Prescription Chips

The Development of Enzyme and Biochemical CMOS Sensor Chips for Biological and Medical Applications May Be Just What the Doctor Ordered

By Yuh-Shyong Yang, Ude Lu, and Ben C.P. Hu

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Following the progress of modern technologies, especially in biotechnology and electronics, a worldwide medical revolution is expected. Recent advancements in molecular biology have moved medical research into the molecular level. Interestingly, the progress of nanotechnology also brings semiconductor research into the scale of a size comparable to that of a molecule. Concurrently, the result of the Human Genome Project has produced new opportunities in all areas of bio-related industries. Therefore, the need for new techniques and instruments for biological research, medical diagnosis, curing diseases, and many other bioanalytical problems has been very urgent. Biochips or other biosensing devices are among the most powerful tools for research in modern biotechnology. They are good examples of how the combination of biotechnology and microelectronic or semiconductor techniques may become the backbone of a new bioelectronics industry. The future of diagnosis, pharmaceuticals, and medical care for the post-genomic era may heavily rely on the application of semiconductor chips. Succeeding its glory days in informational technology and electronics, the next triumph of the semiconductor may be associated with biotechnology. In this article, we discuss the opportunities in bioelectronics based on the progress of modern biology. A light-sensitive CMOS chip is used as an example to illustrate how a semiconductor chip can be very useful in biochemical research and applications.

The Need for Medical and Bioanalytical Diagnosis

There is a general trend toward more decentralized and immediate diagnostics. Thus, development of an accurate, portable, relatively inexpensive, and easy-to-use biosensor has become a high priority in the healthcare industry. On the other hand, high-throughput techniques are also urgently needed for research in genomics and proteomics and for the screening of huge amounts of biological information that cannot be handled with traditional bioanalytical methods. An industrial process that can efficiently integrate small

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and inexpensive sensing devices in large amounts is required to fulfill the above description. The current development of semiconductor chips for biosensors is in position to overcome traditional problems and to fit current and future requirements.

The Complexity of Clinical Analysis

Laboratory testing is one of the largest revenue-producing activities in the healthcare business. In order to obtain a useful measurement, we need to consider the disease correlations, interpretations of data, problem solving, quality assurance, and cost effectiveness; we also need to know not only the "how" of the tests but also the "what," "why," and "when." Bioanalytical problems are among the most challenging due to the variety of substances in biological samples, the complex molecular structures, and time-dependent concentrations. The typical steps required for an analytical procedure or clinical test may illustrate the complexity of the problem:

1) Define the problem.
2) Collect the specimen.
3) Identify the specimen.
4) Transport the specimen to the laboratory.
5) Select an appropriate method.
6) Pretreat the specimen and prepare the sample.
7) Perform the measurements.
8) Compare with reference and quality-control specimens.
9) Calculate statistical parameters.
10) Decide on the performance and reliability of the analysis.
11) Transform data to give an interpretable value.
12) Present the data [1].

The most commonly used point-of-care testing (POCT) devices are the fingerstick blood glucose monitors. Two enzymes, glucose oxidase and peroxidase, are coupled to produce a color whose intensity is measured as a function of the concentration of glucose. Commercial POCT devices generally use electrochemical and optical biosensors for the measurement of glucose, electrolytes, and arterial blood gases [2]. The biosensor can be miniaturized and made available at low cost with the development of microsilicon chip fabrication. An array of biosensors can be produced onto a single silicon wafer that selectively measures a multicomponent of analytes. The high degree of selectivity required for such measurements is based on the process of biomolecular recognition. For example, an enzyme attached to an electrode enables selective monitoring of a substrate of that enzyme. Antibodies immobilized on an optical surface provide a signal indicative of interactions with complementary antigens of the antibody. The concept of a biosensor combined with semiconductor and information technology may be the solution to greatly simplify and enhance the whole bioanalytical procedure.

Molecular Diagnosis and Functional Genomics

Recent progress in biology has revealed many important targets for molecular diagnosis in medical science. The attention of molecular biology has been on the flow of genetic information in the cell, DNA→RNA→Protein, to produce biological

Table 1. Semiconductor sensor as transducers for enzyme chips.

