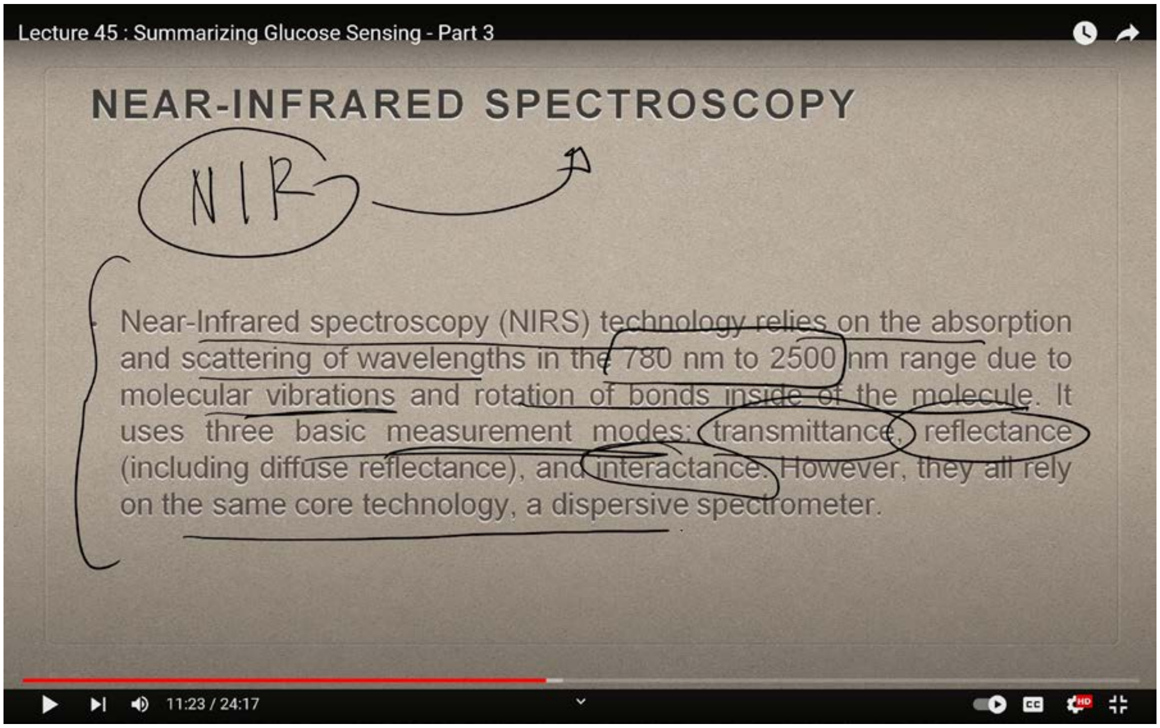
(Refer Slide Time: 10:29)



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.

