# Taşınabilir Biyosensörler için Optik Tanı Yöntemlerinin Değerlendirilmesi
# Assessment of Optical Detection Methods for Compact Biosensors

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***Özetçe*—Toplumda yüksek doğruluklu uygun fiyatlı taşınabilir bir biyosensör için yükselen bir talep bulunmaktadır. Bunun nedeni bu tipteki bir biyosensörün tedavinin önemli bir bileşeni olan tanı sürecini dramatik bir biçimde kısaltabilecek olmasıdır. Ayrıca bu tür bir biyosensör ile tanı sürecinde, yalnızca hastane laboratuvarlarında mevcut olan tesislere ve uzman kullanıcılara da ihtiyaç duyulmayacaktır. Optik biyosensörler oldukça hassas ve yüksek doğruluklu ölçüm yapma imkanı sunabilmektedirler. Bu bildiri farklı optik tanılama metotlarının çalışma prensiplerini ve bunların kompakt biyosensörler üzerindeki uygulamalarının değerlendirilmesini içermektedir.**

***Anahtar Kelimeler*—kompakt biyosensör, patojen tespiti, optik biyosensör**

***Abstract*—There is an increasing demand in the society for an accurate cost sensitive compact biosensor. This is due to the fact that this kind of biosensor can slightly decrease the diagnosis period of diseases, which has major importance on the treatment period. Also it would not require facilities or expertise which is only available in hospital laboratories. Optical biosensors can offer very sensitive and accurate measurements. This paper includes working principles of different optical detection methodologies and evaluation of them for application on compact biosensors.**

***Index terms*—compact biosensor, pathogen detection, optical biosensor**

## I. Introduction

Today, there are many subdivisions of used detection methods in optical biosensors. Detection methods can be classified as labeled and label free methods. Labeled methods are based on a specific antibody-antigen binding. Within this technqiues, there is a specific antibody which is bounded to a label(generally a nanoparticle, which has distinctive physical properties), which eases to discriminate bounded antibodies from other substances. Unbounded antibodies and their labels are somehow eliminated with selective methods(use of magnetic nanoparticles etc.) [1].

Inversely, label-free methods don't depend on any label as the name mentions. Antibodies can be used for their specific binding properties but that's not necessary. There are some methods developed, which uses distinctive physical properties of antigens for their detection [2].

Following section includes the evaluation of labeled and label-free detection methods for application in compact biosensors. Within the "Labeled methods" subsection, Fluorescence Lifetime Measurement, Phosphorescence Lifetime Measurement, Electrochemiluminescence and Absorbance Spectroscopy methods; within the "Label-free methods" subsection, Raman Spectroscopy, Resonance Raman Spectroscopy, Surface Enhanced Raman Spectroscopy and Surface Plasmon Resonance methods are evaluated. Summary of the evaluations are demonstrated within Table I.

Within the "Conclusion" section, result of the evaluation has been reported and some of the detection methods are proposed as more applicable on biosensors.

## II. Detection Methods

### A. Labeled Methods

*1) Fluorescence Lifetime Measurement (FLT):* This technique uses an antibody which is bounded to a fluorescent label to detect a specific antigen. There is a seperation method that is applied to remove label-antibody pairs which is not bounded to an antigen. UV Leds or Lasers are used for excitation of labels in the analyte. Fluorescence light intensity is measured by an optical sensor(Photodiodes, Avalanche Photodiodes, Photomultiplier Tubes, CCD or CMOS Image Sensors) [3]. The relation between fluorescence light intensity level and the label amount in analyte is known; so that the antigen concentration can be determined using obtained light intensity measurement [4].

However, application of the methodology have relatively high hardware requirements; which means increased cost and generally increased form factor. Due to the relative short fluorescence lifetime(mostly at nanoseconds scale) of the labels, hardware's high frequency performance is an important issue. Besides this, hardware should be capable of signal acquisition at high sampling rates(at least a few hundred MHz) [5].

There are many sensitive optical sensor options and even detecting a single photon is possible(Avalanche Photodiodes in Geiger mode, Photomultiplier Tubes etc.). But there are

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some problems due to autofluorescence of the analyte which increases background noise of measurements. There are also techniques to eliminate this problem, but their implementation requires some extra hardware [6].