Semiconductor Sensor	Function as Transducer	Biological Applications (Example)
Light Sensors (Photodiode and array, phototransistor, charge-coupled device)	Light → Current, Voltage	Any enzymatic reaction that produce fluorescence, luminescence, UV/vis absorptions (enzymes are listed in Table 2).
Electrochemical sensors (Amperometric Sensor)	Electron transfer → Current	Enzyme catalyzed redox reactions (Dehydrogenase, oxidase)
Electrochemical Quartz Crystal Microbalance (EQCM)	Mass variation → Current frequency variation	Specific molecular-molecular interaction (DNA or nucleotide binding proteins, protein-protein interaction, Ab-Ag binding, enzyme-inhibitor interaction)
Surface Plasmon Resonance (SPR)	Mass variation → Light absorption band shift	
Surface Acoustic Wave (SAW)	Mass variation → Wave shape shift	
Ion selective FET & Enzyme FET (Potentiometric Sensor)	Electric field variation → Current variation	Enzymatic reactions involved in charge variation. (Hydrase, imides, esterase, protease, amidaes)
Thermal Sensor (Thermistor)	Temperature variation → Resistance	Many biochemical reactions involve thermal sensitivity (Catalase, urease, oxalic acid oxidase, uric acid oxidase)

function. DNA is the hereditary material that carries the information of life. Proteins, including enzymes, are the workforces of the organism. The first step to express information in DNA is the copying of the gene (a DNA sequence that is encoded for a protein) into a strand of messenger RNA, a process called transcription. In the second step of gene expression, the mRNA (using its copied DNA code) directs the synthesis of protein. All the processes are heavily regulated by a variety of proteins and enzymes that are required to catalyze all biological reactions [3]. Analytical tools for these biomolecules (especially DNA, RNA, and protein) are needed to understand the biological process. To understand the biological process is to hold the key to diagnosing and curing a variety of diseases.

Genomics and Proteomics

Genomics is the study of genomes and genes based on DNA sequencing. This study provides new insights into fundamental questions about all biological functions. This information and the technology used to obtain it can also benefit our daily lives. In the genomic era, the gene chip (or DNA chip) is considered as one of the great inventions in recent history. However, to understand the function of genomes, measuring gene expression at the protein level is more informative, but in many situations this cannot be indicated by DNA or mRNA analysis.

The importance of enzymes and other proteins is illustrated by the current trend in biotechnology of moving from genomics to proteomics. Over 90% of the human genome and complete genome sequences of several organisms have been revealed. The next challenge is to compile structural and functional data for all proteins expressed in an organism. Proteomics is the study of the structure, function, and cellular localization of all proteins expressed in a cell at a given time. This understanding will have an immense impact on disease diagnosis, drug development, and our self-knowledge, surpassing that of the genome era [4].

Enzymes as a Tool for Molecular Diagnosis and Medical Care

Enzymes are catalytic proteins that are responsible for catalyzing all the biochemical reactions with only very few exceptions. In the absence of enzymes, the pathways of metabolism and the process of life would become hopelessly congested. Thus, to understand enzymes is to understand how life proceeds. The amino acid sequence of an enzyme is determined by the DNA sequence of the gene, and its magic power as a supercatalyst is determined through a proper three-dimensional structure. Enzymes can accelerate reactions as much as 10^{16} over uncatalyzed rates. Specificity of each enzyme enables only specific reaction to occur in a mixture of metabolites, and a molecule of an enzyme may catalyze over 10^9 reactions in a second.

Following the advancement of modern biotechnology, the use of enzymes in clinical practice has increased rapidly. However, the amount of these enzymes constitutes only a small fraction of enzymes that are potentially useful in medical care. The measurement of enzyme activities is not only important for diagnosis but also as a means of monitoring progress after therapy, recovery after surgery, and detection of transplant rejection. Enzymes are also used as a means of estimating concentrations of substrates as well as for enzyme-replacement therapy, such as treatment for genetic deficiency disease and for cancer therapy [5]. Understanding the function of particular enzymes has enabled the rational design of drugs to inhibit specific enzymes,

Semiconductor sensors are transducers that recognize and convert physical signals, such as chemical, electrical, magnetic, radiant, mechanical or thermal signals, into electrical signals.

and purified enzymes can be used to determine the concentrations of metabolites of clinical importance. A wide range of biological compounds can be measured by enzyme immunoassay, in which the detection of a highly specific antibody is amplified in conjunction with an enzyme.

The enormous applications described above require a suitable sensor to report the biological interaction. Semiconductor sensors and their physical properties that may be used for the design of enzyme chip are summarized in Table 1. Different types of semiconductor chips and many enzymes may be the target for the design and manufacture of enzyme chips. Examples of enzyme systems that may couple with a light-sensitive semiconductor chip for the design of an enzyme chip are listed in Table 2 and will be discussed further.

The Semiconductor Chip as a Transducer of Biological Signals

An Ideal Biosensor

The current need for biosensors requires much more than what is provided with selective detection. The goals of convenience and economics, such as miniaturization, portability, low cost of mass production, and ease of use, need to be achieved. They also need to meet the following basic requirements for a biosensor: eliminate or simplify sample separation steps; provide new options for high selectivity in direct measurements; achieve better accessibility for challenging measurements within the natural location or environment; respond in a continuous, reversible manner; accomplish a measurement with minimum perturbation of the sample; and be compatible with the environment in which the measurement is made [6]. It is not surprising that with so many types of effective biosensors in use today, rarely does one find an individual device that meets all of the above goals.