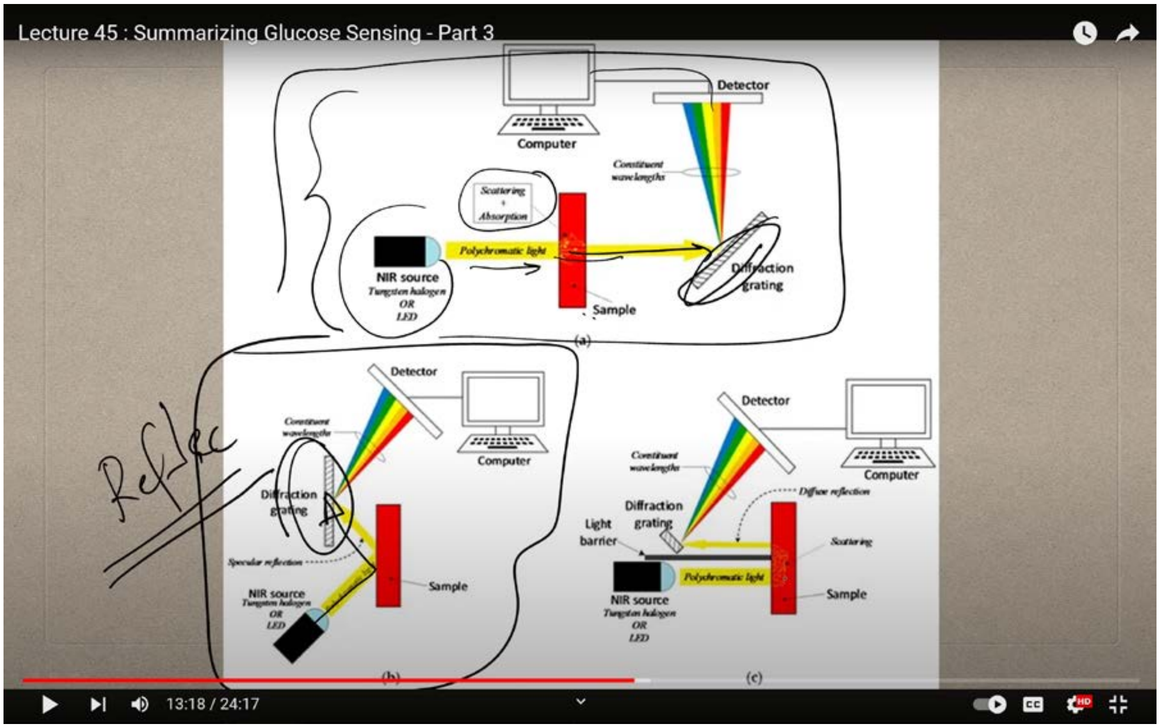
(Refer Slide Time: 11:23)



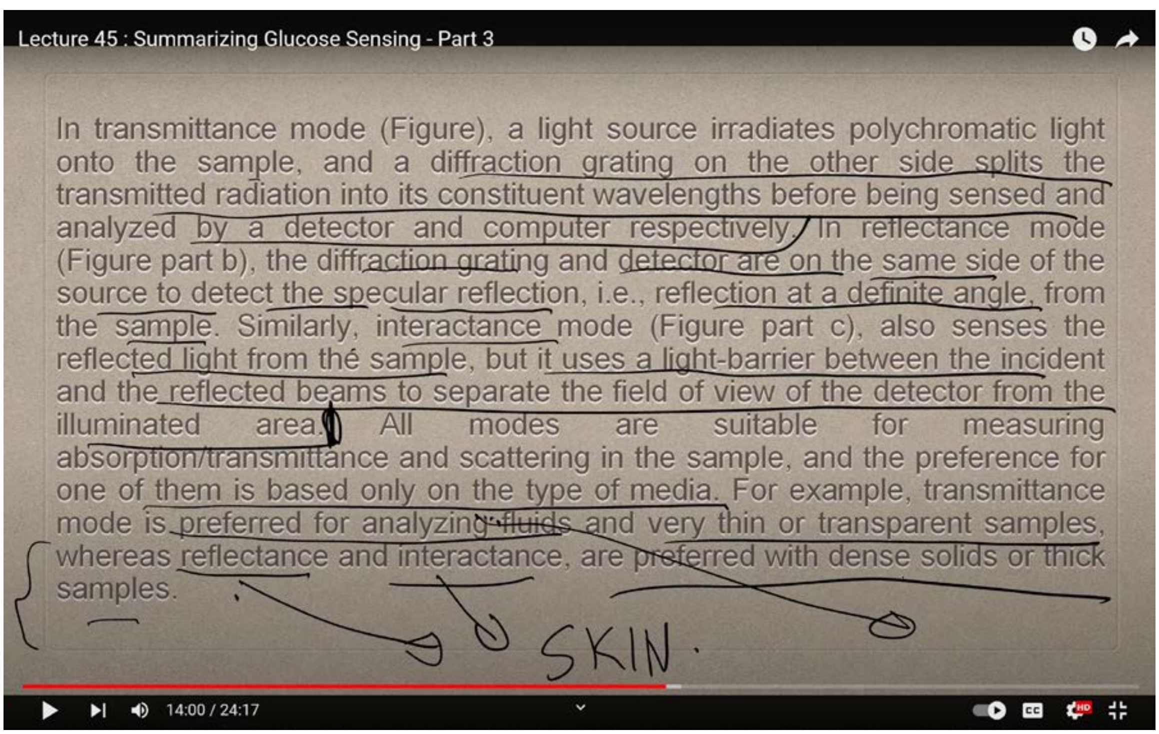
NIR employs three primary measurement modes: transmittance, reflectance, and differences in reflectance. However, all these modes are built upon the same core technology, the dispersive spectrometer. In transmittance mode, scattered light irradiates polychromatic light onto the sample. To illustrate 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)



