Design for Biosecurity

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Lecture 44

Summarizing Glucose Sensing - Part 2

In our last class, we delved into the fundamentals of hexokinase and glucose oxidase sensors. As I concluded that discussion, I mentioned that we would be exploring three-electrode configurations. The concept here is straightforward: whenever we refer to the flow of current, we must also consider the flow of charges. To understand this flow, we need a reference point, something that we can consider as zero, an imaginary reference. It's somewhat akin to how we define pH: at pH 7, the concentrations of H^+ and H^- ions are equal, representing neutrality. From this point, we can classify values below 7 (6, 5, 4, 3, 2, 1) as acidic, while those above 7 (8, 9, 10, 11, 12) are considered alkaline.

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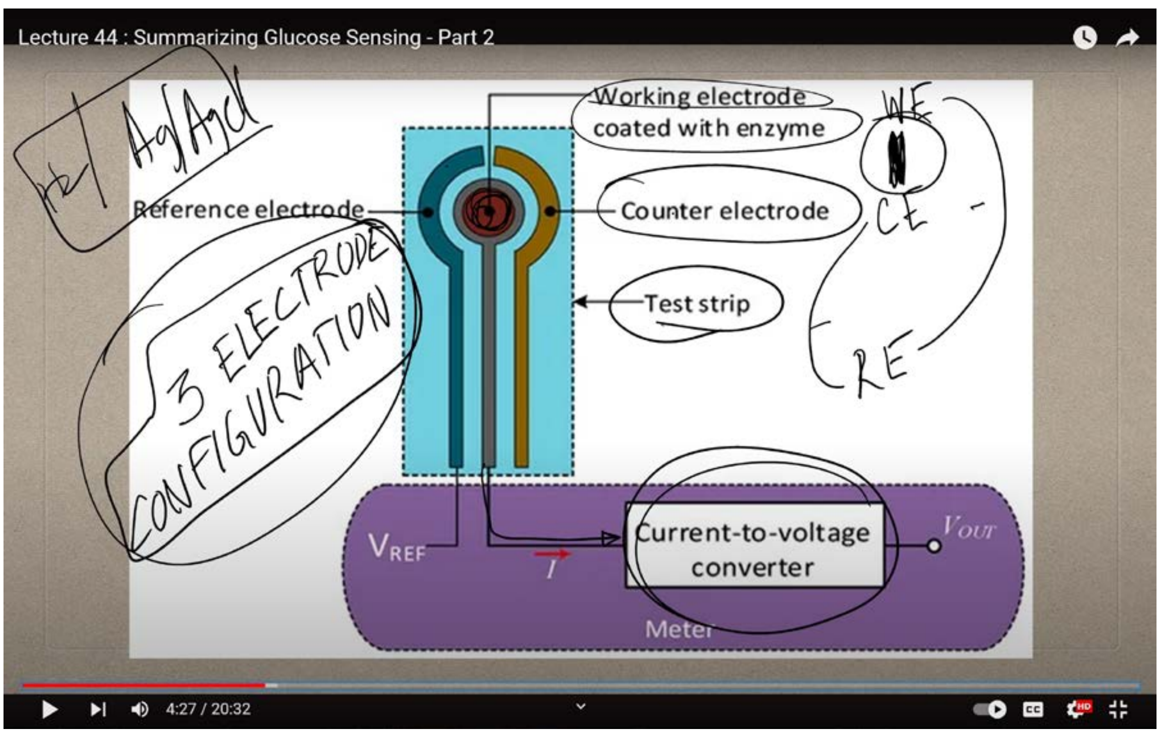

In electrochemistry, this imaginary reference point is vital, as it allows us to measure with respect to a standard electrode. Historically, this reference originated in the early 1900s, around 1901 or 1902. The first reference electrode was the hydrogen electrode, often constructed with mercury. However, the complexity and challenges associated with using hydrogen electrodes led to the development of the silver-silver chloride (Ag-AgCl) electrode, which offered a more practical solution. These electrodes serve as universal references, particularly for aqueous systems, while alternatives exist for non-aqueous environments.

In relation to this reference, we have a working electrode and a counter electrode. The three-electrode configuration is a robust system that allows us to compare the counter electrode with the reference electrode, and the working electrode with the reference electrode. This design effectively eliminates ambiguity in measurements.