The Advantages of a Semiconductor Chip Sensor

The semiconductor sensor is differentiated from other solid-state sensors by its small size and by its manufacturing techniques [7]. Most semiconductor sensors are fabricated by processes that have been developed for integrated circuits (ICs). By using standard batch processing, as in the IC industry, hundreds of thousands of identical semiconductor sensors can be produced in one run, thus substantially improving their performance-to-cost ratio. Semiconductor sensors are potentially cheaper, offer higher performance and reliability, and are smaller in size than their discrete counterparts. The development of silicon micromachining techniques and the utilization of a wide array of technologies already developed for the fabrica-

Table 2. Enzyme systems as biosensors for light-sensitive enzyme chips.

Enzyme/couple enzyme	Reagent	Target	Application
HRP/Cholinesterase/Choline oxidase	Luminol/ 2-methyl Indole/ Isoluminol/ Dioxetane/ Acridinium ester	Cholinesterase	Liver and biliary tract disease.
HRP/Phospholipase/Choline oxidase		Phospholipids	Arteriosclerosis disease, liver function.
HRP/Lipase/Glycerol oxidase		Triglycerides	Fat metabolisms, especially diabetes mellitus, arteriosclerosis disease, renal disease, hypertriglyceridemia.
HRP/Lipase/Glycerol oxidase		Lipase	Acute pancreatitis diagnosis.
HRP/ Phosphorylase/Xanthine oxidase		Inorganic phosphorus	Hypervitaminosis D, renal disease, parathyroid hormone disease, osteoporosis, renal rickets.
HRP / acyl-CoA synthetase /acyl-CoA oxidase		Free fatty acid	Liver disease, diabetes mellitus, parathyroid hormone disease, Addison disease, obesity.
HRP/Lactate oxidase		Lactate	Lactic acidosis, congestive heart failure, serious anemia.
HRP/Kinase/Pyruvate oxidase		Creatine	Muscle disease, parathyroid hormone disease.
Alkaline phosphatase	Luminol-P/ Lucigenin/ CDP-star/ AMPPD/ CSPD	Alkaline phosphatase	Jaundice, Paget disease, bone and skeletal disease diagnosis. ELISA for cancer marker, liver disease, parathyroid hormone disease, drug diagnosis, CKMB Troponin I, Myoglobin, Anti-HBs, HbsAg, Anti-HIV 1/2, Anti-HBc, Anti-HCV, Anti-HBc IgM, Hbe, Anti-Hbe, Anti-HAV IgM, HCV core Ag.
Luciferase	Luciferin	ATP	Detecting ATP production in various enzymatic reactions as well as for detecting low-level bacterial contamination in samples such as blood, milk, urine, soil, and sludge.

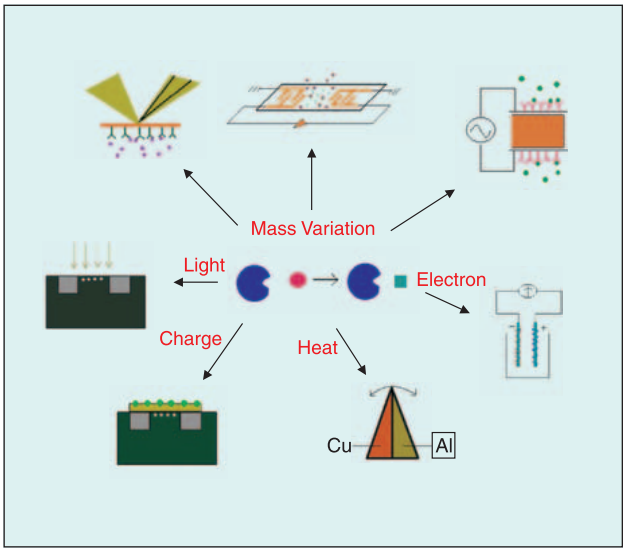
HRP: Horseradish peroxidase

Data obtained from [10] and [2].

tion of integrated circuits will continue to make tremendous progress in the development of a variety of semiconductor sensors. They are being used in a number of application areas, including instrumentation, transportation, health care, industrial processing and manufacturing, avionics and defense, and consumer appliances. There is a large demand, which continues to grow, for low-cost, accurate, and reliable sensors for industrial and consumer-product applications.

Communication Between Semiconductor Chip and Enzyme

Physics is the common language in the study of nature. There is no exception for an enzyme, a biomolecule, and a semiconductor chip, an electronic device. Figure 1 and Table 1 summarize the physical signals that may be produced following enzymatic reactions and the respective semiconductor devices that may receive and report the biological messages. The law of thermodynamics tells us what can and cannot happen according to the status of free energy. Enzymes are super catalysts that boost the rate of a reaction without being consumed by the reaction in a mild condition according the difference in free energies. Reactions catalyzed by enzymes result in the restructuring of chemical composition and also result in the redistribution of energy. The change of energy can be in different forms, as shown in Figure 1 and Table 1.