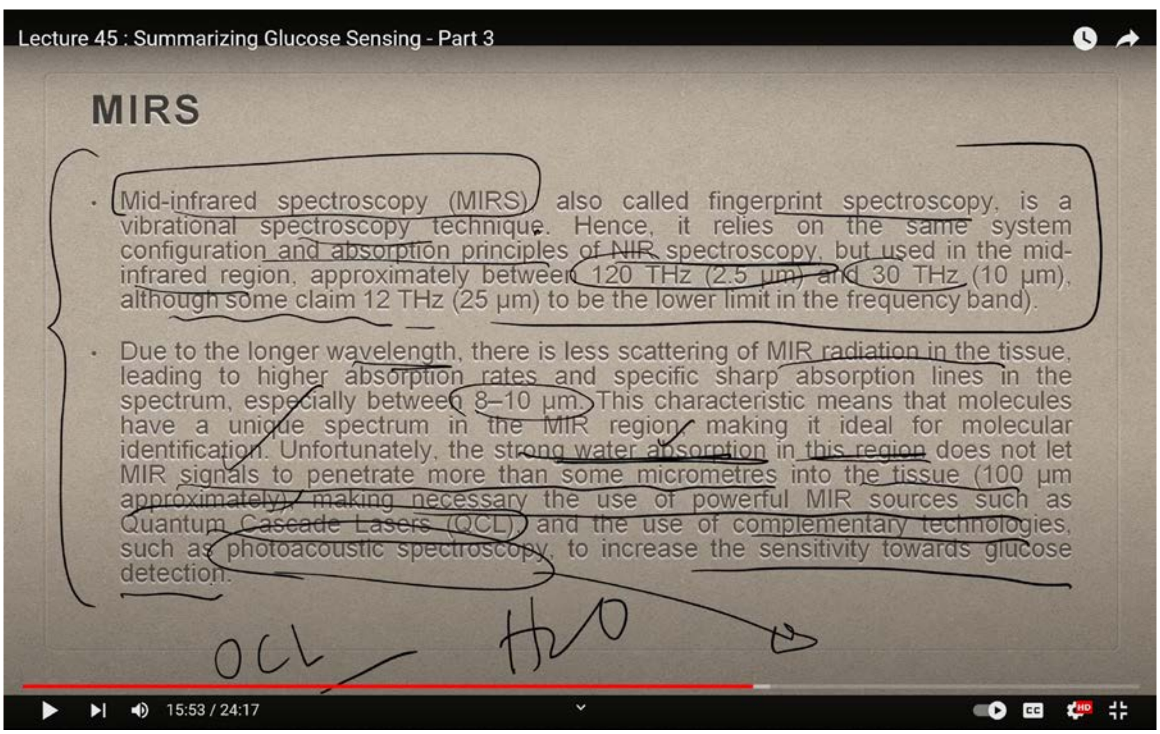
(Refer Slide Time: 14:00)



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)



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.