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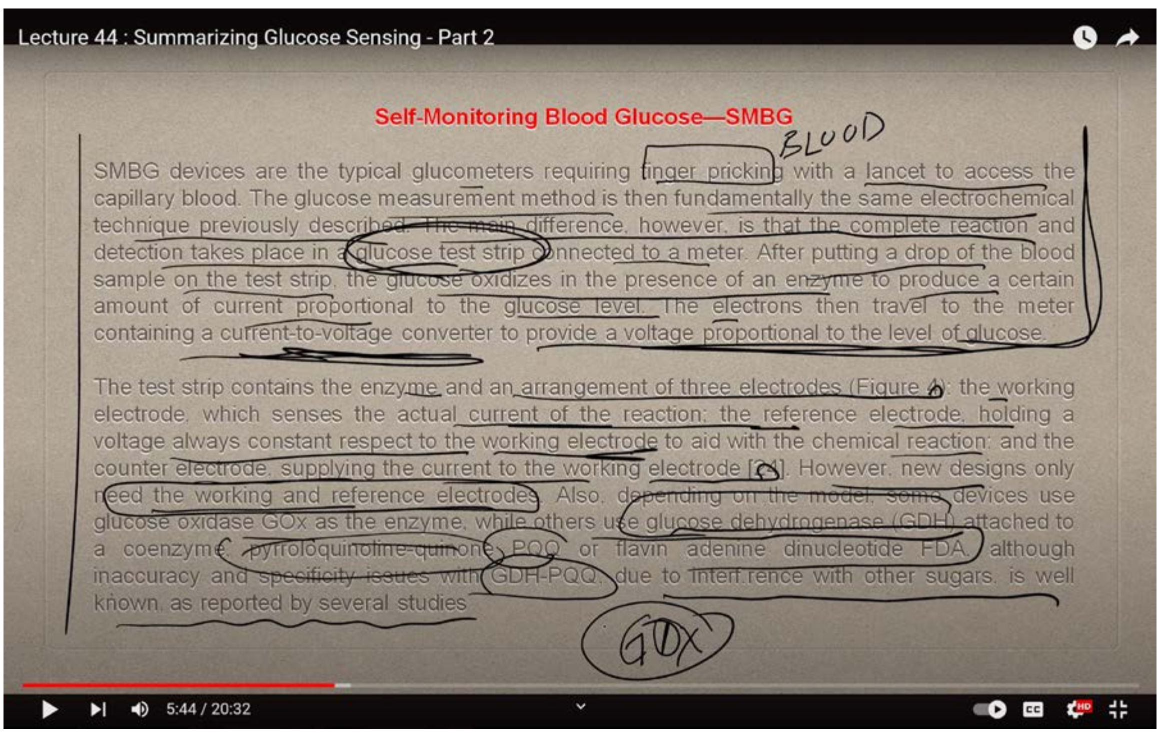

So, we have three components: W for the working electrode, C for the counter electrode, and R_e for the reference electrode. The working electrode is compared to the reference electrode, while the counter electrode is also compared to the reference electrode. This setup balances everything out, and the result obtained between the working and counter electrodes, after accounting for any differences, yields the final measurement.

As we can see, this three-electrode configuration is essential for precise measurements. It adds an extra layer of assurance, ensuring that our measurements remain unambiguous. Now, returning to the test strip: it contains the enzyme and an arrangement of three electrodes, as illustrated in the figure. The working electrode is responsible for sensing the actual current generated by the reaction, the reference electrode maintains a constant voltage relative to the working electrode to facilitate the chemical reaction, and the counter electrode supplies the necessary current to the working electrode. This configuration is crucial for the accurate and reliable functioning of the sensor.