1. Communication between enzyme and semiconductor sensor. This diagram shows examples of the semiconductor sensors that sense the physical signals released following enzymatic reactions. The signal may be the change of mass and may be observed by SPR, SAW, or EQC. A semiconductor temperature sensor may detect the change of heat that is produced by many enzyme-catalyzed reactions. Some enzymatic reactions produce ions and change the conductivity of the solution. A potentiometric semiconductor sensor would be useful to report this type of enzymatic reaction. The exchange of electrons emerges in enzyme-catalyzed redox reactions. This phenomenon can be detected very sensitively with a variety of electrodes. Light may be produced (luminescence), absorbed, or activated (fluorescence) following many enzymatic reactions. A semiconductor light sensor, such as the CMOS described example in this article, would be very useful for measuring enzyme activity.

Semiconductor Sensors that Sense Enzymatic Reactions

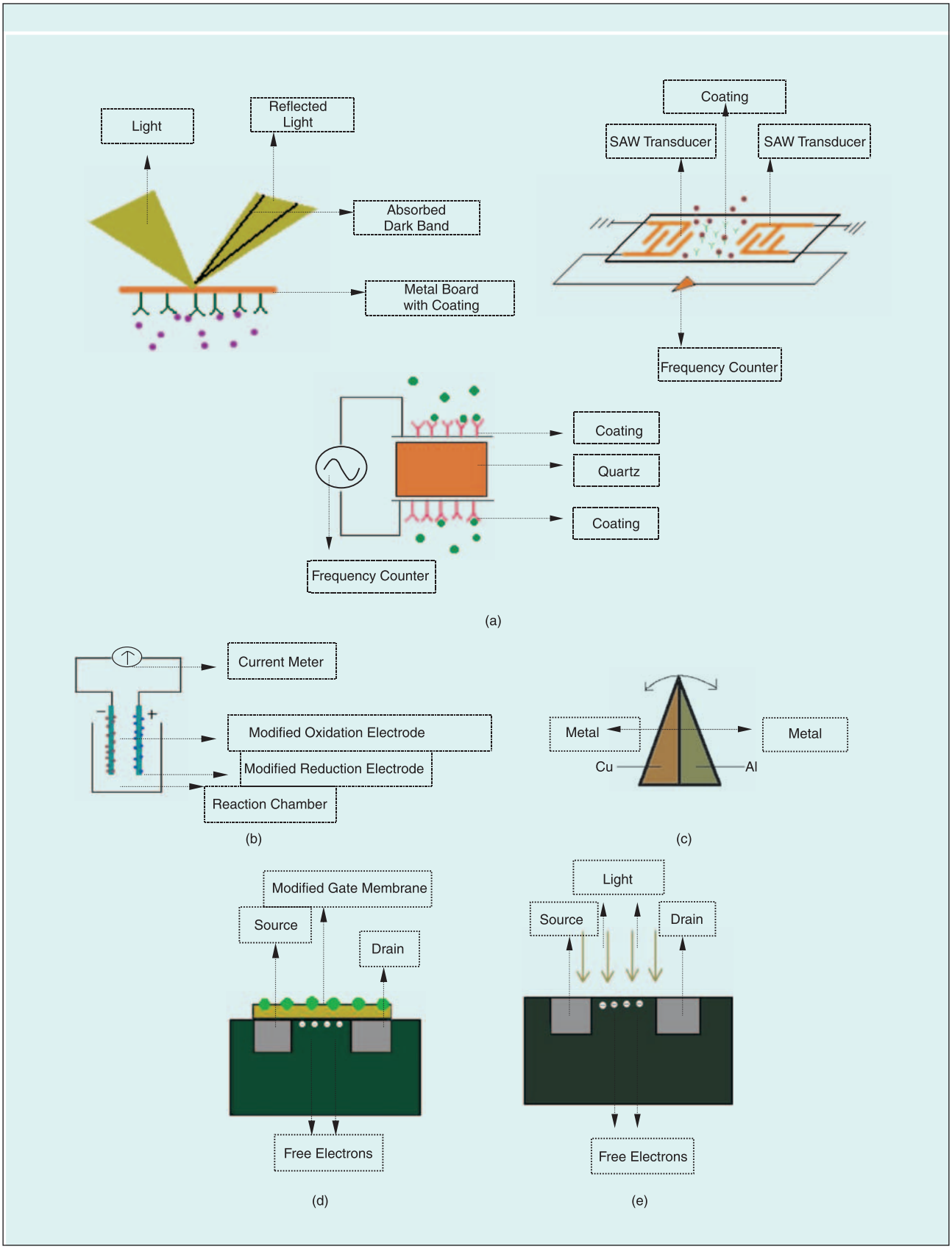
Semiconductor sensors are transducers that recognize and convert physical signals, such as chemical, electrical, magnetic, mechanical, radiant, or thermal signal, into electrical signals. Enzymatic reactions produce physical signals, which may be electrical (electron), magnetic (charge), radiant (light), thermal (heat), or mechanical (mass). Thus, the biological messages received by semiconductor sensors can be translated into electric or digital information. The semiconductor sensors shown in Figure 2(a)-(e) illustrate how enzymatic reactions can be reserved.

