

Science Education Collection

Optical Biosensing

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Abstract

Optical biosensors utilize light to detect the binding of a target molecule. These sensors can utilize a label molecule, which produces a measurable signal such as fluorescence. Or these sensors can be label-free, and use the changes in optical properties, such as refractive index, to sense for the binding of the target molecule. This video introduces both label and label-free optical biosensors, demonstrates their use in the laboratory, and shows some applications of the technology.

Transcript

Optical biosensors are sensors that detect a biological target or targets of interest using light. These devices have found applications in healthcare, pharmaceuticals, environmental monitoring, Homeland Security, and even the battlefield. Optical biosensors are broadly divided into label-based and label-free sensors. An example of label-based sensing is Polymerase Chain Reaction, or PCR, that uses fluorescent labels, or fluorophores, to quantify the amplified target DNA. An example of a label-free method is Surface Plasmon Resonance, or SPR. Here, the unaltered biomolecules interaction with the sensor surface is quantified by measuring a fundamental characteristic of the sensor, like the angle of reflection. This video reviews the basics of both these types of optical biosensing techniques and its critical components, the working principles and common applications of optical biosensors.

First, let us look at the general principles of label-based optical biosensing. Typically, a probe or biorecognition element, like a complimentary antibody, is attached on the sensor surface using traditional immobilization chemistry. The sample solution is then flowed over the sensors surface. The target molecule, which is complimentary to the immobilized biorecognition element, is selectively captured from the complex sample solution. Then the excess sample solution is washed away with buffer or water. Next, to help visualize and quantify the amount of bound target, a secondary molecule that is complimentary to the target and attached to a fluorophore, is flowed through the system. After some time, when binding of the secondary molecule to the target has occurred, the excess unbound fluorophore is washed away. The bound fluorophore's intensity can then be quantified using a fluorescent microscope. This intensity is first plotted for various known target concentrations to create a calibration curve, which then allows for the direct measure of an unknown amount of captured target. Thus, label-based techniques use the fluorescence intensity of extremely sensitive fluorophores to quantify the concentration of the biomolecule of interest.

Now that we have reviewed the principles of label-based optical biosensors, let's take a look at a commonly used example, the current standard for DNA amplification, Quantitative PCR, or qPCR. The qPCR reaction mix includes a labeled probe for real-time reaction quantification. The probe sequence is attached to both fluorescent reporter and quencher molecules, and binds to a specific DNA sequence. The quencher molecule quenches the fluorophore's emission while both of them are attached to the probe. During the PCR reaction, the polymerase enzyme degrades the probe DNA and physically separates the fluorophore reporter, thus preventing quenching and resulting in an increase in fluorescence. To perform qPCR, first add all components of the reaction, including the labeled probes, into a PCR tube or well. Next, place the PCR wells into a specialized thermocycler and enter the parameters for each cycle and the number of cycles. The thermocycler used for qPCR includes a light source with the required optical components to select a wavelength and a detector to measure the fluorescence intensity in real time during the PCR reaction. At the end of each thermal cycle, the fluorescent intensity due to the released Fluorophores is recorded and plotted. The fluorescent intensity is directly proportional to the logarithm of the target concentration in the reaction well, and thus, for a known fluorescence, the unknown target concentration can be calculated.

Let us now look at how sensing can be achieved without any reporters, like fluorophores, using label-free optical sensing techniques. For label-free techniques, the immobilization of the biorecognition element is the same as the label-based technique. Any biomolecule attachment to the surface of these sensors modifies the index of refraction in the immediate vicinity of the optical device. The target molecules bind to the surface of the functionalized optical device forming a thin layer and further modifying the refractive index. These changes in refractive index can be monitored by tracking the change in the natural frequency of vibration, or the resonant frequency of the system. The difference in the resonant frequencies before and after target binding, called the f shift, is directly proportional to the concentration of the captured target.

Now let's take a look at the basic principles of a commonly used label-free optical sensing technique, Surface Plasmon Resonance, or SPR. A typical SPR instrument combines a moveable laser source, an optical detector for measuring intensity shift, and a sensor chip with a glass prism coated on one side with gold. The gold-covered prism is integrated with a fluidic system enabling a flow-through operation. In an experiment, the probe molecule, or biorecognition element, is attached to the gold surface. The target element then flows over the surface and binds to the immobilized probe. Sensing utilizes the SPR phenomenon. This occurs when polarized light is shown on the surface of metal, like gold, at a specific angle, theta SPR. This results in the generation of surface plasmons which are coherent electron oscillations that exist at the interface of two materials that have permittivities of opposite signs. When the mass attached to the sensor surface changes, the resonance condition of the surface plasmons is altered. Consequently, when only the biorecognition element is bound on the surface, the beam is reflected at one angle, theta one. Then, when more mass is attached to the surface, like the captured target, a reduction of the intensity of reflected light at the specific angle, theta SPR is observed, as the angle of reflection changes to theta two. The change in intensity of the reflected light at the resonance angle, theta SPR, is then a function of the angle of reflected light, and can be measured as the difference in angles, theta one and theta two.

Now that we have covered the principles and procedure behind optical biosensors, let's see how researchers are applying these techniques today. The flow cell cytometer is an optical biosensing system that is commonly used for counting cells and measuring a variety of other cellular properties and components. A sample consisting of multiple cell types, each tagged with a unique fluorophore, is prepared in a suspension. Then, the sample is run through the flow cytometer such that the cells run past a laser beam one at a time. As each cell passes through the laser beam, scattered and

emitted fluorescent light by the different fluorophores are separately quantified, which gives a direct count of the various cell types in the sample. Another application of optical biosensors is detection of bacteria in samples. One way to do this is by using optical ring resonator sensors. They are composed of a closed loop coupled to light input and output wave guides. When light of resonant wavelength is applied to the input wave guide, it is passed through the loop and builds up an intensity over multiple round trips due to constructive interference and is output to the output wave guide. Antibodies specific to bacterial surface proteins are immobilized onto the ring resonator surface and a baseline absorption spectrum is obtained. Then the bacterial sample is flowed onto the resonator surface to allow for bacterial binding to the immobilized body, following which a second absorption spectrum is obtained. If the bacterium of interest binds, a visible shift in resonant peaks is observed, which is directly proportional to the concentration of the target bacteria.

You've just watched JoVE's video on optical biosensing. We discussed the basic principles of both label-based and label-free optical sensing along with two prominent examples of these methods and some applications of the techniques. Thanks for watching.