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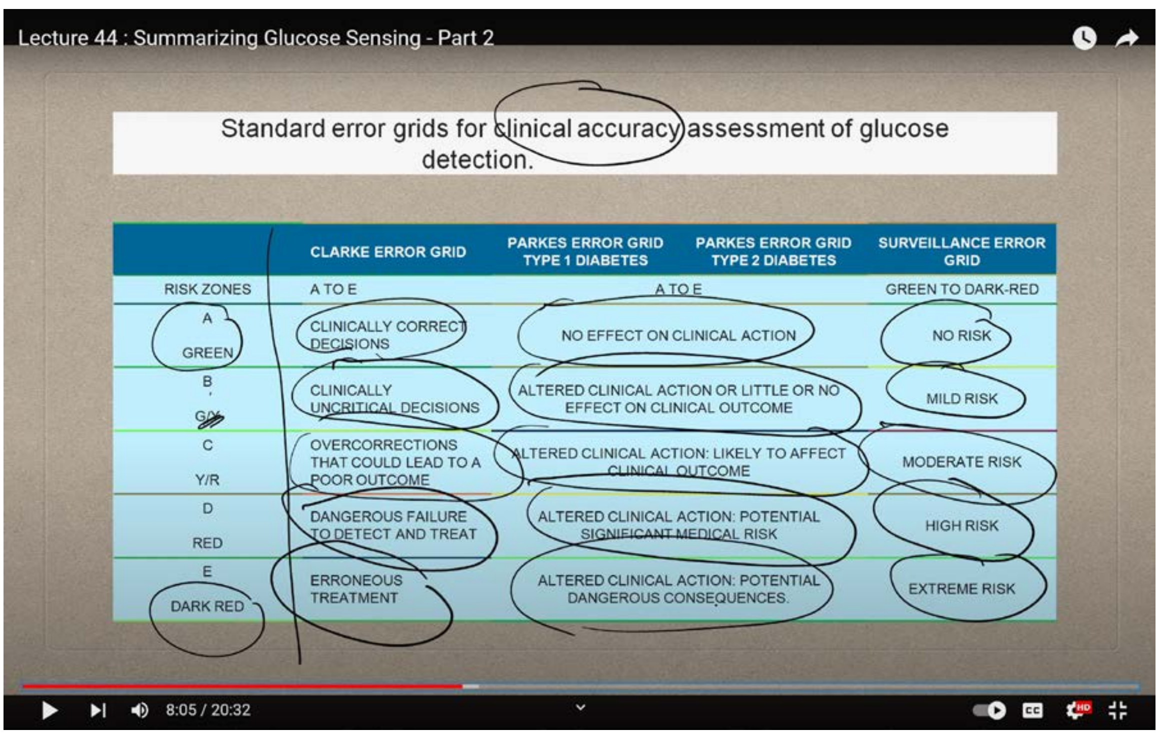

The latest designs of glucose sensors only require the working and reference electrodes, streamlining the setup significantly. Depending on the specific model, some devices utilize glucose oxidase as the enzyme, while others employ glucose dehydrogenase (GDH). This introduces a new dimension to our understanding. When we talk about glucose dehydrogenase, it is typically associated with a coenzyme, such as pyrroloquinoline quinone (PQQ) or flavin adenine dinucleotide (FAD). However, it's important to note that there are known inaccuracies and specific issues with the GDH and PQQ systems, mainly due to interference from other sugars, which have been documented in several studies. Thus far, the glucose oxidase system remains the most efficient choice.

While there are alternative enzymatic systems available, they do not match the efficiency of the glucose oxidase system. Now, let's discuss the standard error grid associated with sensor performance. When evaluating any sensor, standard error grids are established to assess risk. For instance, we categorize the risk zones into sections labeled A, B, C, D, and E. The dark red area represents an extremely erroneous zone, while the green area indicates a go signal.

Zones A to E represent clinically correct decisions that have no adverse effect on clinical actions or risks. Moving down the grid, we enter the realm of clinically uncertain decisions, indicated by green and yellow zones. These areas signify a mild risk of altered clinical action, with little or no effect on clinical outcomes. However, yellow and red zones indicate potential for poor outcomes, where altered clinical actions are likely to affect clinical results with moderate risk.

For every sensor developed, passing through an error grid is essential to ensure clinical accuracy. Another critical aspect to consider is biosensing accuracy, which pertains to the sensor's ability to detect specific targets. Without a standard error grid, the industry is unlikely to accept new technologies due to the inherent risks involved. There are strategic issues at play here, including safety, security, insurance, and numerous other complexities and compliance requirements. Understanding how this business operates is crucial.

The grid also highlights potential dangers: failure to detect and treat altered clinical actions can lead to significant medical risks. The dark red zone represents a scenario of extremely poor signals, indicating high risk and the potential for dangerous consequences from altered clinical actions. This type of error grid is vital for every biosensor developed for detecting specific biological molecules, entities, toxins, hormones, or metabolites.

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As I mentioned at the beginning of class, the opportunities for research, job creation, and industry development in this field are exceptionally high. Understanding the fundamentals and the engineering principles required to develop these sensors is key.

Now, when we consider the technologies currently being developed for minimally invasive (MI) and non-invasive (NI) glucose detection, we can classify them into several categories. For MI technologies, we have optical, thermal, electric, and nanotechnology methods. Examples of minimally invasive techniques include ultrasound and sonophoresis, which we will explore further.

In the electric method category, amperometry is a significant technique, while thermal methods encompass photothermal spectroscopy (PAS), modulated heat conduction (MHC), and thermal spectroscopy.