Interaction among biomolecules is the beginning of many biological processes and is the focus of biological research. Surface plasma resonance (SPR), surface acoustic wave (SAW), and electrochemical quartz crystal microbalance (EQC) sensors may be used as transducers of mass variation produced in an enzymatic reaction or binding among biomolecules [Figure 2(a), (b), and (c)]. The resonant frequency between two SAW transducers will shift with the variation of mass on the coating film. Similarly, the frequency of EQC is dependent on the mass of coating layer. Specific binding with biomolecules immobilized on the coating film can be monitored by change of resonant frequency. In SPR, mass variation of the coating layer affects the absorbed dark band of the reflected light spectrum shift. By measuring the shift angle of the dark band we can monitor the specific binding reaction. There are prism, grating, fiber, and waveguide SPRs. The prism SPR is now commercially available and is becoming an important instrument for measuring bimolecular interaction in biological research. However, prism SPR is not a semiconductor chip. Waveguide SPR, which can be manufactured through a semiconductor procedure, may be the next SPR for studies in biomolecular interactions.

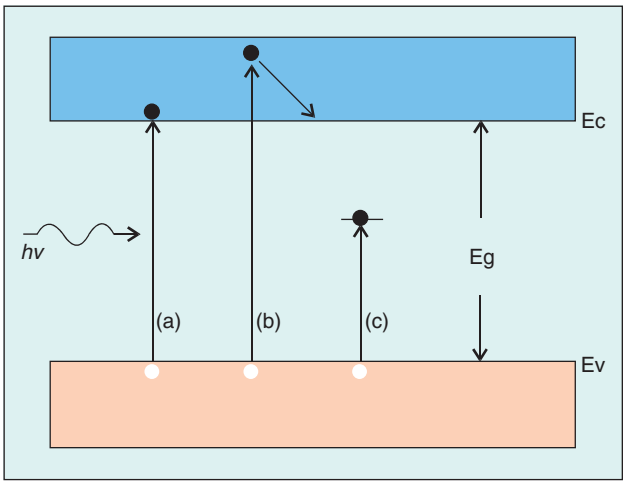
The electrode has been used as a transducer of electrons produced in an enzymatic reaction [Figure 2(b)] for many years. However, the size and efficiency of the enzyme electrode can be greatly improved using a semiconductor chip. Enzymes that catalyze redox reaction can be immobilized on the surface of electrodes. The electron flow generated by enzymatic reaction can be monitored through a current meter to quantify the enzymatic reaction.

Most of the biological reactions result in a change of heat. A thermometer can be a transducer of heat produced in an enzymatic reaction. The thermometer shown in Figure 2(c) is composed of two different metals. The coefficient of expansion of different metal is different. The thermometer will bend left or right depending on the surrounding temperature, and the bending angle determines the change of temperature. An insulated environment is needed to detect the small energy changes produced by enzymatic reactions.

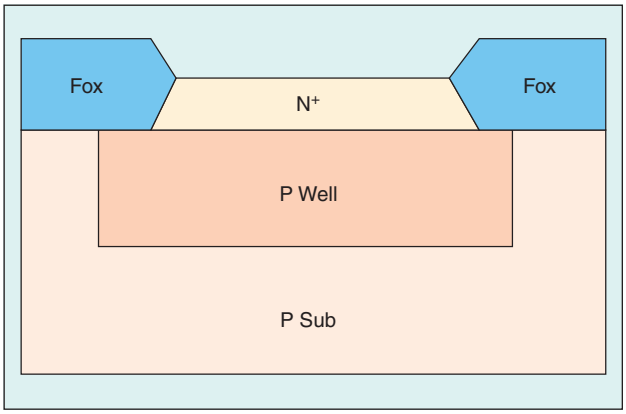
The field effect transistor is used as a transducer of charge produced in an enzymatic reaction [Figure 2(d)]. The gate membrane is modified with an enzyme or antibody. After the enzymatic reaction or ligand binding with the antibody, the total charge of gate membrane may vary and the free electrons generation rate will change. The enzymatic reactions or antibody-antigen binding reactions may be determined by measuring the current.