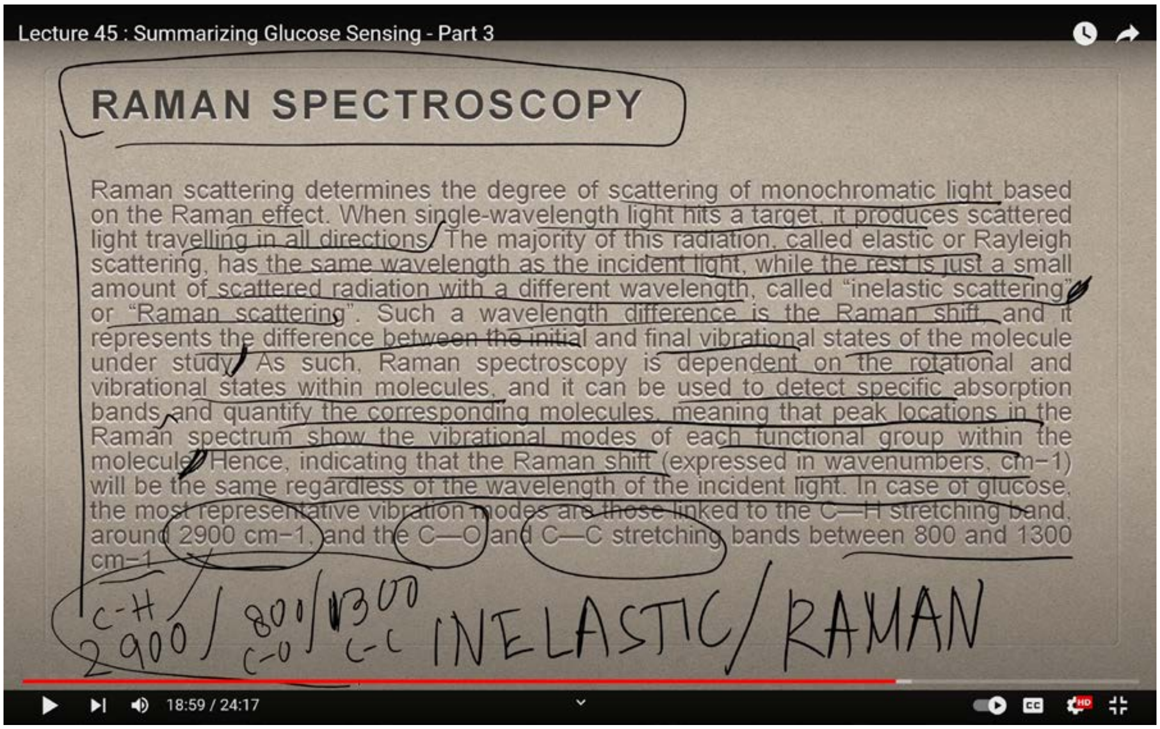
One significant advantage of mid-infrared (MIR) radiation is that its longer wavelengths result in less scattering within tissues, leading to a higher absorption rate and distinctly sharp absorption lines in the spectrum, especially within the range of 8 to 10 micrometers. This characteristic means that 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.

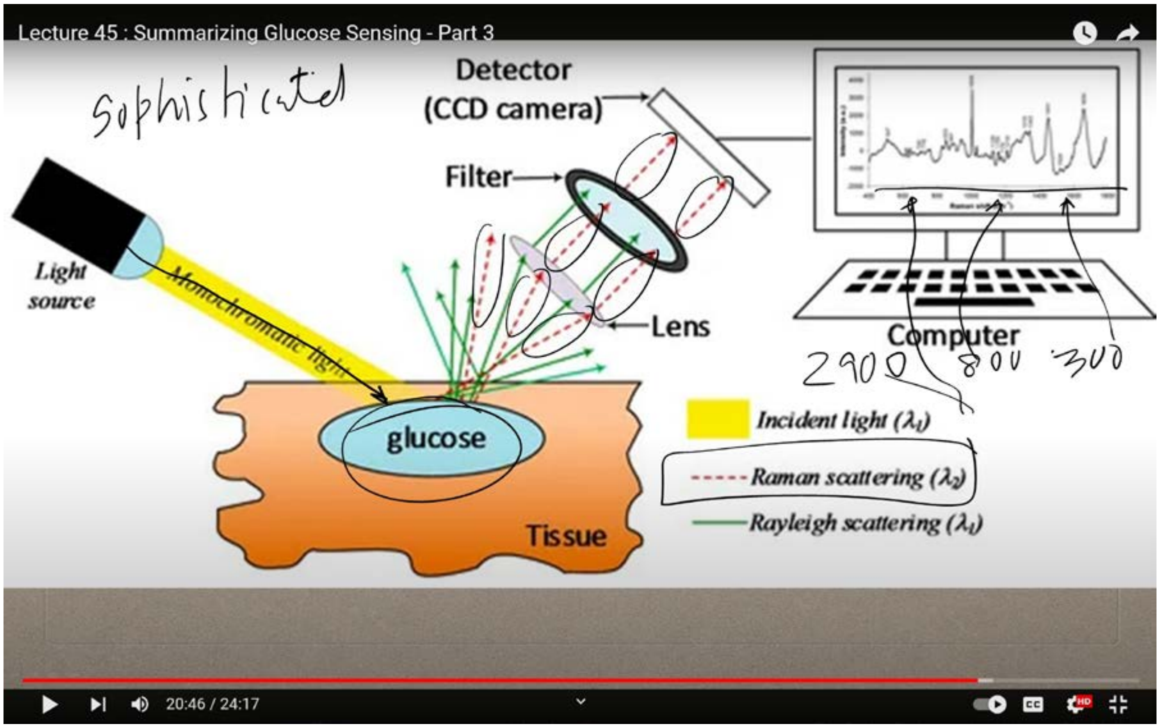
(Refer Slide Time: 18:59)