Optical methods are varied, including time-of-flight mass spectrometry, Fourier-transform infrared (FTIR) spectroscopy, Raman spectroscopy, medium infrared, near-infrared, and tomographic techniques. Additionally, fluorescence methods and surface plasmon resonance (SPR) are noteworthy technologies. We have briefly discussed some of these, and we will delve deeper into others in our upcoming classes.

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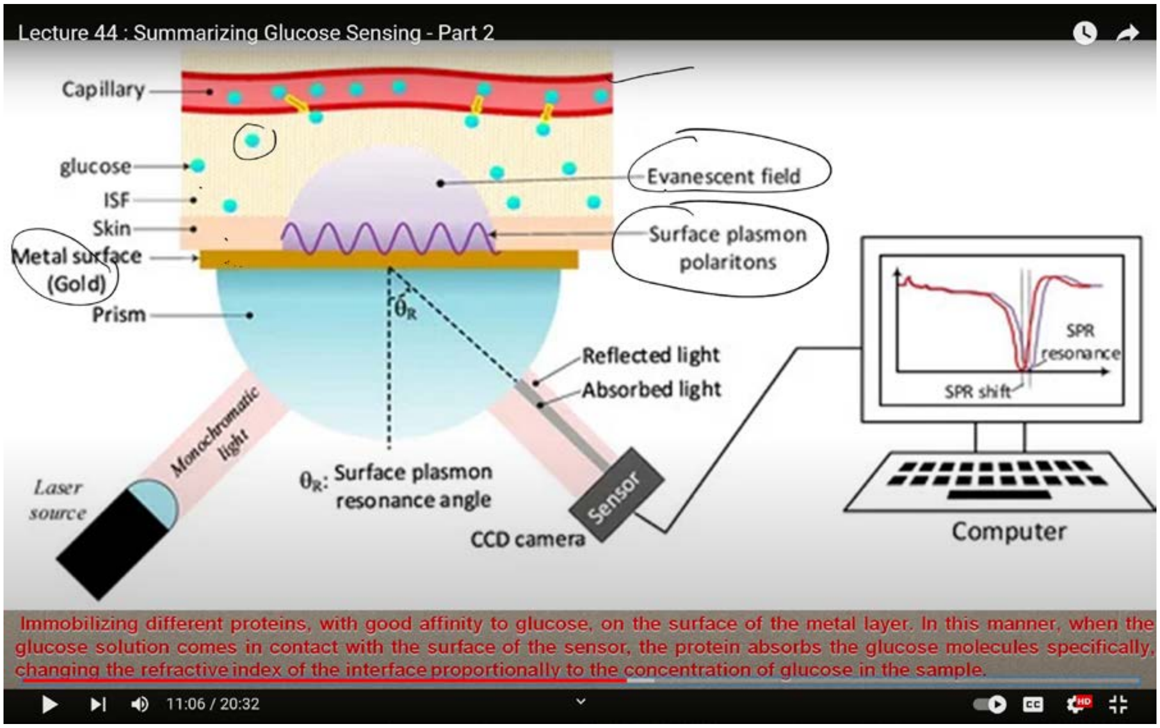

When working with any biosensor, it's essential to recognize that you are navigating a vast spectrum of investigation. This is why I emphasized the importance of corroborating one technology with another. Only when multiple technologies converge and provide a clear understanding can we truly appreciate their advantages and disadvantages, allowing us the freedom to utilize them effectively.

Now, let’s focus on Surface Plasmon Resonance (SPR), a method we've discussed extensively. Today, we will explore its direct application.

Here is what an SPR system looks like: you can see a blood capillary carrying blood, represented here, while the blue dots symbolize glucose molecules found in the interstitial fluid of the skin. On top of the body surface, there’s a metal electrode, typically made of gold. Additionally, there is a prism involved in the detection process, and we previously discussed the evanescent field and the surface plasmon polaritons.