2. Semiconductor sensors as potential transducers for an enzyme chip. (a) Top left: Surface plasma resonance sensor. Top right: Surface acoustic wave sensor. Bottom: Electrochemical quartz crystal microbalance sensor. (b) Modified electrode chemical sensor. (c) Thermometer. (d) Enzyme field effect transistor (EnzymeFET). (e) Photodiode.



3. Activation of electrons by the absorption of light.



4. Side view of N+/P well photodiode structure.

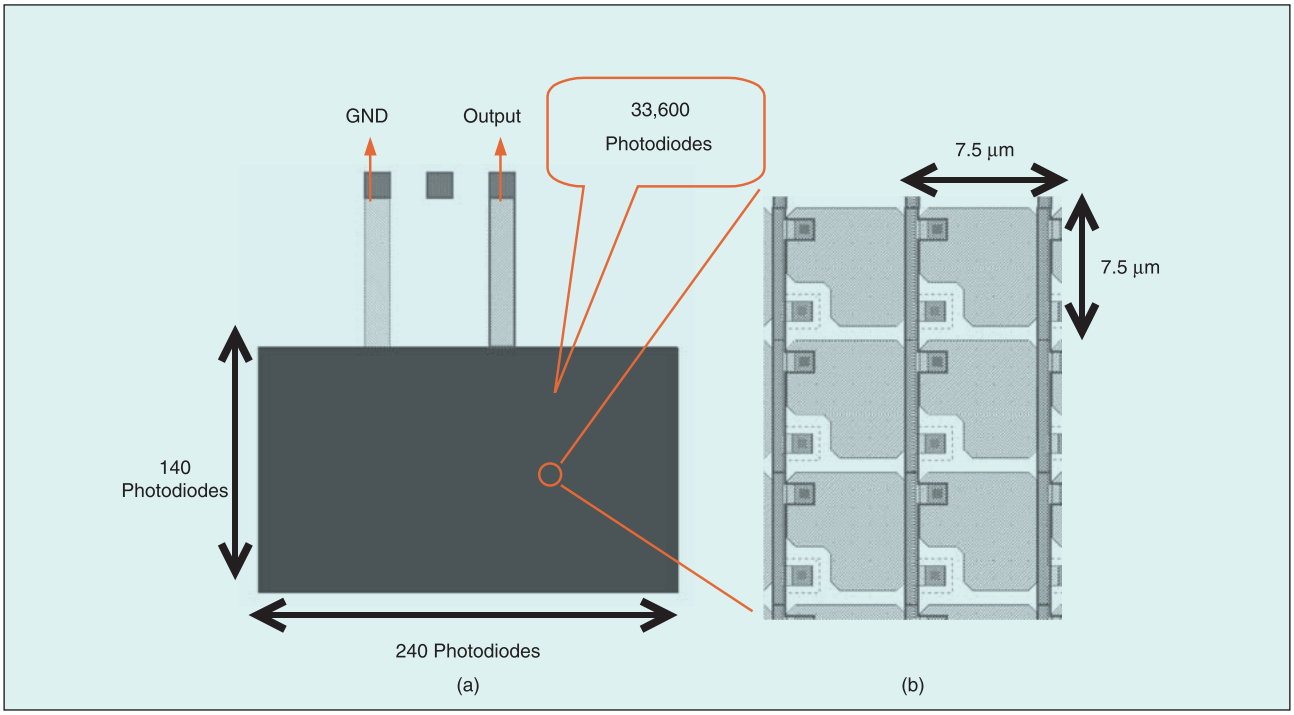
Optical semiconductor devices are widely used in many applications and can be a transducer of light produced in an enzymatic reaction [Figure 2(e)]. The enzymatic reaction could emit visible light, and examples are given in Table 2. In Figure 3, a photodiode is shown as a light sensor. When the photons produced in an enzymatic reaction hit the surface of the photodiode, free electrons may be generated. By measuring the current, we can monitor the reaction.

A Light-Sensitive CMOS Chip as a Transducer for an Enzyme Chip

Optical semiconductor devices are widely used and have become indispensable devices in many equipment and systems. Most optical semiconductor devices are optoelectronic p-n-junction devices, such as laser diodes, light-emitting diodes, and photodiodes. Many kinds of optoelectronic p-n-junction devices have been developed and the main interest in this field has shifted from device physics and operation principles to device applications [8]. The CMOS process is the most used procedure for the production of semiconductor chips. We are reporting a novel application of a light-sensitive CMOS chip as a transducer for enzymatic reaction.

Design of a Light-Sensitive Semiconductor Sensor

The basic idea and design of a light-sensitive semiconductor is illustrated in Figures 3-5. When the semiconductor is illuminated, photons are absorbed to create electron-hole pairs as shown at (a) in Figure 3 if the photon energy is equal to the bandgap energy; that is, hv equals Eg. If hv is greater than Eg, an electron-hole pair is generated and, in addition, the excess energy ($hv - Eg$) is dissipated as heat as shown at (b) in Figure 3. Both process, (a) and (b), are called intrinsic translation (or band-to-band transition). On



5. N+/P-well layout diagram. (a) Bird's-eye view of the layout. (b) Amplified diagram of photodiode array.

the other hand, for hv less than Eg, a photon will be absorbed only if there are available energy states Et in the forbidden bandgap due to chemical impurities or physical defects as shown at (c) in Figure 3. Process (c) is called extrinsic translation.