*2) Phosphorescence Lifetime Measurement (PLT):* This method is very similar to Fluorescence Lifetime Measurement except that the used labels have much longer phosphorescence lifetime. This fact reduces the hardware requirements dramatically. And makes the technique more immune to autofluorescence related background noise. There may also be some phosphorescent substance in the analyte, but by the use of frequency upconverters(there are some lanthanide based labels) background noise can be eliminated dramatically. [7]. However, sensitivity of technique is lower than FLT.

*3) Electrocheluminescence (ECL):* Electrochemiluminescence is a phenomenon which a specific redox couple emit light when they undergo into a electrochemical reaction. Since the electrochemical reactions occur at the surfaces of electrodes, some of the electrode surfaces(depends on the configuration) seem as a light source when this event takes place [4].

Since the excitement of labels are performed electrochemically, there is almost no optical backround noise. Limit of detection property of picomolar concentrations is acquirable [8].

Another advantage of this technique is that the optical hardware requirements within it's application is optimum. Just a converging lens and a sensitive optical sensor are quite sufficient. But the electrode behaviour can be inconsistent, which decreases method's accuracy. In order to increase consistency and emission levels, ultrasonic transducers can be used. Using ultrasonic transducers is effective due to increase of mass transportation at the electrode surfaces [8].

*4) Atomic Absorbance Spectroscopy (AAS):* Absorbance spectroscopy is a widely used technique in the field of analytical chemistry; which can be used to detect chemical compounds of a material.

At these technique a light beam which is composed of light spectra(tungsten lamps used widely as the light source) is passed from the analyte. Incoming light beam's spectral component intensities are measured. One of the generally used configuration is to use a prism to disperse the incoming light beam spatially. Linear image sensor can be used to detect spectrum at once. Since the different molecular structures have different absorption spectrum, substances in a compound can be discriminated and detected by this technique [9].

However, pathogen surface antigens generally don't have dominant specific absorption spectrums so the use of labels with specific optical property is required in biosensing applications. Quantum Dots (QDs) and Gold Nanoparticles (GNPs) are widely used as labels.

Technique doesn't have challenging hardware requirements. But it's sensitivity is quite low compared to other techniques. Besides this, application of the technique require some other optical elements for spatial dispersion(prism, diffractional grating etc.) [10].

*B. Label-free Methods*

*1) Surface Plasmon Resonance (SPR):* Application of SPR technique requires a single wavelength light source (generally a laser), which emits light as a cone form; a metal-dielectric interface and a linear sensor array to detect the angle of reflected light's intensity dip.

Working principle of this technique is based on a quantum mechanical phenomenon. According to this phenomenon, when an electromagnetic wave is applied to a metallic surface, if the momentums of photons and electrons are close, energy transfer occurs between them. When the light is applied to the metal-dielectric interface, tangential component of the reflected light creates an evanescent wave accross the metal-dielectric interface. Evanescent wave is in conjuction with the free electrons of the metal surface. Electron momentums are directly related with the evanescent wave's frequency and amplitude, so change in a refractive index near the surface results in a change at electron resonance frequency, and change of the angle which dip of light intensity appear. So detection mechanism is mainly based on refractive index changes near the metal-dielectric interface. Besides these antibodies are bounded to the metal-dielectric interface also. So when antibody-antigen binding occurs, refractive index near the interface changes. As a result, angle of intensity dip also changes [11].

Methodology requires precision optical equipments. Especially the metal-dielectric interface has major importance on the sensor response; otherwise performance.

Except these, refractive index near the metal-dielectric interface is easily affected from the enviromental variables such as temperature, self assembled layers, contamination etc. This leads into measurement errors and increases the limit of detection [12].

*2) Raman Spectroscopy (RS):* Raman Spectroscopy is a very versatile label free technique. Its based on the fact that different molecules have different kinematics, and their vibrational properties under thermal energies are also different. Each molecule type has different natural vibration frequencies.