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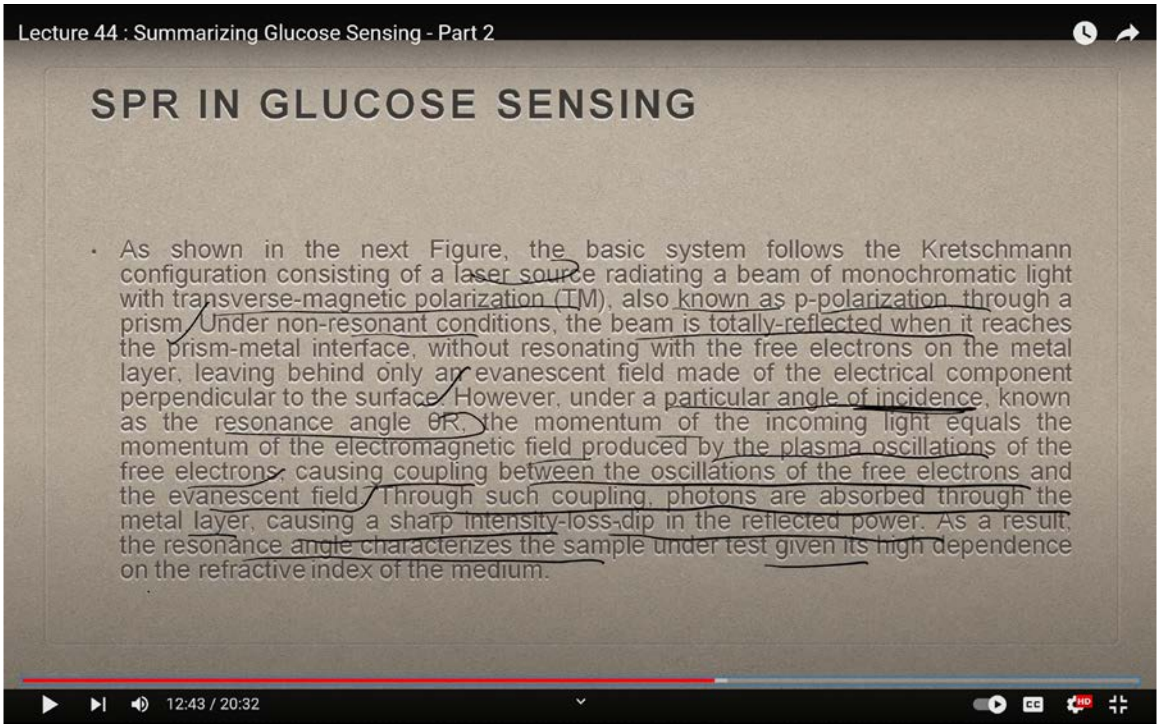

The basic system follows the Kretschmann configuration, which you are already familiar with. This configuration includes a laser source, where a beam of monochromatic light with a transverse magnetic field, also referred to as P polarization, is directed through the prism. As this monochromatic light strikes the surface, it behaves in a specific manner.

Under non-resonant conditions, the beam reflects off the prism-metal surface without resonating with the free electrons in the metal layer. In this scenario, only the evanescent field, an electrical component perpendicular to the surface, remains behind.

However, there is a critical moment that occurs at a particular angle known as the resonance angle, θ_r. At this specific angle, the momentum of the incoming light aligns with the momentum of the electromagnetic field perpendicular to the plasma oscillation of the free electrons. This alignment facilitates coupling between the oscillation of the free electrons and the evanescent field.

As a result of this coupling, photons are absorbed through the metal layer, leading to a sharp dip in the reflected power. This resonance angle is indicative of the sample being tested and is highly dependent on the refractive index of the surrounding medium. Consequently, the reflected light forms the SPR signal that we obtain from the system.

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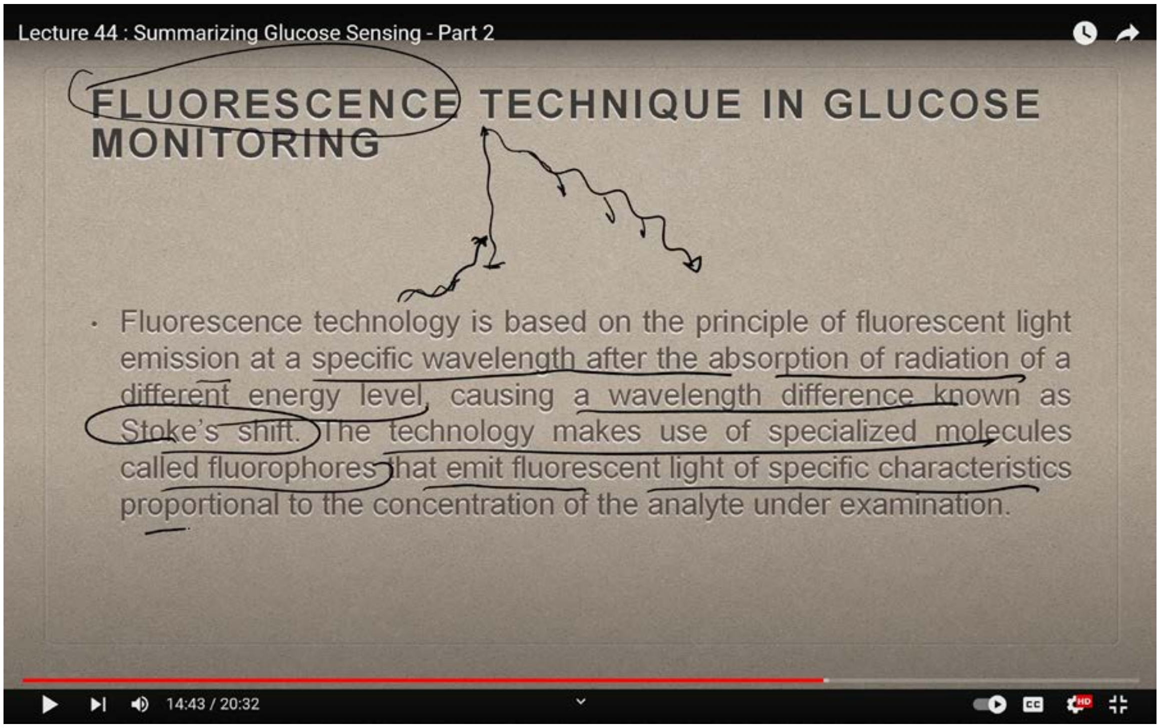