A photodiode is basically a p-n junction operated under reverse bias. When an optical signal impinges on the photodiode, the depletion region serves to separate photogenerated electron-hole pairs, and an electric current will flow in the external circuit. For high-frequency operation, the depletion region must be kept thin to reduce the transit time. On the other hand, to increase the quantum efficiency, the depletion layer must be sufficiently thick to allow a large fraction of the incident light to be absorbed. Thus, there is a trade-off between the response speed and quantum efficiency [7]. Fortunately, the response time of an enzymatic reaction is in the order of seconds for steady-state kinetics. It is quite slow compared to the electric signal. So we only need to focus on the problem of quantum efficiency.

The absorption coefficient a, which is dependent on the material used for the optical device, is a function of hv. The absorption coefficient decreases rapidly at the cutoff wavelength λ_c; that is,

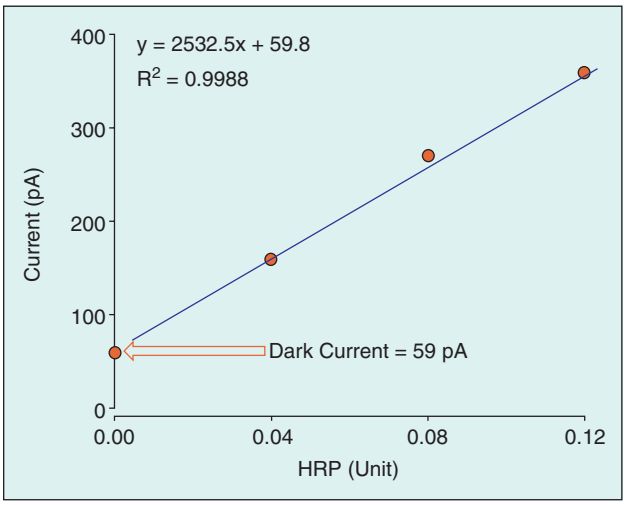
$$\lambda_c = \frac{1.24}{Eg}\mu\text{m} \tag{1}$$

because the optical band-to-band absorption becomes negligible for $hv < Eg$, or $\lambda > \lambda_c$.

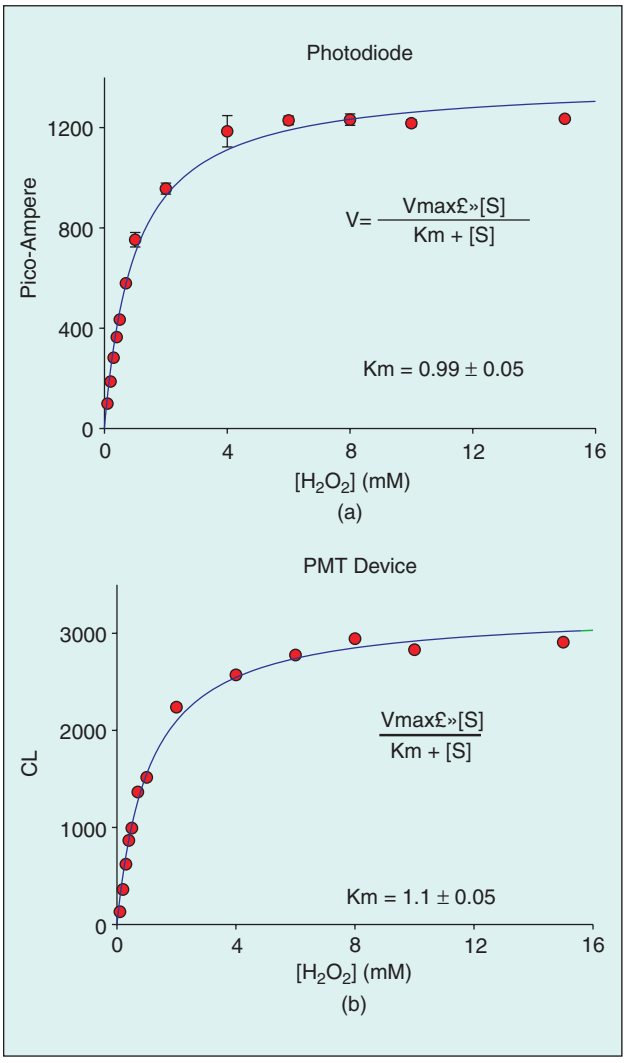
The quantum efficiency is defined as the number of electron-hole pairs generated for each incident photon; that is,