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.

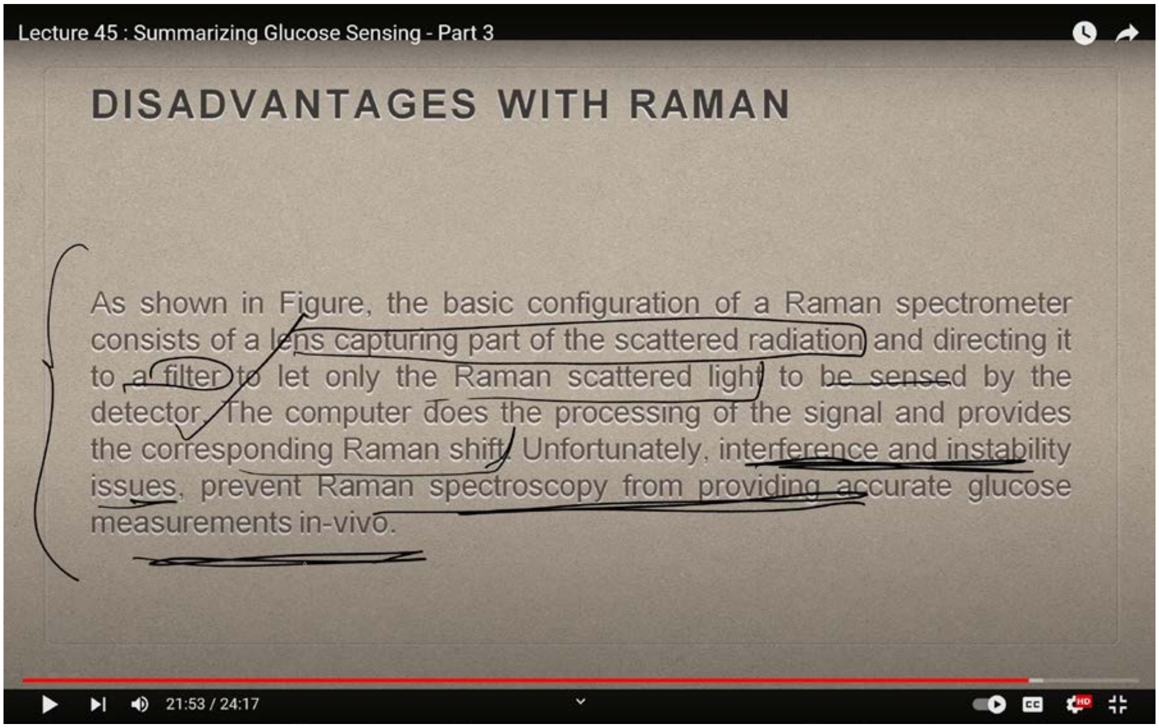
(Refer Slide Time: 20:46)



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)



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!