This technology is advantageous because it doesn't require any conjugation of molecules like glucose oxidase; you simply place the sensor directly on the skin to take measurements. However, it comes with its own challenges, including interference from other molecules and various fine-tuning issues that must be addressed in the SPR setup. Theoretically, it is an excellent technique, but practical implementation involves complex instrumentation, which must be carefully managed.

Now, let’s transition to a technique I haven’t yet discussed: fluorescence.

So, what exactly is fluorescence? When an electron is excited due to irradiation, it jumps out of its valence shell and eventually makes its way back. During this journey, as it returns to its original shell, it releases a significant amount of energy. This energy release includes a specific component known as fluorescent energy. Fluorescence technology is fundamentally based on the principle of fluorescent light emission at a particular wavelength, following the absorption of radiation at a different energy level.

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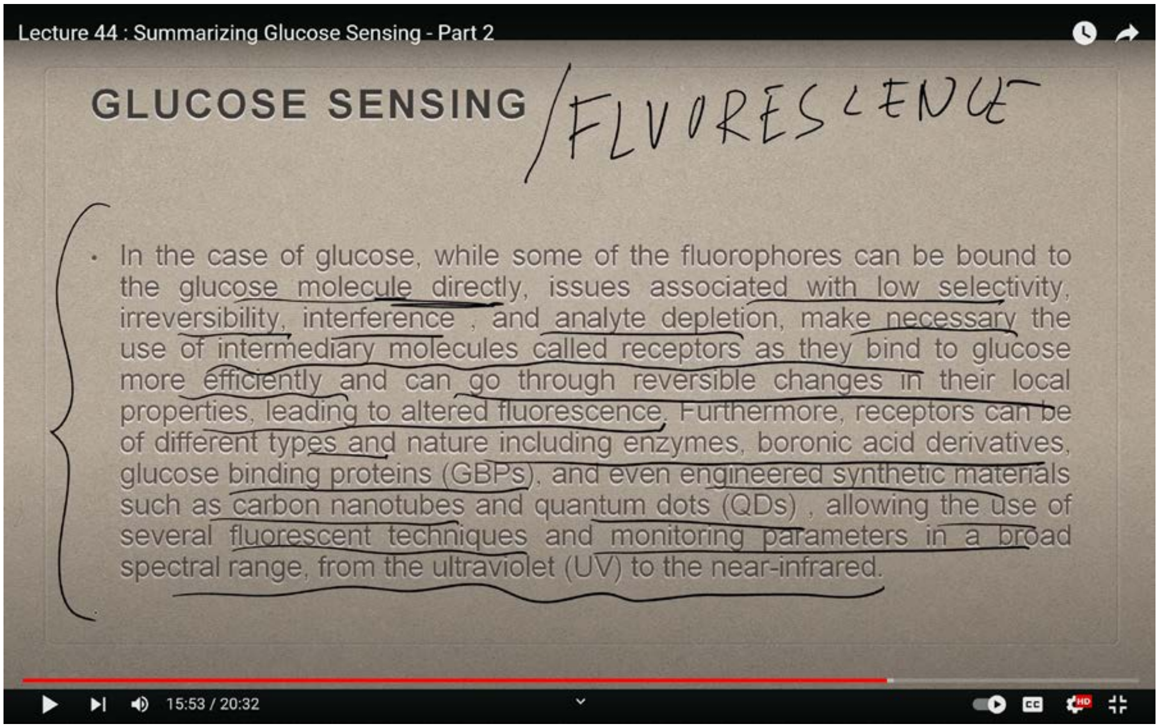