$$\eta = \left(\frac{I_p}{q}\right)\left(\frac{P_{\text{opt}}}{hv}\right)^{-1} \tag{2}$$

where I_p is the photogenerated current from the absorption of incident optical power P_{opt} at wavelength λ (corresponding to photon energy hv). One of the key factors that determine η is the absorption coefficient a. Since a is a strong function of the wavelength, the wavelength range in which appreciable photocurrent can be generated is limited. The long wavelength cutoff λ_c is established by the bandgap (2) and is, for example, 1.1 µm for silicon. For wavelengths longer than λ_c the values of a are too small to give appreciable band-to-band absorption. The short-wavelength cut-off of the photo response comes about because for short wavelengths the value of a is very large (~10^5 cm^{-1}), and hence the radiation is mostly absorbed very near the surface where recombination time is short. Therefore, the photocarriers can recombine before they can be collected in the p-n junction. [7]. The sensor photodiodes are an N^+/P well structure as shown in Figure 4, which is manufactured with a standard 0.5 µm CMOS process. Figure 5 is the layout diagram of the N^+/P well photodiodes, which is a 140 × 240 photodiode array with single pixel size of 7.5 µm × 7.5 µm.



6. Enzyme activities determined by a light-sensitive CMOS chip.

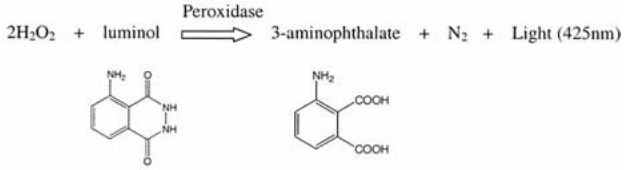


7. Enzyme kinetics observed by a N+/P-well photodiode and photomultiplier tube. The reaction condition is in 0.5 M Tris-HCl (pH 8.6), 0.1 to 15 mM hydrogen peroxide, and 1.5 mM luminol at room temperature. HRP (0.2 unit) is added to start the reaction.

Design of Enzymatic Reaction for a Light-Sensitive Semiconductor Sensor

Bioluminescence in nature is not only fascinating but has practical applications in biotechnology. Table 2 is a partial list of how bioluminescence can be very useful in clinical applications. Some metabolites or enzyme activities (examples listed in Table 2) need to be monitored regularly for large populations of those who suffer from liver disease, diabetes, heart disease, bone and skeletal diseases, and many other diseases. It is also useful for drug diagnosis, determination of bacterial contamination, and many other sensitive measurements of chemicals or biochemicals. It is not very difficult to find applications for a light-sensitive CMOS-enzyme system.

The bioluminescent enzymatic reaction we chose to demonstrate is shown in the following equation. The reaction of the luminol/HRP/H_2O_2 system will emit light of 425 nm. The reaction may be to couple another enzyme system that generates H_2O_2 and to determine a variety of enzyme activities or the concentration of its substrates.



Enzymatic Reactions Monitored by a CMOS Light Sensor

With the CMOS light-sensitive chip, the activity of HRP (Horseradish Peroxidase) can be accurately measured as shown in Figure 6. The data indicate a small dark current, about 55 pA, of the chip relative to the signal produced by HRP. We can observe an excellent linear correlation between the amount of enzyme used and the current induced by the light that is generated in the HRP-catalyzed reaction. This result implies how a CMOS light-sensitive chip can be very useful in the future for determination of many enzymatic activities and for clinical diagnosis.

Enzyme kinetic data obtained by this N^+/P-well photodiode is further compared with a standard biochemical spectrophotometer (Hitachi F4500 fluorometer) that is equipped with a photomultiplier tube as the light sensor. As shown in Figure 7, two very similar kinetic profiles are obtained. The Km, a constant of enzymatic reaction, of H_2O_2 is 1 mM, determined by either method. This example shows a successful system that combines enzymatic reaction and semiconductor sensors. This novel system expands the potential to be an inexpensive, rapid, portable, and easy to use light-detecting system for many applications in diagnosis. This system can also substitute for the fluorescence meter, luminescence meter, and UV/Vis spectrometer in the laboratory for specific enzymatic measurements.

Conclusions

The fusion of biotechnology with the semiconductor and microelectronic industry has great potential for generating advanced technology and novel devices to solve a variety of biological problems. With further miniaturization and integration, enzyme chips will be used not only to monitor biochemical reactions but also to record ongoing biological processes, analyze complex biological reactions, and respond to abnormal biochemical reaction for disease treatment. Rapid advancement in biotechnology has no signs of stopping in the foreseeable future. The information provided by functional genomics and proteomics may be considered as a map for "treasure hunting" in many different applications. One of the most efficient experimental tools for discovering nature's treasure may be the semiconductor biochip.

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