Within the general application of technique, there is a light source, which emits range of spectral components. The light is passed from the analyte. Since the light is an electromagnetic wave, it's electrical field component apply force to the charged particles and dipoles within the analyte. When the light beam's any spectral component's frequency is close to any molecules vibration frequency in some direction, there is a chance that spectral component can do some physical work on the particle (vibrating molecule). And if the electromagnetic wave does work on a particle, waves quanta(photon) losses some energy. There is a much weaker probability that the photon would gain energy from the electron. Anyway, wavelength of the photon is changed except the situation that Rayleigh Scattering takes place.

By spatially dispersing(with using prism, diffractional grating etc.) incoming light from the analyte, power of the spectral components can be measured with a sensor array. CCD or CMOS image sensor chips are widely used for this purpose.

Since chance of Raman scattering to occur is quite low, signal levels are very weak and in order to increase signal levels different solutions have been invented [13].

*Resonance Raman Spectroscopy (RRS)* is based on the fact that, when a spectral component's frequency matches a molecules vibration frequency. Raman signal is strengthened as five-six orders of magnitude. But these technique requires a tunable excitation source (tunable laser mostly). Resonance Raman Spectroscopy is a useful technique, since its label free and the limit of detection of the techqnique can be as low as few molecules. But the tunable lasers aren't cost effective, and big in form factor [14].

*Surface Enhanced Raman Spectroscopy (SERS)* is another modified version of the Raman Spectroscopy. Light beam composed of different wavelength components is applied to the sensing surface. There are metallical nanoparticles on the sensing surface, enhancing the electromagnetic field near the surface. This effect is due to the fact that the nanoparticles have free electrons within their surface, which resonate with the applied electromagnetic field enhancing near electric field. Chance of Raman Scattering to occur near surface is strengthened about six to eleven orders of magnitude, and this makes a single molecule detection possible with sensitive optical instrumentation [1].

## III. Conclusion

Applied methodologies have been investigated and their working principles, limitations, strong and weak spots are evaluated. Optical and electronical hardware requirements are also taken into consideration.

Within the scope of our assessment, it is concluded that the evaluated label-free detection techniques are not suitable to be applied in a compact biosensor, cause they have much higher hardware requirements than labeled ones. Which possibly lead into higher costs and bigger form factor. When labeled techniques are considered, label selection is critical in conjunction with the applied methodology. Except from this fact and within the scope of our assessment, we propose that applying *Phosphorescence Lifetime Measurement* or *Electrochemical Luminescence* techniques on compact biosensors would give a decent compromise between complexity and performance.

| Measurement Method | Sensitivity | Evaluation | Electronic Hardware Requirements | Optical Hardware Requirements |
|---|---|---|---|---|
| Fluorescence Lifetime Measurement | Very High | Can be effected by autofluorescence. Immune to change of enviromental variables. | High | Medium |
| Phophorescence Lifetime Measurement | High | Immune to autofluorescence and change of enviromental variables. But signal levels are lower than FLT about two-three orders of magnitude. | Low | Medium |
| Electrochemiluminescence | High | Gives the best optical isolation. But electrochemical cell behaviour is affected by enviromental variables. Sonication may be used for more accurrate operation. Measurement signal levels are weak, but nearly absent background noise increases the signal to noise ratio. | Medium | Low |
| Absorbance Spectroscopy | Low | Sensitivity is quite low. Enviromental variables may suppress measurement signals quite easily. Light source dominates the measurement signals which decreases the Limit of Detection(LOD). | Low | Medium |
| Surface Plasmon Resonance | High | Sensitive to refractive index variations. Metal dielectric interface should be specially manufactured. Temperature variations effect sensor accuracy. | Low | High |
| Raman Spectroscopy | Low | Measurement signal levels are very low. Excitation light source may suppress the measurement signals. | Medium | High |
| Resonance Raman Spectroscopy | High | Immune to the change of enviromental variables. Precision optical equipment such as Tunable Laser needed. Very selective label free detection mechanism, which provides moderate limit of detection property. | Medium | Very High |
| Surface Enhanced Raman Spectroscopy | Very High | Very high sensitivity, even providing single molecule detection. But sensor response is very dependent on SERS surface. Contamination, self assembled monolayers etc. degrage sensor performance dramatically. | Medium | High |

TABLE I
METHOD COMPARISON CHART

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