It's important to note that the energy level associated with absorption creates a wavelength difference known as the Stokes shift. The technology utilizes specialized molecules called fluorophores, which emit fluorescent light with specific characteristics that are proportional to the concentration of the analyte being examined. This technique is quite popular, provided you have a suitable fluorophore. However, it’s worth mentioning that glucose sensing with fluorescence does not produce much background noise.

In the case of glucose, while certain fluorophores can bind directly to glucose molecules, challenges such as low selectivity, irreversible interference, and analyte depletion necessitate the use of intermediary molecules known as receptors. These receptors bind to glucose more effectively and undergo reversible changes in their local properties, which leads to altered fluorescence.

The receptors can vary in type and nature, including enzymes, boronic acid derivatives, glucose-binding proteins, and even engineered synthetic materials such as carbon nanotubes and quantum dots. This diversity allows for the application of various fluorescent techniques, enabling monitoring across a broad spectral range from ultraviolet to near-infrared light. Despite these advancements, fluorescence technology is not exceptionally effective for several reasons.

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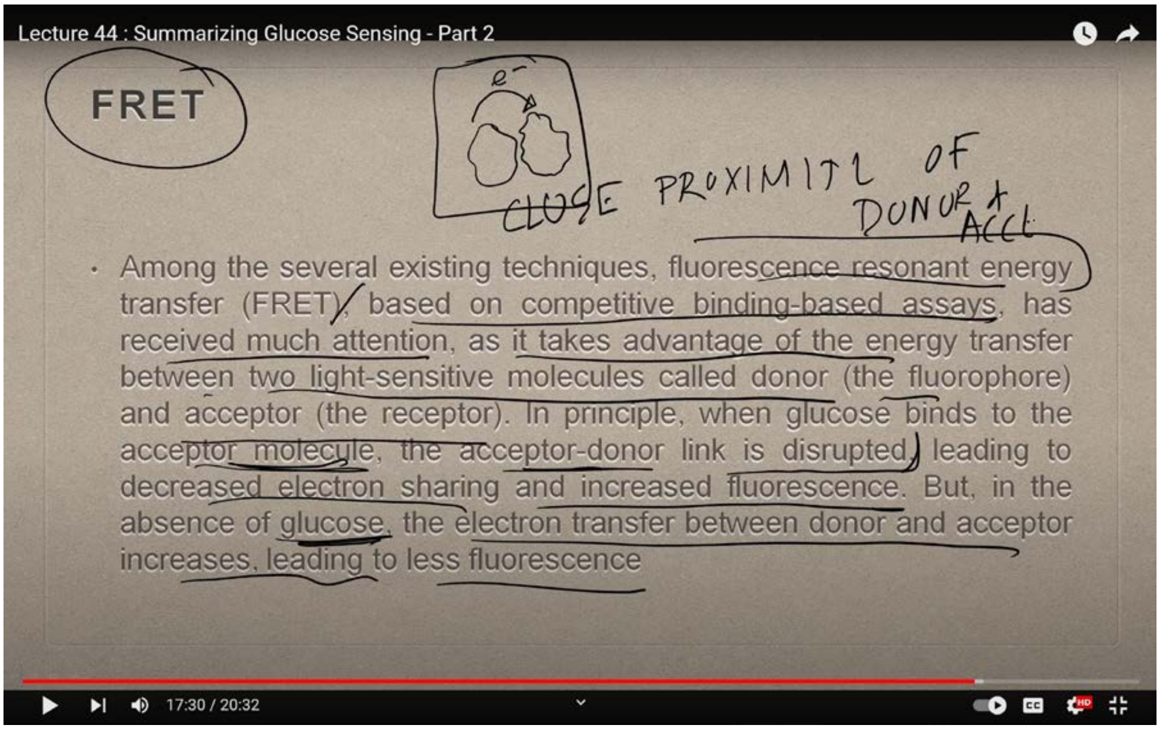

Another related technique is called fluorescence resonance energy transfer, or FRET. This method has garnered significant attention, particularly those based on competitive binding assays, as it leverages energy transfer between two sensitive molecules: a donor fluorophore and an acceptor. In principle, when glucose binds to the acceptor molecule, it disrupts the donor-acceptor link, resulting in decreased energy transfer and increased fluorescence.

Conversely, in the absence of glucose, the electron transfer between the donor and the acceptor increases, leading to reduced fluorescence. Thus, fluorescence resonance energy transfer is a highly specific molecular technique that operates on the principle of electron transfer between closely positioned molecules. It is crucial to maintain the proximity of the donor and acceptor for this technology to function effectively.

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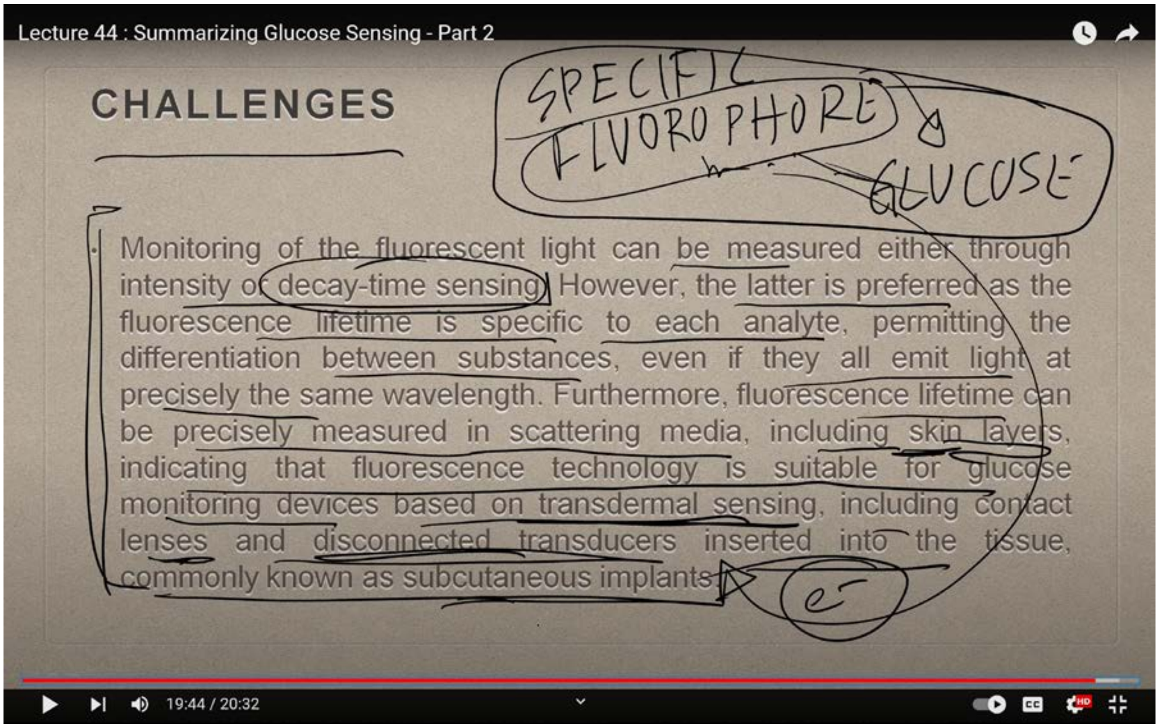

This technology is extensively utilized in cell biology research, and some researchers are exploring its potential as a glucose-sensing mechanism. However, as I mentioned earlier, a significant challenge with fluorescence lies in monitoring the emitted fluorescent light. This light can be quantified either through its intensity or through decay time, although the latter, decay time measurement, is generally preferred. The reason for this preference is that the fluorescence lifetime is specific to each analyte, which allows for the differentiation between substances even if they emit at precisely the same wavelength. Moreover, fluorescence lifetimes can be accurately measured even in scattering media.

This characteristic makes fluorescence technology particularly well-suited for glucose monitoring devices based on transdermal sensing, including innovations such as contact lenses and disconnected transducers that can be implanted subcutaneously within the tissue. Nevertheless, despite its potential, this technology is still gradually gaining significance due to several other technological challenges that I won't delve into here. It is certainly a worthy candidate for glucose sensing, especially if one can develop a highly specific fluorophore that binds to glucose.

If you can achieve this level of specificity, you would be well on your way. However, the issue arises with any fluorophore that lacks specificity; they tend to bind with other interfering molecules. When such nonspecific binding occurs, the entire electron-sharing process becomes compromised, which diminishes the effectiveness of the technique. While fluorescence is indeed a promising method, it requires further refinement to overcome these hurdles.

In our next class, we will explore optical polarimetry and other related techniques. Thank you!